

2022

PREDICTION OF CARDIOMETABOLIC RISK IN CHILDREN

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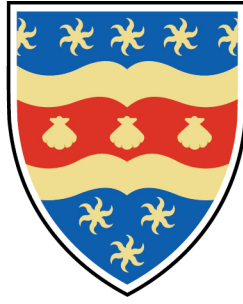
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<http://dx.doi.org/10.24382/543>

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UNIVERSITY OF PLYMOUTH

PREDICTION OF CARDIOMETABOLIC RISK IN CHILDREN

by

OLUBUKOLA AJALA

A thesis submitted to the University
of Plymouth in partial fulfilment
for the degree of

DOCTOR OF PHILOSOPHY

Peninsula Medical School

September 2022

Acknowledgements

I am deeply grateful to my supervisors, Professor Jon Pinkney, Doctor Gail Rees, and Doctor Jo Hosking, for their invaluable support, mentorship, and guidance. Their insightful feedback was critical in formulating the research questions and methodology and encouraged me to put in my best effort

I would like to thank Terence (Terry) Wilkin (1945–2017), Professor of Endocrinology and Metabolism, whose vision and original thinking led to the creation of the EarlyBird Study as well as the establishment of the collaboration with Nestle which made the metabolomic study reported in this thesis possible.

Terry gave me the opportunity of a lifetime when he invited me to join his team; he taught me so much and remained a mentor to the end. I owe him everything.

I would also like to thank Dr. Linda Voss who along with Terry set up the entire study project and kept it going through the years.

Karen Brookes and Val Morgan were the research nurses, who measured all the anthropometry and collected blood samples from the study participants. I acknowledge the radiology technicians who acquired the measurements which was used to derive estimates of body composition

I would like to acknowledge Francois-Pierre Martin (Nestlé Research, Lausanne, Switzerland) who led the collaboration and Ondine Walter the biobanking, sample handling, preparation, and measurement of the metabolites, which allowed metabolomic profiling.

I would like to thank my husband and best friend Babajide for providing me with the freedom to do this; thank you for all the sacrifices you made throughout this process.

For my children: Amy and Ethan, my pride and joy, I love you so much.

My parents: John and Wumi for their unconditional love and support; my big sister, Ade, who excelled in her doctoral study and provided constant encouragement and

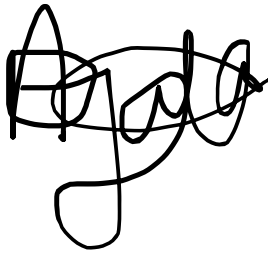
feedback; to my sisters: Ronke and Sola, thank you for supporting me; I will never forget your kindness and love.

To my brother, Seyi, my friends too numerous to count, my mentors: Professor Stuart Ross, and Professor Constance Chik, thank you.

Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

A handwritten signature in black ink, appearing to be 'A. J. G.', with a large loop at the end.

Publications:

Childhood Predictors of Cardiovascular disease in adulthood. A systematic review and meta-analysis. *Obesity Reviews*; 2017; 18 (9):1061-1070

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Oral presentations:

American Association of Clinical Endocrinologists 26th Annual Conference (2017)

Austin, Texas

Postgraduate Research Conference 2017; University of Surrey, United Kingdom

Word count: 60,888

Abstract

Olubukola Ajala

Prediction of Cardiometabolic risk in children

Background: Three decades of clinical research have demonstrated that the risks of atherosclerotic cardiovascular disease (ACVD: coronary disease and ischaemic stroke), and the closely related type 2 diabetes (T2D) are established and influenced by events during childhood. This is at least partly because childhood obesity usually leads to adult adiposity; so, the currently high prevalence of adult cardiometabolic disease, means that obesity needs to be identified as early as possible, ideally in childhood.

Screening for obesity largely relies on anthropometric measurements like body mass index (BMI), however this has many limitations including its inability to distinguish lean from fat mass. There is therefore a need for other reliable predictors of AVCD and T2D

Aim: The focus of this doctoral study was to investigate the ability of various measures of adiposity, nutrition, and metabolomic biomarkers to predict cardiometabolic risk at age 16y.

Methods: A systematic review was conducted to determine the existing evidence on the role of anthropometry in determining future risk of CVD. A continuous metabolic risk score was calculated as a composite of insulin resistance, total/high-density lipoprotein cholesterol ratio, fasting triglycerides, and mean arterial blood pressure. Initial exploratory analysis was conducted followed by longitudinal analyses which included mixed effects modelling when appropriate

Findings: The systematic review indicated that childhood BMI predicts the risk of dysglycaemia, abnormal carotid-intima medial thickness (CIMT), and AVCD events in adulthood; however, its ability to predict hypertension was weak.

A metabolic risk score was designed and utilised in these analyses because there is no universal definition of the metabolic syndrome and in children and adolescents, the prevalence rate of the latter is very low. The score identified those with high adiposity and was also able to detect those most likely to have the metabolic syndrome.

The analysis also showed that simple anthropometric measures of adiposity were at least as effective as fat mass measured by dual energy x-ray absorptiometry in detecting those at an elevated risk of developing cardiometabolic disease in the future

Higher intake of energy and certain macronutrients were predictive of the trajectory of metabolic risk and some of its components, but not of adiposity.

Insulin resistance, Triglycerides, Metabolic risk score, were higher in those who consumed greater energy, lower fibre, higher sugar, and higher saturated fat at 8y.

Also, this unique longitudinal study of homogenous cohort of children and adolescents showed that those who had a high metabolic risk score at 16y had higher levels of specific products of branch-chain amino acid metabolism.

Further studies are required to keep following this cohort to confirm the association of the score with adult-diagnosed metabolic syndrome, type 2 diabetes, and CVD events.

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Abbreviations

AVCD: Atherosclerotic cardiovascular disease

BMI: Body Mass Index

MABP: Mean Arterial Blood pressure

CAD: Coronary artery disease

CIMT: Carotid intima media thickness

CVD: Cardiovascular disease

DBP: Diastolic blood pressure

DRV: Dietary reference values

DEXA: Dual-energy X-ray absorptiometry

EAR: Estimated annual requirements for energy

HDL: High-density lipoproteins

HOMA-IR: Homeostatic Modal Assessment of Insulin Resistance

IGT: Impaired glucose tolerance

IHD: Ischaemic heart disease

IR: Insulin resistance

LBM: Lean body mass

LDL: Low-density lipoproteins

MRS: Metabolic Risk Score

MetS: Metabolic syndrome

MVPA: Moderate-and-Vigorous Physical Activity

PP: Pulse pressure

PVR: Peripheral vascular resistance

PWV: Peak weight velocity

SBP: Systolic blood pressure

SF: Sum of 5 skin folds/Skin folds

SHBG: Sex hormone-binding globulin

T2D: Type 2 Diabetes

TC: Total cholesterol

VLDL: Very low-density lipoproteins

WC: Waist circumference

Chapter 1

Introduction

1.1 Estimating Cardiometabolic Risk

Over the last 50 years, several factors have become accepted as predictors of cardiovascular disease (CVD); these include gender (male sex), lipid profile, blood pressure, smoking, and dysglycaemia. These form the basis of risk prediction scores and algorithms, like the widely used Framingham risk score (Kagan A, 1976).

These scores provide an estimate of the future (typically 10-year) risk of CVD. (Thanassoulis and Vasan, 2010) (Hippisley-Cox J, 2007) and have been shown to be good at identifying those at high risk of CVD; however up to 20% of people who suffer a myocardial infarct do not have any of these risk factors (Cook and Ridker, 2009). So, while the identification of traditional risk factors remains critical in strategies required to predict CVD, there is also a need to identify novel biomarkers and other risk factors to improve the performance of available risk scores.

One of the areas that have not been adequately explored is the influence of events in childhood on the risk of diseases seen in adulthood. There is strong evidence that the presence of some of the traditional risk factors in children can lead to a higher risk for CVD events and Type II Diabetes (T2D) when they are young adults (Lauer RM, 1988) (Juonala M, 2008).

However, the majority of the above studies have only been able to evaluate surrogate measures of CVD events like the presence of atherosclerotic lesions, and carotid intimal-medial thickening (CIMT); this is because long-term studies are difficult to conduct. This does not diminish the importance of exploring these relationships because

the prevalence of CVD events and T2D have increased in the last century and remain among the most pressing health challenges due to the associated high mortality and morbidity (Ford ES, 2004), (Gami AS, 2007) (Ehtisham S, 2004).

Berenson et al showed that the number of atherosclerotic lesions in young adults was correlated with the number of specific risk factors in childhood including, hyperlipidaemia, hypertension, and obesity (Berenson GS, 1998). The Muscatine as well as the Cardiovascular Risk in Young Finns study also reported associations between increased carotid intimal medial thickness (CIMT) and total cholesterol and hypertension in childhood (Juonala M, 2008) (Lauer and Clarke, 1989).

Another mechanism that might explain the association between childhood adiposity and future insulin resistance is the theory of ‘adipose tissue expandability’; white adipose tissue (which forms of bulk of adipose tissue in humans), expands during periods of overnutrition (Caprio S, 2008) through hypertrophy and hyperplasia of adipocytes. The increased adipocyte tissue demands expansion of the vascular network; for this to occur, pro-angiogenic factors like vascular endothelial growth factor (VEGF), need to be produced. The trigger for these factors might be the relative hypoxia that occurs in the expanded adipose tissue (Bouloumie A, 2002), infiltration of the tissue by pro-angiogenic macrophages (Osborn O, 2012) (Wellen KE, 2003), and the increased pro-inflammatory cytokines that accompany excess caloric intake (Han J, 2011)

In animal models, it has been shown that there is a direct link between the increase in adipose tissue vascular networks and hyperinsulinemia (Gurav O, 2014)

In these studies, the consumption of high fat diets directly stimulated the growth of adipose tissue, and increased the expression of several genes, most notably insulin-growth-factor bp4 (IGFbp4) which increases the production insulin-growth-factor-1 (IGF-1); IGF-1 enhances the production of the vascular network required to support the

increase in adipose tissue. In addition, the increase in IGF-1 also leads to increase in insulin secretion.

Adipose tissue is not evenly distributed throughout the body, and there is clear evidence that its function is influenced by its location. In people with higher proportion of adipose tissue in the upper body, there is a higher risk of T2D and CVD events, while in those with a higher proportion in the lower body, the risks of these diseases are lower (Manolopoulos, 2010)

Gender also modulates the distribution of body fat; adult females typically have higher adipose accumulation in the lower body, while men have higher fat in the upper body. This is thought to at least in part, explain higher prevalence of CVD in men (Karastergiou K, 2012)

Even though adipose growth occurs throughout life, the rate is fastest during childhood; therefore, considering the strong association between adipose tissue expandability and the risk of cardiometabolic disease, it is crucial to increase the understanding of the mechanism behind these changes in children (Arner E, 2010).

In 2008, the 'twin cycle hypothesis' was proposed; it postulates that chronic overnutrition leads to accumulation of liver fat which eventually causes inhibition of prandial insulin secretion, ultimately leading to T2D (Taylor R, 2008)

In over-nourished states, any carbohydrate ingested undergoes lipogenesis and is stored in the liver as fat; since insulin stimulates lipogenesis, this process is amplified in those with insulin resistance. The increased liver fat in turn exacerbates hepatic gluconeogenesis, so that there is a constant cycle of hyperinsulinaemia and increased liver fat (Adiels M, 2006)

The finding of high remission rates from T2D in adults who underwent gastric bypass surgery (Guidone C, 2006), validates the twin cycle hypothesis. After bariatric surgery,

there is a sharp reduction in calorie intake; this forces the body to utilise the stores of fatty acid intermediaries. Since these intermediaries usually inhibit glucose metabolism, their reduction leads to more glucose becoming available as a source of energy, ultimately leading to lower blood glucose levels (Guidone C, 2006).

Some studies have measured the risk of T2D and CVD by using components of the metabolic syndrome (MetS); Koskinen et al showed that children with the metabolic syndrome were up to 3 times more likely to have high CIMT and type II diabetes than those without (Koskinen, 2014). Like other studies, the MetS was defined using adult criteria; this is because there are at least 7 definitions of the MetS and the incidence is very low in children (Alberti K, 2005) (Eckel RH, 2005) (Federation, 2005a) (Grundy S, 2004) (Kahn R, 2005) (Hadjiyannakis S, 2005).

Other limitations to the use of the MetS includes the fact that each component is defined by specific cut-offs above which the risk of T2D is deemed high; however, normal ranges for these components are not defined in childhood because children are at an earlier stage in the natural history of the MetS. This is another reason to utilise other methods to detect and characterise metabolic dysfunction in children.

In this thesis, a continuous metabolic risk score (MRS) was derived from components of the MetS; this approach is widely used in epidemiological studies of children (Eisenmann JC, 2010) (Hadjiyannakis S, 2005), and while there are variations in the components, these scores are usually derived from both metabolic and cardiovascular disease risk factors. When compared to dichotomisation of each risk in age-modified definitions of the MetS, the MRS have been shown to have a stronger association with cardio metabolic disease outcomes (Hadjiyannakis S, 2005) (Olfert MD, 2019) (DeBoer MD, 2017). This analysis therefore evaluated a composite MRS and the relationship between its components.

Because the traditional risk factors are not sufficient in predicting the risk of CVD events and T2D, other studies have evaluated the predictive power of genetic risk. Most have evaluated the single nucleotide polymorphisms that are associated with CVD and T2D, and combined them into genetic risk scores (Knowles JW, 2018) and, or into the Framingham risk score (Kagan A, 1976). However, most have not been able to consistently improve risk prediction particularly when compared to the traditional risk factors (Junyent M, 2010).

Overall, genetic risk scores explain less than 5% of the variance in risk for CVD (Ioannidis JP, 2009) so are not yet useful in helping clinicians make meaningful treatment decisions. Therefore, traditional risk factors remain the mainstay of CVD risk prediction and are promoted as the basis of public health screening in children.

The evidence base for screening using traditional risk factors, comes from clinical trials like the Lipid Research Clinics Coronary Primary Prevention and the Hypertension Detection and Follow-up Studies (Freedman DS, 2011) (Lauer RM, 1989); there is however no consensus on whether to screen all children (i.e universal screening), or only those with a family history of premature CVD (i.e targeted screening) (Expert Panel on the Detection, 2001b). There are several limitations to both strategies: universal screening is expensive, and it is not certain that it will lower the future risk of CVD; on the other hand, targeted screening relies on the access of parents to healthcare, as well as their knowledge of their medical history. Also, as highlighted above, parental risk factors are not adequate to identify those most at risk for CVD.

In view of all of the above limitations, as well as the gaps in knowledge of this important subject, this study explored the published literature on available predictors of cardiometabolic risk, the current methods of measuring cardiometabolic risk in children, and the utility of a metabolic risk score in children was presented. In this thesis, the

traditional risk factors were studied, as well as others like anthropometry, nutrition, and more novel areas like metabolomic markers. The discussion in the following sections will highlight the significant limitations of the existing methods for predicting cardiometabolic risk in children and the need for more reliable options.

1.1.1 Lipid Profile

In clinical practice, levels of plasma lipids are considered one of the most important predictors of AVCD in adults. The various fractions of cholesterol and triglycerides (TG) are mostly synthesised in the liver (Figure 1), while less than 20% are dietary in origin (Figure 2).

Lipids are transported in the blood by lipoprotein complexes, which include low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), and intermediate density lipoproteins (IDL); TC includes all these fractions. Triglycerides are produced in the small intestine and hepatic cells (Figure 2) and deliver free fatty acids required for cellular processes.

To maintain equilibrium in cholesterol levels, any excess LDL molecules are ingested by macrophages residing on arterial walls; this sets off an inflammatory process that ends with the macrophages being transformed into ‘foam cells’. The latter forms the basis of atherosclerosis (Owsiany KM, 2019) and explains the association between high LDL and ACVD (Expert Panel on the Detection, 2001b) (Expert Panel on Detection, 2002); for instance, every 1mmol/L reduction in LDL cholesterol levels leads to a 19% reduction in AVCD deaths and 12% reduction in all-cause mortality at 5 years (Soran H, 2017).

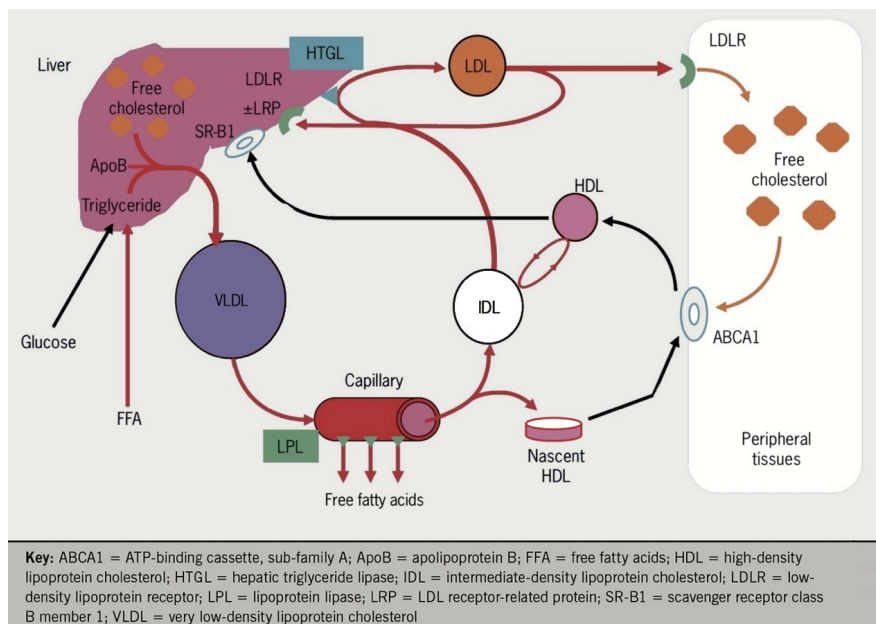


Figure 1. Hepatic Lipoprotein synthesis.
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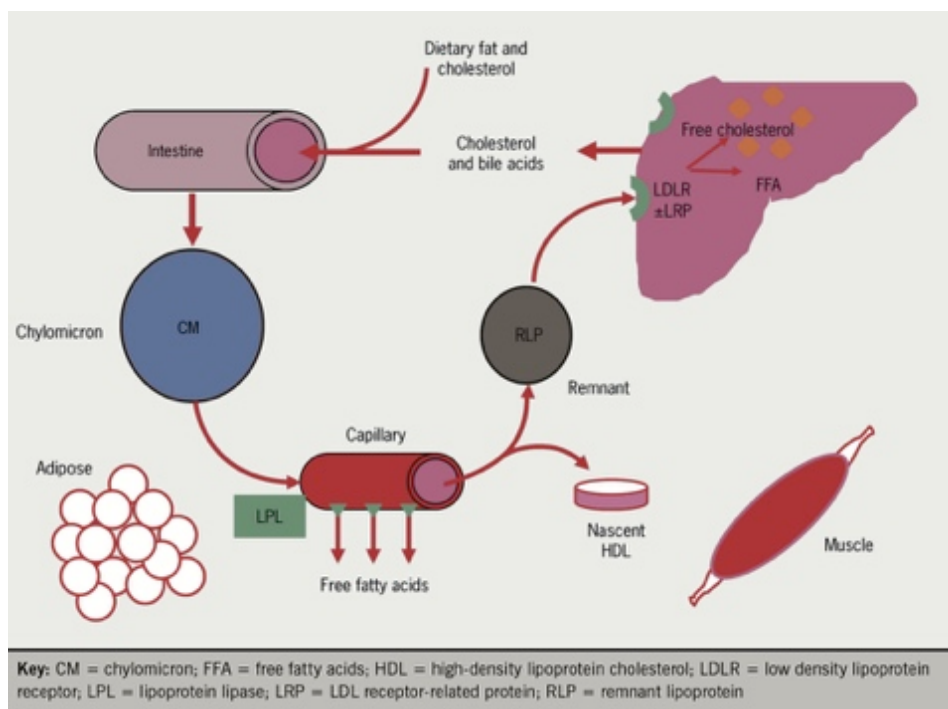


Figure 2. Intestinal Lipoprotein synthesis
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Several researchers argue that LDL needs to be further stratified into its components; this is because even though IDL (which forms the major proportion of LDL) is more

predictive of AVCD events (Soran H, 2017), it is not currently measured in routine clinical practice. However, after statistical adjustments were made for triglycerides and HDL there was no evidence of an increased predictive ability for AVCD events (Gami AS, 2007).

HDL has protective properties when AVCD events are considered (Assmann G, 2004); this occurs through various means: the most important is because of its ability to transport cholesterol from cells to the liver to be excreted. HDL particles also reduce the inflammatory response caused by elevated LDL (described above), and prevents platelet activation (Assmann G, 2004). This explains the 25% increase in AVCD events seen with every 0.13 mmol/L reduction in HDL (A et al., 1976).

Also, at lower LDL levels (<3.2 mmol/L), HDL cholesterol is a stronger predictor of AVCD events; for every 0.26 mmol/L increase in HDL, the rate of AVCD events fell by 29% in those with LDL < 3.2 mmol/L, but only 10% in those with an LDL ≥ 3.2 mmol/L (Sampson UK, 2012). In addition, proinflammatory conditions like diabetes and obesity lead to partial inactivation of functional HDL particles and cause elevation of LDL; the mechanisms involved are complex but include an increase in the hepatic production of apolipoprotein B (apoB). ApoB is the major component of VLDL and LDL, so the net result in these states, is an increase in both (Farbstein D, 2012).

Other mechanisms include the increased lipolysis in adipocytes caused by insulin resistance, which is present in most forms of Type II diabetes and a significant proportion of obese individuals. This leads to high levels of free fatty acids, which are transported to the liver and lead to increased production of VLDL and LDL (DF. van Wijk ES, 2009).

Large epidemiological studies have consistently shown a strong association between triglyceride levels and AVCD (Hokanson JE, 1996); (Miller M, 2011). However, there is no robust evidence that proves that the use of triglyceride-lowering medications leads

to a reduction in ACVD events (Nordestgaard BG, 2014). There are several possible reasons for this; triglycerides usually have a skewed distribution in epidemiological studies (Nordestgaard BG, 2014); also, there are significant differences in the biological variability of triglycerides, and the analytical methods used in these studies (Jacobs DR Jr, 1982). Also, differences in race, gender, lifestyle, and use of certain medications introduces additional variations in the levels of triglycerides; even posture and other phlebotomy-related factors can lead to differences in triglyceride levels (Jacobs DR Jr, 1982)

There appears to be a stronger association between high triglyceride levels and AVCD events at lower levels of HDL and in the presence of T2D (Kugiyana K, 1999); (El HK, 2007); it is difficult to make statistical adjustments for all of these variables in epidemiological studies.

The atherogenic effects of triglycerides are mediated by several mechanisms including the activation of cholesterol ester transfer protein which leads to increased triglyceride attached to LDL and HDL resulting in small dense variants of the latter. This increases the amount of dysfunctional HDL as described above, as well, the denser LDL particles are more prone to oxidative modification compared with less dense forms (Marcel YL, 2008); (Sacks EM, 2002).

The discussion laid out above, explains the pathogenesis of CVD caused by dyslipidaemia in adults; there is however still limited data that shows a clear link between lipids and lipoproteins to the atherosclerotic process in children, or to the incidence of CVD when they become adults (Daniels SR, 2008). To determine this, others have extrapolated information from studies of children who have homozygous familial hypercholesterolemia and receive LDL-lowering therapies from early life; in these studies, there was a reduction in CVD events in adulthood (Wiegman A, 2015). Also, post-mortem findings from the Bogalusa Heart and Pathobiological Determinants

of Atherosclerosis in Youth (PDAY) studies showed that atherosclerotic lesions were present in those who had high levels of TC, non-HDL cholesterol, and LDL; however after adjustments were made for the presence of obesity, hypertension and cigarette smoking, only non-HDL cholesterol was found to be the major risk factor for coronary atherosclerosis (Daniels SR, 2011) (Expert Panel on Detection, 2002, Zieske AW, 2002). Similarly, several studies have also shown that LDL levels in childhood could predict the carotid-intima-media thickness (CIMT) in adulthood (Raitakari OT, 2003) (Juonala M, 2008), however they did not make adjustments for the influence of obesity. Determining the prevalence of abnormal lipid levels in children is difficult for many reasons: gender, ethnicity, and most significantly, puberty, influence the levels of circulating lipids and lipoproteins considerably (Christensen B, 1980, Zieske AW, 2002). Also, the cut-off points used in paediatric studies are based on adult data (Daniels SR, 2008) (Kavey RE, 2003).

Those who recommend the use of adult cut points rely on the fact that lipid levels are fairly constant from childhood, at least till early adulthood; for instance in the Muscatine study, 75% of children who had TC levels > 90th percentile still had elevated TC (≥ 5.2 mmol/L) at 20–25 years of age (Lauer RM, 1988) (Berenson GS, Bogalusa Heart Study Research, 2002).

All these findings show that while lipid parameters can predict AVCD in adults, there is less information on their value as long-term predictors in children. To prove that there is enough evidence to support the role of screening of children for dyslipidaemia, longitudinal studies of contemporary cohorts need to be conducted.

1.1.2 Blood Pressure

Blood pressure is an important and well-established predictor of AVCD events in adults; however less information is available on its role in children.

Blood pressure is the product of cardiac output and peripheral vascular resistance; cardiac output is typically constant, while peripheral vascular resistance varies based on the structure and function of vasculature (Figure 3).

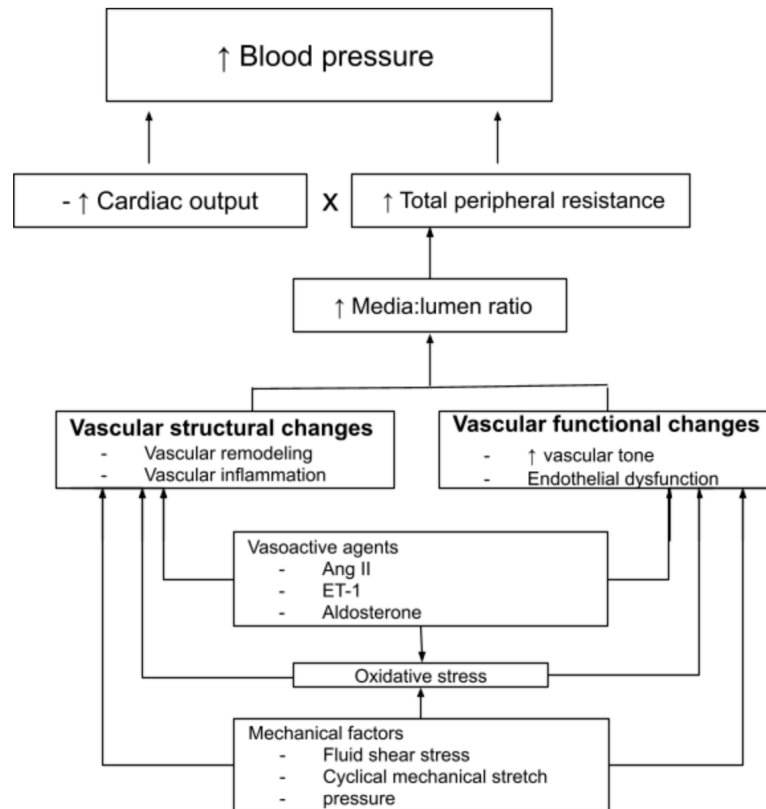


Figure 3. Pathogenesis of elevated blood pressure.

Large epidemiological studies in adults have consistently shown positive associations between both systolic and diastolic blood pressure (SBP, DBP) and the risk of AVCD (Franklin SS, 2009); but because blood pressure is characterized by pulsatile and steady components, recent studies have looked at these aspects of blood pressure in more detail (Glynn RJ, 2000, Staessen JA, 2000, Lewington S, 2002a)

Pulse pressure, is the ‘pulsatile component’, and is determined by blood pressure variations which are caused by changes in left ventricular ejection fraction, large-artery stiffness, early pulse wave reduction, and heart rate (Lewington S, 2002a). The ‘steady

component,' determines mean arterial blood pressure (MABP), which is a function of left ventricular contractility, heart rate, vascular resistance and elasticity averaged over time. MABP has traditionally been measured in terms of peak (systolic) and trough (diastolic) pressures, but also in terms of pulse pressure (Lewington S, 2002a, Palaniappan L, 2002).

Like the other risk factors for AVCD, elevated blood pressure causes endothelial dysfunction, which sets off a cascade of events that elevates oxidative stress and causes vascular remodelling (Vasan R.S., 2001). Although it remains controversial which measures of blood pressure, either alone or in combination, best predict the risk of CVD, systolic blood pressure has been shown in most studies to be the most predictive of CVD and mortality (Domanski MJ, 1999) (Kearney P, 2005) (Palaniappan L, 2002). Irrespective of the type of measurement, it is clear that elevated blood pressure is the most important cause of AVCD with both models (SBP/DBP vs. MAP/PP), being very effective in their predictive value (Franklin SS, 2009).

Defining elevated blood pressure in children is controversial and not consistent amongst clinical trials; some use values greater than the 95th centile, the latter based age, gender and height (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

Others use the adult definitions: Grade 1 hypertension: blood pressure $\geq 140/90$; Grade 2 hypertension: blood pressure $\geq 160/100$ (Unger T, 2020)

The use of blood pressure percentiles is complicated and time-consuming so is rarely used in clinical practice; so if it was the only means of diagnosing hypertension, there would be low detection in children (Hansen ML, 2007). Also, the relationship between blood pressure with age and height is non-linear (Rosner B, 2008) so the conventional regression methods used to determine the percentiles might not be accurate for this purpose (Wang Z, 2012).

In addition, age does not influence blood pressure but height and body size does (Wang Z, 2012); therefore, the current guidelines for assessing blood pressure according to age may be unnecessary.

In adults, there is a continuous relationship between blood pressure (both SBP and DBP) and cardiovascular events (Lewington S, 2002b); for instance, each 20mmhg increase in systolic and 10mmhg increase in diastolic blood pressure above 115/75 leads to a doubling in the risk of death from a CVD event (Joint National Committee on Prevention, 2003). Despite this, threshold-based blood pressure classifications are still widely used to categorise people into stages of hypertension because these classes make it possible to compare the findings of various intervention trials and set treatment targets in clinical practice.

Like adults, the use of categories in children is not evidence-based; this is in part because the onset of hypertension-induced cardiovascular events takes many years, large longitudinal intervention studies in children are lacking, and most of the current recommendations in children are extrapolated from evidence obtained in adults.

There is therefore a critical need for longitudinal studies to fill the gaps in knowledge on this subject.

1.1.3 Insulin Resistance

Insulin resistance (IR) is a well-established risk factor for the development of T2D and AVCD. It occurs when there is a reduction in the cellular response to insulin-mediated actions, and is usually associated with obesity (Levy-Marchal C, 2010).

There are however normal-weight children who develop insulin resistance, typically at the time of peak pubertal development (Goran MI, 2001), and there are also obese children who do not develop insulin resistance (Sinaiko AR, 2005).

The pathogenesis of insulin resistance has been studied using euglycemic hyperinsulinemic clamps in adult populations, and has been shown to mostly occur

when there is an impairment in the response of skeletal muscle to insulin action (Ferrannini E, 1997).

There are significant differences between the severity of insulin resistance between children and adults; in general, children and adolescents appear to have a higher degree of insulin resistance even when they have similar degrees of adiposity and glycaemic control as adults (Arslanian SA, 2018). The mechanisms that drive these differences are still not clear, but one hypothesis is that children and adolescents usually have lower levels of HDL cholesterol; HDL is pivotal in inducing glucose disposal through skeletal muscle, so low HDL induces insulin resistance. Also, there are profound physiological changes in IR during puberty, but little information on the long-term consequences of these changes.

In adults who have other risk factors for AVCD like central obesity, lipid abnormalities, and type 2 diabetes, there is still evidence of an independent association between IR and AVCD events (DeFronzo RA, 1991) (Eckel RH, 2005); this is most apparent in those with T2D who remained at a high risk for developing atherosclerosis even after adjustments were made for confounders like adiposity, dyslipidaemia, and hypertension (Després JP, 1996) (Pyorala K, 1979) (Welborn TA, 1979).

The pathophysiology includes the deleterious effect of insulin on platelet action (Trovati M, 1988) and endothelial function (Kahn AM, 2000) (Steinberg HO, 1994), as well as the inhibitory effect of insulin on fibrinogen synthesis (Kahn AM, 2000). Insulin also enhances oxidation of LDL cholesterol (Steinberg HO, 1994) promotes plasminogen activator inhibitor 1 and endothelin-1 release, and stimulates cholesterol synthesis and LDL receptor expression in the arterial wall (De Feo PP, 1991).

The net effect of the above is a loss of the anti-atherogenic properties of insulin and increased atherosclerosis (Faerch K, 2009).

Insulin acts on hepatic cells, skeletal muscle, and adipose tissue to regulate glucose uptake; in hepatic cells, insulin prevents gluconeogenesis and glycogenolysis, which leads to reduction in the production of glucose and induces glycogen storage. In skeletal muscle and adipose tissue, it enhances glucose uptake and storage. Therefore, insulin resistant states can lead to hyperglycaemia.

The gold standard for the assessment of insulin resistance is the hyperinsulinemic euglycemic clamp, but the intravenous glucose tolerance test and the insulin tolerance test are easier so are more frequently performed (Buchanan TA, 2010). However, all these tests are invasive, expensive, and difficult to perform, so they are not used in clinical practice or large epidemiological studies (Ferrannini E, 1998).

Instead, several surrogate means of measuring IR are utilised, the most popular are fasting plasma insulin, the homeostasis model assessment insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI), all of which have been validated against the hyperinsulinemic-euglycemic clamp (Schwartz B, 2008) (Gungor N, 2004); in addition, these methods are particularly useful in longitudinal studies which require repeated measurements over time.

Several risk factors for IR have been identified including certain ethnicities, the onset of puberty, genetic risk (for T2D, adipose tissue variant depots), and intrauterine foetal growth patterns.

Caucasian children appear to be more affected by IR than African, American, Hispanic, Pima Indian and Asian children (Goran MI, 2002) (Levy-Marchal C, 2010) (Arslanian SA, 2002); these differences are present even at similar body weight. The cause of this difference is not clear, but it has been hypothesized that variations in the proportion of lean mass might be a contributory factor (Kodama K, 2013).

During puberty, there is a 25-50% increase in insulin resistance; this occurs independent of adiposity, and usually resolves at the end of puberty (Moran A, 1999). The

underlying mechanisms are not clear, but might include the increase in growth hormone that occurs during puberty in order to enhance linear growth (Luna AM, 1983) (Moran A, 2002); growth hormone contributes to insulin resistance because it increases lipolysis and free fatty acid levels, as well as being an insulin antagonist (Caprio S, 1989).

The distribution of visceral fat whether determined by genetic factors or lifestyle, is also a strong predictor of insulin resistance (Després JP, 2008); the fat depots secrete free fatty acids which cause endothelial dysfunction, and lead to elevation of pro-inflammatory substances like C-reactive protein, adiponectin, interleukin-6 (IL-6), and plasminogen activator inhibitor-1, all of which cause insulin resistance (Preis SR, 2011).

Insulin resistance usually progresses to T2D (Levy-Marchal C, 2010), which is the main means by which it causes AVCD (Bonora E, 2007) (Jeppesen J, 2009). Some studies have tried to determine if there is a direct association between insulin resistance and AVCD, and they found that it causes endothelial dysfunction, specifically endothelium-dependent vasodilation, but only in those who were obese, or with T2D (Hamburg NM, 2008) (Lee S, 2007).

So, while insulin resistance is associated with a higher risk of T2D, further research is required to determine if it is also a direct cause of AVCD.

1.1.4 Metabolic Syndrome and Metabolic Risk Scores

The metabolic syndrome (MetS) is used to denote the clustering of risk factors associated with the development of atherosclerotic cardiovascular disease (AVCD) and type 2 diabetes (T2D) (National Cholesterol Education Program (U.S.). Working Group on Lipoprotein Measurement. National Institutes of Health, 1995).

MetS is associated with at least double the risk for cardiovascular events and deaths, (Ford ES, 2002) (Gami AS, 2007) and is considered to be present in over 25% of the US adult population (Gami AS, 2007) (ATP III., 2001). A major limitation for its use in

clinical practice is the absence of a universally accepted definition; in adults, there are at least seven definitions in routine use (Alberti K, 2005) (Grundy S, 2004) (Grundy S, 2005) (Federation, 2005a), and most define the MS as subset of at least 3 out of 5 of the following: increased central adiposity, elevated triglycerides, decreased HDL-C, elevated blood pressure, and hyperglycaemia.

The use of the MetS is highly controversial in children for several reasons; first, there is no clear definition in this population, in part due to the multiple definitions used in adults making it difficult for clinicians to decide which to use. Also, most cases of the MetS occur in the presence of obesity so some argue that it does not have any utility above and beyond obesity itself, such that the treatments offered to people with the MetS is usually focused on inducing weight loss (Steinberger J, 2009).

The MetS always occurs in the setting of increased insulin resistance (Lee S, 2007); insulin is produced by the pancreatic β cells and is then transported to the liver where it suppresses glucose production. Therefore, in the insulin-resistant state, the normal suppression of hepatic glucose production is impaired, leading to abnormal glucose metabolism.

Insulin also stimulates lipogenesis, so insulin resistance leads to increased release of free fatty acids and triglycerides into the circulation. This results in dyslipidaemia and increased deposition of adipose tissue (Bremer AA, 2012).

The various adult definitions for the MetS have similarities but also important differences: they all require a minimum number of risk factors, and most exclude those with T2D; on the other hand, there are differences between the types, required number, and the cut points for the criteria. For instance, the NCEP ATP III definition includes hyperglycaemia, hypertriglyceridemia, low HDL-C, hypertension, and increased waist circumference (Expert Panel on the Detection, 2001a); the AHA/NHLBI definition is similar but uses a different glucose cut point down and allows for the use of cholesterol-

lowering therapy (Kahn R, 2005). Also, the IDF definition uses different cut points for waist circumference based on ethnicity, and makes waist circumference a necessary criterion for the diagnosis (Federation, 2005b).

These differences means that for instance, in the presence of hyperglycaemia, hypertriglyceridemia, and low HDL-C, an adult with a normal waist circumference would be diagnosed with MetS by the NCEP criteria but not by the IDF. This therefore significantly affects the diagnostic and prognostic ability of the MetS, as well as the accuracy of prevalence rates in various populations.

When compared to adults, determining the incidence of MetS in children is even more difficult because the different definitions means that even when the same population is studied, prevalence rates range from 4% (Cook S, 2003) to 9% (de Ferranti SD, 2004).

There are also familial influences, including genetic and environmental, which increase the odds of inheriting the risk factors that make up the MetS; evidence for this has been mostly obtained from twin studies that show the significant inheritability of high blood pressure, diabetes, obesity, and dyslipidaemia (Bao W, 1997) (Whitaker RC, 1997). The intrauterine and postnatal environment can also lead to higher likelihood of obesity and other MS components; in particular, maternal gestational diabetes, low birth weight, especially with rapid catch-up growth; restrictive or over-feeding during infancy, have been identified as major factors (Zimmet P, 2007) (Fadzlina AA, 2014).

In a bid to tackle these limitations, the International Diabetes Federation (IDF) developed a consensus definition for children (Alberti K, 2005) by recommending the use of the adult definition in children above the age of 10y. Other groups have made similar recommendations, all based on adult definitions (Weiss R, 2004) (Hadjiyannakis S, 2005) (Grundy S, 2005), there is however no widely accepted definition of the MetS in children.

There are racial differences in the incidence of each component of the metabolic syndrome; for instance compared to other groups, black children and adults have lower rates of dyslipidaemia, higher insulin resistance, and higher blood pressure (Kit BK, 2012) while Hispanic people have higher rates of dyslipidaemia (Kit BK, 2015). This means that even at similar degrees of adiposity, black children have a lower prevalence of the metabolic syndrome compared to other ethnicities (Duncan GE, 2004).

These differences mean the metabolic syndrome may not be accurate means of determining cardiometabolic risk in some ethnic groups.

Considering that there is no consensus on the definition of MetS, the fact that it is affected by puberty therefore not stable throughout childhood, and the uncertainty about its ability to predict future CVD, has led clinicians to use other means to identify those most at risk cardiovascular and metabolic complications.

For this reason several health agencies like the American Academy of Paediatrics (AAP) recommends that clinicians identify those with several CV risk factors, also described as ‘cardiovascular risk factor clustering’, instead of relying on any of the definitions of the MetS (Berenson GS, 1998) (Berenson GS, Bogalusa Heart Study Research, 2002).

Another reason for utilising this approach is the fact that there is a strong association between the incidence of atherosclerotic lesions in children, and increased clustering of atherosclerotic CVD risk factors (Berenson GS, Bogalusa Heart Study Research, 2002). Also while the MetS definitions all use discrete cut points for each risk factor, in reality, these factors are in a continuum so continuous variables are more reliable in predicting risk of cardiometabolic disease than the metabolic syndrome which dichotomises each risk factor (Kelly AS, 2011) (Eisenmann JC., 2010) (McMahan CA, 2005).

1.2 Measures of Obesity

1.2.1 Body Mass Index

Body Mass Index (BMI) is the most popular means of detecting overweight and obesity in children in all settings outside of epidemiological studies and clinical research (Himes JH, 2009). This is because it is derived from 2 simple measurements: height and weight, neither of which are invasive, expensive to obtain, or difficult to measure.

Like other measurements of anthropometry, there are several factors that can affect the accuracy of BMI; these include errors in the accuracy of either component: for instance, variations in the state of hydration, bowel and bladder contents, and position can introduce errors in measurements of height and weight of children (Lampl M, 2001).

The effect of these variations can be significant; one study found a 1.5cm difference in the height of children between morning and afternoon due to changes in the water content of the intervertebral discs (Strickland AL, 1972) and up to 0.75kg difference in weight (Khosla T, 1964).

In a bid to minimise these errors, multiple measurements of height and weight are utilised by researchers; however, it is not realistic for clinicians to undertake this time-consuming task on a regular basis.

BMI is total body weight in kilograms divided by the square of height in meters, so in essence it is a measure of weight adjusted for height; it therefore cannot distinguish fat from lean mass. Understanding this limitation is pertinent in childhood because the rapid rate of linear growth in children leads to significant changes in weight and height that amounts to a 50% increase in BMI. In boys, most of this increase is in fat-free mass/lean mass so BMI can inaccurately misclassify them as overweight/obese. (VanItallie TB, 1990) (Wells JCK, 2006).

Also, there are significant racial differences in the amount of body fat at the same level of BMI. For instance, at similar BMI levels for age, Asians have a higher body fat than

white Caucasian, who in turn, have a higher body fat than Blacks (Wagner DR, 2000) (Freedman DS, 2006).

In view of these limitations, some investigators use the 85th and 95th percentiles of BMI-for-age to define overweight and obese respectively, or link BMI at 18y to the adult classification; however, these cut-offs are not based on associations with disease outcomes. Also, virtually all individuals in the reference population are white Caucasians living in industrialized and wealthy countries, so the findings cannot be generalized (Prospective Studies, 2009). Others have tried comparing weight to height centiles (Cole TJ., 2002), or attempt to validate BMI by comparing it with other anthropometric measures like skin fold thickness (Mei Z, 2007) (Freedman DS, 2009). However, there is no consistency in the cut points chosen in these studies thereby introducing variability in the screening performance. BMI z-scores have also been explored as an alternative, mostly in epidemiological studies; they are standard deviation scores that measure weight adjusted for the child's age and gender. The scores are calculated based on external references (National Centre for health and statistics, CDC growth charts, 2000) (Cole TJ, 1990); when used in epidemiological studies they can be converted into equivalent BMI-for-age percentiles using a normal distribution table, in clinical practice the charts can be used to track weight through childhood.

To evaluate change over time, for instance from childhood to adulthood, BMI needs to be examined as a continuous variable instead of in categories; these scores are well suited for the requirements of statistical analysis of continuous variables (Cole TJ, 2005) (Chinn S, 2002).

A potential drawback however is that the reference data used to create these z-scores were constructed from surveys collected in the 1980s – 1990s when there was already a trend showing higher incidence of childhood obesity; it is therefore probably only

appropriate to use these reference charts on adults who were children at that time (Wright CM, 2002).

Despite being such an imperfect tool, BMI is still the most used means for detecting obesity in adults; however, there is not enough evidence to determine its effectiveness as a screening tool to predict cardiometabolic disease in children

1.2.2 Waist Circumference

In adults, waist circumference (WC) is a reliable marker of intra-abdominal adipose tissue and can predict the development of cardiovascular diseases, type 2 diabetes mellitus, and premature death (Rankinen T, 1999) (Rexrode KM, 1998) (Chan DC, 2003) (Lean ME, 1995); intra-abdominal adipose tissue/visceral fat is a stronger predictor of the metabolic syndrome than peripheral fat (Carr DB, 2004).

Although BMI is thought to be the gold standard for identifying obese and overweight individuals, at least in adults, body fat distribution is a more important risk factor for obesity-related diseases. In adults, excess abdominal fat is associated with an increased risk of cardiometabolic disease, particularly type II diabetes, even more so than BMI.

Even after making statistical adjustment for the influence of BMI in adults, the relative risk of developing diabetes was ten times more in those at the highest category of WC, and two times more for ACVD (Wang J, 2003) (Yusuf S, 2011).

One drawback to the use of WC is the absence of a standardized approach for measurement. Also, WC is influenced by gender, ethnicity, and age (Visscher TL, 2001). Thus, WC is also not free of problems as a tool for assessing adiposity, central fat, and cardiometabolic disease risk.

Accepted methods of measuring total body fat and fat distribution include the use of X-rays (Dual-X-ray Absorptiometry), Computed Tomography (CT), and Magnetic Resonance Imaging (MRI); however the cost and the need for exposure to ionising

radiation (from X-rays and CT) precludes their use in clinical settings (Lohman T, 2005). This has led to widespread use of anthropometric measures like body circumference and skinfolds to estimate body fat and fat distribution. Being able to utilize anthropometry instead of direct measures of fat is critical because for the reasons stated above, the direct measures are not easily accessible.

Some studies conducted in children have shown strong relationships between WC and cardiovascular risk factors like dyslipidaemia and hyperinsulinemia (Cowin I, 2000) (Freedman DS, 1999b) however it is not certain if WC in childhood can independently predict CVD events in adulthood. Also most of the available data is from cross-sectional studies conducted in pubertal children only, so are not able to provide information on the ability of WC to predict risk of future CVD (Khoury M, 2013) (Bluhner S, 2013). In addition, unlike in adults, there is inadequate evidence of a strong relationship between WC in children and direct measures of intra-abdominal fat, which might prevent WC from being used as a substitute. Possible reasons for this include the fact that in children, WC may not be able discriminate between intra-abdominal and truncal subcutaneous adipose tissue. Epidemiological studies in this area have shown conflicting results; some suggest that in children intra-abdominal fat increases in proportion to overall fatness (Goran MI, 1995) while others show that obese children tend to accumulate subcutaneous more than intra-abdominal fat (Fox K, 1992).

Irrespective of how central body fat is distributed, waist circumference may identify children with high amounts intra-abdominal or subcutaneous fat (Freedman DS, 1999a). Adipose tissue is heterogeneous and behaves differently based on its location; for instance in obese adults, it has been shown that adipocytes located in the abdomen have higher lipolytic rates than those located in peripheral areas (Hoffstedt J, 1997) (Berman DM, 1998). The free fatty acids produced from lipolysis in the intra-abdominal fat cells

(like those located in the omentum), drain via the portal vein into the liver where it increases the production of VLDL and triglycerides (Després JP, 1995).

On the other hand, the adipocytes located in the subcutaneous layer have lower rates of lipolysis (Berman DM, 1998), however because children have relatively more fat in this compartment compared to adults, it is still an important source of free fatty acids in children (Fox K, 1992). Together, this suggests that truncal subcutaneous adipocytes make an important contribution to total circulating free fatty acids, and since waist circumference encompasses this as well as intra-abdominal fat, it is important to conduct longitudinal studies to determine if it can identify those at risk of excess central adiposity and CVD risk later in life.

1.2.3 Sum of Skinfolts

Measurement of skinfold thickness is another method of assessing adiposity and body composition; it is used to assess the size of subcutaneous fat depots (Tanner JM, 1975) and can be measured quickly and non-invasively using standardised callipers.

It is not used as often as the other ways of measuring adiposity because it is less accurate and less precise in obese children; this is because they often have thick depots of fat which exceeds the width of the calliper (Rodríguez G, 2005).

Skinfolts are usually used to predict the different compartments of subcutaneous fat through equations designed for specific populations; while there are many of these equations in use, they all include age and gender because these significantly affect the distribution of subcutaneous fat. The scores are calculated from the sum of at least four skinfold thicknesses, and DEXA derived fat mass, but the latter is significantly influenced by gender and pubertal development stage therefore the prediction equation chosen in any analysis has a significant impact on the estimate of fatness obtained.

Therefore in children, the most commonly used equations are associated with large and significant errors (Reilly JJ, 1995).

The other problem with these equations is that they cannot be applied to children affected by diseases/disorders that can affect fat distribution, so are probably only useful in healthy children (Johnston JL, 1998). Skinfold equations are therefore more useful as a relative index of fatness when comparing individuals in a homogenous group but are not accurate as a measure of body fatness at the individual level (Reilly JJ, 1995) (Moreno L, 2002).

For the above reasons, when studying individuals, skinfold thickness is most valuable when the raw values are utilised; in longitudinal studies, they can be converted into standard deviation scores (SDS).

The sum of several skinfolds have been shown to correlate with body fat obtained by DEXA (Goran MI, 1996) (Wells JCK, 2012), so most studies use the sum of both central (subscapular, umbilical) and peripheral (like thigh and hip) skinfolds. Central and peripheral skinfolds are highly correlated with DEXA-measured central and peripheral fat depots respectively (Ketel I, 2007), and at least in adults, also behave similarly in terms of association with CVD: when compared to peripheral, central skinfolds show higher correlation with CVD (Donahue R, 1987) (Katzmarzyk PT, 2006) (Taylor AE, 2010) (Ferreira I, 2004) (Snijder MB, 2004)

Sum of skinfolds are easy and quick to perform, are cost effective, non-invasive, and therefore an effective tool in large studies; also, because different sites of the body can be measured it can be an effective way of assessing body fat distribution (Laurson KR, 2011) (Going SB, 2011) (Goacher PJ, 2012). For skinfolds to become widely used in epidemiological studies reference data needs to become available; this will allow the comparison of body composition of each individual against that of the reference.

1.2.4 Two-Component methods

Two-component methods divide the body into fat mass and fat-free mass (water, bone, and mineral), and assume that all the components of fat-free mass are stable and do not change.

The available two-component methods include Dual energy x ray absorptiometry (DEXA), densitometry, densitometry, isotope dilution, magnetic resonance imaging (MRI), total body electrical conductivity (TOBEC) and whole-body potassium scanning (TBK).

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DEXA) was first developed to measure bone mineral content, but it is now a useful tool for the assessment of body composition. DEXA uses fan-beam x-rays and multi-element solid-state detectors, so it can acquire high-resolution images of the whole body in as little as 20 minutes. Since bone mineral absorbs more x-rays than fat and water, DEXA can differentiate between fat and fat-free mass using specific algorithms, which allows the proportion of each to be estimated.

These algorithms assume that the proportion of soft tissue stays constant irrespective of changes in bone mass, so measurement errors are larger in areas of body with little or no bone mass (Milliken LA, 1996) because the basis of the algorithms no longer holds. For this reason, measuring the composition of the trunk requires substantial prediction rather than measurement, and fat mass estimation in this area is not as accurate as the limbs.

To address this limitation, fan-beam scanners have been introduced; the fan-beam scanner acquires images from the entire area without overlap between adjacent regions thereby allowing the tissue mass to be measured along each ray of the beam (Kelly TL, 1997)

Another limitation of DEXA is its assumption that the body's water content is constant and fixed at 0.73 mL/gram however, an individual's state of hydration can change by as much as 10%, and also changes with age and the amount of muscle mass (Lohman T, 2005) (Tothill P, 1994). Also, since the bone marrow contains fat, variations in the amount can introduce inaccurate measurements of actual fat mass (Svendsen OL, 1993). DEXA requires the use of ionising radiation, which although is very low in dose and deemed safe, concerns about its risks limits its use in children and adolescents. Also, the equipment is expensive, and its size precludes its use in morbidly obese individuals (Brownbill RA, 2005).

Despite these limitations, DEXA remains a reliable method of assessing body composition; the measurements from DEXA are highly reproducible and valid in different populations (O'Connor DP, 2011) (Lang PO, 2015) (Wang J, 1996) (Toombs RJ, 2012) and the use may increase once reference data becomes available to allow valid comparisons between different populations

Densitometry

Densitometry is another two-component method used to measure body composition. The original gold standard method is hydrodensitometry which utilises the ability of a mass to displace an equivalent volume of water (Pupim LB, 2013), the process lasts almost an hour and requires the participant to be submerged in water. For this reason, another method that is used is air displacement plethysmography which measures the volume of air displaced by the individual (Dewit O, 2000). To calculate the amount of fat mass from the total body density (D) measured, formulas have been derived based on the assumption that fat has a constant density of 0.9 kg/L, and the fat-free component has a density of 1.1 kg/L. Body mass is then calculated using 'Siri's equation' for Caucasians [$\% \text{ fat} = 4.95 / (D - 4.5) \times 100$] and 'Shuttle's equation' for African Americans [$\% \text{ fat} = 4.374 / (D - 3.928) \times 100$] (Pupim LB, 2013) (Fomon SJ, 1982).

The main limitation of this method is that while the density of fat is indeed relatively constant, fat-free mass varies according to the amount of body water well as the other components of fat-free mass. These variations are of particular importance in children (Wells JCK, 2012) (Fomon SJ, 1982) in whom there are significant changes in hydration even from one day to the next. Also, in those whose muscle mass is less than the average, there can be overestimation of fat mass.

However, densitometry has been shown to be useful in monitoring changes over time in overweight and obese individuals, and accurate for studies evaluating longitudinal changes in fat mass (Wells JCK, 2006).

Isotope dilution (hydrometry)

Isotope dilution is a two-component method that utilises a specific amount of water which has been labelled with an isotope of hydrogen, deuterium. The process relies on the fact that body water naturally contains a small amount of deuterium so as baseline sample of saliva, blood or urine, is collected and then a specified quantity of deuterium oxide is ingested and allowed to reach equilibrium in the body (usually after 2-5 hours depending on the size of the individual). Another sample of saliva, blood or urine is then collected and analysed using mass spectrometry (Davies PJ, 1994) (Jennings G, 1999) to measure total body water, which then allows fat-free mass to be calculated using the formula: $TBW\ (kg) = \text{dose of deuterium (mg)} / \text{enrichment deuterium in saliva (mg/kg)}$. Like the other methods described earlier, estimation of fat-free mass from total body water requires several assumptions including: that deuterium is distributed only in body water and is equally distributed in all body compartments at a rapid rate of equilibration with no losses out of the body. Because these assumptions are not true, a correction factor (of 1.041) that accounts for the actual volume of distribution is usually included in the formula (Schoeller DA, 2005)

Isotope dilution is a relatively simple means of measuring body composition and is particularly useful in children because of the low compliance required.

Magnetic resonance imaging (MRI)

MRI provides maps of body tissues by utilising a large magnet, which obtains signals from hydrogen protons present in body water and fat. By analysing the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum, MRI produces images based on variations in the phase and frequency of the energy absorbed and emitted from body tissue.

When contrast is utilised, MRI can discriminate adipose and lean tissue and then the amounts can be estimated by the use of an echo imaging method which measures the chemical shift between the different frequencies of hydrogen atoms in water and fat (Thomas EL, 2013).

Unlike DEXA, MRI is not influenced by the amount of body water so accuracy is not affected by the state of hydration of the participant; however, it can underestimate total fat mass because of how fat mass is measured (Napolitano A, 2008): the most accurate method for measuring total body fat involves using contiguous slices covering the entirety of the body. This is however, very time consuming so instead a single slice is usually used at a predetermined level to extrapolate total fat content (Abate NA, 1997); this can introduce some inaccuracy since fat is not evenly distributed. The ‘single slice’ method is adequate for longitudinal studies where changes are being tracked, but may not be ideal for situations where subtle differences are important (Ross RL, 1992).

Despite the high quality, the results obtained by MRI are not easily comparable to those obtained by other techniques like densitometry or hydrometry; this is because the formula used to derive fat mass assumes that the fat content of adipose tissue is constant while it is not. Also, MRI only measures the fat mass in adipose tissue while the other techniques measure total FM not adipose tissue mass.

Despite these limitations, MRI is the most advanced and accurate two-component method for the study of body composition, however access to the equipment, the complexity of data analysis and high cost, limits its use in routine clinical practice (Napolitano A, 2008)

Other techniques

Other techniques two-component techniques include total body electrical conductivity and whole-body potassium scanning. Neither is widely available, there is no significant correlation between the data obtained compared to other methods, and there is no accepted reference data so they are not used in clinical practice (de Bruin NC, 1995) (Duren DL, 2008).

50.560.6 Multi-component models

The multi-component model divides body mass into three to six components: for the three component models, fat mass, water, and fat-free solid mass, the latter comprising bone mass, and protein; for the four-component methods, fat mass, water, bone mass and protein are measured separately

Other models assess 5-6 components: the five-component model divides body mass into water, fat mass, protein mass, bone mineral mass and soft tissue minerals. The six-component model divides body mass into water, fat mass, protein mass, bone mineral mass, soft tissue minerals, and glycogen (Elia M, 1992) (Fuller NJ, 1992) (Heymsfield SB, 2015). Of all of these, the four-component model is considered the reference method for in-vivo assessment of body composition while the others are rarely used. The four-component model does not make the same assumptions as the two-component model which assumes that water, bone, and fat-free mass is constant and predicts the amounts from measurements obtained from infants and (Fomon SJ, 1982). This introduces potential for inaccuracies, while four-component models measure all the components directly.

The five and six component models are regarded as the gold standard for measurement of body composition but require several devices to measure each component; on the other hand, the four-component model requires the same data as two-component and the only additional measurement is of bone mineral density which can be obtained from DEXA (Heymsfield SB, 2015).

Apart from the cost required to measure the mass of each of the five or six components, the use of these models also require additional data collection and analysis which makes them unsuitable for clinical use.

In conclusion, the traditional methods used to assess cardiometabolic risk, such as lipid parameters, blood pressure, IR, and adiposity, have largely been developed for use in adults. There is far less information available on whether these parameters predict cardiometabolic risk in children. Various definitions of the metabolic syndrome have been developed in which measures are combined. However, categorical measures of cardiometabolic risk, such as metabolic syndrome, may be insensitive in children. There is therefore the need for better ways to quantify cardiometabolic disease risk in children.

50.560.6 Novel Methods

The currently available means of quantifying cardiometabolic risk in children is derived from those used in adults and have not been adequately evaluated in longitudinal cohorts of children.

There is therefore growing interest in the ability of other means of measuring future risk of cardiometabolic diseases in children; one evolving area is the study of systems biology known as metabolomics. Metabolomics describes the quantitative analysis of a large number of low-molecular-weight metabolites, which are substrates or products in metabolic pathways of all organism (Nicholson JK, 1999).

The metabolic composition of biological systems can be modified by changes in physiology, gene expression, and environmental stimuli; these changes can be quantified through the analysis of biological fluids like blood, urine, and tissue. Metabolomics profiles can then be generated using mass spectrometry and nuclear magnetic resonance (^1H -NMR) spectroscopy (Cheng S, 2012).

There are very limited studies on the application of metabolomics analysis in children however, it can be a highly effective tool in this population due to the fact that it is safe and simple; while blood sample analysis is invasive, the amount of blood required for metabolomic analysis is very small and other body fluids like urine and saliva that can also be analysed (Buescher JM, 2010).

Studying the metabolic profile identifies biomarkers, which represent the response of individual cells to specific physiological factors, so can provide information on the impact of various factors like obesity and nutrition on human health (Fanos V, 2011).

This thesis investigates the ability of body mass (measured by simple anthropometry), body fat (measured by two-component methods), nutrition, and metabolomic biomarkers to determine the risk of cardiometabolic diseases in children.

1.3 Aims and Objectives of the Study

The purpose of this programme of this study:

- To evaluate the relationship between measures of obesity in childhood and the risk of ACVD and dysglycaemia in adulthood using a systematic review and meta-analysis of currently available longitudinal studies
- To construct a continuous metabolic risk score as a surrogate measure of cardiometabolic risk
- To determine if measures of adiposity in early childhood are predictive of cardiometabolic risk score at 16 years.
- To determine if nutrition in childhood can predict body fat and cardiometabolic risk at age 16 years.
- To examine the metabolomic profiles of children to detect early biomarkers of cardiometabolic disease.

1.4 Study Design

The study comprised a systematic review and meta-analysis, followed by analysis of data from a longitudinal prospective cohort study of children: the EarlyBird study. More details about the study design will be provided, but to summarise, a systematic review and meta-analysis of the literature relevant to the thesis was undertaken. The purpose of the review was to the relationship between obesity in childhood and the risk of ACVD and dysglycaemia in adulthood. This systematic review was the first to be the first published on this subject and is further discussed in chapter two; this was followed by the analyses of the EarlyBird study, which explores the other objectives of the study

1.5 Structure of the Thesis

The thesis is organised into eight chapters.

Chapter One: Introduction to the doctoral work, as well as the construction of the overall dissertation.

Chapter Two: the findings from the systematic review and meta-analysis.

Chapter Three: a description, discussion and justification of the methods chosen to address the aims and objectives of this study.

Chapter Four: description of the metabolic risk score, the main outcome measure in this analysis.

Chapter Five: the analysis of the ability of adiposity to predict cardiometabolic risk.

Chapter Six: presents the analysis of the impact of childhood nutrition on cardiometabolic risk at 16y

Chapter Seven: identifies biomarkers associated with a high cardiometabolic risk.

Chapter Eight: summarises, synthesises and discusses the findings of the entire study and concludes with recommendations for practice and further research.

Chapter 2

Systematic review and Meta-analysis

Published as ‘Childhood Predictors of Cardiovascular disease in adulthood. A systematic review and meta-analysis’.

Obesity Reviews; 2017; 18 (9):1061-1070

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2.1 Abstract

Childhood obesity predicts the risk of adult adiposity, which is associated with the earlier onset of cardiovascular disease and dysglycaemia. It is not known whether childhood obesity directly contributes to the presence of adult atherosclerotic cardiovascular disease-ACVD: hypertension, carotid intima media thickness (CIMT) stroke or ischemic heart disease (IHD).

Data sources were Web of Science, MEDLINE, PubMed, CINAHL, Cochrane, SCOPUS, ProQuest, and reference lists. Studies measuring Body Mass Index, skinfold thickness, or waist circumference were selected.

Childhood BMI predicted CIMT: OR, 3.39 (95% CI, 2.02 to 5.67, $P < 0.001$) and risk of impaired glucose tolerance in adulthood, but its ability to predict ACVD events (stroke, IHD; OR, 1.04; 95% CI, 1.02 to 1.07; $P < 0.001$) and hypertension (OR, 1.17, 95% CI 1.06 to 1.27, $P = 0.003$), was weak-moderate. BMI was not predictive of systolic blood pressure ($r -0.57$, $P = 0.08$) and weakly predicted diastolic blood pressure ($r 0.21$, $P = 0.002$). SF in childhood weakly predicted CIMT in female adults only (spearman rank correlation 0.09, $P < 0.05$).

Childhood BMI predicts the risk of dysglycaemia and abnormal CIMT in adulthood but its ability to predict hypertension and ACVD events was weak and moderate respectively. SF was a weak predictor of CIMT in female adults.

2.2 Introduction

Obesity has become increasingly prevalent worldwide in both children and adults (WHO, 2012) (Dietz WH, 2004). It is detected by specific cut-offs in anthropometric measures – the most popular of which is the body mass index (BMI) (Cole TJ, 1995). Although used less often in clinical practice, measures of adiposity such as waist circumference (WC) and skin fold thickness (SF) are thought to be more predictive of adiposity-related comorbid conditions – perhaps because they more closely correlate with the presence of excess body fat (Freedman DS, 2013) (Prentice AM, 2001).

Irrespective of how it is measured, adult obesity is associated with dysglycaemia and an increased incidence of atherosclerotic cardiovascular disease (ACVD) and type 2 diabetes (T2D) (BHF, 2012) (WHO, 2012) which together are responsible for over half of all deaths from non-communicable diseases worldwide (WHO, 2008). Although the relationship between adult obesity and ACVD risk factors is convincing (Ashwell M, 2012), the association of anthropometric measures of obesity in childhood with disease in adulthood remains uncertain (Lloyd LJ, 2010). This is despite the fact that increased adiposity in childhood is predictive of overweight adults (Singh AS, 2008), and that ACVD risk factors are more prevalent in obese children than those of normal weight (Friedemann C, 2012) (Cote AT, 2015). The predictive ability of anthropometry in childhood may relate to the method used to determine obesity (Health, 2004) (Barton, 2010). For instance, in children, BMI explains just 40% of the variance in insulin resistance (Freedman DS, 2005) (Daniels SSR, 1997), whereas WC appears more predictive of insulin resistance and dyslipidaemia (Lean ME., 1996) (Molarius A, 1998). SF has been reported to increase the sensitivity of WC to detect dysglycaemia by up to 15% (Sievenpiper JL, 2001). To investigate this, we systematically reviewed and pooled data from longitudinal studies that examined the association between

anthropometric measures of childhood adiposity and adult ACVD, prediabetes and type 2 diabetes.

2.3 Methods

A systematic review and meta-analysis of longitudinal cohort studies was conducted, to examine the relationship between anthropometric measures of obesity in childhood and the risk of ACVD and dysglycaemia in adulthood. The Meta-analysis of Observational Studies in Epidemiology guidelines for meta-analyses and systematic reviews of observational studies (MOOSE) (Stroup DF, 2000).

2.3.1 Search Strategy

A systematic approach, using specific eligibility criteria was utilised. The following electronic databases were searched: Web of Science, MEDLINE, PubMed, CINAHL, Cochrane, SCOPUS and ProQuest from inception through September 2014. Bibliographies of the included studies were examined to identify any other relevant articles. Our search strategy utilized medical subject headings (MeSH)/ index terms across databases including the terms: BMI, change in BMI, skinfold, waist circumference, cerebrovascular disease, stroke, hypertension, ischemic heart disease, coronary artery disease, vascular dysfunction, carotid intima media thickness, dysglycaemia, impaired fasting glucose, impaired glucose tolerance, type 2 diabetes, adult, and child. The full search strategy is provided in the supplemental data and on the international prospective register of systematic reviews (PROSPERO, registration number CRD42014014392).

2.3.2 Study Selection (figure 4)

All relevant abstracts were downloaded into an online reference management system (RefWorks; ProQuest LLC). Using a screening tool, two reviewers (O.A, C.B.)

independently evaluated and screened all citations and abstracts for eligibility. Studies included were longitudinal studies of healthy children recruited between 2 and 18 years of age, where BMI, change in BMI, SF or WC were used as predictors of blood pressure, ACVD (carotid intima media thickness, hypertension or events including cerebrovascular disease/stroke ischemic heart disease or vascular dysfunction) or dysglycaemia (impaired fasting glucose, impaired glucose tolerance or T2D) in adulthood (defined as ≥ 18 years). Because of the link between pubertal timing and obesity (Kindblom et al., 2006) studies of peak height and weight velocity were also included. No limits were placed on the searches in terms of sample size, duration of follow-up or region/country of publication. Studies were excluded if the participants were part of an intervention program, if outcomes were self-reported, if anthropometric measures were classified using arbitrary cut-off points, or if children with a diagnosis of malignancy, diabetes, hypertension, preterm birth or low birth weight were included. A third reviewer compared the entered data and resolved inconsistencies by referring to the full text article (M.W.).

2.3.3 Data Extraction

For each study I extracted baseline characteristics of the participants (age, gender and birth cohort) and study characteristics (location, length of follow-up, anthropometric measure and outcome measure). Data were directly extracted from the article or from figures.

2.3.4 Study Quality Assessment

Study quality was assessed using the Newcastle-Ottawa Scale (NOS) (Wells GA, 2014) for cohort studies. This scale assigns ‘star ratings’ based on several criteria including:

- The representativeness of the study population compared to the community average
- Demonstration that the outcome of interest was not present at the start of the study,

- How the outcome was assessed, and
- Whether follow-up was of sufficient duration for outcomes to occur.

2.3.5 Statistical Analysis

The data analysis was performed using the Mantel-Haenszel method by meta-analysis software ‘Comprehensive Meta-Analysis 2.0’ (Biostat, Englewood, NJ, United States). For each study, I calculated the odds ratio (OR) or means for the primary measure. The ORs and means were presented with 95% confidence intervals (Cis), with a P -value < 0.05 considered significant. I estimated the degree of heterogeneity among the trial results using the χ^2 statistic (with a P -value < 0.10 considered significant) and the I^2 test (25%, 50%, and 75% represent low, moderate and high heterogeneity, respectively). Whenever significant heterogeneity ($P < 0.1$ or $I^2 > 50\%$) was detected, I used the random effects model to combine the effect sizes of included studies. Subgroup analyses were performed for systolic and diastolic blood pressure. I assessed the presence of publication bias with a funnel plot of standard error by log odds ratio (see Figure 4). Large studies appear at the top of the graph and cluster near the mean effect size. The absence of significant publication bias is indicated by a symmetrical distribution around the combined effect size.

2.4 Results and Discussion

The search identified 1954 citations (1934 from database searches and 20 from reference lists) from which 18 studies were found relevant to this review. The selection process was represented using a PRISMA flow diagram (Figure 5)

Most of the studies were conducted in industrialized countries: six in North America (Nguyen et al., 2010) (Lauer and Clarke, 1989) (Davis et al., 2001) (Field et al., 2005) (Freedman et al., 2009) (Freedman et al., 2004), five in Finland (Koskinen et al., 2014) (Juonala et al., 2006) (Raitakari et al., 2003) (Tzoulaki et al., 2010) (Eriksson et al.,

2001), four in the United Kingdom (Hardy et al., 2004) (Lawlor et al., 2006) (Li et al., 2007) (Wright et al., 2001), one each in India (Fall et al., 2008), Denmark (Baker et al., 2007), and The Netherlands (Oren et al., 2003). Of the total of 317,133 adults studied, the majority were white Caucasian. The mean age at which baseline childhood data was collected was 10y, and mean follow-up was 25 years. Table 1 shows the characteristics of each study; Five studies were excluded from the meta-analysis because the method of statistical analysis precluded inclusion (Davis et al., 2001), the study subjects were classified based on BMI category/glycaemic function in childhood (Nguyen et al., 2010) (Koskinen et al., 2014) (Tzoulaki et al., 2010), or because there were insufficient studies in within a subgroup for adequate comparison (Tzoulaki et al., 2010). As further analysis of these studies could not be undertaken, a qualitative summary of the results was also prepared.

2.4.1 Childhood Predictors for Adult Atherosclerotic Cardiovascular Disease and/or Dysglycaemia

Studies used one or more of the following: BMI, standardized BMI scores, change in BMI, SF, or combination of BMI and skin folds (SF) (Freedman et al., 2005) as childhood predictors, with the exception of Tzoulaki *et al* (Tzoulaki et al., 2010) in which peak height velocity (PHV) and peak weight velocity (PWV) were used as childhood predictors.

2.4.2 Outcome Measures

Seven studies assessed the presence of ACVD by carotid intima media thickness (CIMT) (Davis et al., 2001) (Freedman et al., 2009) (Freedman et al., 2004) (Juonala et al., 2006) (Raitakari et al., 2003) (Wright et al., 2001) (Oren et al., 2003) , three studied the incidence of ACVD events (Eriksson et al., 2001) (Lawlor et al., 2006) (Baker et al., 2007), while five examined blood pressure (BP) or the presence of hypertension (Lauer

and Clarke, 1989) (Field et al., 2005) (Tzoulaki et al., 2010) (Hardy et al., 2004) (Li et al., 2007). Hypertension was defined as at least one of the following: prescription for blood pressure lowering medication, systolic BP (SBP) ≥ 140 mmHg, and diastolic BP (DBP) ≥ 90 mmHg. Of the three studies that investigated the incidence of dysglycaemia (Nguyen et al., 2010) (Tzoulaki et al., 2010) (Fall et al., 2008), two used both impaired glucose tolerance (IGT) and T2D (Nguyen et al., 2010) (Fall et al., 2008) and one studied the association with T2D alone (Tzoulaki et al., 2010). IGT and T2D were defined by either the American Diabetes Association (ADA; 1997) (Nguyen et al., 2010) (Tzoulaki et al., 2010) or the WHO (1998) (Fall et al., 2008) criteria.

2.4.3 Study Quality and Publication Bias

Study quality was assessed using appropriate elements of the Newcastle-Ottawa scale (Wells GA, 2014) (Illustration 3). All the studies scored at least four ‘stars’ out of a possible six indicating good methodological quality. We assessed the presence of publication bias with a funnel plot of standard error by log odds ratio (Figure 4). The absence of significant publication bias is indicated by a symmetrical distribution around the combined effect size.

2.4.4 Meta-Analysis (Results)

Illustration 1 provides the forest plots with pooled estimates and 95% CI values for the ability of childhood BMI to predict outcomes (detailed below). Illustration 2 provides the relative weight of each study.

2.4.5 BMI/SF and CIMT

Childhood BMI was predictive of CIMT in adulthood (OR, 3.5; 95% CI, 1.1 – 11.1, $P = 0.034$) even after adjusting for adult BMI. With the variability across the studies exceeding what would be expected based purely on sampling error (I^2 98.9%, $n=6$

studies), random effects analysis was conducted. Two studies were not included in the quantitative analysis because the statistical method of analysis used was not compatible for meta-analysis (Davis et al., 2001) (Field et al., 2005). Field *et al* found that a 1-standard deviation increase in SF in childhood was associated with 16 μm increase in CIMT in adulthood while Davis *et al* found very weak correlations between BMI/SF in childhood and adult CIMT in females (BMI: spearman rank correlation r_s 0.18, $P < 0.001$, SF r_s 0.09, $P < 0.05$).

2.4.6 BMI and AVCD Events

Childhood BMI had a negligible ability to predict the development of ACVD events in adulthood, (OR, 1.04; 95% CI, 1.02 to 1.07; $P < 0.001$). None of the studies however, made statistical adjustment for the effect of BMI in adulthood. The variability across the studies did not exceed what would be expected based on sampling error (I^2 11.2%, $n=3$ studies).

2.4.7 BMI, Blood Pressure and Hypertension

After statistical adjustments were made for the impact of adult BMI, childhood BMI was weakly predictive of adult diastolic blood pressure (mean, 0.25; 95% CI, 0.003 to 0.386; $P=0.046$) but not systolic blood pressure (mean, -0.57, 95% CI, -1.22 to 0.07; $P=0.082$). Due to the presence of moderate variability across the studies for diastolic pressure (I^2 53%), random effects analysis was conducted. BMI in childhood predicted the risk of adult hypertension (OR, 1.17, 95% CI 1.06 to 1.27, $P = 0.003$) (Hardy et al., 2004) (Baker et al., 2007), while Tzoulaki *et al* (Tzoulaki et al., 2010) found no relationship between peak weight velocity in childhood and adult hypertension.

2.4.8 BMI and Dysglycaemia

Nguyen *et al* (Nguyen et al., 2010) found that neither BMI nor childhood weight gain predicted the risk of adult diabetes. However the odds ratios of developing prediabetes in

adulthood using baseline BMI and change in BMI in childhood were 1.44 and 1.85 respectively. By contrast, the odds ratios of adult prediabetes for those at the top decile for fasting insulin and IR in childhood were 2.85 and 2.55 respectively. Conversely, Fall *et al* (Fall et al., 2008) found that those who gained weight in childhood were more likely to develop IGT/T2D in adulthood (regression coefficient 1.25, $P=0.01$) as did Koskinen *et al* (Koskinen et al., 2014), who found that overweight youths without the metabolic syndrome were 3.9 times more likely to develop T2D in adulthood, compared to those of with the metabolic syndrome but of normal weight. None of these studies made statistical adjustments for the effect of adult BMI.

2.5 Discussion

This systematic review and meta-analysis indicates that childhood BMI predicts the risk of prediabetes and the surrogate measure of CIMT in adulthood. There was a modest predictive effect of childhood BMI on adult hypertension.

However, the ability of childhood BMI to predict adult ACVD events was weak. This could be explained by the relatively slow progression of atherosclerosis in healthy young adults or to a later change in environment or more significant habits – for instance the uptake of cigarette smoking, a significant risk factor, that tends to occur no earlier than adolescence (Stary et al., 1994). The smoking-related formation of focal plaques in coronary vessels is more predictive of ischemic cardiovascular events than CIMT (Johnsen and Mathiesen, 2009). BMI cannot discriminate fat from lean mass, and the latter has a significant influence on CIMT (Chowdhury et al., 2014); this might explain why some individuals with an abnormal CIMT and high BMI do not develop AVCD (Lorenz et al., 2007).

This analysis also showed that although weight gain in childhood predicted T2D in adulthood, BMI predicted prediabetes only. A possible explanation for this finding is the fact that BMI is a weaker discriminator of visceral fat compared to other measures such as WC, and so this might limit the ability of BMI to predict metabolic risk

(Kissebah et al., 1982). Visceral fat is associated with increased release of free fatty acids (delivered to the liver) and pro-inflammatory cytokines, which lead to worsening insulin resistance (Friedl, 2009). T2D occurs once beta-cell function is no longer able to compensate for this insulin resistance. Inter-individual differences in beta-cell function and/or mass may therefore account for the ability of childhood BMI to predict adult prediabetes rather than T2D. Furthermore a longer duration of follow-up than that reported in the included studies may be required for T2D to develop.

Childhood BMI weakly predicted adult hypertension but did not predict adult systolic blood pressure. This may relate to the relatively low tracking of systolic blood pressure measurements during childhood (Kissebah AH, 1982), or because body composition is does not strongly influence blood pressure.

In most of the included studies, statistical adjustments were not made for the confounding effect of adult BMI on the risk of ACVD events or dysglycaemia (Lloyd et al., 2010). In the studies that did make such adjustments (Raitakari et al., 2003) (Hardy et al., 2004) (Baker et al., 2007), there was a partial attenuation in the effect sizes seen, indicating that adult BMI may have a confounding effect on the outcomes studied. Therefore, it is inherently difficult to investigate the long-term consequences of changing BMI over a long time span.

The weak/modest effect sizes seen in all the outcomes might also be related to the short length of follow up between childhood and adulthood (25 years). For instance, the average age at which a man in a westernized country is diagnosed with an acute coronary event is 65y and just 4-10% of cases of myocardial infarction occur before the age of 45y (Go AS, 2014). A longer duration of follow-up would mean the accrual of more ACVD and diabetes events. More events may increase the power to determine the effect of childhood anthropometric measures in predicting adult disease.

The strengths of this systematic review and meta-analysis include the variety of anthropometric measures of adiposity studied, and the use of a wide range of outcome measures. I performed comprehensive searches of multiple databases and ensured independent study selection and dual data extraction.

Although performing a meta-analysis of observational epidemiological studies is often difficult due to incomplete and unstandardized reporting of studies, the quality of the included studies was good, and random effect analysis was conducted where significant inter-study variations was present. Limitations of this review may be that searches were limited to studies published in English. Few studies examined SF and WC and so more studies are required to confirm the applicability of these measures of adiposity in predicting the risk of adult ACVD and dysglycaemia.

In summary, this systematic review found that anthropometric measures of obesity in childhood predicted CIMT and prediabetes in adulthood. Childhood obesity did not predict ACVD event rates in adults. Whether this relates to inherent inaccuracies of the methods used to measure childhood obesity or to a lack of pathophysiologic effect of childhood obesity requires further study. The prevention and reduction of childhood obesity is likely to remain a priority in order to reduce the risks of cardiometabolic disease in adults. These findings however also suggest that BMI is not a strong predictor so new ways to predict cardiometabolic risk need to be considered.

Table 1. Description of included studies

Author, yr of publication	Participants	Country	Type of study	Age in childhood ^a	Age adult ^a	N	Predictor	Outcome	Results
Cardiovascular disease									
Field, AE. 2005 ²⁷	1963-70 cohort	USA	Prospective	11.7 (1.8)	22.1 (1.8)	269	BMI	Htn	1unit ↑in BMI=0.5mmHg ↑in SBP in males.
Hardy, R. 2004 ³⁵	1946 Birth cohort	England	Prospective	7 (2-15)	43	1241	BMI	Htn/BP	Negative correlation but not significant
Lauer, RM. 1989 ²⁵	Muscatine study	USA	Prospective	11 (7-18)	26-30	339	BMI	Htn/BP	In females, R=0.3 for SBP, 0.34 for DBP; in males: 0.29 and 0.14
Li, L. 2007 ³⁷	1958 Birth cohort	England	Prospective	11 (7-16)	45	7344	BMI	Htn/BP	No significant association after adjusting for adult BMI
Tzoulaki, I. 2010 ³³	1966 Birth cohort	Finland	Prospective	2	31	3246	PWV	Htn/Bp	No significant association after adjusting for adult BMI
Tzoulaki, I. 2010 ³³	1966 Birth cohort	Finland	Prospective	2	31	3069	PHV	Htn/BP	r=1.57 P 0.001 after adjusting for adult BMI
Baker, JL. 2007 ⁴⁰	1930-76 cohort	Denmark	Retrospective	11 (7-13)	25-60	276835	BMI	CVD	RR 1.14 in males, 1.10 in females
Eriksson, JG. 2001 ³⁴	1934-44 cohort	Finland	Retrospective	11	27-63	4574	BMI	CVD	Slight increase in HR 1.03 P 0.0005
Lawlor, DA. 2005 ³⁶	1950 birth cohort	Scotland	Retrospective	4.9 (0.7)	36	11106	BMI	CVD	No increase in HR
Davis, PH. 2001 ²⁶	Muscatine study	USA	Prospective	8-18	33-42	725	SF	CIMT	P=0.04 in males, 0.09 in females

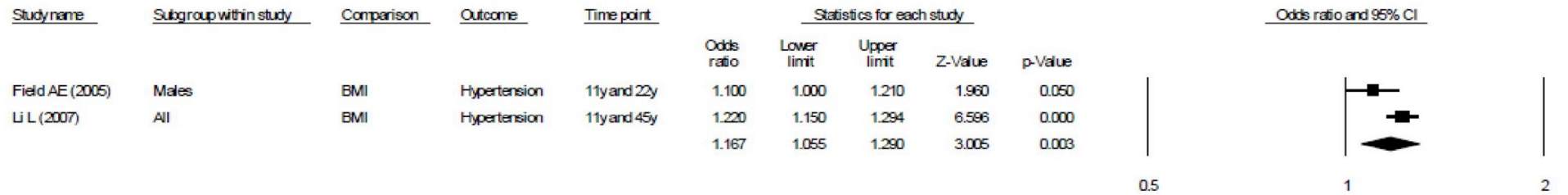
Table 2 continued. Description of included studies

Author, yr of publication	Participants	Country	Type of study	Age in childhood ^a	Age adult ^a	N	Predictor	Outcome	Results
Freedman, DS. 2004 ²⁹	BHS 1959-1968	USA	Prospective	11 (3)	35 (3)	513	SF	CIMT	1 unit ↑ in SSF=16μm ↑ in CIMT
Freedman, DS. 2004 ²⁹	BHS 1959-1968	USA	Prospective	11 (3)	35 (3)	513	BMI	CIMT	1 unit ↑ in BMI=14μm ↑ in CIMT P=0.03 after adjusting for adult BMI
Davis, PH. 2001 ²⁶	Muscantine study	USA	Prospective	8-18	33-42	725	BMI	CIMT	P=0.09 in males, P=0.18 in females
Freedman, DS. 2009 ²⁸	BHS 1959-1968	USA	Prospective	10 (3)	36 (4)	1142	BMI	CIMT	1 unit ↑ in BMI=16μm ↑ in CIMT after adjusting for adult BMI
Juonala, M. 2006 ³¹	YFS	Finland	Prospective	12 (3-18)	33	408	BMI	CIMT	r=0.05(ns) after adjusting for adult BMI
Oren, A. 2003 ⁴¹	ARYA	Netherlands	Prospective	12-16	28.4 (1.6)	750	BMI	CIMT	r=0.9 after adjusting for adult BMI
Oren, A. 2003 ⁴¹	ARYA	Netherlands	Prospective	12-30	28.4 (1.6)	750	BMI Δ	CIMT	r 11.8 P 0.01
Raitakari, OT. 2003 ³²	YFS	Finland	Prospective	14.9 (2.4)	31.7 (5)	1170	BMI	CIMT	Small association: r: 0.009, P=0.007
Wright, CM. 2001 ³⁸	1947 Birth cohort	England	Prospective	9, 13 (9y data)	50	412	BMI	CIMT	r=-0.02 in women, -0.1 in men after adjusting for adult BMI
Dysglycaemia									
Nguyen, QM. 2010 ²⁴	BHS 1981-2000	USA	Prospective	11.6 (3.6)	28.3 (5.1)	1120	BMI Δ	IGT	OR 1.44 P<0.05 for developing IGT

Table 3 continued. Description of included studies									
Author, yr of publication	Participants	Country	Type of study	Age in childhood ^a	Age adult ^a	N	Predictor	Outcome	Results
Nguyen, QM. 2010 ²⁴	BHS 1981-2000	USA	Prospective	11.6 (3.6)	28.3 (5.1)	1120	BMI	IGT	OR 1.85 P<0.01 for developing IGT
Koskinen, J. 2014 ³⁰	YFS	Finland	Prospective	16.2 (5.3)	32-46	1330	BMI	T2D	Obesity associated with 3.9 fold increase in risk of T2D
Nguyen, QM. 2010 ²⁴	BHS 1981-2000	USA	Prospective	11.6 (3.6)	28.3 (5.1)	1120	BMI Δ	T2D	Not predictive
Nguyen, QM. 2010 ²⁴	BHS 1981-2000	USA	Prospective	11.6 (3.6)	28.3 (5.1)	1120	BMI	T2D	Not predictive
Fall, CH. 2008 ³⁹	1969-1972 cohort	India	Prospective		29.2 (1.3)	1492	BMI Δ	T2D/ IGT	OR 1.25 P 0.01 for developing IGT/T2D

^a mean age in years, standard deviation (sd) where available, age range if not. PWV: Peak weight velocity, PHV: peak height velocity, SF: Skin fold thickness; WHR: waist-hip ratio; BP: blood pressure; Htn: Hypertension=systolic ≥ 140 mmHg and/or diastolic ≥ 90 mmHg; IGT/T2D: according to ADA criteria in 2 studies (14,15) and 1999-WHO criteria in one (16). CIMT: carotid intima media thickness; r: regression coefficient; HR: Hazard ratio; OR: Odds ratio; P: Spearman rank correlation; ns: P >0.05. YFS: YoungFinns Study; ARYA: Atherosclerosis Risk in Young Adults study; BHS: Bogalusa Heart Study

Hypertension



Blood pressure

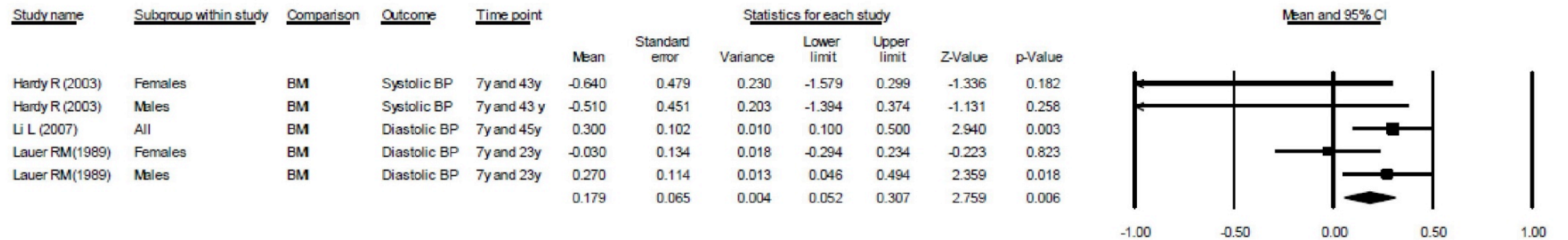
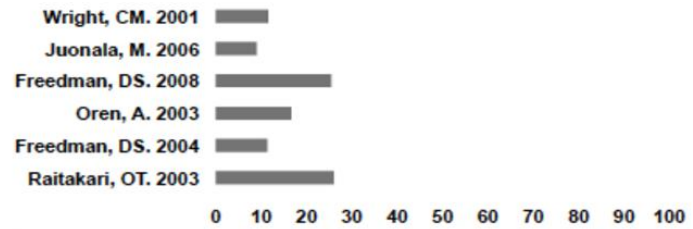
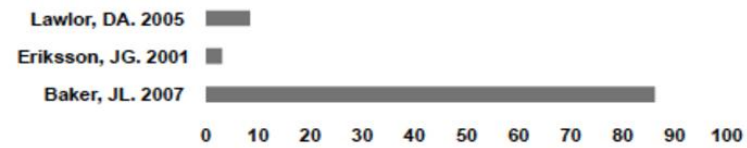


Illustration 1. Forest plots

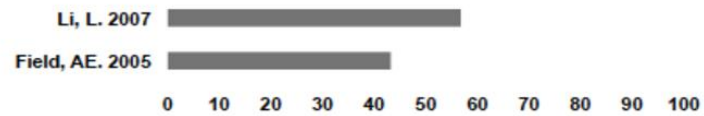
CIMT



CVD



Hypertension



Blood pressure

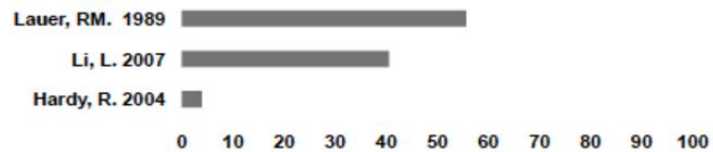


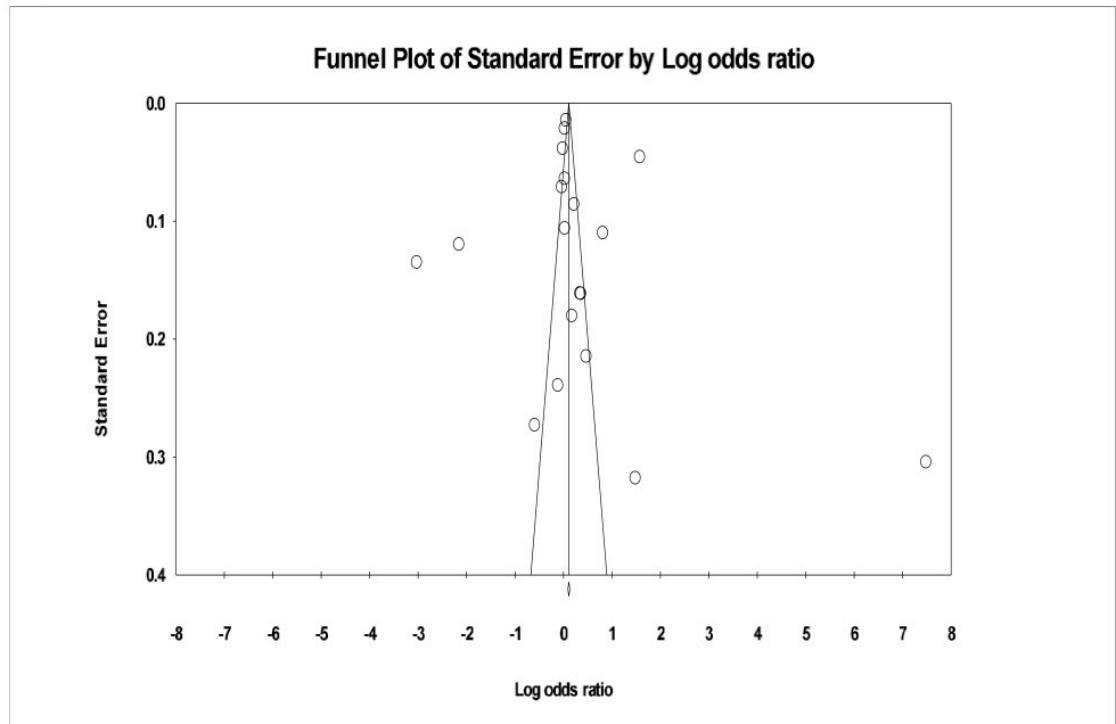
Illustration 2. Relative weight of studies

Author, yr of publication	Representative ^a	Data source ^b	Adjusts for adult measure ^c	Assessment of Outcome ^d	Follow up ^e	Adequacy of follow up ^f
Baker, JL. 2007 ⁴⁰		*		*	*	*
Davis, PH. 2001 ²⁶	*	*		*	*	*
Eriksson, JG. 2001 ³⁴		*		*	*	*
Fall, CH. 2008 ³⁹	*	*		*		*
Field, AE. 2005 ²⁷		*		*	*	*
Freedman, DS. 2004 ²⁹	*	*	*(for BMI not SF)	*	*	*
Freedman, DS. 2009 ²⁸	*	*	*	*	*	*
Hardy, R. 2004 ³⁵	*	*		*	*	*
Juonala, M. 2006 ³¹	*	*	*	*	*	*
Koskinen, J. 2014 ³⁰	*	*		*	*	*
Lauer, RM. 1989 ²⁵	*	*		*	*	*
Lawlor, DA. 2005 ³⁶	*	*		*	*	*
Li, L. 2007 ³⁷	*	*	*	*	*	*
Nguyen, QM. 2010 ²⁴	*	*		*	*	*
Oren, A. 2003 ⁴¹	*	*	*	*	*	*
Raitakari, OT. 2003 ³²	*	*	*	*	*	*
Tzoulaki, I. 2010 ³³	*	*	*	*	*	
Wright, CM. 2001 ³⁸	*	*	*	*	*	*

a Truly representative or somewhat representative = * rating ; b Secure record linkage = * rating; c Study controls for corresponding adult anthropometric measure = * rating

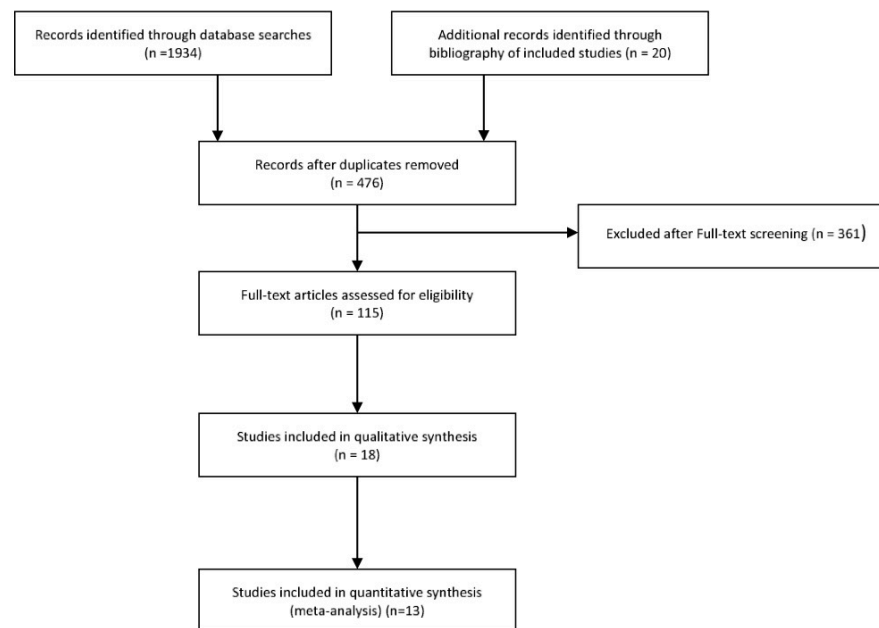
d Independent blind or record linkage = * rating; e Was follow up long enough (16 years) for outcomes to occur=* rating; f All subjects accounted for/those lost to follow up unlikely to introduce bias/small number lost (<30%)/description provided for those lost = * rating

Illustration 3 Quality assessment based on the Newcastle-Ottawa Quality Assessment Scale



Large studies appear at the top of the graph and cluster near the mean effect size.

Figure 4. Publication Bias



Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement*. PLoS Med 6(6) e1000097

Figure 5. Study selection process

Chapter 3

Materials and Methods

3.1 Research Design

Early Bird is a non-intervention prospective cohort study incorporating a 1995/96-birth cohort recruited in 2000/2001 when the children were 5 years old.

3.2 Recruitment Process

All primary schools in Plymouth were identified and their head teachers were asked for agreement to participate in the study. The 54 schools that consented were stratified into quartiles according to the number of children that consumed free school meals; the latter served as surrogate measure of socio-economic status. A random selection of equal numbers of schools was made from each, ensuring a wide socioeconomic mix representative of the Plymouth area (Index of Multiple Deprivation 2004 score: cohort 26.1, Plymouth 26.3, and England 21.7). 98% were white Caucasian, reflecting the racial mix of the area.

In those who provided consent to participate, exclusion criteria including diabetes, pathological states likely to affect growth or body composition, moderate or severe physical disability, and long-term use of oral steroids were applied.

Following ethical approval and written parental consent, 307 children in total (137 girls, 170 boys, mean age 4.9 years), starting school from Jan. 2000 to Jan. 2001, became the original EarlyBird cohort.

After four study years, 30 children had left the study (11 moved out of the area, and 19 withdrew for unknown reasons) representing a low attrition rate of 9.8%. To increase

the sample size and maintain the proportion of both genders, 40 pre-pubertal nine-year-old children were recruited to the study (Thirty-seven girls and three boys).

The relatively high retention rate of over 90% was achieved by regular and close contact between the research team, the participants and their families, as well as a flexible appointment system, free transport to appointments, regular newsletters, social events and gifts after each visit (like T-shirts, water bottles, torches, and stationery).

3.3 Study Protocol

In the first six years of the study, the study participants attended the research centre every six months. A team of research nurses and assistants completed the assessment. When the children entered secondary school, one of the biannual visits was conducted at school. During each visit, two research nurses who also completed venepuncture in the fasted state collected anthropometric measures, blood pressure, pubertal status, and demographic updates.

3.4 Ethical Approval

Local Research Ethics Committee approval was obtained in 1999, with additional approval subsequently granted for dual x-ray absorptiometry (DEXA) scans and further laboratory analyses of archived sera. The Study was subject to routine inspection by COREC. In 2006/7, all the study participants were invited to sign consent forms for their continued participation in the study.

3.5 Exclusion Criteria

Children with a history of pre-existing diabetes, pathological states likely to affect growth or body composition, moderate or severe physical disability and long-term use of oral steroids were excluded from the study.

3.6 Data Collection

3.6.1 Anthropometry

A research nurse within Plymouth hospital's department of child health examined the children at 8am in the fasted state. Anthropometric measures and blood pressure were measured every six months and all the data were anonymised and electronically archived.

Height was measured to the nearest 1mm (Leicester Height Measure, Child Growth Foundation, London), and weight to the nearest 200g (Tanita Solar 1632W electronic scales, West Drayton, Middlesex). Skinfold thickness was measured at five sites (biceps, triceps, subscapular, suprailiac and para-umbilical) by callipers (Holtain Ltd, Crosswell, Crymych, Pembrokeshire). Mid-arm, waist (measured at the narrowest part between the lower border of the ribs and the upper border of the pelvis), and hip circumference were measured by metal tape (Chasmors Ltd, London).

A minimum of two 'blind' repeats was made of each anthropometric measure at each visit. The precision and reliability of all anthropometric measures was regularly assessed, particularly following the training of new research assistants.

3.6.2 Body composition analysis

Total body fat was obtained using a two-component model which utilised DEXA (Lunar Prodigy fan beam densitometer, GE Medical Systems, previously Lunar DPX-L pencil beam); body composition analysis was by a Tanita Body Composition Analyser, (TBF-300M, Tanita Corp.of America Inc., Arlington Heights, Illinois) and several values were derived: total fat mass (kg), percent body fat, fat mass index (fat mass in kilograms divided by height in metres squared (BFI), fat free mass index in kilograms, divided by height in metres squared (FFMI).

Android, and Gynoid fat mass were defined using EnCore 2004 software:

- Android fat mass = Lower boundary at Pelvis cut. Upper boundary above Pelvis cut by 20% of the distance between Pelvis and Neck cuts. Lateral boundaries are the Arm cuts.
- Gynoid fat mass = Upper boundary below the Pelvis cut line by 1.5 times the height of the Android region of interest. Gynoid region of interest height equals to 2 times the height of the Android region of interest. Lateral boundaries were the outer Leg cuts.

3.6.3 Physical activity

Physical activity was measured objectively using actigraph accelerometers (Model 7164, formerly MTI/CSA, Fort Walton Beach, FL). The accelerometers were worn around the child's wrist and set to run continuously for 7 days at each annual time-point. Only recordings that captured at least four weekdays of monitoring (defined as ≥ 9 hours wear time each day) and 1 weekend day was included in the analysis. Each 1-minute epoch of activity data was labelled as sedentary if < 1000 counts/minute, light intensity if 1000-2499 counts/minute, or moderate-and-vigorous intensity (MVPA) if ≥ 2500 counts/minute.

3.6.4 Demography

Parents completed a baseline questionnaire detailing medical history and socio-economic circumstances (occupation, income band, eligibility for free school meals and family tax credit, post-code, educational level of the parents). This was updated annually.

Measures of socio-economic status are relatively stable and widely used in research studies. In this study, the Index of Multiple Deprivation (IMD) was used (Smith T, 2015); IMD takes into account, the income, employment, health, education, housing,

environment & crime in the neighbourhood; the higher the score, the greater the deprivation.

3.6.5 Blood pressure

Blood pressure was measured by an automated sphygmomanometer (Welch-Allyn, Beaverton, Oregon, US), with the participant seated and rested. The mean of the second and third reading was used.

3.6.6 Laboratory analysis (table 2)

A research nurse collected venous blood from each participant at 0800 after a 10 hour fast. Topical anaesthesia (EMLA- Astra Zeneca Luton or Ethyl chloride BP fine spray- Acorus Therapeutics Ltd, Chester) was applied before venepuncture.

Insulin and sex hormone-binding globulin (SHBG) were measured by immunometric assay on a DPC Immulite analyser, using kits measured by Diagnostic Products Corporation (Los Angeles, CA). Insulin cross-reactivity with proinsulin was less than 1%, and the detection limit of the assay was 2.0 mU/l.

Glucose, cholesterol, HDL cholesterol, triglycerides and uric acid were measured on a Cobas Integra 700 analyser (Roche Diagnostics, Lewes, UK).

A haematological profile was recorded (full blood count, haemoglobin, haematocrit). Glycated haemoglobin was measured by automated high performance liquid chromatography using a Menarini Biomen HA 8140 analyser.

Follicle-stimulating hormone (FSH) and luteinising hormone (LH) were measured by automated 74ort he74nd74scent sandwich immunoassay on an Advia Centuar analyser (Bayer Diagnostics, Newbury, Berks, UK).

Insulin resistance was derived from fasting glucose and insulin using the homeostatic model assessment (HOMA) method and described as 'HOMA-IR'. HOMA-IR has been validated against euglycaemic and hyperglycaemic clamps in the minimal model with

correlations of $r > 0.9$ in children (Gungor N, 2004). In order to derive a HOMA value from samples where the insulin concentration lay below the threshold of assay detection (2mU/l), an arbitrary value of 1.5mU/l was given.

Table 2. Precision data for EarlyBird tests (Derriford Hospital Combined Laboratory, 2010)

Analyte	Concentration	Within-run CV (%)	Total CV (%)
Insulin (mu/L)	7.67	5.5	7.3
	12.5	4.0	4.9
	17.2	3.3	4.1
	26.4	3.9	5.0
	100	3.8	4.2
	291	3.7	5.3
Glucose (mmol/L)	3.7	1.1	
	15.2	0.8	
	6.5		1.9
	14.0		1.9
Cholesterol (mmol/L)	3.04	1.0	
	5.98	0.8	
	2.97		2.1
	5.44		1.7
Triglyceride (mmol/L)	0.9	1.4	1.9
	2.06	1.3	
	2.01		1.7
HDL (mmol/L)	0.89	1.0	
	2.08	0.6	
	0.82		1.3
	1.64		1.2
HbA1c (%)	6.15		2.3
	7.17		2.8
SHBG (nmol/L)	14.9	1.1	2.3
	219	1.7	2.8
FSH (IU/L)	6.0	2.6	
	178	2.5	
	5.3		3.6
	229		4.5
LH (IU/L)	0.5	1.8	5.2
	50.7	0.8	2.0

3.6.7 Nutrition

Dietary intake was assessed by validated food frequency questionnaires (FFQ) (Hammond J, 1993). The frequency of foods consumed per week was derived from the

responses. At each annual visit, FFQ was completed by the parents on behalf of their children to profile habitual intake of food, beverages, and cooking fat during the previous 12 months. Foods eaten outside of the home were also included. Each item or food group was allocated to one of ten frequency categories ranging from ‘never’, ‘once per month’, ‘once per fortnight’ and ‘1 day a week’ to ‘7 days a week’. The selected frequency choice for each item was then converted into a weekly intake for analysis.

3.6.8 Metabolomics

Bioinformatic analysis of raw metabolomic output was undertaken in collaboration with the Nestle Institute of Health Sciences (NIHS), in order to prepare the data output for this project. Metabolic profiling was carried out by means of proton nuclear magnetic resonance spectroscopy (^1H NMR) spectroscopy. Briefly, 400 μL of blood serum were mixed with 200 μL of deuterated phosphate buffer solution 0.6M KH_2PO_4 . ^1H NMR metabolic profiles of serum samples were acquired with a Bruker Avance III 600 MHz spectrometer equipped with a 5mm cryoprobe at 310K (Bruker Biospin, Rheinstetten, Germany) and processed using TOPSPIN (version 2.1, Bruker Biospin, Rheinstetten, Germany) software package. Based on an internal database of reference compounds, representative signals of metabolites were integrated. The signals are expressed in an arbitrary unit according to a peak area normalized to total metabolic profiles. ^1H NMR spectroscopy being a quantitative method, metabolite peak area is proportional to metabolite concentration, and thus changes are representative of absolute change in metabolite concentrations in the serum.

3.7 Data Analysis

Body Mass Index Standard Deviation Score (BMI-SDS) was calculated from the British 1990 standards (Cole TJ, 1995), and Waist Circumference Standard Deviation Score (WC-SDS) from the British 2001 standards (McCarthy HD, 2001). Participants were

categorized as 'overweight/obese' or 'normal weight' according to BMI-SDS (91st centile) from the 1990 British standards (Cole TJ, 1995), overweight obese was BMI-SDS \geq 91st centile while normal weight was $<$ 91st centile.

Metabolic Risk Score: A continuous metabolic risk score was calculated as a composite of total: high-density lipoprotein cholesterol ratio, fasting triglycerides (mmol L⁻¹), mean arterial blood pressure and the internal z-score of HOMA-IR; the z score was derived by subtracting the mean IR for the cohort from individual IR values, and then dividing this difference by the standard deviation of the cohort.

Appropriate summary statistics were used to describe data and all effect sizes were presented with 95% confidence intervals (CI) as a measure of their precision. The distributions of all continuous variables were checked for outliers and normality and transformed appropriately (eg. Log transformation), to allow the following analyses to be undertaken:

Cross-sectional analyses: Initial exploratory analyses included simple correlations between continuous variables at each cross-sectional time point. For example, the association between the different measures of adiposity and between adiposity and metabolic risk were determined by Pearson's correlation.

Longitudinal analyses: The relative stability (tracking) of variables within children over the course of childhood and adolescence was established by year-on-year correlation and longitudinal tracking co-efficient. Tracking coefficients were calculated using the method suggested by Twisk (Twisk JE, 2004) which uses a generalised estimating equation (GEE) model in which the first measurement is regressed on subsequent measurements (measured annually till 16y). The tracking coefficient ranges from 0 to 1, with 1 indicating perfect tracking and 0 indicating no tracking. There is no accepted cut-off to differentiate good from poor tracking, but I considered tracking coefficients of \geq 0.40 to indicate 'moderate' tracking.

Time-lagged correlations were used to explore associations between measurements made early in childhood and outcomes later on (at 16y). Specifically, time-lagged correlations were defined as the relationship between the variable of interest (eg. Adiposity) at each age from 5y to 15y, and the final measurement of the outcome variable (eg. Metabolic risk score) at 16y.

Mixed effects modelling was used to analyse longitudinal relationships between variables. A mixed effects model incorporates both fixed and random effects and allows modelling of individual as well as population change over time; it also incorporates all available longitudinal data simultaneously and enables children to be included in the analysis even if they don't have data at all of the time points.

All analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS Inc, Chicago, IL,USA).

Power: This project was based on the existing data from the EarlyBird study and as such it was not possible to undertake *a priori* power calculations specifically for the analyses presented here. Results are thus interpreted within the context of all analyses undertaken and no adjustment for multiple comparisons was made. It is therefore acknowledged that further confirmatory studies may be needed for testing of hypotheses generated.

Power calculations had previously been performed using *SamplePower* version 2 (SPSS UK Ltd, Surrey). A difference of 24% or 0.2 units in insulin resistance between boys and girls would be significant at the 5% level with 80% power in a sample of 170 boys and 137 girls. A correlation of $r=0.16$ observed in a sample of 307 would be significant at the 5% level with at least 80% power. When analysing boys (girls) only, a correlation of $r=0.21$ ($r=0.24$) observed in a sample of 170 would be significant at the 5% level with at least 80% power.

Chapter 4

Metabolic Risk Score

4.1 Abstract

Aims and objectives:

This chapter describes the process through which a continuous metabolic score (MRS) was calculated, and how it compared to the only available definition of childhood metabolic syndrome (MetS).

The MRS is a useful alternative to the adult definition of the MetS and can be used to identify children at a future risk of atherosclerotic cardiovascular disease (ACVD)/type 2 diabetes (T2D); this is because when the adult definitions of the MetS are used, only few children meet these criteria; this highlights the need for alternative means of estimating metabolic risk in children.

Methods: The MRS is a composite of total/high-density lipoprotein cholesterol ratio, fasting triglycerides, mean arterial blood pressure, and insulin resistance; the latter was presented as the internal z score of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

The only available definition of childhood MS is from the International Diabetes Federation (IDF) which defines MetS as 3 or more of: WC \geq 90th percentile, triglycerides \geq 1.7mmol/L, HDL $<$ 1.03mmol/L, systolic blood pressure \geq 130 mmhg / diastolic blood pressure \geq 85 mmhg, fasting glucose \geq 5.6mmol/L or known type 2 diabetes. In the children aged 16y or over: WC \geq 94cm in males/80cm in females, plus any 2 of triglycerides \geq 1.7mmol/L, HDL $<$ 1.03mmol/L in males/ $<$ 1.29mmol/L in

females, treatment for hypertension or systolic = 130mmhg/ diastolic =85mmhg, fasting glucose =5.6mmol/L or known type 2 diabetes

Results: The MRS was higher in girls than boys and significantly higher in overweight /obese children. The score showed tracking from childhood (age 5y) to adolescence (16y). The proportion of children who met the criteria for the MetS was less than 5%

Conclusion: Because there is no universal definition of the MetS, metabolic risk scores are potentially more useful in epidemiological research. These scores are more relevant in children because the current definitions of the MetS cannot detect all of those at a high metabolic risk.

Further studies are required to keep following the EarlyBird cohort to confirm the association of the score with adult-diagnosed MetS, T2D, and ACVD.

4.2 Introduction, Aims and Objectives

The metabolic syndrome (MetS) is widely used to define the clustering of risk factors that can lead to development of atherosclerotic cardiovascular disease (AVCD) and type 2 diabetes (T2D) (Kahn R, 2005). MS is associated with at least double the risk for cardiovascular events and deaths (WHO, 2008), and is present in over 25% of the US adult population (Ford ES, 2004).

Perhaps the most important limitation of the MetS is the absence of a single definition; there are at least seven definitions in routine use (Alberti K, 2005) (Eckel RH, 2005) (Federation, 2005a) (Grundy S, 2004) (Kahn R, 2005). Another problem with the MetS is the fact that only few children meet the criteria for any of the available definitions (Hadjiyannakis S, 2005); this is at least in part because normal ranges for each component of the syndrome are not well defined in childhood, and also because children are at an earlier stage in the natural history of the MetS. It is therefore crucial to utilise other methods to detect and characterise metabolic dysfunction in children.

One approach is to define a continuous metabolic risk score (MRS), derived from components of the MetS; this approach has been used in epidemiological studies of cardiometabolic risk in children (Eisenmann JC, 2010) (Hadjiyannakis S, 2005).

These scores are derived from both metabolic and cardiovascular disease risk factors and have been shown to have a stronger association with cardio metabolic disease outcomes than the MetS which divides each risk factor into categories (Hadjiyannakis S, 2005) (Olfert MD, 2019) (DeBoer MD, 2017).

This analysis evaluated a composite MRS, the relationship between its components, and compared it to the IDF definition of the MetS.

4.3 Methods

Data from the EarlyBird study was analysed; details of the study methods are described in chapter 3.

4.3.1 Measures

A continuous metabolic risk score was calculated as a composite of the total/high-density lipoprotein cholesterol ratio, fasting triglycerides (mmol/L), mean arterial blood pressure, and Insulin Resistance (IR); the internal z score of the homeostasis model assessment (HOMA-IR) represented IR. To calculate this score, the mean IR for the cohort was subtracted from individual IR values, and the difference was then divided by the standard deviation.

Trends and gender differences of each component of the score were described, as was the relationship between each component of the score.

The presence of the MetS was identified based on the IDF (International Diabetes Federation) definition: Children aged 10-15y as: 3 or more of WC \geq 90th percentile, triglycerides \geq 1.7mmol/L, HDL cholesterol $<$ 1.03mmol/L, systolic blood pressure \geq 130/ diastolic \geq 85, fasting glucose \geq 5.6mmol/L or known type 2 diabetes; in children \geq

16y – 18y: waist circumference (WC) $\geq 94\text{cm}$ in males/ 80cm in females, plus any 2 of triglycerides $\geq 1.7\text{mmol/L}$, HDL cholesterol $< 1.03\text{mmol/L}$ in males/ $<1.29\text{mmol/L}$ in females, treatment for hypertension or systolic = 130mmHg / diastolic = 85mmHg , fasting glucose $\geq 5.6\text{ mmol/L}$ or known type 2 diabetes (International Diabetes Federation, 2005b).

4.4 Statistics

Tracking of the components of the MRS and MetS was investigated, in boys and girls separately, using year-on-year correlations and longitudinal tracking coefficients as described in chapter 3.

For each variable, the trend over time was characterised by plotting the mean and its standard error as well as the box plots of their distribution at each time point. Gender differences were tested using independent samples *T*-test. Pearson correlations were obtained to determine the strength of the relationship between the components of the MRS and the components of the MetS.

4.5 Results

4.5.1 Trends in the Metabolic Syndrome and Its Components

High density Lipoprotein (Figure 6 and appendix A1)

There was a steady increase in HDL from 5y to the peak at 9-10y of age, followed by a drop back to baseline. Until 14y, boys had a slightly higher HDL than girls; from 15-16, however the reverse was the case. None of these differences were statistically significant ($p > 0.5$). To meet the MetS criteria, HDL had to be < 1.03 in 10-15y old boys and girls, and $< 1.03\text{mmol/L}$ in the 16y boys; $< 1.29\text{ mmol/L}$ in 16y old girls. Very few children met the criteria for HDL; the highest number was at 9y when just 4 girls met these criteria.

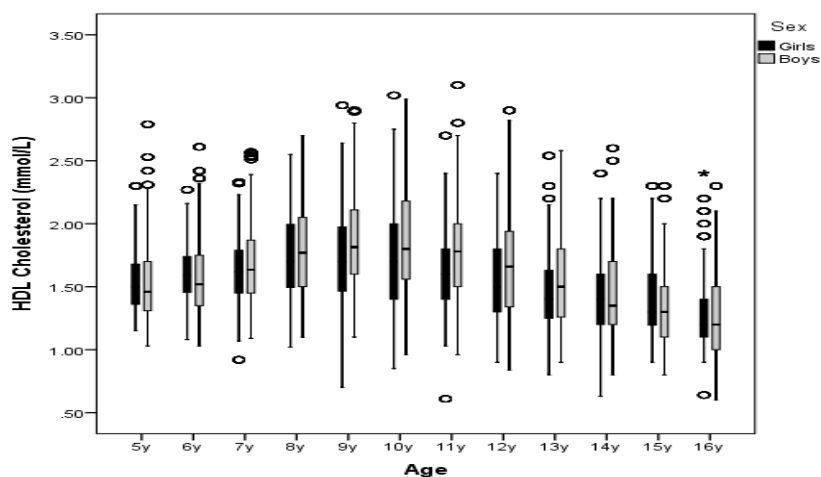


Figure 6. HDL from 5y to 16y in boys and girls

Glucose (Figure 7 and appendix A2)

Fasting glucose increased steadily from 5y to 16y and was consistently higher in boys. The gender difference became significant at 16y ($P<0.05$). Criteria for the MetS were a fasting glucose of $\geq 5.6\text{mmol/L}$; unlike with HDL, 26 children met the criteria for glucose at 14years (about 10% of the participants), similar numbers at 15y and 16y. Mean Glucose was also higher in boys than girls ($P<0.01$ at 15 and 16y) despite a much higher average IR in the girls.

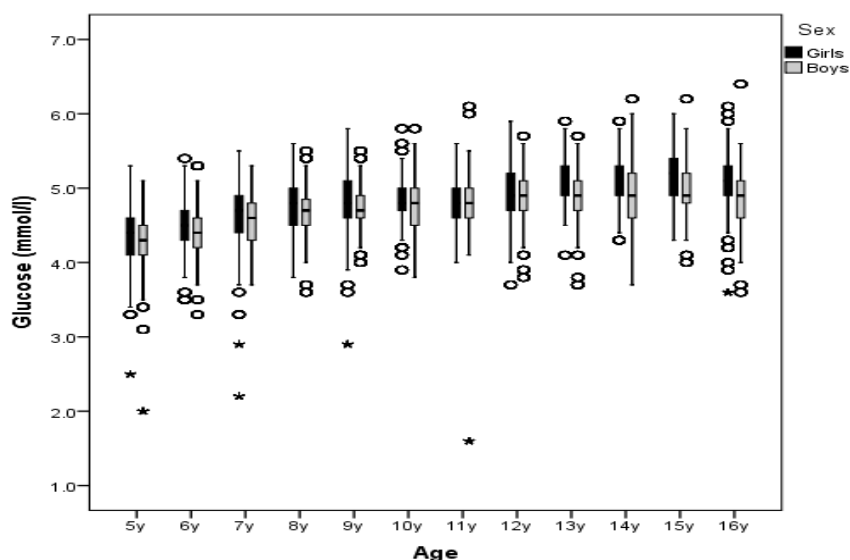


Figure 7. Glucose from 5y to 16y in boys and girls

Blood Pressure (Figures 8-9, and appendix A3)

SBP increased steadily till 12y of age when there was acceleration till the peak at 15y. SBP was consistently higher in boys with the gender difference becoming significant from 14y onwards ($P<0.001$). Similarly, DBP increased steadily, and accelerated from 12y. There were however no significant gender differences.

Blood pressure criterion for the MetS was systolic blood pressure $\geq 130\text{mmhg}$, diastolic $\geq 85\text{mmhg}$, or a previous diagnosis of hypertension.

Twelve 16y-old boys ($\approx 10\%$) met the systolic blood pressure criteria; overall only few children (majority of whom were boys) met the systolic blood pressure criteria.

Very few of the participants met the diastolic blood pressure criteria and virtually all were girls.

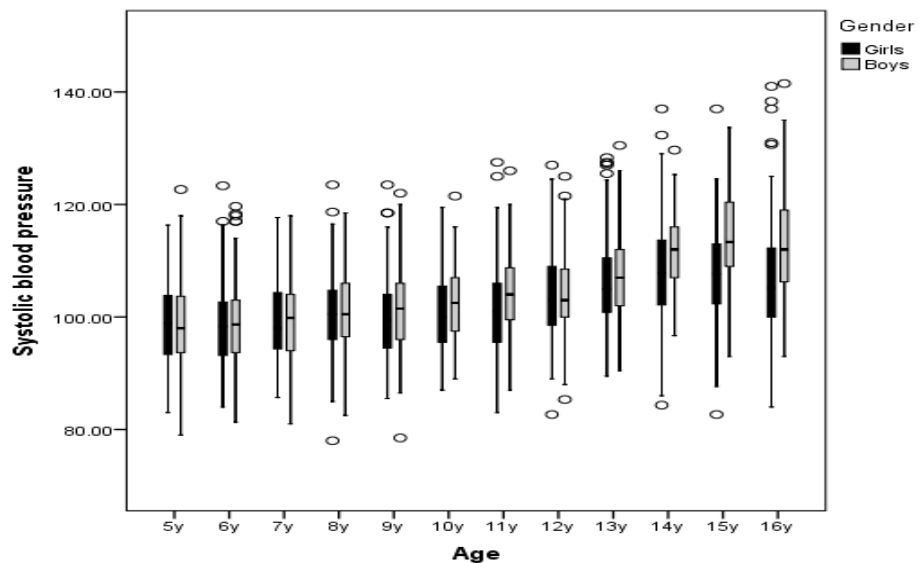


Figure 8. Systolic Blood Pressure from 5y to 16y in boys and girls

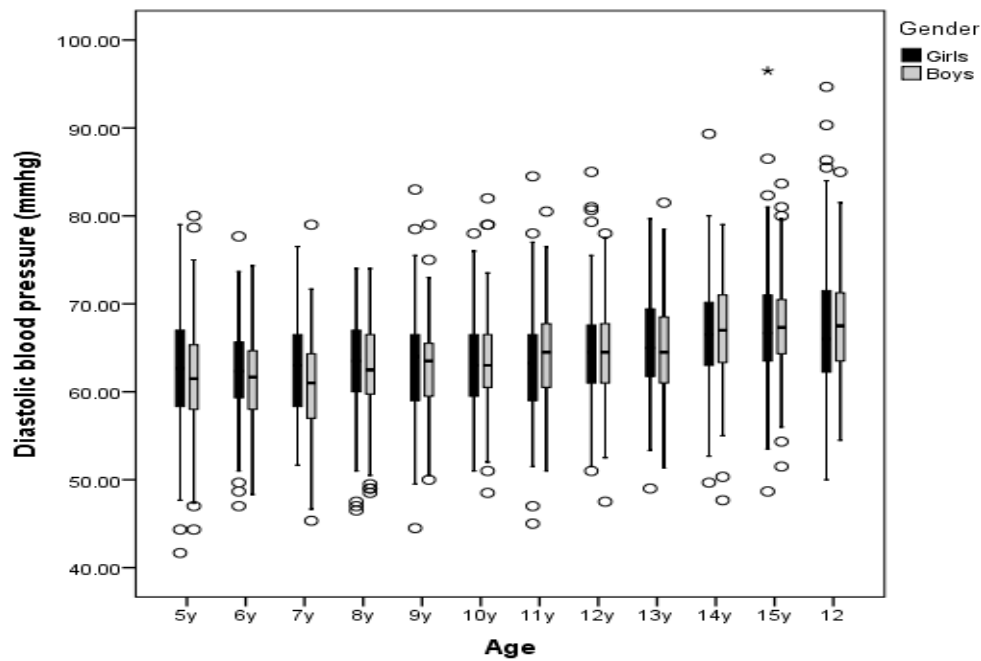


Figure 9. Diastolic Blood Pressure from 5y to 16y in boys and girls

Triglycerides (Figure 10, and appendix A4)

There was a steady increase in triglycerides with age, peaking at 12-14y of age. The levels were higher in girls ($p < 0.01$), till 16y when gender differences disappeared. Triglycerides had to be $\geq 1.7\text{mmol/L}$ to meet the definition of the MetS. It was around the typical age for the onset of puberty ($\geq 9\text{y}$) that a significant number of children met the criteria for Triglycerides ($P < 0.01$); the peak was at 14y at 6% of the cohort. Overall, most of those who met the above definition were girls.

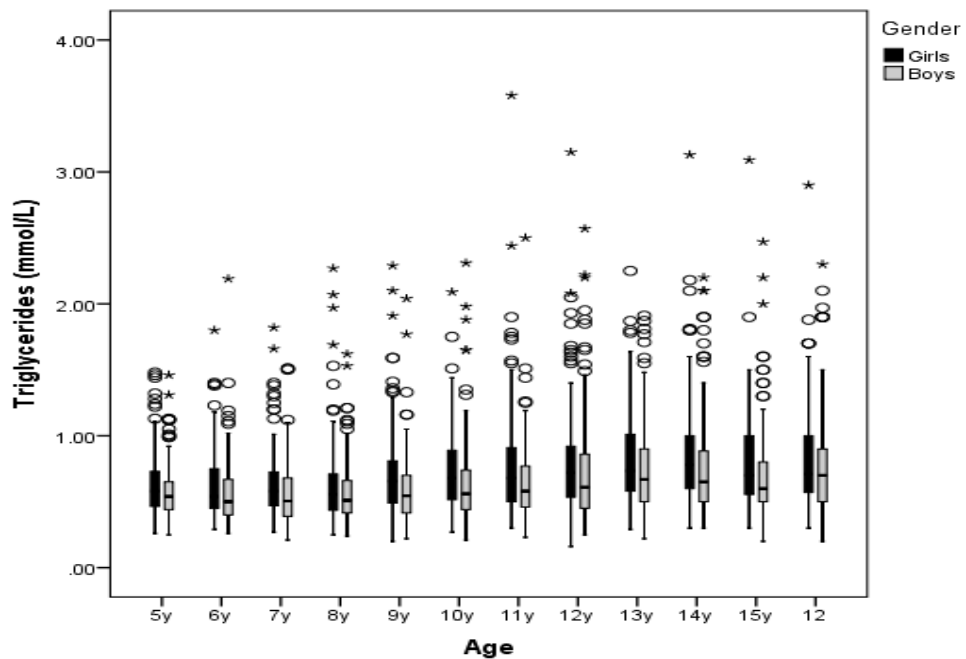


Figure 10. Triglycerides from 5y to 16y in boys and girls

Waist circumference (Figure in appendix A5)

There was a steady increase in WC from 5y-16y, with no significant gender difference.

In 10-15y olds, the criteria for the MS were $WC \geq 90^{\text{th}}$ centile; in 16yolds boys it was $\geq 94\text{cm}$, in 16y old girls, $\geq 80\text{cm}$.

Like triglycerides, there was an association between the number of participants who met the above criteria and the typical age of onset of puberty. The highest proportion was at 16y when 8% of the participants met the WC definition.

4.5.2 Tracking of the Components of the Metabolic Syndrome

Table 3 shows the year-on-year correlations and Table 4 shows the tracking coefficients controlled for age, for all the components of the MetS.

4.5.3 HDL Cholesterol

The year-on-year correlation was high (ranged from 0.64 to 0.87 in boys, and 0.64 to 0.82 in girls) and was significant ($p < 0.001$) for all eleven measurements. The

longitudinal tracking coefficient derived from GEE was $B=0.49$ (95% CI 0.294 – 0.649) in boys and $B=0.52$ (95% CI 0.162-0.509) in girls.

4.5.4 Glucose

The year-on-year correlation was moderate (ranged from 0.17 to 0.56 in boys, and 0.16 to 0.57 in girls) and was significant ($p<0.05$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B=0.139$ (95% CI 0.024-0.9) in boys and $B=0.126$ (95% CI 0.057-0.395) in girls.

4.5.5 Systolic Blood Pressure

The year-on-year correlation was moderate (ranged from 0.33 to 0.58 in boys, and 0.44-0.62 in girls) and was significant ($p<0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B=0.150$ (95% CI 0.16-0.20) in boys and $B=0.265$ (95% CI 0.12-0.41) in girls.

4.5.6 Diastolic Blood Pressure

The year-on-year correlation was moderate (ranged from 0.19 to 0.53 in boys, and 0.28-0.59 in girls) and was significant ($p<0.01$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B=0.126$ (95% CI 0.09-0.20) in boys and $B=0.376$ (95% CI 0.176 -0.54) in girls.

4.5.7 Triglycerides

The year-on-year correlation was moderate – high (ranged from 0.29 to 0.63 in boys, and 0.34-0.70 in girls) and was significant ($p<0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B=0.2$ (95% CI 0.05-0.26) in boys and $B=0.404$ (95% CI 0.180-0.602) in girls.

Table 3. Year-on-year correlation coefficients for each component of the metabolic risk score

	HDL (mmol/L)		Glucose (mmol/L)		Systolic Blood Pressure (mmHg)		Diastolic Blood Pressure (mmHg)		Triglycerides (mmol/L)		Waist Circumference (cm)	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Age (years)												
5 and 6	0.65**	0.64**	0.16*	0.17*	0.51**	0.42**	0.28**	0.19**	0.34**	0.29**	0.89**	0.91**
6 and 7	0.72**	0.74**	0.37**	0.22**	0.47**	0.55**	0.42**	0.40**	0.43**	0.48**	0.93**	0.92**
7 and 8	0.74**	0.79**	0.22**	0.36**	0.51**	0.57**	0.42**	0.53**	0.65**	0.50**	0.94**	0.93**
8 and 9	0.72**	0.78**	0.28**	0.43**	0.52**	0.55**	0.44**	0.41**	0.64**	0.42**	0.95**	0.93**
9 and 10	0.80**	0.79**	0.53**	0.27**	0.62**	0.49**	0.59**	0.37**	0.65**	0.46**	0.96**	0.95**
10 and 11	0.73**	0.79**	0.41**	0.31**	0.54**	0.33**	0.52**	0.38**	0.58**	0.60**	0.97**	0.95**
11 and 12	0.74**	0.82**	0.38**	0.48**	0.54**	0.39**	0.46**	0.43**	0.49**	0.59**	0.96**	0.96**
12 and 13	0.82**	0.87**	0.53**	0.41**	0.53**	0.46**	0.50**	0.42**	0.47**	0.58**	0.95**	0.93**
13 and 14	0.77**	0.80**	0.57**	0.56**	0.58**	0.44**	0.59**	0.34**	0.70**	0.51**	0.96**	0.92**
14 and 15	0.71**	0.81**	0.43**	0.50**	0.48**	0.44**	0.55**	0.40**	0.63**	0.63**	0.95**	0.93**
15 and 16	0.64**	0.86**	0.33**	0.40**	0.44**	0.58**	0.47**	0.48**	0.41**	0.64**	0.95**	0.94**

**<0.001; * <0.05

Table 4. Longitudinal tracking coefficients for each component of the metabolic risk score

	HDL (mmol/L)		Glucose (mmol/L)		Systolic Blood Pressure (mmHg)		Diastolic Blood Pressure (mmHg)		Triglyceride s (mmol/L)		Waist Circumference (cm)	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Coefficient	0.52**	0.49**	0.74**	0.69**	0.62**	0.62**	0.60**	0.64**	0.66**	0.54**	0.91**	0.93**

**<0.001; * <0.05

4.5.8 Associations between the components of the Metabolic Syndrome (Cross-Sectional correlations)

The strength of association between the variables (HDL, Glucose, SBP, DBP, triglycerides, and WC) was similar for boys and girls and consistent from 5y-16y. The strongest cross-sectional associations were between HDL and triglycerides (r 0.5-0.7 p <0.001), and between systolic and diastolic pressure (r 0.5 -0.64, P <0.001). The strength of association between the other variables was 0.20 – 0.7 P <0.05.

4.5.9 Trends in the Metabolic Risk Score and its components

Total Cholesterol: HDL (Figure 11 and appendix A6)

There was no significant gender difference in total cholesterol: HDL ratio; the mean was relatively stable from 5y to 16y. The components of the ratio were examined separately: total cholesterol was similar in boys and girls till 14y onwards when it became significantly higher in girls. In addition, the peak total cholesterol was at 9y in both boys and girls. HDL was lower in girls (P < 0.05) from 5y-14y after which the reverse became the case.

Insulin Resistance (Figure 12 and appendix A7)

Mean Insulin Resistance (IR) was higher in girls than boys (P < 0.001), and increased rapidly from 7y of age till it peaked at 12y in girls; the peak was 2 years later in boys.

Mean Arterial Blood Pressure (Figures in appendix A8)

Mean arterial blood pressure (MABP) was similar in boys and girls till 13y of age when it became significantly higher in boys (P < 0.05). The components of the MAP, Systolic Blood Pressure and Diastolic Blood Pressure, are described in detail above.

Triglycerides (Figure 13 and appendix A9)

Triglycerides were higher in girls than boys ($P < 0.001$) and increased from 9y of age till it peaked at 14y in both girls and boys.

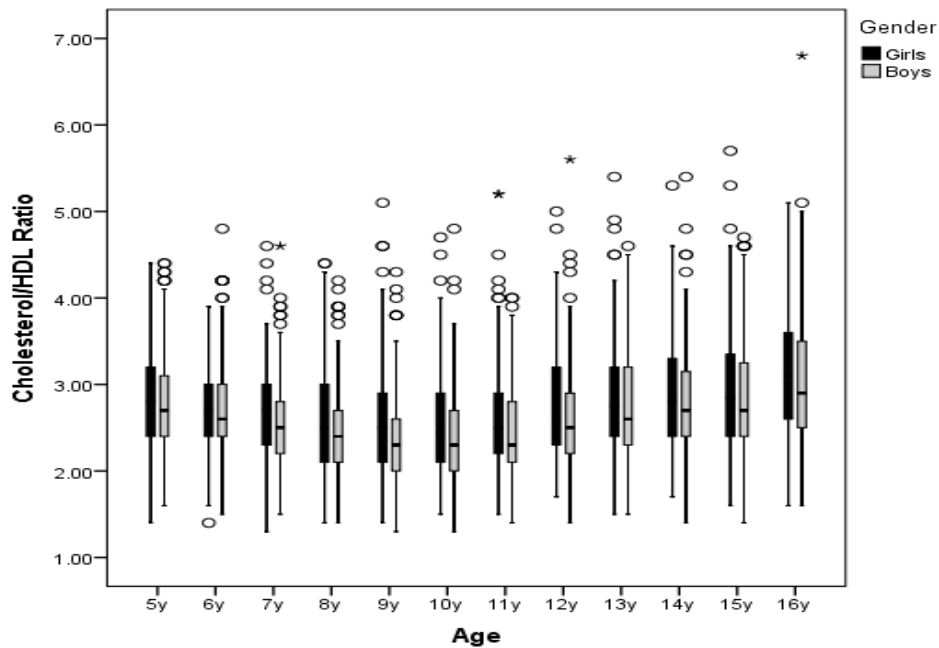


Figure 11. Total Cholesterol: HDL from 5y to 16y in boys and girls

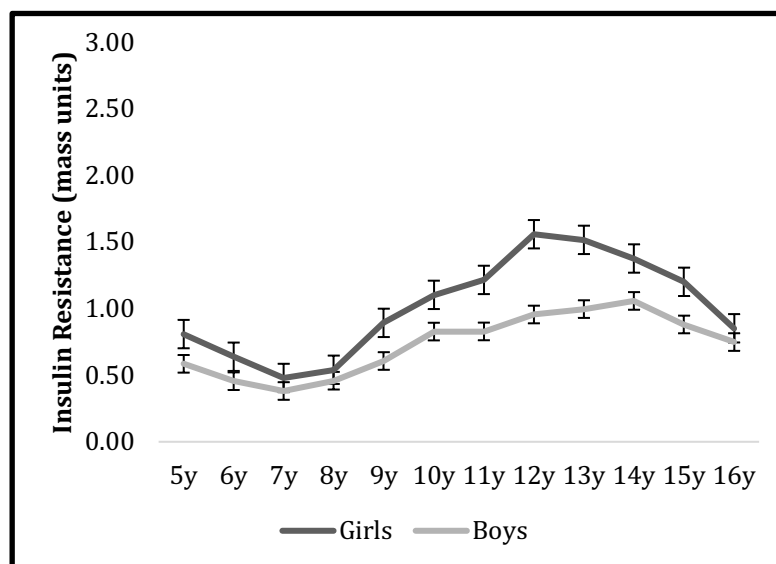


Figure 12. Insulin resistance from 5y to 16y in boys and girls

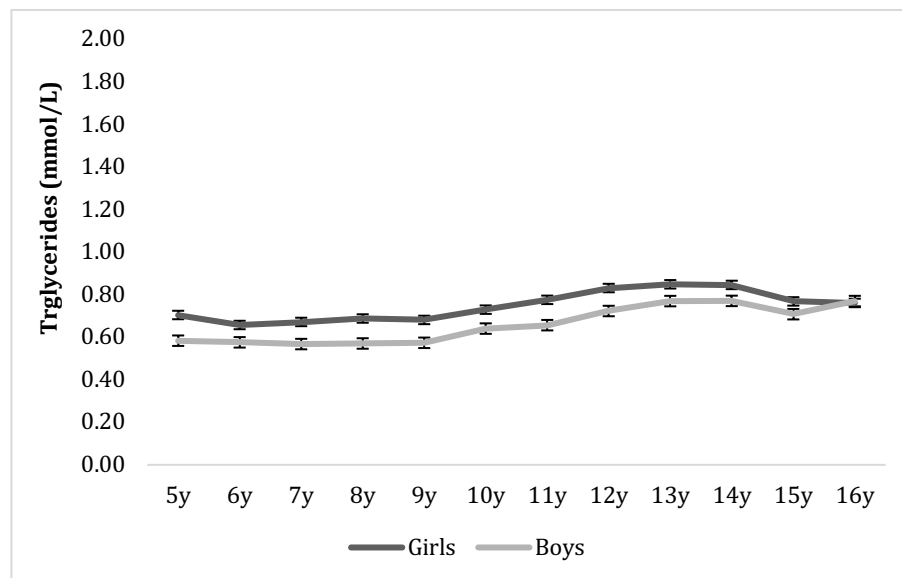


Figure 13. Triglycerides from 5y to 16y in boys and girls

4.5.10 Metabolic Risk Score (*Figure 14 and appendix A10*)

MRS was higher in girls than boys ($P < 0.001$), until 16y of age when there was an overlap (Figure 8).

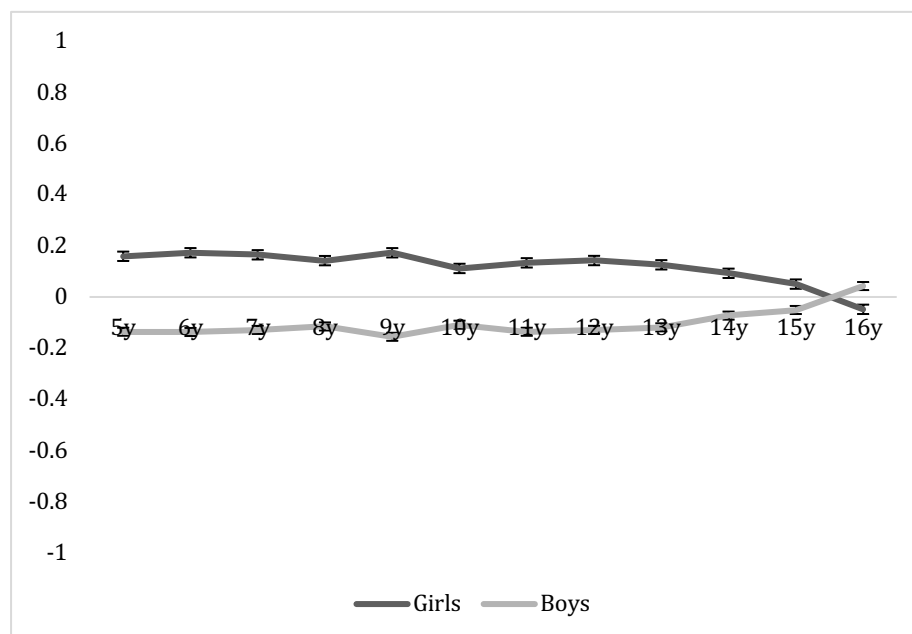


Figure 14. Trend in MRS from 5y to 16y in boys and girls

4.5.11 Tracking of the Metabolic Risk Score and Its Components

Table 5 shows the year-on-year correlation coefficients controlled for age, for all the components of the metabolic risk score, at the first and subsequent measurements.

Total Cholesterol: HDL

The year-on-year correlation was high (ranged from 0.64 to 0.84 in boys, and 0.64 to 0.86 in girls) and was significant ($p < 0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B = 0.66$ (95% CI 0.25-0.75) in boys and $B = 0.56$ (95% CI 0.26-0.81) in girls.

Insulin Resistance

The year-on-year correlation was moderate-high (ranged from 0.28 to 0.56 in boys, and 0.34 to 0.80 in girls) and was significant ($p < 0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B = 0.37$ (95% CI 0.27-0.71) in boys and $B = 0.37$ (95% CI 0.21-0.53) in girls.

Mean Arterial Blood Pressure

The year-on-year correlation was moderate (ranged from 0.26 to 0.55 in boys, and 0.35 to 0.64 in girls) and was significant ($p < 0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was $B = 0.41$ (95% CI 0.10-0.60) in boys and $B = 0.48$ (95% CI 0.30-0.86) in girls.

Triglycerides

The year-on-year correlation was moderate – high (ranged from 0.29 to 0.63 in boys, and 0.34 to 0.70 in girls) and was significant ($p < 0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE ranged was $B = 0.42$ (95% CI 0.26 – 0.64) in boys and $B = 0.44$ (95% CI 0.18-0.62) in girls.

Metabolic Risk Score

The year-on-year correlation was moderate – high (ranged from 0.46 to 0.75 in boys, and 0.48 to 0.75 in girls) and was significant ($p < 0.001$) for all eleven measurements.

The longitudinal tracking coefficient derived from GEE was $B = 0.57$ (95% CI 0.11-0.70) in boys and $B = 0.63$ (95% CI 0.10-0.74) in girls.

Table 5. Year-on year correlation coefficients between the components of the Metabolic risk score.

Age (years)	Tchol: HDL		Insulin Resistance		Mean Arterial Blood Pressure (mmHg)		Triglycerides (mmol/L)		Metabolic risk score	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
5 and 6	0.66**	0.64**	0.34**	0.39**	0.35**	0.26**	0.34**	0.29**	0.48**	0.46**
6 and 7	0.65**	0.78**	0.34**	0.28**	0.44**	0.48**	0.43**	0.48**	0.48**	0.61**
7 and 8	0.81**	0.80**	0.53**	0.32**	0.48**	0.55**	0.65**	0.50**	0.74**	0.60**
8 and 9	0.86**	0.80**	0.50**	0.31**	0.49**	0.48**	0.64**	0.42**	0.75**	0.58**
9 and 10	0.84**	0.78**	0.80**	0.56**	0.64**	0.43**	0.65**	0.46**	0.74**	0.68**
10 and 11	0.78**	0.79**	0.80**	0.53**	0.56**	0.38**	0.58**	0.60**	0.72**	0.67**
11 and 12	0.81**	0.83**	0.68**	0.62**	0.51**	0.45**	0.49**	0.59**	0.69**	0.69**
12 and 13	0.86**	0.84**	0.63**	0.56**	0.51**	0.44**	0.47**	0.58**	0.66**	0.65**
13 and 14	0.82**	0.82**	0.62**	0.54**	0.60**	0.37**	0.70**	0.51**	0.75**	0.59**
14 and 15	0.78**	0.84**	0.74**	0.48**	0.53**	0.43**	0.63**	0.63**	0.69**	0.75**
15 and 16	0.66**	0.81**	0.64**	0.47**	0.48**	0.53**	0.41**	0.64**	0.55**	0.72**

**<0.001; * <0.05

4.5.12 Associations between the components of the Metabolic Risk Score (cross-sectional correlations); table 6

The strength of association between the variables (Tchol: HDL, IR, SBP, DBP, Trigs) was similar for boys and girls and consistent from 5y-16y. The strongest cross-sectional associations were between TC: HDL and Triglycerides (r 0.5-0.7 p <0.001), and between systolic and diastolic pressure (r 0.5 -0.64, P <0.001). The strength of association between the other variables was 0.20 – 0.39 P <0.05.

Table 6. Correlations between each component of the MRS at 16y

Boys						
		TC: HDL	IR	SBP	Trigs	DBP
TC:HDL	r	1	.271**	.196*	.664**	.242**
	P		.001	.021	.000	.004
	N	139	139	139	139	139
IR	r	.271**	1	.327**	.363**	.376**
	P	.001		.000	.000	.000
	N	139	139	139	139	139
SBP	r	.196*	.327**	1	.220**	.665**
	P	.021	.000		.009	.000
	N	139	139	141	139	141
Trig	r	.664**	.363**	.220**	1	.347**
	P	.000	.000	.009		.000
	N	139	139	139	139	139
DBP	r	.242**	.376**	.665**	.347**	1
	P	.004	.000	.000	.000	
	N	139	139	141	139	141
Girls						
		TC:HDL	IR	SBP	Trigs	DBP
TC:HDL	r	1	.266**	.106	.509**	.134
	P		.002	.227	.000	.125
	N	133	132	133	133	133
IR	r	.266**	1	.251**	.196*	.274**
	P	.002		.004	.024	.002
	N	132	132	132	132	132
SBP	r	.106	.251**	1	.034	.708**
	P	.227	.004		.697	.000
	N	133	132	137	133	137
Trigs	r	.509**	.196*	.034	1	.015
	P	.000	.024	.697		.863
	N	133	132	133	133	133
DBP	r	.134	.274**	.708**	.015	1
	P	.125	.002	.000	.863	
	N	133	132	137	133	137

** . Correlation is significant (P) at the 0.01 level (2-tailed). * . Correlation is significant at the 0.05 level (2-tailed).

4.5.13 Number of children meeting the criteria for the Metabolic Syndrome

Few children fulfilled the definition of the MetS. At 16y, only 3 children met three or more criteria. Twelve children met two or more criteria at 16y, and nine of them were boys.

4.6 Discussion and Conclusion

This analysis describes a continuous metabolic risk score, a composite of known predictors of T2D and CVD, and compared it to the International Diabetes Federation's definition of the metabolic syndrome (MetS).

The MetS is a clustering of several risk factors for the development of T2DM, hypertension, dyslipidaemia, and atherosclerotic cardiovascular disease (ACVD) (Eckel RH, 2005) (Fall CH, 2008). It is associated with at least double the risk for ACVD, and is also a predictor for (Grundy S, 2005), certain cancers, vascular dementia and Alzheimer's disease (Reaven G, 2008) (Cornier MA, 2008).

The pathogenesis of the MetS is complex, but starts with increased visceral adiposity which leads to increased insulin resistance; insulin resistance leads to mitochondrial dysfunction which in turn alters glucose metabolism and causes early atherogenesis.

MetS affects over 25% of the adult population in westernised countries (Ford ES, 2004), but controversy exists regarding the various definitions of the syndrome and its ability to predict cardiometabolic events (Weiss R, 2004).

Similarly, defining the MetS in children is difficult, at least in part due to the low incidence of T2D and cardiovascular events in children, and due to the impact of race and puberty on the latter.

Few children in the EarlyBird cohort met the IDF criteria for the MetS. The low incidence of the MetS in children is due to several reasons including the fact that unlike in adults, the components of the MetS are not as well defined in childhood; also, since

children are at an earlier stage in the natural history of the MetS, those at a high risk may not be immediately apparent.

The absence of a single definition for the MetS is problematic, and even though the IDF provides the only definition specific to children, it is similar to the adult definition (International Diabetes Federation, 2005b). This significant limitation explains why the uptake of any of the MetS definitions in epidemiological studies of children is very low (DeBoer MD, 2017) (Eisenmann JC, 2010); in addition to the above reasons, another limitation is the low incidence of the MetS in children. Therefore, if the MetS is the only means of identifying those at high risk of future ACVD and T2D, the detection of children at a high risk for these conditions will be low.

Another limitation of the MetS is the fact that the definition is based on the binary nature/presence or absence of each diagnostic criteria; this precludes an accurate estimation of the risk in situations where the values at the margins of the cut-off for the definition. In addition to the above, the binary definitions used in the MetS requires the dichotomization of continuous outcome variables; this reduces statistical power and leads to lower detection of those at an increased risk of cardiometabolic disease (Franks PW, 2004). This is particularly important because the risk of developing ACVD and T2D is due to the progressive increase in several risk factors and not simply the presence or absence of the latter.

The metabolic risk score solves this problem because it is a composite of a continuous range of values (Wijndaele K, 2006) (Kahn R, 2005) which form a scale of severity of metabolic derangements.

For these reasons most epidemiological studies of metabolic risk in children utilise risk scores instead of the MetS definition. Similar to others, the score described in this analysis includes important risk factors for CVD events (Eisenmann JC., 2010) (DeBoer

MD, 2017) (Olfert MD, 2019), and also utilised internally-derived Z scores to standardise the variables included in the scores.

Again, like other studies, each component of the metabolic risk score is allocated equal weighting (DeBoer MD, 2017) (Olfert MD, 2019) (Vukovic R, 2017) (Heshmat R, 2017) (Wijndaele K, 2006) (Ibrahim MS, 2019); this leads to a balanced contribution of each component, and ensures accurate statistical analysis.

In this chapter, the MRS was described in detail, including all the components, relationship between each, and then compared to the IDF definition of the MetS.

The MRS score utilised in this study includes both metabolic and cardiovascular disease risk factors. Other researchers have compared similar risk scores in adults to several components of the MetS and found an association between a higher score and incidence of the MetS. Most of these scores however, either included metabolic or cardiovascular disease risk factors, but not both (Vukovic R, 2017) (Weiss R, 2004).

Previous studies have identified some factors that influence metabolic risk scores including ethnicity (Walker SE, 2012) and physical activity (DeBoer MD, 2019). This analysis adds to the existing body of evidence by identifying the impact of gender on the metabolic risk score. Girls had consistently higher scores and that this gender difference was driven by the presence of higher insulin resistance in girls.

Also, the high tracking coefficients in this analysis confirmed that the metabolic risk score was consistent from childhood to adolescence and early adulthood. This, and the fact that there are significant associations between markers of the metabolic syndrome in childhood and T2D/CVD in adulthood (Alberti K, 1998) (Bao W, 1994), provides additional evidence of the predictive utility of this scores on disease outcomes.

Using the IDF definition (International Diabetes Federation, 2005b), the incidence of the MetS was very low in this cohort, at less than 5%. This is also similar to many other

studies of children (DeBoer MD, 2017) (Heshmat R, 2017) (Katzmarzyk PT, 2001), and highlights the role of the MRS as a more useful tool in epidemiological studies.

The MRS is specific to the EarlyBird cohort and cannot be applied to other populations unless the data distribution, demographic characteristics, and variability of data is similar. To address this, instead of the cohort-specific Z score approach, individual variables could be compared to their respective population median. For instance, the 75th percentile of age- and sex-specific reference values. This will allow a standardized method of calculating the score allowing comparison between several populations.

This analysis has shown that metabolic risk scores track from childhood to adolescence and early adulthood. Further studies are required to keep following the EarlyBird cohort to confirm the association of the score with adult-diagnosed MetS, T2D, and ACVD.

In summary, this chapter provides an overview of the derivation and utility of a continuous metabolic risk score in a cohort of children and adolescents. This and similar scores are important in epidemiological research because there is no universal definition of the MetS in children, and the prevalence rate in this population is very low. The score described in this chapter, allowed each study subject to have a continuous value; with lower values indicating a better metabolic profile and higher values indicating a poorer metabolic profile compared to the sample studied.

Chapter 5

Metabolic Risk Score and Measures of Adiposity

5.1 Abstract

Aims and Objectives: In this chapter, the relationship between measures of adiposity in early childhood and the metabolic risk score (MRS) at 16y of age was determined, as a means of ascertaining if a high MRS at 16y is associated with high adiposity at an early age.

Methods: Adiposity was measured by anthropometry (Body Mass Index, Sum of Skin Folds, Waist Circumference) fat mass (via a two-component body composition method that utilised Dual-energy X-ray absorptiometry). Cross-sectional correlations were utilised to assess the relationship between the measures of adiposity and the MRS at each age. Time-lagged correlations and mixed effect models were used to establish the association between measures of adiposity from 5y to 16y and the presence of high metabolic risk score at 16y.

Results: All the measures of adiposity showed high tracking coefficients confirming stability over time. The association between measures of adiposity and the MRS was present early in life: those with high MRS at 16y had higher measures of adiposity as early as 5y of age. Simple anthropometric measures of adiposity were at least as effective as the more sophisticated two-component method in identifying those with high metabolic risk scores.

Conclusions: This study showed that measures of adiposity were able to identify those at an elevated risk of developing cardiometabolic disease; also, simple anthropometry was at least as effective as two-component methods.

5.2 Introduction

Atherosclerotic lesions have been shown to appear in young adults who had risk factors for cardiovascular disease (CVD) when they were children (Berenson GS, 1998) (Bogalusa Heart Study Research, 2002). The risk factors were hyperlipidaemia, high blood pressure, and obesity. The Muscatine Iowa Study and the Cardiovascular Risk in Young Finns study both reported that the presence of CVD risk factors in childhood was associated with increased carotid intimal medial thickness (CIMT) and hypertension in adolescence and early adulthood (Juonala M, 2008) (Lauer RM, 1989).

The findings from these studies support the role of screening children for CVD risk factors to allow early intervention to prevent progression to CVD events; however, there is no universally accepted screening method across different populations. For instance, the Expert Panel on Blood Cholesterol in Children and Adolescents and the American Academy of Paediatrics recommend targeted screening of children who have a family history positive for premature CVD or in those whose parents have hypercholesterolemia (≥ 6.2 mmol/l) (Expert Committee, 2007).

The problem with this screening criterion is the fact that it excludes children whose parents do not have access to healthcare, and those who don't know the medical history of their relatives.

Other authorities recommend measurement of BMI at the start of formal education to screen for obesity (Barton M, 2010) (US Preventative Services Task Force, 2010) (The UK National Screening Committee policy on Obesity, 2006); this is however not based on any research data, and it is not certain if BMI is the best screening method (Ashwell M, 2012) (Balkau B, 2007).

Childhood obesity predicts adult adiposity (Barton, 2010) (DS Freedman, 2011) (US Preventative Services Task Force, 2010) (Wright CM, 2001), and adult adiposity accounts for over a third of cardiovascular diseases (Wilson PW, 2002) (Alberti K,

1998) (Pettitt DJ, 1982) (Lew EA, 1979). However, anthropometric measures of adiposity are not identical in what they measure; for instance, waist circumference (WC) is useful in measuring central adiposity, which is predictive of insulin resistance and dyslipidaemia (Balkau B, 2007) (Chan DC, 2003), while the less widely used skin fold thickness (SF) measures subcutaneous fat (Freedman DS, 2009). Like WC and SF, body mass index (BMI) is a simple and cheap surrogate measure of body fat (Taylor AE, 2010); however it is not able to distinguish lean from fat mass.

Analysing data from the EarlyBird study provides information from a single cohort of contemporary children and allows the relationship between early measures of body composition and later cardiovascular status to be examined. Establishing such a link adds importance to the monitoring of children's growth. In addition, studying a homogenous, cohort of children over a significant period will provide useful information to answer the questions raised above. It will also inform primary prevention programmes aimed at reducing the incidence of cardiovascular disease and type 2 diabetes.

The aim of this analysis was to evaluate the ability of anthropometric measures of adiposity to predict metabolic risk, and to compare the predictive ability of these anthropometric measures to fat mass.

5.3 Methods

Details on the Methodology and Statistical analysis are discussed in Chapter 3. Insulin resistance (IR) was calculated using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ($\text{FPG (mmol/L)} \times \text{FPI (mU/L)} / 22.5$) (Matthews DR., 1985) and the presence of IR was defined as $\text{HOMA-IR} \geq 3.4$ (van der Aa MP, 2014).

Total percent fat mass was calculated as $\text{total fat mass} / \text{total body mass} \times 100$. The distribution of all continuous variables was checked for outliers and normality and transformed appropriately (natural log transformation for skin folds, and percent fat

mass). Participants were categorised as ‘overweight/obese’ or ‘normal weight’ according to WC ($\geq 90^{\text{th}}$ centile = overweight/obese) and BMI-SDS centiles ($\geq 91^{\text{st}}$ centile = overweight/obese). High metabolic risk score at 16y was defined as having a metabolic risk score $\geq 85^{\text{th}}$ centile.

Tracking of the measures of adiposity was investigated, in boys and girls separately, using year-on-year correlation and longitudinal tracking coefficients, as described in Chapter 3. Pearson correlations were obtained to determine the strength of the relationship between the measures of adiposity and the metabolic risk score at each time-point. Time-lagged correlations were used to explore the association between early adiposity and later metabolic risk score.

The trajectories of BMI, WC, and SF were described from age 5y to 16y, and fat mass from 9 to 16y. For each measure of adiposity, the trend over time was characterised by plotting the mean as well as with boxplots of their distributions at each time-point. Gender differences were tested using independent samples *T*-test.

The trajectories of adiposity from 5y-16y were also examined according to the presence high MRS at 16y, and the results were presented both graphically and in mixed effects models. Mixed effects modelling was carried out for each measure of adiposity – random intercepts were included as well as age (categorized to allow for non-linear change in adiposity over time), MRS group ($< 85^{\text{th}}$ centile at 16y or $\geq 85^{\text{th}}$ centile at 16y), and the ‘MRS group x age’ interaction as fixed effects.

5.4 Results

5.4.1 Trends in the Measures of Adiposity

Body Mass Index

As expected in a contemporary UK cohort, the mean BMI-SDS of children was well above the 50^{th} centile of the 1990 UK standards (Table 7).

BMI-SDS was consistently higher in girls than boys, ($p < 0.01$) and in both, increased over time (Figures 15 & Appendix A14).

Table 7. Adiposity of children at 5y, 8y and 16y mean (\pm SEM/IQR)

	5y		8y		16y	
	Boys	Girls	Boys	Girls	Boys	Girls
BMI -SDS	0.2 (0.1)	0.5 (0.1)	0.3 (0.1)	0.6 (0.1)	0.4 (0.1)	0.8 (0.1)
BMI (kg/m ²)	16 (1.56)	16.5 (1.8)	16.5 (2.3)	17.5 (3.0)	21.6 (4.7)	23.3 (4.9)
Ln % Fat	-	-	3 (0.0)	3.2 (0.0)	3.1 (0.0)	3.2 (0.0)
Ln SSF	0.62 (0.1)	0.59(0.1)	0.72 (0.2)	0.70 (0.2)	0.59 (0.1)	0.69 (0.1)
SSF	3.9 (1.14)	4.8 (10.6)	4.9 (2.3)	6.3 (12.1)	5.7 (3.9)	8.2 (3.7)
WC-SDS	-0.29 (0.1)	0.06 (0.1)	0.35 (0.1)	0.53 (0.1)	0.45 (0.1)	1.1 (0.1)
WC	51.0 (4.4)	51.3 (4.0)	56.5 (5.8)	57.3 (8.9)	75.0 (12.2)	72.6 (12)
IR	0.6 (0.0)	0.8 (0.0)	0.5 (0.0)	0.54 (0.0)	0.8 (0.1)	0.9 (0.1)
MRS	-1.06 (0.1)	-0.81 (0.0)	-1.8 (0.2)	-0.8 (0.2)	1.3 (0.2)	1.0 (0.2)

SEM (Standard error of the mean) for SD scores and Ln, IQR (interquartile range for SF, WC; BMI (Body mass index in kg/m²); BMI-sds (Standard deviation scores); % Fat percent body fat measured by DXA; SSF (Sum of 5 skin folds in cm); WC (Waist circumference in cm, BMI)); IR (natural log Insulin resistance measured by HOMA); MRS: Metabolic risk score.

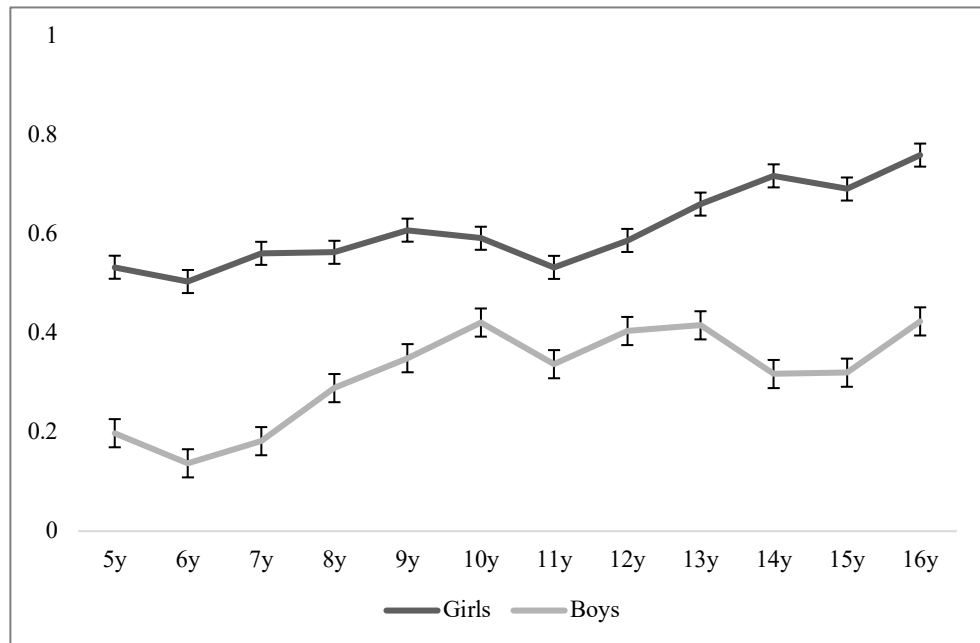


Figure 15. Mean BMI_SDS from 5y to 16y in boys and girls

Waist Circumference

Mean WC-SDS was higher in girls than in boys ($p < 0.05$), and steadily increased over time (Figures 16 & Appendix A15) from 5y to the peak at 14y and was stable up to 16y. In boys, it peaked at 10y, and was stable till 16y.

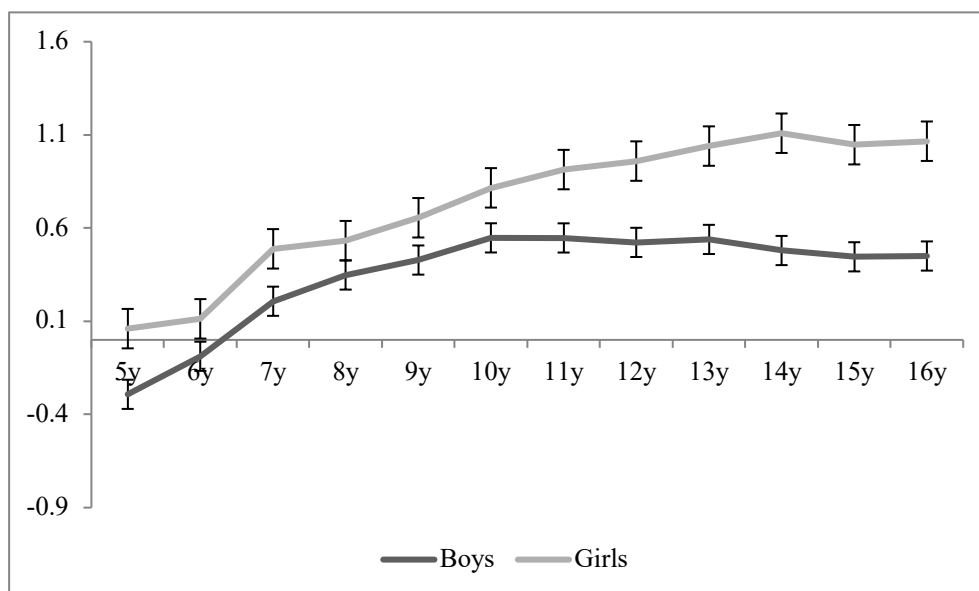


Figure 16. Mean WC-SDS from 5y-16y in boys and girls

Sum of Skin Folds

Ln SF (natural log of the sum of skin folds) was consistently higher in girls than boys ($p < 0.001$), and apart from a slight downward trend between 15y and 16y, it increased steadily from 5y (Figures 17 & Appendix A16).

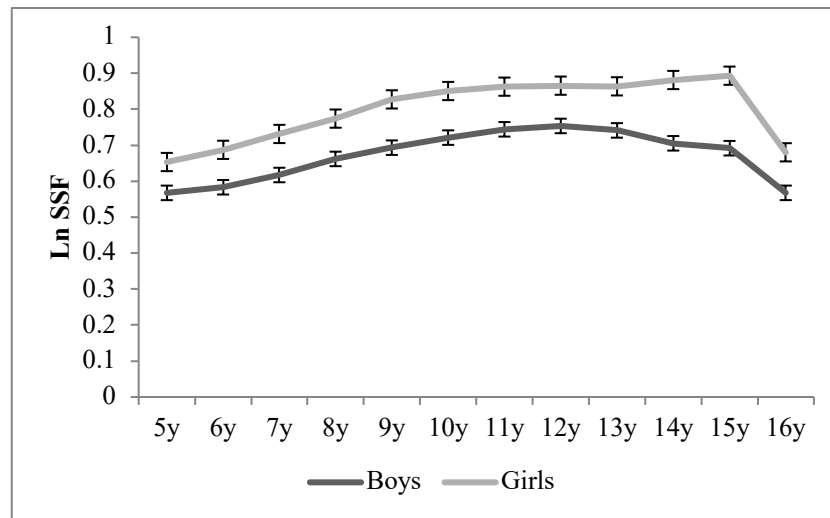


Figure 17. Mean Ln SSF from 5y-16y in boys and girls.

Fat mass

Percent Fat mass fat mass was significantly higher in girls than boys ($p < 0.001$) and increased steadily from 9y to 16y of age in both (Figures 18 & Appendix A17).

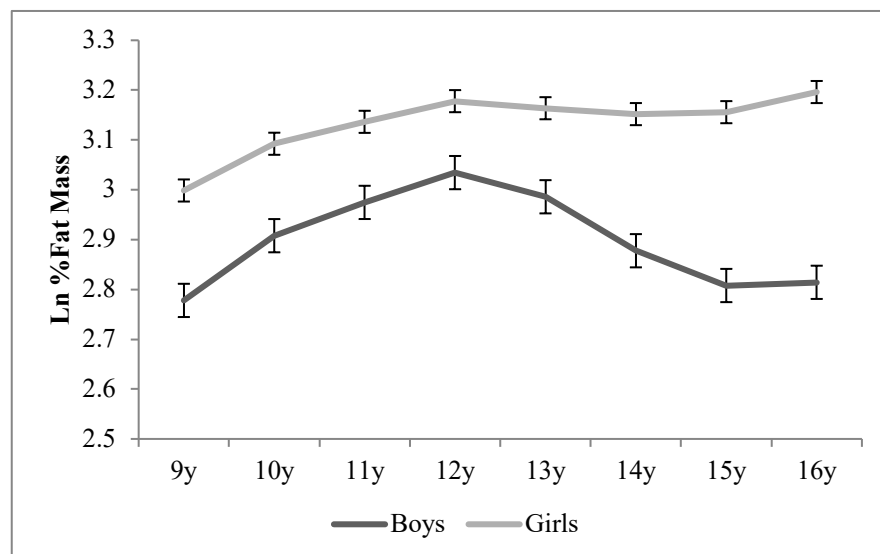


Figure 18. Total percent fat mass in boys and girls from 9y to 16y. (Ln= natural logarithm)

Irrespective of the area measured, fat mass was consistently higher in girls than boys. The highest proportion of fat was in the trunk, then legs, and then arms. Fat mass in the arms legs, and trunk (Appendix A18-A20) increased steadily in both boys and girls, peaking at 16y.

The distribution of adipose tissue around the trunk and upper body (android fat distribution) was similar in boys and girls, while the fat in the lower part of the body (Gynoid: hips, gluteal region and thighs) was much higher in girls (figures in Appendix A18-A20)

Tracking of the measures of adiposity

Table 8 shows the year-on-year correlations and table 9 shows the tracking coefficient controlled for age for all the measures of adiposity.

BMI

The year-on-year correlations in BMI-SDS were strong and consistent in both boys (0.92-0.96) and girls (0.93-0.96) and were significant ($p < 0.001$) for all eleven measurements. The longitudinal tracking coefficient derived from GEE was 0.760 in girls and 0.800 in boys, $p < 0.001$ for both.

Waist Circumference

Year-on-year correlations in WC-SDS were strong and consistent in boys (0.8-0.95) and girls (0.8-0.93), $p < 0.001$. The longitudinal tracking coefficient derived from GEE was 0.490 in girls and 0.750 in boys, $p < 0.001$ for both.

Skin Folds

Year-on-year correlations in Ln-SSF were strong and consistent in both boys (0.8-0.93) and girls (0.72-0.88). The longitudinal tracking coefficient derived from GEE was 0.600 in girls and 0.720 in boys, $p < 0.001$ for both.

Total Fat Mass

Year-on-year correlations in Ln-Percent Fat Mass were strong > 0.9 and consistent in both boys and girls $p < 0.001$ for both. The longitudinal tracking coefficient derived from GEE was 0.800 in girls and 0.820 in boys, $p < 0.001$ for both.

Relationship between measures of adiposity (cross-sectional correlations)

There were strong correlations between all the measures of adiposity from 5-16y. The correlation coefficient r between BMI SDS / WC –SDS and BMI SDS / Ln SF was > 0.8 in girls and > 0.7 in boys, $p < 0.001$ for both.

Between Ln SF / WC, r was > 0.7 in girls and > 0.6 in boys, $p < 0.001$.

There was a strong relationship between Ln percent fat mass and all of the anthropometric measures of adiposity:

With SF: $r > 0.7$ in boys, > 0.6 in girls, $p < 0.001$ for both

With WC-SDS: $r > 0.6$ in boys, > 0.7 in girls, $p < 0.001$ for both

With BMI-SDS: $r > 0.5$ in boys, > 0.7 in girls, $p < 0.001$ for both

The relationship between WC, BMI, SF, and the individual areas of fat mass was also evaluated, as well as the relationship between WC, BMI and the individual areas of fat mass. The relationship BMI-SDS and the individual skin folds were strong ($r > 0.6$, $p < 0.001$) but highest with truncal areas of skinfolds (Hip: $r > 0.8$ in boys and girls, subscapular > 0.7 in boys, > 0.8 in girls, $p < 0.001$ for all). The pattern seen between WC-SDS and the individual skinfolds was virtually identical to the latter (Hip, subscapular and suprailiac: $r > 0.9$ in boys and girls, $p < 0.001$).

The relationships between individual areas of SF and fat mass, and between individual areas of fat mass and WC were of similar magnitude.

Table 8. Year-on-year tracking correlation coefficients between each measure of adiposity.

Age (years)	BMI-SDS		Waist circumference-SDS		Ln Skinfolts		Ln % Fat mass	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
5 and 6	0.930**	0.916**	0.931**	0.911**	0.828**	0.850**	-	-
6 and 7	0.940**	0.910**	0.799**	0.917**	0.715**	0.900**	-	-
7 and 8	0.950**	0.951**	0.859**	0.930**	0.875**	0.890**	-	-
8 and 9	0.960**	0.900**	0.848**	0.929**	0.822**	0.800**	-	-
9 and 10	0.956**	0.951**	0.899**	0.905**	0.824**	0.860**	0.969**	0.962**
10 and 11	0.940**	0.959**	0.845**	0.950**	0.869**	0.800**	0.927**	0.958**
11 and 12	0.940**	0.947**	0.799**	0.844**	0.859**	0.820**	0.901**	0.968**
12 and 13	0.922**	0.929**	0.660**	0.816**	0.832**	0.870**	0.910**	0.930**
13 and 14	0.928**	0.900**	0.713**	0.852**	0.855**	0.890**	0.951**	0.902**
14 and 15	0.940**	0.920**	0.714**	0.908**	0.863**	0.900**	0.953**	0.928**
15 and 16	0.900**	0.935**	0.774**	0.809**	0.823**	0.885**	0.949**	0.938**

**<0.001; * <0.05

Table 9. Longitudinal tracking coefficients for measures of adiposity

	BMI-SDS		Waist circumference-SD		Ln Skinfolds		Ln % Fat mass	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Coefficient	0.760**	0.800**	0.490**	0.750**	0.600**	0.720**	0.800**	0.820**

**<0.001; * <0.05

5.4.2 Association Between BMI and the MRS

Cross-sectional correlations; Figure 19; scatter plots in Appendix A23-A26

In exploring the relationship between BMI-SDS and the individual components of the MRS, there were weak-moderate correlations between BMI and total cholesterol: HDL) in both boys and girls (r from 5y-16y ranged from 0.1 to 0.4 in girls, -0.2 to 0.5 in boys). The relationship was significant from 6y in girls and 5y in boys, with the strongest correlation at 16y.

The relationship between BMI and triglycerides became significant at 7y in girls but was already significant at 5y in boys (r 0 to 0.4 in girls, 0.2 to 0.5 in boys), stronger over time in both boys and girls.

A significant relationship between BMI and insulin resistance was present from 7y onwards in both boys and girls, and the peak was at 7y-9y in both boys and girls (r 0.2 to 0.54 in girls, r 0.2 to 0.5 in boys).

The relationship between BMI and mean arterial blood pressure was weak-moderate r 0.2 to 0.5 in both boys and girls, strongest at 8y and at 15y.

The relationship between BMI and the MRS was moderate – strong, peaked at 10y in girls (r 0.6, $p < 0.001$) and at 13y in boys (r 0.64, $p < 0.001$).

In summary, amongst all the components of the MRS, IR had the strongest relationship with BMI, closely followed by Tchol: HDL, then Triglycerides, and then MAP. The relationship between BMI and the components of the MRS was generally stronger with age.

5.4.3 Association Between BMI and the MRS

Time-Lagged, Figure 19

In exploring the relationship between BMI-SDS from 5-15y and the individual components of the MRS at 16y, there were moderate and significant correlations between BMI and total cholesterol: HDL in both boys and girls (r from 5y-15y ranged

from 0.2 to 0.4 in girls 0.2 to 0.512 in boys, $p < 0.01$ for both). The relationship was stable till 9y and steadily increased from then.

A significant relationship between BMI and insulin resistance was present from 9y onwards in both boys and girls.

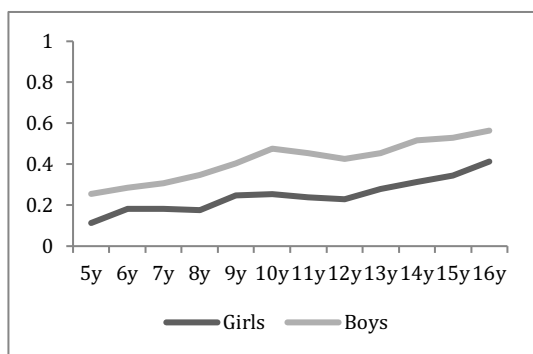
Correlation coefficients ranged from 0.2 at 5y in boys, to 0.43 at 16y and in girls, from 0.1 at 5y to 0.43 at 16y

The relationship between BMI from 5-15y and triglycerides at 16y was not significant in girls till 15y (r 0.2, p 0.008), but was weak – moderate in boys (r 0.3 to 0.5, $p < 0.01$).

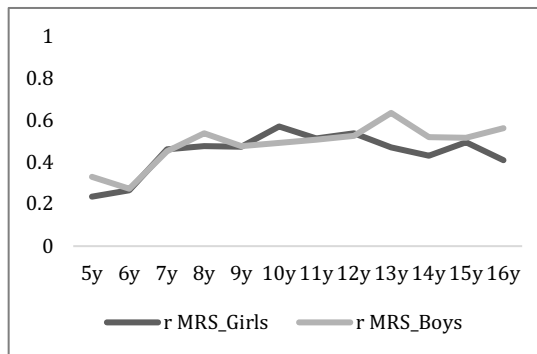
The relationship between early BMI and mean arterial blood pressure at 16y was not significant in girls but present in boys from 13-16y.

The relationship between BMI and the MRS was moderate – strong, peaked at 15y in girls (r 0.4, $p < 0.001$) and boys (r 0.6, $p < 0.001$).

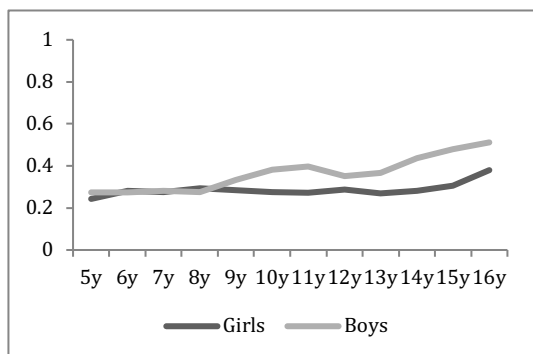
In summary, amongst all the components of the MRS, IR had the strongest relationship with BMI, closely followed by Tchol: HDL, then Triglycerides, and then MAP. The relationship between BMI and the components of the MRS was generally stronger with age.



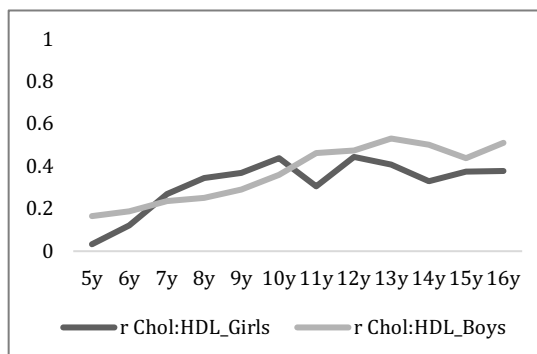
Time-Lagged
Correlations between BMI-SDS and MRS



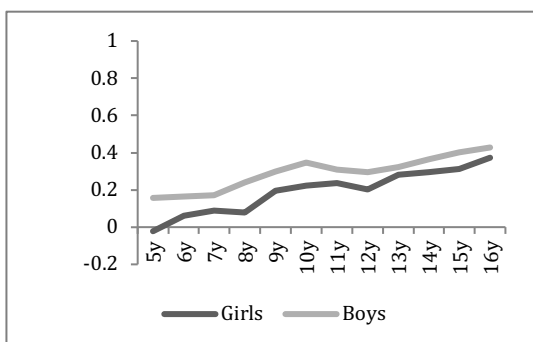
Cross-sectional



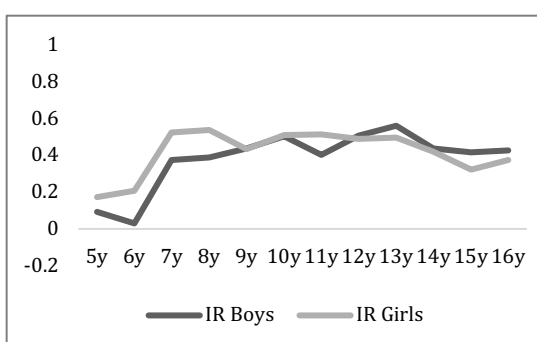
Time-Lagged
Correlations between BMI-SDS and Tchol: HDL



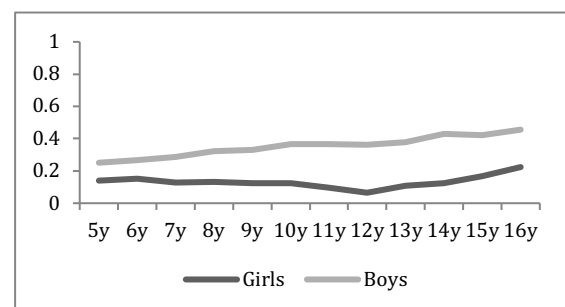
Cross-sectional



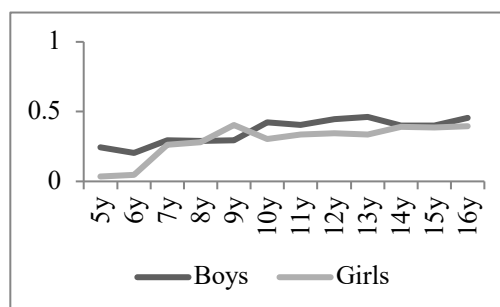
Time-Lagged
Correlations between BMI-SDS and IR



Cross-sectional

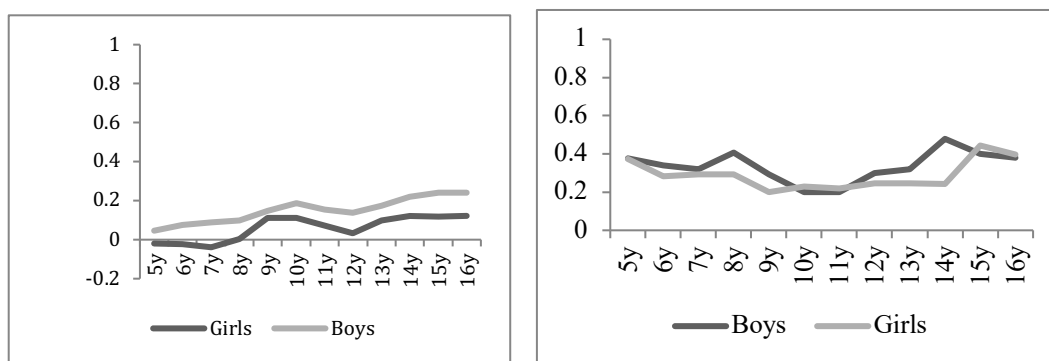


Time-Lagged
Correlations between BMI-SDS and Triglycerides



Cross-sectional

Figure 19. Association between BMI and the MRS



Time-Lagged
Cross-sectional
Correlations between BMI-SDS and Mean Arterial Blood Pressure
Figure 19 continued. Association between BMI and the MRS

5.4.4 Association Between Waist Circumference and the MRS

Cross-sectional (Figure 20)

In boys and girls, the strongest contributor to the relationship between WC –SDS and the MRS was IR, followed by Tchol: HDL, Triglycerides, and then MAP.

For the most part, all these cross-sectional correlations got stronger with time except for MAP which was stable over time, and Triglycerides in boys, which peaked at 10y and dropped subsequently.

The correlations between WC-SDS and the MRS were strongest at 16y in both boys and girls, significant from 9y-16y in girls, and from 5-16y in boys.

The relationship between WC-SDS and the MRS was much stronger than that seen with BMI. This was particularly true in girls in whom the correlations were stronger overall.

5.4.5 Association Between Waist Circumference and the MRS

Time-Lagged (Figure 20)

In exploring the relationship between WC-SDS from 5-16y and the individual components of the MRS at 16y, there were moderate and significant correlations between WC and total cholesterol: HDL in both boys and girls (r from 5y-16y ranged from 0.2 to 0.4 in girls 0.2 to 0.512 in boys, $p < 0.01$ for both). The relationship was stable till 9y and steadily increased from then, with the strongest correlation at 16y.

A significant relationship between WC and Insulin resistance was present from 8y onwards in both boys and girls, also strongest at 16y in both.

Correlation coefficients ranged from 0.2 at 5y in boys, to 0.5 at 16y. In girls, 0.1 at 5y to 0.5 at 16y

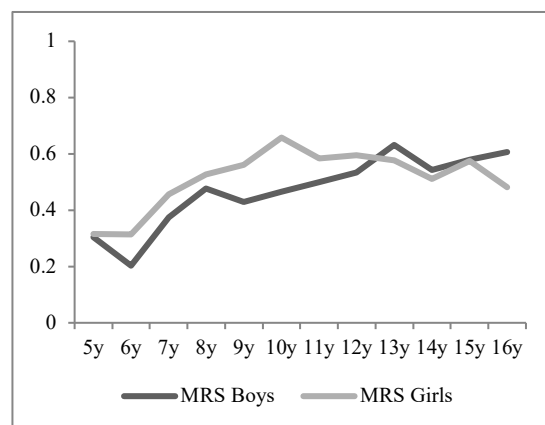
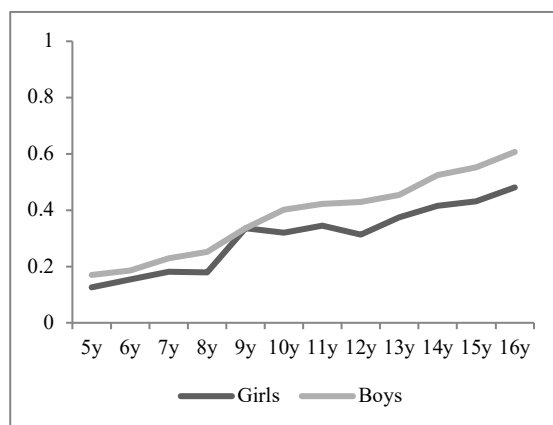
The relationship between WC-SDS and triglycerides was not significant in girls till 15y ($r\ 0.2$, $p\ 0.008$), but was moderate in boys ($r\ 0.3$ to 0.5 , $p < 0.01$), also strongest at 16y.

The relationship between WC-SDS and mean arterial blood pressure was weak and only significant in from 14-16y, in both boys and girls, strongest at 16y.

The relationship between WC-SDS and the MRS was moderate – strong, peaked at 16y in girls ($r\ 0.5$, $p < 0.001$) and boys ($r\ 0.6$, $p < 0.001$).

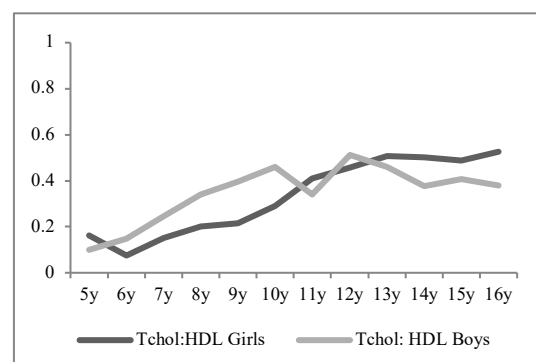
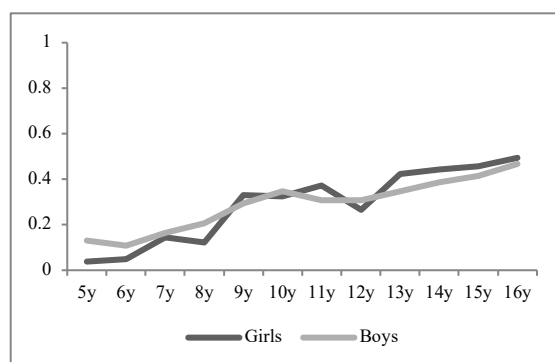
In summary, amongst all the components of the MRS, IR had the strongest relationship with WC-SDS, closely followed by Tchol: HDL, then Triglycerides, and then MAP.

The relationship between WC and the components of the MRS was generally stronger with age.



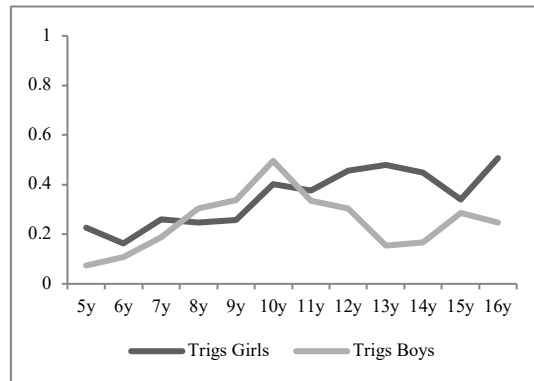
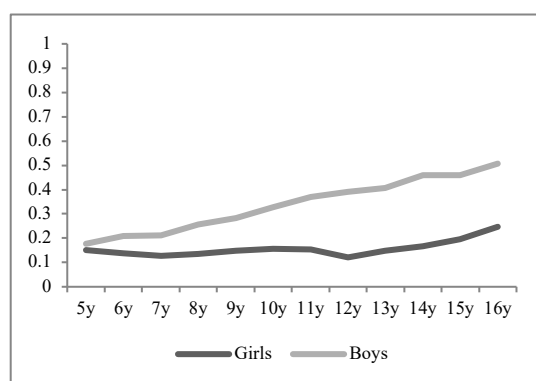
Time Lagged
Correlations between WC-SDS and metabolic risk score

Cross-sectional



Time-Lagged
Correlations between WC-SDS and Tchol:HDL

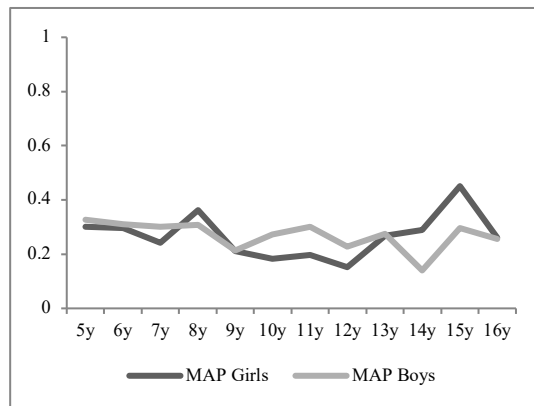
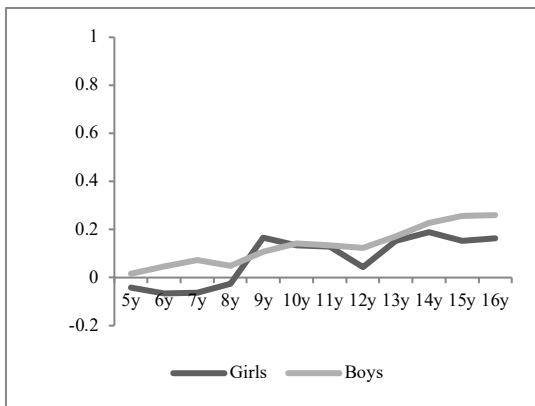
Cross-sectional



Time-Lagged
Correlations between WC-SDS and Triglycerides

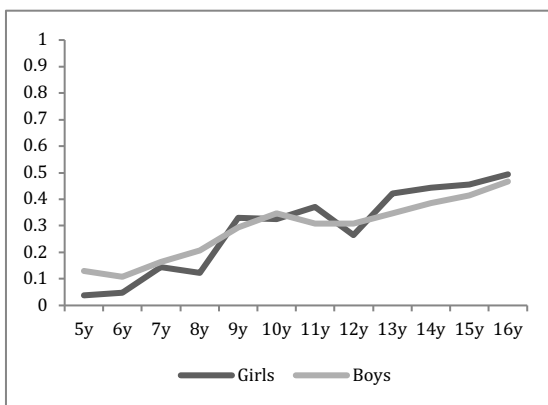
Cross-sectional

Figure 20. Association between Waist Circumference and the MRS

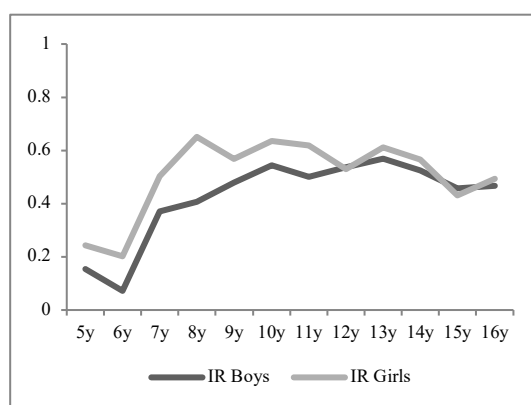


Time-Lagged
Correlations between WC-SDS and Mean Arterial Blood pressure

Cross-sectional



Time-Lagged
Correlations between WC-SDS and Insulin Resistance



Cross-sectional

Figure 20 continued. Association between Waist Circumference and the MRS

5.4.6 Association Between Skin Folds and the MRS

Cross Sectional (Figure 21)

In examining the components of the MRS, Ln SSF and TC: HDL were most correlated, significant from 6-16y ($p < 0.001$).

In girls there were weak correlations between SF and triglycerides, but in boys, the relationship was stronger and significant from 5-16y, strongest at 16y.

In both girls and boys, there was a significant relationship between SF and IR, but there were weak correlations between Systolic Blood pressure and SF in both boys and girls.

The individual areas included in the sum of skin folds were analysed and overall, truncal and hip SF was most correlated with IR.

The strongest contributor to the relationship between SF and MRS was IR, followed by Tchol: HDL, Triglycerides, and then MAP.

Apart from MAP, and triglycerides in boys, all these cross-sectional correlations got stronger with time. For MAP, it was relatively stable, while for Trigs in boys, it peaked at 10y and dropped subsequently.

The relationship between SF and the MRS was moderate – strong, peaked at 16y in girls ($r\ 0.4$, $p < 0.001$) and boys ($r\ 0.6$, $p < 0.001$).

Finally, although the trends in BMI were similar in girls and boys, unlike with WC and BMI, the relationship between SF and MRS was much stronger in boys overall.

5.4.7 Association Between Skin Folds and the MRS

Time Lagged (Figure 21)

In exploring the relationship between Ln SSF from 5-16y and the individual components of the MRS at 16y, there were moderate and significant correlations between WC and total cholesterol: HDL in both boys and girls (r from 5y-16y ranged from 0.2 to 0.4 in girls 0.2 to 0.512 in boys, $p < 0.01$ for both). The relationship was stable till 9y and steadily increased from then, with the strongest correlation at 16y.

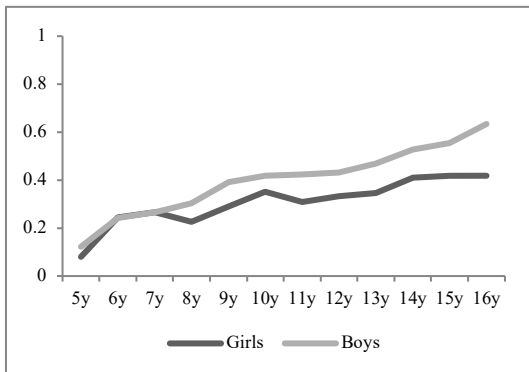
A significant relationship between SF and insulin resistance was present from 7y onwards in both boys and girls, also strongest at 16y in both. Correlation coefficients ranged from 0.2 at 5y in boys, to 0.5 at 16y. In girls, 0.1 at 5y to 0.5 at 16y

The relationship between SF and triglycerides was not significant in girls till 15y ($r\ 0.2$, $p\ 0.008$), but was moderate in boys ($r\ 0.35$ to 0.53 , $p < 0.01$), also strongest at 16y.

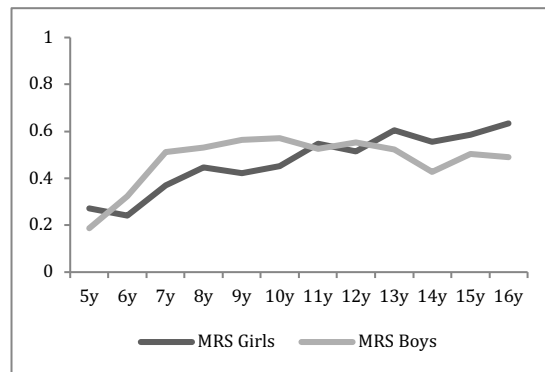
The relationship between SF and mean arterial blood pressure was weak and only significant in from 14-16y, in both boys and girls, strongest at 16y.

In summary, amongst all the components of the MRS, IR had the strongest relationship with SF, closely followed by Tchol: HDL, then triglycerides, and then MAP. The

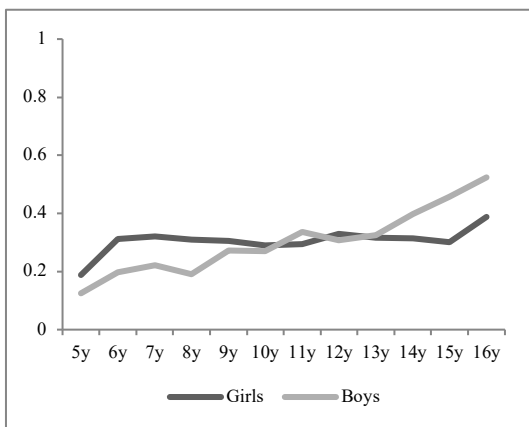
relationship between BMI and the components of the MRS was generally stronger with age.



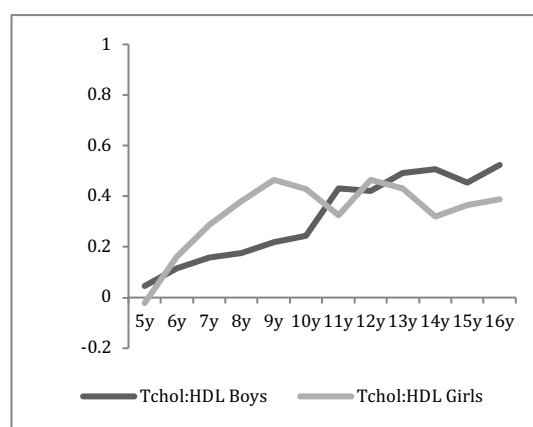
Time-Lagged
Correlations between Ln_SSF and Metabolic Risk Score



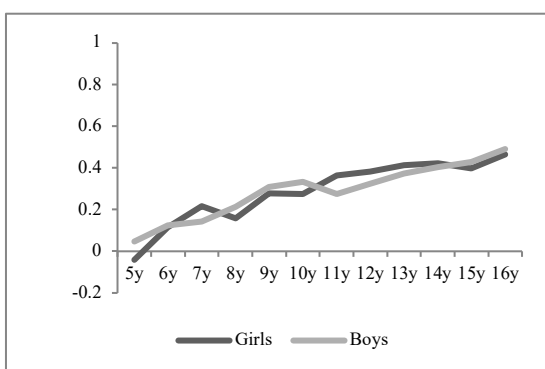
Cross-sectional



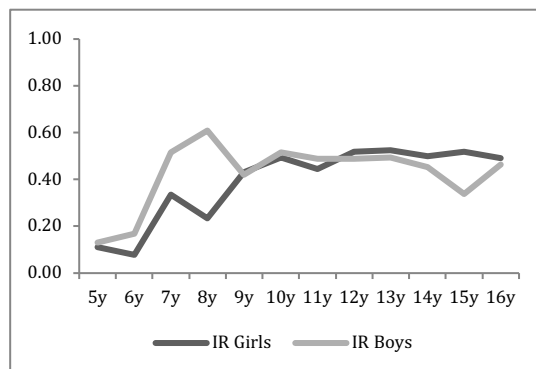
Time-Lagged
Correlations between Ln_SSF and Tchol:HDL



Cross-sectional

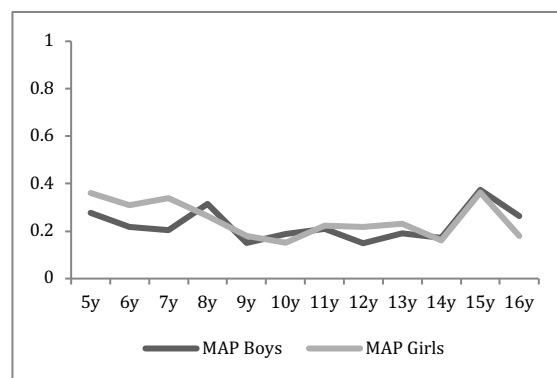
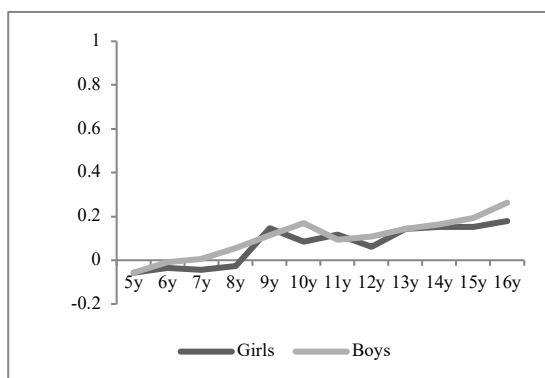


Time-Lagged
Correlations between Ln-SSF and Insulin Resistance



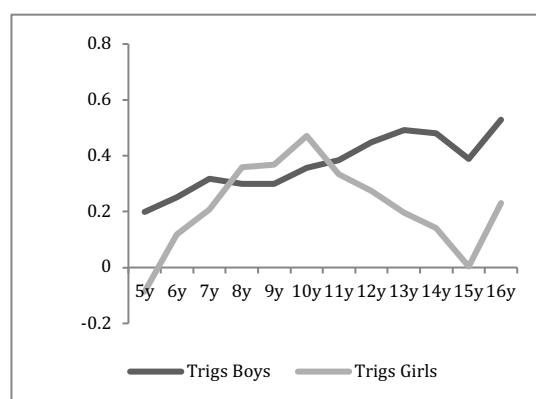
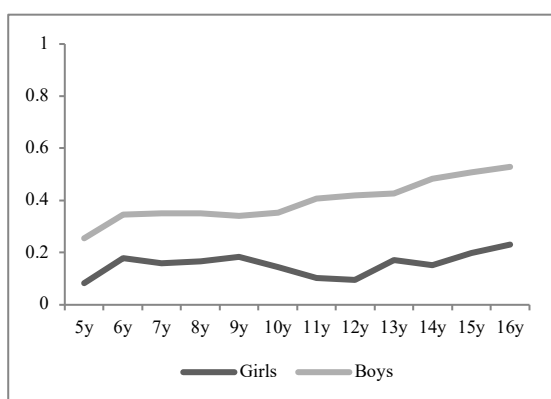
Cross-sectional

Figure 21. Association between Skin Folds and the MRS



Time-Lagged
Correlations between Ln-SSF and Mean Arterial Blood Pressure

Cross-sectional



Time-Lagged
Correlations between Ln-SSF and Triglycerides

Cross-sectional

Figure 21 continued. Association between Skin Folds and the MRS

5.4.8 Association Between Fat Mass and the MRS (*Figure 22*)

Cross-sectional

There were moderate – strong correlations between the MRS and Ln Percent Fat mass.

The relationship between fat mass in the arms, legs, and trunk, and the MRS was similar in boys and girls, and did not change much over time.

The relationship between android and gynoid fat distribution and the MRS was quite similar, although overall, the relationship in boys got stronger over time. In girls, the correlation was strongest at 10y of age, and slightly weaker subsequently. Cross sectional correlations were similar, and stable in both boys and girls.

As expected, the strongest correlations were seen between total fat mass and IR in both boys and girls. Correlations were moderate – strong, and as high as 0.6, peaking at 13y in both girls and boys (boys r : 0.3 to 0.5, girls r : 0.2 to 0.53) ($p < 0.001$).

The correlations between total fat mass and mean arterial blood pressure was weak in boys and girls, although slightly stronger in girls (peaked at 11y for both, r 0.4, < 0.01).

The relationship with triglycerides was weak in girls, but moderate in boys (boys 0.24-0.4; strongest r was at 12y $p < 0.01$; in girls, the range was 0.1 to 0.3 strongest at 9y $p < 0.01$).

There were weak – moderate correlations between Tchol: HDL and total fat mass: in boys, r 0.2 to 0.4, strongest at 11y, $p < 0.01$; in girls, r 0.1 to 0.3, strongest at 9y $p < 0.01$.

Of all the individual areas measured, fat mass in the trunk had the strongest relationship with IR in both girls and boys with correlations coefficients r ranging from 0.4 to 0.6.

The relationship in girls was stronger ($p < 0.001$) than boys and peaked at 10-11y of age.

The weakest relationship was between fat mass in all areas and blood pressure; in boys the correlations coefficients were low ($r < 0.2$) but slightly stronger in girls (0.1 to 0.3)

5.4.9 Association Between Fat Mass and the MRS (*Figure 22*)

Time Lagged

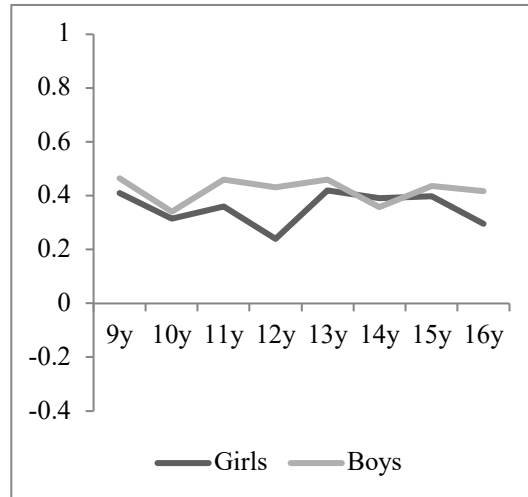
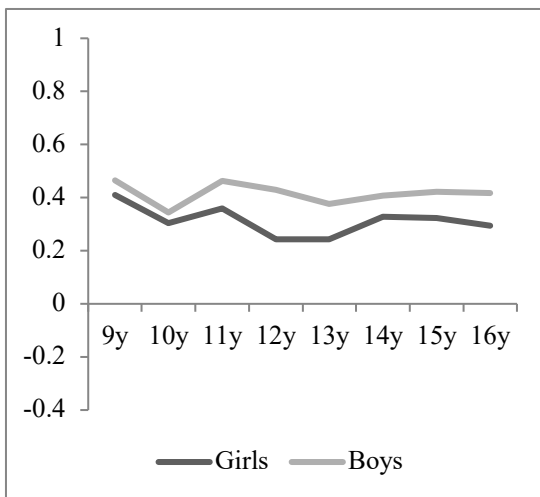
In exploring the relationship between Fat Mass from 9-16y and the individual components of the MRS at 16y, there were weak-moderate correlations between Total Fat Mass and total cholesterol: HDL in both boys and girls (r from 9y-16y ranged from 0.1 to 0.3 in girls 0.2 to 0.3 in boys, $p < 0.05$ from 13y in girls and from 10y in boys). The relationship was stable till 12y and steadily increased from then, with the strongest correlation at 16y.

A significant relationship between Total Fat Mass and Insulin resistance at 16y was present from 13y onwards in both boys and girls, also stable from then till 16y in both. Correlation coefficients ranged from 0 at 9y to 0.24 at 16y in girls, and in boys, 0.14 at 9y to 0.34 at 16y

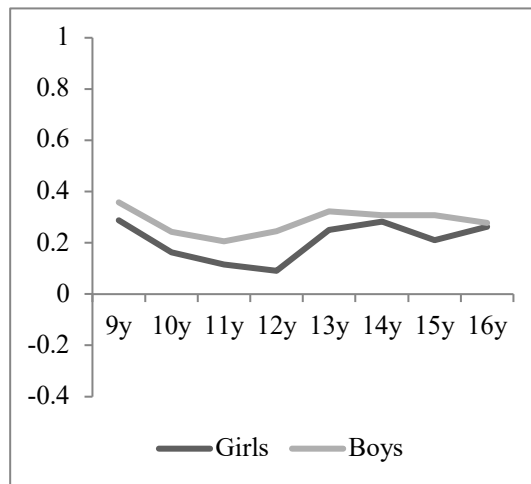
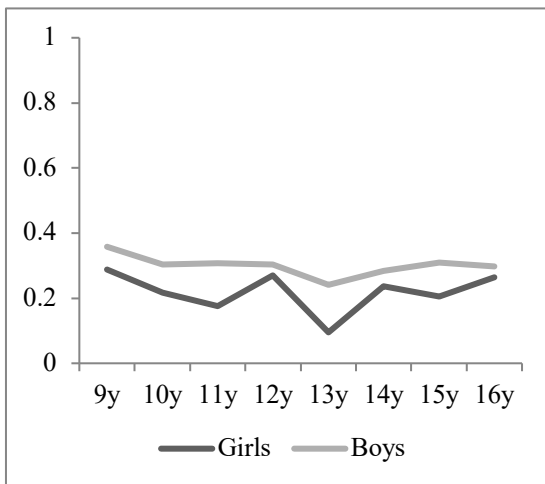
The relationship between Total Fat Mass and triglycerides was not significant in girls. In boys, it became significant at 13y ($r\ 0.34$, $p < 0.05$) till 16 y ($r\ 0.4$, $p < 0.01$).

The relationship between Total Fat Mass and mean arterial blood pressure was weak and not significant in both boys and girls.

In summary, the time-lagged correlation between the components of the MRS and fatness was weak – moderate, but slightly stronger with IR. The time-lagged relationship between Fat Mass and the components of the MRS was generally stronger with age.

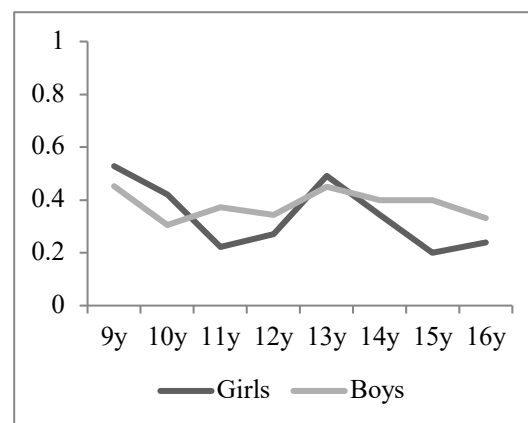
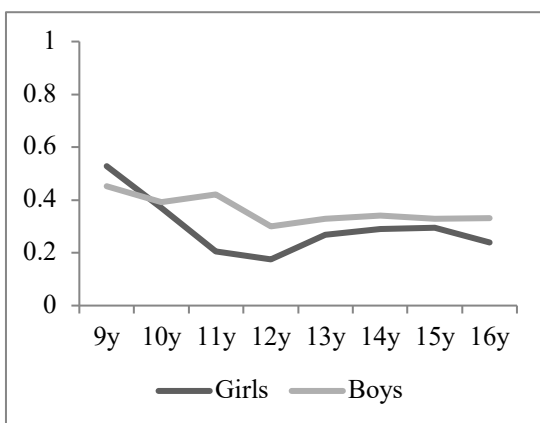


Time-Lagged Cross-sectional
Correlations between total fat mass and the metabolic risk score



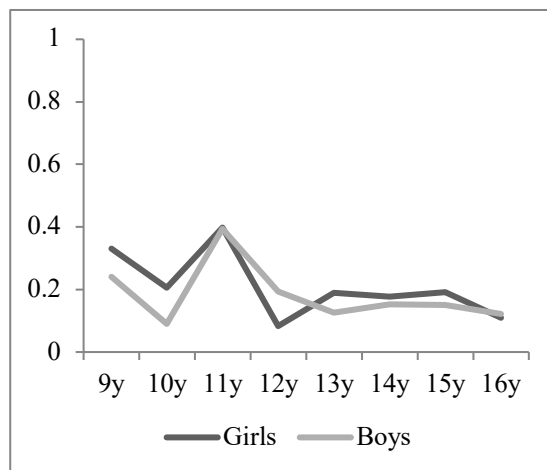
Time-Lagged
Correlations between total fat mass and Tchol:HDL

Cross-sectional

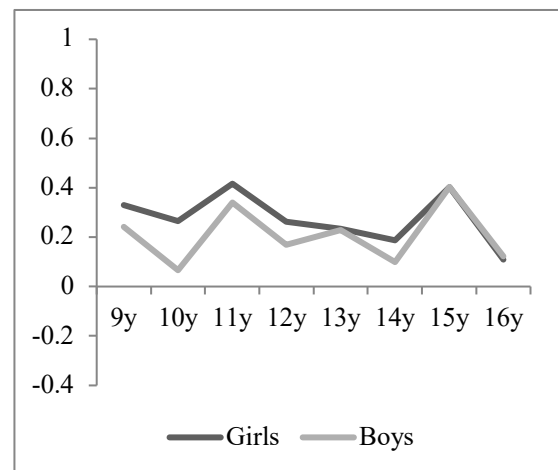


Time-Lagged Cross-sectional
Correlations between total fat mass and insulin resistance

Figure 22. Association between Fat Mass and the MRS

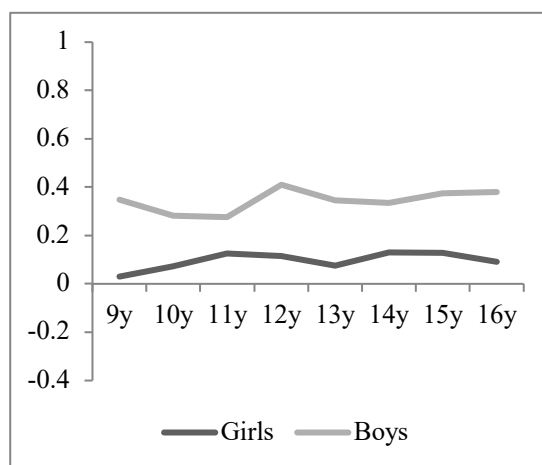


Time-Lagged

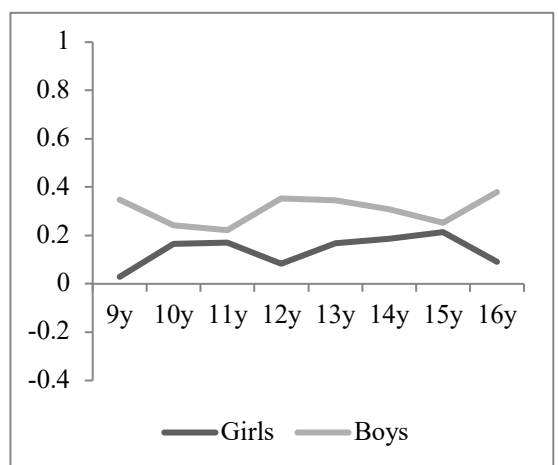


Cross-sectional

Correlations between total fat mass and Mean Arterial Pressure



Time-Lagged



Cross-sectional

Correlations between total fat mass and triglycerides

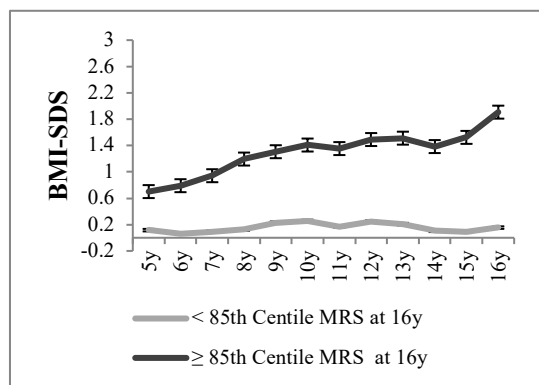
Figure 22 continued. Association between Fat Mass and the MRS

5.4.10 Adiposity according to metabolic risk score at 16y

5.4.11 BMI-SDS. (*Figure 23*)

The BMI-SDS of the boys with a high metabolic risk score was much higher than the rest of the cohort ($p < 0.001$). Similarly, the BMI-SDS in girls with a high metabolic risk score at 16y was much higher than the rest of the cohort ($p < 0.001$). These differences were already present at 5y and increased rapidly over time.

Boys



Girls

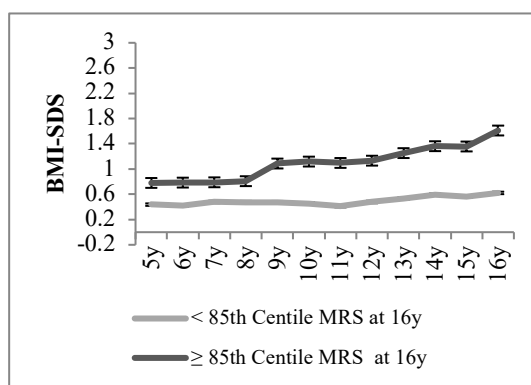
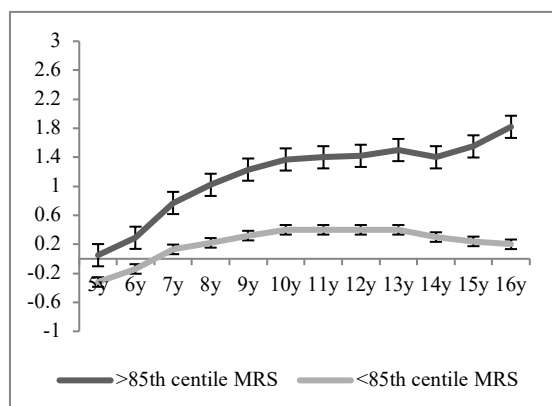


Figure 23. Adiposity (BMI-SDS) classified according to metabolic risk at 16y

5.4.12 Waist Circumference. (Figure 24)

Compared to the rest of the cohort, the WC-SDS was much higher in those with a high metabolic risk score ($p < 0.001$). These differences were already present at 5y of age, and increased over time, peaking at 16y.

Boys



Girls

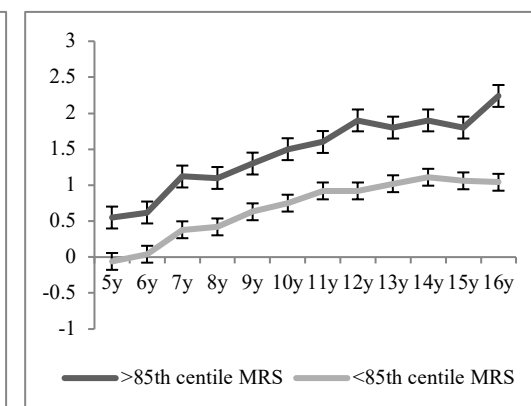


Figure 24. Adiposity (Waist Circumference) classified according to metabolic risk at 16y

5.4.13 Skin Folds. (Figure 25)

Compared to the rest of the cohort, Ln SSF was higher in those with a high metabolic risk score ($p < 0.01$). The differences were also present from 5y, and increased over time particularly in boys, to a lower degree in girls. These differences were not as significant as the other anthropometric measures of adiposity.

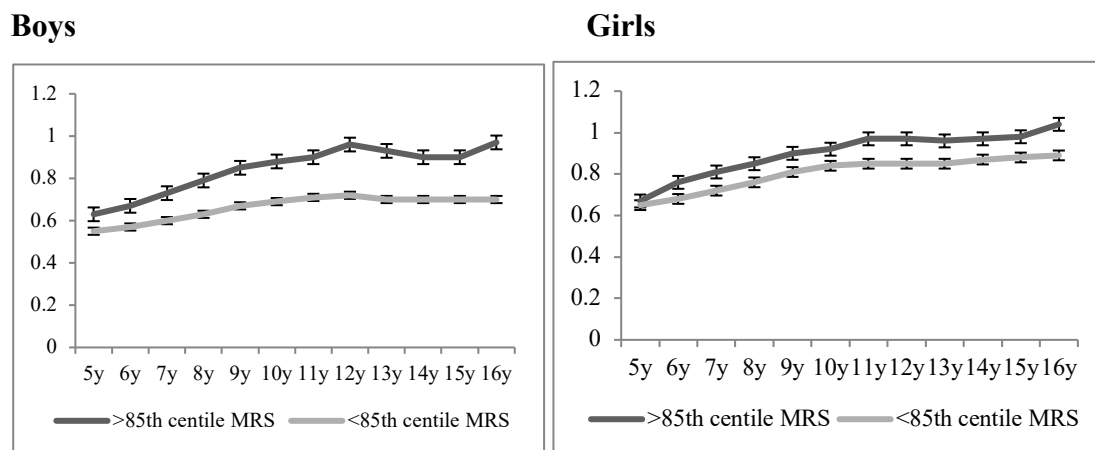


Figure 25. Adiposity (Skin Folds) classified according to metabolic risk at 16y

5.4.14 Fat Mass. (Figure 26)

Ln Percent total Fat Mass was significantly higher in boys with a high Metabolic Risk Score compared to the rest of the cohort. These differences were significant at 9y $P < 0.001$ and were constant till 16y. In girls, the differences were less significant ($p < 0.05$).

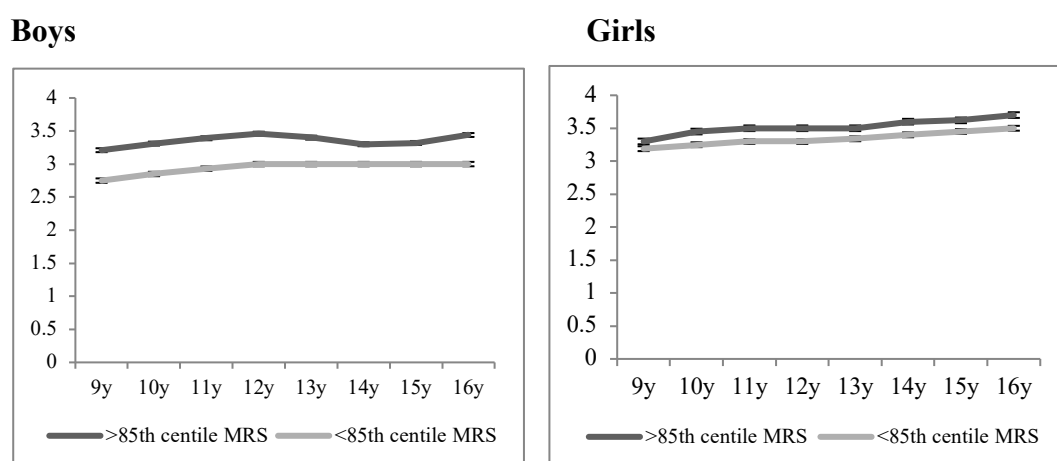


Figure 26. Adiposity (Fat Mass) classified according to metabolic risk at 16y

5.4.15 Mixed Effects Models (tables 10-17)

Mixed effects models were built to quantify the association between each measure of adiposity and the presence of a high metabolic risk score.

Irrespective of age, BMI was higher in those with a high metabolic risk score (F 25.56, $P < 0.001$ in boys, and F 7.76, $p < 0.001$), as was Skin folds (F 35.2, $P < 0.001$ in boys, and F 4.5, $P < 0.001$ in girls), and waist circumference (F 189.5, $P < 0.001$ in boys and F 11.7 $P < 0.001$ in girls). Total Fat mass was also higher in those with a high metabolic risk score.

Table 10. Mixed effect model quantifying the association between BMI and the presence of a high metabolic risk score in boys.

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	142.139	41.603	.000
Age_By_Visit	11	1480.633	10.175	.000
MRS_Boys_Gp_85	1	142.139	25.559	.000
Age_By_Visit * MRS_Boys_Gp_85	11	1480.633	7.431	.000

a. Gender = Boys
b. Dependent Variable: ch_bmisd.1: Child Bmi (sds).

Table 11. Mixed effect model quantifying the association between BMI and the presence of a high metabolic risk score in girls.

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	133.836	40.017	.000
Age_By_Visit	11	1271.860	7.679	.000
MRS_Girls_Gp_85	1	133.836	7.761	.006
Age_By_Visit * MRS_Girls_Gp_85	11	1271.860	1.074	.379

a. Gender = Girls
b. Dependent Variable: ch_bmisd.1: Child Bmi (sds).

Table 12. Mixed effect model quantifying the association between WC and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	1610	400.872	.000
MRS_Boys_Gp_85	1	1610.000	189.542	.000
Age	11	1610	9.435	.000
MRS_Boys_Gp_85 * Age	11	1610	2.103	.018

a. Gender = Boys

b. Dependent Variable: WC_SDS.

Table 13. Mixed effect model quantifying the association between WC and the presence of a high metabolic risk score in girls

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	133.845	52.753	.000
Age_By_Visit	11	1253.067	50.086	.000
MRS_Girls_Gp_85	1	133.845	11.697	.001
Age_By_Visit * MRS_Girls_Gp_85	11	1253.067	2.370	.007

a. Gender = Girls

b. Dependent Variable: WC_SDS.

Table 14. Mixed effect model quantifying the association between SSF and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	136.525	2249.210	.000
Age_By_Visit	11	1454.605	61.037	.000
MRS_Boys_Gp_85	1	136.525	35.246	.000
Age_By_Visit * MRS_Boys_Gp_85	11	1454.605	10.625	.000

a. Gender = Boys

b. Dependent Variable: LnSSF.

Table 15. Mixed effect model quantifying the association between SSF and the presence of a high metabolic risk score in girls

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	100.332	1930.806	.000
Age_By_Visit	11	1260.840	83.365	.000
MRS_Girls_Gp_85	1	100.332	4.489	.037
Age_By_Visit * MRS_Girls_Gp_85	11	1260.840	1.724	.063

a. Gender = Girls
b. Dependent Variable: LnSSF.

Table 16. Mixed effect model quantifying the association between fat mass and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	15112	408060.693	.000
MRS_Boys_Gp_85	1	15112	2927.562	.000
Age	9	15112.000	52.948	.000
MRS_Boys_Gp_85 * Age	9	15112.000	12.890	.000

a. Gender = Boys
b. Dependent Variable: LnPercFatMass.

Table 17. Mixed effect model quantifying the association between fat mass and the presence of a high metabolic risk score in girls

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	10684.000	686641.205	.000
Age	9	10684	127.568	.000
MRS_Girls_Gp_85	1	10684.000	310.488	.000
Age * MRS_Girls_Gp_85	9	10684	.627	.775

a. Gender = Girls
b. Dependent Variable: LnPercFatMass.

5.5 Discussion/Conclusion

In this chapter, the ability of childhood adiposity to predict the metabolic risk score at 16y was examined. It was important to conduct this analysis because there was otherwise limited information from a single cohort of contemporary children that examined this subject. Establishing the link between adiposity and future metabolic risk justifies the importance of screening for obesity in children.

This analysis showed that all the measures of adiposity increased over time, becoming stronger with age, and peaked at puberty. Virtually all the measures of adiposity were higher in girls, and those with a high MRS consistently had higher measures of adiposity, and these differences were already present at 5 years of age.

Over the last century, there has been a trend to earlier onset of puberty (Patton GC, 2007) (Marshall WA, 1970) probably due to the high prevalence of obesity, particularly in girls (Aksglaede L, 2009) (St George IM, 1994) (Kelly Y, 2017). The pathophysiology includes the obesity-induced increase in insulin resistance, which causes a reduction in sex hormone binding protein (Patton GC, 2007); the latter leads to an increase in the levels of circulating oestrogen which in turn accelerates the onset and progression of puberty (Ahmed ML, 2009) (Shimizu H, 2007). Also, excess adiposity is associated with a proinflammatory state, leading to higher levels of cytokines, which promote the production of puberty-inducing androgens. Aside from the above, another explanation of link between excess adiposity and early puberty is the role of Leptin. Obese children have higher Leptin levels and Leptin stimulates the gonadotropin hormone-releasing receptors thereby inducing the onset of puberty (Junfen F, 2014) (Ahima R, 1997).

Conversely, premature puberty is also associated with the development of obesity, diabetes, and breast cancer in adulthood (Lakshman R, 2008) (Velie EM, 2005).

The fact that an association between excess adiposity and the onset of puberty was shown in this study is therefore an important finding and has significant implications for the future risk of significant diseases.

When the various forms of adiposity were studied, this analysis showed that waist circumference, the measure of visceral adiposity, was consistently higher in girls. On the other hand, the measure of subcutaneous fat, skinfold thickness, was higher in boys. There were other gender differences in fat distribution: truncal fat mass, truncal skinfold thickness and gynoid pattern of fat distribution (hips and thighs) were higher in girls compared to boys.

There are not many studies that have examined gender differences in fat distribution in children; the few available studies were cross-sectional and showed conflicting results. In one, boys had higher abdominal circumferences than girls of the same age, while skinfolds were higher in girls (Weinand C, 2000); in another cohort, there were no gender differences (Komiya S, 2000); and in yet another, girls had higher waist circumference and skinfolds compared to boys (Mast M, 1998).

The findings in this analysis are novel and provide the first longitudinal study showing the trends as well as gender differences in the body composition of a cohort of children from childhood to adolescence. All the measures of adiposity had high tracking coefficients, confirming stability over time. This finding is important because it adds to the body of evidence which shows that adiposity in childhood is predictive of obesity in adulthood (Lloyd LJ, 2010) (Vanitallie TB, 1994). In this study, those who were obese at 5y old remained obese throughout childhood and adolescence; this is similar to other longitudinal studies of children (Starc G, 2011) (Craigie AM, 2011).

A meta-analysis that included over 200,000 participants showed that obese children were five times more likely to be obese in adulthood than those who were not obese (Simmonds M, 2016). Although, three-quarters of the obese adults in these studies were

not obese in childhood, the studies included in this meta-analysis used BMI as the measure of adiposity. This might have led to under-detection of obesity in the cohorts studied. Another strength of the analysis presented in this chapter, is the use of several measures of adiposity and not just BMI.

In this analysis, those with a high MRS consistently had higher measures of adiposity, and these differences were already present at 5 years of age. This illustrates the usefulness of screening for obesity in childhood and indicates that the metabolic consequences of excess adiposity are already present early in life and persist till adulthood. Therefore, early interventions are needed to tackle childhood obesity, and if these are effective, it might be possible to reduce the incidence of CVD in adulthood.

This analysis also provides the data to back up the current practice screening school-age children for obesity (US Preventative services task force, 2010) (Ehtisham S, 2004) (Barton M, 2010), and confirms that BMI is a valid screening measure for obesity, albeit not as sensitive as WC in detecting those with adverse cardio-metabolic risk.

This is similar to adults in whom strong associations were also found between central adiposity and CVD events (Wang J, 2003) (Visscher TL, 2001) (Friedl KE, 2009). This is not surprising since WC is able to measure central and visceral adiposity, an established predictor of CVD in adults. BMI on the other hand, measures both total body mass and cannot differentiate lean from fat mass (Himes JH, 2009); SF measures subcutaneous fat only (Sievenpiper JL, 2001), and is not reliable in those with the highest amount of body fat (Tanner JM, 1975) (Mei Z, 2007).

One of the drawbacks to the use of WC is the need to define gender, age, and ethnic-specific cut-points. For instance, in certain lean populations from Hong Kong (Ko GT, 1996), WC is not sensitive or specific in detecting visceral adiposity. Also, it may not be an accurate measure of central obesity in the elderly (Visscher TL, 2001) who typically have lax abdominal muscles thereby making the measurement inaccurate.

There are several reasons why WC is more strongly associated with the risk of cardiometabolic disease; adipose tissue stored in visceral regions has been shown to confer greater disease risk than adipose tissue in subcutaneous depots (Visscher TL, 2001). Also, animal studies indicate that fat cells from visceral abdominal regions are more sensitive to lipolytic stimuli and less likely to be suppressed by insulin compared to other areas of the body (Wahrenberg H, 1989) (Terry RB, 1991). This means that individuals with increased central adiposity produce higher concentrations of free fatty acids compared to those with lower abdominal fat, leading to increased triglyceride synthesis and then decreased hepatic insulin clearance (Winter Y, 2008)

The fact that the anthropometric measurements (BMI, SF, and WC) were at least as effective as two-component methods in predicting those with a high metabolic risk is of great clinical relevance; anthropometry is cheap, relatively easy to measure and also easy to interpret. On the other hand, DEXA is expensive, not readily available and so cannot be routinely used in clinical settings (Wells JCK, 2006) (Wang J, 1996) (Tucker LA, 1997).

Overweight and obesity have become prevalent in most societies, and also presents earlier in life; this means the odds of the associated complications from excess adiposity children is higher (Han JE, 2010) (Kelly AS, 2017)

This analysis confirms the link between excess adiposity in early life and higher metabolic risk from childhood to early adulthood. The main contributor to this elevated risk is the presence of insulin resistance, which is arguably the earliest sign of abnormal glucose metabolism (Castro A.). It manifests as relatively high level of insulin (Levy-Marchal CAS, 2010), which then typically progresses to dysglycaemia (Kurtoğlu S, 2010) (Lee JM, 2006).

The gold standard to determine IR is from euglycaemic clamp studies, however this is these are invasive so is not used in clinical practice; surrogate methods are therefore the norm in epidemiological studies and in clinical practice (van der Aa MP, 2014)

Like previous studies, this analysis found that the presence of IR, defined as a HOMA IR of > 3.4 (van der Aa MP, 2014) was not sufficient to identify those at a high metabolic risk; this reinforces the need for other means of detecting those at a high risk of cardiometabolic disease.

The Metabolic Syndrome definition is often used in adults for this purpose; but with at least seven different definitions of the Syndrome (Kahn R, 2005) (Grundy S, 2005) (Grundy S, 2004) (Gami AS, 2007) (International Diabetes Federation, 2005b), and no consensus definition that is specific to children, continuous risk scores like the one used in this analysis are critical for epidemiological studies of children. The components of the score utilised in this study includes both metabolic and cardiovascular disease risk factors.

Predictably perhaps, previous studies have shown a stronger association between exposure and outcome when similar scores are used instead of the dichotomisation of each risk factor (Wijndaele K, 2006).

Similar scores have also been evaluated in adults and were able to identify those with the metabolic syndrome (Olfert MD, 2019); also, there was a linear relationship between the score and the number of components of the metabolic syndrome.

In this study, high metabolic risk was defined as a value equal or above the 85th percentile; although z-scores have the advantage of being standardized, comparable across gender and age, and studied as continuous variables, they are not easily used in clinical settings. This makes the use of percentiles increasingly popular; selecting the appropriate centile to indicate high risk is difficult but several authorities recommend the 85th centile (Preedy NR, 2012).

This study has limitations; the EarlyBird cohort is predominantly white Caucasian (98%) and was recruited from a single city. While this has the obvious benefit of homogeneity, it significantly limits the option to generalise these findings to other populations. Another limitation of this study is that fat mass was only measured from 9 to 16 years, while the anthropometric measures of adiposity were measured from 5 to 16 years.

Due to the pulsatile nature of insulin secretion, the time of the day the blood sample is drawn can lead to variations in the level of plasma insulin; as well, there are day-to-day differences in levels of exercise, and other stressors that can cause differences of up to 10% (Van der Aa MP, 2015) (Henríquez S, 2013). To minimise this, all the blood samples were collected at 8am, after a 12-hour fast.

Finally, the results of ROC analysis, which compared the Metabolic Syndrome definition with the metabolic risk score, were not presented due to the low incidence of the metabolic syndrome in this cohort.

5.5.1 Conclusions

All the measures of adiposity were strongly associated with the metabolic risk score; of these, anthropometric measures particularly waist circumference, were the most useful in identifying those at high metabolic risk. These measures of adiposity should therefore be treated like other established CVD risk factors.

The metabolic risk score has been shown to be a useful tool in identifying those at a high risk for future cardiometabolic disease; however, further longitudinal studies are required to follow this cohort of children till adulthood to see the association between the measures of obesity and actual CVD events.

Current guidelines recommend screening for obesity in all children and adolescents, typically at school entry; this analysis confirms that there are already significant

differences in metabolic risk in overweight children as early as 5y of age. Considering these findings, considerations should be made to start screening for obesity from early childhood, in order to identify and treat children most at risk for cardiometabolic disease.

Chapter 6

Influence of Macronutrient Intake on Cardiometabolic Risk

6.1 Abstract

6.1.1 Objective

This chapter presents the analysis of the impact of nutrition in childhood on body mass index, waist circumference, skin folds, body fat, and metabolic risk at 16y of age

6.1.2 Design and Methods

The participants were classified based on macronutrient intake at 8y and then the relationship between this and subsequent adiposity, lipid profile, insulin resistance, and metabolic risk score was examined.

6.1.3 Results

The children who reported higher overall caloric intake, lower fibre, higher sugar, and higher saturated fat at 8 years of age were more likely to have higher metabolic risk score; there was however no difference in the measures of adiposity. The factors that drove the higher metabolic risk score were insulin resistance and triglycerides.

6.1.4 Conclusions

Further areas of research should evaluate the thresholds at which intake of specific macronutrients cause a negative impact on the risk of adiposity and CVD risk factors.

6.2 Introduction

The impact of macronutrient intake on the risk of adiposity and cardiometabolic disease in adults has been extensively studied, and some associations have been established; for instance it has been shown that adults who replaced the saturated content of dietary fat with unsaturated forms, reduced their risk of cardiovascular events by 14% (Frantz JR, 1989, Sacks FM, 2017) (Forouhi NG, 2018). There was however no evidence as to whether this led to a reduction in cardiovascular mortality (Forouhi NG, 2018).

In children however, only few studies have evaluated this subject; it has been shown that those who consumed energy dense diets had higher body fat (Tucker LA, 1997) (Johnson L, 2008) (Emmett PM, 2015) and those whose diets were low in saturated fat had slightly lower blood pressure (Simons-Morton DG, 1997) (Li L, 2007) (Lauer LM, 1989).

Several intervention trials seeking to prevent obesity by reducing dietary fat in children showed reduction in intake (Hingle DM, 2010), (Jaime PC, 2009) but the long-term benefits could not be evaluated largely because the modifications implemented were not sustained (Forouhi NG, 2018). Also, there is no data that links dietary modification in children in sustained change in the future risk of adiposity or cardiometabolic disease.

In Chapter 5 of this thesis, data was presented that confirms that several cardiovascular disease risk factors were constant from 5 to 16 years of age. This chapter extends this investigation in the same cohort of children to determine if this phenomenon might be partly explained by nutrition/food choices.

There is a commonly held notion that eating habits and food choices are established in early life, and persist into adulthood (Aggett PJ, 1997). This impression is however based on very few studies, which had few participants, and the period of observation only lasted a few years. Also, these studies showed moderate tracking (r 0.38 to 0.65) in early childhood and moderate to high in adolescence (r 0.25 to 0.70), but neither

examined the same cohort from early childhood to adolescence. These are therefore not adequate to draw conclusions that can be extrapolated to the general population (Briefel RR, 2004).

It is important to confirm if eating habits indeed tracks from childhood to adulthood, as this forms the basis of the commonly held notion that it dictates dietary choices later in life. This is even more crucial because it has been shown that the eating habits of adults influence their development of cardiovascular diseases (Baranowski T, 1990) (Jacques PF, 2001).

A few short-term cross-sectional studies have examined the association between intake of specific food groups like sugar, on weight and lipid profile (Schwarz JM, 2017); some other studies have shown an association between high calorie diets and increased fat mass in adolescence (Emmett PM, 2015). However, none of the studies to date have examined the impact of nutrient intake in childhood on the future risk of cardiovascular disease.

Therefore, this study tested the hypothesis that dietary habits in early childhood can influence cardiometabolic risk in subsequent years; the intake of each macronutrient at 8y, 12y, and 16y of age was calculated, and the relationship between macronutrient intake and measures of adiposity and cardiometabolic risk from age 9y to 16y was examined.

6.3 Methods

At each visit, food frequency questionnaires (FFQ) were completed by the parents on behalf of their children. The information on the FFQ was used to create a profile on the habitual intake of food, beverages and cooking fat during the previous 12 months. Foods eaten outside of the home were also included. Each item or food group was allocated to one of ten frequency categories ranging from ‘never’, ‘once per month’,

‘once per fortnight’ and ‘1 d a week’ to ‘7 d a week’. The selected frequency choice for each item was then converted into a weekly intake for analysis.

The portion sizes of various foods were defined for the 3 age groups studied (8, 12, and 16 years of age). Portion sizes were based on the weighted average portion sizes in taken from the British National Diet and Nutrition Survey of young people aged 4–18y (Foster E, 2009) (Gregory J, 2000). All food items were classified into main food groups; for instance, ‘Beef’ included hamburger, roast beef, mince and stew; ‘high fibre breakfast cereals’ included bran flakes, Weetabix and shredded wheat. Using the Dietplan 7 Analysis Software and data from McCance & Widdowson (McCance RA, 2014), each food on the FFQ was analysed to give the nutrient content per 100g.

Each of the food items in the database was assigned the unique McCance and Widdowson code (McCance RA, 2014) to ensure accuracy for subsequent analysis. Averages for all the foods combined for each item on the FFQ were then calculated and presented in three summary tables of nutrient values in Excel– one for each age group.

With the use of an online random number generator (Random.org) 10% of the data was randomly selected and checked for errors. To confirm accuracy, the process was chronologically followed back to the values obtained from the original FFQ.

The energy and nutrient intake of each child was classified based on the British Dietary Reference Values. These values are age-specific and are an estimate of the nutritional requirements of a healthy population. The Scientific Advisory Committee on Nutrition (SACN) sets the values and advises the British government on diet and health (Gatenby SJ, 1995) (Scientific Advisory Committee on, 2012)

The relevant reference values are summarised below in tables 18-19:

6.3.1 Estimated Nutrition Requirements

Macronutrients – Energy, fat, carbohydrates and protein

Table 18. Estimated Average Requirements for Energy

Age (yrs)	Males		Females	
	MJ/d	kcal	MJ/d	kcal
8	7.3	1745	6.8	1625
12	9.4	2247	8.8	2103
16	12.4	2964	10.1	2414

Table 19. Dietary reference values for Carbohydrate and Fat

DRVs for carbohydrate and fat as a percentage of energy intake	
% Daily Food Energy	
Total Carbohydrate*	50%
<i>of which</i> free sugars*	Not more than 5%
Total Fat†	Not more than 35%
<i>of which</i> Saturated Fat‡	Not more than 11%

Dietary reference values (DRVs) for total fat, saturated fat, total carbohydrates and sugars are given as a percentage of daily energy intake.

6.3.2 Statistical Analyses

Tracking of dietary intake measures was investigated, in boys and girls separately, using year-on-year correlation and longitudinal tracking coefficients, as described in Chapter 3. Only those who completed an FFQ on all 3 visits were included in the analyses.

Pearson correlations were obtained to determine the strength of the relationship between dietary measures and adiposity and the metabolic risk score at each time point. Time-lagged correlations (as described in Chapter 3) were used to explore the association between early dietary intake and later adiposity and metabolic risk score.

For each measure of dietary intake, the trend over time was characterised using boxplots of their distributions at 8y, 12y, and 16y. Gender differences were tested using independent samples *T*-test.

The trajectories of adiposity and metabolic risk score from 9y-16y were examined according to the classification of children according to energy and nutrient intake at 8y of age both graphically and using mixed effects models. Mixed effects modelling was carried out for adiposity and metabolic risk score – random intercepts were included as well as age (categorized to allow for non-linear change in outcome over time), physical activity, dietary intake (as a continuous variable), and the ‘dietary intake x age’ interaction as fixed effects.

6.4 Results

6.4.1 Total Energy and Proportion of Macronutrients

At 8y of age, there was no difference in total energy intake between boys and girls. Over time however, energy intake became higher in boys and increased significantly with age ($p < 0.01$) (Table 20-22, and Figure 27).

The proportions of the macronutrients were relatively stable with time; at 54-55% (Figures 28-33, Tables 20-22), carbohydrates formed the bulk of energy consumed, followed by fat (around 33% of dietary energy) and then protein. Around 22% of energy intake was from sugar.

At 8y of age, of the 145 boys, 5 (3%) reported intake of ≥ 1745 kcal of energy (estimated average requirements, EAR) daily, while 12 (10%) of the 115 girls reported ≥ 1625 kcal or EAR.

31 (21%) boys and 24 (21%) girls consumed ≤ 20 g of fibre per day, 27 (19%) boys and 17 (15%) girls reported consuming saturated fat intake that was less than 11% of dietary energy; 16 (11%) of the boys and 8 (7%) of the girls reported a total sugar intake that was $\leq 5\%$ of energy intake.

Table 20. Mean dietary intake for all children at 8, 12 and 16 years of age

Age	8 (0.3)	12 (0.3)	16 (0.3)
N	260		
Total energy intake (kcal)	1300 (258)	1495(307)	1703 (400)
Carbohydrates (grams)	180.7(37.0)	208.7 (44.5)	233 (56.4)
Carbohydrates (% energy)	55.4 (4)	55.4(4.3)	54.7 (4.6)
Fat (grams)	48.4(10.9)	54.3 (12.8)	63.5 (17.5)
Fat (% energy)	34.4 (3.2)	32.7 (3.4)	33.4 (3.7)
Protein (grams)	46.8 (11.7)	57.2(12.1)	65.0(17.4)
Protein (% energy)	14.6 (2)	15.3(2)	15.3(2)
Sat Fat (grams)	18.5 (4.6)	20.5 (5.5)	23.7 (7.1)
Sat Fat (% energy)	12.7 (1.7)	12.3 (1.9)	12.4 (1.9)
Fibre (g/day)	11.9 (3)	15.1 (4)	16.9 (4.8)
Sugar (% energy)	22.8 (3.4)	21.3 (3.5)	20.4 (3.9)
Sugar (grams)	74.3 (18.8)	86.6 (21.1)	86.6 (25.1)

Figures shown are mean (SD) and for 1day; each macronutrient is represented in grams and as a percentage of daily energy intake; Age in years (SD)

Table 21. Mean dietary intake for girls at 8, 12 and 16 years of age

Age	8 (0.3)	12 (0.3)	16 (0.3)
N	115		
Total energy intake (kcal)	1318 (266)	1472(323)	1597 (391)
Carbohydrates (% energy)	55.5 (3.6)	55.4(4.0)	55.0 (4.4)
Carbohydrates (grams)	182.8 (38.0)	204.0(46.3)	219.5 (50.9)
Fat (% energy)	33.2 (2.9)	32.6 (3.2)	33.0 (3.7)
Fat (grams)	48.8 (11.4)	53.6 (13.8)	59.1 (17.5)
Protein (% energy)	15.0(2)	15.4(2)	15.0(2)
Protein (grams)	47.4(12.3)	56.2(12.8)	60.5(16.9)
Sat Fat (% energy)	12.5 (1.5)	12.3 (1.9)	12.4 (2.1)
Sat Fat (grams)	18.5 (4.7)	20.2 (5.8)	22.2 (7.2)
Fibre (g/day)	12.1 (2.9)	14.7 (3.7)	15.9 (4.4)
Sugar (% energy)	22.7 (3.5)	21.5 (3.5)	20.4 (4)
Sugar (grams)	75.1 (20.0)	79.5 (22.2)	82.9 (24.6)

Figures shown are mean (SD) and for 1day; each macronutrient is represented in grams and as a percentage of daily energy intake; Age in years (SD)

Table 22. Mean dietary intake for boys at 8, 12 and 16 years of age

	8 (0.3)	12 (0.3)	16 (0.3)
N	145		
Total energy intake (kcal)	1285 (251)	1518(289)	1804 (389)
Carbohydrates (% energy)	55.3 (3.8)	55.4(4.4)	54.2 (4.7)
Carbohydrates grams)	179.1 (36.3)	213(42.5)	245.7 (58.6)
Fat (% energy)	33.6 (3.1)	32.7 (3.6)	33.7 (3.6)
Fat (grams)	48.1 (10.6)	55.0 (11.7)	67.8 (16.5)
Protein (% energy)	14.5 (1.7)	15.3(2)	15.4(2.1)
Protein (grams)	46.0 (11.2)	58.2(11.4)	69.3(17.0)
Sat Fat (% energy)	12.9 (1.9)	12.3 (1.9)	12.5 (1.8)
Sat Fat (grams)	18.4 (4.5)	20.8 (5.3)	25.1 (6.8)
Fibre (g/day)	11.7 (3)	15.4 (4)	17.7 (4.9)
Sugar (% energy)	22.9 (3.4)	21.1 (3.6)	20.0 (3.7)
Sugar (grams)	73.6 (17.8)	80.3 (20.2)	90.1 (25.1)

Figures shown are mean (SD) and for 1day; each macronutrient is represented in grams and as a percentage of daily energy intake; Age in years (SD)

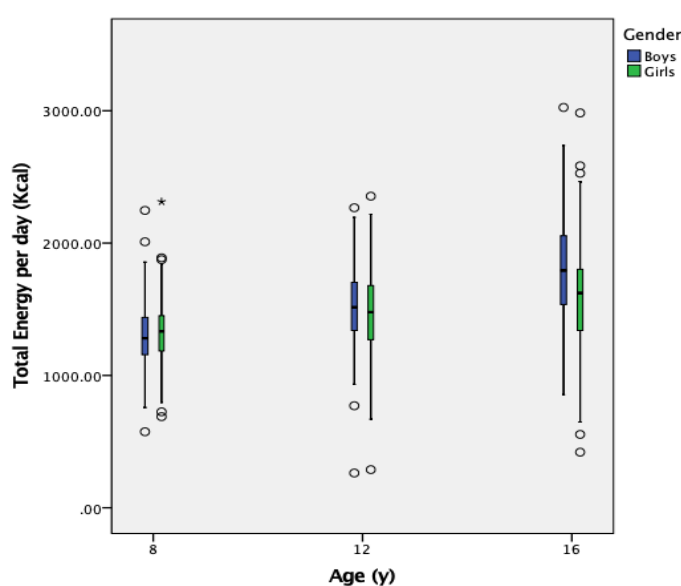


Figure 27. Total Energy intake in boys and girls

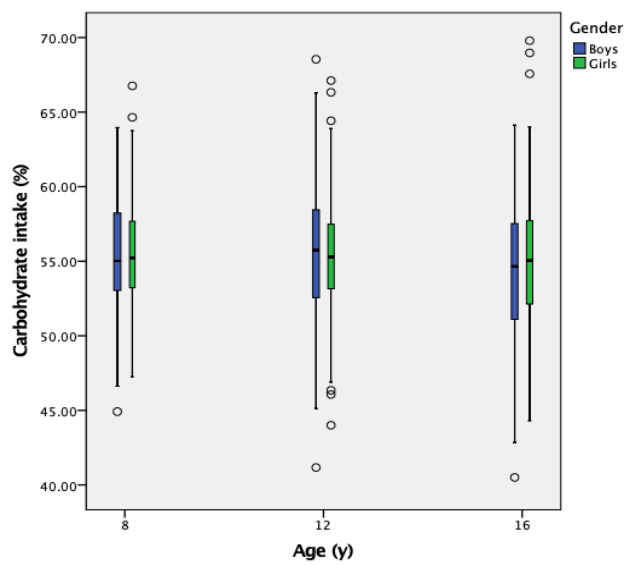


Figure 28. Proportion of energy obtained from carbohydrates

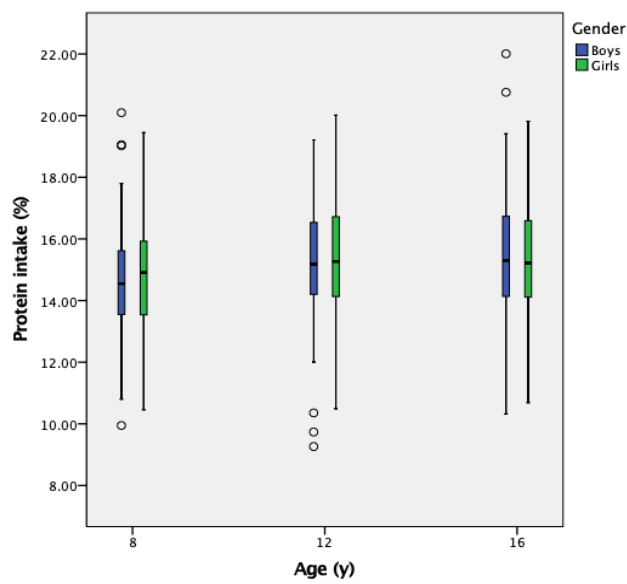


Figure 29. Proportion of energy obtained from protein

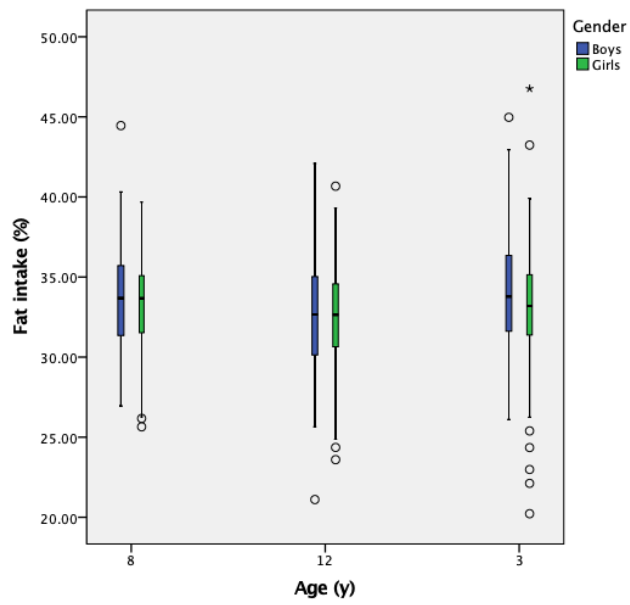


Figure 30. Proportion of energy obtained from fat

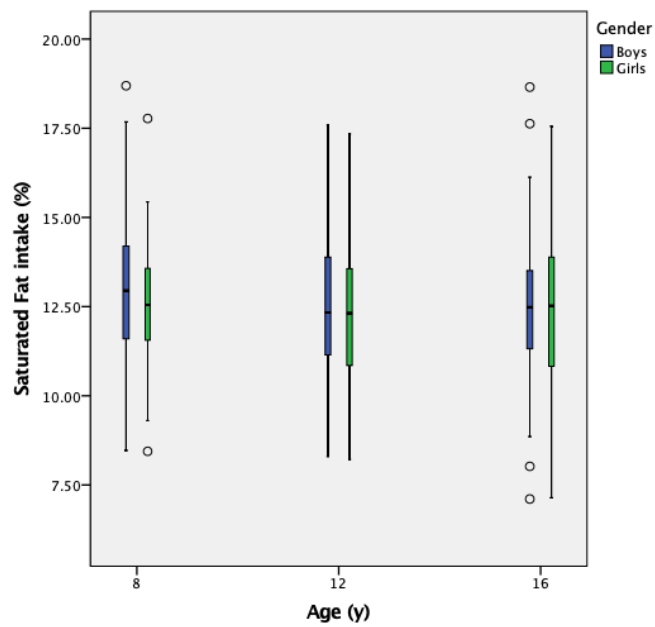


Figure 31. Proportion of energy obtained from saturated fat

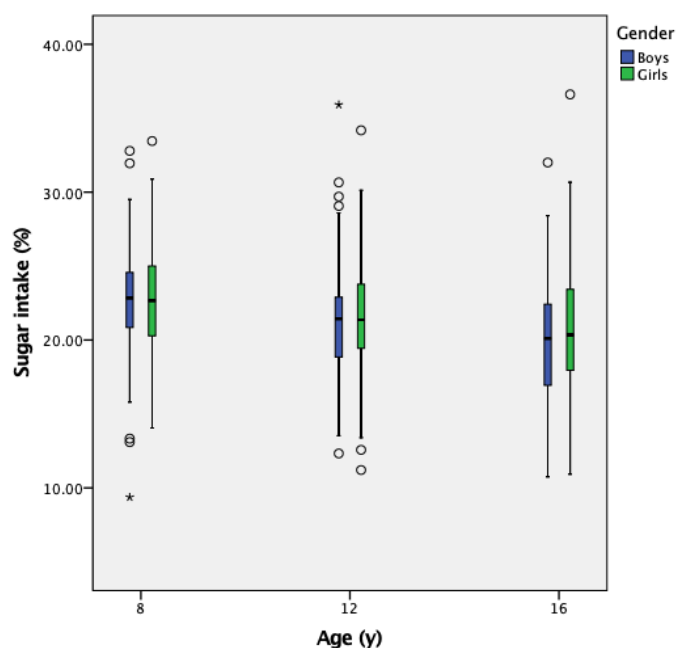


Figure 32. Proportion of energy obtained from added sugar

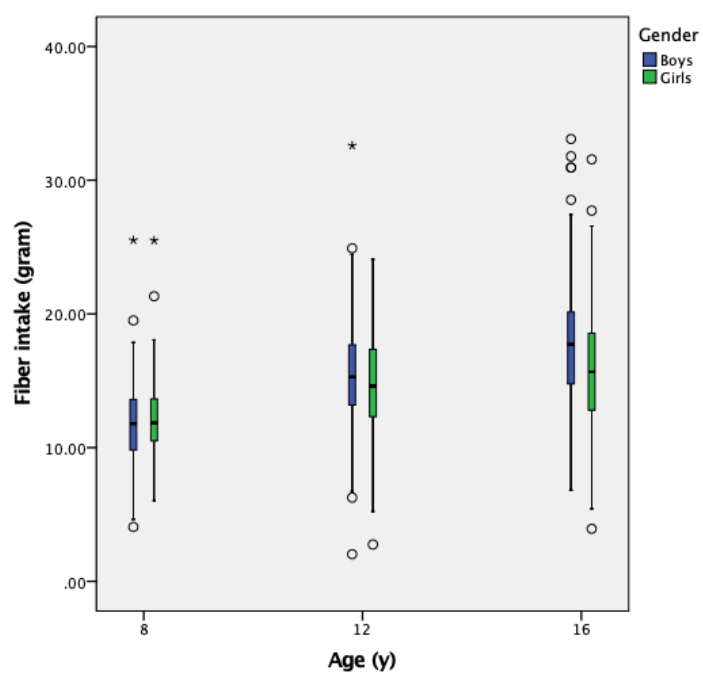


Figure 33. Proportion of energy obtained from fibre

6.4.2 Trends in Macronutrient Intake over Time; tables 23-24

The proportion of fat, carbohydrate, and protein, as a percentage of dietary energy, was consistent over time, as evidenced by the high tracking coefficient for the intake of these macronutrients.

The year-on-year correlation coefficient for total energy intake from 8y-16y was >0.7 ($p < 0.001$) for boys and >0.6 ($p < 0.001$) for girls (Table 17) and ranged from 0.6-0.8 for the major macronutrients.

In boys, the correlation coefficient of total energy and fat intake over time was higher compared to girls. After fat, the next strongest correlation in boys was in carbohydrates, and then protein; for girls it was for protein and then carbohydrates.

The results were similar when the analysis was completed for the macronutrient intake measured in grams (as compared to percentage of total energy intake).

Table 23. Year-on-year correlation between energy, carbohydrates, protein and fat

Age (y)	Total Energy		Carbohydrate intake		Protein Intake		Fat intake	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
8 & 12	0.741**	0.798**	0.708**	0.797**	0.750**	0.750**	0.758**	0.807**
8 & 16	0.650**	0.713**	0.627**	0.657**	0.695**	0.695**	0.658**	0.732**
12 & 16	0.652**	0.703**	0.628**	0.663**	0.613**	0.613**	0.653**	0.760**

** <0.001 ; * <0.05

Table 24. Year-on-year correlation between fibre, saturated fat and sugar

Age (y)	Fibre Intake		Saturated Fat		Sugar Intake	
	Girls	Boys	Girls	Boys	Girls	Boys
8 & 12	0.777**	0.822**	0.773**	0.795**	0.701**	0.645**
8 & 16	0.674**	0.638**	0.651**	0.673**	0.702**	0.535**
12 & 16	0.656**	0.681**	0.643**	0.731**	0.649**	0.528**

6.4.3 Cross-sectional relationships between macronutrient intake and measures of adiposity (correlation matrix in Appendix A27-A28)

Carbohydrates

In 8y, 12y and 16y old girls, there were weak and negative correlations ($r -0.2$ to -0.25 $p < 0.01$) between BMI-SDS, WC, and Ln Percent fat and carbohydrate intake (total and

proportion of total intake). Also, there was no significant correlation between either total or proportion of carbohydrate intake, and skin folds (r -0.042-0.064, $p > 0.2$).

In the 8y old boys, there was no relationship between any of the measures of adiposity and carbohydrate intake (total or proportion of total intake, r 0.004-0.099, $p > 0.2$).

In 12y old boys however, there were weak-moderate negative correlations (r -0.2 to -0.3 $p < 0.01$) between BMI-SDS and Ln Percent fat and the total intake of carbohydrates (in grams). However, there was no relationship between the percentage of total energy intake from and these parameters (r -0.144 – 0.084, $p > 0.094$). There was no relationship between either total or proportion of carbohydrate intake with skin folds at any age (r -0.057 - .006, $p > 0.2$).

Sugar

There was no relationship between sugar intake in boys and any of the measures of adiposity (r . -0.114 – 0.170, $p > 0.09$). In girls however, there were weak correlations between sugar intake at 8y and percent body fat at 8y (r 0.2, $p < 0.05$).

Fibre

There was no significant relationship between total fibre intake and any of the measures of adiposity (r -0.157 – 0.143, $p > 0.14$).

Protein

In boys, there was no significant relationship between protein intake and any of the measures of adiposity at any age (r -0.113 – 0.177, $p > 0.075$); while in girls, there were weak correlations between skin folds and WC at 8y and the proportion of protein intake (r 0.3, $p < 0.01$).

Fat

There were no substantial relationships between total fat intake and any of the measures of adiposity in both boys and girls, (r -0.23 – 0.12, $p < 0.01$), there were weak correlations between the total intake of saturated fat and BMI-SDS in girls (at 8yr, r 0.3, $p < 0.001$; 12y r 0.2, $p < 0.01$, 16y, r 0.3, $p < 0.01$)

The correlation between total energy intake and the measures of adiposity was similar to that between total carbohydrate intake and these measures.

So, to **summarise**, the cross-sectional relationship between measures of adiposity and proportions of macronutrient intake was weak and not substantial.

6.4.4 Differences in Measures of Adiposity Based on Nutrient Intake at 8y

Based on the Dietary Reference Values described above, the study participants were classified based on energy and nutrient intake at 8y of age, and then the differences in BMI, skin folds, waist circumference, and percent body fat from 8y to 16y were examined.

6.4.5 Total Energy Intake

At 8y of age, of the 145 boys, only 5 (3%) reported total energy intake of ≥ 1745 kcal daily, while 12 (10%) of the 115 girls consumed ≥ 1625 kcal. The difference in energy intake between the 2 groups was: 411 ± 10 kcal/day for boys and 423 ± 14 kcal for girls.

In the boys who consumed more energy at 8y, BMI was higher from 9y-14y of age (Figure 34a). In girls however (Figure 34b), BMI was significantly lower in those who consumed more than 1625 kcal daily.

A similar pattern was seen in WC-SDS (Figure 35 a-b); in boys who exceeded 1745 kcal a day at 8y of age, WC-SDS from 9y-14y was significantly higher.

When the sum of skin folds was examined, there was no difference between both boys and girls who consumed more or less than the EAR for energy. Percent body fat was higher in boys who consumed > 1745kcal at 8y compared to those who consumed less. To **summarise**, most of the measures of adiposity were slightly higher in boys who consumed more energy at 8y, but for girls the opposite was found.

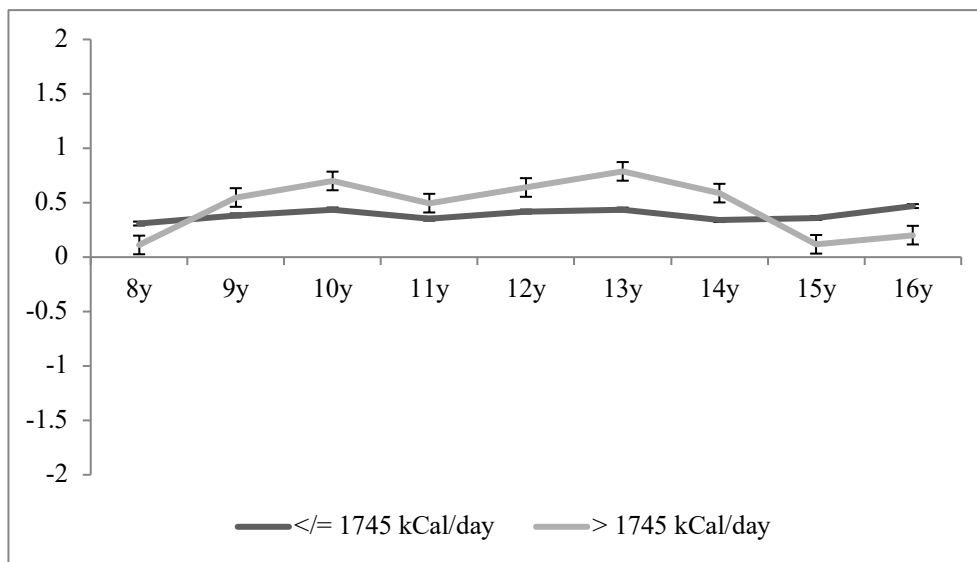


Figure 34a. Difference in BMI according to energy intake in boys

Figures shown are mean (\pm SEM) and represent the difference in BMI from 8y-16y based on mean energy intake at 8y

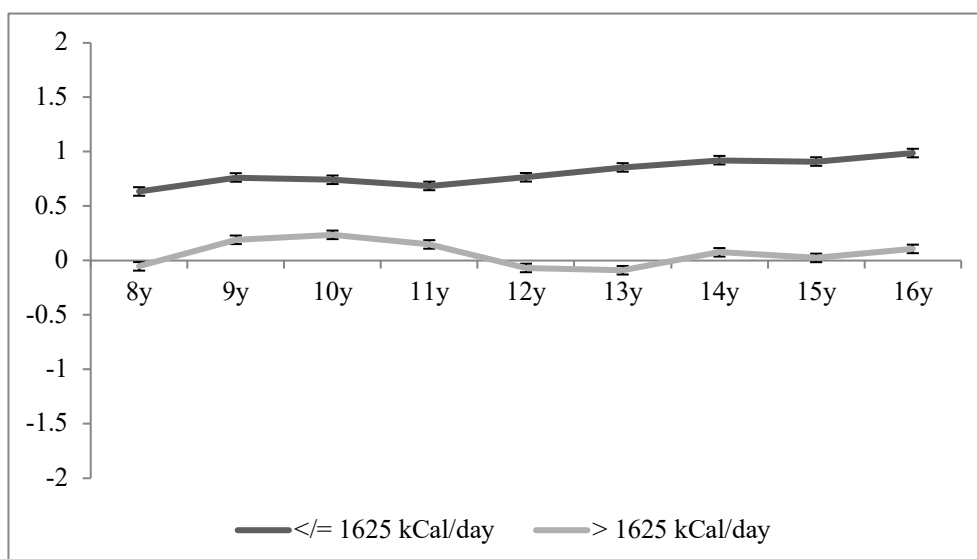


Figure 34b. Difference in BMI according to energy intake in girls

Figures shown are mean (\pm SEM) and represent the difference in BMI from 8y-16y based on mean energy intake at 8y

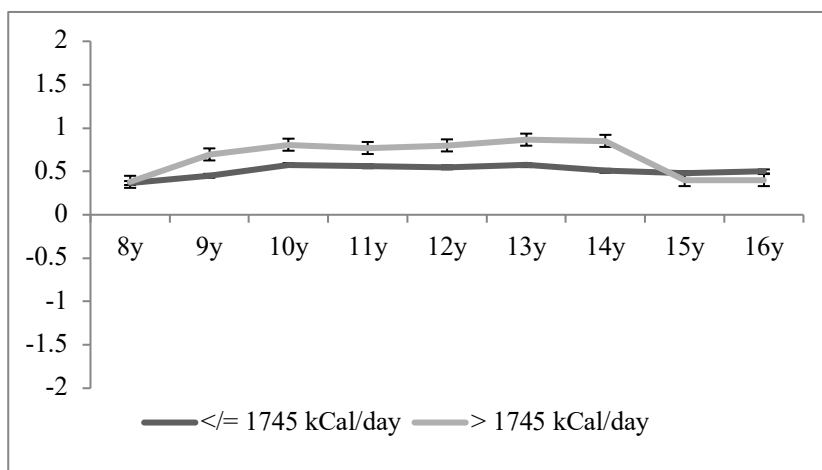


Figure 35a. Difference in WC according to energy intake in boys

Figures shown are mean (\pm SEM) and represent the difference in WC from 8y-16y based on mean energy intake at 8y

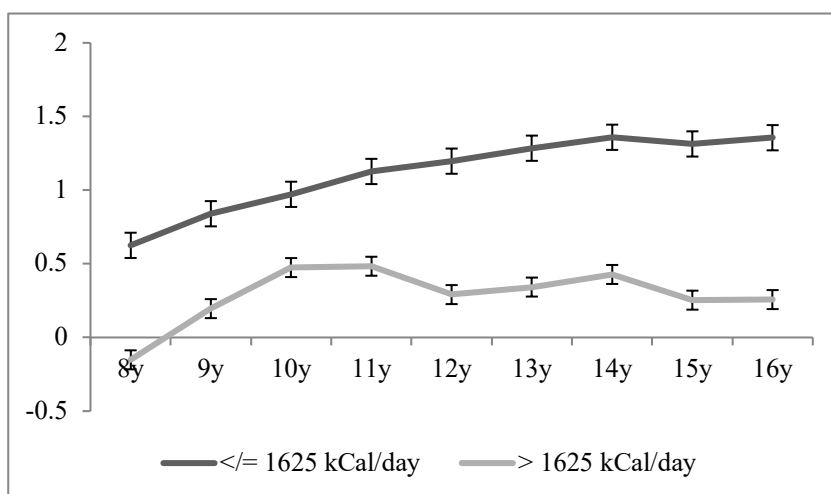


Figure 35b. Difference in WC according to energy intake in girls

Figures shown are mean (\pm SEM) and represent the difference in WC from 8y-16y based on mean energy intake at 8y

6.4.6 Fibre Intake

BMI, and WC from 8y-16y was higher in the boys who consumed < 20g of fibre a day at 8y of age; the findings were similar when skin folds and body fat was examined, but the magnitude was smaller.

In girls, the differences were not as substantial and not consistent: BMI and skin folds were only higher at 15-16y; also, there was no difference in WC and percent fat between the groups

6.4.7 Saturated Fat

All the measures of adiposity were higher in those whose saturated fat intake at 8y was less than 11% of total energy intake. The findings in boys and girls were similar, but the magnitude was greater when WC and BMI were examined.

6.4.8 Sugar

All the measures of adiposity were higher in those whose total sugar intake at 8y was less than 5% of total energy intake. The findings in boys and girls were similar, but the differences were only significant when BMI was examined. For the other measures of adiposity, the differences were small and not significant.

To **summarise**, boys who consumed >20g of fibre had lower measures of adiposity but the girls who consumed less energy, lower saturated fat, and lower sugar, at 8y had higher measures of adiposity from 8y to 16y.

6.4.9 Influence of Macronutrient Intake on the Trajectory of Metabolic Risk

Total Energy Intake

IR was higher in the boys who exceeded 1745 kcal/day and girls who exceeded 1625 kcal/day at 8y of age (Figure 36). These differences were present from 10y – 16y of

age. Triglycerides were higher in the girls who exceeded the EAR for energy, compared to those who did not.

In boys however, there was no significant difference in triglycerides between the 2 groups. In both boys and girls, there was no difference in Mean Arterial Pressure, and total cholesterol: HDL between those who consumed more than the EAR for energy intake at 8y and those who did not. However, Metabolic Risk Score was higher from 11- 16y in boys who consumed more energy than the EAR; in girls, these differences were present from 9-16y (Figure 37).

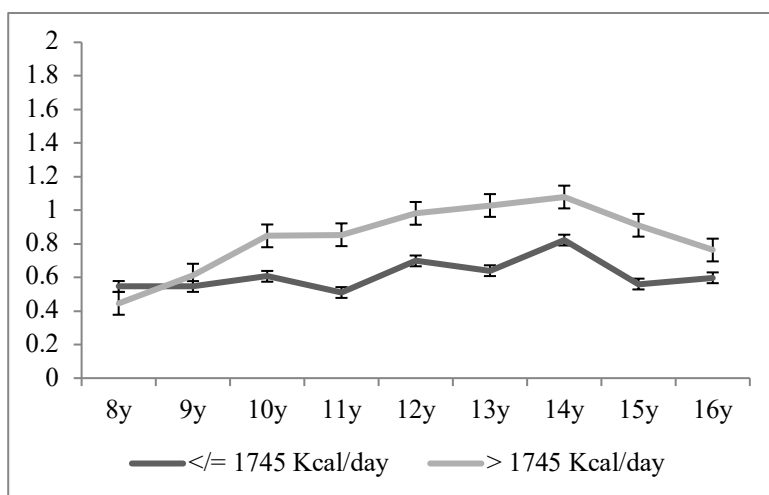


Figure 36a. Difference in Insulin Resistance according to energy intake in boys

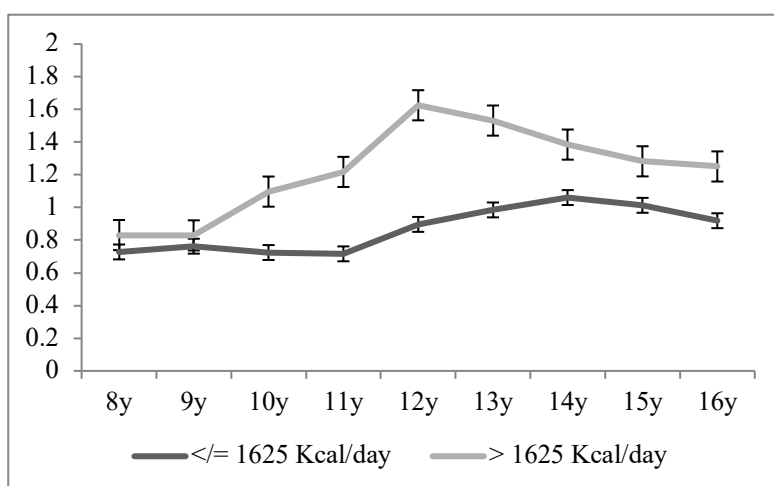


Figure 36b. Difference in Insulin Resistance according to energy intake in girls

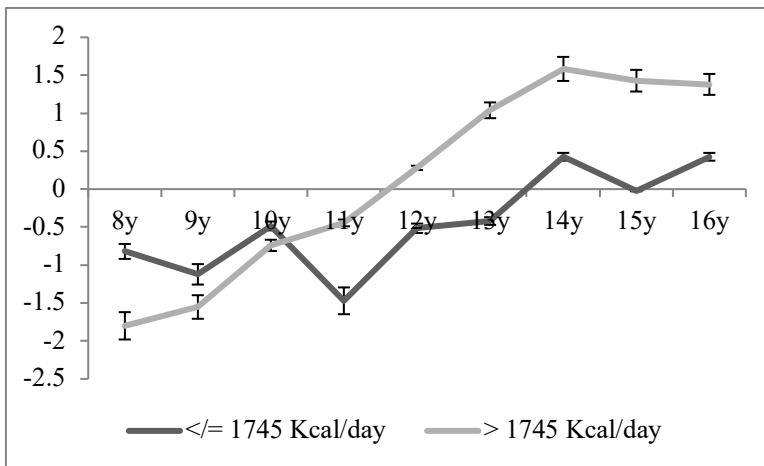


Figure 37a. Difference in Metabolic Risk Score according to energy intake in boys

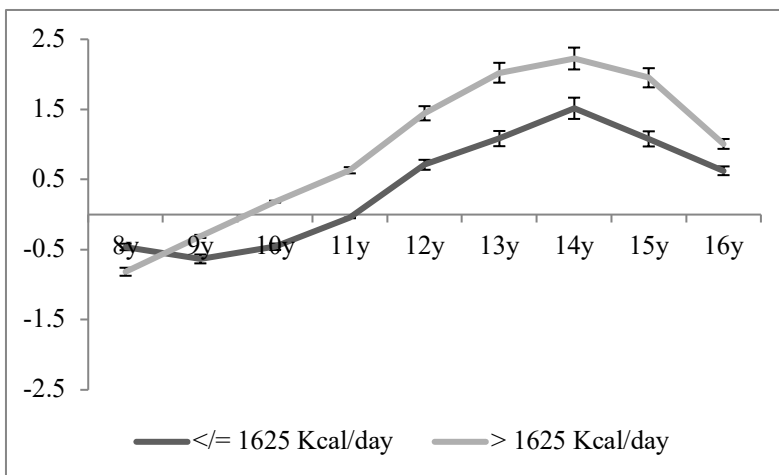


Figure 37b. Difference in Metabolic Risk Score according to energy intake in girls

Fibre Intake

The study participants were classified based on fibre intake at 8y of age, and then the differences in mean metabolic risk score, as well as the individual components were examined

In the boys who consumed more than 20g of fibre a day (n=114), triglycerides were higher only from 8y-10y. In girls who consumed more than 20g of fibre (n=98), triglycerides were lower from 10-14y but not at other time points. On the other hand, in both boys and girls, there was no difference in Total Cholesterol: HDL, and Mean Arterial Pressure between the groups. From 10 -16y, insulin resistance was higher in

boys who consumed less than 20g of fibre at 8y; in girls however, there was no difference between the groups (Figure 38).

Metabolic risk score was lower in the girls who consumed less than 20g of fibre, on the other hand, there was no difference in the boys (Figure 39).

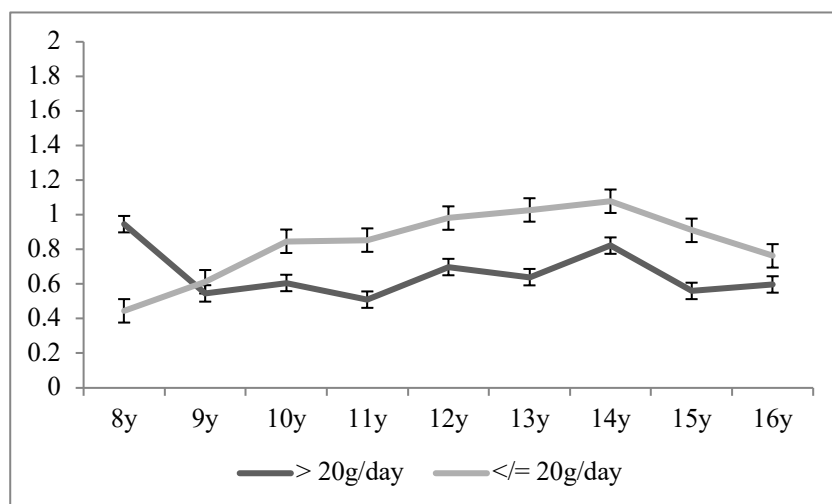


Figure 38a. Difference in Insulin Resistance according to fibre intake in boys

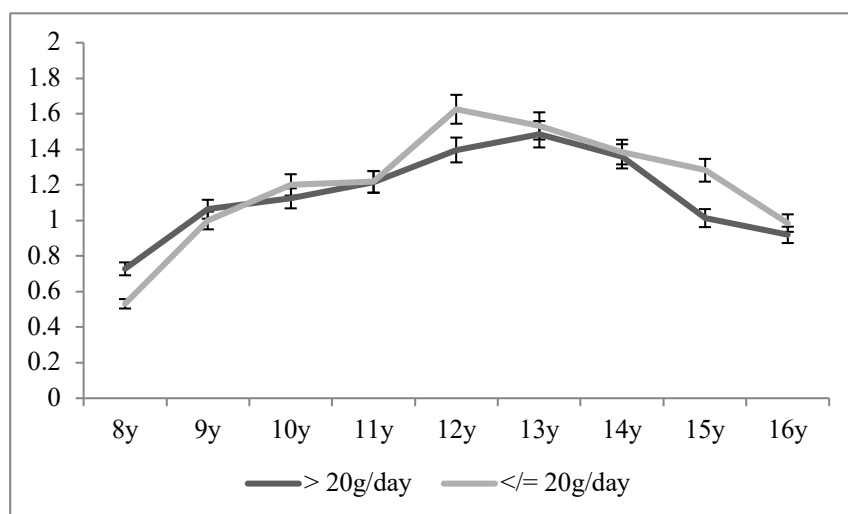


Figure 38b. Difference in Insulin Resistance according to fibre intake in girls

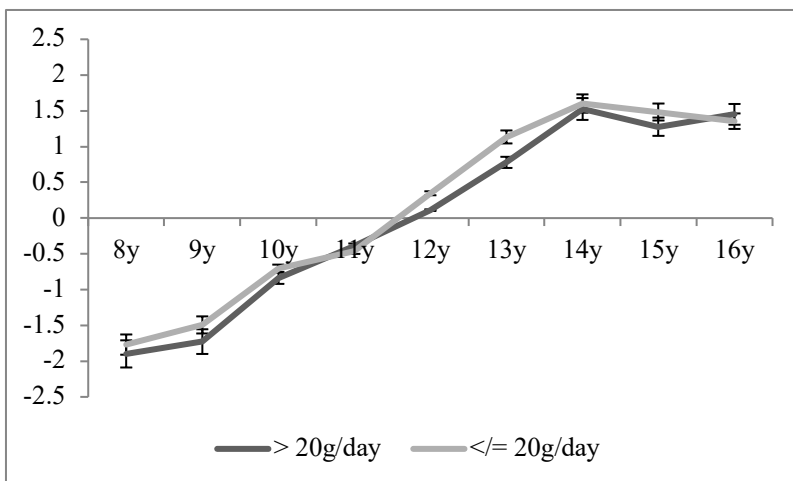


Figure 39a. Difference in Metabolic Risk Score according to fibre intake in boys

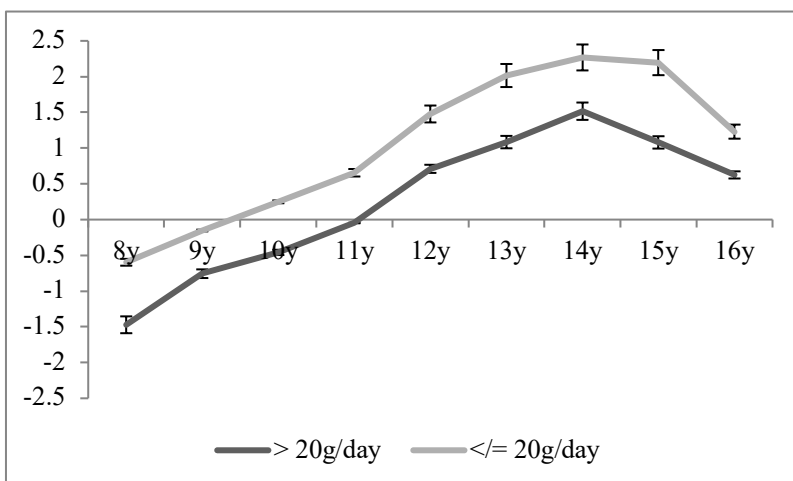


Figure 39b. Difference in Metabolic Risk Score according to fibre intake in girls

Saturated Fat Intake

The study participants were classified based on saturated fat intake at 8y of age, and then the differences in mean metabolic risk score, as well as the individual components were examined.

Insulin Resistance from 10-16y was lower in both boys and girls whose saturated fat intake at 8y was less than 11% of total energy intake (n=27 for boys, 17 for girls; figure 40). On the other hand, in both boys and girls, there was no difference in Total Cholesterol: HDL, or in Mean Arterial Pressure between the groups. From 10-14y, triglycerides were higher in the girls whose saturated fat intake at 8y was more than 11% of total energy intake. In boys, a similar difference was seen but only from 13-16y of age (figure 41).

In both boys and girls, Metabolic Risk Score was higher in those whose saturated fat intake was greater than 11% of total energy intake. In boys, this difference was only present from 13y – 16y, while in girls from 10y -16y ($p<0.01$; figure 42).

Overall, the trend was for those children with intake of saturated fat above 11% of dietary energy to have higher IR, triglycerides, and total cholesterol: HDL.

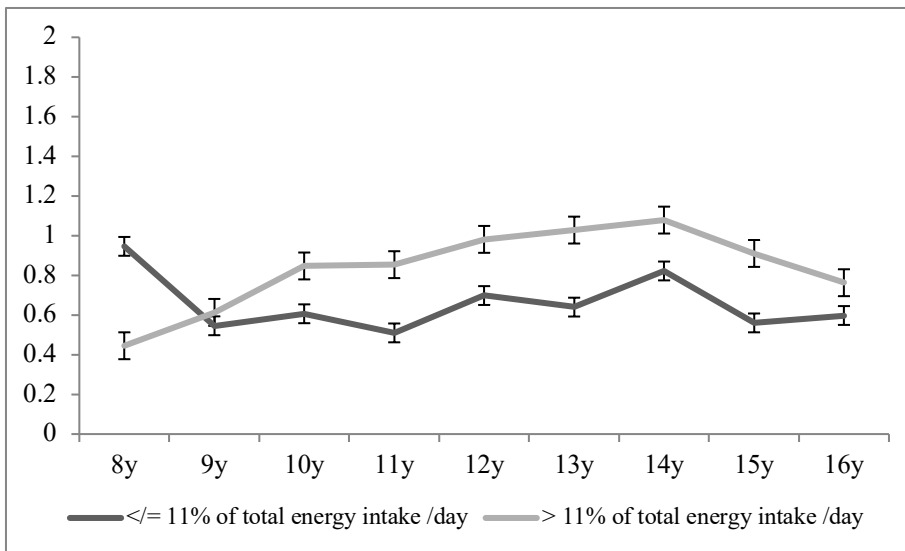


Figure 40a. Difference in insulin resistance according to saturated fat intake in boys

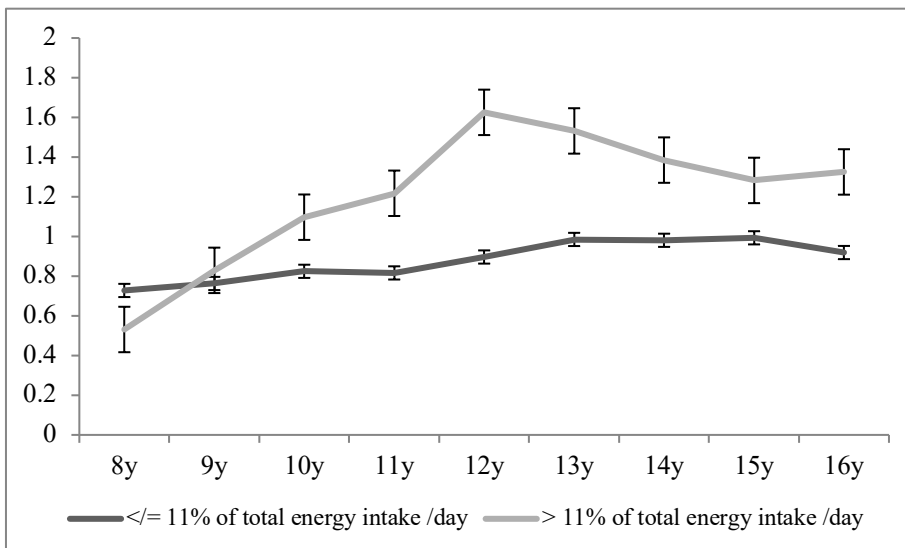


Figure 40b. Difference in insulin resistance according to saturated fat intake in girls

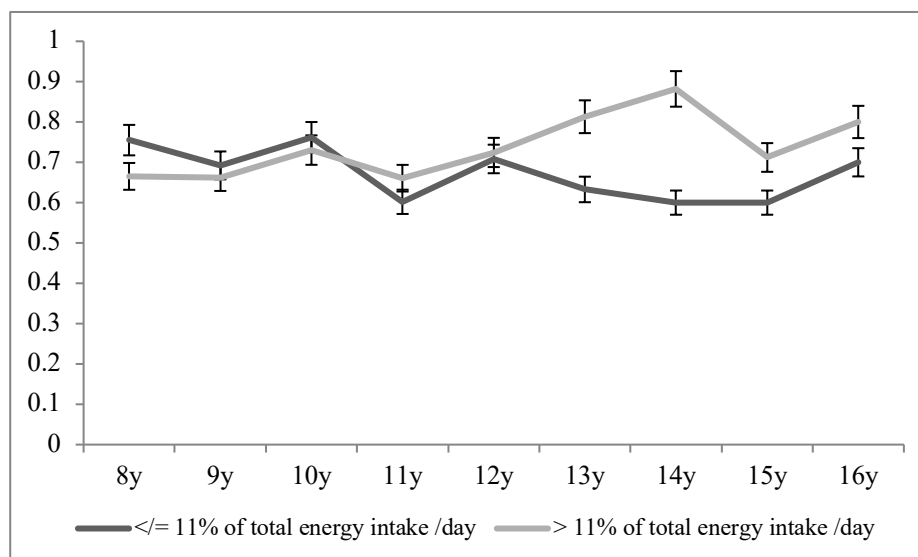


Figure 41a. Difference in triglycerides according to saturated fat intake in boys

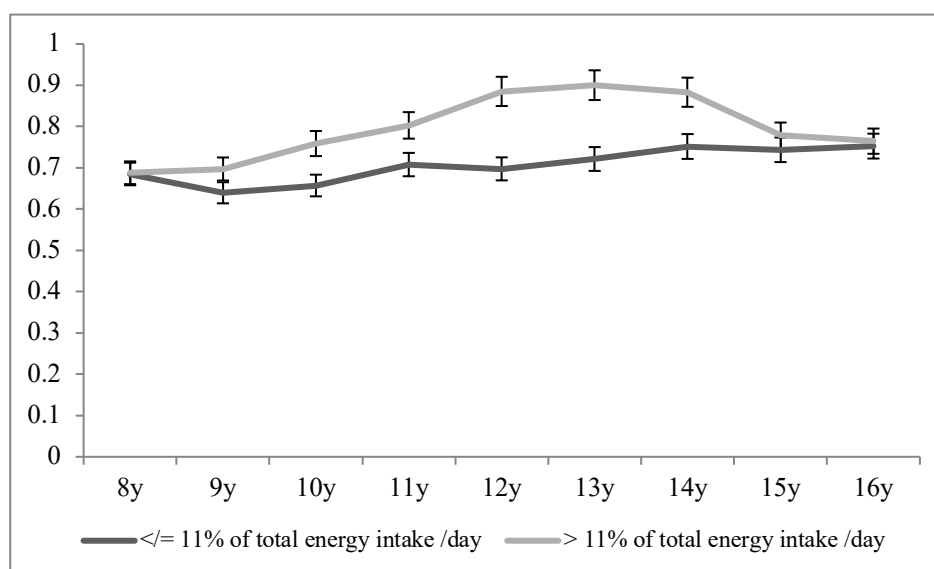


Figure 41b. Difference in triglycerides according to saturated fat intake in girls

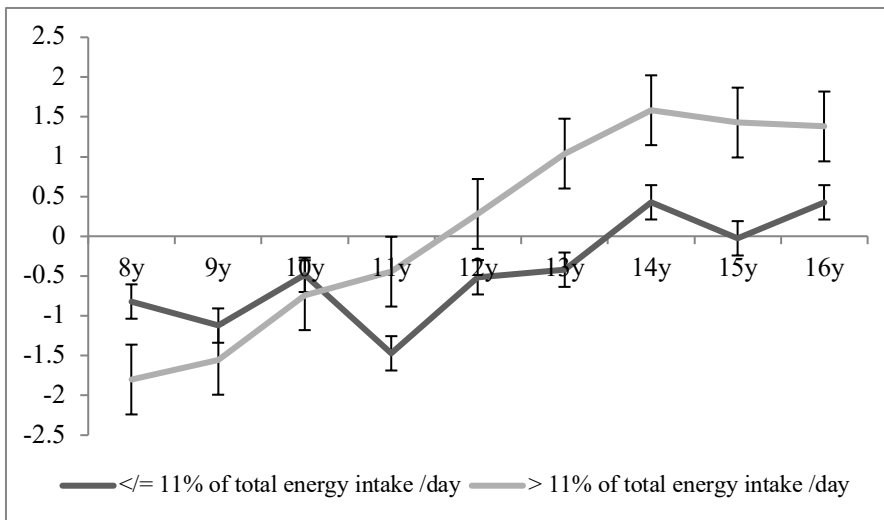


Figure 42a. Difference in metabolic risk score according to saturated fat intake in boys

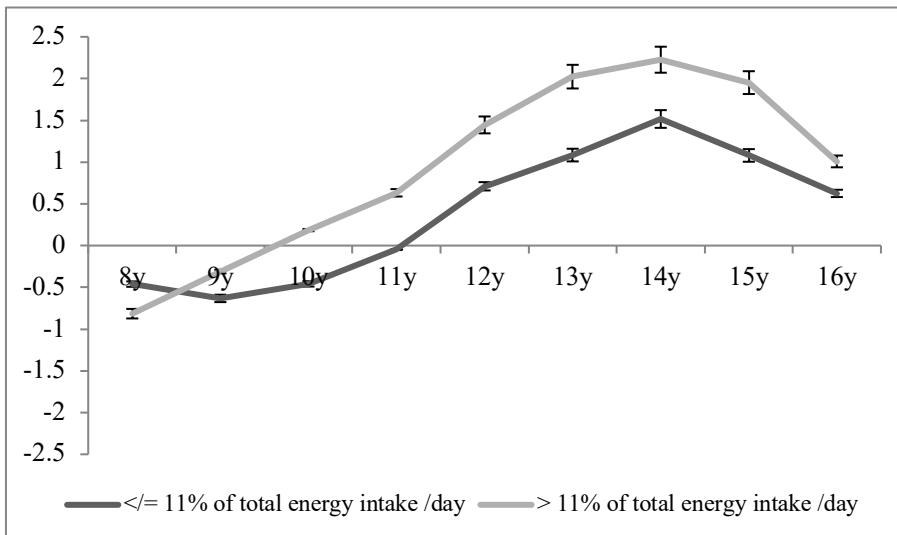


Figure 42b. Difference in metabolic risk score according to saturated fat intake in girls

Sugar Intake

The study participants were classified based on macronutrient intake at 8y of age, and then the differences in mean metabolic risk score, as well as the individual components were examined

In boys whose sugar intake at 8y was greater than 5% of total energy (n=129), IR was higher from 9y-16y; there was no difference in girls (figure 43).

Also, in both boys and girls, there was no difference in MAP or Tchol: HDL between the 2 groups.

Triglycerides from 9-14y was higher in girls whose sugar intake at 8y was greater than 5% of total energy; in boys a difference was only present at 13 and 14y of age (figure 44).

Metabolic risk score was significantly higher from 11- 16y in boys who consumed more sugar than the above daily limits; in girls, the trend was the same, but only significant from 12-15y (figure 45).

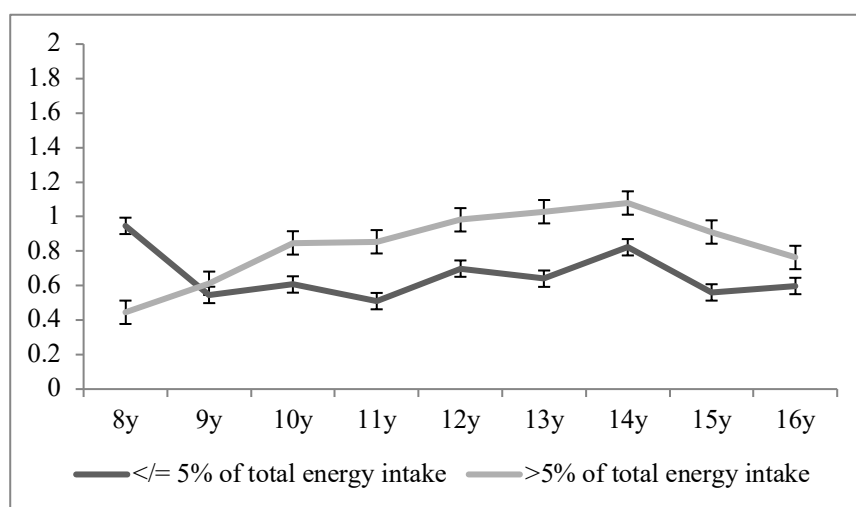


Figure 43a. Difference in insulin resistance according to sugar intake in boys

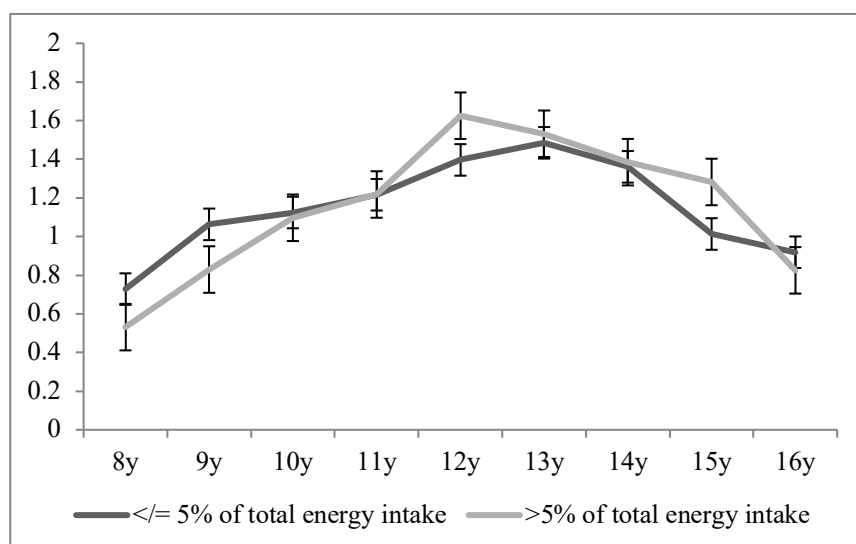


Figure 43b. Difference in insulin resistance according to sugar intake in girls

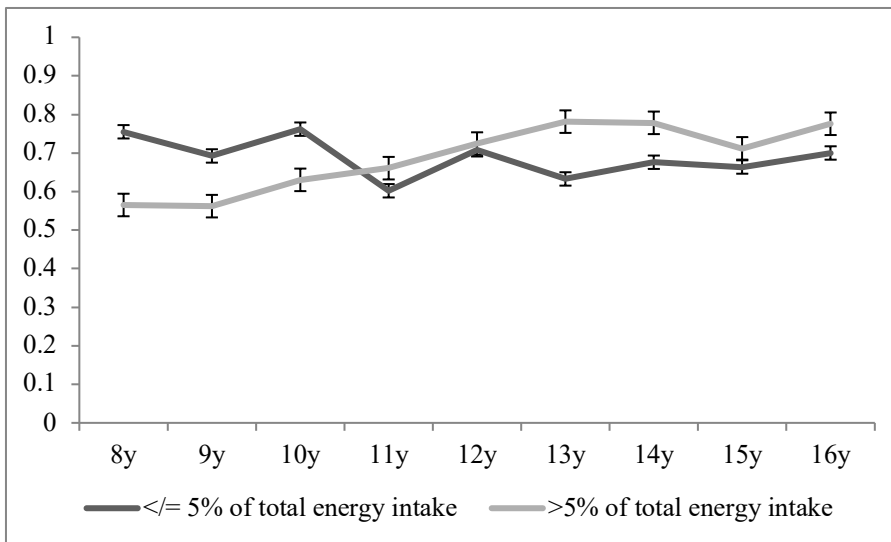


Figure 44a. Difference in triglycerides according to sugar intake in boys

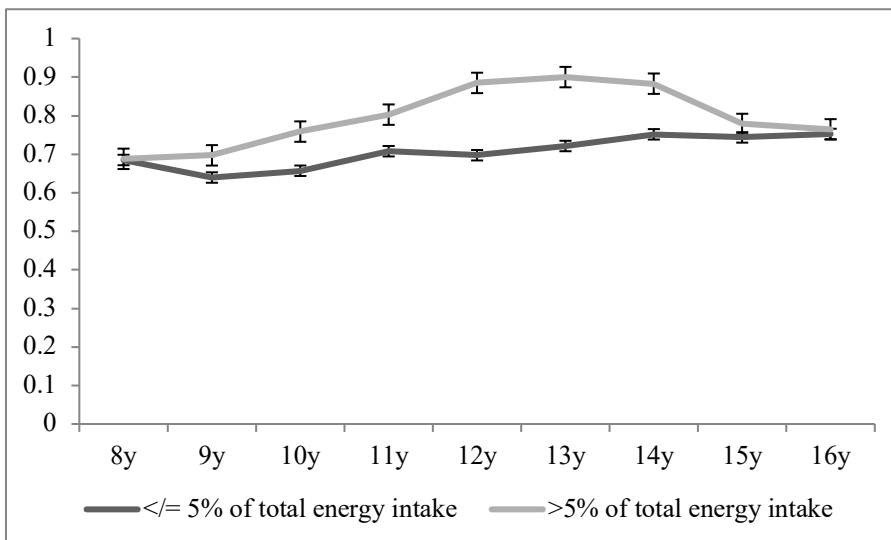


Figure 44b. Difference in triglycerides according to sugar intake in girls

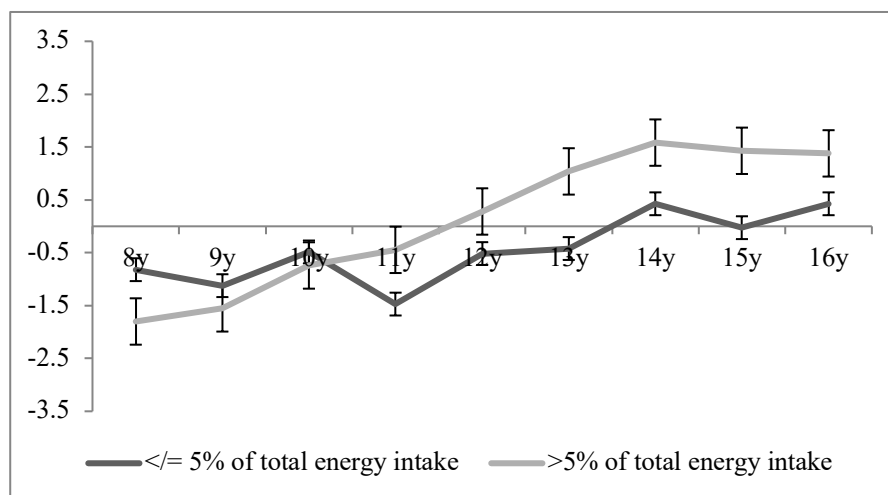


Figure 45a. Difference in metabolic risk score according to sugar intake in boys

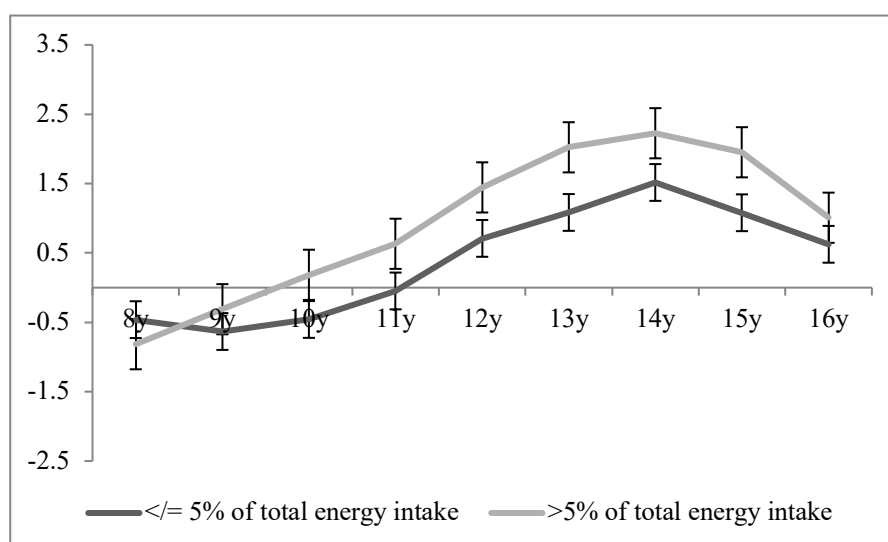


Figure 45b. Difference in metabolic risk score according to sugar intake in girls

To **summarise**, children who consumed higher than average energy, saturated fat, and sugar intake at 8y, and lower fibre, had higher Insulin resistance, Triglycerides, and Metabolic risk score, compared to those who did not.

6.4.10 Time-Lagged correlations between macronutrient intake and measures of adiposity

Carbohydrates

In both boys and girls, weak inverse correlations were observed between the intake of carbohydrates at 8y and BMI-SDS at from 9y to 16y, as well as between carbohydrate intake at 12y and BMI-SDS from 13-16y, (r -0.2 to -0.27 $p < 0.01$).

However, there was no substantial relationship between carbohydrate intake, and skin folds (r -0.164 – 0.084, $p > 0.06$). In 12y old boys, there was a weak relationship between total carbohydrate intake and percent fat from 13-16y (r -0.220 – 0.200, $p \leq 0.05$).

Sugar

There was no significant relationship between sugar intake in boys and any of the measures of adiposity (r -0.103 – 0.166, $p > 0.3$). In girls however, there were weak positive correlations between sugar intake at 8y and percent body fat at 8-13y (r 0.2-0.3, $p < 0.05$).

Fibre

There was no significant relationship between total fibre intake and any of the measures of adiposity (r -0.109 – 0.049, $p > 0.09$).

Protein

In boys, there was no significant relationship between protein intake and any of the measures of adiposity (r -0.063 – 0.054, $p > 0.7$). While in girls, there were weak correlations between skin folds and WC at 8y and the proportion of protein intake at 9-12y (r 0.3, $p < 0.01$).

Fat

Although there was no relationship in both boys and girls between total fat intake and any of the measures of adiposity, there were weak negative correlations between the total intake of saturated fat at 8y and BMI-SDS 12-16y (boys: $r = -0.3$ $p < 0.001$; girls $r = -0.3$, $p < 0.001$), and between saturated fat intake at 12y in girls and BMI-SDS at 13-16y ($r = 0.2$ $p < 0.01$).

To **summarise**, there was no strong or consistent relationships between measures of adiposity and proportion or macronutrient intake, whether cross-sectional or time lagged.

6.4.11 Cross-Sectional Relationships between metabolic risk score, and its components, and Macronutrient Intake

Carbohydrates

In boys, there was a positive correlation between proportion of carbohydrate intake at 12y and HDL cholesterol ($r = 0.2$, $p < 0.05$). In girls, there was a significant positive correlation between proportion of carbohydrate intake at 12y and LDL cholesterol at 12y ($r = 0.2$, $p < 0.01$).

Sugar

There was a significant positive correlation between sugar intake and IR and MRS in 12y old girls ($r = 0.2$, $p < 0.05$).

Fibre

There was no relationship between total fibre intake and any of the components of the metabolic risk score (MAP: $r = -0.043 - 0.03$, $p > 0.23$; Tchol: HDL $r = -0.191 - 0.07$, $p > 0.07$; IR $r = -0.022 - 0.05$, $p > 0.6$; Triglycerides $r = -0.089 - 0.142$, $p > 0.2$).

Protein

In 12y old boys, there was a significant positive correlation between the proportion of protein intake and LDL ($r\ 0.3$, $p < 0.01$).

Fat

Although in both boys and girls there was no relationship between total fat intake and any of the components of the MRS, there were weak positive correlations between the total intake of saturated fat and HDL (in 8y old girls, $r\ 0.26$, $p < 0.01$; 16y old boys $r\ 0.2$ $p < 0.01$).

6.4.12 Time-Lagged Correlations between Macronutrient Intake, Cardiovascular risk factors, and the metabolic risk score

Carbohydrates

The only significant relationships were in girls: between proportion of carbohydrate intake at 12y and LDL cholesterol at 13-16y ($r\ 0.3$, $p < 0.01$) and MRS at 14-16y ($r\ 0.3$, $p < 0.01$)

Sugar

There was a significant correlation between sugar intake at 12y and triglycerides from 13-15y in girls ($r\ 0.2$, $p < 0.05$)

Fibre

There was no significant relationship between total fibre intake and any of the components of the metabolic risk score (Triglycerides $r\ -0.036$ to -0.014 , $p > 0.14$; Tchol:HDL $r\ -0.116$ to -0.057 , $p > 0.5$; IR $r\ -0.104$ to -0.03 , $p > 0.1$; MAP $r\ -0.103$ to -0.097 , $p > 0.2$).

Protein

In 12y old boys, there was a significant relationship between proportion of protein intake and LDL from 13-16y ($r\ 0.3$, $p<0.01$).

Fat

Although there was no relationship in both boys and girls between total fat intake and any of the components of the MRS, there were weak correlations between the total intake of saturated fat at 12y and HDL at 13-14y ($r\ 0.2-0.3$, $p<0.01$) in boys and in girls between total saturated fat intake at 8y and HDL at 12y ($r\ 0.3$, $p<0.01$). Also. The relationship between **total energy intake** and the components of the MRS was identical to the findings observed between total carbohydrate intake and these measures.

To **summarise**, there was no substantial relationship between measures of adiposity and proportion or macronutrient intake, or between lipid profile and the proportion of macronutrient intake whether cross-sectional or time lagged.

6.5 Mixed Effects Models

6.5.1 Longitudinal Analysis of the Effect of Macronutrient Intake on Adiposity

Mixed effects models were built to quantify the association between the intake of each macronutrient at 8y and the measures of adiposity from 9y to 16y. Each macronutrient was examined as a continuous variable.

Age was included in each model as a covariate to determine its effect; physical activity was also evaluated but did not significantly influence any of the models.

In boys, the tests of fixed effects showed that total energy intake at 8y had no significant effect on any of the measures of adiposity (BMI, WC, SSF, and percent fat) from 9y to 16y ($F=0.000\ p=1.000$).

In girls, total energy intake at 8y had a significant effect on BMI and percent Fat from 9y-16y, however the estimate of fixed effects was low, indicating that higher energy

intake was not predictive of increased adiposity (BMI Estimate -0.000188, F 4.897, p0.029; WC Estimate -0.000157 F 3.643 p 0.059; SSF Estimate -0.000018, F 2.841 p 0.094, Percent Fat Estimate -0.000060, F 4.227, p 0.04).

In both boys and girls, carbohydrate intake at 8y had no significant effect on any of the measures of adiposity from 9-16y.

In girls, apart from SSF, total fat intake at 8y had a significant effect on the measures of adiposity from 9y – 16y; the estimates were however low, indicating that fat intake was not predictive of increased adiposity. (BMI Estimate -0.004578, F 5.935 p 0.016; WC Estimate -0.004049, F 4.418, p 0.038; SSF Estimate -0.000457, F 3.531, p 0.063; percent fat Estimate -0.001596, F 5.574, p 0.018).

In boys, fat intake at 8y had no significant effect on any of the measures of adiposity.

In girls, protein intake at 8y had no significant effect on any measure of adiposity. In boys however, protein intake at 8y had a significant effect on percent body fat, but not on the other measures of adiposity from 9y – 16y (in boys, percent fat Estimate 0.001238, F 3.739, p0.05).

In girls, saturated fat intake at 8y had a significant effect on percent body fat but not the other measures of adiposity; the estimate was however low, indicating that Saturated fat intake was not predictive of increased body fat: percent fat Estimate -0.003216, F 3.738 p0.05.

In boys, saturated fat intake at 8y had no significant effect on any of the measures of adiposity.

In girls, sugar intake at 8y had a significant effect on all the measures of adiposity; the estimates were however low, indicating that free sugar intake was not predictive of increased body fat: BMI Estimate -0.002765, F 6.843, p 0.01; WC Estimate -0.002335, F 4.609, p 0.034; SSF Estimate -0.000332, F 5.863, p0.017; percent fat Estimate -0.001056, F 10.420, p 0.002.

In boys, sugar intake at 8y had no significant effect on any of the measures of adiposity at 9y-16y.

In both boys and girls, fibre intake at 8y had no significant effect on any of the measures of adiposity.

In **summary**, the only macronutrient that was predictive of increased adiposity was protein intake at 8y in boys.

6.5.2 Longitudinal Analysis of the Effect of Macronutrient Intake on Metabolic Risk

Mixed effects models were built to quantify the association between the intake of each macronutrient at 8y and the metabolic risk score and its components from 9y to 16y.

To allow for its effect, age included in each model as a factor, as there was an increase in insulin resistance with age.

In both boys and girls, the tests of fixed effects showed that total energy intake at 8y had no significant effect on the MRS from 9y to 16y.

Also, in both boys and girls, total energy intake had no significant effect on any of the individual components of the MRS.

In girls, carbohydrate intake at 8y had a significant effect on the MRS; however, the estimate was low, indicating that carbohydrate intake was not predictive of increased MRS (Estimate -0.003930, F 5.910, p 0.015). There was no significant effect when the individual components of the MRS were examined.

In boys, carbohydrate intake had no significant effect on the MRS or any of its components.

In boys and girls, protein intake at 8y had no significant effect on the MRS or any of its components.

Although total fat intake had no significant effect on the MRS in boys and girls, of all the components of the latter, fat intake had a significant effect on the MAP. However, the estimates were low, indicating that fat intake was not predictive of increased MAP

(Boys: Estimate -0.023740, F 5.330, p 0.021; Girls: Estimate 0.023156, F 3.978, p 0.047). Similarly, saturated fat intake had no significant effect on the MRS in boys; of its components however, saturated fat influenced MAP. The estimate of this effect was however small, indicating that it was not predictive of an increased MAP (Estimate -0.054966, F 5.148, p 0.024). In girls, saturated fat intake had no significant effect on the MRS or its components.

In both boys and girls, fibre intake at 8y had no significant effect on the MRS or any of its components.

In girls, sugar intake at 8y had a significant effect on the MRS; the estimate of this effect was however small, indicating that it was not predictive of an increased MRS (Estimate -0.054966, F 5.148, p 0.024)

Among the components, IR (estimate -0.001830, F 5.104, p 0.024), triglycerides (estimate -0.001016, F 5.264, p 0.022), and Tchol: HDL (estimate -0.001469, F 5.273, p 0.022), were significant contributors to the above.

In boys, even though sugar intake did not have a significant effect on the MRS, one of its components did (IR estimate 0.001850, F 6.092, p 0.014).

In **summary**, in girls, intake of none of the macronutrients at 8y was predictive of the MRS or any of its components from 9y -16y. In boys, sugar had significant effect on IR from 9-16y.

6.6 Discussion/Conclusion

Utilising data from a longitudinal cohort of children studied from age 8y to 16y, this chapter presents the impact of macronutrient intake in early childhood, on adiposity and metabolic risk from childhood to adolescence.

This analysis showed that when the EAR for energy or DRV for macronutrients classified the boys, higher intake of carbohydrates, fat, sugar, saturated fat, and lower intake of fibre was associated with higher measures of adiposity as well as a higher

metabolic risk score. However, when the cohort was examined as a whole, the above variables were not significant predictors of adiposity or the metabolic risk score.

In adults, the link between adiposity and various cardiometabolic diseases is clear, and was established after several cohort studies showed a direct relationship between an increased risk of cardiovascular diseases and the number of cardiometabolic risk factors present (Hubert HB, 1983) (Stern MP, 1990) (Pettitt DJ, 1982) (Bays, 2011) (Wilson PW, 2002). These associations have not been evaluated in children.

Some cross-sectional studies have shown that overweight and obese children have higher levels of markers of inflammation (Kenchiah S, 2002). These markers are also associated with endothelial dysfunction and the initial stages of atherosclerosis (Ndumele CE, 2016). In another analysis, adolescents who were overweight/obese and or met the adult criteria for the metabolic syndrome had a higher risk of type 2 diabetes; they also had evidence of endothelial dysfunction thickness in adulthood (Freedman DS, 2001).

In adults, the proportion of macronutrients consumed is not always predictive of adiposity; for instance, there was no difference in weight loss when low fat diets were compared with moderate or high fat diets (Romieu I, 1988) (Astrup A, 2000). Other studies have shown that low carbohydrate and high protein diets were associated with short-term weight loss, but neither of these diets was sustainable or superior to the other (Sacks FM, 1986) (Stern MP, 2004)

Atherosclerosis of the coronary arteries has been detected as early as 6 years of age, so it can be inferred that early exposure to relevant risk factors is at least partially responsible for the onset of cardiovascular disease (Berenson GS, and Bogalusa Heart Study Research, 2002). Therefore, evaluating the impact of macronutrient intake on later adiposity and metabolic status is worthwhile.

There are no universally accepted for guidelines on the recommended quantities of macronutrient intake in children, and most are derived from those of adults, and also from consensus statements (Grundy SM, 1982) (Gatenby SJ, 1995); this is due to the limited evidence base for dietary recommendations in children in whom only small reductions in weight have been seen in several intervention trials (Ells LJ, 2018). There is insufficient data on the impact of various macronutrients on adiposity or cardiometabolic risk in children and the few studies had limited number of participants (Grundy SM, 1982) (Ells LJ, 2018), were not intervention studies (Gidding SS, 1996) and did not study diverse populations (Fisher EA).

Over the last 5 decades, several factors have influenced food choices in children: increased availability of energy-dense foods, relatively lower food prices, and with busier lifestyles, increased dependence on convenience foods (Jeffery RW, 1998) (Story M, 2004) (Shrewsbury V, 2008) (WHO, 2017) which are typically higher in added sugar and saturated fat (Drewnowski A, 2005).

Epidemiological surveys in the 1990s showed that the proportion of convenience foods consumed by children and adolescents increased by 20% compared to the preceding decade (Jahns L, 2015) (Nielsen SJ, 2003). Also a direct relationship has been identified between the frequency of visits to fast food restaurants and total energy and sugar intake in children; as well, there is an inverse relationship between the intake of vegetables and the number of visits to these restaurants (Story M, 2004). Measures like publicly funded meals provided at school cafeterias have been adopted in an attempt to improve the above; initially without success perhaps because the meals contained high amounts of added sugar, saturated fat, and other energy-dense foods (Taveras EM., 2005). Improvements in these programs however have led to lower intake of obesogenic foods (Robinson-O'Brien R, 2010) (Welker SE, 2016), but it remains to be seen if this will translate to lower rates of obesity.

In a bid to restrict intake of added sugar, saturated fat, and trans-fat, most authorities recommend that children consume a diet that is predominantly composed of fruits, vegetables, whole grains, low-fat dairy, and lean meat (Grundy SM, 1982) (Gidding SS, 1996) (WHO, 2012) (Scientific Advisory Committee, 2012). These guidelines are also not based on rigorously conducted longitudinal studies. Several attempts have been made to establish the relationship between macronutrient intake and adiposity, but the available studies have significant limitations including the use of dietary recall, varying methods of measuring adiposity, and lack of long-term follow up. Also similar to the findings in this analysis, the proportion of carbohydrate intake was inversely related to body fat particularly in those that were obese (Tucker LA, 1997) (Maffeis C, 1996); possible explanations for these findings include the fact that carbohydrates are less energy dense than fat and also, in the rested state, there is preferential oxidation of carbohydrates over fat to produce energy in skeletal muscle (Hargreaves M, 2012).

The increased prevalence of childhood obesity over the last 40 years has coincided with a significant increase in total energy intake in children (Ogden C, 2002) (Nielsen SJ, 2002) (Centre for Disease Control, 1993); but even though the latter is assumed to be the cause, there are limited longitudinal studies that have been able to evaluate this relationship.

The analysis presented in this chapter indicates that only the boys, who consumed higher than the DRV of the main macronutrients and lower fibre, had higher measures of adiposity in subsequent years. On the other hand, when the entire cohort was examined, higher macronutrient intake was not predictive of increased adiposity. A possible explanation for the latter includes the very small number of children who consumed above the EAR for energy; thereby preventing the detection of statistically significant differences in the risk of adiposity.

When the cohort was examined as a whole, there was no relationship between total energy intake and adiposity and metabolic risk; however, the children who consumed more than the EAR of energy at 8y had higher measures of adiposity and metabolic risk compared to the others. These findings although novel, suggest that there might be a threshold beyond which caloric intake in early childhood can influence future risk of adiposity and a high metabolic risk.

Similarly, the boys who consumed more than the recommendation for fibre intake at 8y had lower measures of adiposity and metabolic risk in later years.

The results from other studies in this area are inconsistent; a cross-sectional study showed modest reduction in body fat (4%) in overweight children who consumed 3g more fibre than comparators (Davis JN, 2009). A similar study of overweight children showed lower risk of the metabolic syndrome in those who consumed 1g more soluble fibre than comparators (Ventura EE, 2008). As well, Low fibre diets were associated high body fat in a cross-sectional study of a small cohort of British children (Johnson L, 2008). On the other hand, another study did not find any association between dietary fibre intake and obesity (Davis JN, 2007); in others, higher fibre intake was associated with increased adiposity in a cohort of overweight and obese children (Cheng G, 2009). Explanations for these inconsistencies include differences in the background characteristics of the populations studied, the types (soluble vs. insoluble vs. total fibre) and quantity of fibre evaluated, and the possibility that the other macronutrients consumed by the study subjects might have influenced the outcome.

Fibre is a bulking agent, which increases satiety and slows gastric emptying; it also influences the rate of glucose diffusion across the aqueous layer of the gastrointestinal tract, and in the fasted state, it alters the rate of peristalsis (Howard P, 1989). Also, increased intake of insoluble fibre increases insulin sensitivity (Bell SJ, 2003). The exact mechanism for these effects is unclear, but several hypotheses have been proposed; in

animal studies, higher intake of soluble fibre was associated with an increase in the production of short-chain fatty acids in the gut due to increased colonic fermentation of ingested fibre. As well, in the animals fed insoluble fibre, there was increased fatty oxidation in the liver due to changes in gene expression (Isken F, 2010).

Another study found that soluble fibre intake affects gut flora such that there is an increase in the production of short chain fatty acids (SCFA). The increased production of SCFA then reduces blood glucose levels by inhibiting gluconeogenesis (Weitkunat K, 2015).

Like fibre, saturated fat intake in childhood may be a predictor of later adiposity, as well as a driver of increased future risk of CVD events. In adults, the mechanism by which the increased CVD risk occurs appears to be driven by the effect of saturated fat intake on lipid profile (Mensink RP, 1989).

In children, no mechanism has been established. It is known however, that excessive intake of saturated fat leads to an increased production of free fatty acids and triglycerides, which then leads to hyperinsulinemia, which in turn exacerbates insulin resistance (Maron DJ., 1991). The result from this analysis is also consistent with the above: compared to the rest of the cohort, the children who consumed higher than the DVR of saturated fat at 8y had higher levels of IR in later years.

Just like the relationship between saturated fat and lipids in adults (Mensink RP, 1989), this analysis showed that higher intake of saturated fat at 8y was associated with higher triglycerides and lower Tchol: HDL in later years.

In this analysis, the relationship between saturated fat intake and insulin resistance was stronger than with lipids; this indicates that at least in children IR plays a more significant role in the pathogenesis of cardiometabolic disease.

This analysis also showed that the girls who consumed more sugar at 8y were more likely to show higher measures of increased adiposity and metabolic risk in subsequent years. In boys, sugar intake was associated with higher risk of insulin resistance.

The role of added sugar in the pathogenesis of obesity has been studied extensively; there is a strong relationship between the increased intake of sugary drinks, and the prevalence of obesity in children (Strong WB, 1992) (Della Torre SB, 2016). One study found an almost doubling in the risk of obesity in those who consumed one additional serving (265 mL) of a sugar-sweetened beverage, which was associated with an extra 188 kCal/day of energy intake over that of comparators (Ludwig DS, 2001). Other studies have also shown that in those with higher milk intake instead of sugar-sweetened beverages, even if energy intake was similar, weight gain was lower (St-Onge MP, 2003). These studies showed that there was a difference in the risk of adiposity between children who consume drinks with added sugar and those who consume drinks that were of equivalent calories but higher in fat and protein. Possible explanations for these findings include the fact that high sugar drinks lead to reduction in satiety as well as reduction in energy expenditure, thereby stimulating weight gain (Teegarden D, 2003).

Most added sugars are consumed in liquid forms; and liquids are typically less satisfying than if an equivalent amount of calories was consumed in solid form (Pan A, 2011). This leads to an increased intake of calories overall.

This analysis did not show an association between sugar intake and adiposity in boys; this could be explained by the way sugar intake was measured: in this analysis, the frequency of sugary food intake was used in calculating the estimated sugar intake and so could lead to underestimation of the actual amount of sugar consumed. Also, in this analysis total sugar was estimated, as it was not possible to estimate added sugar which is what the guideline of <5% dietary energy is based on (Scientific Advisory Committee

on, 2012) (WHO, 2012). So <5% of the dietary energy may not be the best way of classifying high vs low sugar intake, a higher cut-off might have more accurately differentiated the high consumers. However, the 75th centile was also used as another method of classifying the intake of the children and irrespective of the macronutrients studied, the results were similar.

This analysis has shown that the girls who consumed higher amounts of sugar at 8y had a higher metabolic risk in later years, and the boys who consumed higher amounts of sugar at 8y had higher risk of IR in later years. Although data in children is limited, several studies have also suggested a link between sugar intake and the risk of the metabolic syndrome in adults (Ludwig DS, 2002).

In a study of adult men, a diet supplemented with 200 g sucrose per day was associated with higher triglycerides, but no difference was seen if the sugar was replaced by an equivalent amount of complex carbohydrates (Akinyanju PA, 1968). Other studies have shown similar findings (Raben A, 2002) (Faeh D, 2010) with high sugar intake leading to increased Insulin resistance and higher levels of triglycerides. Possible mechanisms for these findings could be extrapolated from animal studies where the intake of fructose led to hypertrophy of cells in the kidney, thickening of the renal afferent arterioles, and narrowing of the latter, with the net result being glomerular hypertension (Sánchez-Lozada LG, 2007). Also, fructose intake is associated with lower satiety probably due to its inability to stimulate the rapid production of ghrelin, which is necessary for enhancement of satiety (Yudkin J, 1980). Other possible explanations include the fact that fructose slows the basal metabolic rate (Teff KL, 2004), and raises uric acid levels. Uric acid is now thought to be an independent trigger for CVD events (Nakagawa T, 2006) and in this and other studies, lowering uric acid led to resolution of features of the metabolic syndrome (Yudkin J, 1980).

Uric acid has been found to have several effects on the structure and function of blood vessels: it stimulates the proliferation of vascular smooth muscle, it also increases the production of pro-inflammatory substances (Rao GN, 1991) (Watanabe S, 2002) (Choi YJ, 2014) and impairs the oxidative stress pathway of adipocytes, which leads to an impairment in the secretion of adiponectin (Sautin YY, 2007).

Finally, in this analysis, there were gender differences in the proportion of macronutrient intake; from puberty onwards, total energy intake was significantly higher in boys. In adults, the results of various studies show that men consume higher calories (Bennett CM, 1991) (Whitton C, 2011) but others showing otherwise (Imamura F, 2009). Also, there is inconsistency in the differences in macronutrient intake: most studies indicate that women consume less fat and added sugar but others indicate otherwise (Bennett E, 2018).

Reasons for these inconsistencies include the fact that most researchers use data from national health surveys, which are often based on self-report, consistently proven to be a reason for under-reporting of food intake. In this analysis, FFQ questionnaires were utilised, and were completed by the parents so could also be affected by under-reporting (Brunner E, 2001). Also, the questionnaires did not include several foods such as pizzas or pies, nor did it include portion sizes for the included foods so, the latter had to be estimated.

There might be behavioural reasons for these gender differences including the possibility that like women (Wardle J, 2000), girls may prefer to snack on processed foods, which are typically high in sugar. Apart from the gender differences in energy, this analysis also showed differences with age. So, at 8y, caloric intake was higher in girls, however at 12y and 16y, it was much higher in boys.

A possible explanation is that during puberty, boys require more energy than girls due to their typically larger muscle mass (Caprio SP, 2008).

There are major limitations to this analysis; dietary recall is associated with significant reporting bias, most apparent in the underreporting of intake in those who are obese and overweight (Stice E, 2015) (Fricker J, 1992) (Klesges RC, 1995).

In this cohort, the pattern of food intake was consistent over time; however, there are usually variations in food intake from day-to-day, which is typically not captured in FFQs. As well, the use DRVs of macronutrients to classify the participants is not based on research on the threshold at which there is increased risk of adiposity/cardiometabolic disease; instead, it is based on the average requirement for energy, and on healthy eating recommendations for macronutrients in the United Kingdom. Also, total fibre intake was utilised in the analysis, but not the specific types of fibre (i.e soluble vs. insoluble).

There are day-day biological variations in inter and intra individual energy and macronutrient requirement based on energy expenditure, and levels of physical activity. This was not accounted for in this analysis and introduces another significant limitation. There are several risk factors for cardiovascular diseases and the pathogenesis is complex. This analysis adds to the increasing body of evidence on the role of various macronutrients in the pathogenesis of cardiometabolic diseases and revealed novel findings that higher intake of energy and certain macronutrients were predictive of the trajectory of metabolic risk and some of its components, as well as future adiposity.

Insulin resistance, triglycerides, and metabolic risk score, were higher in those who consumed greater energy, lower fibre, higher sugar, and higher saturated fat intake at 8y.

Further areas of research should evaluate the thresholds at which the macronutrients show a negative impact on the risk of adiposity and CVD risk factors, detect a dose dependent relationship between these macronutrients and increased cardiovascular risk and obesity.

Chapter 7

Influence of the Metabolome on Cardiometabolic Risk

7.1 Abstract

7.1.1 Objective

Metabolomic studies have increased the understanding of the pathogenesis of cardiometabolic diseases in adults; there are however very limited studies in children. This aim of this analysis was to investigate whether changes in metabolomic profiles could be used to identify children with a higher risk cardiometabolic risk score

7.1.2 Methods

The association between individual metabolites (isoleucine, leucine, valine, and lactate) as well as their tracking from 5 to 16 years was established. Both cross-sectional and longitudinal association analyses between the metabolic risk score and individual metabolites were performed from age 5 to 16 years.

7.1.3 Results and Conclusion

In both boys and girls, the relationship between each metabolite was strong, with cross-sectional correlations ranging from 0.50 – 0.84, $p < 0.005$.

The relative level of each metabolite was consistent within individuals over time, as evidenced by the moderate-high year-on-year correlation coefficients of 0.4 – 0.8, $p < 0.01$.

There were significant positive cross-sectional correlations between MRS and isoleucine in boys at age 5, 6, 8, 10, 12, and 13y (r 0.25-0.51, $p < 0.03$), as well as in girls at 10y (r 0.52, p 0.007) and 15y (r 0.42 p 0.016). There were also significant

correlations between MRS and lactate in boys at 14-16y (r 0.28-0.41, $p < 0.001$), and in girls at 6y, 9y, and 15y (r 0.4-0.6, $p < 0.03$).

In boys, the only significant correlations were between MRS at 16y and lactate at 8y-15y (r 0.24 – 0.34, $p < 0.05$).

All the metabolites were higher in the children who had a high metabolic risk score at 16y; however, mixed effects models showed that when statistical adjustments were made for the impact of adiposity, the only significant positive association was found between high MRS and isoleucine.

In conclusion, this study showed that the children who had a higher metabolic risk score at the age of 16y already exhibited higher levels of specific branch-chain amino acids and lactate in early childhood. Therefore, the metabolites studied in this analysis may be early markers of cardiometabolic risk in children who are overweight/obese.

7.2 Introduction

In adults, a variety of risk factors for cardiometabolic diseases have been established, including excess adiposity, genetic influences, and a range of environmental factors (Yusuf S, 2011) (Hargreaves W, 2002) (Welborn TA, 1979). In children however, the exact mechanisms by which obesity, and other acquired risk factors, cause cardiometabolic disease is less clear and remains the subject of various studies.

The metabolome represents a collection of metabolites including sugars, organic acids and amino acids, which are produced during cellular metabolic reactions (Dunn WB, 2005). The study of the metabolome, also known as metabolomics, seeks to understand cellular function at the most fundamental level, in part by tracking changes in specific metabolites over time (Fiehn O, 2000) (Fell DA, 2005).

Prior to the advent of metabolic profiling, the function of cells was often investigated and characterised in terms of single metabolites whereas metabolomic profiling has allowed the study of multiple cellular functions in greater detail. Such metabolomic

analyses can be metabolome-wide, which potentially identifies all detectable metabolites, or targeted on specific molecules.

In adults, certain metabolites including branch-chain and aromatic amino acids (BCAA) have been shown to be consistently and positively associated with the presence of insulin resistance and dysglycaemia. The associations were present even after the influence of adiposity was removed (Wang TJ, 2011) (Cheng S, 2012).

In this study, four BCAAs were examined; they were chosen based on the results of previous studies which identified them as metabolites of interest in relation to dysglycaemia (Zhao X, 2016) (Hosking J, 2019) (Wang TJ, 2011) (Cheng S, 2012) (Fiehn O, 2000): a meta-analysis of eight studies conducted in adults showed that the levels of isoleucine, leucine and tyrosine was associated with an almost 40% higher risk of Type II diabetes (T2D). Valine and phenylalanine were associated with about 30% increased risk of T2D, while the levels of glycine and glutamine were inversely associated with T2D risk (Wang TJ, 2011). This indicates that at least in adults, BCAA metabolites may be predictive of the risk of dysglycaemia (Hu W, 2016).

It is still not unclear if there are similar associations between certain metabolites and cardiometabolic diseases. Examining this area is important because if similar relationships are found in children, this information may also shed light on biochemical pathways and mechanisms involved in the pathogenesis of cardiometabolic disease from childhood.

A recently conducted systematic review of 10 studies showed that BCAA, aromatic amino acids (AAA) and acylcarnitines were associated with IR in children, while BCAA and tyrosine were associated with other markers of metabolic risk (Zhao X, 2016). Most studies conducted in children, however, were cross-sectional, and were predominantly of obese children; in contrast, longitudinal studies have been lacking, and normal weight children have not been evaluated. Therefore, the changes described

in previous studies could simply represent association, and not be related to causal mechanisms. Therefore, there is a need to determine whether there might be cause and effect relationships between the levels of these metabolites and the development of risk factors for cardiometabolic diseases.

To investigate this, a detailed longitudinal analysis was conducted to evaluate how specific metabolites relate to the metabolic risk score during childhood and adolescence. This analysis is novel and considers the effects of important covariates like age, and adiposity.

7.3 Methods

A subset of the EarlyBird cohort was selected for metabolomics analysis. For a previous study, 150 children (105 boys and 45 girls) were selected based on those who had showed impaired fasting glucose between age 5y and 16y and age/gender matched controls.

Serum samples collected from each child every year from age 5 to 16 years; the samples were subjected to metabolomics analysis. Metabolic profiling was carried out by means of Proton nuclear magnetic resonance spectroscopy (^1H NMR) spectroscopy. 400 μL of blood serum were mixed with 200 μL of deuterated phosphate buffer solution 0.6 M KH_2PO_4 . ^1H NMR metabolic profiles of serum samples were acquired with a Bruker Avance III 600 MHz spectrometer equipped with a 5 mm cryoprobe at 310 K (Bruker Biospin, Rheinstetten, Germany) and processed using TOPSPIN (version 2.1, Bruker Biospin, Rheinstetten, Germany) software package. Based on an internal database of reference compounds, representative signals of metabolites were integrated. The signals are expressed in an arbitrary unit corresponding to a peak area normalized to total metabolic profiles. ^1H NMR spectroscopy being a quantitative method, metabolite peak area is proportional to metabolite concentrations, and thus their changes are representative of absolute change in metabolite concentration in the serum. This

metabolomics approach covers major metabolic pathways, including lipoproteins, amino acids, carboxylic acids, and central energy metabolism in a highly reproducible manner across more than 1700 serum samples. In particular, ^1H -NMR spectrum of human blood serum enables the monitoring of signals related to lipoprotein bound fatty acyl groups found in triglycerides, phospholipids and cholesteryl esters, together with peaks from the glyceryl moiety of triglycerides and the choline head group of phosphatidylcholine. The analysis presented here focused on a small number of specific metabolites found by previous studies to be of interest in relation to IR, and therefore potentially predictive of MRS.

7.3.1 Statistical Analysis

Tracking of metabolites was investigated, in boys and girls separately, using year-on-year correlation and longitudinal tracking coefficients; details of the methodology are described in Chapter 3. Pearson correlations were obtained to determine the strength of the relationships both among the metabolites and between the metabolites and adiposity and the metabolic risk score at each time-point. Time-lagged correlations (as described in Chapter 3) were used to explore the association between metabolites and later metabolic risk score.

For each metabolite, the trend over time was characterised using boxplots of their distributions at each time-point. Gender differences were tested using an independent sample *T*-test.

In order to illustrate the trajectories in the metabolites from age 5y according to their metabolic risk at 16y, the participants were classified according to metabolic risk score (MRS) at 16y: ‘high MRS’ was arbitrarily defined as any participant whose MRS at 16y was at or above the 85th centile. The metabolite trajectories from 5y-16y were examined according to classification of children according to metabolic risk score at 16y, both

graphically and using mixed effects models. Mixed effects modelling was carried out for each metabolite – random intercepts were included as well as age (categorized to allow for non-linear change in outcome over time), adiposity, physical activity, MRS group ($<85^{\text{th}}$ centile or $\geq 85^{\text{th}}$ centile at 16y), and the ‘MRS group x age’ interaction as fixed effects.

7.4 Results

7.4.1 Trends in Metabolites over Time

Summary statistics for each of the metabolites from 5y to 16y are shown in table 25 (boys) and table 26 (girls). In both boys and girls, isoleucine was at its highest at 5y, dropped around 7y, and then remained stable until 16y (Figure 46); a similar pattern was seen with leucine (figure 47). The levels of valine (Figure 48) remained steady from 5y to 16y, while that of lactate (Figure 49) increased with age.

Table 25. Average level of metabolites in boys

Boys				
Age	Isoleucine	Leucine	Valine	Lactate
5y	0.00268	0.00983	0.00608	0.03793
6y	0.00240	0.00901	0.00580	0.03752
7y	0.00238	0.00902	0.00577	0.03789
8y	0.00233	0.00875	0.00567	0.04143
9y	0.00236	0.00924	0.00598	0.04453
10y	0.00235	0.00891	0.00576	0.04644
11y	0.00238	0.00914	0.00591	0.04643
12y	0.00236	0.00915	0.00604	0.04283
13y	0.00237	0.00909	0.00599	0.04554
14y	0.00238	0.00926	0.00603	0.04698
15y	0.00233	0.00933	0.00617	0.04617
16y	0.00232	0.00936	0.00618	0.04742

Table 26. Average level of metabolites in girls

Girls				
Age	Isoleucine	Leucine	Valine	Lactate
5y	0.00273	0.00956	0.00581	0.04112
6y	0.00254	0.00919	0.00560	0.03795
7y	0.00246	0.00918	0.00586	0.04015
8y	0.00249	0.00923	0.00582	0.04582
9y	0.00241	0.00911	0.00578	0.04753
10y	0.00257	0.00964	0.00611	0.04805
11y	0.00250	0.00943	0.00598	0.04886
12y	0.00248	0.00918	0.00560	0.04737
13y	0.00249	0.00932	0.00583	0.04787
14y	0.00253	0.00922	0.00556	0.05222
15y	0.00245	0.00905	0.00568	0.04932
16y	0.00241	0.00896	0.00575	0.04820

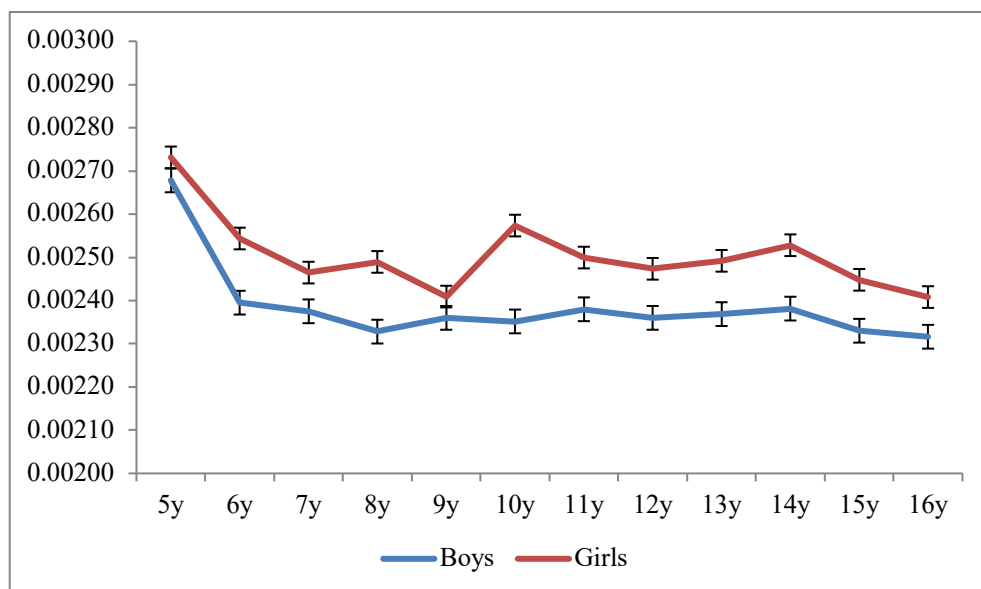


Figure 46. Trends in Isoleucine from 5y-16y

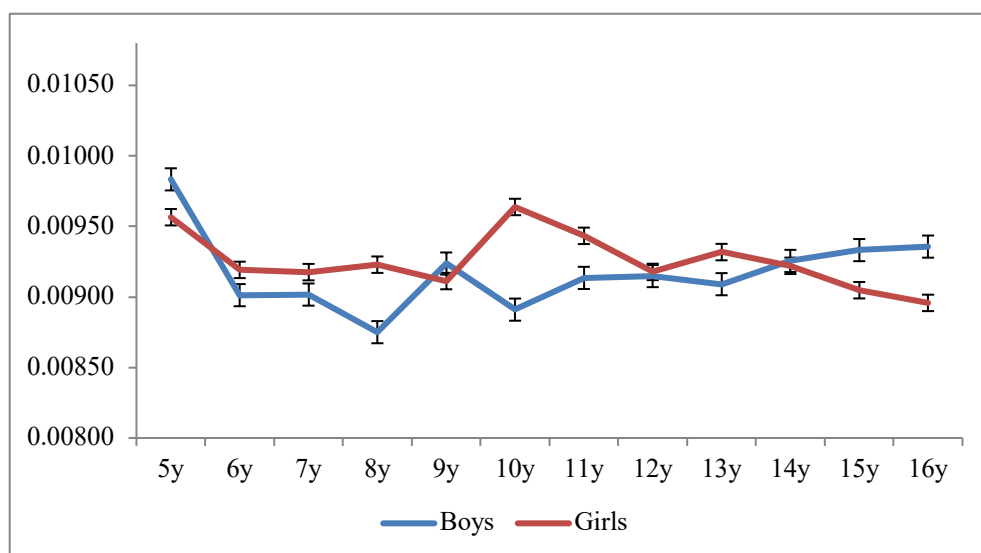


Figure 47. Trends in Leucine from 5y to 16y

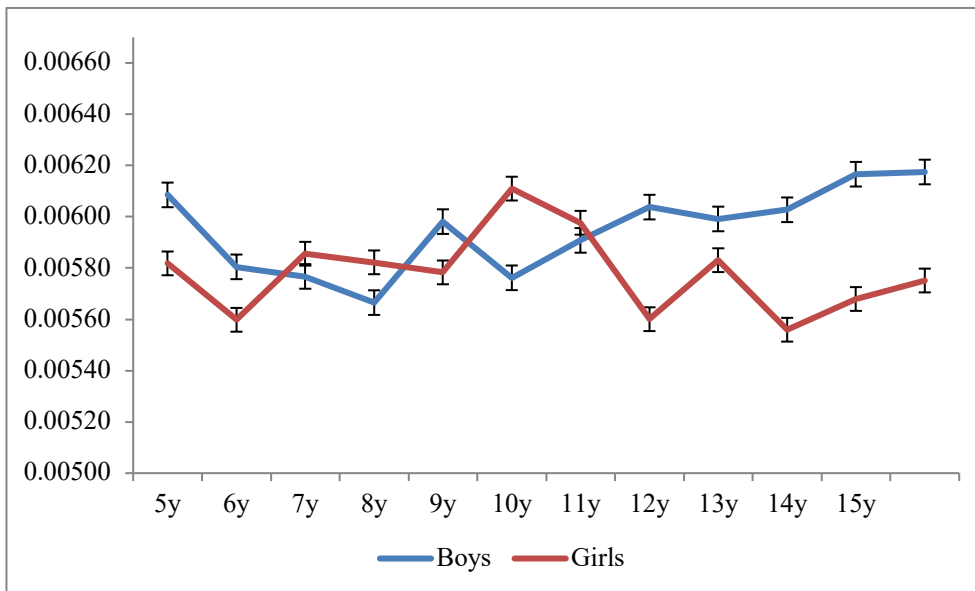


Figure 48. Trends in Valine from 5y to 16y

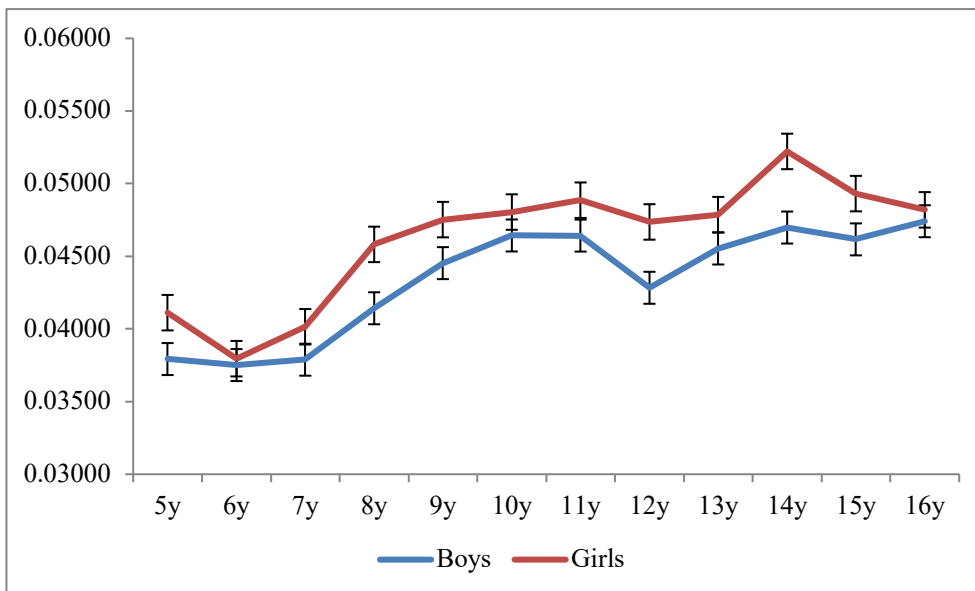


Figure 49. Trends in Lactate from 5y to 16y

Tracking of serum metabolites over time

The tracking of each metabolite within individuals over time, was evidenced by the moderate-high year-on-year correlation coefficients (table 27). For isoleucine, correlation coefficients ranged from 0.5 to 0.7 in boys and girls; for leucine, 0.4 to 0.8, valine 0.4 to 0.7, and lactate 0.4 to 0.6.

Table 27. Year-on-year correlation between metabolites

Age (years)	Isoleucine		Leucine		Valine		Lactate	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
5 and 6	0.578**	0.523**	0.590**	0.520**	0.437**	0.467**	0.456*	0.452*
6 and 7	0.524**	0.582**	0.622**	0.631**	0.582**	0.510**	0.608**	0.430*
7 and 8	0.563**	0.684**	0.521**	0.506**	0.549**	0.556**	0.603**	0.580**
8 and 9	0.574**	0.638**	0.677**	0.509**	0.528**	0.564**	0.591**	0.402*
9 and 10	0.592**	0.595**	0.635**	0.560**	0.646**	0.524**	0.691**	0.554**
10 and 11	0.604**	0.696**	0.785**	0.564**	0.657**	0.551**	0.539**	0.502**
11 and 12	0.509**	0.669**	0.666**	0.516**	0.586**	0.530**	0.609**	0.467**
12 and 13	0.528**	0.534**	0.652**	0.541**	0.539**	0.506**	0.539**	0.598**
13 and 14	0.509**	0.513**	0.583**	0.592**	0.534**	0.570**	0.574**	0.554**
14 and 15	0.536**	0.567**	0.579**	0.559**	0.588**	0.574**	0.534**	0.460**
15 and 16	0.597**	0.587**	0.664**	0.609**	0.523**	0.515**	0.594**	0.520**

**<0.001; * <0.05

7.4.2 Associations between Individual Serum Metabolites

In both boys and girls, and from 5y to 16y, the relationship between each metabolite was strong: For isoleucine and leucine, correlation coefficients r ranged from 0.50 to 0.84 ($p < 0.001$) in boys, and 0.56 to 0.80 ($p < 0.001$) in girls.

The correlation coefficient between isoleucine and valine ranged from r 0.31 to 0.76, p (< 0.005) in boys, and 0.51 to 0.8 ($p < 0.001$) in girls. For isoleucine and lactate, correlation coefficients ranged from 0.4 to 0.8 ($p < 0.001$) in both boys and girls, while the correlation coefficients for leucine and valine ranged from 0.7 to 0.8 ($p < 0.001$) in boys, and 0.7 to 0.9 ($p < 0.001$) in girls.

7.4.3 Associations between MRS and Serum Metabolites (Cross-Sectional and Time-Lagged)

Cross-sectional

When the entire cohort was examined, there were significant correlations between MRS and isoleucine in boys at age 5, 6, 8, 10, 12, and 13y (r 0.25 to 0.51, $p < 0.03$), as well as in girls at 10y (r 0.52, p 0.007) and 15y (r 0.42 p 0.016).

There were also significant correlations between MRS and lactate in boys at 14-16y (r 0.28 to 0.41, $p < 0.001$), and in girls at 6y, 9y, and 15y (r 0.4 to 0.6, $p < 0.03$).

There were no other significant cross-sectional correlations between the metabolites and the MRS.

Time-Lagged

There were significant correlations in boys, between MRS at 16y and lactate at 8y to 15y (r 0.24 to 0.34, $p < 0.05$); there were no other significant time-lagged correlations between any of the metabolites and MRS at 16y.

7.4.4 Longitudinal Analysis of the Trajectory in the Levels of Metabolites Based on MRS at 16y

The study participants were classified based on MRS at 16y of age, into ‘high’ and ‘low’ MRS; then the differences in isoleucine, leucine, valine, and lactate from 5y to 16y were examined. High MRS was defined, as MRS at or above the 85th centile while low was MRS below the 85th centile

At 16y of age, of the 105 boys, 19 (18%) and 10 (22%) of the 45 girls had MRS at or above the 85th centile.

7.4.5 Isoleucine

In girls, Isoleucine was higher in the low MRS group 6,7, and 14y (Figure 50, $p < 0.05$) but the difference in average levels of isoleucine between both groups was small.

On the other hand, in boys isoleucine was higher from 9y-16y in those with a high MRS at 16y (Figure 51)

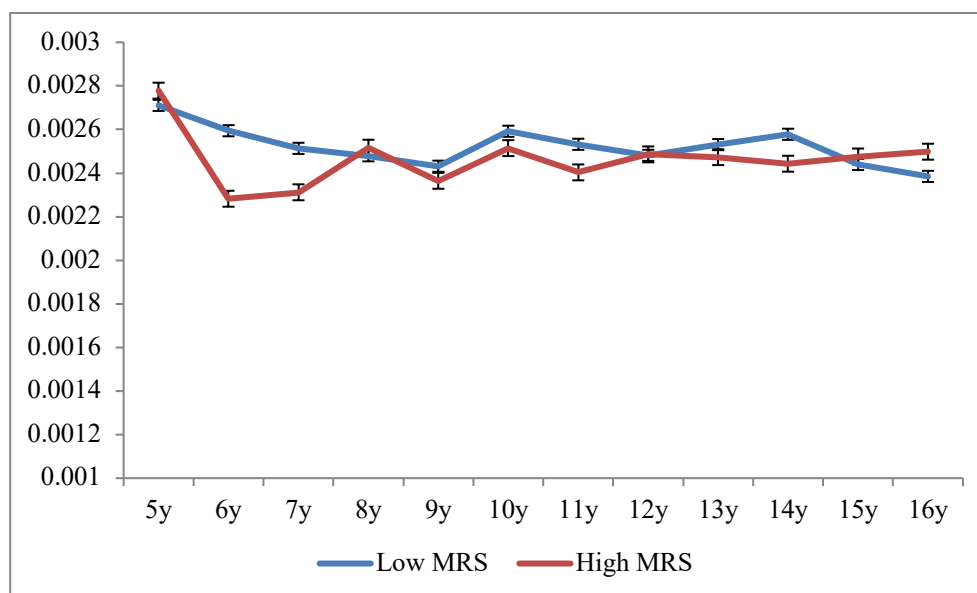


Figure 50. Level of Isoleucine in according to metabolic risk score in girls

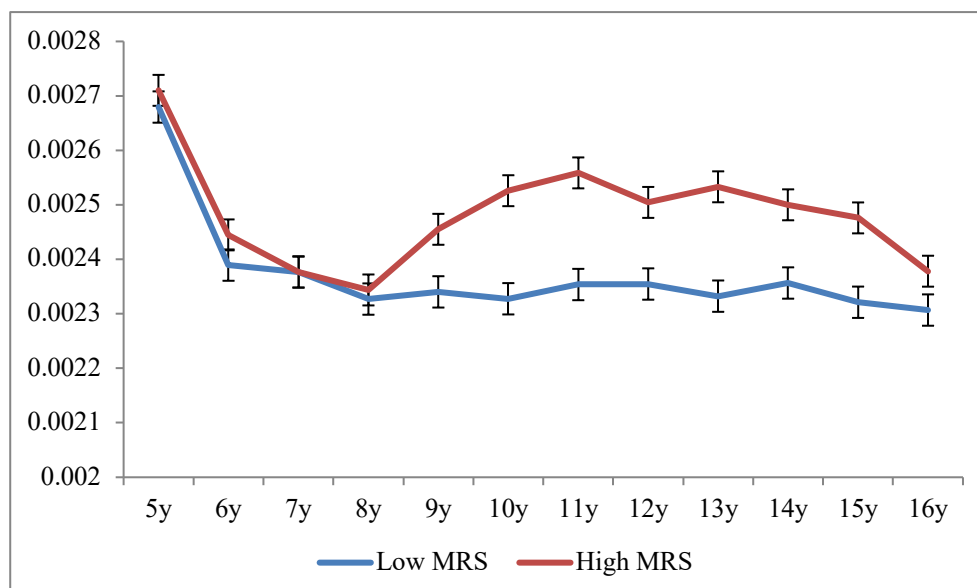


Figure 51. Level of Isoleucine in according to metabolic risk score in boys

7.4.6 Leucine

In girls, leucine levels were similar between the two groups (Figure 52); while in boys there was a consistently higher level of leucine in those with high MRS at 16y (figure 53, $p<0.001$).

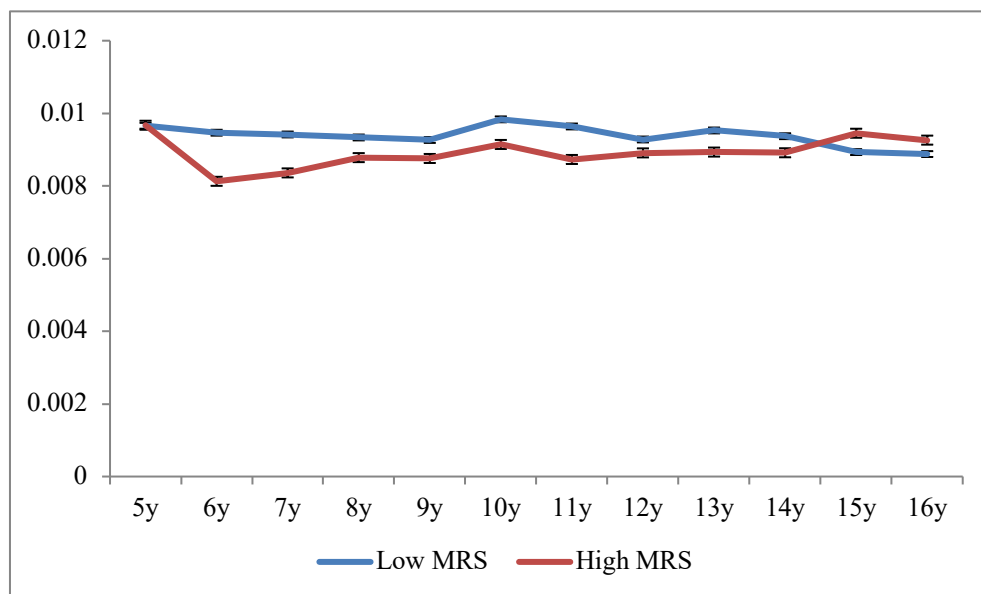


Figure 52. Level of Leucine in according to metabolic risk score in girls

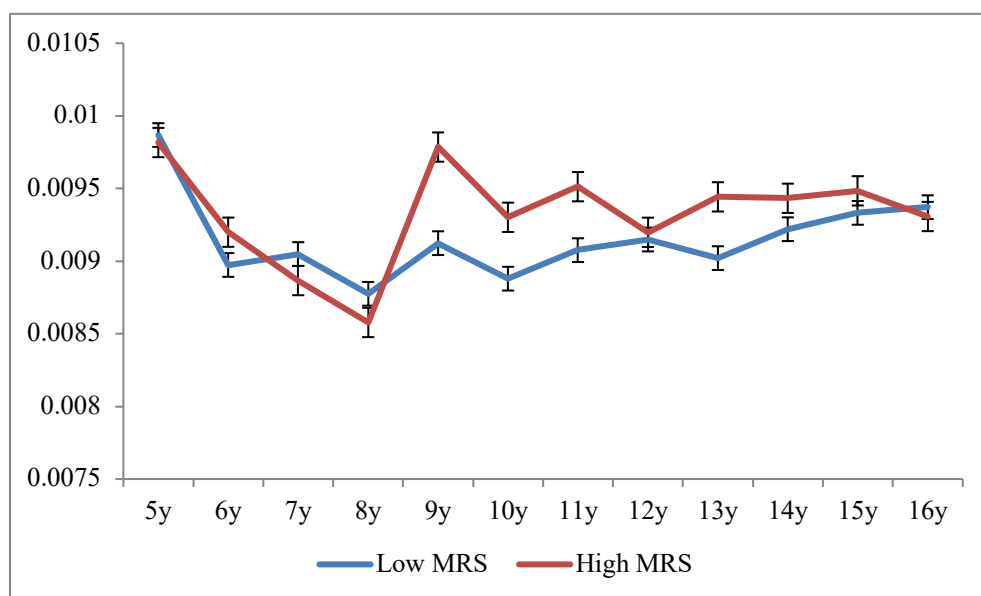


Figure 53. Level of Leucine in according to metabolic risk score in boys

7.4.7 Valine

In girls, valine was lower in those with a high MRS at 16y (Figure 54, $p < 0.02$); on the other hand, in boys (Figure 55, the level of valine was similar between the groups.

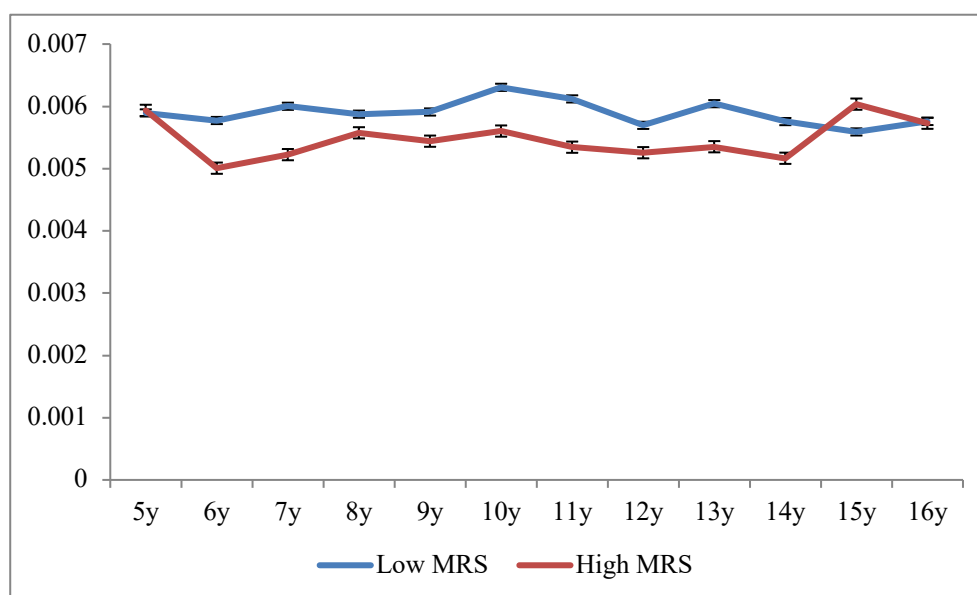


Figure 54. Level of Valine in according to metabolic risk score in girls

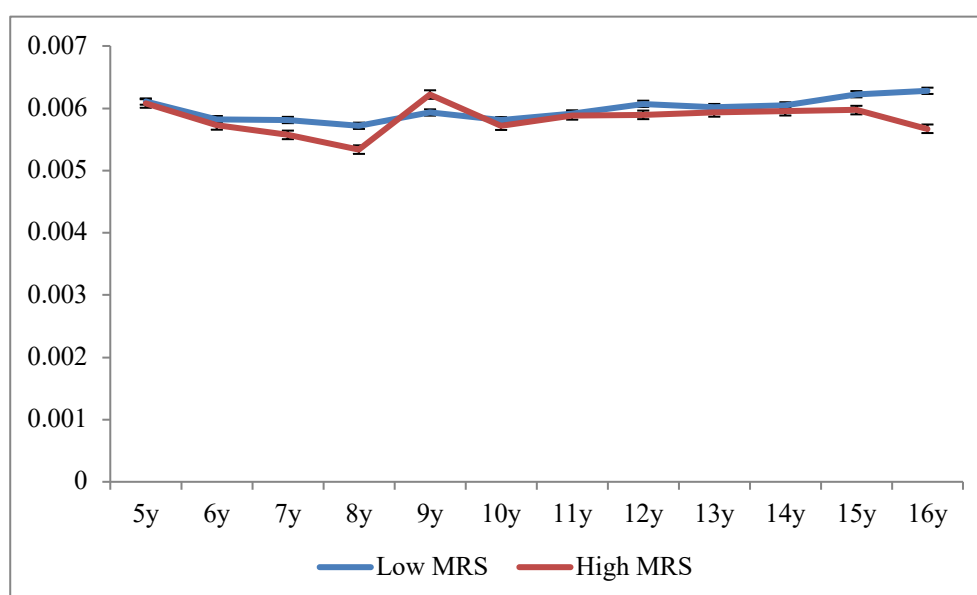


Figure 55. Level of Valine in according to metabolic risk score in boys

7.4.8 Lactate

In both boys and girls, lactate was higher in those with a high MRS at 16y; however, the difference was at a higher magnitude in boys and was significant from 8y to 16y ($p < 0.01$), compared to just 12-14y in girls (Figures 56 and 57).

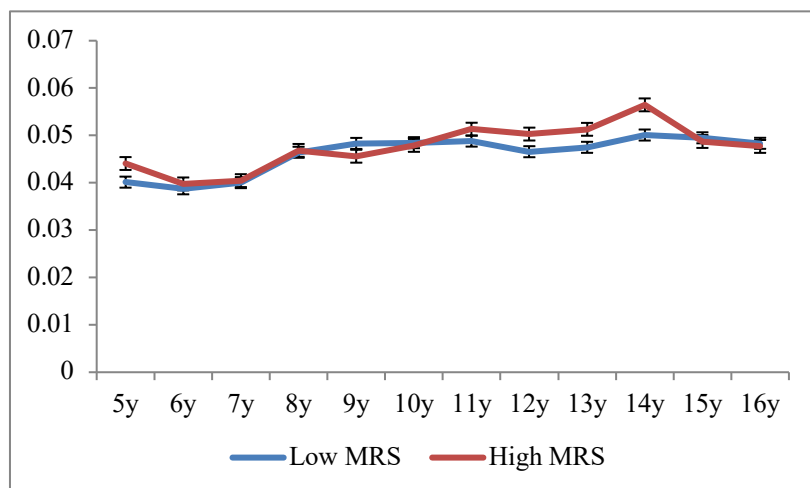


Figure 56. Level of Lactate in according to metabolic risk score in girls

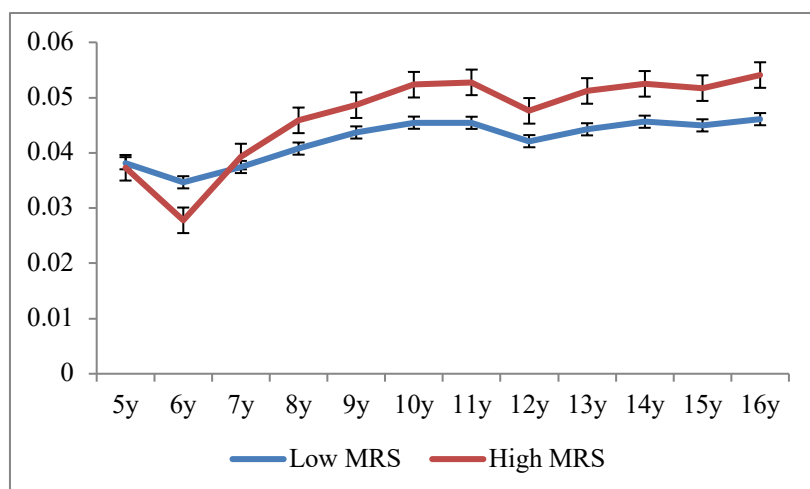


Figure 57. Level of Lactate in according to metabolic risk score in boys

In summary, isoleucine, leucine, and lactate was higher in boys with a high MRS compared to those with low MRS; while in girls, lactate was higher in those with a high MRS, no significant difference was seen in leucine, the difference in isoleucine was not of significant magnitude. In both boys and girls, lactate was higher in those with a high MRS at 16y.

7.4.9 Mixed Effects Models (tables A32 - A39 in appendix)

Mixed effects models were built to quantify the association between each metabolite and the presence of a high metabolic risk score.

When adjustments were made for the influence of age and adiposity (BMI), the only significant finding was higher isoleucine in the girls with a high metabolic risk score (F 28, P 0.024).

7.5 Discussion

Utilising data from a longitudinal cohort of children studied from age 5y to 16y, this chapter investigated the relationship between metabolic risk and changes in the metabolites associated with cellular metabolism.

The results show that high MRS at 16 years is predictive of the levels of specific products of branch-chain amino acid metabolism; mixed effects models indicate that these effects appear to be modulated by the presence of adiposity.

In this analysis, four products of branch-chain amino acid (BCAA) metabolism were studied: isoleucine, leucine, lactate and valine; these were selected because they have been associated with hyperglycaemia in adults (Guasch-Ferré M, 2016) (Tai ES, 2010) and because we have observed associations between IR and BCAAs (Hosking J, 2019) .

In these studies, the associations between these four metabolites were consistent

throughout the cohorts studied and persisted even when statistical adjustments were made for the impact of adiposity.

Apart from the studies described above, research on this subject is limited with very few studies done in children. In animal studies, there was also an association between insulin resistance and isoleucine; but this was only observed in the presence of a high fat diet (Nishimura J, 2010).

A meta-analysis of eight studies in adults showed that one standard deviation difference in isoleucine, leucine, and valine was associated with at least a 35% higher risk of type II diabetes (Zhao X, 2016); raising the possibility that disorders of BCAA metabolism might contribute to the development of high insulin resistance and type II diabetes.

A few cross-sectional studies in children have shown positive associations between some products of BCAA metabolism and insulin resistance. However, a major limitation is that these studies were only in obese children, were cross-sectional, and included few participants (Zhao X, 2016). On the other hand, the findings of Hosking et al also from the longitudinal Earlybird cohort, showed that in healthy, predominantly normal weight children, IR was associated with lower levels of BCAA, but elevated lactate concentrations preceded the onset of IR (Hosking J, 2019). In this study however, the analysis goes further to show the relationship between products of BCAA metabolism and lactate and the MRS.

By using the MRS, which is a composite score of several risk factors for future cardiometabolic diseases, this analysis indicates that there might be other factors apart from IR, that influence the levels of these products of BCAA metabolism.

One of the key steps in BCAA metabolism is inactivation by branched- chain keto acid dehydrogenase (BCKD) (Tomita M, 1978); so BCKD is responsible for the breakdown of BCAAs but unlike other amino acids, the majority of BCAA catabolism occurs in skeletal muscle (Brosnan JT, 2006). The activity of BCKD in muscle cells is impaired

by insulin (Han N, 2018) (Sh A, 2011) so in insulin resistant states which cause hyperinsulinemia, BCKD is inhibited leading to higher levels of BCAA. This might provide an explanation for the findings described in this chapter.

In a small study of obese women, there were significant associations between leucine and markers of inflammation like Tumour necrosis factor-alpha (TNF- α) and HOMA-IR (Du S, 2017). Similarly, in this analysis, apart from isoleucine in girls, the differences in the levels of the metabolites were attenuated once the influence of adiposity was eliminated.

Another possible explanation for the association between leucine and metabolic risk could be inferred from animal studies in which markers of inflammation like TNF- α prevented the uptake of BCAA in fat tissue, leading to a further increase in serum BCAA levels (Burrill JS, 2015).

Like leucine, the relationship between valine and cardiometabolic risk has not been evaluated in children. In a small study of obese women, it was found to be elevated in those who were insulin resistant (Fiehn O, 2000). Other studies have shown similar results in adult men (Hu W, 2016), and along with leucine and isoleucine, valine levels were observed to predict the incidence of Type II diabetes 12 years before occurrence (Wang TJ, 2011).

In animal studies, a similar association was found between insulin resistance and higher levels of isoleucine, however this was only observed in those fed a high fat diet (Newgard CB, 2009). These studies also suggested associations between the level of isoleucine and HOMA-IR although not statistically significant ($P = 0.09$) and with markers of inflammation like Interleukin-6 (Reddy P, 2018). Therefore, isoleucine may be a predictor of increased metabolic risk. In this analysis, other metabolites: lactate and leucine were also predictive of higher MRS.

This analysis showed that lactate levels were higher in those with higher metabolic risk, but the effect was not present when the impact of adiposity was eliminated.

Lactate has not previously been recognised as a predictor of increased metabolic risk; however, it has been recognised as a marker of oxidative capacity, and is well established as a prognostic marker of the severity of acute illness (Andersen LW, 2013). In studies that examine the changes that occur in children born with errors in gluconeogenesis, several organic acids like lactate were directly associated with dysglycaemia (Goldberg GR, 1992).

The findings of increased plasma lactate levels in insulin resistant patients may also support an association between increased lactate levels and higher metabolic risk (Andersen LW, 2013) (Cox K, 2012) (Lovejoy J, 1992). Similarly, adults with a recent diagnosis of Type II Diabetes (Crawford SO, 2010), and non-diabetic but obese adults (Lovejoy J, 1992) have also been found to have high levels of lactate. The underlying mechanism may be similar in both scenarios and could include the increased oxidative stress in metabolic diseases (Crawford SO, 2010). Insulin resistance is associated with impaired insulin action on both glucose transport and intracellular glucose metabolism; several studies have shown that in animals fed a high fat diet, there is suppression of intracellular glucose metabolism which then impairs the ability of insulin to transport glucose into skeletal muscle (Kim JK, 1996) (Choi YJ, 2002).

In these studies, lactate elevation preceded the onset of insulin resistance in skeletal muscle which supports the idea that elevated lactate may precede the onset of insulin resistance.

BCAAs are produced from the metabolism of dietary proteins by gut microbes (Layman DK, 2003), so another possible explanation for the association of BCAA with insulin resistance can be drawn from studies of the gut microbiome (Qin LQ, 2011). In the study participants with insulin resistance, the concentration of BCAAs was associated

with the levels of specific gut flora including *Prevotella copri* and *Bacteroides vulgatus*. As well, these bacteria were identified as the main synthesizers of BCAAs (Qin LQ, 2011) (LA Lotta, 2016). Similarly altered gut microbiome has been seen in people with Type II diabetes (Walford GA, 2013).

Most of the evidence on metabolomic profiles of IR has come from studies in adults but the analysis presented in this chapter provides confirmation of similar associations in children and adolescents. Knowledge of these profiles also has clinical relevance; for instance, several studies have shown that certain anti-diabetes medications led to lower levels of BCAAs (Irving BA, 2015) (Preiss D, 2016), and so these have been considered as possible biomarkers for earlier detection of diabetes (Wang TJ, 2011)

This analysis has several limitations; first the sample size was relatively small which explains why some of correlations between metabolites and the metabolic risk score were not statistically significant. Clinical studies with larger sample sizes should be performed to confirm the findings described in this chapter.

The study only followed children to the age of 16 years, and we have not had the opportunity to include longer-term data. At the age of 16y, most but not all children have reached final height and completed puberty. It is not known whether MRS at age 16y reflects emerging cardiometabolic risk of young adults, but there was an assumption in this research that MRS at age 16y will have long term implications for health. Clearly, it is likely that MRS will be modified by subsequent lifestyle-related factors including weight gain. Therefore, longer-term follow-up of the cohort will be required. Other limitations of this analysis include the absence of data on linear growth. No adjustments were made for the impact of puberty (as opposed to age) on the metabolites. However, the complexity of accounting for pubertal development (eg Tanner Stage, Age at Peak Height Velocity, etc) was beyond the scope of the present work.

Insulin resistance was calculated with the HOMA method, which has several limitations; although it has been shown to correlate highly with clamp-derived measurements (Nguyen QM, 2010). Finally, although the 10-year period of follow-up is possibly the longest reported in literature, it may not be sufficiently long enough to detect the long-term effects of high metabolic risk on the metabolome. Ultimately the present study shows an association between MRS and a metabolite pattern throughout childhood, but this evidence falls short of proving cause and effect. The predictive potential of the study would be greatly enhanced by longer-term follow up, as the participants age, become more overweight and obese, and begin to develop cardiometabolic diseases.

The strength of the Earlybird cohort includes the longitudinal design, the study of healthy children, with detailed annual biochemical and anthropometric measurements from age 5 to 16 years.

In conclusion, this unique longitudinal study of a homogenous cohort of children and adolescents shows that those who developed a higher metabolic risk score at the age of 16y exhibited higher levels of specific BCAA and lactate during childhood, suggesting that these molecules may be early markers of cardiometabolic risk in children. However, these effects were largely attenuated by the influence of adiposity, suggesting that the latter is at least partly responsible for these findings.

Chapter 8

Conclusion

Cardiometabolic diseases, including atherosclerotic cardiovascular disease (ACVD) and type 2 diabetes (T2D) are among the leading causes of mortality and morbidity in adults so preventing these diseases is critical. To achieve this, individuals at risk need to be identified early in life, ideally in childhood, and then effective strategies to reduce the incidence should be implemented. There is limited research in this area, so this doctoral study was undertaken to investigate the ability of measures of adiposity, nutrition, and metabolomic biomarkers to predict cardiometabolic risk.

First, through a systematic review and meta-analysis of other relevant studies, an exploration of the available literature on predictors of cardiometabolic risk in children was undertaken; the review confirmed that high body mass index in childhood predicts the presence of high carotid intima media thickness, impaired glucose tolerance, and hypertension in adulthood. The overwhelming majority of the studies included in the review used BMI as the measure of adiposity; this emphasised the need to study alternative methods for early identification of long-term risk of ACVD.

One of the most important aspects of this doctoral study involved deriving a metabolic risk score, to make it easier to identify those at a high risk for future cardiometabolic disease. This score is important in epidemiological research because there is no universal definition of the metabolic syndrome in children, and the prevalence rates in this population is very low. The score derived and utilised in this study allowed each study subject to have a continuous value; lower values indicated a better metabolic syndrome profile and higher values indicated a poorer metabolic syndrome profile.

Next, different parameters were examined to determine if they could predict high metabolic risk score. The studies confirmed that those who had higher measures of adiposity had higher metabolic risk scores by the age of 16 years. In addition, simple anthropometric measures of adiposity (BMI, WC, SF) were at least as effective as the more sophisticated two-component method of measuring body composition, in identifying those most at risk of developing cardiometabolic risk in the future. Therefore, adiposity in early childhood predicts high metabolic risk score in subsequent years.

The next study evaluated the ability of nutrition to predict cardiometabolic risk. Higher intake of total calories, higher intake of sugar, and higher intake of saturated fat was predictive of an increase in metabolic risk score. Also, children who consumed low amounts of fibre were more likely to have increased metabolic risk. Higher intake of carbohydrates, fat, sugar, saturated fat, and lower intake of fibre was associated with higher measures of adiposity as well as higher metabolic risk score. Insulin resistance and triglycerides was higher in those who consumed greater energy, Lower fibre, higher sugar intake, and higher saturated fat intake at 8y.

In the metabolomic study, it was observed that adolescents who had higher metabolic risk at the age of 16y already exhibited higher levels of isoleucine and lactate from the age of 5y, suggesting that these molecules may be early biomarkers of increased cardiometabolic risk.

By showing that overweight children already exhibited higher metabolic risk at 5y of age, this analysis supports the role of early screening for childhood obesity. Detecting obesity early in life offers the opportunity to introduce interventions to reverse it.

Prior to this work, the evidence base for dietary recommendations in children was limited and there was insufficient data on the impact of various macronutrients

on adiposity and cardiometabolic risk in children. The findings from this doctoral work represent and advance in understanding.

The final chapter of this thesis presents the results of the examination of metabolomics in predicting the risk of cardiometabolic diseases. The metabolome represents a collection of metabolites produced during cellular metabolic reactions, so the metabolomic profiling utilised in this analysis allowed the examination of the relationship between specific molecules and the risk of CVD. The results indicate that a high MRS at 16 years is predictive of the levels of specific products of branch-chain amino acid metabolism.

The origin of cardiovascular disease is complex, and the risk factors in children have not been established; this analysis adds to the body of evidence on the role of various measures of adiposity, macronutrients, and metabolites in children, on the pathogenesis of cardiometabolic diseases. This doctoral work identifies novel findings, which will prove useful in identifying the children most at risk of CVD in the future.

The strength of the Earlybird cohort includes its longitudinal design, and the detailed biochemical and anthropometric measurements from a healthy homogenous cohort of children for over a decade. Also, the stability of the predictors and outcome measures over time made them reliable for all the analyses performed.

There are several limitations to this analysis; first, the metabolic risk score was internally derived and therefore cannot be directly compared to other cohorts. Also, dietary recall is associated with significant reporting bias, including underreporting of food intake in those who are obese and overweight.

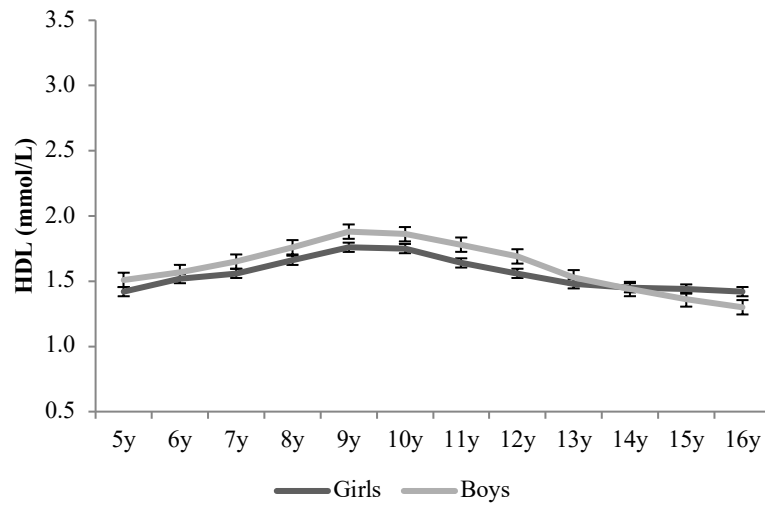
In some of the analysis no statistical adjustments were made for the influence of puberty, which is known to influence the onset of adiposity. Also, energy expenditure and levels of physical activity are different for each child, introducing variability in

energy and macronutrient requirements; it was not possible to account for this in this analysis.

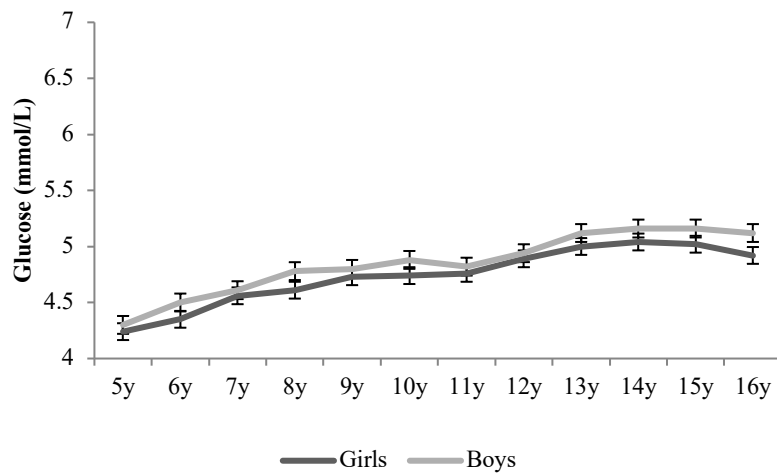
The sample size was relatively small, and this alone can reduce the chances of identifying statistically significant differences in some of the analysis; therefore clinical studies with larger sample sizes should be performed. Also, the study only followed children to the age of 16 years, and we have not had the opportunity to include longer-term data. At the age of 16 years, most but not all children have reached final height and completed puberty, and there is a possibility that the future trajectory of weight and other lifestyle related factors might change in some.

Finally, although the 10-year period of follow-up is possibly the longest reported in literature, it may not be sufficiently long enough to detect the long-term effects of adiposity, macronutrient intake, and the metabolome on the risk of CVD.

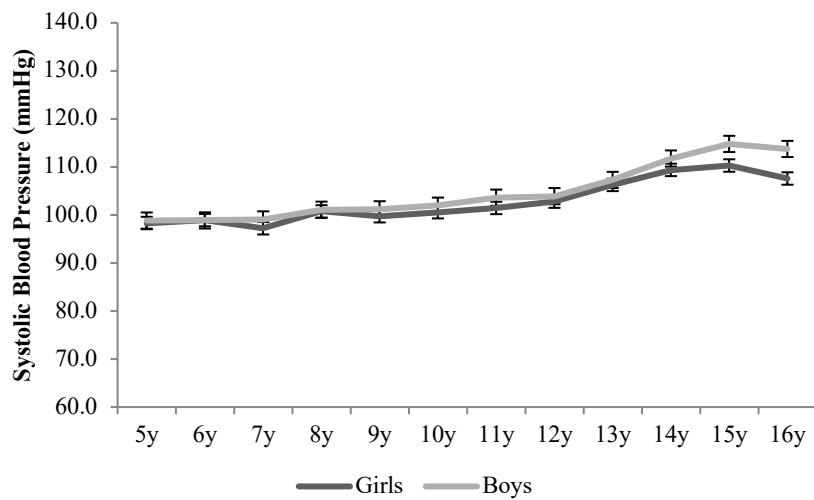
Appendix



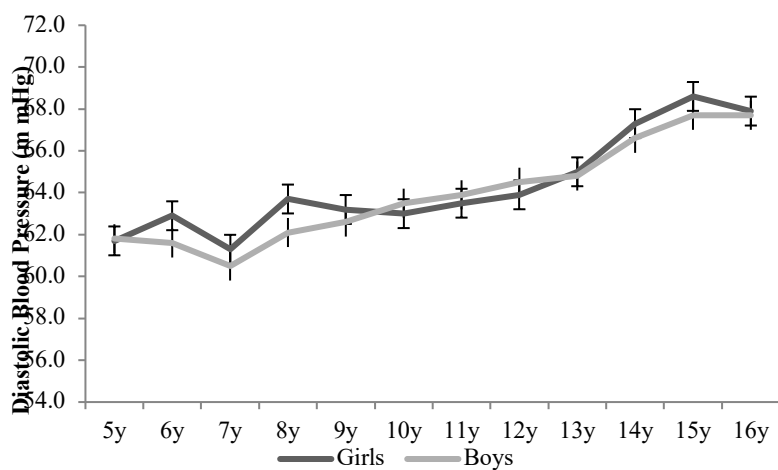
A1. Trends in HDL from age 5y to age 16y



A2. Glucose from 5y to 16y in boys and girls



A3. Systolic Blood Pressure from 5y to 16y in boys and girls



A4. Diastolic Blood Pressure from 5y to 16y in boys and girls

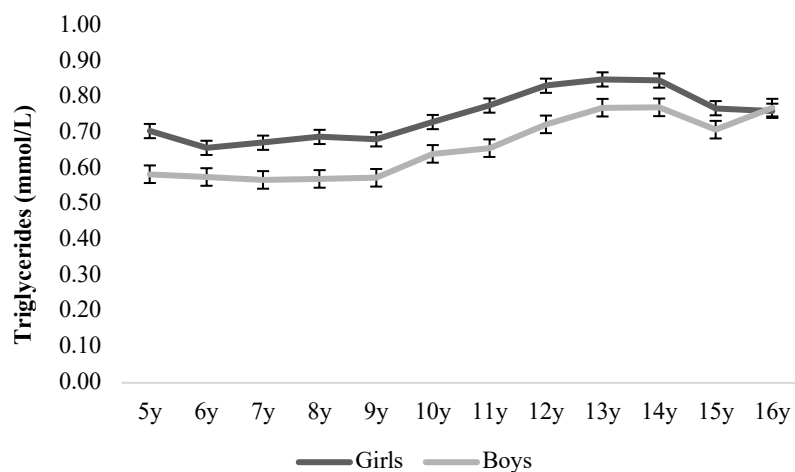
A5. Number of children who met the criteria for the MS

HDL												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	1	1	4	0	0	0	1	1	1	0
Boys	1	1	0	0	0	0	0	0		0	0	0
Total	1	1	1	1	4	0	0	0	1	1	1	0
Glucose												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	0	0	0	2	2	3	4	9	6	6
Boys	0	0	0	2	3	3	1	11	12	17	16	16
Total	0	0	0	2	3	5	3	14	16	26	22	22
Systolic BP												

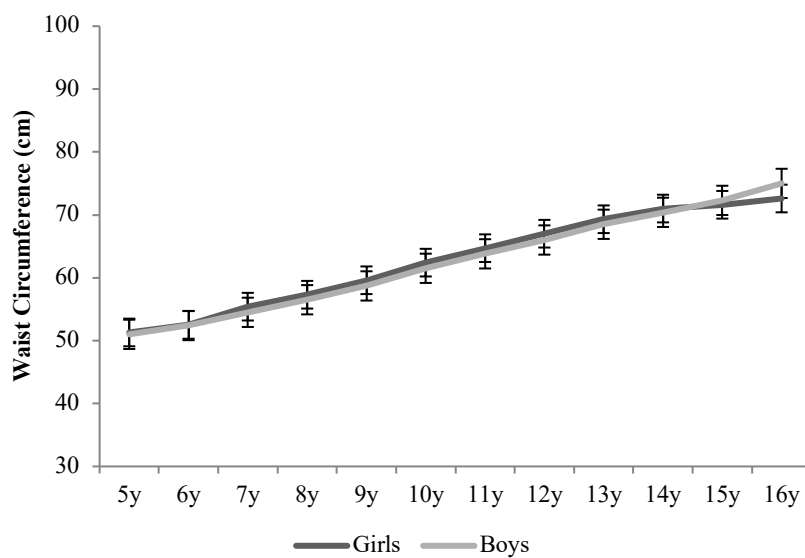
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	0	0	0	0	0	0	0	1	1	4
Boys	0	0	0	0	0	0	0	0	1	0	4	12
Total	0	0	0	0	0	0	0	0	1	1	5	16

Diastolic BP												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	0	0	0	0	1	1	1	1	2	4
Boys	0	0	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	0	0	0	1	1	1	1	2	5
WC												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	0	0	0	2	4	4	6	11	9	13
Boys	0	0	0	0	0	0	1	1	3	5	7	12
Total	0	0	0	0	0	2	5	5	9	16	16	25
Triglycerides												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	2	2	5	9	2	6	8	5	9	2	5
Boys	0	0	0	1	3	5	1	7	5	7	3	7
Total	0	2	2	6	12	7	7	15	10	16	5	12
3 or more MS criteria:												
	5y	6y	7y	8y	9y	10y	11y	12y	13y	14y	15y	16y
Girls	0	0	0	0	0	1	2	1	1	2	1	1
Boys	0	0	0	0	0	0	0	0	2	0	1	2
Total	0	0	0	0	0	1	2	1	3	2	2	3

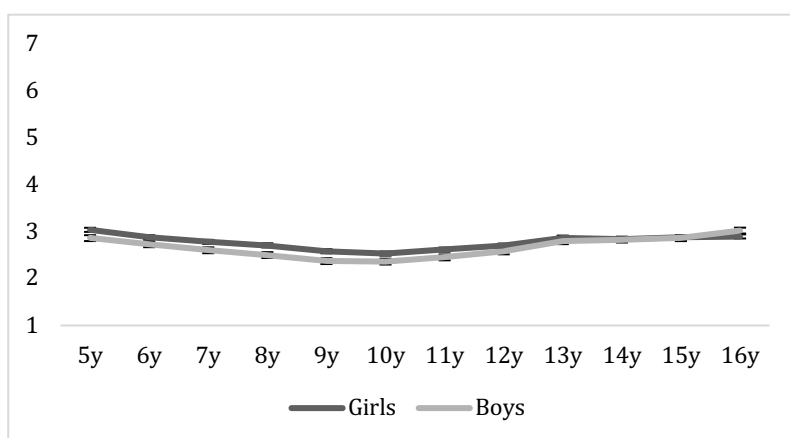
A5 continued. Number of children who met the criteria for the MS



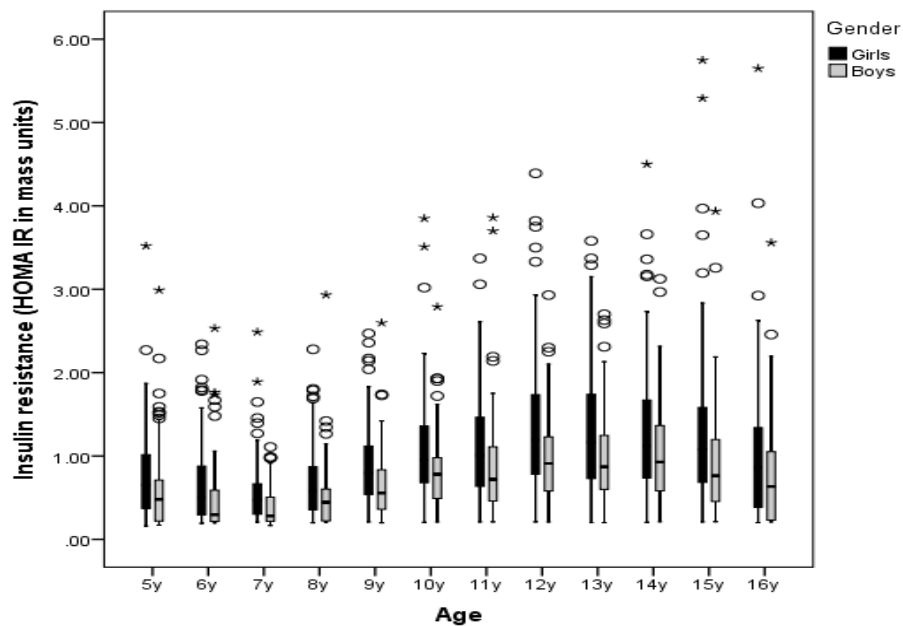
A6. Triglycerides from 5y to 16y in boys and girls



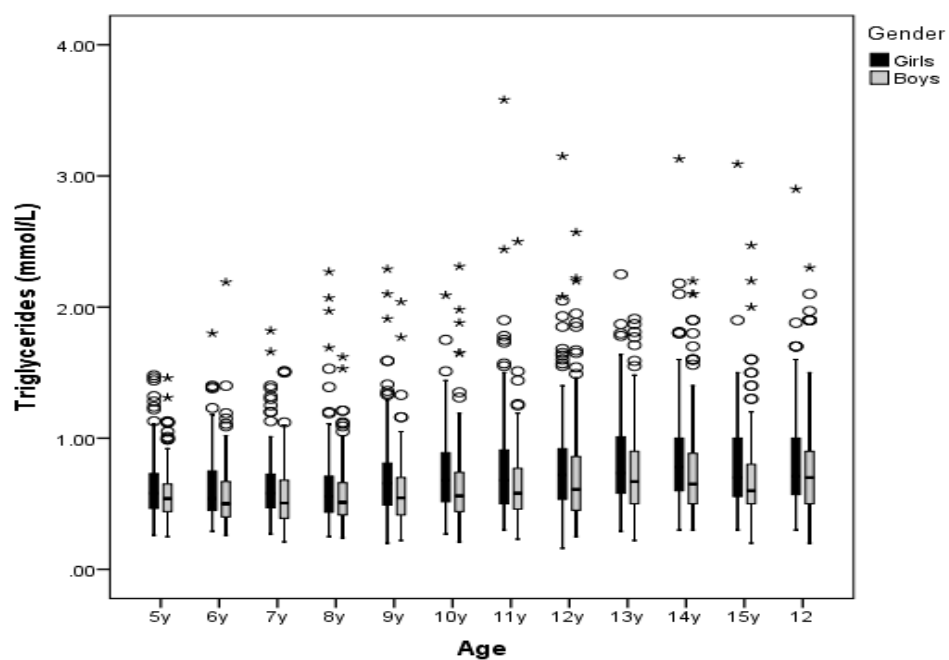
A7. Waist Circumference from 5y to 16y in boys and girls.



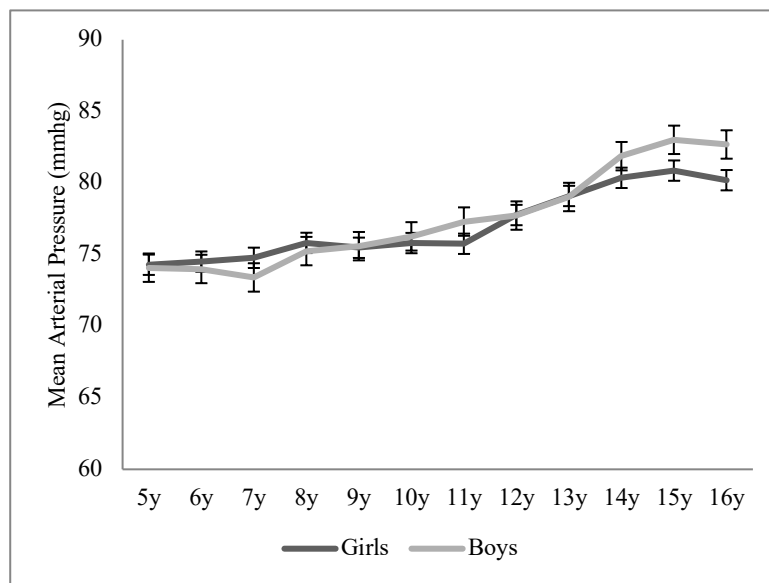
A8. Total Cholesterol: HDL from 5y to 16y in boys and girls



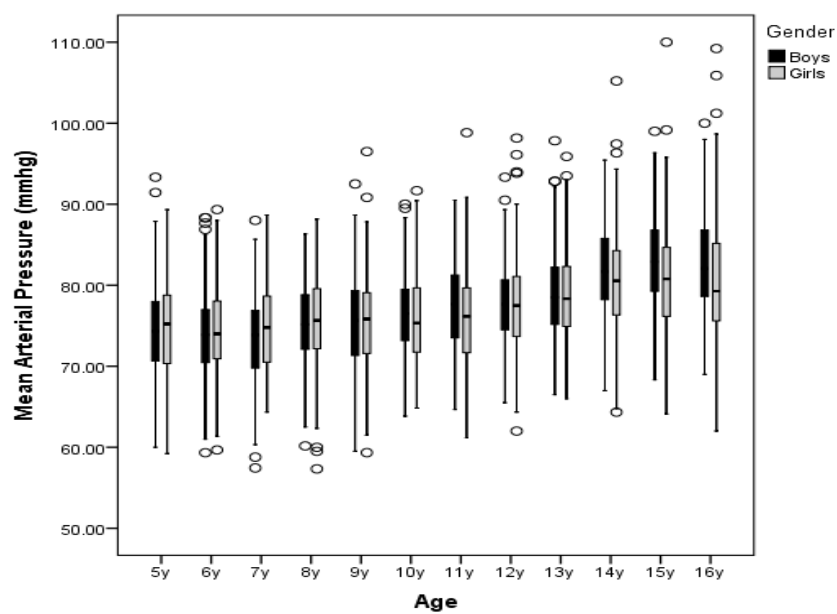
A9. Insulin resistance from 5y to16y in boys and girls



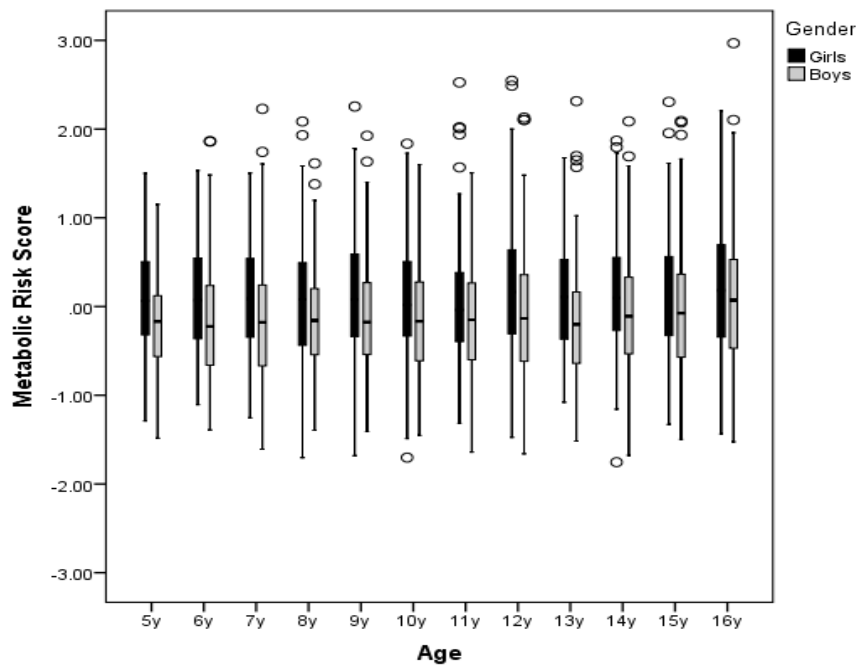
A10. Triglycerides from 5y to16y in boys and girls



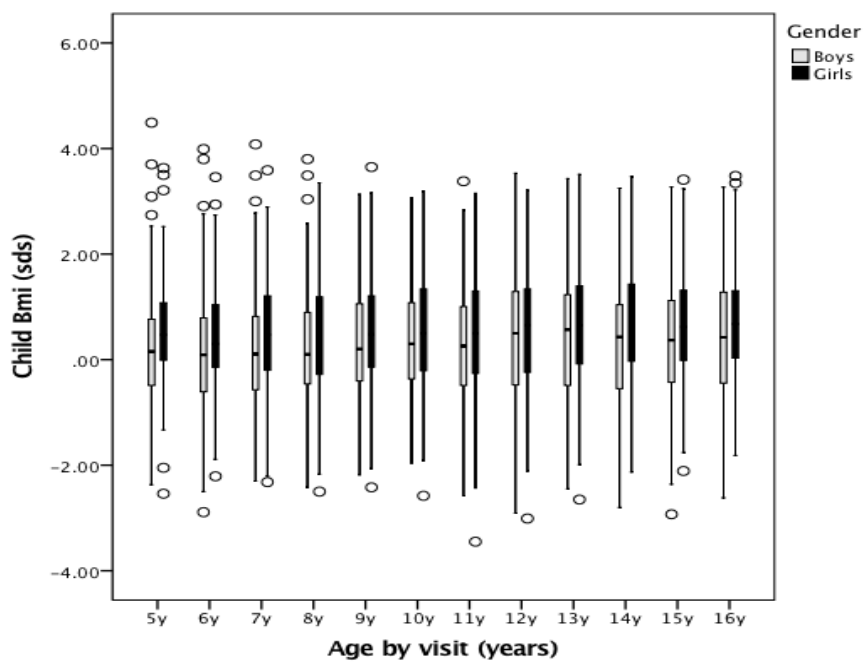
A11. Mean Arterial Blood Pressure from 5y to16y in boys and girls



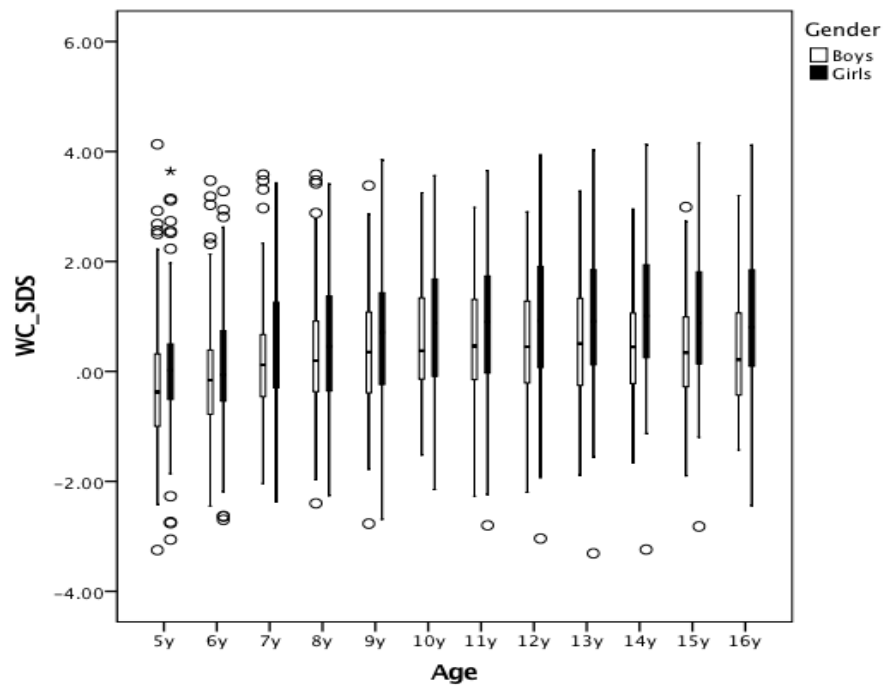
A12 Mean Arterial Blood Pressure from 5y to16y in boys and girls



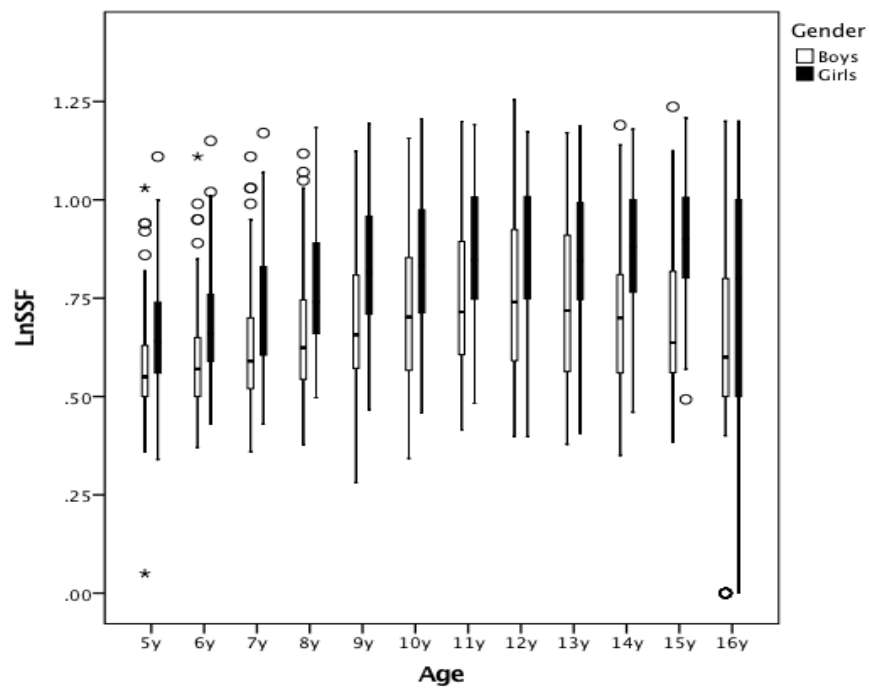
A13. Trend in MRS from 5y to 16y in boys and girls



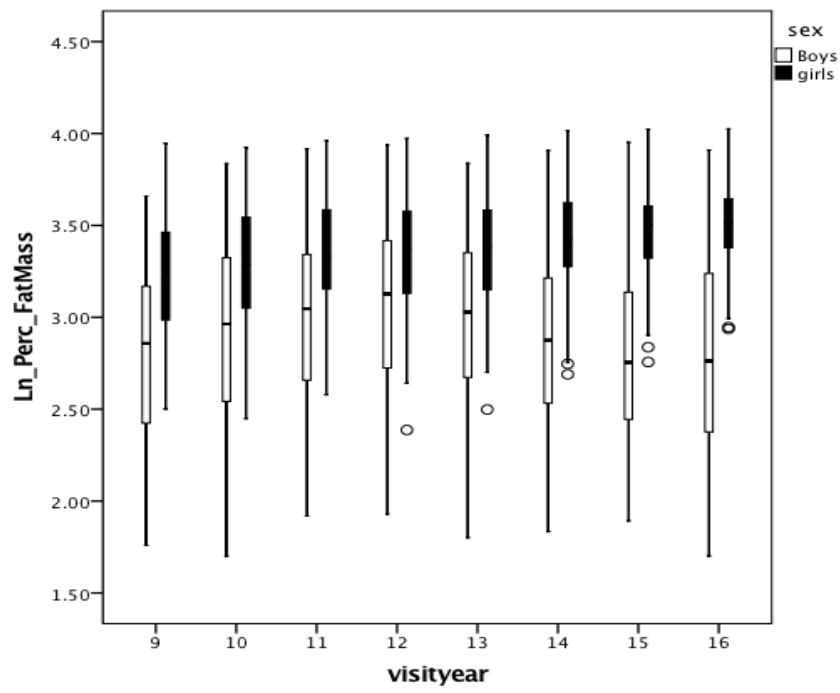
A14. Distribution of BMI-SDS in boys and girls from 5y to 16y



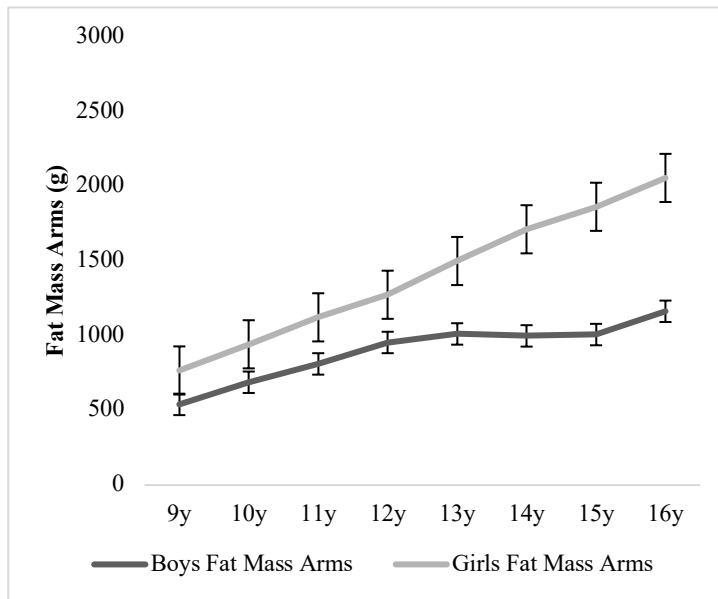
A15. Distribution of WC-SDS in boys and girls from 5y to 16y



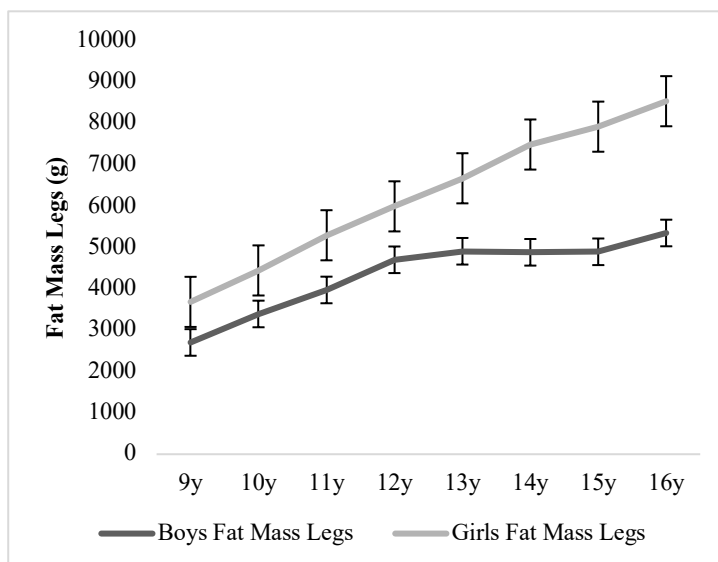
A16. Distribution of SSF in boys and girls from 5y to 16y; (natural logarithm)



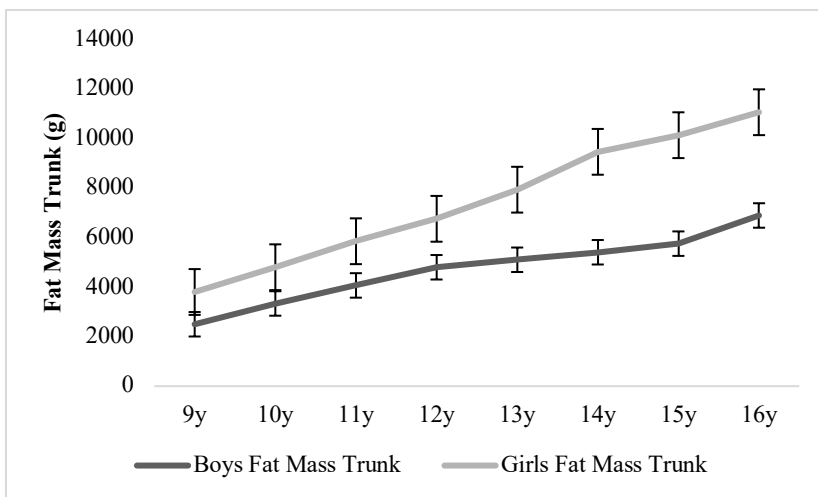
A17. Distribution of Ln percent fat mass in boys and girls from 9y to 16y)



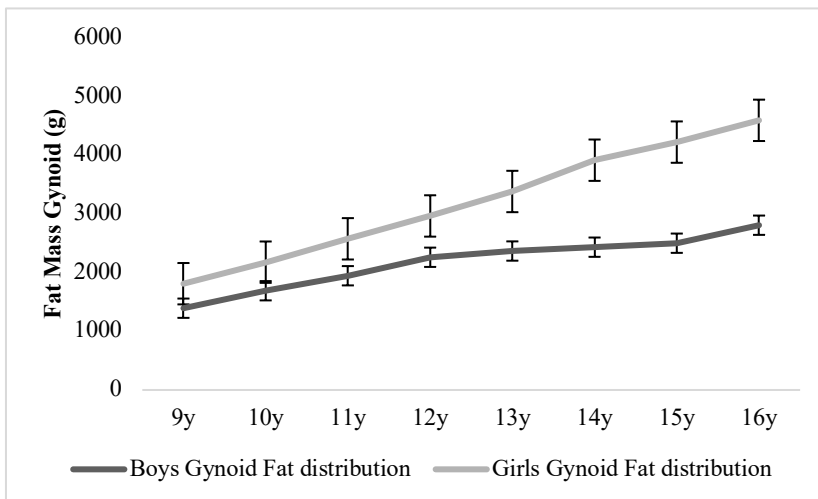
A18. Fat mass in the arms in boys and girls from 9y to 16y



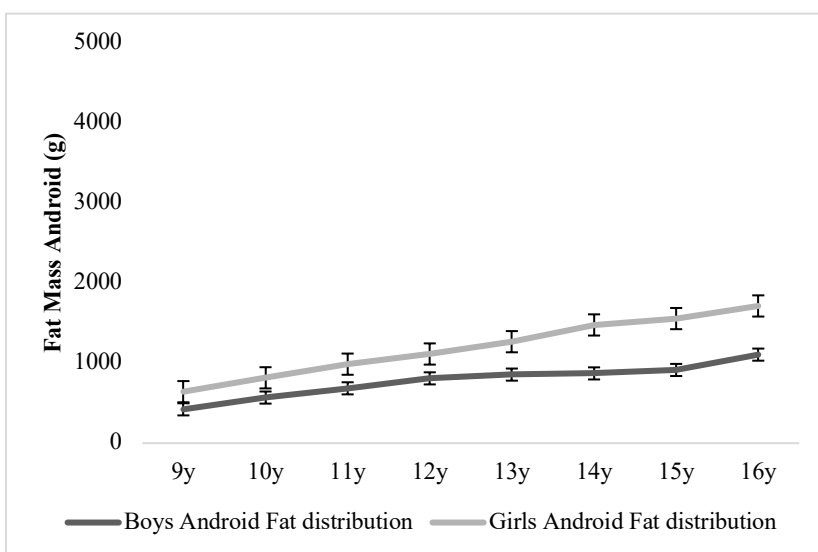
A19. Fat mass in the legs in boys and girls from 9y to 16y



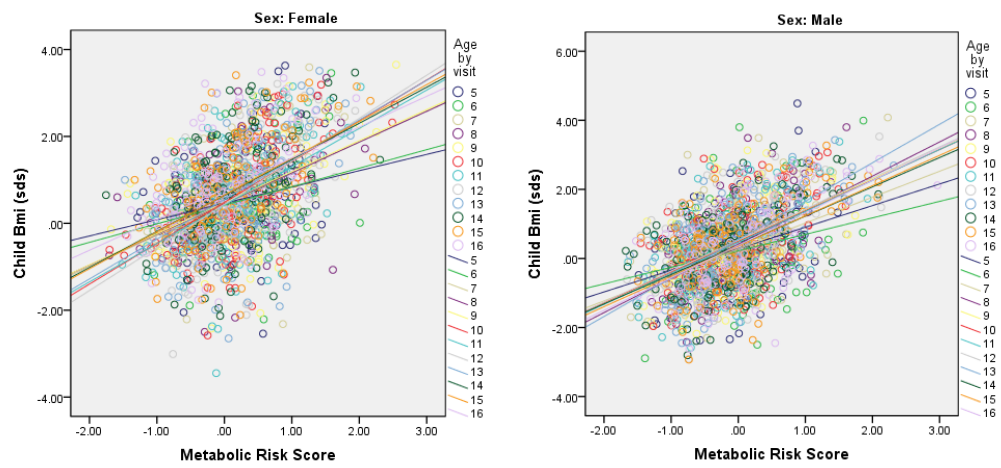
A20. Fat mass in the Trunk in boys and girls from 9y to 16y



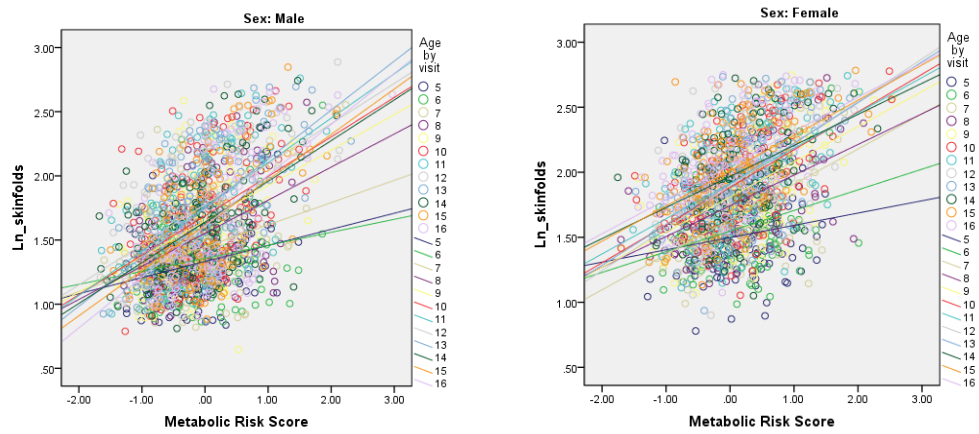
A21. Gynoid fat distribution



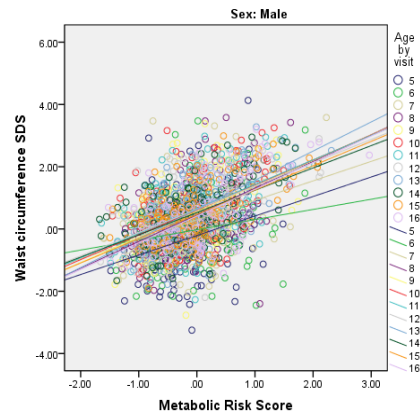
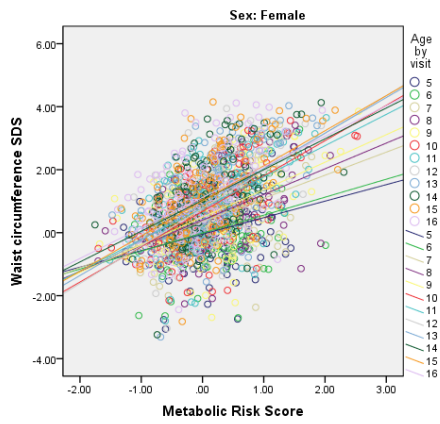
A22. Android fat distribution



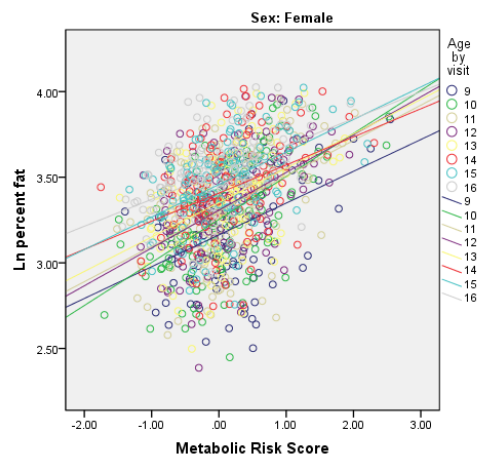
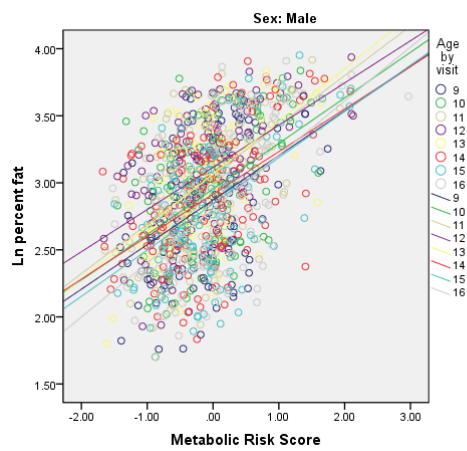
A23. Correlation between BMI and MRS



A24. Correlation between SF and MRS



A25. Correlation between WC and MRS



A26. Correlation between fat mass and MRS

A 27a. Correlation matrix of macronutrients and measures of adiposity in boys

	Energy 8y			CHO (g) 8y			CHO (%) 8y			
BMI 16y	-.150	.090	129	-.119	.179	129	.071	.421	129	** Correlation
BMI 15y	-.121	.173	129	-.098	.267	129	.057	.523	129	(r) is
BMI 14y	-.072	.413	130	-.068	.442	130	.012	.895	130	significant at the 0.01 level
BMI 13y	-.063	.471	133	-.061	.486	133	.004	.960	133	(2-tailed), *
BMI 12y	-.098	.262	133	-.098	.264	133	-.012	.888	133	Correlation
BMI 11y	-.036	.674	139	-.020	.814	139	.048	.571	139	(r) is
BMI 10y	-.053	.533	139	-.033	.699	139	.056	.516	139	significant at the 0.05 level
BMI 9y	-.069	.422	138	-.038	.662	138	.081	.344	138	(2-tailed).
BMI 8y	-.104	.215	145	-.068	.417	145	.099	.236	145	Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy
CHO (%) 8y	-.031	.712	145	.301**	.000	145	1		145	(%) BMI: Body mass index; SF: Sum of 5 skinfol
CHO (g) 8y	.941**	.000	145	1		145	.301**	.000	145	
Energy 8y	1		145	.941**	.000	145	-.031	.712	145	
	r	p	n	r	p	n	r	p	n	

A27b. Correlation matrix of macronutrients and measures of adiposity in Girls

	Energy 8y			CHO_Gram_8y			CHO_%_8y		
BMI 16y	r	p	n	r	p	n	r	p	n
BMI 15y	-.199	.051	96	-.165	.109	96	.087	.401	96
BMI 14y	-.026	.026	98	-.203*	.045	98	.041	.692	98
BMI 13y	-.010	.010	97	-.223*	.028	97	.090	.382	97
BMI 12y	-.004	.004	99	-.268**	.007	99	.031	.758	99
BMI 11y	-.018	.018	104	-.201*	.041	104	.049	.619	104
BMI 10y	-.019	.019	108	-.193*	.045	108	.054	.576	108
BMI 9y	-.010	.010	110	-.233*	.014	110	-.007	.942	110
BMI 8y	-.021	.021	115	-.200*	.032	115	.002	.984	115
CHO (%) 8y	-.065	.489	115	.221*	.018	115	1		115
CHO (g) 8y	.957*	.000	115	1		115	.221*	.018	115
Energy 8y	1		115	.957**	.000	115	-.065	.489	115

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%) BMI: Body mass index; SF: Sum of 5 skinfolds

A28a. Correlation matrix of macronutrients and measures of adiposity in Boys

	SF.16y	SF.15y	SF.14y	SF.13y	SF.12y	SF.11y	SF.10y	SF.9y	SF.8y	CHO(%) 8y	CHO (g) 8y	Energy 8y
	-.096	-.059	-.014	.002	-.053	-.071	.019	.061	-.053	-.031	.941*	1
	.279	.508	.873	.983	.544	.407	.824	.466	.525	.712	.000	
	129	128	130	133	133	139	145	145	145	145	145	145
	-.071	-.042	-.018	.003	-.050	-.059	.057	.088	-.034	.301**	1	.941**
	.424	.636	.836	.968	.569	.490	.495	.290	.686	.000		
	129	128	130	133	133	139	145	145	145	145	145	145
	.071	.059	.003	.013	.007	.036	.122	.087	.054	1	.301**	-.031
	.422	.509	.971	.883	.938	.673	.142	.301	.516		.000	.712
	129	128	130	133	133	139	145	145	145	145	145	145
Energy 8y	r	p	n	r	p	n	r	p	n	r	p	n
CHO (g) 8y	r	p	n	r	p	n	r	p	n	r	p	n
CHO (%) 8y	r	p	n	r	p	n	r	p	n	r	p	n

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%) BMI: Body mass index; SF: Sum of 5 skinfolds

A28b. Correlation matrix of macronutrients and measures of adiposity in Girls

SF.16y	-.184	.071	97	-.146	.153	97	.108	.291	97
SF.15y	-.172	.096	95	-.136	.188	95	.092	.376	95
SF.14y	-.155	.129	98	-.132	.194	98	.063	.536	98
SF.13y	-.172	.092	97	-.134	.189	97	.105	.308	97
SF.12y	-.258**	.010	99	- .226*	.025	99	.055	.587	99
SF.11y	-.201*	.041	104	-.180	.067	104	.020	.841	104
SF.10y	-.191*	.041	115	- 197*	.040	115	-.051	.591	115
SF.9y	-.129	.169	115	-.123	.189	115	-.003	.974	115
SF.8y	-.194*	.038	115	- 187*	.045	115	-.011	.911	115
CHO (%) 8y	-.065	.489	115	.221*	.018	115	1		115
CHO (g) 8y	.957**	.000	115	1		115	.221*	.018	115
Energy 8y	1		115	.957* *	.000	115	-.065	.489	115
Energy 8y			CHO_Gram_8y			CHO_%_8y			
	r	p	n	r	p	n	r	p	n

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%) wc: waist circumference; BP: mean arterial blood pressure.

A29a. Correlation matrix of macronutrients and measures of adiposity and blood pressure in Boys

	BP16y	BP15y	BP14y	BP13y	BP12y	BP11y	BP10y	BP9y	BP8y	wc16y	wc15y	wc14y	wc13y	wc12y	wc11y	wc10y	wc9y	wc8y	CHO (%) 8y	CHO (g) 8y	Energy 8y
BP16y	-.023	.798																			
BP15y	.012	.897																			
BP14y	.052	.557																			
BP13y	-.097	.267																			
BP12y	-.135	.123																			
BP11y	-.045	.598																			
BP10y	-.143	.095																			
BP9y	-.056	.514																			
BP8y	-.066	.429																			
wc16y	-.092	.298																			
wc15y	-.080	.367																			
wc14y	-.017	.847																			
wc13y	-.042	.627																			
wc12y	-.060	.493																			
wc11y	-.022	.797																			
wc10y	-.021	.802																			
wc9y	-.026	.763																			
wc8y	-.046	.583																			
CHO (%) 8y	-.031	.712																			
CHO (g) 8y	.941*	.000																			
Energy 8y	1																				
CHO_Gram_8y																					
CHO_%_8y																					

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%) wc: waist circumference; BP: mean arterial blood pressure

A29b. Correlation matrix of macronutrients and measures of adiposity and blood pressure in Girls

	BP16y	BP15y	BP14y	BP13y	BP12y	BP11y	BP10y	BP9y	BP8y	wc16y	wc15y	wc14y	wc13y	wc12y	wc11y	wc10y	wc9y	wc8y	CHO (%) 8y	CHO (g) 8y	Energy 8y
BP16y	.061	.550	.97	.060	.558	.97	-.001	.994	.97												
BP15y	-.152	.138	.97	-.174	.087	.97	-.113	.271	.97												
BP14y	-.164	.106	.98	-.167	.100	.98	-.021	.839	.98												
BP13y	-.225*	.027	.97	-.183	.073	.97	.148	.149	.97												
BP12y	-.174	.085	.99	-.141	.164	.99	.099	.331	.99												
BP11y	-.134	.173	.104	-.079	.423	.104	.168	.088	.104												
BP10y	-.092	.348	.107	-.064	.512	.107	.055	.575	.107												
BP9y	-.150	.120	.109	-.174	.070	.109	-.105	.276	.109												
BP8y	-.173	.065	.115	-.123	.190	.115	.142	.129	.115												
wc16y	-.188	.065	.97	-.150	.144	.97	.108	.295	.97												
wc15y	-.172	.091	.97	-.140	.170	.97	.085	.411	.97												
wc14y	-.178	.080	.98	-.157	.122	.98	.050	.625	.98												
wc13y	-.198	.052	.97	-.166	.104	.97	.077	.455	.97												
wc12y	-.241*	.016	.99	-.219*	.030	.99	.038	.708	.99												
wc11y	-.202*	.040	.104	-.173	.080	.104	.056	.572	.104												
wc10y	-.201*	.037	.108	-.164	.091	.108	.082	.398	.108												
wc9y	-.209*	.029	.109	-.191*	.046	.109	.022	.818	.109												
wc8y	-.209*	.025	.115	-.192*	.040	.115	.018	.851	.115												
CHO (%) 8y	-.065	.489	.115	.221*	.018	.115	1		.115												
CHO (g) 8y	.957**	.000	.115	1		.115	.221*	.018	.115												
Energy 8y	1		.115	.957**	.000	.115	-.065	.489	.115												

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%); IR: HOMA IR

A30a. Correlation matrix of macronutrients and insulin resistance in Boys

	IR.16y	IR.15y	IR.14y	IR.13y	IR.12y	IR.11y	IR.10y	IR.9y	IR.8y	CHO (%) 8y	CHO (g) 8y	Energy 8y
	-.042	-.027	.023	.016	-.092	-.013	.070	.032	.033	-.031	.941**	1
	.637	.763	.806	.856	.303	.882	.425	.710	.703	.712	.000	
	128	125	121	129	128	132	131	135	138	145	145	
Energy 8y												r
												p
												n
	-.054	-.056	.042	-.020	-.097	.004	.081	.074	.003	.301**	1	.941**
	.546	.534	.644	.824	.277	.964	.356	.395	.975	.000		.000
	128	125	121	129	128	132	131	135	138	145	145	145
CHO_Gram_8y												r
												p
												n
	-	-	.081	-	-	.056	.034	.109	-	1	.301**	.031
	.712	.478	.376	.355	.609	.523	.698	.207	.437		.000	.712
	128	125	121	129	128	132	131	135	138	145	145	145
CHO_%_8y												r
												p
												n

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%); MRS: metabolic risk score; Fat: Natural log of percent total body fat; IR: HOMA IR

A30b. Correlation matrix of macronutrients and insulin resistance in Girls

	Energy 8y			CHO_Gram_8y			CHO_%_8y		
	r	p	n	r	p	n	r	p	n
IR.16y	-.041	.701	92	.068	.522	92	.291**	.005	92
IR.15y	-.106	.312	93	-.075	.472	93	.070	.506	93
IR.14y	-.018	.863	93	.032	.764	93	.168	.107	93
IR.13y	-.163	.116	94	-.104	.318	94	.142	.171	94
IR.12y	-.059	.570	94	-.049	.640	94	.023	.826	94
IR.11y	-.156	.122	100	-.123	.222	100	.064	.530	100
IR.10y	-.069	.488	104	-.034	.731	104	.128	.195	104
IR.9y	-.069	.485	104	-.091	.359	104	-.087	.377	104
IR.8y	-.135	.163	108	-.085	.382	108	.150	.122	108
CHO (%) 8y	-.065	.489	115	.221*	.018	115	1		115
CHO (g) 8y	.957**	.000	115	1		115	.221*	.018	115
Energy 8y	1		115	.957**	.000	115	-.065	.489	115

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%); MRS: metabolic risk score; Fat: Natural log of percent total body fat; IR: HOMA IR

A31a. Correlation matrix of macronutrients, adiposity, insulin resistance, and metabolic risk in Boys

	Fat.16y	Fat.15y	Fat.14y	Fat.13y	Fat.12y	Fat.11y	Fat.10y	Fat.9y	Fat.8y	MRS.16y	MRS.15y	MRS.14y	MRS.13y	MRS.12y	MRS.11y	MRS.10y	MRS.9y	MRS.8y	IR.16y	IR.15y	IR.14y	IR.13y	IR.12y	IR.11y	IR.10y	IR.9y	IR.8y	CHO (%) 8y	CHO (g) 8y	Energy_8y
Fat.16y																														
Fat.15y	-.061																													
Fat.14y	-.036	.503																												
Fat.13y	.013	.884																												
Fat.12y	.022	.806																												
Fat.11y	-.073	.415																												
Fat.10y	.004	.964																												
Fat.9y	-.011	.900																												
Fat.8y	-.022	.803																												
MRS.16y	-.037	.690																												
MRS.15y	-.015	.866																												
MRS.14y	-.008	.933																												
MRS.13y	.004	.966																												
MRS.12y	-.053	.553																												
MRS.11y	-.069	.440																												
MRS.10y	.051	.558																												
MRS.9y	.005	.955																												
MRS.8y	-.010	.906																												
IR.16y	-.040	.644																												
IR.15y	-.044	.618																												
IR.14y	-.058	.518																												
IR.13y	.014	.882																												
IR.12y	.033	.713																												
IR.11y	-.072	.417																												
IR.10y	.012	.891																												
IR.9y	.067	.447																												
IR.8y	.047	.591																												
CHO (%) 8y	.012	.893																												
CHO (g) 8y	-.031	.712																												
Energy_8y	.941**	.000																												
CHO(g)8y																														
CHO(%)8y																														
Energy_8y																														

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%); MRS: metabolic risk score; Fat: Natural log of percent total body fat; IR: HOMA IR

A31b. Correlation matrix of macronutrients, adiposity, insulin resistance, and metabolic risk in Girls

	Fat.15y	Fat.14y	Fat.13y	Fat.12y	Fat.11y	Fat.10y	Fat.9y	Fat.8y	MRS.16y	MRS.15y	MRS.14y	MRS.13y	MRS.12y	MRS.11y	MRS.10y	MRS.9y	MRS.8y	IR.16y	IR.15y	IR.14y	IR.13y	IR.12y	IR.11y	IR.10y	IR.9y	IR.8y	CHO (%) 8y	CHO (g) 8y	Energy_8y
Fat.15y	-174	.101																											
Fat.14y	-.148	.169																											
Fat.13y	-.231*	.026																											
Fat.12y	-.259*	.013																											
Fat.11y	-.236*	.024																											
Fat.10y	-.250*	.018																											
Fat.9y	-.210*	.032																											
Fat.8y	-.192*	.049																											
MRS.16y	-.221*	.029																											
MRS.15y	-.137	.193																											
MRS.14y	-.132	.207																											
MRS.13y	-.104	.320																											
MRS.12y	-.096	.360																											
MRS.11y	-.239*	.021																											
MRS.10y	-.237*	.018																											
MRS.9y	-.080	.420																											
MRS.8y	-.188	.057																											
IR.16y	-.211*	.028																											
IR.15y	-.071	.503																											
IR.14y	-.111	.290																											
IR.13y	.048	.647																											
IR.12y	-.107	.306																											
IR.11y	-.083	.428																											
IR.10y	-.189	.059																											
IR.9y	-.061	.538																											
IR.8y	-.104	.295																											
CHO (%) 8y	-.129	.185																											
CHO (g) 8y	-.065	.489																											
Energy_8y	.957**	.000																											
CHO(g)8y																													
CHO(%)8y																													
Energy 8y																													

** Correlation (r) is significant at the 0.01 level (2-tailed), * Correlation (r) is significant at the 0.05 level (2-tailed). Energy: total daily calorie intake; CHO: carbohydrate intake in grams (g), proportion of total energy (%); MRS: metabolic risk score; Fat: Natural log of percent total body fat; IR: HOMA IR

A32. Mixed effect model quantifying the association between Isoleucine and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	8.E+32	630.899	1.000
Age_By_Visit	1	7.E+39	0.000	1.000
BMI_SDS	1	0.000	1.339	1.000
MRS_Boys_Gp_85	1	0.000	0.560	1.000
Age_By_Visit * MRS_Boys_Gp_85	1	1.E+31	0.482	1.000

a. Gender = Boys

b. Dependent Variable: **Isoleucine**

A33. Mixed effect model quantifying the association between Isoleucine and the presence of a high metabolic risk score in girls

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	28.000	1.824	0.188
Age_By_Visit	1	28.000	3.401	0.076
BMI_SDS	1	28.000	2.594	0.118
MRS_Girls_Gp_85	1	28.000	5.688	0.024
Age_By_Visit * MRS_Girls_Gp_85	1	0.000	5.828	1.000

a. Gender = Girls

b. Dependent Variable: **Isoleucine**

A34. Mixed effect model quantifying the association between Leucine and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	83.000	5.385	0.023
Age_By_Visit	1	83.000	0.039	0.845
BMI_SDS	1	83.000	0.001	0.974
MRS_Boys_Gp_85	1	83.000	0.092	0.762
Age_By_Visit * MRS_Boys_Gp_85	1	83.000	0.047	0.830

a. Gender = Boys

b. Dependent Variable: **Leucine**

A35. Mixed effect model quantifying the association between Leucine and the presence of a high metabolic risk score in girls

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	28.000	0.560	0.461
Age_By_Visit	1	66004.68	0.077	0.782
BMI_SDS	1	28.933	0.020	0.887
MRS_Girls_Gp_85	1	0.482	0.000	1.000
Age_By_Visit * MRS_Girls_Gp_85	1	0.000	3.746	1.000

a. Gender = Girls

b. Dependent Variable: **Leucine.**

A36. Mixed effect model quantifying the association between Lactate and the presence of a high metabolic risk score in boys

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	69.491	25.615	0.000
Age_By_Visit	1	71.725	22.676	0.000
BMI_SDS	1	0.000	0.003	1.000
MRS_Boys_Gp_85	1	0.000	16.872	1.000
Age_By_Visit * MRS_Boys_Gp_85	1	0.000	0.000	1.000

a. Gender = Boys

b. Dependent Variable: **Lactate.**

A37. Mixed effect model quantifying the association between Lactate and the presence of a high metabolic risk score in girls.

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	28.001	1.506	0.230
Age_By_Visit	1	1.351E+9	0.000	0.992
BMI_SDS	1	0.000	0.000	1.000
MRS_Girls_Gp_85	1	0.003	0.000	1.000
Age_By_Visit * MRS_Girls_Gp_85	1	4607386.94	0.002	0.963

a. Gender = Girls

b. Dependent Variable: **Lactate.**

A38. Mixed effect model quantifying the association between Valine and the presence of a high metabolic risk score in boys.

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	1E+43	2.258	1.000
Age_By_Visit	1	6E+48	0.000	1.000
BMI_SDS	1	0.000	0.123	1.000
MRS_Boys_Gp_85	1	2E+43	0.077	1.000
Age_By_Visit * MRS_Boys_Gp_85	1	0.000	0.084	1.000

a. Gender = Boys

b. Dependent Variable: **Valine**

A39. Mixed effect model quantifying the association between Valine and the presence of a high metabolic risk score in girls.

Type III Tests of Fixed Effects ^{a,b}				
Source	Numerator df	Denominator df	F	Sig.
Intercept	1	1.E+31	0.742	1.000
Age_By_Visit	1	0.000	2.066	1.000
BMI_SDS	1	5.E+35	0.003	1.000
MRS_Girls_Gp_85	1	0.000	1.146	1.000
Age_By_Visit * MRS_Girls_Gp_85	1	0.000	0.000	1.000

a. Gender = Girls

b. Dependent Variable: **Valine.**

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