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ARTERIAL STIFFNESS, OBESITY AND METABOLIC SYNDROME IN CHILDREN AND ADOLESCENTS.

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A submission presented in partial fulfilment of the
requirements of the University of Glamorgan/Prifysgol Morgannwg
for the degree of Doctor of Philosophy

January 2012

Abstract.

Atherosclerotic cardiovascular disease (CVD) is the leading cause of death in Western Societies. The pathological processes and risk factors associated with its development begin in childhood, long before clinical consequences emerge. Cardiovascular risk factors have been shown to cluster together in adults and children, particularly in the presence of obesity. Exposure to these risk factors in the first decade of life has been shown to cause vascular endothelial dysfunction and autopsy documented atherosclerosis. Childhood overweight and obesity is increasing in worldwide, Western populations and is strongly associated with vascular dysfunction and arterial stiffness. The aim of this research program was to collect anthropometrical, haematological and physiological data from a large number of apparently healthy Welsh children and adolescents with the purpose of examining central and peripheral haemodynamic indices of arterial stiffness and their association with CVD risk factors. In addition it sought to examine the prevalence of overweight and obesity, the prevalence of metabolic syndrome and examine relationships with emerging risk factors. This study has shown that the prevalence of overweight and obesity in children and adolescents is high yet varies greatly dependent on the measurement methods and cut-off criteria applied. Prevalence estimates for metabolic syndrome ranged from 0% to 3.5%. All measures of adiposity showed significant associations with insulin resistance and with 2 or more components of the metabolic syndrome. Aortic pulse wave velocity increased with increasing BMI status. A negative association was found between arterial stiffness and aerobic fitness. The overall prevalence of hypertension was 9.8% with 4.4% of individuals identified with isolated systolic hypertension. The mechanisms underlying isolated systolic hypertension could not be confirmed through this study. Elevations in alanine aminotransferase were highly prevalent and strongly associated with insulin resistance, fasting insulin, body composition, clustering of metabolic risk factors and inversely related to aerobic fitness.

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Certificate of Research

This is to certify that, except where specific reference is made, the work described in this thesis is the result of the candidate's research. Neither this thesis, nor any part of it, has been presented, or is currently submitted, in candidature for any degree at any other University.

Signed ..... *Candidate*

Date *23rd January 2012*

Signed ..... *Director of Studies*

Date *23rd January 2012*

Acknowledgements.

Anyone who knows me will not be surprised how long it took me to write this thing. All of you that *really* know me showed no doubt that I would eventually write this thing, it is for that support and encouragement that I would truly like to thank you all.

Thank you to Dr Simon Williams for his confidence in me over the many years that I have known him. If it were not for him I would not be where I am now. He was always ready to offer advice, guidance and assistance. He has become a close colleague and an enduring friend. A great deal of thanks goes to all of the colleagues at the University of Glamorgan, University of Cambridge and the Wales Heart Research Institute at Cardiff University's School of Medicine. Foremost would be Professor John Cockcroft who provided a great deal of knowledge (and funding) in the early part of the research program. Dr Barry McDonnell and Maggie Munnery who were great friends and guides while at WHRI and were invaluable during the data collection. Dean Whitcombe, omnipresent throughout the last few years. Dr Carmel McEniery deserves special thanks for her help, guidance and indefatigable replies to many, many emails. Dr Ian Wilkinson who made me feel welcome to be a part of the team that made up the Anglo-Cardiff Collaborative Trial. There are many others who deserve thanks, in no particular order: Sharon Wallace, Kaisa Maki-Petaya, Professor Bruce Davies, Lewis Fall, Dave Hullin, my colleagues at the University of Glamorgan, and everyone else I have shamefully forgotten. A particular thank you to my friends: Gwynnie, Kim, TJ, Dave, Livi, Taff, Sharon, Kiwi, Jo, Indigo; and my family: Dad, Jean, Richard, Sally, Ben, Will and Abby (sorry I forgot your birthdays).

Finally, and most importantly my thanks go to my wife Rachel who proved an unending source of love and support. Without her, this PhD would have taken much longer and would not have been anything as enjoyable.

Live long and prosper.

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Glossary of Terminology and Nomenclature

AASI,	ambulatory arterial stiffness index
ABPM,	ambulatory blood pressure monitoring
ACE,	after the common era
AIx,	augmentation index
AIx_{75},	augmentation index adjusted to a heart rate of 75 bpm
ALT,	alanine aminotransferase
ANOVA	analysis of variance
AS,	arterial stiffness
AST,	aspartate transferase
AP,	augmentation pressure
BCE,	before the common era
BMI,	body mass index
BP,	blood pressure
CCA,	common carotid artery
c-f PWV,	carotid-to-femoral pulse wave velocity
CI,	cardiac index or confidence interval
CO,	cardiac output
cDBP,	central diastolic blood pressure
cMAP	central mean arterial pressure
cPP,	central pulse pressure
cSBP	central systolic blood pressure
CHD,	coronary heart disease
CRF,	cardio-respiratory fitness
CRP,	C-reactive protein
c-r PWV,	carotid-to-radial pulse wave velocity
CV,	cardiovascular

CVa,	co-efficient of analytical variation
CVD,	cardiovascular disease
d,	diastole
D,	diameter point
DBP,	diastolic blood pressure
DM,	diabetes mellitus
ECG,	electrocardiogram
EH,	essential hypertension
E_{inc},	incremental elastic modulus
ET,	endothelin
FFA,	free fatty acid
FG,	fasting glucose
FMD,	flow-mediated dilatation
GTN,	glyceryl trinitate
h,	wall thickness
HC,	hypercholesterolaemic
HDL,	high-density lipoprotein cholesterol
HOMA(-IR),	homeostatic model assessment of insulin resistance
HR,	heart rate
hsCRP,	high-sensitivity C-reactive protein
Htn,	hypertension
i,	inflection
IFG,	impaired fasting glucose
IGT,	impaired glucose tolerance
IMT,	intima-media thickness
IR,	insulin resistance
IRS,	insulin resistance syndrome

ISH,	isolated systolic hypertension
ln,	natural logarithm
LDL,	low-density lipoprotein cholesterol
MAP,	mean arterial pressure
MetSyn,	metabolic syndrome
MRI,	magnetic resonance imaging
MSFT,	multi-stage fitness test
NAFLD,	non-alcoholic fatty liver disease
NO,	nitric oxide
Ob,	obesity
OGTT2,	oral glucose tolerance test at 2 hours
Ow,	overweight
P,	pressure
P_1,	systolic inflection point
P_2,	pressure of the systolic peak
PP,	pulse pressure
pPP,	peripheral pulse pressure
PVR,	peripheral vascular resistance
PWA,	pulse wave analysis
PWV,	pulse wave velocity
RR,	relative risk
s,	systole
SBP,	systolic blood pressure
SD,	standard deviation
SSH,	spurious systolic hypertension
SV,	stroke volume
T1DM,	type 1 diabetes mellitus

T2DM,	type 2 diabetes mellitus
TC,	total cholesterol
TG,	triglyceride
Tr,	time to wave reflection
TS,	Tanner stage
V,	volume
VLDL,	very low-density lipoprotein cholesterol
WC,	waist circumference
WHR,	waist-to-hip ratio
WHtR,	waist-to-height ratio
%ile,	percentile

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Research Communications Associated with this Thesis

Elevations in alanine aminotransferase are associated with insulin resistance, overweight and obesity and low aerobic fitness levels in children and adolescents.

Retallick, C.J., Whitcombe, D.M., Davies, B., Hullin, D.A., McDonnell, B., Munnery, M., Cockcroft, J.R. and S.R.P. Williams[1].

Association for the Study of Obesity, Diabetes and Obesity Conference, University College London, November 2011. Appetite (in press).

Abstract

Introduction: Alanine aminotransferase (ALT) is the liver enzyme with the closest association with liver fat accumulation and consequently has been used as a circulating marker of non-alcoholic fatty liver disease (NAFLD). This study examined the association of elevated ALT with insulin resistance (IR), body composition and aerobic fitness in a cohort of apparently healthy children and adolescents from Wales, UK. **Methods:** 158 children and adolescents (91 male) aged 11-14 years were assessed for ALT levels, body mass index (BMI), waist circumference (WC) and IR (HOMA). Aerobic fitness was assessed using a progressive 20m shuttle run test (n = 70). **Results:** Elevated ALT levels (>30 U/L) were found in 12%, with a higher prevalence in boys than in girls (13.3% and 10.3% respectively). Using the recently identified lower limits of >25 U/L for 12-17 year old males and >22 U/L for 12-17 year old females, overall prevalence of elevated ALT was found to be 43.7%. Conversely, with a lower prevalence in males than females (36.7% and 52.9%, respectively). ALT was significantly correlated with elevated fasting insulin (r = 0.3, p <0.001), IR (r = 0.3, p <0.001), BMI (r = 0.3, p <0.001) and WC (r = 0.3, p <0.001). ALT in the children that displayed the lowest fitness was significantly greater than those with higher fitness (p for trend = 0.001). In multiple stepwise regression analysis IR was the major independent predictor of ALT levels. Other independent predictors were age and WC. **Conclusions:** We have shown for the first time in apparently healthy Welsh school

children that elevated ALT levels were highly prevalent and were strongly associated with insulin resistance, body composition and low aerobic fitness. This study suggests that children and adolescents displaying elevated cardiovascular risk factors are at danger of developing NAFLD.

Association of alanine aminotransferase with aerobic fitness in healthy children and adolescents in Wales, UK.

C.J. Retallick, S.R.P. Williams, D.M. Whitcombe, B. Davies, D.A. Hullin, B. McDonnell, M. Munnery, J.R. Cockcroft.

3rd International Congress on Pre-Diabetes and the Metabolic Syndrome, Nice, April 2009 and *Journal of Diabetes,* (2009), 1(Supplement 1), A179.

Abstract

Introduction: Alanine aminotransferase (ALT) is the liver enzyme with the closest association with liver fat accumulation and consequently has been used as a circulating marker of non-alcoholic fatty liver disease (NAFLD). This study examined the prevalence of elevated ALT in apparently healthy children and adolescents from Wales, UK and subsequently assessed the association of ALT with physical fitness. **Methods:** 107 children (70 male) aged 11-14 years were assessed for ALT and VO_{2max}. Aerobic fitness was assessed using a progressive 20m shuttle run test. Test scores were converted using the equation of Mahar *et al.* (2006). VO2max values were arranged into quintiles (Q1, lowest fitness -Q5, highest fitness). **Results:** Elevated ALT levels were found in 13.1%, with a higher prevalence in boys than in girls (14.7% and 10.3% respectively). ALT in the children that displayed the lowest fitness (Q1) was significantly greater than those with higher fitness (Q3-5; $p = 0.002$, $p = 0.001$, $p = 0.012$). Multivariate regression analysis showed that aerobic fitness explained ~16% of the variation in ALT ($r^2 = 0.160$; $p < 0.0001$). **Conclusions:** In conclusion, we have shown for the first time in apparently healthy Welsh school children that elevated ALT levels were highly prevalent and were associated with low aerobic fitness. These data suggest that children and adolescents with the lowest aerobic fitness may be at risk of developing NAFLD.

Association between aerobic fitness and prevalence of metabolic syndrome in children and adolescents in Wales, U.K.

C. Retallick, S.R.P. Williams, D.A. Rowe, J.S. Baker, B. Davies, N.E. Thomas, J.R. Cockcroft, M. Munnery, B. McDonnell, R. Williams, M.B. Gravenor.

Oral poster presentation at the 2nd International Congress on Pre-Diabetes and the Metabolic Syndrome, Barcelona, April 2007 and *Diabetes and Vascular Disease Research* (2007) 4(1): s47-s48.

Abstract

This study examined the association between aerobic fitness and the prevalence of metabolic syndrome (MetSyn) in children and adolescents aged 11-14 years. Fasting plasma glucose, HDL-cholesterol and triglycerides, waist girth and blood pressure were measured in 148 boys and 139 girls [mean ± SD heights and weights: 155.6 ± 9.4 cm and 51.0 ± 12.1 kg (boys); 155.3 ± 6.3 cm and 50.7 ± 10.9 kg (girls)] who also completed a progressive 20-metre shuttle run test of aerobic fitness. Test scores were converted to VO_{2max} (ml/kg/min) using the equation of Mahar *et al.* (2006). MetSyn was defined using the criteria of Ford *et al.* (2005). VO2max values were arranged into sex-specific quintiles (Q1-Q5). Odds ratios were calculated to compare prevalence of MetSyn according to fitness quintile. Prevalence of MetSyn was significantly ($p < 0.05$) higher in the lowest fitness quintile (Q1, 30.8%) than Q2 (15.2%), Q3 (3.4%), Q4 (1.9%) and Q5 (0%). Results were similar when the data were analyzed separately for boys and girls. The odds ratios for presence of MetSyn in Q1 were 2.49, 12.44 and 22.67 in comparison to Q2, Q3 and Q4 respectively ($p < 0.05$ for trend). There were no individuals with MetSyn in Q5 so it was not possible to calculate an odds ratio for this group comparison. In conclusion, this study suggests the prevalence of MetSyn is higher in children and adolescents with low aerobic fitness, and that the risk of MetSyn is inversely related to aerobic fitness.

Obesity prevalence estimates in children and adolescents in Wales, UK: A comparison of standards based on BMI and waist girth.

Retallick CJ, Rowe D, Thomas N, Cockcroft JR, McDonnell B, Munnery M, Williams R, Gravenor MB, Davies B, Baker JS, Whitcombe D, Williams SRP.

IASO International Congress on Obesity, Sydney, Australia, September 2006 and *Obesity Reviews* (2006) 7: S2; 234.

Abstract

Whilst there is no universally accepted criterion for classifying obesity in childhood and adolescence, standards that rely on body mass index (BMI) and waist girth (WG) percentiles predominate. This study aimed to compare prevalence estimates of obesity in a child and adolescent population in Wales using IOTF obesity standards (Cole *et al.*, 2000) and two proposed threshold values based on WG percentiles (75th percentile, de Ferranti *et al.*, 2004; and 98th percentile, McCarthy *et al.*, 2003). Boys (n = 230) and girls (n = 229), aged 11–14 years from three large schools participated voluntarily in a series of measurements including weight, height and waist girth (midway between lowest rib and superior iliac crest). Prevalence estimates were: 32% (overweight or obese, IOTF); 8.3% (obese; IOTF); 54.5% (obese, WGPR75); and 19.4% (obese, WGPR98). Using the IOTF obesity classification as the comparison standard, the sensitivity of WGPR98 was 92% and specificity was 87%. For WGPR75, sensitivity was 100% but specificity was only 50%. These data show that estimates of obesity prevalence in this population are highly dependent on the method used to determine obesity and that the recently proposed 75th percentile of WG leads to a large number of false positive cases of obesity in comparison to the IOTF BMI standards. Further criterion-referenced evidence is required to determine standards and measures that accurately predict obesity-related co-morbidity.

Prevalence estimates of the metabolic syndrome in children and adolescents in Wales, UK.

Retallick CJ, Rowe D, Thomas N, Cockcroft JR, McDonnell B, Munnery M, Williams R, Gravenor MB, Davies B, Baker JS, Whitcombe D, Williams SRP.

IASO International Congress on Obesity, Sydney, Australia, September 2006 and *Obesity Reviews* (2006) 7: S2; 234.

Abstract

In children and adolescents, diagnosis of metabolic syndrome (MetS) relies on modified adult criteria. This study aimed to estimate the prevalence of MetS in a cohort of children and adolescents using three published definitions of paediatric and adolescent MetS. Participants (n = 369, 192 male) aged 11–14 years, had their waist girth, resting blood pressure and fasting plasma glucose, HDL-C and triglycerides measured. Prevalence of MetS was estimated using the criteria of Cook *et al.* (2003), de Ferranti *et al.* (2004) and Ford *et al.* (2005). Data presented as mean ± SD. For males and females respectively, waist girth was 71.4 ± 10.7 cm and 67.5 ± 9.1 cm ($p < 0.05$); systolic BP was 115 ± 12 mmHg and 113 ± 10 mmHg ($p < 0.05$); diastolic BP was 66 ± 9 mmHg and 70 ± 9 mmHg ($p < 0.05$); HDL-C was 1.49 ± 0.31 mmol/L and 1.56 ± 0.33 mmol/L ($p < 0.05$). For both males and females, glucose was 4.9 ± 0.4 mmol/L and triglycerides were 0.88 ± 0.45 mmol/L and 0.95 ± 0.41 mmol/L (P > 0.05). Prevalence estimates of MetS using the three criteria were 8.9%, 18.2% and 9.2% respectively. Prevalence estimates in males and females were 9.9% and 7.9% (Cook et al. 2003), 18.9% and 17.5% (de Ferranti *et al.* 2004) and 10.4% and 7.9% (Ford *et al.* 2005). The three proposed definitions all identified a high prevalence of MetS in this cohort. Prevalence estimates based on the criteria of de Ferranti et al. were considerably higher than those based on the criteria of Cook *et al.* and Ford *et al.*, principally due to the lower cut-off point (>75th percentile) for identifying central obesity (waist girth) in the de Ferranti criteria.

1 Chapter One: Introduction

"In extreme old age, the arteries themselves, the grand instrument of the circulation, by continual apposition of earth, become hard, and as it were bony, till, having lost the power of contracting themselves they can no longer propel the blood, even through the largest channels, in consequence of which death naturally ensues" (John Wesley, 1703-1791) (Jackson, 1872).

"When you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind" [Sir William Thompson (Lord) Kelvin. Popular Lectures and Addresses 1891-1894, vol. 1, "Electrical Units of Measurement", 1883].

1.1 General introduction

Atherosclerotic cardiovascular disease (CVD) is the leading cause of adult death in Western Societies (Rosamond *et al.*, 1998; Najjar *et al.*, 2005). The pathological processes and risk factors associated with its development have been shown to begin in childhood (Berenson *et al.*, 1998; Li *et al.*, 2004; Aggoun *et al.*, 2005) long before its clinical consequences emerge. Cardiovascular risk factors have been shown to cluster together in both adults (Reaven, 1988) and children (de Ferranti *et al.*, 2004) particularly in the presence of obesity (Weiss *et al.*, 2004b). This clustering of risk factors occurs to a greater extent than would be expected by chance alone and has been termed the insulin resistance syndrome (Steinberger and Daniels, 2003) or metabolic syndrome (National Cholesterol Education Program, 2001a). Cardiovascular disease and all-cause mortality are increased in men with the metabolic syndrome, even in the absence of baseline CVD and diabetes (Lakka *et al.*, 2002). Moreover, individual risk factors have been shown to track from childhood in to adulthood. This has been shown to be true for childhood obesity (Srinivasan *et al.*, 2002; Eisenmann *et al.*, 2004), serum lipid and lipoprotein levels (Nicklas *et al.*, 2002), blood pressure (Bao *et al.*, 1995; Lambrechtsen *et al.*, 1999; Lane and Gill, 2004), and insulin levels (Srinivasan *et al.*, 2002). In particular, childhood obesity

has been shown to track within childhood from the first year of life (Vogels *et al.*, 2006) and also to predict for the metabolic syndrome in adulthood (Vanhala *et al.*, 1999). Early life exposure to these risk factors (from the first decade onwards) has been shown to cause dysfunction of the vascular endothelium and autopsy documented atherosclerosis (Celermajer *et al.*, 1992; McGill *et al.*, 1997).

Excess adiposity is a central feature of many (National Cholesterol Education Program, 2001a; Zimmet *et al.*, 2005) but not all (Balkau and Charles, 1999; Einhorn *et al.*, 2003) definitions of metabolic syndrome. Whether total adiposity (body mass index) or regional adiposity (waist circumference) contribute better to the prediction of CVD or type 2 diabetes (T2DM) risk is a matter of debate (Safar *et al.*, 2006). A long-term follow-up study has shown that weight gain is an independent predictor for increasing CVD risk (Czernichow *et al.*, 2002). Furthermore, a meta-analysis of 26 observational studies reports the relative risks in obese individuals for death caused by coronary heart disease (CHD), and death caused by cardiovascular disease (CVD) to be 1.57, and 1.48, respectively, when compared with the those within the lowest BMI category (McGee, 2005). Moreover, it has recently been shown that childhood overweight and obesity is increasing in several worldwide, Western populations (Wang and Lobstein, 2006). Obesity in childhood is strongly associated with vascular dysfunction and arterial stiffness (Tounian *et al.*, 2001; Mimoun *et al.*, 2008).

Data from several large, prospective studies demonstrate that aortic pulse-wave velocity predicts both cardiovascular and all-cause mortality in a number of patient populations (Blacher *et al.*, 1999b; Laurent *et al.*, 2001; Meaume *et al.*, 2001a; Cruickshank *et al.*, 2002; Sutton-Tyrrell *et al.*, 2005; Mattace-Raso *et al.*, 2006; Willum-Hansen *et al.*, 2006). Compared to lean individuals, arterial stiffness is increased in obese children (Aggoun *et al.*, 2008) and children with metabolic syndrome (Iannuzzi *et al.*, 2006) even from a young

age. Most studies of arterial stiffness in children have involved overweight or obese populations or children with known pathologies (see Table 2.6).

Systolic (SBP) and diastolic (DBP) blood pressure is traditionally measured by cuff sphygmomanometry at the brachial artery, yet it has long been recognised that the arterial pressure wave travelling through arteries with differing elastic properties, coupled with reflection of the forward travelling pressure wave leads to an amplification of pulse pressure in the distal arteries compared to the aorta. Arterial stiffening leads to a faster transmission of the forward travelling wave, an increased pulse wave velocity (PWV). In normal healthy arteries the reflected wave returns to the heart during diastole leading to an augmentation of diastolic pressure, aiding coronary perfusion to the myocardium. In conditions where PWV is increased the reflected wave returns to the heart earlier, during systole, leading to an augmentation of systolic pressure and increasing myocardial workload (Nichols and O'Rourke, 2005). Thus, the accurate measurement of central blood pressure becomes important. Aortic pressure can be measured invasively with cardiac catheterisation with a mounted pressure sensor. O'Rourke (O'Rourke, 1993) developed a device (Sphygmocor) whereby central blood pressure could be determined non-invasively by applanation tonometry and pulse wave analysis of superficially accessible arteries (typically the radial, carotid and femoral arteries).

1.2 Experimental aims and objectives

The aim of this research program was to collect anthropometrical, haematological and physiological data from a large number of apparently healthy children and adolescents with the purpose of examining central and peripheral haemodynamic indices of arterial stiffness and their association with risk factors for cardiovascular disease in young individuals. In addition it sought to examine the prevalence of overweight and obesity, the prevalence of

metabolic syndrome and other associated cardiovascular risk factors and examine the relationships with emerging risk factors.

1.3 Chapter overviews

Chapter Two: Review of the Literature

This chapter reviews previous research and establishes the theoretical basis for the research program undertaken. Specifically, it contains a review of: metabolic syndrome history, its definition in adult, child and adolescent populations and the identification of abnormalities in its individual components; blood pressure, including hypertension and pseudo-systolic hypertension of youth; and arterial stiffness, its methods of measurement and focussing on pulse wave amplification, pressure wave reflection and the non-invasive method of applanation tonometry for the determination of arterial stiffness and central blood pressure, and arterial stiffness in children and adolescents.

Chapter Three: General Methodology

This chapter provides detailed descriptions for all of the measurements undertaken during the data collection for the research program and the studies presented in the subsequent chapters.

Chapter Four: Prevalence and Agreement of Methods for the Determination of Overweight and Obesity.

This chapter reports the prevalence overweight and obesity examining the various measures and definitions of excess weight currently utilised in the United Kingdom and internationally. It specifically identifies the sensitivity and specificity of the methods in use. This study has shown that the prevalence of overweight and obesity, and obesity alone in children and adolescents in Wales, UK is high yet varies greatly dependent on the measurement methods employed and the cut-off criteria applied.

Chapter Five: Prevalence of metabolic syndrome in children and adolescents.

Chapter five reports the prevalence of metabolic syndrome applying several proposed definitions in use worldwide. It also identifies prevalence estimates of individual components of the syndrome and their associations with other cardiovascular risk factors outside of metabolic syndrome definitions. This study has shown prevalence estimates for metabolic syndrome ranging from 0% to 3.5% and that all measures of adiposity showed significant associations with 2 or more components of the metabolic syndrome. Insulin resistance displayed significant positive associations with all measures of adiposity.

Chapter Six: Arterial Stiffness, Metabolic Syndrome and Aerobic Fitness in Children and Adolescents.

Chapter six examines the associations between metabolic syndrome, its individual components and non-invasive measurement of arterial stiffness using the gold standard measure of aortic pulse wave velocity and augmentation index. This study has shown a graded increase in aortic pulse wave velocity with increasing BMI status and with increasing blood pressure status. It has also shown a negative association between arterial stiffness and aerobic fitness.

Chapter Seven – Pseudo-Systolic Hypertension of Youth

The purpose of this chapter was to identify the prevalence of brachial hypertension and its subtypes in children and adolescents and to study the determinants of isolated systolic hypertension through examination of pulse pressure amplification, arterial stiffness, aortic pulse wave velocity, cardiac output and an objective measure of aerobic fitness. This study identified that the overall prevalence of hypertension, using age, gender and height specific blood pressure cut-off criteria, was 9.8% with 4.4% of individuals identified with isolated

systolic hypertension. The occurrence of pseudo-systolic hypertension could not be confirmed through this study.

Chapter Eight: Association between Alanine Aminotransferase and Metabolic Risk Factors in Children and Adolescents.

Non-alcoholic fatty liver disease and elevations in the circulating marker alanine aminotransferase are identifiable in children, yet data on the association of liver enzyme abnormalities with insulin resistance and physical fitness are lacking. This chapter examined the association of elevated ALT with insulin resistance, body composition and aerobic fitness in healthy children and adolescents. It was found that elevations in ALT are highly prevalent and were strongly associated with insulin resistance, fasting insulin, body composition, clustering of metabolic risk factors and inversely related to aerobic fitness.

Chapter Nine: Overall Conclusions

The major results and novel findings of the thesis are summarised in this chapter. The implications of the findings, together with suggestions for future research are also discussed.

2 Chapter Two: Review of the Literature

2.1 Metabolic syndrome

2.1.1 Introduction: History of the metabolic syndrome

In the scientific literature the term "metabolic syndrome" came into common usage in the late 1970s to describe the various associations of cardiovascular and metabolic risk factors with diabetes. The first observations that overeating and obesity lead to poor metabolic outcomes, including diabetes mellitus, were made in the 1920s (Joslin, 1921; Kylin, 1923) and a clustering of abnormalities (hypertension, hyperglycaemia and hyperuricaemia) in patients was later described (Kylin, 1923). Thereafter, Jean Vague (Vague, 1947) observed that upper body obesity appeared to predispose patients to diabetes, atherosclerosis, gout and calculi (hard lumps produced by the concretion of mineral salts that forms in hollow organs or ducts of the body) and that upper body (android) obesity in particular was associated with metabolic disturbances, premature atherosclerosis and diabetes (Vague, 1956). In the 1960s, Albrink and Meigs (1964) also emphasized the relationship of central obesity with hypertriglyceridaemia and hyperglycaemia, and later obesity and hyperlipidaemia were further ensconced in this cluster by Avogaro *et al.* who described six moderately obese patients with diabetes, hypercholesterolaemia and marked hypertriglyceridaemia, all of which improved when the patients were placed on a hypocaloric, low carbohydrate diet (Avogaro *et al.*, 1967).

In 1977, Hermann Haller used the term "metabolic syndrome" to describe the additive associations of obesity, diabetes mellitus, hyperlipoproteinaemia, hyperuricaemia and hepatic steatosis on atherosclerosis (Haller, 1977). He had previously concluded that the combination of obesity, hypertension, dyslipidaemia, and disturbed glucose metabolism, with a concomitant increase of cardiovascular disease risk, occurs more often than might be expected by chance alone (Haller and Hanefeld, 1975). Moreover, Haller recognized that hyperuricaemia and hepatic steatosis were associated with the syndrome, although not

as risk factors, but as a consequence. Furthermore, He proposed obesity to be the common causative factor. Also in 1977, Singer used the same term to describe the associations of obesity, gout, diabetes, mellitus and hypertension with hyperlipoproteinaemia (Singer, 1977).

In 1976 Gerald B. Phillips observed a high incidence of hyperoestrogenaemia in men under 44 years of age with myocardial infarction (Phillips, 1976). Further investigations in 1977 and 1978, led to Phillips developing the concept that some risk factors for myocardial infarction (i.e. glucose intolerance, hyperinsulinaemia, hyperlipidaemia [hypercholesterolaemia and hypertriglyceridaemia] and hypertension) coincide to provide a "constellation of abnormalities" (Phillips, 1977) which he termed the "glucose-insulin-lipid defect", and that this defect was associated not only with heart disease, but also with aging, obesity and other clinical states. Phillips went on to suggest that there must be a linking factor underlying this clustering of abnormalities, the identification of which could lead to the prevention of cardiovascular disease. Phillips hypothesized that this factor was an imbalance in the sex hormones, specifically an elevation in the estradiol-17β to testosterone ratio (Phillips, 1978). In 1980, Margaret Albrink was perhaps the first to identify a cluster of factors including obesity, HDL and hypertriglyceridaemia that was associated with increased risk for coronary artery disease (Albrink *et al.*, 1980). In his much cited 1988 Banting Lecture, Gerald M. Reaven proposed that insulin resistance was the underlying factor for a group of disorders, consisting of impaired glucose tolerance, hyperinsulinaemia, high levels of very low density lipoprotein (VLDL), low levels of high-density lipoprotein (HDL) and hypertension. He named this constellation of abnormalities as Syndrome X (Reaven, 1988). Reaven's contribution was to provide an explanation for how insulin resistance, and its compensatory hyperinsulinaemia, could predispose individuals to many of the above conditions and thus was the underlying cause of much CVD and type 2 diabetes mellitus. Reaven, however, did not include abdominal obesity,

which has also been hypothesised as the underlying factor, in place of insulin resistance, as part of the condition; nor did he comment significantly on a potential clinical role for his observations. The next year, Norman Kaplan added central adiposity to Reaven's list of disorders and named the four main characteristics of the syndrome (central adiposity, impaired glucose tolerance, hypertriglyceridaemia and hypertension) as "the deadly quartet" (Kaplan, 1989). The term "insulin resistance syndrome" was utilised in the following years to reflect the belief that insulin resistance rather than obesity had a causal role in the development of the syndrome (DeFronzo and Ferrannini, 1991; Haffner *et al.*, 1992).

In the years following Reaven's lecture many studies have documented the clustering of cardiovascular disease risk factors and their relationship with insulin resistance. A PubMed search in 2009 for the term "metabolic syndrome" alone returns over 13 000 related documents (date of search: 24/02/2009), in 2011 the same search returned over 37 000 related documents (date of search: 01/11/2011) (http://www.ncbi.nlm.nih.gov/pubmed/). The terms "metabolic syndrome", "insulin resistance syndrome", and "syndrome X" are now widely used in the medical literature to define a constellation of abnormalities that are associated with increased risk for the development of type 2 diabetes and atherosclerotic cardiovascular diseases.

2.1.2 Definition of metabolic syndrome

In an attempt to present a unified, international definition for the metabolic syndrome, several organisations have developed differing diagnostic criteria to enable the clinical identification of the metabolic syndrome in adults. The principle organisations of note include the World Health Organisation (WHO) (Alberti and Zimmet, 1998; World Health Organisation, 1999a) and the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (NCEP ATP III) (Meaume *et al.*, 2001a; NCEP, 2001;

Grundy *et al.*, 2004b). Other institutions have also developed similar, but not identical criteria including the European Group for the Study of Insulin Resistance (EGIR) (Balkau and Charles, 1999; Balkau *et al.*, 2002), the American Association of Clinical Endocrinologists (AACE) (Einhorn *et al.*, 2003) and the International Diabetes Federation (IDF) (Zimmet *et al.*, 2005; Alberti *et al.*, 2006) (Table 2.1).

Table 2.1: Criteria used for the identification of the metabolic syndrome in adults.

	WHO, 1998	EGIR, 1999	NCEP ATP III, 2001	AACE, 2003	IDF, 2006
Reference	(Alberti and Zimmet, 1998; World Health Organisation, 1999a)	(Balkau and Charles, 1999; Balkau *et al.*, 2002)	(NCEP, 2001)	(Einhorn *et al.*, 2003)	(Zimmet *et al.*, 2005; Alberti *et al.*, 2006)
Definition	Hyperinsulinaemia, IFG, IGT or diabetes and two or more of the following four criteria:	Insulin resistance or fasting hyperinsulinaemia and two or more of the following four criteria:	Three or more of the five following criteria:	In an individual with risk factors, two or more of the four following criteria:	Central obesity (ethnicity and gender specific waist circumference) and two out of the four following criteria:
Obesity	Waist-hip ratio Males: >0.90 Females: >0.85 and/or BMI 30 $kg \cdot m^2$	Waist circumference Males: ≥94 cm Females: ≥80 cm	Waist circumference Males: >102 cm Females: >88 cm		As above
Blood pressure (mm Hg)	≥160/90	≥140/90 **or** treated for hypertension	≥130/≥85	>130/85	SBP: ≥130 or DBP: ≥85 or treatment of previously diagnosed hypertension
Triglycerides ($mmol \cdot l^{-1}$)	≥1.7 **and/or**	> 2.0 **or**	≥1.7	>1.7	≥1.7 **or** specific treatment for this lipid abnormality.
HDL cholesterol ($mmol \cdot l^{-1}$)	Males <0.9 Females <1.0	< 1.0 **or** treated for dyslipidaemia	Men <1.03 Women < 1.3	Men <1.03 Women < 1.3	Men <1.03 Women <1.29 **or** specific treatment for this lipid abnormality.
Fasting plasma glucose ($mmol \cdot l^{-1}$)		≥6.1 $mmol \cdot l^{-1}$, but non-diabetic	≥ 6.1 $mmol \cdot l^{-1}$	6.1-6.9 $mmol \cdot l^{-1}$ **or** 120 min post-glucose challenge (75 g) 7.8-11 $mmol \cdot l^{-1}$	≥5.6 **or** previously diagnosed Type 2 diabetes
Microalbuminuria	urinary albumin excretion rate ≥20 $\mu g.min^{-1}$ or albumin:creatinine ratio ≥30 $mg.g^{-1}$				

DBP, diastolic blood pressure; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; SBP, systolic blood pressure

The WHO definition of the metabolic syndrome was only ever proposed as a working definition when included as part of the report on the diagnosis and classification of diabetes mellitus (Alberti and Zimmet, 1998; World Health Organisation, 1999a). The WHO definition (Table 2.1) includes insulin resistance (IR) or abnormalities in glucose regulation (impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diabetes mellitus (DM)) as a prerequisite for definition of the syndrome although they do note some heterogeneity in the strength of the IR relationship with the different components between and within populations. It is worthy of note, and perhaps indicative of the uncertainty of the data available at the time, that the WHO definition was changed slightly between the preliminary report and the definitive statement: the systolic blood pressure threshold was lowered from $\geq$160 to $\geq$140 mmHg and microalbuminuria, as assessed by albumin/creatinine ratio, was changed from $\geq$20 to $\geq$30 $mg.g^{-1}$ (Alberti and Zimmet, 1998; World Health Organisation, 1999a).

The definition of the EGIR came to being in the form of a letter of response to the WHO provisional report and suggested that because there were non-metabolic components to the syndrome a more appropriate name would be the insulin resistance syndrome (IRS). Citing the complexity of the measurement of IGT in the WHO definition and the high negative correlation between fasting insulin and insulin sensitivity the EGIR suggested a definition of a fasting insulin of >75th percentile plus two additional components (Table 2.1). Furthermore, the EGIR stated that microalbuminuria was not a necessary component of the syndrome and should be removed on the ground there was no consensus that it was linked to insulin concentrations. Interestingly, it could be noted that the EGIR definition was also changed slightly between it's original comment on the WHO criteria (Balkau and Charles, 1999) and their later comparison of the frequency of the metabolic syndrome when defined by the WHO criteria and by their own criteria (Balkau *et al.*, 2002). Hypertension was

originally imprecisely defined as "systolic/diastolic blood pressures ≥140/90mmHg or treated for hypertension" (Balkau and Charles, 1999, p442) but later changed to "systolic blood pressure ≥ 140 mmHg **and/or** diastolic blood pressure (DBP) ≥ 90 mmHg and/or treatment for hypertension" (Balkau *et al.*, 2002, p366).

The NCEP ATPIII report (NCEP, 2001) was focussed on the detection, evaluation and treatment of high blood cholesterol and described the metabolic syndrome as a secondary target of risk-reduction therapy, after LDL reduction (and seemingly suggested that insulin resistance is at the root of the problem (Reaven, 2005)). One of their stated objectives in the management of the metabolic syndrome was to reduce the underlying causes i.e. obesity and physical activity. The report goes on to say that therapeutic lifestyle change should stress the importance of weight management and increased physical activity in the reduction of all the metabolic syndrome risk factors. In an addition to the original report (Grundy *et al.*, 2004a, b), specifically addressing the definition of the metabolic syndrome, the primary clinical outcome of the metabolic syndrome was given as coronary heart disease/cardiovascular disease. They explain their less conservative cut-off criteria on the basis that multiple marginal risk factors can impart significantly greater risk for CVD (Grundy *et al.*, 2004b, p435). The NCEP ATPIII criteria were developed in the context of increasing worldwide rates of obesity and decreasing physical activity as a tool to identify individuals at high CV risk (Malik *et al.*, 2004) and designed with clinical utility in mind, thus not requiring a measure of insulin sensitivity. Moreover, it afforded all components equal weighting. The NCEP ATPIII provided a loose modification of their 2001 definition by introducing a lower boundary to define elevated fasting plasma glucose of ≥5.6 mmol/L (≥100 mg/dL) (Genuth *et al.*, 2003; Grundy *et al.*, 2004a, b).

The AACE position statement (American College of Endocrinology, 2003) and preceding executive summary (Einhorn *et al.*, 2003), while using the title of the insulin resistance

syndrome, was focussed on identifying insulin resistant/hyperinsulinaemic individuals who are at risk of CVD and T2DM. To meet its description of the insulin resistance syndrome, the AACE conclude that for epidemiological purposes an individual with risk factors should also be above the threshold value in at least two out of four criteria. The risk factors specifically identified by the AACE that an individual should possess before identification of the metabolic syndrome were:

- Diagnosis of CVD, hypertension, poly-cystic ovarian syndrome, non-alcoholic fatty liver disease, or acanthosis nigricans
- Family history of type 2 diabetes, hypertension, or CVD
- History of gestational diabetes or glucose intolerance
- Non-Caucasian ethnicity
- Sedentary lifestyle
- BMI >25.0 kg/m2 (or waist circumference >40 inches in men, >35 inches in women)
- Age >40 years

It has been noted that the AACE deliberately does not provide a specific definition of the syndrome, but rather allows the diagnosis to remain a clinical judgement (Alberti *et al.*, 2006). The AACE may be viewed as a modification of the NCEP ATPIII criteria reflecting their belief that insulin resistance was the core feature.

The IDF definition (Zimmet *et al.*, 2005) arose from the recognition that published prevalence's for different populations are difficult despite attempts to reach agreement on definition (Eckel *et al.*, 2005). Zimmet *et al.* (2005) placed central obesity at the core of the definition and went further by defining ethnicity specific waist circumference cut-off points reflecting the differences that exist between populations when assessing CVD and T2DM risk (Grundy *et al.*, 2004b). Other components were defined similar to previous definitions (Table 2.1). Furthermore, by attributing a greater weighting to central obesity over other components, it seemed likely that rather than being universally accepted, this

definition would serve to create further discussion among proponents of alternative underlying pathophysiologies (Sarafidis and Nilsson, 2006).

In 2005 the IDF and the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) attempted to reconcile differences in their respective definitions (Alberti *et al.*, 2005; Grundy *et al.*, 2005). However, consensus was not found over the specific criteria for the identification of abdominal obesity. The IDF recommended WC cut-off points of ≥94 cm and ≥80 cm for European origin males and females, respectively. This approximated to a BMI of 25 kg/m^2 in males. The AHA/NHLBI recommended ≥102 cm and ≥88 cm in males and females, respectively. Approximating a BMI of 30 kg/m^2. More recently an attempt was made to harmonise the differing definitions and individual criteria used between organisations. A joint statement from the IDF; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society and the International Association for the Study of Obesity (Alberti *et al.*, 2009) identified that there should be no obligatory components (e.g. abdominal obesity, insulin resistance) and that three abnormal findings out of five criteria would identify individuals with the metabolic syndrome. A single set of cut-off points were agreed for all diagnostic criteria with the exception of waist circumference, for which the diagnostic criteria became the presence of an "elevated waist circumference" until more data become available.

The utility of the terms (metabolic syndrome, insulin resistance syndrome, etc.), the components of the syndrome that enable a clinical diagnosis, and indeed the concept that the "syndrome" itself actually exists have been regularly challenged, perhaps most notably by Gerald Reaven himself (Reaven, 2005; Kahn, 2007). The debate would seem to centre on two clear areas: 1) it's definition, and 2) the ability of the current definitions of the metabolic syndrome to predict the risk of developing cardiovascular disease or type 2

diabetes mellitus to a greater extent than can be predicted from individual risk factors alone (Kahn *et al.*, 2005b; Kahn, 2007; Cornier *et al.*, 2008). A third area of debate exists if it is assumed that the "syndrome" exists, 3) as to whether there is an underlying pathophysiology.

Firstly, a syndrome has been defined previously as "a group of sign and symptoms that occur together and characterise a particular abnormality or condition" (Beaser and Levy, 2007, p1813). It has long been recognised that the risk factors that make up the metabolic syndrome occur together more frequently than would be expected by chance alone (Yki-Jarvinen, 2004) so from this perspective the term "syndrome" may be warranted. However, the term "cluster" is often substituted (Kahn *et al.*, 2005b), or even used interchangeably (Sarafidis and Nilsson, 2006; Reaven, 2008). As to whether the syndrome/cluster represents a particular abnormality or condition, the debate continues (Reaven, 2005; Kahn, 2007; de Zeeuw and Bakker, 2008; Reaven, 2008). Insulin resistance continues to be strongly advocated as the explanation for the clustering of risk factors (Reaven, 2008) whereas others believe abdominal obesity is the key factor (Zimmet *et al.*, 2005).

Second, the ability of the current definitions of the metabolic syndrome to predict the risk of developing cardiovascular disease or type 2 diabetes mellitus to a greater extent than can be predicted from individual risk factors alone has been debated in several articles (Kahn *et al.*, 2005b; Beaser and Levy, 2007; Kahn, 2007; de Zeeuw and Bakker, 2008). A meta-analysis of 37 studies found that the metabolic syndrome indicated an increased relative risk (RR) of 2.18 (95% CI 1.63 – 2.93) for a cardiovascular event (Gami *et al.*, 2007). Interestingly, in 7 of the studies analysed, which provided risk estimates for males and females separately, the risk of cardiovascular events was higher in women than in men (RR 2.63 vs. 1.98, $P = 0.09$). The joint statement from the American Diabetes Association and the European Association for the Study of Diabetes reviewed a number of studies and

reported that the increased cardiovascular disease risk in patients with the metabolic syndrome ranged from 30 to 400% (Kahn *et al.*, 2005b). Conversely, the Casale Monferrato Study (Bruno *et al.*, 2004) evaluated the ability of the WHO definition to predict all-cause and cardiovascular disease mortality in 1565 elderly subjects with type 2 diabetes mellitus. This study found that in subjects with the metabolic syndrome hazard ratios were no different than subjects without the syndrome. Using data from the PREVEND study (Pinto-Sietsma *et al.*, 2000), de Zeeuw and Bakker (2008) constructed receiver operating characteristic curves comparing the sensitivity and specificity of the metabolic syndrome (using dichotomous and continuous variables), the Framingham Risk Score, systolic blood pressure and hypertension (dichotomous) as predictors of cardiovascular events. The Framingham Risk Score had the highest predictive power (area under the curve, 0.81, 95% CI 0.79-0.83) compared to the current dichotomised metabolic syndrome criteria (0.60, 95% CI 0.57-0.62) which provided the lowest predictive power of all the risk predictors. Data from the San Antonio Heart Study and the Mexico City Diabetes Study, a population-based sample of 1,709 and 1353 initially non-diabetic subjects, compared the NCEP ATPIII metabolic syndrome criteria with the Diabetes Predicting Model and the Framingham Risk Score and concluded that "the metabolic syndrome is inferior to established predicting models for either type 2 diabetes or CVD" and even combining the metabolic syndrome criteria with either of the other two risk predictors offered no improvement (Stern *et al.*, 2004). The more recent IDF metabolic syndrome definition has also been shown to identify a surplus of individuals whose cardiovascular disease risk is no different than those without the metabolic syndrome (Boronat *et al.*, 2009).

Finally, the underlying pathophysiology to the metabolic syndrome is an area of continuing debate, as discussed above. In 1988 Reaven proposed that insulin resistance was central to the aetiology of type 2 diabetes mellitus, hypertension and coronary artery disease

(Reaven, 1988). Many others have since identified insulin resistance and/or compensatory hyperinsulinaemia as the central factor (Ferrannini *et al.*, 1987; Reaven, 1988; DeFronzo and Ferrannini, 1991; DeFronzo, 1992; Reaven, 2005; 2008). A possible molecular mechanism for the effect has been suggested via the phosphatidylinositol 3-kinase (PI-3K) and the mitogen-activated protein (MAP) kinase insulin signalling pathways activated after binding of insulin to the insulin receptor. A response to PI-3K activation is translocation of the glucose transporter GLUT4 to the cell membrane. MAP kinase signalling is associated with mitogenic and pro-inflammatory effects (Miranda *et al.*, 2005).

Obesity, and in particular abdominal obesity, has been placed centrally in the development of the metabolic syndrome by some groups (Zimmet *et al.*, 2005), and is believed to be a key feature by many others (Alberti and Zimmet, 1998; Balkau and Charles, 1999; World Health Organisation, 1999a; NCEP, 2001; Balkau *et al.*, 2002). A possible mediating effect has been suggested as an increased free fatty acid (FFA) turnover from an expanded adipose tissue mass (Randle, 1998). Under normal conditions, insulin inhibits adipose tissue lipolysis, but with insulin resistance and increased adipose tissue triglyceride stores the process of lipolysis is accelerated and there is a greater release of FFAs in to plasma (Kahn and Flier, 2000). In the liver FFAs are either stored or oxidised and in insulin resistant subjects triglyceride synthesis and storage are increased and excess triglyceride is released as very low-density lipoprotein cholesterol (VLDL) (Lewis and Steiner, 1996). It has been suggested that the dyslipidaemia associated with insulin resistance is a consequence of the increased VLDL secretion by the liver (Ginsberg *et al.*, 2005). Cornier *et al.* (2008) have provided an extensive review of the metabolic syndrome and possible contributing pathophysiologies.

2.1.3 Paediatric and adolescent definitions of metabolic syndrome

Table 2.2 summarises studies identifying criteria used for the identification of the metabolic syndrome and CVD risk factor clustering in children and adolescents (supporting references are found in Appendix 1). Most studies are cited as modifications of existing adult metabolic syndrome criteria, principally NCEP ATPIII. Many others are modifications of earlier studies, which themselves are modifications of adult criteria. Only a very few studies choose to propose their specific criteria as definitions for the identification of paediatric and adolescent metabolic syndrome (Cruz and Goran, 2004; de Ferranti *et al.*, 2004; Zimmet *et al.*, 2007).

Table 2.2: Summary of studies identifying criteria (and sources of evidence) used for the identification of the metabolic syndrome and CVD risk factor clustering in children and adolescents.

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Webber *et al.* (1979)[116]	[A]	3 of 3	Weight/height$^{2.00}$ index: >75th (2.5-5.5 yrs) [H] Weight/height$^{2.77}$ index: >75th (5-14 yrs) [H]	>75th [H]	TC: >75th [H]				USA, Bogalusa; 3770; 2.5-5.5 yrs (preschool cohort) and 5-14 yrs	3.33 (5-14 yrs) 1.56 (2.5-5.5 yrs)
Raitakari *et al.* (1994)[101]	[A]	3 of 3	obesity index: ≥75th (sum of biceps, triceps and subscapular skinfolds) [B, E]	≥75th (SBP) [B, E]	LDL: ≥75th [B, E]				Finland; 3457; 3-18 yrs	All: 3.1
Bavdekar *et al.* (1999)[32]	[A]	IR plus 1-3 of 3		>75th (SBP)[B]	TG: >75th [B] HDL: <25th [B]		HOMA-IR: >75th [B]		India; 477; 8.5 ± 0.1 yrs	All: 5 (IR plus ≥2 of 3)
Chu *et al.* (1999)[39]	[A]	Increased BMI plus 1-3 of 3	BMI: ≥85th [B, E [94, 112]]	≥90th [B, E] or treatment for hypertension	TG: ≥90th [B, E] TC: ≥90th [B, E] or treatment for dyslipidaemia	FG: ≥90th [B, E] or treatment for hyperglycaemia			Taiwan, China; 1366; 12-16 yrs	Lean: ~0.4 Ob: ~3 (3 of 3 risk factors)
Freedman *et al.* (1999)[58]	[A]	≥3 (not explicitly stated) of 5		≥95th [B,H]	TG: ≥1.47 [20, 76] HDL: <0.91 [20, 76] LDL: ≥3.367 [20]		≥95th [B]		USA, Bogalusa; 9167; 5-17yrs	All: 2 Ow: 10.1
Morrison *et al.* (1999a)[86]	[A]	≥3 (not explicitly stated) of 5		90th (SBP), or 90th (DBP) [H [85]]	TG: 90th [84] HDL: 10th [84] LDL: 90th [84]				USA; 536 (males); 10-15 yrs	Ow: 4.2 (white), 11.8 (black) Lean: 2.1 (white), 4.2 (black)
Morrison *et al.* (1999b)[87]	[A]	≥3 (not explicitly stated) of 5		115 or 117 (90th), or 74 or 75 (90th) (SBP, DBP) [D [3]]	TG: 1.18 [2] HDL: 1.036 [2] LDL: 3.263 [2]				USA; 1870 (females); 9-10 yrs	Ow: 14.5 (white), 6.8 (black) Lean: 3 (white), 1.2 (black)

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Csábi *et al.* (2000)[46]	[A]	4 of 4		>95th (MAP) [G] [109]	TG: >1.1 (<10 yrs), 1.5 (>10 yrs) [106], or HDL: <0.9 [106], or TC: >5.2 [106]	FG: 2 or more values >97th [65]	>112.2 (95th [C])		Hungary; 180 (obese), 239 (control); ~13±2.5 yrs	Ob: 8.9 Lean: 0 (control)
Torok *et al.* (2001)[111]	[A]	≥4 of 5	Weight exceeds expected weight-for-height: >20%, and Body fat: >25% (males) (>30%, females)[53, 96]	>95th (mean ABPM) [G] [109]	TG: >1.5 [107], or HDL: <0.9 [107], or TC: >5.2 [107]	IGT: <7.0 and OGTT2 ≥7.8 but ≤ 11.1[16]	>112.2 (mean + 2SD of a control population)		Hungary; 39 (obese), 29 (control); ~14.16±2.5 yrs	NA
Andersen *et al.* (2003)[27]	[A]	≥4 of 5	Sum of 4 skinfolds: >75th [E]	>75th (SBP) [E]	TG: >75th [E] HDL/TC: <25th [E]		>75th [E]		Denmark; 1020; 9-16 yrs	All: 5.4
Cook *et al.* 2003[42]	NCEP[88, 90]	≥3 of 5	WC: ≥90th [B, E]	≥90th [B, F] [8] or treatment for hypertension	TG: ≥1.24 (90th [B]) [4] HDL: ≤1.03 (10th [B]) [4]	FG ≥6.1 [12]			USA, NHANES III; 2430; 12-19 years	All: 4.2 BMI <85th: 0.1; BMI 85th-<95th: 6.8; BMI ≥95th: 28.7
Invitti *et al.* (2003)[71] (abstract)	[A]	≥3 of 5	BMI: ≥97th [C, E]	Htn ≥95th [C, E]	TG: ≥95th [C, E] HDL: ≤5th [C, E]	IGT or IFG (undefined) or IR	HOMA-IR: ≥2.5 or IGT or IFG		Italy; 748 (obese); 6-18yrs	31.6
Cruz and Goran (2004)[44]	NCEP[88, 90]	≥3 of 5	WC: 90th [H]	90th [E] [8]	TG: 90th [E] [67] HDL: 10th [E] [67]	FG: ≥5.6, or IGT: ≥7.8 [24, 25, 43]			NA	NA
Cruz *et al.* 2004[45]	NCEP[88, 90]	≥3 of 5	WC: ≥90th [E] (for Hispanic ethnicity)	>90th [F] [8]	TG: ≥90th [E] [67] HDL: ≤10th [E] [67]	IGT: OGTT2 ≥7.8 but ≤ 11.1 [25]			US, Hispanic, family history of T2DM, overweight; 126; 8-13 yrs	30.2
de Ferranti *et al.* 2004[49]	NCEP[88, 90]	≥3 of 5	WC: >75th [E] [48, 121]	>90th (SBP) [F] [8]	TG: ≥1.1 [1] HDL: <1.3; boys aged 15-19yrs, <1.17 [1]	FG ≥6.1 [90]			USA, NHANES III; 1960; 12-19 yrs	All: 9.2 Ow/Ob: 31.2

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Duncan *et al.* (2004)[52]	NCEP[88, 90], same criteria as Cook *et al.* (2003)[42]	≥3 of 5	WC: ≥90th [B, E]	≥90th [B, F [8]] or treatment for hypertension	TG: ≥1.24 [B [4]] HDL: ≤1.03 [B [4]]	FG ≥6.1 [12]			USA, NHANES IV; 991; 12-19 yrs	All: 6.4 BMI <85th: 0; BMI 85th-<95th: 7.1 BMI ≥95th: 32.1
Goodman *et al.* (2004)[62]	WHO[17, 31]	Hyperinsulinaemia, hyperglycaemia or DM plus 2 of 3	BMI: ≥95th [112], or ≥30, or WC: ≥102cm (males), ≥88cm (females)	≥130/≥85	TG: ≥1.7, or HDL: ≤0.91 (males), ≤1.01 (females)	FG: ≥6.1 or known DM or hyperinsulinaemia [17]	>75th [B], or known DM or hyperglycaemia		USA; 2710; 15.2 ±1.6 yrs	All: 8.4 Ow: 1.9 Ob: 38.9
	NCEP[88]	≥3 of 5	WC: ≥102cm (males), ≥88cm (females), or BMI ≥30	≥130/≥85	TG: ≥1.7 HDL: ≤1.036 (males), ≤1.295 (females)	FG: ≥6.1 or known DM[17]			USA; 2710; 15.2 ±1.6 yrs	All: 4.2 Ow: 1.1 Ob: 19.5
Katzmarzyk *et al.* (2004)[74]	[A]	≥3 of 6		>80th [B, H]	TG: >80th [B, H] HDL: <20th [B, H] LDL: >80th [B, H]	FG: >80th [B, H]	>80th [B, H]		USA, Bogalusa; 2597; 5-18 yrs	All: 18.2/17.3 (white/ black males) 16.5/16.6 (white/ black females)
Lambert *et al.* 2004[77]	[A] (IRS1)	≥3 of 6	BMI: ≥85th [B, E [68]]	≥75th (SBP) [B, F [37, 102]]	TG: ≥75th [B, E [37, 102]] HDL: ≤25th [B, E [37, 102]]	IFG: ≥6.1 and <7.0 [55]	≥75th [B, E [37, 102]]		Canada; 2244; 9, 13 and 16 yrs	All: 14.0
	[A] (IRS2)	Insulin plus ≥2 of 5	BMI: ≥85th [B, E [68]]	≥75th (SBP) [B, F [37, 102]]	TG: ≥75th [B, E [37, 102]] HDL: ≤25th [B, E [37, 102]]	IFG: ≥6.1 and <7.0 [55]	≥75th [B, E [37, 102]]		Canada; 2244; 9, 13 and 16 yrs	All: 11.5
	[A] (IRS1 modified)	≥3 of 6	BMI: Overweight [41]	≥90th (SBP) [8]	TG: ≥75th [B, E [37, 102]] HDL: ≤25th [B, E [37, 102]]	IFG: ≥6.1 and <7.0 [55]	≥75th [B, E [37, 102]]		Canada; 2244; 9, 13 and 16 yrs	Similar to IRS1
	[A] (IRS2 modified)	Insulin plus ≥2 of 5	BMI: Overweight [41]	≥90th (SBP) [8]	TG: ≥75th [B, E [37, 102]] HDL: ≤25th [B, E [37, 102]]	IFG: ≥6.1 and <7.0 [55]	≥75th [B, E [37, 102]]		Canada; 2244; 9, 13 and 16 yrs	Similar to IRS2

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Rodriguez-Moran *et al.* 2004[104]	NCEP[88, 90]/WHO[17]/ AACE[21]/E GIR[31]	≥3 points[I]	BMI: ≥90th [E]	≥90th [E] [8]	TG: ≥90th [E] [4] HDL: ≤10th (not stated explicitly) [4]	FG: ≥6.1 [33]			Mexico; 965; 10-18 yrs	All: 7.8
Weiss *et al.* 2004[117]	NCEP[89]/W HO[17]	≥3 of 5	BMI: >97th (z score ≥2.0) [E]	>95th [E] [8]	TG: >95th [H] [9] HDL: <5th [H] [9]	IGT: OGTT2 >7.8 but <11.1 [22]			USA; 439 (obese); 4-20 yrs	Moderate ob: 38.7; Severe ob: 49.7
Boney *et al.* 2005[34]	NCEP[88, 90]	≥2 of 4	BMI: >85th [D]	>95th [D] [3]	TG: >95th [D] [6], or HDL: <5th [D] [6]	FG: ≥5.6; or IGT: OGTT2 > 7.8 [90]			USA; 179; 5 year follow-up at 6,7,9 and 11 yrs	Children of large birth-weight and maternal gestational DM: 50
Butte *et al.* 2005[35]	NCEP[88, 90]	≥3 of 5	WC: >90th [H] [56]	>90th [F] [8]	TG: >90th (for ages 12-19yrs and ethnicity) [67] HDL: ≤10th [F] [67]	FG: ≥5.6 [25]			USA; 1030 (Hispanic); 4-19 yrs	Ow: 20 (males); 19 (females)
	NCEP[88, 90]	≥3 of 6	WC: >90th [H] [56]	>90th [F] [8]	TG: >90th (for ages 12-19yrs and ethnicity) [67] HDL: ≤10th [F] [67]	FG: ≥5.6 [25]		ALT: >97.5th %ile [E] [79]	USA; 1030 (Hispanic); 4-19 yrs	Ow: 28 (males); 27 (females) Ob: 40
Da Silva *et al.* (2005)[47]	Modificatio n of Invitti *et al.* 2003)[71]	≥3 of 5/6	BMI: 97th [E] [41]	>95th [E] [14]	TG: ≥1.47 [13] HDL: ≤0.91 [13]	IFG: 5.6-6.9, or IGT: OGTT2 7.8-11.1, or T2DM[26]	HOMA-IR: >2.5		Brazil; 99 (family history of T2DM); 10-19 yrs	All: 6 Lean: 0 Ow: 0 Ob: 26.1
Ford *et al.* 2005[57]	NCEP[88, 90]/Cook *et al.* (2003)[42]	≥3 of 5	WC: ≥90th (for gender)	≥90th [F] [8]	TG: ≥1.24 [4] HDL: <1.03 [4]	FG ≥6.1			US, NHANES IV; 1366; 12-17 yrs	5.9
	NCEP[88, 90]/ Grundy *et al.* (2004)[64]	≥3 of 5	WC: ≥90th (for gender)	≥90th [F] [8]	TG: ≥1.24 [4] HDL: <1.03 [4]	FG ≥5.6 [64]			US, NHANES IV; 1366; 12-17 yrs	6.2

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Klein-Platat *et al.* (2005)[75]	NCEP[88], modification of Cruz *et al.* (2004)[44]	≥3 of 5	WC: >95th E [82]	>90th E [92])	TG: >90th E [4] HDL: <5th E [4]	FG >6.1[24, 25]			France; 60 (ow), 60 (nw); 11.5±0.1 yrs	Ow: 25% Lean: 0%
Ogawa *et al.* (2005)[95]	A	≥3 of 6		≥120/≥70 to ≥140/≥85 D [7, 29]	TG: ≥1.36 [29] HDL: <1.036 [29] LDL: ≥3.626 [29]		≥90 [29]	ALT: >30 IU/ml [29]	Japan; 100 (obese); 8-13 yrs	18.8 (high adiponectin) 57.6 (low adiponectin)
Park *et al.* (2005)[97]	NCEP[88]	≥3 of 5	WC: ≥90th B, E	≥90th E	TG: ≥90th (≥1.54) E HDL: <10th (<1.05) E	FG ≥90th (≥6.05) E			Korea; 1594; 10-19 yrs	All: 3.3
Viner *et al.* 2005[114]	WHO[17]	≥3 of 4	BMI: ≥95th E [40]	≥95th (SBP) E [3]	TG: ≥1.75 [67], or HDL: <0.9 [67], or TC: ≥95th [67]	IFG: ≥6.1, or IGT: OGTT2 ≥7.8 [17], or hyperinsulinaemia	≥90 (pre-pubertal); ≥180 (mid-puberty); ≥120 (post-pubertal) [17, 63], or IFG or IGT		UK, obese; 103; 2.3-18 yrs	Ob: 33
Whincup *et al.* (2005)[118]	NCEP[88, 90], modification of Cruz *et al.* (2004)[45]	≥3 of 5	WC: >90th B, E	>90th B, F	TG: >90th B, E HDL: <10th B, E	FG >6.1			UK; 383; ~15.5yrs;	All: 2
Yoshinaga *et al.* (2005)[120]	NCEP[88, 90]	≥3 of 5	WC: ≥90th [28]	≥120/≥70 to ≥130/≥80 (depending on school age)	TG: >1.34 [29] HDL: <1.036 [29]	FG: >5.6 [59]			Japan; 471 (ow/obese); 6-11 yrs	Ow: 8.7 Ob: 17.7
Agirbasli *et al.* 2006[15]	NCEP[88, 90]	≥3 of 5	BMI: Overweight or obese E [41]	≥95th [8, 93, 108]	TG: ≥90th [9, 42] HDL: ≤10th [9, 42]	IFG ≥5.6 [59]			Turkey; 1385; 10-17 yrs	All: 2.2 Ow/Ob: 21

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Atabek *et al.* (2006)[30]	WHO[17]	≥3 of 4	BMI: >95th [E]	>95th (SBP) [E]	TG: >1.2 (<10 years of age), >1.5 (≥10 years of age), or HDL: <0.9, or TC: >95th	IFG: ≥6.1, or IGT: OGTT2 ≥7.8 and <11.1)[54], or hyperinsulinaemia	>90 (prepubertal) >180 (puberty)[63], or IFG or IGT		Turkey; 169 (obese); 7-18yrs (10.8 ± 3 yrs)	All: 27.2 Prepubertal: 20 Pubertal: 37.6
Druet *et al.* (2006)[50]	NCEP[88, 90]	≥3 of 5	WC: ≥75th [E] [99]	≥90th [E] [8]	TG: ≥90th [H] [38] HDL: ≤1.03	FG: ≥6.1 and <7.0 [119]			France; 308 (ow and ob); 7-17 yrs	Ow/ob: 15.9
	WHO[17]	IR plus ≥2 of 5	BMI: IOTF[41] defined ow and ob [105]	≥90th [E] [8]	TG: ≥90th [H] [38] HDL: ≤1.03	IGT: OGTT2 ≥7.8 [119]	HOMA-IR: ≥75th [E] [19]			Ow/ob: 42.5
Gilardini *et al.* (2006)[60]	WHO[119], modification of Invitti *et al.* (2006)[72]	IR, IFG, IGT or DM plus ≥2 of 4	BMI: ≥97th and WC: ≥97th [C]	≥95th [C]	TG: ≥95th [C], and/or HDL: ≤5th [C]	IFG: ≥6.1 and <7.0, or IGT: OGTT2 ≥7.8 [119], or IR or DM	HOMA-IR: 2.4, 2.8, 3.0, 4.1 and 3.0 for Tanner stages 1-5 respectively [70], or IFG, IGT or DM	Microalbuminuria: excretion rate of 20-200 µg/min	Italy; 162 (obese); 9-18 yrs	Ob: 25
Golley *et al.* 2006[61]	Lambert *et al.* (2004)[77] (IRS2)	Insulin plus ≥2 of 5	BMI: ≥85th [40]	≥95th (SBP) [F] [8]	TG: M: ≥0.9; F: ≥1.0 (75th) [77] HDL: ≤1.2 (25th) [77]	FG: 6.1-7.9 [55]	M: ≥35.0; F: ≥40.6 (75th) [77] [L]		Australia; 99 (ow or mild ob); 6-9 yrs	Ow/ob: 60 Ob: 63 Ow: 48
	Lambert *et al.* (2004)[77] (IRS2) modified	Insulin plus ≥2 of 5	BMI: ≥85th [40]	≥95th (SBP) [F] [8]	TG: ≥1.8 (75th)[2] HDL: ≤0.8 [5]	FG: 6.1-7.9 [55]	M: ≥35.0; F: ≥40.6 (75th) [77] [L]			Ow/ob: 39 Ob: 42 Ow: 29
	EGIR[31]	Insulin plus ≥2 of 5	WC: ≥91st [E] [82, 83]	≥95th (SBP) [F] [8]	TG: ≥1.8 (75th), or HDL: ≤0.8 [5]	FG: 6.1-7.9 [55]	M: ≥35.0; F: ≥40.6 (75th) [77] [L]			Ow/ob: 39
	NCEP[88, 90]	≥3 of 5	WC: ≥91st [82, 83]	≥95th (SBP) [F] [8]	TG: ≥1.8 (75th). HDL: ≤0.8 [5]	FG: 6.1-7.9 [55]				Ow/ob: 3

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
Iannuzzi *et al.* (2006)[69]	NCEP[88, 90], modification of de Ferranti *et al.* 2004[49]	≥3 of 5	WC: >75th [E]	>90th [F] (SBP or DBP)	TG: >1.13 HDL: <1.166 (males), <1.295 (females)	FG: >6.1			Italy; 100 (obese); 6-14 yrs	Ob: 38
Invitti *et al.* 2006[72]	WHO[10]	IFG, IGT, DM or IR plus ≥2 of 4	BMI: ≥97th [C] [36] and WC: ≥97th [C] [36]	≥95th [C]	TG: ≥95th [C] [10] HDL: ≤5th [C] [10]	IFG: ≥6.1 and <7.0 or IGT: OGTT2 ≥7.8 [10] or IR	HOMA-IR: 2.4, 2.8, 3.0, 4.1 and 3.0 for Tanner stages 1-5 respectively [70]		Italy; 588 (obese); 6-16 yrs	Ob: 23.3
López-Capapé *et al.*(2006)[80]	NCEP[88, 90], Cook *et al* (2003)[42] modified	≥3 of 5	BMI: z score ≥2.0 [E, J] [66]	>95th [F] [91]	TG: ≤1.24 [42] HDL: ≤1.03 [42]	IFG: ≥5.6 and <7.0, or IGT: OGTT2 ≥7.8 - 11, or DM [26]			Spain; 429 (obese); 4-18 yrs	Ob: 18
Park *et al.* (2006)[98]	NCEP[88, 90]	≥3 of 5	WC: ≥90th [F] [11]	≥90th [F] [8]	TG: ≥1.24 [5, 110] HDL: ≤1.03 (or ≤1.05)[5, 110]	FG ≥6.1 (or ≥6.05) [23]			Korea; 229; 11-19 yrs	All: 10.3 (males), 1.9 (females)
Platat *et al.* (2006)[100]	NCEP[88], modification of Cruz and Goran (2004)[44]	≥3 of 5	WC: 95th [82]	>90th [E] [8, 92]	TG: >90th [E] [4] HDL: <5th [E] [4]	FG: >6.1			France; 640; 12 yrs	All: 5.8 Lean: 0 Ow: 26.2
Retnakaran *et al.* (2006)[103]	NCEP[88, 90], modification of de Ferranti *et al.* 2004[49]	≥3 of 5	WC: ≥90th [E] [56]	≥90th [F] [91]	TG: ≥1.1 [49] HDL: <1.2, boys aged 15-19yrs; <1.3 all other children [49]	FG: ≥6.1[49]			Canada (Native population); 236; 10-19 yrs	All: 18.6
Vikram *et al.* (2006)[113]	NCEP[88, 90]	≥3 of 7	BMI: >85th, and BF%: >30.1 (males), >34.7 (females), or WC: ≥90th	≥90th or treatment for hypertension	TG: ≥90th [42] HDL: <10th [42]	FG: ≥6.1 and <7.0, or diabetes (FG ≥7.0)[59]	>120μU/ml[114]		India; 793; 14-19 yrs	All: 10.2

(Continued)

Table 2.2: *(Continued)*

	Modified adult criteria	Diagnostic criteria	Obesity	BP (%ile or mmHg)[K]	Dyslipidaemia (mmol/l or %ile)	Glucose (mmol/l)	Insulin (%ile or pmol/L) or IR	Other	Population; *N*; Age	MetS prevalence (%)
DuBose *et al.* (2007)[51]	[A]			MS score: WC, MAP, HOMA-IR, HDL and TG standardised by regression onto age, gender and ethnicity. Z-scores for individual components summed to create a continuous MS score						Ow (BMI ≥95th): 3.33 At risk for ow: 0.15 Lean (BMI <85th): -1.42
Jolliffe and Janssen (2007)[73]	NCEP[88, 90]	≥3 of 5	WC: 92nd (males); 72nd (females), (elevated WC assumed if BMI ≥89th (males), ≥84th (females))	M: 92nd/97th F: 93rd/99th (SBP/DBP)	TG: 89th HDL: 26th (males), 43rd (females)	FG: 5.6			US, NHANES III-IV; 6067; 12-20 yrs	All: 7.6 (*n*=1820)
	IDF[18]	obesity plus ≥2 of 4	WC: 83rd (males); 50th (females) (elevated WC assumed if BMI ≥89th (males), ≥84th (females))	M: 92nd/97th F: 93rd/99th (SBP/DBP)	TG: 89th HDL: 26th (males), 43rd (females)	FG: 5.6			US, NHANES III-IV; 6067; 12-20 yrs	All: 9.6 (*n*=1820)
Zimmet *et al.* 2007 [122]	IDF[18]		WC: 6-<10yrs: ≥90th [H] [42, 45, 57, 81]	MetS cannot be diagnosed but further investigation if family history of MetS, T2DM, dyslipidaemia, CVD, HTN, and/or obesity.						
	IDF[18]	10-<16yrs: obesity plus ≥2 of 4	WC: ≥90th [H] [42, 45, 57, 81]	≥130 (SBP) or ≥85 (DBP) [90]	TG: ≥1.7 HDL: <1.03	FG: ≥5.6, or known T2DM				
	IDF[18]	16+yrs: IDF adult criteria, obesity plus ≥2 of 4	WC: ≥94cm (males); ≥80cm (females)[78] (ethnicity specific) (elevated WC assumed if BMI >30)	≥130 SBP or ≥85 DBP or previously diagnosed Htn	TG: ≥1.7 HDL: <1.03 (males), <1.29 (females) or specific treatment for abnormalities	FG: ≥5.6 or known T2DM				

%ile, percentile; ABPM, ambulatory blood pressure monitoring; ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; DM, diabetes mellitus; EGIR, European Group on Insulin Resistance; FG, fasting glucose; HDL, high-density lipoprotein cholesterol (for age and gender); Htn, hypertension; IDF, International Diabetes Federation; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LDL, low density lipoprotein cholesterol; MetS, metabolic syndrome; *N*, sample size; NCEP, National Cholesterol Education Program Adult Treatment Panel III; OGTT2, oral glucose tolerance test at 2 hours; T2DM, type-2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHO, World Health Organisation.

Standard international conversion factors: to convert triglycerides to mg/dL, divide by 0.0113; to convert total cholesterol to mg/dL, divide by 0.0259; to convert glucose to mg/dL, divide by 0.0555; to convert HDL to mg/dL divide by 0.0259; to convert HDL to mg/dL divide by 0.0259; to convert insulin to μU/mL divide by 6 [115].

[A] not stated as a modification of existing criteria; [B] of sample population; [C] of controls; [D] for age; [E] for age and gender; [F] for age, gender and height; [G] for gender and height; [H] for age, gender and ethnicity; [I] points based on two steps 1) family history of obesity, T2DM and/or hypertension (1pt); high or low birth weight (1pt); high BP (1pt); high BMI (1pt); if 2 or more points then step 2) high glucose (1pt); high TG (1pt); low HDL (1pt); [J] of Spanish normative charts; [K] SBP and/or DBP unless otherwise stated; [L] quoted as pmol/L but more likely to be μU/L.

Overall prevalence of child and adolescent metabolic syndrome in varying populations has been reported to be between 2% (Freedman *et al.*, 1999a; Whincup *et al.*, 2005) and 16.5 – 18.2% (Katzmarzyk *et al.*, 2004) of subjects. In lean, overweight and obese individuals metabolic syndrome has been reported between 0% (Csabi *et al.*, 2000; da Silva *et al.*, 2005; Klein-Platat *et al.*, 2005; Platat *et al.*, 2006) and 1.2 – 4.2% (Morrison *et al.*, 1999a; Morrison *et al.*, 1999b); 0% (da Silva *et al.*, 2005) and 48% (Golley *et al.*, 2006); and ~3% (Chu *et al.*, 1998) and 63% (Golley *et al.*, 2006), respectively. Much of the variation in prevalence between studies is likely to be due to differences between population ethnic groups studied (Morrison *et al.*, 1999a; Morrison *et al.*, 1999b). Much more of the variation is likely to be due to the diagnostic criteria applied. For example, a study of 99 overweight or mildly obese children aged 6 – 9 years, which applied multiple definitions, reported metabolic syndrome prevalence's of between 3 and 60% depending on the criteria employed (Golley *et al.*, 2006). Overall, metabolic syndrome occurs infrequently in lean individuals but is highly prevalent in the presence of overweight and obesity.

The prevalence of child and adolescent metabolic syndrome in UK cohorts has previously been estimated (Viner *et al.*, 2005; Whincup *et al.*, 2005). These studies have predominantly been conducted in an English cohort and have identified an overall metabolic syndrome prevalence of 2% (Whincup *et al.*, 2005), while in obese children prevalence increases markedly (33%) (Viner *et al.*, 2005). In adults, cardiovascular disease rates are known to vary by region within individual countries (Scarborough *et al.*, 2010) and it is likely that cardiovascular disease risk factors in children are also likely to vary. Overall, this means that metabolic syndrome prevalence's estimated from an English child and adolescent population may not be representative of the Welsh population.

The need for a globally applicable metabolic syndrome definition has been repeatedly called for (Cook *et al.*, 2003; Jolliffe and Janssen, 2007; Zimmet *et al.*, 2007) to facilitate

global comparisons and enable early detection and treatment. The studies summarised in Table 2.2 broadly suggest a developing consensus definition for child and adolescent metabolic syndrome of: waist circumference >90th percentile for age and gender; SBP and/or DBP >90th percentile for age, gender and height; triglyceride > 90th percentile for age and gender; HDL <10th percentile for age and gender; and fasting plasma glucose of >5.6 mmol/L.

2.1.4 Components of metabolic syndrome in children and adolescents

2.1.4.1 Identification of overweight and obesity in children and adolescents

2.1.4.1.1 Body mass index

In the UK growth reference curves have been available for many years and used as tools to identify normal/abnormal growth rates. Widely used reference data used in the past include those by Tanner *et al.* for head circumference, weight, height and the velocity of both (1966a, b), for skinfolds thickness (Tanner and Whitehouse, 1975) and for stages of puberty as well as longitudinal data for height, weight and velocities (Tanner and Whitehouse, 1976); and Gairdner and Pearson (1971) for height weight and head circumference. A deficiency of these reference data sets has been their inability to assess child fatness. Weight and height measures are not useful in children because weight is highly correlated with height in early development and is more representative of body size rather than body shape or composition. The body mass index (BMI; also known as the Quetelet index (Quetelet, 1869) or Kaup index (Kaup, 1921)) is a measure of an individual's weight *relative to* their height, expressed as weight/height^2. BMI as a measure of body fatness has been used extensively in adults (Berrington de Gonzalez *et al.*, 2010) but its use in children and adolescents is problematic because BMI changes substantially in the early years, rising rapidly in infancy, falling in early childhood and rising again during adolescence and into adulthood (Cole *et al.*, 1995). For this reason age-specific BMI

reference curves must be used. Furthermore, because of developmental differences between boys and girls, the age-specific curves must be gender-specific equally.

In the UK there are currently three different classification systems commonly in use to define child overweight and obesity. Firstly, the 1990 UK National BMI percentile charts (UK 1990) (Cole *et al.*, 1995) are the most commonly used to report the national picture and this classification arbitrarily uses the age- and sex-specific 85th and the 95th percentiles of the UK 1990 data cut-off points to identify overweight and obesity respectively. However, the published growth charts do not explicitly identify either of these percentiles although they can be calculated using the available database (Pan, H. and Cole, T. (n.d.). lmsGrowth [online]. Available at: http://www.healthforallchildren.co.uk/pro.epl?DO=PRODUCT&WAY=INFO&ID=185. [Accessed 21st October 2009]). Second, in clinical practice the age- and sex-specific 91st and 98th percentiles of these same BMI percentile reference charts are used, with the higher percentile cut-off points used in an attempt to improve the sensitivity of the identification of excess adiposity. More recently the International Obesity Task Force (IOTF) has attempted to establish a standard, worldwide definition for overweight and obesity using reference data collected from six countries to enable international comparisons to be made (Cole *et al.*, 2000). The UK 1990 and the IOTF definitions are developed using the same latent moderated structural equations (LMS) method (Cole and Green, 1992) which adjusts the BMI distribution for skewness and allows BMI in individual subjects to be expressed as an exact centile or standard deviation (SD) score.

The reference sample of children for the UK 1990 data is a combination of data from 11 distinct surveys in the UK from 1978 – 1990 forming a dataset consisting of BMI for 15 636 boys and 14 899 girls, for ages between 33 weeks of gestation and 23 years. The BMI growth curves are divided into 9 centiles each two-thirds of a SD apart (percentiles: 0.4, 2,

9, 25, 50, 75, 91, 98, and 99.6; SD: -2.67, -2, -1.33, -0.67, 0, 0.67, 1.33, 2, 2.67). The rationale for the 9 centiles, and the spacing between them, is so that there would be some similarity with the previously used percentiles (3, 10, 25, 50, 75, 90, and 97). As mentioned above the Department of Health uses the 91st and 98th percentiles as clinical cut-off points, whereas for public health monitoring the 85th and 95th are preferred. The 0.4 and 99.6 percentiles allow for improved screening cut-offs where only the very underweight or very obese can be identified.

The IOTF study (Cole *et al.*, 2000) utilised six large nationally representative cross sectional growth surveys from Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the United States. Each survey had over 10 000 subjects, with ages ranging from 6-18 years. The IOTF has questioned the use of the 85th and the 95th percentiles obtained from nationally representative survey data in the United States and subsequently recommended for wider use (Cole *et al.*, 2000, p1240). The use of American data to specify cut-off points is clearly problematic and the use of the 85th or 95th percentile (or the 90th, 91st, 97th, or 98th centile) as a cut-off point can be criticised as arbitrary. Therefore, its purpose was to develop an internationally acceptable definition of child overweight and obesity, specifying the measurement, the reference population, and the age and sex specific cut off points. Similar to the UK 1990 data, centile curves for body mass index were constructed for each dataset by gender using the LMS method (Cole and Green, 1992). Superimposing the curves of the six datasets leads to a cluster of centile curves that all pass through the adult BMI cut-off values of 25 kg/m^2 and 30 kg/m^2 at 18 years of age. The data was then averaged to provide a series of smooth percentile curves.

More recently a fourth classification system has been developed by the World Health Organisation (WHO). Following the release of the WHO Child Growth Standards for Preschool Children in 2006, which is based on a prescriptive approach (World Health

Organisation 2006a), the WHO subsequently released the 2007 BMI-for-age Growth Reference Data for 5-19 Years (de Onis *et al.*, 2007). Using data from the 1977 National Center for Health Statistics (NCHS)/WHO growth reference (1-24 years), the WHO BMI-for-age growth standards identifies a cut-off point for overweight as greater than plus one standard deviation (+1 SD) from the 50th percentile (at 19 years this is equivalent to a BMI of 25.4 kg/m^2 for males and 25.0 kg/m^2 for females) and obesity as greater than +2 SD (at 19 years this is equivalent to a BMI 29.7 kg/m^2 for both sexes). For reference purposes thinness and severe thinness are defined as less than -2 SD and -3 SD, respectively.

Internationally, other methods for identification of childhood and adolescent overweight and obesity are in use. Many studies use the Centers for Disease Control and Prevention (CDC) clinical age- and sex-specific growth charts (Centers for Disease Control and Prevention (2000). Clinical growth charts. Available at: http://www.cdc.gov/nchs/nhanes/growthcharts/clinical_charts.htm [Accessed 23.07.2009]). Similar to the UK 1990 growth charts, overweight is defined as a BMI at or above the 85th percentile but below the 95th percentile, and obesity is defined as a BMI at or above the 95th percentile. A recent study (Mei *et al.*, 2002) evaluating the validity of BMI for the assessment of body fatness in children and adolescents used data collected from two large data sets. To compare skinfold thicknesses with measures of height-adjusted weight, data on 11096 children aged 2-19 years from the third National Health and Nutrition Examination Survey (NHANES III) were used, and to compare dual-energy x-ray absorptiometry measurements of fatness with height-adjusted weight measures data from three international studies were pooled. The results showed that BMI-for-age was significantly better than other measures (weight-for-height and the Rohrer index, weight/height^3) for detecting overweight when average skinfold thicknesses were used as the standard. When percentage body fat or total fat mass was used as the standard, BMI-

for-age remained better than the Rohrer index in detecting overweight but performed similarly to weight-for-height.

The use of BMI as a measure of overweight or obesity may be criticised on the basis that it does not measure body fat directly. However, research has shown that BMI correlates well with more direct measures of body fat such as underwater weighing and dual-energy x-ray absorptiometry (Mei *et al.*, 2002). Childhood overweight and obesity also shows a strong relationship with the presence of other traditional cardiovascular risk factors such as increased blood pressure, high total cholesterol and triglyceride and increased fasting insulin concentrations (Freedman *et al.*, 1999a). Although there is a fairly weak link between infant weight and subsequent adult BMI (Parsons *et al.*, 1999) substantial evidence suggests that overweight or obese children are more likely to be overweight or obese as adults (Zack *et al.*, 1979; Stark *et al.*, 1981; Rolland-Cachera *et al.*, 1987; Mossberg, 1989; Clarke and Lauer, 1993; Serdula *et al.*, 1993; Guo *et al.*, 1994) and that obesity in childhood lays the metabolic groundwork for adult cardiovascular disease (Srinivasan *et al.*, 1996). A recent study in the US (Nader *et al.*, 2006) showed that children that were identified as being overweight (>85th percentile for BMI) at least once at ages 24, 36 or 54 months were more that 5 times as likely to be overweight at 12 years of age when compared to children who were below the 85th percentile. A very large study of 276 835 children aged 7-13 years with 5 063 622 person-years of follow-up showed that higher BMI during childhood was associated with an increased risk of CHD in adulthood. The associations were found to be stronger in boys than in girls and increase with the age of the child in both genders (Baker *et al.*, 2007). Further research suggests that this link is stronger for overweight in adolescence compared to childhood overweight (Serdula *et al.*, 1993; Guo *et al.*, 1994) and for those with a higher childhood body weight (Troiano and Flegal, 1998). Some studies show the positive relationship to be stronger for males than females (Casey *et al.*, 1992; Baker *et al.*, 2007). While others have found the reverse to be

true (Guo *et al.*, 1994). Furthermore, two studies both spanning six decades of follow-up showed that adults who were overweight in adolescence had an increased risk of morbidity and mortality from cardiovascular diseases, independently of adult weight (Must *et al.*, 1992; Gunnell *et al.*, 1998).

A representative survey in England found 18% of schoolchildren to be overweight and a further 6% to be obese (Her Majesty's Stationery Office, 2002). On this basis, some 1.8m children in the United Kingdom are overweight and a further 700 000 children are obese (Lobstein and Leach, 2004). Moreover, a recent systematic review and comparison of the prevalence of childhood overweight and obesity used data from the 2001-2002 *Health Behaviour in School-Aged Children Study* (HBSC) (Janssen *et al.*, 2005). This study was conducted in 34 (mainly European and North American) countries, where BMI was calculated and international age- and gender-specific child BMI cut-points were used to define overweight and obesity (Cole *et al.*, 2000). Welsh school children ranked third highest in terms of the total prevalence of overweight and obesity (21.5%; 16.7% overweight, 4.8% obese). However, the HBSC study data collection method consisted of a questionnaire administered in a classroom setting with height and body weight based on self-report measures. It has been previously identified that there is significant underestimation of BMI when self-report measures are used in children and adolescents (Himes and Story, 1992; Brener *et al.*, 2003; Himes *et al.*, 2005).

2.1.4.1.2 Waist circumference

It has been suggested that BMI may be a less sensitive indicator of body fatness in children (Reilly *et al.*, 2000) and BMI gives no indication about fat distribution. In the adult population, waist circumference measurement has been used to assess risk for obesity-related diseases such as cardiovascular disease (Lemieux *et al.*, 2000). Furthermore, waist circumference has been shown to correlate well with intra-abdominal fat mass (Lean *et al.*,

1995), which in turn has been shown to be related to an atherogenic lipoprotein profile (Han *et al.*, 1995). Waist circumference is associated with insulin resistance and increase in waist circumference is associated with a worsening of insulin resistance (Park *et al.*, 2010).

The health risks associated with an excessive abdominal fat accumulation in children in comparison to adults remain unclear. Waist circumference in children, consistent with the situation in adults, has been shown by some to be an independent predictor of insulin resistance, lipid levels, and blood pressure (Bacha *et al.*, 2006; Lee *et al.*, 2006a). In a cross-sectional study of 2996 children aged 5-17 years, central adiposity was measured by WC and waist-to-hip ratio (Freedman *et al.*, 1999b). A central or abdominal distribution of body fat was shown to be related to adverse concentrations of triglyceride, LDL cholesterol, HDL cholesterol, and insulin; these associations were independent of race, sex, age, weight, and height. These associations were observed whether fat patterning was characterized by using waist circumference alone (after adjustment for weight and height) or waist-to-hip ratio.

McCarthy *et al.* (2001) developed waist circumference percentiles derived from data collected from 8355 children aged 5 – 16.9 years, which they state was a representative sample of British children in 1988. However, they do note that the sample was not truly representative of non-Caucasian children. Using the same LMS method as described for BMI (above), smoothed waist circumference percentile curves were constructed. They tentatively proposed that the 85th and 95th percentiles could be used provisionally for both clinical and possibly epidemiological studies. Confusingly, McCarthy *et al.* (2001, p903) state that "the waist circumference measurement was taken midway between the tenth rib and the iliac crest and was recorded to the nearest millimetre" while also calling this the "natural waist" and suggesting an additional measurement site at the level of the umbilicus. Wang *et al.* (2003) identified 14 different definitions for waist circumference measurement

and compared 4 commonly used ones. They found that midway between the tenth rib and the iliac crest produced significantly different results in males and females when compared to the natural (narrowest) waist.

2.1.4.1.3 Identification of hypertension in children and adolescents

Most studies identifying the metabolic syndrome in children and adolescents explicitly use the criteria of the National High Blood Pressure Education Program (NHBPEP) Working Group on Hypertension Control in Children and Adolescents (National High Blood Pressure Education Program, 1996), an update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents. The 1996 update itself has since been updated (National High Blood Pressure Education Program, 2005 (revised)). This report combined data collected on children from the 1988 - 1991 United States National Health and Nutrition Examination Survey III plus nine additional US data sets to develop normative BP tables. This report defines hypertension as average SBP or DBP $\geq 95^{th}$ percentile for age, gender and height, "high normal" is defined as SBP or DBP $\geq 90^{th}$ percentile for age, gender and height. Interestingly, the 2005 update now recommends that, as with adults, children and adolescents with BP levels at 120/80 mmHg or above, but less than the 95th percentile, should be considered prehypertensive.

Some early studies advocated the use of greater than or greater than or equal to the 75^{th} percentile (Webber *et al.*, 1979; Raitakari *et al.*, 1994; Bavdekar *et al.*, 1999). Several others define absolute cut points for SBP and DBP with values ranging between 115-140 mmHg and 74-85 mmHg, respectively (Morrison *et al.*, 1999b; Ogawa *et al.*, 2005). However, while some studies using the NHBPEP (National High Blood Pressure Education Program, 1996) criteria apply the US derived age, gender and height percentiles others use age, gender and height percentiles derived from their sample populations (Cook *et al.*, 2003; Duncan *et al.*, 2004; Whincup *et al.*, 2005), or just age and gender (Cruz and

Goran, 2004; Rodriguez-Moran *et al.*, 2004; Weiss *et al.*, 2004b; da Silva *et al.*, 2005; Druet *et al.*, 2006). The remainder derive cut-off criteria for blood pressure derived: from the sample population (Bavdekar *et al.*, 1999); from controls (Gilardini *et al.*, 2006; Invitti *et al.*, 2006); by age (Boney *et al.*, 2005); by gender and height (Csabi *et al.*, 2000; Torok *et al.*, 2001); by age, gender and ethnicity (Morrison *et al.*, 1999a), or a mixture of the above.

In the UK blood pressure centile curves for males and females aged 4-24 years have been published (Jackson *et al.*, 2007) and are commercially available (Harlow Printing Limited, South Shields, Tyne and Wear). Developed using data collected from seven national health and social surveys carried out between 1995 and 1998 which were obtained from the UK Data Archive (http://www.data-archive.ac.uk/), 22974 subjects were measured using an automatic oscillometric method. Sex-specific smoothed centiles were derived using the LMS method for age and sex as described previously (Cole and Green, 1992). The relationship of systolic and diastolic blood pressure, weight and height was investigated through the multiple regression of blood pressure on weight and height. Weight was found to have a large and positive effect on blood pressure (p <0.001), whereas height had a smaller negative effect (p <0.005). Jackson *et al.* (2007) explain that a 1 SD increase in weight was associated with a 0.3 SD and 0.08 SD increase in systolic and diastolic pressure, respectively. Whereas a 1 SD increase in height was associated with a 0.03 SD reduction in both systolic and diastolic pressure. Thus, for any given weight, a taller (and thinner) individual had lower blood pressure.

2.1.4.1.4 Identification of dyslipidaemia in children and adolescents

2.1.4.1.4.1 Triglyceride

Most studies cite differing sources of evidence to define cut-off criteria to identify hypertryglyceridaemia. The most frequently cited sources are from the NCEP Report of the

Expert Panel on Blood Cholesterol Levels in Children and Adolescents (National Cholesterol Education Panel, 1991; American Academy of Pediatrics, 1992; National Cholesterol Education Program, 1992). However, despite citing these reports the actual values reported as being applied within the studies vary from a value >90th percentile (Platat *et al.*, 2006), >95th percentile (Boney *et al.*, 2005), or a value of ≥1.24 mmol/L (Cook *et al.*, 2003; Duncan *et al.*, 2004; Ford *et al.*, 2005; Park *et al.*, 2006). Most other studies that state, or suggest, they are modifications of NCEP ATPIII criteria (National Cholesterol Education Program, 2001b) cite percentile cut-offs ranging from 89th (Jolliffe and Janssen, 2007) to >95th (Weiss *et al.*, 2004b), and values ranging from >1.1 mmol/L (Retnakaran *et al.*, 2006) to >1.8 mmol/L (Golley *et al.*, 2006). Within the ranges given in all of the studies in Table 2.2 the percentiles can be with reference to the sample population (Bavdekar *et al.*, 1999), the controls (Invitti *et al.*, 2006), age (Boney *et al.*, 2005), age and gender (Cruz and Goran, 2004), or age, gender and ethnicity (Katzmarzyk *et al.*, 2001).

The NCEP Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (National Cholesterol Education Panel, 1991; American Academy of Pediatrics, 1992; National Cholesterol Education Program, 1992) states that hypertriglyceridaemia in children and adolescents is identified for children aged 10 – 19 years as a borderline high range of 1.02 – 1.46 mmol/L, the midpoint value for triglycerides has been identified as ≥1.24 mmol/L (Cook *et al.*, 2003) and taken as the 90th percentile value for age.

2.1.4.1.4.2 High-density lipoprotein cholesterol

Similar to the identification of elevated triglyceride, the criteria for the identification of low HDL also varies, from ≤0.8 mmol/L (Golley *et al.*, 2006) to <1.3 mmol/L (de Ferranti

et al., 2004) and <5th percentile (Boney *et al.*, 2005) to the 26th and 43rd percentiles for males and females, respectively (Jolliffe and Janssen, 2007).

Again, the NCEP Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (National Cholesterol Education Panel, 1991; American Academy of Pediatrics, 1992; National Cholesterol Education Program, 1992) is perhaps the most widely cited and states that the borderline low range for HDL in children and adolescents is 0.91 – 1.16 mmol/L for all ages and both genders. The mid-point value, also identified as the 10th percentile, has been given as ≤1.03 mmol/L (Cook *et al.*, 2003).

2.1.4.1.4.3 Fasting plasma glucose

The identification of dysglycaemia in children and adolescents in metabolic syndrome studies is made based on an elevated fasting plasma glucose level (FG), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or diagnosed diabetes mellitus. Studies using elevated fasting plasma glucose typically use values of ≥6.1 mmol/L or, more recently, ≥5.6 mmol/L following a lowering of the adult FG cut point (American Diabetes Association, 2002; Genuth *et al.*, 2003). IFG is frequently identified as a fasting plasma glucose level ≥6.1 mmol/L and <7.0 mmol/L, again more recent studies apply a range of ≥5.6 - <7.0 mmol/L. The terms for FG and IFG are frequently used interchangeably in the literature. IGT is identified following a 2 hour oral glucose tolerance test (OGTT) and is most often defined as a plasma glucose concentration of ≥7.8 and <11.1 mmol/L. Type 2 diabetes mellitus is identified as a fasting plasma glucose concentration >7.0 mmol/L or OGTT ≥11.1 mmol/L (Genuth *et al.*, 2003). As with all previous classical components of the metabolic syndrome the defining criteria for glucose handling abnormalities varies, with some authors choosing percentile cut-off points of ≥90th (Chu *et al.*, 1998; Park *et al.*, 2005b) or >97th (Csabi *et al.*, 2000). Others choose small, but potentially significant modifications of plasma glucose levels (e.g. 6.1 – 7.9 mmol/L (Golley *et al.*, 2006)).

2.1.4.1.5 Potential additions to metabolic syndrome definition criteria

2.1.4.1.5.1 Alanine aminotransferase

Unexplained elevations in aminotransferase concentrations have been strongly associated with adiposity and thus may represent non-alcoholic fatty liver disease (NAFLD) (Clark *et al.*, 2003). Alanine aminotransferase (ALT) is the liver enzyme with the closest association with liver fat accumulation (Schindhelm *et al.*, 2006) and consequently has been used as a circulating marker of NAFLD. Moreover, liver fat accumulation predicts the development of metabolic syndrome, type 2 diabetes and cardiovascular disease (Kotronen and Yki-Jarvinen, 2008). In adults, NAFLD is considered to be the hepatic manifestation of the metabolic syndrome and is associated with insulin resistance (Marchesini *et al.*, 1999; Hanley *et al.*, 2007) and reductions in insulin sensitivity (Marchesini *et al.*, 2001). Furthermore, elevated ALT predicts future incidence of type 2 diabetes (Sattar *et al.*, 2004), metabolic syndrome (Hanley *et al.*, 2005), atherothrombotic cardiovascular disease (Marchesini *et al.*, 2001), risk of mortality from liver diseases (Kim *et al.*, 2004) and predicts coronary heart disease events (Schindhelm *et al.*, 2007).

The prevalence of NAFLD in adults has been estimated to range from 3% to 24% (Clark, 2006). In children there have been few population based studies identifying the prevalence of NAFLD although it has been reported to be 2.6% in Japanese children (Tominaga *et al.*, 1995) and 3% in American children (Strauss *et al.*, 2000). Autopsy findings of children and adolescents adjusted for age, gender and ethnicity have found a prevalence of 9.6% increasing with age (Schwimmer *et al.*, 2006). The prevalence of elevated ALT in adults has been reported between 2.8% (Ruhl and Everhart, 2003) and 11.1% (Kariv *et al.*, 2006), depending on the definition employed while in children and adolescents it has been reported to range from 7.4% to 11.5%, depending on ethnicity, with a greater prevalence among males than females (Fraser *et al.*, 2007). The identification of elevated ALT concentrations in children depends on the cut-off criteria utilised and studies have used

values of >30 U/L (Strauss *et al.*, 2000), >40U/L (Park *et al.*, 2005a), >75th percentile of the sample population (Patel *et al.*, 2011) or ≥95th percentile (England *et al.*, 2009). Recently, using data from the National Health and Nutrition Examination Survey (NHANES), it has been suggested that cut-off values are set too high for the reliable detection of childhood liver disease and 95th percentile values of >25 U/L and >22 U/L should be applied, for males and females aged 12-17 years, respectively (Schwimmer *et al.*, 2010). Insulin resistance (Kahn *et al.*, 2005b) and adiposity (Zimmet *et al.*, 2005; Alberti *et al.*, 2006) are believed to play important roles in the pathogenesis of the metabolic syndrome. The rising prevalence of the metabolic syndrome and its adverse effects has prompted growing concern about this condition not only in adults but also in adolescents (Weiss *et al.*, 2004a). Moreover, elevations in ALT (Park *et al.*, 2005a; Patel *et al.*, 2011) and NAFLD in overweight and obese children (Schwimmer *et al.*, 2008) are adversely associated with all of the major components of the metabolic syndrome. Physical activity has also been found to be inversely associated with metabolic risk, independent of potential confounders, and this relationship may be stronger in children with low cardiorespiratory fitness (Brage *et al.*, 2004).

2.1.5 Utility of metabolic syndrome in children and adolescents

Although there is ongoing debate (Kahn *et al.*, 2005b; Beaser and Levy, 2007; Kahn, 2007; de Zeeuw and Bakker, 2008) the metabolic syndrome has been shown to be a predictor for cardiovascular disease and type 2 diabetes mellitus (Kahn *et al.*, 2005b; Gami *et al.*, 2007). Several studies have examined the hypothesis that the metabolic syndrome is a developmental disorder (McMillen and Robinson, 2005) influenced by exposure to risk factors during the first two decades (Batey *et al.*, 1997; Reaven *et al.*, 1998) and others have demonstrated that risk factor clustering in apparently healthy children remains stable into adulthood (Bao *et al.*, 1994; Chen *et al.*, 2000; Katzmarzyk *et al.*, 2001). In addition, individual risk factors also track from childhood in to adulthood. This has been shown to

be true for childhood obesity (Srinivasan *et al.*, 2002; Eisenmann *et al.*, 2004), serum lipid and lipoprotein levels (Nicklas *et al.*, 2002), blood pressure (Bao *et al.*, 1995; Lambrechtsen *et al.*, 1999; Lane and Gill, 2004), and insulin levels (Srinivasan *et al.*, 2002). In particular, childhood obesity has been shown to track within childhood from the first year of life (Vogels *et al.*, 2006) and also to predict for the metabolic syndrome in adulthood (Vanhala *et al.*, 1999). Parental obesity more than doubles the risk of adult obesity among both obese and non-obese children under 10 years of age (Whitaker *et al.*, 1997). Moreover, in obese children the prevalence of type 2 diabetes has been recorded in 0.14% to over 4%, with impaired glucose tolerance found in 4.5% to 14.9% (Sinha *et al.*, 2002; Invitti *et al.*, 2003a; Lobstein *et al.*, 2004). It has been suggested that the degree of clustering in metabolic syndrome components is most likely influenced by age-related changes in obesity and insulin resistance (Chen *et al.*, 2000). Interestingly, young adults who maintained a stable BMI over a 15 year follow-up period, regardless of baseline BMI, were shown to have minimal progression of risk factors and a lower incidence of the metabolic syndrome (Lloyd-Jones *et al.*, 2007).

2.2 Hypertension

2.2.1 Introduction

2.2.1.1 Blood pressure

Blood pressure occurs as a consequence of the heart pumping blood into the closed circuit of the vascular system. The level of the blood pressure is determined by cardiac output, the product of heart rate and stroke volume, and total peripheral vascular resistance to blood flow. If the heart ejected blood at a constant rate into the vasculature and if the resistance to flow provided by the peripheral vessels remained constant then blood pressure would remain at a single constant value. However, the heart provides a pulsatile ejection of blood into a vasculature with heterogeneous morphology, and variable tone. This produces a blood pressure that cyclically fluctuates between an upper limit, each time the heart contracts, and lower limit, when the heart is relaxed in-between contractions, the levels of which may change dependent on the body's requirements for homeostasis. In the presence of disease states or other unidentified disturbances in homeostasis the blood pressure can rise and be maintained at higher levels to such a degree that detrimental physical remodelling of the cardiovascular system can occur, including ventricular hypertrophy, particularly of the left ventricle (Levy *et al.*, 1996); increased intima-media thickness and accelerated fatigue fracture of vascular elastin filaments (Nichols and O'Rourke, 2005). This increase in blood pressure is termed hypertension.

With ageing there is a steep rise in brachial SBP from childhood to approximately 18 years of age (Uiterwaal *et al.*, 1997) followed by a plateau until approximately 40 years of age followed by a subsequent steady rise. This effect is particularly evident in males (Figure 2.1 and Figure 2.2) and to a lesser effect in females (Figure 2.2). Brachial DBP displays a steady rise until the age of 50 years followed by a plateau or even a decline. Brachial pulse pressure increases markedly from childhood to early adulthood, yet while SBP plateaus from 18 – 40 years of age, pulse pressure appears to decrease due to the rise in DBP (Burt

et al., 1995; O'Rourke, 1999). From the age of 40 years there is a widening of pulse pressure with age. Conversely central (aortic) SBP displays a steady increase throughout the life course (Nichols *et al.*, 1985).

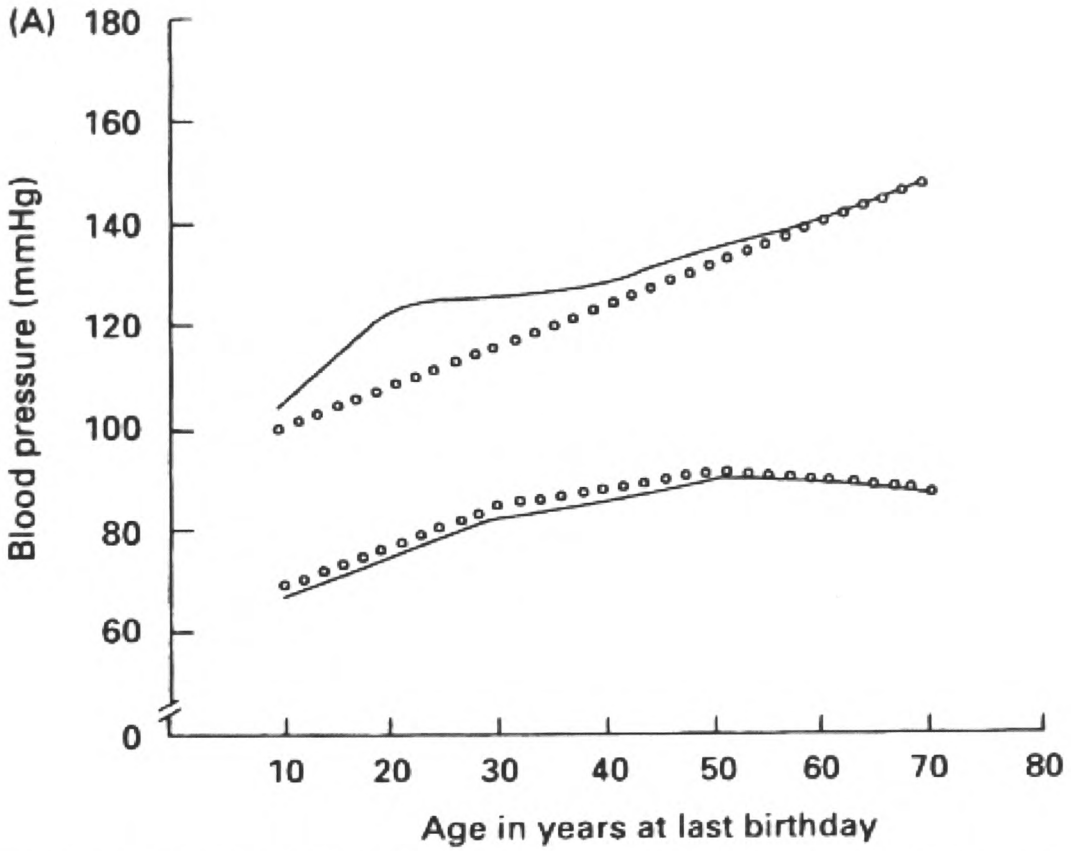

Figure 2.1: Change in arterial blood pressure with age for males (US National Health Survey, 1977). Solid lines represent published epidemiological data, dotted lines represent estimated aortic pressure values (subsequently confirmed by invasive measurement (Nichols *et al.*, 1985). Reproduced from O'Rourke (2000).

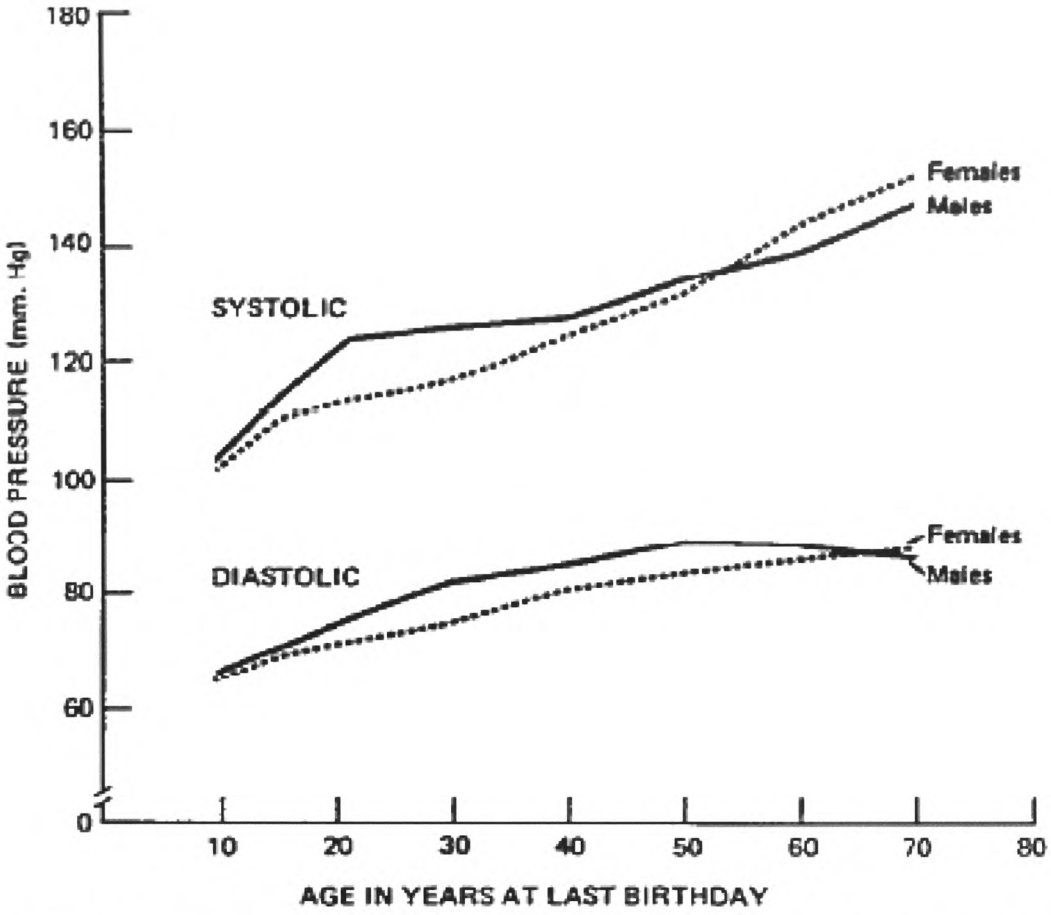

Figure 2.2: Mean systolic and diastolic blood pressure of persons 7-74 years, by age and gender. Reproduced from US National Health Survey (Roberts and Maurer, 1977; US National Health Survey, 1977).

Age related changes in brachial blood pressure have been explained by examination of the pulse wave (Nichols and O'Rourke, 2005). With ageing there is a progressive stiffening of the predominantly elastic central arteries leading to a concomitant increase in the pulse wave velocity (see Chapter 2, section 2.3: Arterial stiffness). In young adults the return of the reflected pressure wave arrives during the diastolic period augmenting diastolic pressure and myocardial blood flow. With ageing and increasing stiffness of the central arteries the reflected wave returns sooner and augments the systolic pressure. Such augmentation is the principal cause of increased systolic pressure, of decreasing diastolic pressure, and increasing pulse pressure over the age of 50.

The constancy of brachial SBP between the ages of 18 – 40 years (and the decline in PP) may also be explained by examination of the pulse wave and in particular its transmission from the aorta to the brachial artery. In adolescence there is an amplification of the pressure wave from central to peripheral arteries. This amplification can lead to brachial SBP and PP measuring 15-20 mmHg higher than central pressures. With ageing and arterial stiffening this amplification is reduced and brachial pressures may then be within ~5 mmHg of central pressures (Nichols and O'Rourke, 2005).

Hypertension is a common condition, affecting ~25% of the overall population (Burt *et al.*, 1995; Colhoun *et al.*, 1998) and is a major risk factor for cardiovascular disease and a component risk factor for the metabolic syndrome. Hypertension may be broadly divided into primary hypertension where the underlying cause is unknown and secondary hypertension where the hypertension itself is secondary to a known underlying disease or condition. Epidemiological data has shown that the incidence of hypertension increases with age, and that the form of hypertension is also modified with aging (Colhoun *et al.*, 1998; Franklin *et al.*, 2001a). Hypertension may be further subdivided dependent on which component of the blood pressure is elevated. Essential hypertension (EH) describes the condition in which the systolic and the diastolic blood pressures are both elevated, or the diastolic pressure alone is raised. Isolated systolic hypertension (ISH) describes individuals who exhibit an increased systolic blood pressure while maintaining normal diastolic blood pressure. In younger individuals (less than 50 years of age) the predominant form of hypertension is essential hypertension, whereas in older individuals isolated systolic hypertension becomes the most common form affecting ~50% of individuals aged over 60 years (Nielsen *et al.*, 1995; Colhoun *et al.*, 1998). Interestingly, recent data suggest that isolated systolic hypertension is also prevalent in younger individuals and that the cause of this condition in these individuals may not be the same as that for older individuals.

The aetiology of hypertension differs with the differing forms described. Essential hypertension is characterised by an increased peripheral vascular resistance, whereas isolated systolic hypertension is thought to be mainly caused by larger artery stiffening. The precise component of blood pressure that best predicts risk has been the subject of considerable debate. It has been historically suggested that the diastolic pressure had the greater influence on cardiovascular events following the publication of the third edition of Sir James Mackenzie's text (published after his death) where a statement was included suggesting increased SBP was a sign of a strong heart and an increased DBP a sign of increased peripheral vascular resistance (Nichols and O'Rourke, 2005, p373). Subsequently, the findings of several landmark studies (Framingham, Multiple Risk Factor Intervention Trial (MRFIT), Systolic Hypertension in the Elderly Program (SHEP)) stress the influence of systolic pressure rather than DBP (Kannel *et al.*, 1971; Kannel *et al.*, 1981; Amery *et al.*, 1985; Rutan *et al.*, 1988; SHEP, 1991; Domanski *et al.*, 2002), or the difference between systolic and diastolic pressure, the pulse pressure (Franklin *et al.*, 1999), as the best predictor of cardiovascular risk. However, given that the incidence of the different forms of hypertension change with age, it follows that the component that best predicts risk will also change with age. At young ages brachial SBP and PP does not accurately reflect central SBP and PP explaining the greater predictive value of DBP for cardiovascular disease at younger ages.

Treatment to reduce hypertension in younger and older individuals leads to improvements in cardiovascular risk factor profiles, whether by lifestyle intervention or by pharmacological intervention

2.2.1.2 Hypertension and cardiovascular risk

Present guidelines for starting blood pressure lowering treatment are based on the absolute risk estimate of coronary heart disease (Chobanian *et al.*, 2003). It has been well

established that in young participants DBP is a better predictor than SBP, whereas in older age the reverse is true (Franklin *et al.*, 1999; Franklin *et al.*, 2001b; Wilkinson *et al.*, 2001a). This observation is explained by the fact that at young age DBP correlates best with central pressures, whereas SBP can vary greatly when measured centrally or brachially as seen in spurious systolic hypertension (see below). The presence of a higher brachial SBP with a normal central pressure in a young individual may therefore not reflect an increased risk (Hulsen *et al.*, 2006).

2.2.1.3 Pseudo systolic hypertension of youth

Hypertension in young people is uncommon yet has been shown to occur in 12% of subjects aged 17 – 27 years (McEniery *et al.*, 2005b). Several studies have now described elevated brachial systolic pressure in young individuals (O'Rourke *et al.*, 2000; Mahmud and Feely, 2003; McEniery *et al.*, 2005b; Hulsen *et al.*, 2006). Data from a number of studies suggest that isolated systolic hypertension is prevalent both in adolescence (Sorof *et al.*, 2002) and early adulthood (Mallion *et al.*, 2003). Although the mechanisms underlying isolated systolic hypertension in younger individuals are poorly understood, two recent studies suggest that an exaggerated amplification of the pulse pressure from the central to the peripheral arteries may be responsible, and this being the case the terms "spurious systolic hypertension" (O'Rourke *et al.*, 2000), "pseudo-systolic hypertension" (Mahmud and Feely, 2003) and "pseudo hypertension of youth" (Cockcroft *et al.*, 2003) have been applied. Pseudo hypertension has been previously described as a condition affecting older individuals. It is characterised by a calcification and concomitant hardening of the arteries to such an extent that they become resistant to compression. Traditional sphygmomanometric blood pressure measurement is dependent on how much force it takes to compress the brachial artery, having thick stiffened arteries falsely elevates the sphygmomanometer reading or peripheral blood pressure. In younger individuals this age

related calcification and stiffening of the arteries is unlikely to have occurred, and it would seem likely that alternative mechanisms are responsible.

O'Rourke *et al.* (2000) described a case study in which six young, otherwise healthy, male subjects aged from 14 to 23 years presented with consistently elevated systolic pressure measured repeatedly by sphygmomanometer cuff in the brachial artery (mean, 161 ± 9 mmHg). These subjects were referred for further assessment which included measurement of radial and carotid artery waveforms with applanation tonometry, and synthesis of the ascending aortic waveform using a generalised transfer function. In each case diastolic pressure was normal, elevated systolic pressure was associated with, and due to, a high narrow peak of the pressure wave recorded in the radial artery. Mean pressure was normal, and the synthesised aortic pressure wave was described as being of normal amplitude and contour, and with a "normal" aortic systolic pressure (mean, 119 ± 6 mmHg). The carotid pulse wave form was normal for their respective ages, and the ascending aortic waveform synthesised from the carotid pulse was identical to the ascending aortic waveform synthesised from the radial pulse.

These subjects displayed an elevated brachial systolic blood pressure with a normal diastolic pressure which would classify these individuals as having isolated systolic hypertension. However, the further assessment identified normal ascending aortic systolic pressure. O'Rourke *et al.* termed this condition "spurious systolic hypertension" of youth (2000, p.142) because the brachial systolic pressure was falsely elevated compared to the aortic systolic pressure, and it has been suggested that it is the pressure that the heart is subject to that is of consequence for adverse cardiac remodelling (McEniery *et al.*, 2006). They further hypothesised that in young adults this condition represents an extreme of the normal situation for this age group, where elevated brachial and radial systolic pressures represent nothing more than an unusually high amplification of the initial pressure wave, in

part due to very distensible arteries. However, central to peripheral pressure amplification was not specifically measured in these subjects. O'Rourke *et al.* (2000) suggested that if their hypothesis and postulated mechanism was correct, then evidence would be found in population studies that systolic pressure should be unusually high in all young adults, and more so in males than in females because of their greater stature. Examining data from the 1977 USA National Health Survey, and the Australian National Heart Foundation study for adult males combined with a similar study in Australian children, O'Rourke *et al.* (2000) highlights the steep rise in brachial systolic pressure from the ages of 5-20 years, with a plateau between ages 20 to 40 years, followed by a progressive rise thereafter. When compared to female data from the same studies, the pattern was described as similar although the variations were not as marked.

In an attempt to extend the work of O'Rourke *et al.* (2000), and examine pulse pressure amplification in young adults with elevated brachial systolic blood pressures, Mahmud and Feely (2003) used the same technique of pulse wave analysis to compare brachial blood pressure to aortic blood pressure. They studied 174 young, male and female adults aged 23 ± 0.5 years, measuring radial artery waveforms and synthesising aortic waveforms and aortic pressures, again with applanation tonometry. Furthermore, they calculated pulse pressure amplification by subtracting the aortic pulse pressure from the brachial pulse pressure. Of the 174 subjects, 11 were identified who fitted the criteria for spurious systolic hypertension (elevated brachial systolic pressure but normal brachial diastolic and aortic systolic blood pressure). All 11 of these subjects, in addition to having higher brachial systolic pressures, were slightly taller, had lower resting heart rates, had greater aortic systolic pressures and increased pulse pressure amplification when compared to age and gender matched normotensive controls. Moreover, all were male, non-smokers, and physically active in sports. Therefore lending support to the hypothesis of O'Rourke *et al.* (2000) that spurious systolic hypertension of youth, in young men, is due to exaggerated

pulse pressure amplification. Recently, McEniery *et al.* (2005b) investigated the prevalence and mechanisms of ISH in a large cohort of young adults. They hypothesised that EH and ISH result from different haemodynamic processes: ISH resulting from an elevated cardiac output and/or arterial stiffness, and EH as a result of increased peripheral vascular resistance (PVR). In a cross-sectional cohort of 1008 young subjects aged 17 – 27 years from the ENIGMA study, in addition to blood pressure, measurements were also made of aortic stiffness, cardiac output, stroke volume (SV), PVR and physical activity levels. McEniery *et al.* (2005b) report an overall hypertension prevalence of 12% with an ISH prevalence of 8%. Moreover, they confirmed their hypothesis that ISH and EH result from different underlying haemodynamic mechanisms with ISH involving elevations of SV and/or aortic stiffness with a normal PP amplification, and EH involving an elevated PVR, decreased SV, normal aortic stiffness and reduced PP amplification. Furthermore, although ISH subjects had a normal PP amplification ratio, central SBP was higher than in normotensive individuals suggesting that ISH is not a condition of PP amplification and may not be benign, as previously thought.

In a more recent report, Hulsen *et al.* (2006) aimed to investigate the prevalence and determinants of spurious systolic hypertension in a population based sample of young adults. In addition, they estimated their 20 year risk score for coronary heart disease based on the Framingham risk score. From a population of 750 men and women aged 26-31 years they identified 57 men and 3 women with elevated systolic pressure, normal diastolic pressure and normal central systolic pressure. The three female subjects were excluded from further analysis because of the low numbers. The study of Hulsen *et al.* (2006) differed from the studies of O'Rourke *et al.* (2000) and Mahmud and Feely (2003) in that they did not consider the shape of the pulse waveform for the identification of spurious systolic hypertension. Rather, they used a figure at, or below the 90th percentile score of the central systolic pressure for the group to identify those subjects with low central

pressures. Although, the authors do note that because of this differing diagnostic methodology it is possible that a number of their subjects may differ on certain characteristics from the previously reported studies. Pulse pressure amplification was slightly higher in the spurious systolic hypertension group compared to normotensive controls and the majority of subjects identified were male. This finding lends support of the hypothesis proposed by O'Rourke *et al.* (2000) and the findings of Mahmud and Feely (2003). However, when Hulsen *et al.* (2006) examined some of the factors that are determinant of pulse pressure amplification (in this case arterial stiffness, heart rate and height), they found that there was no difference in pulse wave velocity (a measure of arterial stiffness) between the groups and no difference in heart rate. Additionally, although the subjects with spurious systolic hypertension were slightly taller than normotensive controls, this did not reach statistical significance.

Pulse pressure is the product of stroke volume and arterial stiffness, and varies throughout the arterial tree. Normally, there is amplification of systolic blood pressure moving from the aorta to the peripheral arteries (Nichols and O'Rourke, 2005), with little change in diastolic pressure and mean arterial pressure. Therefore, an increased peripheral pulse pressure could either reflect an increased central pressure with a normal degree of amplification, or a relatively normal central pulse pressure but with an exaggerated amplification (Cockcroft *et al.*, 2003).

2.3 Arterial stiffness

2.3.1 Introduction

Assessment of the arterial pulse has always been an important part of clinical examination. Indeed, an Egyptian papyrus dating from the 17th Century BCE notes:

> "... examining is like one counting a certain quantity with a bushel, or counting something with the fingers ... like measuring the ailment of a man in order to know the action of the heart. There are canals in it [the heart] to every member. Now if the priests of Sekhmet or any physician put his hands or his fingers upon the head, upon the two hands, ... upon the two feet, he measures to the heart ... because its pulsation is in every vessel of every member ... Measure ... his heart in order to recognize the indications which have arisen therein; meaning ... in order to know what is befalling therein" (Edwin Smith Papyrus, 17th Century BCE).

More recently, Galen (130-200 ACE) palpated the pulse and classified it in terms of strength, rate and rhythm. With the development of the sphygmograph by Marey (1860) and its subsequent refinement by Mahomed (1872), Broadbent (1890) and Mackenzie (1902), important contributions were made to the interpretation of the pressure wave and the shape of the arterial waveform. Although it had been noted that changes in the contour of the arterial pressure waveform changed with age and accompanied various disease states such as hypertension (Bright's disease) (Mahomed, 1872), gradual stiffening of the arteries was generally thought to be an inevitable consequence of aging. Roy (1881) suggested that this may have significance for the health of the individual and commented that, "only in the case of young children do we find that the elasticity of arteries is so perfectly adapted to the requirements of the organism as it is in the case of the lower animals." Roy further pointed out that "With old age the elasticity of the arteries is found greatly modified in its characters, becoming less and less fitted to enable the arteries to fulfil their function in the

economy" (Roy, 1881, p.159). Together these findings suggest that the implications of arterial stiffening with respect to cardiac load were appreciated at the time, together with the relentless stiffening that occurs in humans with age (Nichols and O'Rourke, 2005).

The arterial pulse is a fluctuation caused by heart contraction and occurs at the same frequency as the heart rate. The ejection of blood from the left ventricle through the aortic valve and into the ascending aorta leads to flow, pressure and diameter pulsations throughout the systemic arterial system (Nichols and O'Rourke, 2005). Blood flow can now be measured in a number of ways as can arterial diameter (Nichols and O'Rourke, 2005). Blood pressure, as previously described, can be measured invasively via a catheter-manometer methodology (Nichols and O'Rourke, 2005), or as is more common in clinical settings, non-invasively via auscultatory sphygmomanometry or oscillometric sphygmomanometry. Any of these fluctuations can be considered as the 'pulse', but most clinicians refer to the pulse only as the arterial pressure pulses which can be palpated in large accessible arteries (Asmar, 1999) in the periphery, typically at the radial artery.

2.3.2 The human arterial system

The arterial wall is divided into three concentric regions, the tunica intima, tunica media and tunica adventitia. The innermost region, the tunica intima is composed of the vascular endothelium, a single layer of cells that lines the entire vascular system, a thin layer of cells and a thin layer of elastin and collagen fibres that anchor it to the internal elastic lamina. Elastin and collagen fibres are the predominant material in the vascular wall with elastin presenting in continuous sheets. The internal elastic lamina marks the demarcation between the tunica intima and the tunica media. The tunica media forms the major part of the artery wall and determines the artery's mechanical properties (Nichols and O'Rourke, 2005). It is composed in lamellar units or layers which have a fibrous structure (elastin and collagen) with the fibres running circularly or in a tight spiral. In between these layers lie

smooth muscle cells that for the most part lie parallel to the elastin fibres although some lie longitudinally. The closely interlocked elastin, collagen and smooth muscle in the tunica media leads to the artery acting mechanically in a homogeneous manner. The outer elastic lamina separates the tunica media from the outermost tunica adventitia. The tunica adventitia is a region of collagen fibres and some elastin that merges with the surrounding connective tissues, small blood vessels and nerves.

As stated previously, the structure of the tunica media determines an individual artery's mechanical properties. In the proximal aorta elastin is the predominant fibre (approximately 60% in canine thoracic aorta) while in the distal aorta the content reverses, and in the peripheral arteries collagen becomes the predominant fibre (approximately 70% in canine extrathoracic arteries)(Harkness *et al.*, 1957). The elastic modulus of collagen [0.75 mmHg/cm (1000 x 10^6 dyne/cm^2)] is much higher than that of elastin [0.002 mmHg/cm (3 x 10^6 dyne/cm^2)], so that as the distance from the heart increases, the elastic modulus (stiffness) increases (Nichols and O'Rourke, 2005). The importance of the elastic fibres within the arteries becomes apparent when one considers the pulsatile load that they have to bear. Elastin fibres are arranged as concentric plates and serve to accommodate and dampen the pulsatile expulsion of blood from the left ventricle (Bergel, 1961). Collagen fibres are arranged circumferentially and are laid down in the vessel walls with some slackness in an un-stretched state. This arrangement allows the elastin fibres to stretch and the artery as a whole to stretch at physiologic distending pressures (Roach, 1977; Dobrin, 1978). Whereas, at higher pressures, the stiffer collagen fibres are recruited and serve as a safety net to prevent vessels rupturing. As a consequence, the stress-strain relationship of the arterial wall is non-linear with elastin bearing a portion of the circumferential and longitudinal load at low pressures (Ho *et al.*, 1972) and collagen assuming responsibility for circumferential load at higher pressures (Wolinsky and Glagov, 1964; Armentano *et al.*, 1991).

Elastin and collagen are not solely responsible for vascular tone within arteries. Smooth muscle cells within the tunica media are innervated by sympathetic neurons of the autonomic nervous system leading to vasoconstriction when sympathetic stimulation is increased. Vasodilatation occurs when sympathetic stimulation decreases. Furthermore, the vascular endothelium is now known to produce a number of chemical substances that play important roles in the regulation of vascular tone, haemostasis, immune and inflammatory responses and lead to various physiological (e.g. inflammation) and pathological (e.g. atherosclerosis) responses (Vane *et al.*, 1990). Indeed, the pivotal role of the vascular endothelium in the pathogenesis and progression of atherosclerosis is now well established (Ross, 1993).

For many years the tunica adventitia received minor attention as a contributor to vascular control. However, there is evidence to suggest that adventitial regulation of vascular function may be important in several circumstances such as subintimal cell proliferation that precedes atherosclerosis, vascular calcification and the release of neurotransmitters from sympathetic and vagal efferent fibres that regulate vasomotor tone (Gutterman, 1999). Interestingly, periadventitial adipose tissue, and more specifically cytokines released from this adipose tissue depot has been shown to contribute to coronary vascular regulation by impairing endothelial function through direct inhibition of endothelial nitric oxide synthase (Payne *et al.*, 2009).

The function of the systemic arterial system is to deliver blood at pressure and in a continuous stream to various peripheral (and central) vascular beds. The arterial system itself can be thought of as having three distinct regions (Nichols and O'Rourke, 2005): 1) The large arteries, especially the elastic arteries which serve as a cushioning reservoir that stores blood during left ventricular systole and expels it to the periphery during left

ventricular diastole; 2) The long muscular, conduit arteries that serve to deliver blood to the periphery and vital organs. These arteries are able to modify pulse wave propagation by alterations in smooth muscle tone; 3) The arterioles, which by changes in diameter are able to modify peripheral vascular resistance and therefore serve to maintain mean arterial pressure and the delivery of blood to in a steady flow to the organs in need. As a consequence of the non-uniform nature of the vessels that make up the arterial system, analysis of the stiffness of the arteries becomes problematic. The result is that numerous indices have been introduced to quantify arterial stiffness.

2.3.3 Vasodilatation and vasoconstriction

Endothelial derived nitric oxide (NO) is a potent vasodilator which diffuses from the vascular endothelium to act on the layers of smooth muscle tissue within the vascular media causing muscle relaxation and a reduction in vascular tone. Endothelin (ET) is a vasoconstrictor with the opposite effect of NO. However, studies have shown no change in forearm blood flow with ET receptor blockade in healthy humans, but vasodilatation in hypertensive and hypercholesterolaemic patients. This suggests ET receptor activity is different in healthy humans (McEniery and Wilkinson, 2002; McEniery *et al.*, 2002). There is substantial evidence that many endothelial functions are sensitive to the presence of reactive oxygen species and subsequent oxidative stress. Exogenous antioxidants can normalise the endothelium-dependent vasodilatation response in healthy subjects as well as in several conditions at risk for atherosclerosis (Pratico, 2005).

2.3.4 Indices of arterial stiffness

In simple terms, arterial stiffness describes the rigidity of the arterial walls. However, in the field of arterial stiffness, terminology can be confusing and terms are frequently used interchangeably. No one index has proved superior and all have problems associated with measurement and interpretation. Table 2.3 contains terms and definitions that have been

used to describe arterial stiffness and are generally agreed as an interim measure (O'Rourke, 1995; Asmar, 1999; Mackenzie *et al.*, 2002; Nichols and O'Rourke, 2005). However, use of these terms to assess arterial stiffness may be problematic since the properties are different in different arteries, in the same artery at different distending pressures, and with activation of smooth muscle in the vessel wall (O'Rourke, 1995).

Table 2.3: Indices of arterial stiffness

Term	Definition
Arterial compliance	Absolute change in diameter (or area) for a given change in pressure $\Delta D/\Delta P\ (cm/mmHg, or\ cm^2/mmHg)$
Arterial distensibility	Relative change in diameter (or area) for a given change in pressure (inverse of the elastic modulus) $\Delta D/(\Delta P \times D)\ (mmHg^{-1})$
Capacitative compliance (C_1, or large artery elasticity index)	Relationship between volume change and pressure change in the arteries during the exponential component of diastolic pressure decay $\Delta V/\Delta P\ (cm^3/mmHg)$
Oscillatory compliance (C_2, or small artery elasticity index)	Relationship between the oscillating volume change and oscillating pressure change around the exponential component of diastolic pressure decay $\Delta V/\Delta P\ (cm^3/mmHg)$
Peterson's Elastic modulus	The pressure change required for a theoretical 100% stretch from the resting diameter $(\Delta P \times D)/\Delta D\ (mmHg)$
Young's Elastic modulus	Elastic modulus per unit area, wall tension per cm wall thickness for 100% diameter increase (Young's elastic modulus represents the slope of the stress-strain curve) $(\Delta P \times D)/(\Delta D \times h)\ (mmHg/cm)$
Volume elastic modulus	The pressure change required for a theoretical 100% increase in volume $\Delta P/(\Delta V/V)\ (mmHg)$
Pulse wave velocity	Velocity of travel of the pulse along a length of artery $Distance/\Delta t\ (cm/s)$
Pressure augmentation	The difference between the peak systolic pressure and the pressure at the inflection point caused by a reflected wave $(P_s - P_i)\ (mmHg)$
Augmentation index	The difference between the second and first systolic peaks as a percentage of pulse pressure $[(P_s - P_i)/\Delta P] \times 100\ (\%)$
Pulse pressure	The difference between the systolic and diastolic pressures $(P_s - P_d)\ (mmHg)$
Stiffness index (β)	Ratio of natural log(systolic/diastolic pressures) to (relative change in diameter) $\beta = \dfrac{\ln(P_s - P_i)}{(D_s - D_d)/D_d}$
Characteristic impedance	The ratio of oscillatory pressure to flow at input of a tube in which no reflected wave return to the origin $(P_i - P_d)/peak\ flow\ (or\ velocity)\ (mmHg/L/min.)$

D, diameter; *P*, pressure; *V*, volume; *h*, wall thickness; *s*, systole; *d*, diastole; *i*, inflection point; *ln*, natural logarithm. Adapted from O'Rourke (1995), O'Rourke *et al.* (2002) and Mackenzie *et al.* (2002).

2.3.5 Methods for determining arterial stiffness

Following the indices of arterial stiffness several methods have been developed to measure these indices. Broadly speaking the methods of measurement rely either on imaging techniques to identify the wall bordering the lumen of the target artery and measure lumen diameter, lumen volume and arterial wall thickness or techniques to assess properties of the pressure waveform (Table 2.4).

Table 2.4: Methods for determining arterial stiffness

Term	Methods of measurement
Arterial compliance**	Ultrasound*, MRI
Arterial distensibility**	Ultrasound*, MRI
Capacitative compliance	Pressure waveform
Oscillatory compliance	Pressure waveform
Peterson's Elastic modulus**	Ultrasound*, MRI
Young's Elastic modulus**	Ultrasound*, MRI
Volume elastic modulus	Ultrasound*, MRI
Pulse wave velocity	Pressure waveform*, volume waveform, ultrasound, MRI
Pressure augmentation	Pressure waveform
Augmentation index	Pressure waveform
Pulse pressure	Pressure waveform, blood pressure*
Stiffness index (β)**	Ultrasound
Characteristic impedance	Catheterisation

*Most common method of measurement; **Also requires pressure measurement.
MRI, magnetic resonance imaging.

In recent years a number of additional methods have been developed to provide a non-invasive measure of arterial stiffness e.g. ambulatory arterial stiffness index (AASI), digital volume pulse, photoplethysmography, etc.

2.3.6 Pressure wave amplification

Each time the ventricles of the heart contract a pressure wave is initiated and subsequently propagates throughout the arterial system. The Reverend Stephen Hales conducted the first invasive measurements of blood pressure nearly 300 years ago and famously inserted a brass cannula attached to a glass tube in to the femoral artery of a horse, measuring the blood rising to a height of over 8 feet (8 ft/H_20 = 179 mmHg) (Hales, 1733). Since the development of the modern mercury sphygmomanometer by Scipione Riva-Rocci (1863-1937) and its combination with auscultation after Nikolai Korotkoff (1874-1920), for the last century clinical attention has been focussed on measuring brachial systolic (SBP) and diastolic (DBP) blood pressures, and the difference between the two identified as the brachial pulse pressure (PP). However, it is important to note that there is amplification of PP downstream of the aorta (Remington and Wood, 1956) meaning that pressure recorded conventionally in the brachial artery is not representative of pressure in the larger central arteries.

As noted in section 2.3.2 the arterial system is non-uniform in size and structure and the effect of PP amplification may be ascribed to pressure wave transmission and wave reflection (see below) through vessels with differing viscoelastic properties. This amplification of the pressure wave results in a gradual and significant increase in SBP and PP as the pressure wave moves distally from the heart, whereas DBP and mean arterial pressure (MAP) remain relatively constant or decreases slightly (1-2 mmHg) (Nichols and O'Rourke, 2005). At rest, the amplification of SBP from the aorta to the brachial artery may be 20 mmHg or more (Nichols and O'Rourke, 2005), and 10-35 mmHg from the aorta to the radial artery (Pauca *et al.*, 1992).

Pulse pressure amplification is represented by the difference between, or the ratio of, the peripheral to central pulse pressure (Wilkinson *et al.*, 2001a). The major factors

contributing to a change in the shape of the arterial pressure wave are the pattern and duration of ventricular ejection (cardiac factors) and the velocity of wave travel in large arteries, degree of vasomotor tone and wave reflection in peripheral beds (vascular factors) and also MAP. Amplification is greatest when wave velocity is low and ejection duration short. With ageing there is a progressive decrease in amplification of the central (aortic) pressure wave (O'Rourke *et al.*, 1968). Indeed, in children around 10 years of age PP measured at the femoral artery may be 50% greater than ascending aortic PP, whereas at age 65 femoral and ascending aortic PP are virtually the same. This change with age can be explained by an increase in aortic pulse wave velocity (due to age related arteriosclerosis) causing and earlier return of wave reflection, leading to an increase in the amplitude of the aortic PP, thus central PP increases relative to peripheral PP. Similarly, pressure waves in the upper limb are less amplified with age yet, in contrast, any changes are almost entirely due to the change in the aortic pressure wave contour rather than to changes in the structure of the upper limb arteries. This is because the reflected pressure wave returning from the lower body substantially affects the contour of the brachial or radial pulse contour (Kelly *et al.*, 1989), whereas reflection from the upper limb has little effect on the lower body pressure wave.

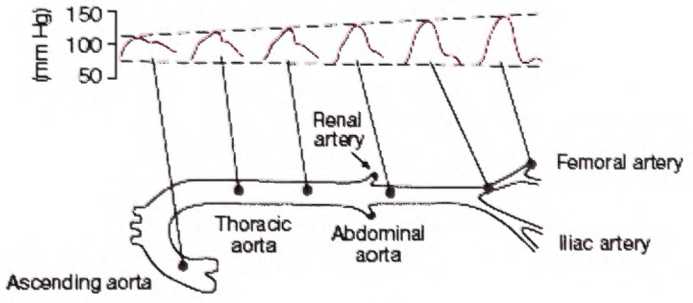

Figure 2.3: Wave amplification of systolic blood pressure and pulse pressure along the aorta of a 24-year-old (Nichols and O'Rourke, 2005).

Pressure waves show little or no perceptible change during transmission to the upper descending thoracic aorta and to the branching of the braciocephalic and subclavian

arteries with patterns of pressure being virtually identical in the proximal part of these vessels (Nichols and O'Rourke, 2005). This suggests that measurement of the pressure wave at, or close to, the braciocephalic arteries would provide a close approximation of central (aortic) blood pressures.

In young, apparently healthy individuals around 20 years of age the peripheral PP (pPP) to central PP (cPP) amplification ratio (pPP:cPP) is approximately 1.7 decreasing steadily with age to almost 1.0 in the eighth decade (Wilkinson *et al.*, 2001a; McEniery *et al.*, 2005a). As stated previously, this age related reduction is attributed to an increased aortic PWV augmenting wave reflection and subsequently central SBP. Paradoxically, infants and children display decreased brachial pressure wave amplification similar to that of older adults (Nichols and O'Rourke, 2005, p363). However, this can be explained on the basis of body length and the timing of the reflected wave. Wave reflection returns early not because of stiff arteries and increased PWV but because body length is short and reflection sites are closer.

In addition to ageing, pulse pressure amplification is also known to be reduced with hypercholesterolaemia (Wilkinson *et al.*, 2002c), vasoconstriction (Wilkinson *et al.*, 2001b) and sometimes after cardiac surgery (Nichols and O'Rourke, 2005, p179). Pulse pressure amplification may be augmented with exercise (Kroeker and Wood, 1955; Rowell *et al.*, 1968; Sharman *et al.*, 2005), postural change (Kroeker and Wood, 1955; Rowell *et al.*, 1968) and increased heart rate (Wilkinson *et al.*, 2002b). Brachial pulse pressure amplification has been found to be very sensitive to changes in the duration of ventricular ejection (O'Rourke, 1971). In addition, pressure amplification has been shown to be linearly related to heart rate (Wilkinson *et al.*, 2002b) and inversely related to mean arterial pressure (Wilkinson *et al.*, 2001b). For subjects aged 20 to 25 years, amplification is generally <26 mm Hg (Cockcroft *et al.*, 2003). Amplification of the pressure wave has also

been recorded in children (O'Rourke *et al.*, 1968) where it was found that the amplitude of the pressure wave increased progressively as it passed along the aorta, and a prominent diastolic wave appeared in the distal aorta and iliac artery.

Taken together these results suggest that: BP (SBP, DBP, MAP and PP) measured conventionally from a peripheral (brachial) artery is not representative of central BP, the pressure that the body's organs are exposed to; PP amplification differs dependant on the site of measurement with peripheral measures (e.g. brachial) typically higher than central (aortic) measures; and that PP amplification is modified with age and disease among other factors.

In normal circumstances, the arterial pressure wave is markedly amplified in transit from the ascending aorta to the radial artery while mean pressure falls by 2.0 mmHg at most. The factors responsible for amplification are similar to those described for the femoral artery (age, MAP, height, PWV). Age, however, appears to be less important in the upper limb, presumably because of the different flow wave contour in the brachiocephalic and subclavian arteries compared with the descending thoracic aorta, and the smaller change in upper limb than in aortic PWV that occurs with age (Nichols and O'Rourke, 2005).

2.3.7 Pressure wave reflection

As discussed above, contraction of the heart propagates a pressure wave through the arterial tree. When this wave is measured and recorded two principle components may be identified, an initial outgoing wave generated by ventricular contraction, and a reflected wave returning to the heart from peripheral sites of reflection. In normal healthy arteries the reflected wave returns to the heart during diastole leading to an augmentation of diastolic pressure, aiding coronary perfusion to the myocardium. In conditions where PWV is increased the reflected wave returns to the heart earlier, during systole, leading to an

augmentation of systolic pressure and increasing afterload. Afterload is a term that may be used to describe the external vascular factors that oppose ventricular systolic ejection (Nichols and O'Rourke, 2005). With increased afterload there is a consequent increase in myocardial workload and oxygen demand. With long-term elevations in afterload cardiac remodelling occurs in the form of increased left ventricular thickness.

With normal ageing there is an increase in aortic PWV leading to an earlier return of the reflected wave. The reflected wave is, therefore, principally responsible for the age related increase in amplitude of central PP. Techniques to distinguish between the outgoing pressure wave and the reflected wave are based on the identification of the foot of the reflected wave. The time from the foot of the outgoing pressure wave to the foot of the reflected wave tends to be less than the ejection duration of the heart making it identifiable during systole (Nichols and O'Rourke, 2005).

With ageing, in individuals with hypertension, diabetes, end-stage renal disease and other vascular diseases, the elastic arteries become stiffer. An increase in arterial stiffness causes an increase in PWV and an early return of the reflected wave to the ascending aorta during ventricular ejection. Such timing is detrimental, since the augmentation caused by the reflected wave increases systolic pressure and ventricular afterload (Nichols and O'Rourke, 2005, p 206).

2.3.8 Applanation tonometry

Tonometry is widely used for recording intra-ocular pressure for the diagnosis of glaucoma and is based on the theoretical principle that when the surface of a rounded chamber or vessel is flattened, tangential pressures are normalised, and a sensor placed on the outer surface will record the pressure within the chamber or vessel (Kelly *et al.*, 1989; O'Rourke and Gallagher, 1996). Applanation tonometry, applied to arteries involves the use of a

high-fidelity micromanometer placed on the surface of the skin over an accessible artery (radial, carotid, femoral, etc.) and compressing the artery against the solid structures (e.g. bone) underneath. The presence of tissue between the sensor and the vessel is known to degrade the theoretical principle somewhat. However, differences in pressure (pulse pressure) may be recorded with a good degree of accuracy and pressures recorded are identical to those recorded within the artery (Chen *et al.*, 1996) under ideal conditions. The actual systolic and diastolic pressures obtained vary with the pressure applied to the sensor by the operator and as a consequence the absolute pressure must be calibrated with a brachial blood pressure obtained with a sphygmomanometer. Although there is some amplification of pressure between the brachial and radial sites, this is small and is usually ignored.

In this thesis the technique of applanation tonometry has been used for pulse wave analysis and the measurement of pulse wave velocity using the Sphygmocor system (Atcor Medical, Sydney, Australia).

2.3.9 Pulse wave analysis

As described previously the arterial pressure waveform is a composite of the forward travelling pressure wave caused by ventricular ejection and a reflected wave. The reflected wave originates in the periphery at arterial branches and sites of impedance mismatch (O'Rourke and Gallagher, 1996). Applanation tonometry is used to record pressures at a superficial artery site (e.g. radial, carotid and femoral arteries) and a generalised transfer function (Kelly *et al.*, 1989; Karamanoglu *et al.*, 1993; Chen *et al.*, 1996) is applied to synthesise a central, aortic pressure waveform. From the derived aortic waveform a number of properties can be obtained, including the augmentation index (see below) and central arterial pressures. The waveform is calibrated using systolic and diastolic pressure values from conventional cuff measurement. Moreover, pulse wave analysis has been

adapted as a method for the non-invasive assessment of endothelial function *in-vivo* (Wilkinson *et al.*, 2002a) and is accepted as a good measure of central arterial pressures (Laurent *et al.*, 2006).

2.3.10 Augmentation pressure and augmentation index

Analysis of the aortic pressure waveform enables the identification of the point at which the reflected pressure wave is first detected, the systolic inflection point (P_1), and the pressure of the systolic peak (P_2) (Figure 2.4). In highly elastic arteries P_1 occurs during diastole, serving to augment diastolic pressure and coronary artery perfusion. However, in stiffer arteries P_1 occurs earlier causing augmentation of systolic pressure. The difference in pressure between P_1 and P_2 is the augmentation pressure (AP). The amount of augmentation increases as arteries stiffen and in the young (15 – 25) years it is common to see no augmentation (Nichols and O'Rourke, 2005). The augmentation index (AIx) is calculated as the difference between the first and second systolic peaks expressed as a percentage of the pulse pressure. AIx is considered to be a measure of wave reflection and an indirect, surrogate measure of systemic arterial stiffness (Laurent *et al.*, 2006).

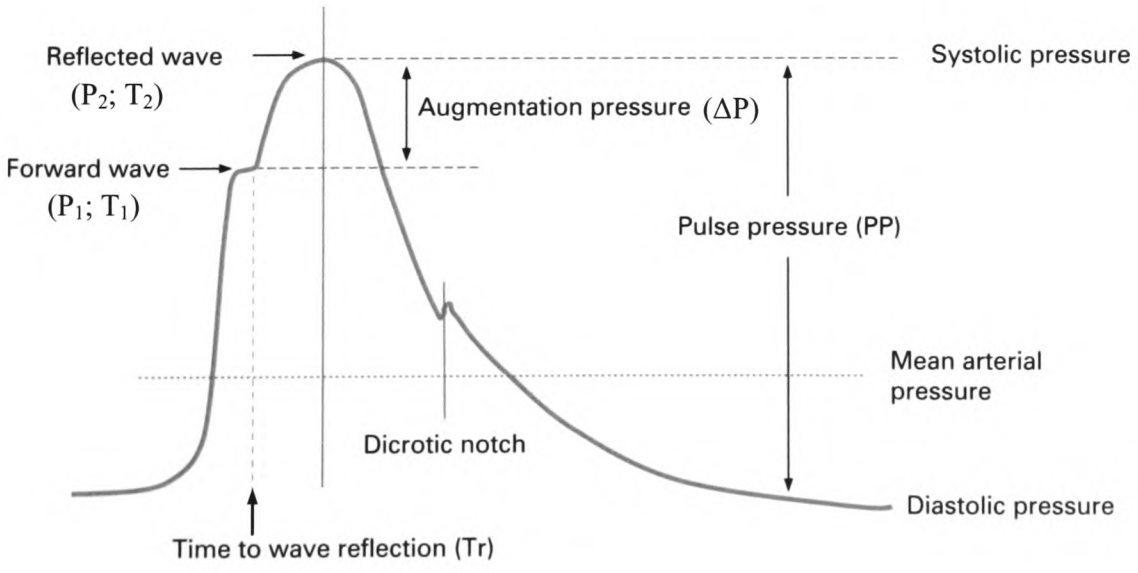

Figure 2.4: Illustration of a typical aortic pressure waveform.

2.3.11 Pulse wave velocity

Pulse wave velocity (PWV) is the speed at which the forward travelling pressure wave is transmitted from the aorta throughout the vasculature (Nichols and O'Rourke, 2005). A pressure wave ejected in to an elastic artery causes the artery to distend thus damping the pressure wave, with the purpose of converting a pulsatile flow in the conduit arteries in to a smooth, non-pulsatile flow in the more fragile distal vasculature. Conversely, a pressure wave ejected in to a stiff, inelastic artery has a reduced damping effect and the pulse wave is transmitted more rapidly. PWV increases with progressive arterial stiffness and is related to the mechanical properties of an arterial segment, the mathematical model is represented by the Moens-Korteweg equation (Nichols and O'Rourke, 2005):

$$PWV = \sqrt{\frac{Eh}{2R\rho}}$$

Where E is the elastic modulus of the arterial wall, h is the wall thickness, R is arterial radius and ρ is blood density. Pulse wave velocity is also inversely related to distensibility (D) by the Bramwell-Hill formula (Bramwell *et al.*, 1923; Van Bortel *et al.*, 2002):

$$PWV = \sqrt{\frac{1}{\rho D}}$$

Calculation of PWV is made by measuring the time taken for the pulse wave to travel between two points a given distance apart. Readings may be made simultaneously or sequentially gating the readings to a fixed point on the cardiac cycle, the R wave on an electrocardiogram (ECG). The most popular method for determining PWV is the foot-to-foot method where the foot of the upstroke of proximal and distal waveforms is identified,

thus eliminating any influence from reflected waves (Izzo and Shykoff, 2001; Pannier *et al.*, 2002; Laurent *et al.*, 2006) (Figure 2.5).

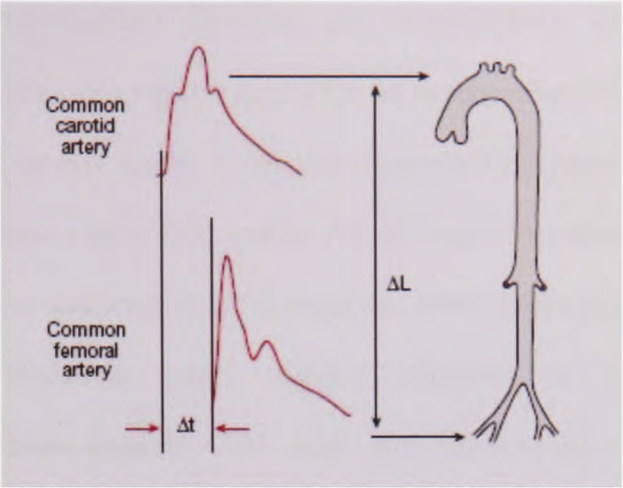

Figure 2.5: Principle of arterial stiffness measurement by pulse wave velocity with the foot-to-foot method (Laurent *et al.*, 2006). L, distance; t, time.

2.3.12 Causes of arterial stiffening

Arterial stiffness is determined by structural and functional components related to the intrinsic elastic properties of the artery and the endothelium. Elastic tissue within the intimal and medial layers and vascular smooth muscle contribute to arterial stiffness, as stated previously. Arterial stiffening occurs as a result of ageing (McEniery *et al.*, 2005a) and arteriosclerosis. Ageing results in the fatigue-fracture of elastin fibres leading to a gradual decline in elasticity. Arteriosclerosis is characterised by fibrosis of the intima and calcification of the media (Nichols and O'Rourke, 2005). However, it is now recognised that arteriosclerosis is actually pathological and is neither inevitable nor irreversible (Najjar *et al.*, 2005). Indeed, some indigenous human populations do not show any age-related rise in pulse pressure with age (Truswell *et al.*, 1972; Poulter *et al.*, 1985). Furthermore, within populations, arterial stiffness and the rate of arterial stiffening varies considerably (Benetos *et al.*, 2002). Many other conditions and risk factors are associated with arterial stiffness and lead to premature vascular ageing (McEniery *et al.*, 2005a; McEniery *et al.*, 2007), these include genetic background, cardiovascular risk factors (e.g.

obesity, smoking, hypertension), cardiovascular diseases, kidney disease and rheumatoid arthritis (Laurent *et al.*, 2006)(Table 2.5). Moreover, evidence is largely indirect coming from cross-sectional studies which are only able to determine correlational relationships rather than causal relationships. However, the Atherosclerosis Risk In Communities (ARIC) study of middle-aged subjects (age 45 to 64 years), using ultrasound examination of the left common carotid artery, found that 1 standard deviation increase in arterial stiffness was associated with a 15% greater risk of future hypertension, independent of established risk factors and level of BP (Liao *et al.*, 1999). More recently, using multiple linear regression modelling, aortic stiffness measured by echocardiography in normotensive individuals aged 35 – 93 years, was found to be a predictor of future hypertension after correcting for systolic BP, age, sex, body mass index, heart rate, total cholesterol, diabetes, smoking, alcohol consumption, and physical activity; these finding were noted for both young and old subjects and for both sexes (Dernellis and Panaretou, 2005). This has led to the suggestion that the relationship between arterial stiffness and hypertension may be two-way (Franklin, 2005). It is worthy of note at this point that obesity is associated with greater arterial stiffness (Wildman *et al.*, 2003) in both younger and older adults and visceral adiposity is particularly detrimental (Resnick *et al.*, 1997). The aortic media contains layers of smooth muscle and changes in muscle tone provide functional regulation of arterial stiffness. Endothelial nitric oxide has been shown to regulate larger artery stiffness *in vivo* (Schmitt *et al.*, 2005).

A recent systematic review examined the independent associations of arterial stiffness, specifically carotid-femoral PWV, with cardiovascular risk factors in predominantly middle- to older aged adults (Cecelja and Chowienczyk, 2009). This review found that the contribution of risk factors other than age and blood pressure was small or insignificant. No similar review has been carried out for younger individuals.

Table 2.5: Clinical conditions associated with increased arterial stiffness and/or wave reflections.

Ageing	**CV risk factors**
Other physiological conditions	Obesity
Low birth weight	Smoking
Menopausal status	Hypertension
Lack of physical activity	Hypercholesterolaemia
Genetic background	Impaired glucose tolerance
Parental history of hypertension	Metabolic syndrome
Parental history of diabetes	Type 1 diabetes
Parental history of MI	Type 2 diabetes
Genetic polmorphisms	High CRP
CV diseases	**Primarily non-CV diseases**
Coronary heart disease	End-stage renal disease
Congestive heart failure	Moderate chronic kidney disease
Fatal stroke	Rheumatoid arthritis
	Systemic vasculitis
	Systemic lupus erythematosus

Adapted from Laurent *et al.* (2006)

2.3.13 Consequences of arterial stiffening

The consequences of arterial stiffening may be viewed in respect of its mechanical effects on the cardiovascular system. Increased aortic PP, which is correlated with increased intima-media thickness (Boutouyrie *et al.*, 1999), in turn leads to increased cyclical stress and increased left ventricular afterload and decreased myocardial perfusion. Perhaps the most common clinical manifestation of arterial stiffening is the development of isolated systolic hypertension (McEniery *et al.*, 2005b) and CHD, stroke and heart failure (Nielsen *et al.*, 1995; Staessen *et al.*, 2000). Arterial stiffness correlates closely with the extent of atherosclerotic disease (Hirai *et al.*, 1989), with cardiovascular risk factors (even from a young age) (Tounian *et al.*, 2001) and with cardiovascular risk (Blacher *et al.*, 1999a).

2.3.14 Arterial stiffness and cardiovascular risk

Arterial stiffness and, more specifically, aortic stiffness, is recognised as an independent determinant of cardiovascular risk in a variety of patient groups (McEniery *et al.*, 2007). Data from several large, prospective studies demonstrate that aortic pulse-wave velocity

predicts both cardiovascular and all-cause mortality in a number of patient populations (Blacher *et al.*, 1999b; Laurent *et al.*, 2001; Meaume *et al.*, 2001a; Cruickshank *et al.*, 2002; Sutton-Tyrrell *et al.*, 2005; Mattace-Raso *et al.*, 2006; Willum-Hansen *et al.*, 2006). In the elderly, aortic stiffness is an independent marker of cardiovascular risk (Meaume *et al.*, 2001a; Meaume *et al.*, 2001b; Sutton-Tyrrell *et al.*, 2005). Aortic PWV is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients (Laurent *et al.*, 2001; Boutouyrie *et al.*, 2002), an independent predictor of cardiovascular mortality in diabetes mellitus (Cruickshank *et al.*, 2002), an independent predictor of all-cause and cardiovascular mortality in end-stage renal disease patients (Blacher *et al.*, 1999a; Blacher *et al.*, 1999b; Blacher *et al.*, 2002; Blacher *et al.*, 2003) and in end-stage renal disease patients with diabetes mellitus (Shoji *et al.*, 2001). In this same patient group carotid arterial stiffness gives additional predictive value, over and above the extent of vascular calcification, in cardiovascular and all-cause mortality (Blacher *et al.*, 2001). Similarly, augmentation index and arterial wave reflection are also independent predictors of CV and all-cause mortality in end-stage renal disease patients (London *et al.*, 2001). Aortic pulse pressure independently predicts outcome in patients with end-stage renal failure (Safar *et al.*, 2002) and cardiovascular events in patients with hypertension (Williams *et al.*, 2006). Even after renal transplantation carotid arterial stiffness predicts CV risk (Barenbrock *et al.*, 2002). Blacher *et al.* have comprehensively reviewed the landmark studies that have contributed to the prognostic significance of arterial stiffness measurements in end-stage renal disease patients (Blacher *et al.*, 2002). A recent analysis reviewing 97 studies selected 12 which encompassed end-stage renal disease, hypertension, diabetes and the elderly and had measured aortic PWV (Khoshdel *et al.*, 2007). All studies showed an increasing mortality risk with increased PWV. Specifically, pooled data showed that a one level increment in PWV produced a RR of 2.41 (1.81-3.20) for all-cause mortality and 1.69 (1.35-2.11) for cardiovascular events. Moreover, a 1 standard deviation increment in PWV is equivalent to 10 years of ageing, or approximately

1.5 - 2 times the RR of a 10 mmHg increase in systolic blood pressure. Moreover, it has more recently been demonstrated that aortic PWV independently predicts outcome in unselected, middle-aged and older adults (Sutton-Tyrrell *et al.*, 2005; Mattace-Raso *et al.*, 2006; Willum-Hansen *et al.*, 2006).

2.3.15 Arterial stiffness in children and adolescents

Pulse wave contour changes with age (Kelly *et al.*, 1989) and at younger and older ages the initial systolic peak is of low amplitude compared with the second peak (Figure 2.2 and Figure 2.6).

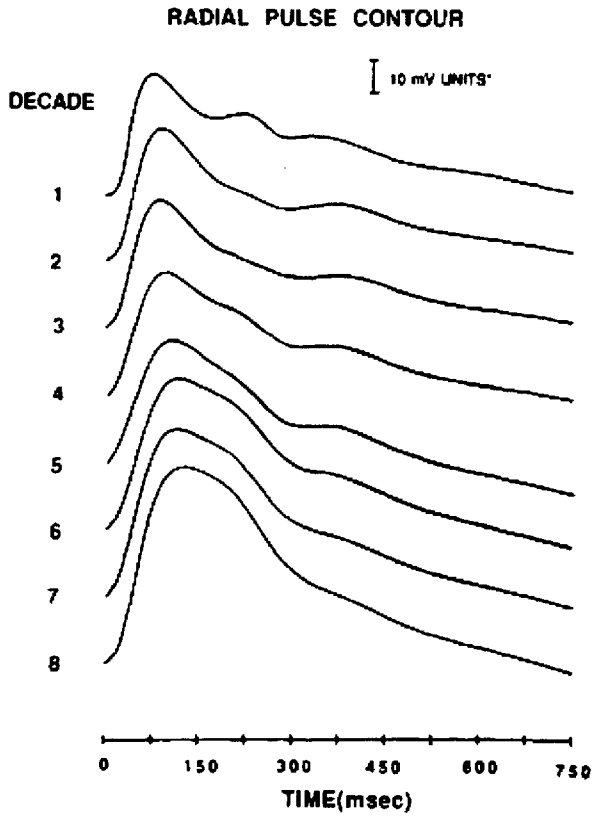

Figure 2.6: Contours showing averaged radial waves from 420 subjects. Radial waveforms are displayed above each other from infants in the first decade (1), through children, to adults up to the eighth decade (8). Each pulse is average of 40-70 individual pulses. *Amplitude is expressed in uncalibrated voltage (mV) units. Reproduced from Kelly *et al.* (1989).

This wave formation in children and older adults is believed to be due to an earlier return of wave reflection from peripheral sites, in children because of their shorter stature and in

older adults because of an increased aortic pulse wave velocity as a consequence of age related arterial stiffening. In adolescents, relative to children and older adults, the amplification of the brachial pressure wave is greater as a consequence of a later return of the reflected wave. This is because adolescents will have attained or be near to attaining full adult height, yet still with very distensible arteries and a low aortic pulse wave velocity.

Arterial stiffness in children has been measured using several different indices of measurement (Table 2.3), several different methods of measurement (Tables 2.4) and several different devices, with non-invasive procedures tending to predominate (Table 2.6, supporting references are found in Appendix 2). Differing methodologies and differing devices have led to a wide range of values for central, peripheral and systemic arterial stiffness.

Table 2.6: Summary of studies of arterial stiffness in children and adolescents.

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Bercu *et al.*, 1979)	63; 7 mo-18 yrs; cardiac and/or metabolic abnormalities.	USA	Pressure waveform	QK_D interval (timing of Korotkoff sounds) PWV	Brachial	PWV increases with age. Identifies progressive loss of functional elasticity of the major arteries throughout childhood.
(Avolio *et al.*, 1983)	57; 6.7 (1-10) yrs. 51; 17 (11-20) yrs. (study age range 3-89 yrs)	China (urban)	Ultrasound	PWV	Aorta	Aging rather than atherosclerosis is the dominant factor in reduced compliance.
(Stella *et al.*, 1984)	28; 9.3±5.9 yrs; T1DM. 28; 8.9±3.4 yrs; control.	Italy	Ultrasound	Elastic modulus PWV	Leg	Elastic modulus changes with duration of disease and age; PWV not reported or discussed.
(Avolio *et al.*, 1985)	54, ~6.2 (0-10) yrs. 71, ~15.5 (11-20) yrs. (study age range 2 mo–94 yrs)	China (rural)	Ultrasound	PWV	Aorta	After the 1st decade PWV is lower than age and pressure matched urban dwellers.
(Avolio *et al.*, 1986)	32, 10.4 (2-19) yrs. 16 low salt diet, 16 control diet (study age range 2-66 yrs).	Australia	Ultrasound	PWV	Aorta Leg Arm	Leg PWV only lower in low salt diet. Lower aortic PWV in older low salt diet groups.
(Lanne *et al.*, 1992)	76; 5-71 yrs; males. (25 aged ~5-15 yrs)	Sweden	Ultrasound	Elastic modulus Stiffness index (β)	Aorta	Stiffness of the abdominal aorta increases exponentially with age in males.
(Sonesson *et al.*, 1993)	22; ~4-16 yrs; females. (study age range 4-74 yrs)	Sweden	Ultrasound	Elastic modulus Stiffness index (β)	Aorta	Stiffness of the abdominal aorta increases almost linearly with age in females.
(Hsieh *et al.*, 1996)	24; 6 mo-13 yrs; congenital heart disease 11; 4mo-12 yrs; control.	Taiwan	Pressure waveform	Compliance PWV	Arm	Data are comparable to more invasive measurements from other studies.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(de Simone *et al.*, 1997)	373; ~9.7±3 yrs; 77% white, 21% black.	USA/Italy	Ultrasound/ sphygmomanometry	Compliance (SV/PP ratio)	Systemic	Compliance decreases progressively with aging.
(Gosse *et al.*, 1999)	27; 17±3 (10-19) yrs. (study age range 10-78 yrs)	France	BP (ambulatory)	QK_D interval	N/A	Confirms the reduction in distensibility with age.
(Iannuzzi *et al.*, 1999)	37; 96.4±32.0 mo (3-14 yrs); HC. 30; 92.9±33.4 mo (3-14 yrs); NC.	Italy	Ultrasound	Elastic modulus Stiffness index (β)	Aorta	HC in children reduces the effect of aging on the elastic properties of the aorta.
(Meaney *et al.*, 1999)	100; ~14.5±3 (10-21) yrs; 50% parental history of hypertension.	Mexico	Ultrasound	Stiffness index (β)	Aorta, carotid	Increased carotid stiffness in children of hypertensive parents.
(Leeson *et al.*, 2000b)	361; 11±0.36 yrs.	UK	Ultrasound	Distensibility	Brachial	Inverse relationship between cholesterol and distensibility.
(Martin *et al.*, 2000)	44; 9±1.3 yrs; 22 low birth weight for age.	Sweden	Ultrasound	Stiffness Index (β)	Aorta, carotid	Low birth weight children show a trend toward increased carotid artery stiffness.
(Miyai *et al.*, 2001)	1495; 9-17 yrs.	Japan	Volume waveform	d/a ratio of 2nd derivative (analogous to AIx)	Finger	Low d/a ratio related to atherosclerotic risk factors.
(Okubo *et al.*, 2001)	40; 2.4±2.1 (3 mo-7) yrs; KD. 168; 7.7±7.6 (7 days-31) yrs; control.	Japan	Ultrasound	Distensibility Elastic modulus	Aorta	Distensibility is low in infants, increases to peak at age10-15yrs and thereafter decreases with age.
(Tounian *et al.*, 2001)	48; 12.6 (4.2-16.3) yrs; obese. 27; 12.0 (6.0-17.0) yrs; control.	France	Ultrasound	Compliance Distensibility Elastic modulus	Carotid	Severe childhood obesity is associated with increased stiffness of elastic arteries.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Salaymeh and Banerjee, 2001)	13; 9.1±3.2 (3-12) yrs; Williams syndrome. 16; 7.6±1.3 (3-12) yrs; control.	USA	Ultrasound/tonometry	Compliance Stiffness Index (β)	Aorta	AS is increased in children with Williams syndrome.
(Levent *et al.*, 2002)	25; 12.4±1.4 yrs; hypertensive obese. 25; 11.9±1.5 yrs; normotensive obese. 25; 12.1±1.8 yrs; control.	Turkey	Ultrasound	Elastic modulus PWV	Aorta	Aortic velocity and elastic modulus was increased in hypertensive obese children.
(Singhal *et al.*, 2002)	294; 13-16 yrs. 216 pre-term birth, 78 born at term.	UK	Ultrasound	Distensibility	Brachial	Elevated leptin inversely associated with impaired distensibility. No difference in distensibility between groups.
(Cheung *et al.*, 2002)	13; 4.9-16 yrs; with polyarteritis nodosa. 155; 6-18 yrs; control.	Hong Kong	Volume waveform	PWV	Arm	Distensibility is decreased in children with vasculitis which is amplified during inflammatory exacerbation.
(Senzaki *et al.*, 2002)	112; 6mo – 20 yrs; data collected during diagnostic cardiac catheterisation.	Japan	Invasive micromanometer X-ray aortogrphy	Compliance Elastic modulus	Aorta	Compliance (normalised to BSA) decreases with age with the most rapid changes occurring before 3-7yrs of age, stiffness of the proximal aorta increases with age.
(Argyropoulou *et al.*, 2003)	31; 13.6 (3.4-26.2) yrs; juvenile idiopathic arthritis. 28; 13 (3.2-26.5) yrs; control.	Greece	MRI	Distensibility PWV	Aorta	Increased PWV and decreased distensibility in patients compared to controls.
(Ahimastos *et al.*, 2003)	58; 10.3±0.1 yrs; 32/26; prepubescent. 52; 15.9±0.3 yrs; 22/30; postpubescent.	Australia	Ultrasound/ Pressure waveform	Compliance PWV	Systemic Aorta, leg	Large artery stiffness varies intrinsically between genders but is also modified by sex hormones.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Kwok *et al.*, 2003)	30; 9.5±2.8 yrs; primary snorers. 30; 9.7±2.7 yrs; control.	Hong Kong	Volume waveform	PWV	Arm	Children with primary snoring have reduced distensibility.
(Lurbe *et al.*, 2003)	219; 7-18 (mean 11.3) yrs.	Spain	Pressure waveform	AIx	Radial	AIx increased in children with the lowest birth weights (<3kg).
(Sandor *et al.*, 2003)	6; 15.7 (10.1-17.2) yrs; Marfan's syndrome. 9; 13.7 (9.8-17.7) yrs; ICTD. 14; 12.3 (7.4-18.2) yrs; control.	Canada	Ultrasound	Elastic modulus PWV	Aorta	Increased PWV in children with Marfan's and inflammatory connective tissue disease.
(Schutte *et al.*, 2003)	1244; 10-15 yrs; mixed ethnic groups.	South Africa	Blood pressure	Compliance	Finger (systemic)	White and Indian children had higher compliance than black and mixed-origin children at all ages.
(Cheung *et al.*, 2004a)	86; 8.2 ± 1.7 yrs. Group 1: 7.4 ± 1.4 yrs, pre-term and small for gestational age. Group 2: 8.3 ± 1.8 yrs, pre-term but normal weight. Group 3: 8.4 ± 1.6 yrs, born at term and normal weight.	Hong Kong	Volume waveform	PWV	Arm	PWV significantly greater in children born pre-term and small for gestational age.
(Cheung *et al.*, 2004b)	37; 9.0±3.1 yrs; KD and coronary aneurysms. 29; 8.9±3.2 yrs; KD and no aneurysms. 36; 9.1±2.6 yrs; control.	Hong Kong	Volume waveform	PWV	Arm	Adverse CV risk profile and increased PWV in children after KD. PWV higher in children with aneurysms.
(Haller *et al.*, 2004)	98; 10-18 yrs; type 1 diabetes. 57; 10-18 yrs; control.	USA	Pressure waveform	AIx, AIx_{75}	Radial	Children with type 1 diabetes have increased AS.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Litwin *et al.*, 2004)	49; 14.5 (6-20) yrs; hypertensive. 61; 13.5 (6-20) yrs; control.	Poland	Ultrasound	Compliance Distensibility Elastic modulus Stiffness index (β)	Carotid	Increased stiffness observed in hypertensive children along with functional and anatomical changes in the vasculature.
(Jourdan *et al.*, 2005)	247; 10-20 yrs.	Germany, Poland	Ultrasound	Distensibility Elastic modulus Stiffness index (β)	Carotid	AS changes with body size and is affected by BP and relative body mass.
(Schack-Nielsen *et al.*, 2005)	93; ~10 yrs; 78.5% of cohort were prepubertal.	Denmark	Volume waveform	PWV	Arm, aorta	AS inversely associated with PA.
(Whincup *et al.*, 2005)	471; ~15.5±0.6 (13-15) yrs.	UK	Ultrasound	Distensibility	Brachial	Decreased arterial distensibility associated with adiposity and components of the MS.
(Yasuoka and Harada, 2005)	103; 9.1±3.8 (1mo-17) yrs.	Japan	Ultrasound	Stiffness index (β)	Aorta	Aortic stiffness was constant with age.
(Covic *et al.*, 2006)	18; 14.1±2.6 yrs; haemodialysis patients 15; 12.7±3 yrs; control.	Romania	Pressure waveform	PWV AIx	Aorta Carotid	Children on dialysis have structural arterial wall abnormalities and increased AS.
(Donald *et al.*, 2006)	16; 13 (7-17) yrs; males.	UK	Pressure waveform	AIx	Radial	Acute endothelial dysfunction is detectable with AIx.
(Iannuzzi *et al.*, 2006)	100; 6-14 yrs; obese with (38) and without (62) MS.	Italy	Ultrasound	Stiffness index (β)	Carotid	Obese children with MS had higher CCA stiffness than obese children without MS.
(Khan *et al.*, 2006)	44; 15-18 yrs; poor glucose handlers (≥7.7mmol/l 2-h post feeding) vs. good glucose handlers (≤5mmol/l).	Scotland, UK	Pressure waveform	AIx	Radial	Increased AS (decrease in Tr) in children with poor glucose handling.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Niboshi *et al.*, 2006)	970; 14.7 ± 2.6 (9-17) yrs.	Japanese	Volume waveform	PWV	Brachial-ankle	baPWV influenced by age and gender.
(Aggoun *et al.*, 2008)	48; 8.9±1.5 yrs; obese. 23; 8.5±1.5 yrs; lean.	Switzerland	Ultrasound	Compliance Distensibility Elastic modulus	Carotid	Impaired endothelial and smooth muscle functions, and altered wall material develop before puberty in obese children.
(Briese *et al.*, 2008)	36; 14±3.4 yrs; renal transplant. 49; 13.3±3.3 yrs; control.	Germany	Pressure waveform	PWV AIx	Aorta Carotid	Increased AS in renal transplant patients.
(Cheung *et al.*, 2008a)	167; 8.9±4.1yrs; KD, 73 with coronary aneurysms 124; 9.7±4.3 yrs; control	China	Ultrasound	Stiffness index (β)	Carotid	Carotid stiffness greater in KD patients.
(Cheung *et al.*, 2008b)	51; 13.4±0.6 yrs; KD, 32 with coronary aneurysms. 32; 14.6±0.6 yrs; control.	China	Ultrasound	Stiffness index (β)	Carotid	Oxidative stress increased in KD patients with aneurysms and associated with stiffness.
(Collins *et al.*, 2008)	205; 15.9 (12-21) yrs; 65% black.	USA	Volume waveform	PWV	Brachial-ankle	Differences in compliance among gender (M>F) and ethnic groups (B>W) are already detectable in adolescence.
(Kyvelou *et al.*, 2008)	55; 14-30 yrs; 31 offspring of parent(s) with arterial hypertension.	Greece	Pressure waveform	PWV AIx	Arm, aorta Radial	Higher AS in offspring with parental hypertension.
(Mimoun *et al.*, 2008)	384; 2.5-18 yrs; obese.	France	Ultrasound	Compliance Distensibility Elastic modulus	Carotid	No relationship between AS and MS in children/adolescents.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Polat *et al.*, 2008)	56; 11.5 ± 2.3 (4.6-15.8) yrs; obese. 30; 10.1 ± 2.3 yrs; control.	Turkey	Ultrasound	Elastic modulus Stiffness index (β)	Aorta	Stiffness parameters were all higher in obese children and correlated highly with visceral fat thickness.
(Simonetti *et al.*, 2008a)	79; 11.4 (8.4-14.8) yrs.	Switzerland	Volume waveform	Stiffness index	Finger	Digital volume pulse analysis has only a weak correlation with PWV.
			Ultrasound	PWV	Aorta	
(Simonetti *et al.*, 2008b)	114; 12.0±3.6 yrs; hypertensive. 71; 12.1±2 yrs; normotensive.	Switzerland	Ambulatory BP	Arterial stiffness index	Brachial	AASI is elevated in hypertensive children and is correlated with the duration of disease.
(Sakuragi *et al.*, 2009)	573; 10.1 ± 0.3 (9-10) yrs; prepubescent.	Australia	Pressure waveform	PWV	Aorta	Increased body mass and adiposity and decreased CRF associated with arterial stiffening.
(Cseprekál *et al.*, 2009)	25;15.1 (13.5-16.7) yrs (CI); renal transplant patients. 188; 6-23 yrs; control.	Hungary	Pressure waveform	PWV	Aorta	PWV may be reduced after renal transplantation.
(Haller *et al.*, 2009)	51; 10-21 yrs; T1DM.	USA	Pressure waveform	AIx_{75}	Radial	Potential reduction of arterial stiffness following atorvastatin therapy.
(Heilman *et al.*, 2009)	30; 13.1±3.6 (4.7-18.6) yrs; T1DM. 30; 13.2±3.9 yrs; control.	Estonia	Pressure waveform	PWV AIx, AIx_{75}	Aorta Radial	Children with T1DM display atherosclerosis-related structural and functional changes to the arterial wall.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Farpour-Lambert *et al.*, 2009)	44; 8.9±1.5 yrs; obese. 22; 8.5±1.5 yrs; lean.	Switzerland	Pressure waveform	Elastic modulus	Radial	Obese children had higher AS compared to lean.
(Galler *et al.*, 2009) abstract	93; 3.0 – 17.9 yrs; T1DM. 85; 3.5 - 18 yrs; control.	Germany	Ultrasound	Compliance Distensibility Stiffness index (β)	Aorta	AS increased in children with T1DM; no association with adiponectin concentrations.
(Kallio *et al.*, 2009)	386; 11yrs.	Finland	Ultrasound	Distensibility Elastic Modulus Stiffness index (β)	Aorta, carotid	tobacco smoke exposure has a dose-dependent association with decreased aortic elasticity in healthy children.
(Iannuzzi *et al.*, 2010)	52; 6 – 14 yrs.	Italy	Ultrasound	Stiffness index (β)	Carotid	Association between AS and proximity to heavily trafficked roads
(Reusz *et al.*, 2010)	1008; 6 – 20 yrs.	Hungary, Italy, Algeria	Pressure waveform	PWV	Aorta.	Provides reference values for PWV.
(Urbina *et al.*, 2010a)	195; 18.3±3.2 yrs; T2DM. 234; 18.1±3.3 yrs; obese. 241; 17.8±3.5 yrs; lean.	USA	Pressure waveform	Distensibility PWV AIx_{75}	Arm. Aorta, arm, leg. Radial.	AS increased across groups (T2DM > obese >lean)
(Urbina *et al.*, 2010b)	535; 14.6±3.3 yrs; T1DM. 241; 17.8±3.5 yrs; control.	USA	Pressure waveform	Distensibility PWV	Arm. Aorta, arm, leg.	Increased AS in T1DM with peripheral abnormalities predominating especially in males.
(Wadwa *et al.*, 2010)	535; 14.6±3.3 yrs; T1DM. 60; 17.4±2.7 yrs; T2DM.	USA	Pressure waveform	Distensibility PWV AIx_{75}	Brachial. Aorta. Radial.	Poorer AS values in T2DM than T1DM mediated through increased central adiposity and blood pressure.

(Continued)

(Table 2.6, continued)

Reference	*N*; age (range); population characteristics	Country	Method;	Measure of arterial stiffness	Site of measure	Main findings
(Pandit *et al.*, 2011)	208; 6-17 yrs; 95 obese, 44 overweight, 69 lean.	India	Ultrasound	Compliance Elastic modulus Stiffness index (β) PWV	Carotid.	AS is increased in overweight and obese children.
(Urbina *et al.*, 2011b)	531; 17.4±3.1; NT (21.9% T2DM). 65; 19.3±3.5; Pre-HTN (40% T2DM). 127; 20.0±3.1; HTN (55.9% T2DM).	USA	Pressure waveform	Distensibility PWV AIx_{75}	Carotid Aortic Carotid	AS increased across groups (HTN > Pre-HTN > NT) (high prevalence of T2DM and obesity due to study design).

Data presented as means ± SD unless otherwise specified. Subjects are healthy unless other wise specified. Age range is given where possible. AASI, ambulatory arterial stiffness index; AIx, augmentation index; AIx_{75}, augmentation index corrected to heart rate of 75 bpm; AS, arterial stiffness; B, black; CCA, common carotid artery; CI, confidence interval; CRF, cardio-respiratory fitness; E_{inc}, incremental elastic modulus; F, female; FMD, flow-mediated dilatation; HC, hypercholesterolaemic; HTN, hypertension; ICTD, Inflammatory connective tissue disease; IMT, intima-media thickness; KD, Kawasaki disease; M, male; mo, months; MS, metabolic syndrome; NC, normal cholesterol; NT, normotension; NTG-MD, nitroglycerin-mediated dilatation; Pre-HTN, pre-hypertension; PWV, pulse wave velocity; T1DM, type-1 diabetes mellitus; T2DM, type-2 diabetes mellitus; W, white; yrs, years.

The studies summarised in Table 2.6 show that most investigations in children utilise ultrasound measurements to determine arterial compliance, distensibility and stiffness index. Moreover, most studies have described arterial stiffening in diverse worldwide populations and principally among differing patient groups including: overweight and obesity (Tounian *et al.*, 2001; Iannuzzi *et al.*, 2006; Aggoun *et al.*, 2008; Mimoun *et al.*, 2008; Polat *et al.*, 2008; Farpour-Lambert *et al.*, 2009; Pandit *et al.*, 2011), type 1 and type 2 diabetes (Stella *et al.*, 1984; Haller *et al.*, 2004; Galler *et al.*, 2009; Haller *et al.*, 2009; Heilman *et al.*, 2009; Urbina *et al.*, 2010a; Urbina *et al.*, 2010b; Wadwa *et al.*, 2010; Urbina *et al.*, 2011a), cardiac abnormalities (Bercu *et al.*, 1979; Hsieh *et al.*, 1996; Senzaki *et al.*, 2002), low birth weight or premature birth (Martin *et al.*, 2000; Singhal *et al.*, 2002; Cheung *et al.*, 2004a) and genetic disorders (Okubo *et al.*, 2001; Salaymeh and Banerjee, 2001; Sandor *et al.*, 2003; Cheung *et al.*, 2004b). Overall, arterial stiffness has been found to increase in children with aging (Bercu *et al.*, 1979; Avolio *et al.*, 1983; Stella *et al.*, 1984; Lanne *et al.*, 1992; Sonesson *et al.*, 1993; de Simone *et al.*, 1997; Gosse *et al.*, 1999; Okubo *et al.*, 2001; Senzaki *et al.*, 2002; Niboshi *et al.*, 2006), in overweight and obesity (Tounian *et al.*, 2001; Levent *et al.*, 2002; Jourdan *et al.*, 2005; Whincup *et al.*, 2005; Aggoun *et al.*, 2008; Polat *et al.*, 2008; Farpour-Lambert *et al.*, 2009; Sakuragi *et al.*, 2009; Urbina *et al.*, 2010a; Pandit *et al.*, 2011), in hypertension (Levent *et al.*, 2002; Litwin *et al.*, 2004; Simonetti *et al.*, 2008b; Urbina *et al.*, 2011b) and in children with a parental history of hypertension (Kyvelou *et al.*, 2008), in type 1 and type 2 diabetes mellitus (Galler *et al.*, 2009; Heilman *et al.*, 2009; Urbina *et al.*, 2010a; Urbina *et al.*, 2010b) with poorer values in especially in type 2 diabetes mellitus mediated through increased adiposity and blood pressure (Wadwa *et al.*, 2010), and in children with low birth weight (Martin *et al.*, 2000; Lurbe *et al.*, 2003; Cheung *et al.*, 2004a). Increased arterial stiffness is also found in children with low physical activity (Schack-Nielsen *et al.*, 2005) and low cardiorespiratory fitness (Sakuragi *et al.*, 2009). Positive associations have also been found between arterial stiffness and metabolic syndrome (Whincup *et al.*, 2005; Iannuzzi *et al.*,

2006) whereas others report no associations (Mimoun *et al.*, 2008). In recent years pressure waveform analysis has become the predominant method in use yet there is still little data available solely in apparently healthy paediatric or young adult populations and specifically in UK children and adolescents. Of the UK investigations two described ultrasound determined brachial distensibility (Leeson *et al.*, 2000b; Whincup *et al.*, 2005) and two others used pressure waveform analysis to identify AIx but only with small subject numbers (Donald *et al.*, 2006; Khan *et al.*, 2006).

Table 2.7 details those studies that specifically identify PWV and AIx in children and adolescents (supporting references are found in Appendix 2).

Table 2.7: Summary of studies measuring pulse wave velocity and augmentation index in children and adolescents.

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure		Values		
					PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Bercu *et al.*, 1979)	63; 7mo-18yrs; cardiac and/or metabolic abnormalities	USA	PWV (brachial)		~4.4-5.5 between ages 10-15 yrs		
(Avolio *et al.*, 1983)	57, 6.7 (1-10) yrs; 51, 17 (11-20) yrs (study age range 3-89 yrs)	China, urban	PWV (aortic arch-femoral)	1st decade: 2nd decade:	6.0–6.4; 6.5–10.2		
(Stella *et al.*, 1984)	28; 9.3±5.9 yrs; T1DM 28; 8.9±3.4 yrs; control	Italy	PWV (femoral-tibial)		Not given		
(Avolio *et al.*, 1985)	54, ~6.2 (0-10) yrs; 71, ~15.5 (11-20) yrs (study age range 2 mo 94 yrs)	China, rural	PWV (aortic arch-femoral)	1st decade: 2nd decade:	4.4–5.9; 5.1–13.9		
(Avolio *et al.*, 1986)	32, 10.4 (2-19) yrs; 16 low salt diet, 16 control diet (study age range 2-66yrs).	Australia	PWV (aorta; femoral-post-tibial; and brachial-radial)		Not clearly stated		
(Hsich *et al.*, 1996)	24; 6 mo-13 yrs; congenital heart disease 11; 4 mo-12 yrs; control	Taiwan	PWV (brachial-radial)	Patients: Control:	10.0 ± 3.7 9.1 ± 2.5		
(Levent *et al.*, 2002)	Group 1: 25; 12.4±1.4 yrs; hypertensive obese. Group 2: 25; 11.9±1.5 yrs; normotensive obese. Group 3: 25; 12.1±1.8 yrs; control	Turkey	PWV (aorta)	Obese: Group 1: Group 2: Control: Group 3:	11.44 ± 1.76 9.86 ± 1.16 9.67 ± 0.85		

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure		Values		
					PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Cheung *et al.*, 2002)	13; 4.9-16 yrs; with polyarteritis nodosa aged, 155; 6-18 yrs; controls	Hong Kong	PWV (brachial-radial)	Patients: Control:	0.99 ~0 *(z-score)*		
(Argyropoulou *et al.*, 2003)	31; 13.6 (3.4-26.2) yrs; juvenile idiopathic arthritis 28; 13 (3.2-26.5) yrs; control	Greece	PWV (aorta)	Patients: Control:	3.68 ± 1.59 1.38 ± 0.54		
(Ahimastos *et al.*, 2003)	58; 10.3±0.1 yrs; prepubertal. 52; 15.9±0.3 yrs; postpubertal	Australia	PWV (carotid-femoral) (femoral-dorsal pedis)	Males: Prepubertal: c-fPWV: f-dPWV: Postpubertal: c-fPWV: f-dPWV: Females: Prepubertal: c-fPWV: f-dPWV: Postpubertal: c-fPWV: f-dPWV:	 4.1 ± 0.1 5.9 ± 0.1 ~5.6 8.2 ± 0.2 5.9 ± 0.2 7.3 ± 0.2 ~5.3 7.2 ± 0.2		
(Kwok *et al.*, 2003)	30; 9.5±2.8 yrs; primary snorers 30; 9.7±2.7 yrs; control	Hong Kong	PWV (brachial-radial)	Patients: Controls:	9.7 ± 1.6 7.9 ± 2.0		
(Lurbe *et al.*, 2003)	219; 11.3 (7-18) yrs.	Spain	AIx (radial) (Sphygmocor, Atcor Medical)	Birth weight groups: <2.50kg: 2.50-2.99kg: 3.00-3.50kg: >3.50kg:		3.9 ± 1.3 3.7 ± 0.8 0.9 ± 0.6 0.7 ± 0.5	

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure		Values		
					PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Sandor *et al.*, 2003)	6; 15.7 (10.1-17.2) yrs; Marfan's syndrome 9; 13.7 (9.8-17.7) yrs; ICTD 14; 12.3 (7.4-18.2) yrs; control	Canada	PWV (aorta)	Marfan's syndrome: ICTD: Control:	4.96 (3.5-7.67) 5.33 (2.17-6.95) 3.62 (2.6-5.8) *(median (range))*		
(Cheung *et al.*, 2004a)	86; 8.2 ± 1.7 yrs: Group 1: 7.4±1.4 yrs, pre-term and small for gestational age, Group 2: 8.3±1.8 yrs, pre-term but normal weight, Group 3: 8.4±1.6 yrs, born at term and normal weight;	Hong Kong	PWV (brachial-radial),	Pre-term: Group 1 : Group 2: Term: Group 3:	9.45 ± 1.79 7.29 ± 1.85 7.09 ± 1.2		
(Cheung *et al.*, 2004b)	102; Group 1: 9.0±3.1 yrs; KD and coronary aneurysms. Group 2: 8.9±3.2 yrs; KD and no aneurysms. Group 3: 9.1±2.6 yrs; controls	Hong Kong	PWV (brachial-radial),	KD: Group 1: Group 2: Control: Group 3:	7.17 ± 1.79 6.71 ± 1.82 5.89 ± 1.35		
(Haller *et al.*, 2004)	98; 10-18y rs; T1DM. 57; 10-18 yrs; controls.	USA	AIx, AIx_{75} (radial) (Sphygmocor, Atcor Medical)	T1DM: Controls:		1.11 ± 10.15 -0.47 ± 9.79	1.88 ± 10.75 -3.31 ± 10.36
(Schack-Nielsen *et al.*, 2005)	93; ~10 yrs; 78.5% prepubertal	Denmark	PWV (aorto-radial), PWV (aorto-femoral)	Males: a-rPWV: a-fPWV: Females: a-rPWV: a-fPWV:	3.8 ± 0.4 2.6 ± 0.2 3.9 ± 0.5 2.6 ± 0.3		

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure	Values			
					PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Covic *et al.*, 2006)	18; 14.1±2.6 yrs; dialysis patients 15; 12.7±3 yrs; control	Romania	PWV (carotid-femoral) AIx (carotid) (Sphygmocor, Atcor Medical)	Patients: Control:	6.6 ± 1.0 5.4 ± 0.6	29.7 ± 15.4 8.3 ± 8.0%	
(Donald *et al.*, 2006)	16; 13 (7-17) yrs; males	UK	AIx (radial) (Sphygmocor, Atcor Medical)			42.7 ± 9.7 (peripheral AIx)	
(Khan *et al.*, 2006)	44; 15-18 yrs; poor glucose handlers (≥7.7mmol/l 2-h post feeding) vs. good glucose handlers (≤5mmol/l)	Scotland, UK	AIx (radial) (Sphygmocor, Atcor Medical)	Poor glucose handlers: Good glucose handlers:		-1 ± 8 2 ± 10	
(Niboshi *et al.*, 2006)	970; 14.7±2.6 (9-17) yrs	Japanese	PWV (brachial-ankle)	Males: 9-11yrs: 12-14yrs: 15-17yrs: Females: 9-11yrs: 12-14yrs: 15-17yrs:	9.41 ± 1.02 9.47 ± 1.17 10.41 ± 1.07 9.19 ± 1.04 9.32 ± 1.18 9.52 ±1.03		
(Briese *et al.*, 2008)	36; 14±3.4 yrs; renal transplant. 49; 13.3±3.3 yrs; control.	Germany	PWV (carotid-femoral) AIx (carotid)	Transplant: c-fPWV: Control: c-fPWV:	5.43 ±0.9 4.68 ±0.7	-14.3 ± 15.2 -26.3 ± 13.5	
(Collins *et al.*, 2008)	205; 15.9 (12-21) yrs; 65% black	USA	PWV (brachial-ankle)	Black: White: Male: Female:	10.8 ± 1.34 10.4 ± 1.35 10.96 ± 1.36 10.39 ± 1.29		
(Kyvelou *et al.*, 2008)	55; 14-30 yrs; 31 offspring of parent(s) with arterial hypertension	Greece	PWV (carotid-radial, carotid-femoral) AIx (radial)	Subjects: c-fPWV: c-rPWV: Controls: c-fPWV: c-rPWV:	6.55 ± 0.88 8.51 ± 1.0 5.43 ± 0.8 7.39 ± 0.95	5.93 ± 14.03 -6.27 ± 11.28	

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure	Values		
				PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Simonetti *et al.*, 2008a)	79; 11.4 (8.4-14.8) yrs	Switzerland	PWV (carotid-femoral)	5.9 ± 0.9		
(Cseprekál *et al.*, 2009)	25;15.1 (13.5-16.7) yrs (CI); renal transplant patients 188; 6-23 yrs; controls	Hungary	PWV (carotid-femoral)	Patients: 15.1 yrs: 5.46 Controls: 6-<8yrs: 4.42 8-<10 yrs: 4.55 10-<13 yrs: 4.76 13-<16 yrs: 4.92 16-<19 yrs: 5.70 19-<21 yrs: 6.60		
(Sakuragi *et al.*, 2009)	573; 10.1±0.3 (9-10) yrs; prepubescent	Australia	PWV (carotid-femoral) (Sphygmocor, Atcor Medical),	All: 4.4 ± 0.5 Tertile of %BF: 1st: 4.2 ± 0.4 2nd: 4.4 ± 0.4 3rd: 4.6 ± 0.5		
(Haller *et al.*, 2009)	51; 10-21 yrs; T1DM	USA	AIx_{75} (radial)			4.37 ± 13
(Heilman *et al.*, 2009)	30; 13.1±3.6 (4.7-18.6) yrs; T1DM 30; 13.2±3.9 yrs; control	Estonia	PWV (carotid-femoral) AIx, AIx_{75} (radial)	T1DM: 5.42 ± 0.7 Control: 5.16 ± 0.6	2.85 ± 11.6 3.66 ± 8.85	4.787 ± 13 -2.22 ± 10.6
(Reusz *et al.*, 2010)	1008; 6 – 20 yrs	Hungary, Italy, Algeria	PWV (carotid-femoral)	Males: 11yrs (50th %ile): 4.615 14yrs (50th %ile): 5.014 Females: 11yrs (50th %ile): 4.783 14yrs (50th %ile): 4.987		

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure	Values				
						PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Urbina *et al.*, 2010a)	195; 18.3±3.2 yrs; T2DM. 234; 18.1±3.3 yrs; obese. 241; 17.8±3.5 yrs; control.	USA	PWV (carotid-femoral, -radial, -dorsalis pedis) AIx_{75} (radial) (Sphygmocor, Atcor Medical),	T2DM:	PWV aorta: PWV arm: PWV leg:	6.7 ± 1.2 7.6 ± 1.1 8.3 ± 1.6		2.07 ± 10.9
				obese:	PWV aorta: PWV arm: PWV leg:	6.3 ± 1.1 7.3 ± 1.0 8.1 ± 1.4		2.7 ± 11.6
				Lean:	PWV aorta: PWV arm: PWV leg:	5.4 ± 0.7 7.4 ± 1.1 8.0 ± 1.2		-0.52 ± 10.8
(Urbina *et al.*, 2010b)	535; 14.6±3.3 yrs; T1DM 241; 17.8±3.5 yrs; controls	USA	PWV (carotid-femoral, -radial, -dorsalis pedis) (Sphygmocor, Atcor Medical),	T1DM:	PWV aorta: PWV arm: PWV leg:	5.3 ± 0.8 6.9 ± 0.9 7.5 ± 1.4		2.07 ± 10.9
				Control:	PWV aorta: PWV arm: PWV leg:	5.4 ± 0.7 7.4 ± 1.1 8.0 ± 1.2		-0.52 ± 10.8
(Wadwa *et al.*, 2010)	535; 14.6±3.3 yrs; T1DM 60; 17.4±2.7 yrs; T2DM	USA	PWV (carotid-femoral) AIx_{75} (radial) (Sphygmocor, Atcor Medical)	T1DM: T2DM:		5.3 ± 0.8 6.4 ± 1.3		2.2 ± 10.2 6.4 ± 9.9%
(Pandit *et al.*, 2011)	208; 6-17 yrs; 95 obese, 44 overweight, 69 lean.	India	PWV (carotid)	Boys:	Ob: Ow: Lean:	4.24 ± 0.5 3.97 ± 0.5 3.74 ± 0.5		
				Girls:	Ob: Ow: Lean:	4.40 ± 0.8 4.0 ± 0.4 3.72 ± 0.4		

(Continued)

(Table 2.7, continued)

Reference	*N*; age (range); population characteristics	Country	Measure and site of measure		Values		
					PWV ($m.s^{-1}$)	AIx (%)	AIx_{75} (%)
(Urbina *et al.*, 2011b)	531; 17.4±3.1; NT (21.9% T2DM).	USA	PWV (carotid-femoral) AIx_{75} (carotid) (Sphygmocor, Atcor Medical)	NT:	5.75 ± 0.92		0.69 ± 11.52
	65; 19.3±3.5; Pre-HTN (40% T2DM).			Pre-HTN:	6.38 ± 1.06		3.89 ± 10.21
	127; 20.0±3.1; HTN (55.9% T2DM).			HTN:	7.12 ± 1.25		9.35 ± 10.62

Data presented as means ± SD unless otherwise specified. Subjects are healthy unless other wise specified. Age range is given where possible. Studies using the Sphygmocor device (Atcor Medical) are specifically identified. %ile, percentile; AIx, augmentation index; AIx_{75}, augmentation index corrected to heart rate of 75 bpm; CI, confidence interval; HC, hypercholesterolaemic; ICTD, Inflammatory connective tissue disease; KD, Kawasaki disease; mo, months; NC, normal cholesterol; Ob, obese; Ow, overweight; PWV, pulse wave velocity; T1DM, type-1 diabetes mellitus; T2DM, type-2 diabetes mellitus; yrs, years.

For children aged approximately 10 – 16 years of age central (aortic) PWV values range between 1.38 $m.s^{-1}$ (Argyropoulou *et al.*, 2003) and 9.67 $m.s^{-1}$ (Levent *et al.*, 2002). Discarding the very highest and very lowest values gives an average aortic PWV (for males and females combined) of 4.77 $m.s^{-1}$. However, the path length over which aortic PWV is measured in these studies (Table 2.7) are not identical and include the proximal aorta, the carotid-femoral region, the distal aorta and the abdominal aorta. A recent study (Reusz *et al.*, 2010) has attempted to provide reference ranges for age and height specific carotid-femoral PWV in 1008 children and young adults aged 6 – 20 years using data collected from three countries. PWV was obtained via the pressure waveform using the technique of applanation tonometry (see Section 2.3.8). Calculating a mean of the 50^{th} percentile values for both males and females at ages 10 – 16 years (inclusive) an average of 4.90 ± 0.23 $m.s^{-1}$ is obtained. The specific 50^{th} percentile values at ages 11 and 14 years, respectively, are 4.62 and 5.01 $m.s^{-1}$ for males, and 4.78 and 4.99 $m.s^{-1}$ for females. Relatively few studies that have included measures of AIx in apparently healthy children (including controls) and most employ differing methodologies. These studies have reported carotid AIx values of -26.3% (Briese *et al.*, 2008) and 8.3% (Covic *et al.*, 2006), radial AIx values between -6.3% (Kyvelou *et al.*, 2008) and 2% (Khan *et al.*, 2006) and radial AIx, standardised to a heart rate of 75 bpm, of -2.22% (Heilman *et al.*, 2009) and -0.52% (Urbina *et al.*, 2010a; Urbina *et al.*, 2010b). Similar to those studies listed in Table 2.6 which utilised varying methods for measuring arterial stiffness, Table 2.7 shows that aortic PWV increases with ageing (Avolio *et al.*, 1983; Avolio *et al.*, 1985; Ahimastos *et al.*, 2003; Reusz *et al.*, 2010), increased body fat (Levent *et al.*, 2002; Sakuragi *et al.*, 2009; Pandit *et al.*, 2011), hypertension (Levent *et al.*, 2002), parental hypertension (Kyvelou *et al.*, 2008) and both type 1 (Heilman *et al.*, 2009; Urbina *et al.*, 2010b) and type 2 diabetes mellitus (Urbina *et al.*, 2010a; Wadwa *et al.*, 2010).

2.3.16 Arterial stiffness and metabolic syndrome risk factors in children and adolescents

2.3.16.1 Overweight and obesity

In a study of 384 obese French children aged 2.5 – 18 years (Mimoun *et al.*, 2008) arterial stiffness was assessed by ultrasound imaging of the common carotid artery to identify cross-sectional compliance and distensibility, intima-media thickness and incremental elastic modulus. Children were categorised as obese for inclusion to the study if the BMI z-score was at least 2.5 standard deviations above national normative data. Abdominal obesity was identified as an abdominal fat to lower-limb fat ratio >0.85, suggested by the authors as similar to the waist-to-hip ratio. In addition, metabolic syndrome was identified using criteria based on the World health Organisation and National Cholesterol Education Programme definitions (Balkau and Charles, 1999; National Cholesterol Education Program, 2001b) and similar to the definition of Weiss *et al.* (2004a) differing only in the criteria for abdominal obesity. In this cohort of obese children only cross-sectional compliance was found to be negatively correlated with obesity (r = -0.22; p = 0.02). Metabolic syndrome was found not to be related to any arterial variables, whereas some of its individual components and other metabolic factors (LDL and HDL) were associated with vascular alterations. The suggestion is that although obesity is associated with deleterious vascular alterations, metabolic syndrome does not predict cardiovascular risk better than each of its individual components and may not be clinically useful in this population.

A Turkish study of 56 obese children, with obesity identified using the criteria of Cole *et al.* (2000), aged 4.6 – 15.8 years undergoing echocardiographic measurement of the abdominal aorta and ultrasound measurement of visceral fat thickness found that abdominal adipose tissue accumulation was significantly associated with BMI, fasting glucose, insulin and HOMA-IR. Furthermore, visceral fat thickness was also found to be

strongly correlated with the elastic properties of the abdominal aorta (aortic strain, r = 0.83, $p < 0.001$; elastic modulus, r = -0.67; $p < 0.001$; and β-stiffness index, r = -0.56; $p <$ 0.001) (Polat *et al.*, 2008).

A study of 48 severely obese children with BMI z-score greater than 3 standard deviations above French normative national data and aged 12.6 years (median; range 4.2 – [1]6.3) underwent ultrasound measurements of the common carotid artery to identify intima-media thickness, cross-sectional compliance and distensibility and elastic modulus. Subjects also had endothelial function assessed by flow-mediated dilatation of the brachial artery. Obese children were found to have significantly impaired flow-mediated and glyceryl trinitate (GTN) -mediated dilatation ($p < 0.001$) and increased arterial stiffness (cross-sectional compliance, $p = 0.02$; elastic modulus, $p = 0.0001$) when compared to controls. The authors suggest that the vascular dysfunction and arterial stiffness identified may represent an early step in the development of atherosclerosis (Tounian *et al.*, 2001).

An Italian investigation of 100 obese children aged 6 – 14 years, where obesity was defined as a BMI > 95th percentile of US growth charts (Kuczmarski *et al.*, 2000), found 38% of subjects to meet their criteria for metabolic syndrome. Moreover, individuals with metabolic syndrome had significantly greater stiffness (β) of the common carotid artery as assessed by ultrasound than obese children without metabolic syndrome. In addition, metabolic syndrome children had higher C-reactive protein plasma concentrations than obese children without metabolic syndrome (Iannuzzi *et al.*, 2006).

Similar findings have been found in other studies of obese European children (Levent *et al.*, 2002; Urbina *et al.*, 2010a) and obese Indian boys and girls compared to normal weight children (Pandit *et al.*, 2011). Taken together the data suggest that obese children are at risk of developing arterial stiffness and atherosclerotic disease although recent data

suggests that, at least in younger children, vascular remodelling is not yet present. The study by Aggoun *et al.* (2008) of 48 obese pre-pubertal children aged 6 -11 year (8.8 ± 1.5 years) assessed arterial stiffness and structure of the common carotid artery by high-resolution ultrasound and brachial artery smooth muscle function by flow-mediated dilatation. Pre-pubertal obese children, compared to controls, were found to have an increased incremental elastic modulus ($p < 0.01$), lower flow-mediated and GTN-mediated dilatation. However, no differences were found in intima-media thickness between obese individuals and controls. The authors suggest that arterial (endothelial) dysfunction may be a first marker of atherosclerosis before structural vascular alterations occur.

2.3.16.2 Hypertension

As with hypertensive adults (Cecelja and Chowienczyk, 2009) children and adolescents also display significant associations between elevated blood pressure and measures of arterial stiffness (Litwin *et al.*, 2004; Aggoun *et al.*, 2008; Simonetti *et al.*, 2008b). Furthermore, in comparison to controls, young individuals (mean age 18 years) with only moderately elevated blood pressure have been shown to have increased carotid artery intima-media thickness, augmentation index and carotid-femoral PWV in addition to an increased left ventricular mass index (Urbina *et al.*, 2011b). Moreover, children with even a single hypertensive parent have been shown to have significantly increased carotid artery stiffness and smaller carotid diameter when compared to children with normotensive parents (Meaney *et al.*, 1999). Children with at least one hypertensive parent have been shown to have significantly increased carotid-radial PWV, carotid-femoral PWV and AIx_{75} (Kyvelou *et al.*, 2008).

2.3.16.3 Fasting glucose and diabetes

The association between plasma glucose and arterial stiffness was investigated using pulse wave analysis obtained by the Sphygmocor system (Atcor Medical, Sydney, Australia) to

measure AIx and the timing of the reflected wave (Tr), a surrogate measure for PWV in a study of 145 children from Scotland aged 15 – 18 years (Khan *et al.*, 2006). Subjects were divided in to quintiles based on plasma glucose levels obtained from a standard 2-hour oral glucose tolerance test. The 23 children in the lowest quintile were termed excellent glucose handlers and the 21 children in the highest quintile termed poor glucose handlers. While no significant differences were found between the two groups for AIx, Tr was marginally but significantly shorter (p = 0.04) in the poor glucose handlers suggesting a higher PWV. In addition, significant associations were found between intraventricular septal thickness, SBP, DBP and BMI in the poor glucose handling group but not in the excellent glucose handling group. While not clinically significant this may suggest the beginnings of a relationship between BMI, BP and altered ventricular structure in this group and an early predisposition to cardiovascular diseases.

To assess the effect of T2DM and obesity in young individuals 670 subjects aged 10 – 24 years with a mean age of approximately 18 years subjects were classified as either lean (BMI <85th percentile), obese (BMI >95th percentile) or type 2 diabetic (93% with BMI >85th and 80% with BMI >95th percentile). Arterial stiffness was assessed using the Sphygmocor system (Atcor Medical, Sydney, Australia) to measure AIx and carotid-radial, carotid-femoral and femoral-foot PWV. Brachial artery distensibility was also assessed from brachial artery pressure curves (DynaPulse, Pulse Metric Inc., San Diego, USA). Results showed a progressive increase in AIx (p <0.01) and carotid-to-femoral PWV (p <0.01) with a progressive decline in brachial artery distensibility (p <0.001; obese vs. T2DM, p =0.02) from lean individuals to obese individuals to subjects with obesity related T2DM. Group status was found to be an independent predictor of arterial stiffness even after adjustment for cardiovascular risk factors (Urbina *et al.*, 2010a).

Type 1 (T1DM) and T2DM patients aged 10 – 23 years were studied to assess the relationship between arterial stiffness and the type of diabetes (Wadwa *et al.*, 2010). The authors found that young individuals with T2DM had significantly worse brachial artery distensibility (6.1 ± 1.2 vs. 5.2 ± 0.9 %/mmHg; p <0.0001), AIx_{75} (2.2 ±10.2 vs. 6.4 ± 9.9 %; p =0.003) and carotid-femoral PWV (5.3 ± 0.8 vs. 6.4 ± 1.3 m/s; p <0.0001) than T1DM. Moreover, the authors concluded that these differences were largely accounted for through increased central adiposity and higher BP in youth with T2DM.

Several other studies have found that children with T1DM have increased arterial stiffness compared to controls (Haller *et al.*, 2004; Heilman *et al.*, 2009; Galler *et al.*, 2010) and one study (Urbina *et al.*, 2010b) in children aged 14.6 ± 3.3 years found that increased peripheral stiffness was more common in children than central stiffness with reduced brachial distensibility being found in 33% of subjects versus elevated carotid-femoral PWV being found in only 9.9%, with males displaying a higher of abnormalities than females.

2.3.16.4 Dyslipidaemia

Post-mortem studies have identified that aortic fatty streaks in young individuals (aged from 3 months to 30 years) were strongly correlated with LDL cholesterol levels determined before death (Newman *et al.*, 1986). Studies have also demonstrated that hypercholesterolaemic children have increased intima-media thickness compared to normal controls (Pauciullo *et al.*, 1994; Martino *et al.*, 2008). A U.K. study of 361 young children aged 9 – 11 years identified significant inverse correlations between brachial artery distensibility and both total cholesterol and LDL cholesterol (both p <0.01). No consistent associations were found for HDL cholesterol and triglyceride (Leeson *et al.*, 2000a). Whereas, a study from China of 86 obese children and adolescents found significant positive correlations between intima-media thickness and triglyceride (p <0.001) and LDL

cholesterol (p <0.01) and a negative correlation with HDL cholesterol (p <0.001) (Fang *et al.*, 2010).

3 Chapter Three: General Methodology

3.1 Introduction

This study was designed and conducted by the author and in collaboration with the Wales Heart Research Institute, School of Medicine, Cardiff University and the Clinical Pharmacology Unit, Addenbrooke's Hospital, University of Cambridge. The author and researchers from both collaborating institutions were all members of the Anglo-Cardiff Collaborative Trial (ACCT). Ethical approval was applied for in September 2003 and full approval was granted 8th March 2004 by the South East Wales Local Research Ethics Committee, reference number 04/5439. The author collected, compiled and analysed all data but was assisted with the collection of data for: anthropometry, cardiovascular measurements, venous blood collection and physical fitness measurements by researchers from the University of Glamorgan and the ACCT. Blood analysis was carried out by both the Department of Clinical Biochemistry, Royal Glamorgan Hospital, Llantrisant, UK and at the Diabetes Research Unit, Llandough Hospital, Cardiff, UK.

3.2 Study participants

Between June 2004 and February 2005 data was collected in two local secondary schools. Subject recruitment was on a voluntary basis following presentations to the 9-11 year groups (ages 11-14 years). In an effort to maximise participation the collection of data was divided in to two parts: part 1 consisted of descriptive and anthropometric data only; part 2 comprised all measurements. Potential participants were all given parental information letters (Appendices 3 and 4) accompanied by parental information sheets (Appendices 5 and 6). Informed parental consent was then gained for participation in either part 1 of the data collection (Appendix 7) or for part 2 (Appendix 8). Specific inclusion criteria for participation in the study were identified as: children should be aged 9 – 18 years and apparently healthy. Specific exclusion criteria were identified as: known cardiovascular disease (with the exception of primary hypertension) abnormal lipid profile or known

familial hypertriglyceridaemia and diabetes. Parents/guardians of the children were further asked to complete a Health and Fitness Questionnaire (Appendix 9) to identify children with past or current medical conditions.

Physical activity questionnaires (Appendix 10; see section 3.6) were explained and administered in a classroom setting prior to the main measurement days. Data collection for parts 1 and 2 took place within a dedicated classroom(s) at each school. Following receipt of completed parental consent forms subjects then attended once for anthropometry, cardiovascular and arterial stiffness measures and venous blood collection. A lifestyle questionnaire was then completed by each child in private in an attempt to gather data on smoking habits and alcohol and drug consumption (Appendix 11). Self-administered pubertal stage questionnaires were also completed in private after explanation by a member of the research team (Appendix 12; see section 3.7). The multi-stage fitness test was administered, and results recorded, within 1 week of the data collection, within each school and overseen by the author and additional members of the research team (see section 3.8).

All participants were assigned a unique identification number upon arrival for first measurements, enabling all data to be anonymised. Venous blood was collected in to aliquots and labelled with the participant's identification number and date of birth. Participant data and measurements were recorded on data collection sheets (Appendices 13 and 14).

In total 231 apparently healthy children and adolescents aged 11 – 14 years were recruited to the study.

3.3 Anthropometric measurements

3.3.1 Stature

Height was measured using a calibrated free-standing stadiometer (Seca 225; Seca Ltd, Birmingham). Subjects removed their footwear and were encouraged to stand erect, facing away from the stadiometer. The head was positioned in the Frankfort plane and the sliding head piece was then positioned at the highest position on the cranium. Subjects then crouched down and moved away and height was recorded to the nearest millimetre.

3.3.2 Body mass

Body mass was measured using a calibrated mechanical balance beam column scale (Seca; Seca Ltd, Birmingham). Subjects were dressed only in lightweight trousers or skirt, cotton shirt or blouse and socks. Body mass was recorded to the nearest 0.1kg.

3.3.3 Body mass index

Body mass index (BMI) was calculated as the subjects weight in kilograms divided by height in metres squared:

$$BMI = \frac{body\ mass\ (kg)}{height\ (m^2)}$$

Children were defined as being overweight or obese according to international criteria and cut-off points (Cole *et al.*, 2000). Subjects were considered underweight if the BMI was less than the age and gender specific paediatric equivalent for the adult definition of 18.5 kg/m^2; normal weight if the BMI was within the range of the age and gender specific paediatric equivalent for the adult definition of 18.5 – 24.99 kg/m^2; overweight if the BMI was greater than, or equal to the age and gender specific paediatric equivalent for the adult definition of 25 kg/m^2; obese if the BMI was greater than, or equal to the age and gender specific paediatric equivalent for the adult definition of 30 kg/m^2.

3.3.4 Triceps skinfold thickness

Triceps skinfold thickness was measured using calibrated Harpenden Skinfold Callipers (Baty International, Burgess Hill, West Sussex) by an experienced operator. The triceps skinfold is a vertical fold on the posterior midline of the upper arm, over the triceps muscle, halfway between the acromion process of the scapula and olecranon process of the ulna. Measurements were taken on apparently healthy, undamaged dry skin. All measurements were taken on the right side of the body. A tape measure was used to identify the midpoint and marked using a pen with water soluble ink. Subjects were then instructed to extend the elbow and keep the muscles relaxed during the test. The skinfold was grasped using the tips of the thumb and index finger and pulled lightly away from the body. The calliper was placed perpendicular to the fold, on the site marked, with the dial facing up, at approximately 1 cm below the finger and thumb. While maintaining the grasp of the skinfold, the calliper was released so that full tension was placed on the skinfold. The reading was taken, to the nearest 0.50 mm, 1 to 2 seconds after the calliper was fully released. A minimum of two measurements were taken and if repeated tests varied by more than 1 mm, the measurement was repeated. The final value recorded was an average of the two that seemed best to represent the skinfold fat site (Eston and Reilly, 2009). The measurement of the triceps skinfold thickness has been reported to have a better correlation with percent body fat ($r = 0.6$) than other measurement sites (Rolland-Cachera, 1995).

3.3.5 Waist circumference

Waist circumference was measured by an experienced operator using a standard methodology (World Health Organisation, 1987; Lean *et al.*, 1996). Subjects removed heavy outer clothing restricting easy access to the waist and abdomen. Measurements were taken over light, loosely fitting cotton shirts or blouses. Tight belts were loosened. Subjects stood relaxed with their hands at their sides and feet 12-15cm apart. With the operator

situated in front of the subject the right iliac crest was identified by palpation and at a level mid-way between the lower rib margin and the iliac crest, a flexible, inelastic measuring tape (Seca Ltd, Birmingham) was positioned around the waist and held horizontal to the ground. Subjects were asked to breathe normally and the reading of the measurement was taken at the end of gentle exhalation. This prevented subjects from contracting their abdominal muscles or from holding their breath.

3.3.6 Hip circumference

Hip circumference was measured at the site of maximal circumference over the buttocks. Measurements were taken over light trousers or skirts. Tight belts were loosened. Subjects stood relaxed with their hands at their sides and feet 12-15cm apart. With the operator standing to the right of the subject a flexible, inelastic measuring tape (Seca Ltd, Birmingham) was located around the maximal circumference. Subjects were asked to breathe normally and the reading of the measurement was taken at the end of gentle exhaling.

3.3.7 Waist-to-height ratio

The waist-to-height ratio (WHtR) was calculated as subject waist circumference in centimetres divided by subject height in centimetres and expressed as a ratio:

$$WHtR = \frac{waist\ circumference\ (cm)}{height\ (cm)}$$

In adults a waist-to-height ratio between 0.4 and 0.5 may be inferred to be optimal (Ashwell, 2005; Ashwell and Hsieh, 2005), a ratio >0.5 to 0.6 indicates increased CHD risk (Hsieh and Yoshinaga, 1995a, b; Ashwell *et al.*, 1996b; Cox and Whichelow, 1996). A ratio >0.6 indicates a substantially increased risk (Ashwell, 1997). A cut-off point of 0.5

has been proposed as an indicator of increased obesity-related morbidity in children and adolescents (McCarthy and Ashwell, 2006)

3.3.8 Waist-to-hip ratio

The waist-to-hip ratio (WHR) was calculated as subject waist circumference in centimetres divided by subject hip circumference in centimetres and expressed as a ratio:

$$WHR = \frac{waist\ circumference\ (cm)}{hip\ circumference\ (cm)}$$

A waist-to-hip ratio <0.9 for men and <0.8 for women is considered optimal (Dobbelsteyn *et al.*, 2001).

3.4 Cardiovascular measurements

3.4.1 Blood pressure

3.4.1.1 Brachial systolic and diastolic blood pressure

All measurements were conducted in accordance with British Hypertension Society (BHS) guidelines (Ramsay *et al.*, 1999). Systolic (SBP) and diastolic (DBP) blood pressure was measured in the brachial artery of the non-dominant arm using the clinically validated (O'Brien *et al.*, 1996) automated Omron HEM-705CP oscillometric sphygmomanometer (Omron Corporation, Kyoto, Japan). The Omron HEM-705 series is clinically validated in adults (El Assaad *et al.*, 2003; Coleman *et al.*, 2006), in obesity (Altunkan *et al.*, 2007) and in children (Stergiou *et al.*, 2006).

For seated blood pressure measurement subjects removed bulky and obstructive clothing and sat quietly for 5 minutes. Immediately after the resting period an experienced operator placed an appropriately sized cuff on the non-dominant arm. Measurements were carried out in duplicate or triplicate, where first and second measurements were found to be different, and mean values were used in subsequent analyses. For supine blood pressure measurement subjects rested quietly for 15 minutes before measurement was carried out as detailed above.

Blood pressure status was defined as normal, high normal or hypertension in accordance with the UK1990 age and gender specific paediatric blood pressure reference curves (Jackson *et al.*, 2007). Additionally, blood pressure status was also assessed using the CDC age, gender and height specific reference curves (National High Blood Pressure Education Program, 1996).

3.4.1.2 Mean arterial pressure

Mean arterial pressure (MAP) was calculated from measured brachial systolic and diastolic blood pressure using:

$$MAP\ (mmHg) = DBP + \frac{1}{3}(SBP - DBP)$$

3.4.1.3 Pulse pressure

Pulse pressure (PP), both measured (peripheral) brachial PP (pPP) and measured central PP (cPP) was calculated as the difference between the peak systolic pressure and the end-diastolic pressure.

$$PP\ (mmHg) = SBP - DBP$$

cPP was calculated using the central SBP and central DBP values generated by the Sphygmocor device (Atcor Medical, Sydney, Australia) described below.

3.4.2 Applanation tonometry

Applanation tonometry was used to record high fidelity wave forms from the radial, carotid and femoral arteries. Arterial waveforms were recorded with a high-fidelity strain gauge micromanometer (SPC-301, Millar Instruments, Houston, Texas) in the flattened end of a pencil-type probe that can be held in the hand and applied over an artery. The tip of the probe consists of a 0.5 x 1 mm piezoelectric crystal with a frequency response rate in excess of 2 kHz. The crystal is co-planar with a 7 mm face allowing direct and accurate applanation of the target artery and registration of arterial pressure waves (O'Rourke *et al.*, 1992)

3.4.3 Pulse wave analysis

Peripheral artery waveforms obtained by applanation tonometry were analysed using the Spygmocor device and integral software (Sphygmocor, Atcor Medical, Sydney, Australia). Pulse wave analysis was then used to generate a corresponding central (aortic) waveform using fast-Fourier analysis in a validated generalised transfer function (Karamanoglu *et al.*, 1993; Chen *et al.*, 1997; Pauca *et al.*, 2001). Calibration of the arterial wave form pressures was performed using brachial systolic and diastolic blood pressure obtained from the dominant arm using a clinically validated automated sphygmomanometer (HEM-705CP, Omron Corporation, Kyoto, Japan) (see section 3.4.1.1). After 10 seconds of sequential waveforms had been acquired, an averaged peripheral waveform, and a corresponding central waveform, was generated. Recordings were excluded from analysis after examination of in-built quality control criteria. Recordings were excluded if the systolic or diastolic variability of the waveforms exceeded 5%, or the amplitude of the waveform, a measure of the quality of the tracing, was <100 mV.

3.4.4 Augmentation index

Augmentation index (AIx), a measure of systemic arterial stiffness, was measured with a high-fidelity strain gauge micromanometer (SPC-301, Millar Instruments, Houston, Texas) and using the Sphygmocor device (Atcor Medical, Sydney, Australia) and integral software. AIx is calculated as the difference between the first and second systolic peaks of the central waveform, in systole, expressed as a percentage of the pulse pressure and is a measure of systemic arterial stiffness and wave reflection (see Figure 2.4). AIx was measured at the radial artery with the corresponding central waveform generated using the generalised transfer function. Carotid AIx was measured at the carotid artery and the waveform was calibrated using the mean arterial pressure, rather than systolic and diastolic pressures, which results in a central waveform produced without the use of the generalised transfer function. Additionally, because AIx is influenced by heart rate (Wilkinson *et al.*,

2002b), AIx was also adjusted to a standardised heart rate of 75 bpm (AIx_{75}) using the integral software of the Sphygmocor device.

3.4.5 Carotid-femoral pulse wave velocity

Carotid-femoral pulse wave velocity (PWV) was measured with a high-fidelity strain gauge micromanometer (SPC-301, Millar Instruments, Houston, Texas) and using the Sphygmocor device (Atcor Medical, Sydney, Australia) and integral software. Subjects were connected to a three-lead ECG for determination of the R-wave. Self-adhesive single use electrodes were placed either at the suprasternal notch, below the xyphoid process inferior to the sternum and manubrium and at a point between the 9th and 10th left ribs, or inferior to the left and right clavicle and at a point between the 9th and 10th left ribs. The purpose of the ECG is for determination of the heart rate (see below) and for determination of the R-wave. Path length was calculated as the superficial distance from the suprasternal notch to the carotid measurement site minus the distance from the suprasternal notch to the femoral measurement site. Sequential pressure waveform measurements were then recorded at the carotid artery and at the femoral artery while simultaneously being gated to the R-wave. PWV was therefore measured as the time taken from the R-wave to the foot of the upstroke of the forward travelling pulse wave divided by the distance covered (path length). The foot of the pulse wave is used because it is not influenced by wave reflection. The intersecting tangents algorithm was used to identify the foot of the upstroke.

3.4.6 Carotid-radial pulse wave velocity

Carotid-radial pulse wave velocity (PWV) was measured using the Spygmocor device (Atcor Medical, Sydney, Australia) and integral software. Subjects were connected to a three-lead ECG for determination of the R-wave. Path length was calculated as the distance from the suprasternal notch to the carotid measurement site minus the distance from the suprasternal notch to the radial measurement site. Sequential pressure waveform

measurements were then recorded at the carotid artery and at the radial artery while simultaneously being gated to the R-wave as described above.

3.4.7 Central systolic and diastolic blood pressure

Central systolic and diastolic blood pressure values were measured using the Sphygmocor device (Atcor Medical, Sydney, Australia) and the integral validated generalised transfer function (Karamanoglu *et al.*, 1993; Chen *et al.*, 1997; Pauca *et al.*, 2001) from analysis of the radial waveform. In addition, central systolic and diastolic blood pressure was measured from the carotid waveform calibrated using the mean arterial pressure resulting in a central blood pressure values produced without the use of the generalised transfer function (Kelly and Fitchett, 1992; Verbeke *et al.*, 2005). Carotid artery tonometry requires a higher degree of technical expertise (Laurent *et al.*, 2006), but a transfer function is not necessary, since the arterial sites are very close and waveforms are similar (Chen *et al.*, 1996).

3.4.8 Cardiac output

Cardiac output was measured using an inert gas rebreathing technique (Innocor; Innovosion, Odense, Denmark). After a minimum of 15 minutes supine rest and following measurement of arterial haemodynamics detailed above. Inert gas rebreathing is a pulmonary gas exchange method for determination of cardiac output and a number of other hemodynamic parameters. Subjects were fitted with a nose clip and, using a mouthpiece, inhaled a fixed volume of gas from a rebreathing bag prefilled with an O_2 enriched mixture containing 0.5% nitrous oxide (N_2O) and 0.1% sulphur hexafluoride (SF_6). During the rebreathing test the Innocor device measures the relative levels of the two inert gases, one blood soluble and one insoluble, over a few respirations (about 5 breaths or 15 seconds). The method relies on the principle that the rate of disappearance of the blood soluble gas from the alveolar space is proportional to the flow of blood perfusing the ventilated parts

of the lungs (effective pulmonary blood flow). This is equal to cardiac output in the absence of a significant intrapulmonary shunt. The blood insoluble gas is measured to determine the lung volume from which the soluble gas disappears and to account for other factors that affect the distribution of the blood soluble gas. The gases and CO_2 are measured continuously and simultaneously at the patient's mouth by a fast responding photoacoustic infrared gas analyzer inside the Innocor device.

3.4.9 Heart rate

Heart rate was monitored using the Spygmocor device (Atcor Medical, Sydney, Australia) following placement of the 3-lead ECG as described above.

3.5 Haematological measurements

3.5.1 Collection of venous blood

Venous blood was collected in accordance with the University of Glamorgan, Division of Sport, Health and Exercise Science, Standard Operating Procedures for blood sampling via venepuncture. All blood collection was completed by staff who had completed an accredited practical and theoretical venepuncture course and certified as competent by an accredited assessor. Briefly, the procedure was first explained to the subject. Hands were washed and sterilised and surgical gloves worn. Subject rested in a supine position for a minimum of 20 minutes before collection and restrictive clothing was removed. A tourniquet was positioned above the puncture site which was sterilised with an alcohol swab rotating outwards from the puncture site. Appropriately sized butterfly needles were connected to a luer lock adaptor. The tourniquet was tightened and the needle was inserted into an antecubital vein. Vacutainers were then placed sequentially into the luer lock. When full, vacutainers were removed and inverted 6 times and placed in a secure ice-cooled box. All vacutainers were centrifuged within 2 hours of collection and serum and plasma were pipetted in to 2 ml and 5 ml aliquots which were subsequently stored at -80 °C prior to analysis.

3.5.2 Blood sampling and analysis

Blood analyses were completed at the Department of Clinical Biochemistry, Royal Glamorgan Hospital, Llantrisant, UK (hsCRP, urea and electrolytes, liver function tests) and at the Diabetes Research Unit, Llandough Hospital, Cardiff, UK (glucose, insulin, blood lipids). Brief analysis methodologies and coefficient of analytical variation are given below.

High-sensitivity CRP, urea and electrolytes and liver function tests were all conducted using the VITROS 950 Chemistry System (Ortho-Clinical Diagnostics, Amersham, UK).

This is an automated clinical chemistry system used for discrete quantitative measurements of analyte concentrations in human fluid specimens. The system includes colorimetric, potentiometric, immuno-rate and rate test methodologies using multi-layer VITROS Chemistry Slides (Ortho-Clinical Diagnostics, Amersham, UK). Insulin was measured using a microtitre plate luminometer (MicroLumat LB96P, Berthold Technologies, Wildbad, Germany). Glucose and blood lipids were measured by direct photometry using the Sapphire 180 analyser (Audit Diagnostics, Carrigtwohill, Ireland).

3.5.3 Glucose

Blood glucose was measured in plasma using the Diasys Glucose Hexokinase assay (Diasys Diagnostic Systems, Holzheim, Germany) which provides a rapid measurement of glucose in plasma when run on the Sapphire 180 analyser (Audit Diagnostics, Carrigtwohill, Ireland). The instrument uses direct photometry to measure a coloured endpoint, from 2 reactions:

1) $Glucose + ATP \xrightarrow{hexokinase} glucose\text{-}6\text{-}phosphate + ADP$

2) $Glucose\text{-}6\text{-}phosphate + NAD^{+} \xrightarrow{G\text{-}6\text{-}PDH} gluconate\text{-}6\text{-}phosphate + NADH + H^{+}$

Wavelength:

340 nm

Reagent ingredients:

Reagent 1 contains TRIS buffer, Mg^{2+}, ATP and NAD. Reagent 2 contains Mg^{2+}, Hexokinase and Glucose-6-phosphatedehydrogenase (G-6-PDH).

Calibration:

Standards and reagents were supplied by Bio-Stat, Stockport, UK, which were introduced to the analyser prior to blood samples (25ul). The Sapphire 180 is calibrated daily and gives a printout of the results. Printed adjacent to this is the expected range so an abnormal calibration may be noticed before an assay is performed.

Reportable range (SI Units):

2 - 30 mmol.l^{-1})

Within assay co-efficient of analytical variation (CVa) was < 2% for 5.3, 9.7 and 11.9mmol.l respectively.

3.5.4 Insulin

Plasma insulin was measured in plasma using a specific immunochemiluminometric assay (Invitron Limited, Monmouth, UK). The Invitron Insulin assay is a two-site immunoassay, employing an insulin-specific solid phase antibody immobilised on microtitre wells, and a soluble antibody labelled with a chemiluminescent acridinium ester. The plasma sample was incubated simultaneously with the labelled antibody solution in the microtitre well, followed by a wash step to remove unbound labelled antibody before measurement. The bound luminescence was quantified by a microtitre plate luminometer (MicroLumat LB96P, Berthold Technologies, Wildbad, Germany) capable of *in situ* reagent addition. The luminescent reaction is a rapid flash type (>95% completes in 1 second) which permitted the entire plate to be read in approximately 5 minutes. Standards, quality controls and blood samples (25ul) were added to a 96 well plate in duplicate.

Approximate incubation time and temperature:

2 hours, 37°C

Microtitre plate:

Coated with a specific monoclonal antibody.

Standards, concentrates, diluent and buffer ingredients:

Chemiluminescent labelled antibody concentrate in a protein matrix including preservatives and 0.05% sodium azide, labelled antibody diluent in a protein matrix including preservatives and 0.05% sodium azide, hosphate buffered saline wash containing a detergent and 0.09% sodium azide.

Reportable range (SI Units):

0 – 200 $mU.l^{-1}$ (0 - 1200 $pmol.l^{-1}$)

The within assay CVa was <5% for low, medium and high tertile concentrations.

3.5.5 Total cholesterol

Total cholesterol (TC) was measured in plasma using the Audit Sapphire 180 (Audit Diagnostics, Carrigtwohill, Ireland) Cholesterol 'Trinder' assay, which provides a rapid measurement of TC in both serum and plasma samples. Quality control samples and reagents were supplied by Bio-Stat, Stockport, UK.

Principle

The instrument uses direct photometry to measure a coloured end point, from 3 reactions:

1) $Cholesterol\ ester + H_2O \xrightarrow{cholesterol\ esterase} cholesterol + fatty\ acids$

2) $Cholesterol + O_2 \xrightarrow{cholesterol\ oxidase} 4\text{-}cholesten\text{-}3\text{-}one + H_2O_2$

3) $H_2O_2 + phenol\text{-}4\text{-}aminoantipyrine \xrightarrow{peroxidase} red\ quinone + 4H_20$

Calibration:

Standards and reagents were supplied by Chemtrak Platinum, Bio-Stat, Stockport, UK, which were introduced to the analyser prior to blood samples (25ul). The Sapphire 180 is calibrated daily and gives a printout of the results. Printed adjacent to this is the expected range so an abnormal calibration may be noticed before an assay is performed.

The within assay CVa was <5% for low, medium and high tertile concentrations.

3.5.6 Triglyceride

Triglyceride (TG) was measured in plasma using the Diasys Triglyceride assay (Diasys Diagnostic Systems, Holzheim, Germany) which provides a rapid measurement of TG in both serum and plasma (3ul) when measured on the Sapphire 180 (Audit Diagnostics, Carrigtwohill, Ireland). Quality control samples and reagents were supplied by Bio-Stat, Stockport, UK.

Principle

The instrument uses direct photometry to measure a coloured endpoint, from 4 reactions:

1) $Triglycerides + H_2O \xrightarrow{lipoprotein\ lipase} glycerol + fatty\ acids$

2) $Glycerol + ATP \xrightarrow{glycerol\ kinase} glycerol\text{-}3\text{-}phosphate + ADP$

3) $Glycerol\text{-}3\text{-}phosphate + O_2 \xrightarrow{G\text{-}3\text{-}P\ oxidase} dihydroxyacetone\ phosphate + H_2O_2$

4) $H_2O_2 + 4\text{-}aminoantipyrine + 4 - chlorophenol \xrightarrow{peroxidase} red\ quinone + 4H_2O$

Wavelength:

500 nm

Calibration:

Standards and reagents were supplied by Chemtrak Platinum, Bio-Stat, Stockport, UK, which were introduced to the analyser prior to blood samples (25ul). The Sapphire 180 is calibrated daily and gives a printout of the results. Printed adjacent to this is the expected range so an abnormal calibration may be noticed before an assay is performed.

The within assay CVa was <5% for low, medium and high tertile concentrations.

3.5.7 High-density lipoprotein cholesterol

High-density lipoprotein cholesterol (HDL) was measured in plasma using the Audit Sapphire 180 HDLc assay (Genzyme Diagnostics, West Malling, UK) provides a rapid measurement of HDLc in both serum and plasma samples (3ul). Quality control samples and reagents were supplied by WAKO Diagnostics (Neuss, Germany).

Principle

The instrument uses direct photometry to measure a coloured end point.

1)

HDL, LDL, VLDL, chylomicrons

$$\xrightarrow[+DSBmT+peroxidase]{accelerator+chol.\ oxidase} \textit{non-reactive LDL, VLDL, chylomicrons}$$

2) $HDL \xrightarrow{HDL\ specific\ detergent} HDL\ disrupted$

3) $HDL \xrightarrow{cholesterol\ esterase+cholesterol\ oxidase} \Delta^4 cholesterone + H_2O_2$

4) $H_2O_2 + DSBmT + 4\text{-}AAP \xrightarrow{peroxidase} colour\ development$

(DSBmT, N, N-bis (4-Sulfobutyl)-m-toluidine disodium; 4-AAP, 4-aminoantipyrene)

The within assay co-efficient of analytical variation was 1.8% for 25, 35 and 60 mg/dl.

Reportable range (SI Units):

3.7 – 200 mg/dl (mmol.l^{-1})

3.5.8 Low-density lipoprotein cholesterol

Total low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald equation (Friedewald *et al.*, 1972):

1) $LDL = total\ cholesterol - HDL - \left(\frac{triglyceride}{2.2}\right)$

This equation was deemed suitable as all participants were free of hypertriglyceridaemia (Friedewald *et al.*, 1972).

3.5.9 High sensitivity C-reactive protein

High sensitivity C-reactive protein (hsCRP) was measured in serum where the sample is reacted with a buffer and anti-CRP coated latex (Full Range CRP. Randox Laboratories, County antrim, UK). The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is absorbed at 570nm. By constructing a standard curve from the absorbance of the standards, CRP concentration of the sample is determined.

The within assay CVa was 2.8, 1.8 and 1.0% for 1.07, 72.7 and 150 $mg.l^{-1}$ respectively.

3.5.10 Sodium

Sodium was measured in serum using VITROS Na^+ slides and the VITROS Chemistry Products Calibrator Kit 2 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support that uses direct potentiometry for measurement of sodium ions. The slide consists of two ion-selective electrodes, each containing methyl monensin (an ionophore for sodium), a reference layer, a silver layer and a silver chloride layer coated on a polyester support.

A drop of patient sample and a drop of VITROS Reference Fluid on separate halves of the slide results in migration of both fluids toward the centre of the paper bridge. A stable liquid junction is formed that connects the reference electrode to the sample electrode. Each electrode produces an electrochemical potential in response to the activity of sodium. The potential difference between the two electrodes is proportional to the sodium concentration of the sample.

Approximate incubation time and temperature:

3 mins, 25°C

Reactive slide ingredients per cm^2:

Silver, 0.4 mg; sliver chloride, 0.2 mg; sodium chloride, 0.3 mg; and methyl monensin, 50 µg.

Other slide ingredients:

Binders, buffer, plasticisers, surfactants, stabiliser and nickel.

Calibration:

- When the slide lot number changes
- When the VITROS Reference Fluid lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range (SI Units):

75 – 250 $mmol.l^{-1}$

The within assay CVa was 1.0, 1.1 and 0.9% for 121, 132 and 134$mmol.l^{-1}$ respectively.

3.5.11 Potassium

Potassium was measured in serum using VITROS K^+ slides and the VITROS Chemistry Products Calibrator Kit 2 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a

multi-layered, analytical element coated on a polyester support that uses direct potentiometry for measurement of potassium ions. The slide consists of two ion-selective electrodes, each containing valinomycin (an ionophore for potassium), a reference layer, and a silver chloride layer coated on a polyester support.

A drop of patient sample and a drop of VITROS Reference Fluid on separate halves of the slide results in migration of both fluids toward the centre of the paper bridge. A stable liquid junction is formed that connects the reference electrode to the sample electrode. Each electrode produces an electrical potential in response to the activity of potassium applied to it. The potential difference poised between the two electrodes is proportional to the potassium concentration in the sample.

Approximate incubation time and temperature:

3 mins, 25°C

Reactive slide ingredients per cm^2:

Silver, 0.4 mg; sliver chloride, 0.2 mg; potassium chloride, 63 µg; and valinomycin, 55 µg.

Other slide ingredients:

Binders, plasticisers, surfactants, stabiliser and nickel.

Calibration:

- When the slide lot number changes
- When the VITROS Reference Fluid lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range

- After certain service procedures have been performed.

Reportable range (SI Units):

1 – 14 mmol.l^{-1}

The within assay CVa was 2.0, 2.0 and 1.8% for 2.9, 5.5 and 3.8mmol.l^{-1} respectively.

3.5.12 Creatinine

Creatinine was measured in serum using VITROS CREA slides and the VITROS Chemistry Products Calibrator Kit 1 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. A drop of the patient sample is deposited on the slide and is evely distributed by the spreading layer to the underlying layers. Creatinine diffuses to the reagent layer, where it is hydrolysed to creatine in the rate-determining step. The creatine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidised to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidise-catalysed oxidation of a leuco dye to produce a coloured product.

Following addition of the sample, the slide is then incubated. During the initial reaction phase, endogenous creatine in the sample is oxidised. The resulting change in reflection density is measured at two time points. The difference in reflection density is proportional to the concentration of creatinine present in the sample.

1) $creatinine + H_2O \xrightarrow{creatinine\ amidohydrolase} creatine$

2) $creatine + H_2O \xrightarrow{creatine\ amidohydrolase} sarcosine + urea$

3) $sarcosine + O_2 + H_2O \xrightarrow{sarcosine\ oxidase} glycine + formaldehyde + H_2O_2$

4) $sarcosine + H_2O_2 \xrightarrow{peroxidase} dye + 2H_2O_2$

Wavelength:

670 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

Creatinine amidohydrolase, 0.20 U; creatine amidinohydrolase, 4.7 U; sarcosine oxidase, 0.55 U; peroxidise, 1.6 U; and 2-(3,5-dimethoxy-4-hydroxyphenyl)-4,5-bis-(4-dimethylaminophenyl) imidazole (leuco dye) 32µg.

Other slide ingredients:

Pigment, binders, surfactants, stabiliser, scavenger, chelator, buffer, dye solubiliser and cross-linking agent.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range [SI Units (conventional units)]:

4 – 1238 μmol.l^{-1} (0.05 – 14 mg.dl^{-1})

The within assay CVa was 5.4, 1.2 and 1.0% for 18, 80 and 495 μmol.l^{-1} respectively.

3.5.13 Urea

Urea or blood urea nitrogen (BUN) was measured in serum using VITROS BUN/UREA slides and the VITROS Chemistry Products Calibrator Kit 1 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. A drop of serum is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Water and non-proteinaceous components then travel to the underlying reagent layer, where the urease reaction generates ammonia. The semipermeable membrane allows only ammonia to pass through to the colour-forming layer, where it reacts with the indicator to form a dye. The reflection density of the dye is measured and is proportional to the concentration of urea in the sample.

1) $H_2NCONH_{2+}H_2O \xrightarrow{urease} 2NH_3 + CO_2$

2) $NH_3 + ammonia\ indicator \rightarrow dye$

Wavelength:

670 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

Urease, 1.2 U; and N-propyl-4-(2, 6-dinitro-4-chlorobenzyl)-quinolonium ethane sulphonate (ammonia indicator) 0.26 mg.

Other slide ingredients:

Pigment, binders, buffer, surfactants, stabilisers, chelator and cross-linking agent.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range [SI Units (conventional units)]:

0.71 – 42.83 $mmol.l^{-1}$ (2.0 – 120 $mg.dl^{-1}$)

The within assay CVa was 2.3, 2.0 and 1.9% for 5.7, 6.5 and 14.0 $mmol.l^{-1}$ respectively.

3.5.14 Total protein

Total protein was measure in serum using VITROS TP slides and the VITROS Chemistry Products Calibrator Kit 4 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. The method of analysis is based on the biuret reaction (), which produeces a violet complex when protein reacts with cupric ion (Cu^{2+}) in an alkaline medium. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. When the fluid

penetrates the reagent layer, the reagent diffuses up to the spreading layer and reacts with protein. The reaction between protein and copper tartrate takes place largely in the spreading layer where the protein is confined because of its high molecular weight. The amount of coloured complex formed is proportional to the amount of total protein in the sample and is measured by reflectance spectrophotometry.

1) $protein + copper\ tartrate \xrightarrow{lithium\ hydroxide} coloured\ complex$

Wavelength:

540 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

Copper sulphate, 0.9 mg; tartaric acid, 1.2 mg; and lithium hydroxide, 1.3 mg.

Other slide ingredients:

Polymer beads, binders and surfactants.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range [SI Units (conventional units)]:

20 – 110 $g.l^{-1}$ (2 – 11 $g.dl^{-1}$)

The within assay CVa was 3.5, 3.7 and 2.3% for 45, 52 and 74 $g.l^{-1}$ respectively.

3.5.15 Albumin

Albumin was measured in serum using VITROS ALB slides and the VITROS Chemistry Products Calibrator Kit 4 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. When the fluid penetrates the reagent layer, the bromcresol green (BCG) dye diffuses to the spreading layer and binds to albumin from the sample. This binding results in a shift in wavelength of the reflectance maximum of the free dye. The colour complex that forms is measured by reflectance spectrophotometry. The amount of albumin bound dye is proportional to the concentration of albumin in the sample.

1) $albumin + bromcresd\ green(BCG) \rightarrow BCG\text{-}albumin\ complex$

Wavelength:

630 nm

Approximate incubation time and temperature:

2.5 mins, 37°C

Reactive slide ingredients per cm^2:

Bromcresol green dye 104 µg.

Other slide ingredients:

Polymer beads, binders, buffer and surfactants.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range [SI Units (conventional units)]:

10 – 60 $g.l^{-1}$ (1 -6 $g.dl^{-1}$)

The within assay CVa was 1.4, 1.5 and 1.3% for 28, 39 and 45 $g.l^{-1}$ respectively.

3.5.16 Alkaline phosphatase

Alkaline phosphatise was measured in serum using VITROS ALKP slides and the VITROS Chemistry Products Calibrator Kit 3 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the *p*-nitrophenyl phosphate substrate and other components needed for the reaction. The alkaline phosphatise in the sample catalyses the hydrolysis of the *p*-nitrophenyl phosphate to *p*-nitrophenol at alkaline pH. The *p*-nitrophenol diffuses into the underlying layer, and it is monitored by reflectance spectrophotometry. The rate of change in reflection density is converted to enzyme activity.

1) $p-nitrophenyl\ phosphate \xrightarrow{alkalinephosphatase,Mg^{2+},AMP} p-nitrophenol + H_3PO_4$

Wavelength:

400 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

p-nitrophenyl phosphate, 55 µg; 2-amino-2-propanol (AMP), 0.1 mg; and magnesium sulphate, 1.6 µg.

Other slide ingredients:

Pigment, binders, buffers, surfactants, cross-linking agent and stabiliser.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range (SI Units):

20 – 1500 $U.l^{-1}$.

The within assay CVa was 2.6 and 1.9% for 83 and 496 $U.l^{-1}$ respectively.

3.5.17 Alanine aminotransferase

Alanine aminotransferase was measured in serum using VITROS ALT slides and the VITROS Chemistry Products Calibrator Kit 3 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the alanine aminotransferase substrates L-alanine and sodium α-ketogluterate. Alanine aminotransferase catalyses the transfer of the amino group of L-alanine to α-ketogluterate to produce pyruvate and glutamate. Lactate dehydrogenase then catalyses the conversion of pyruvate and NADH to lactate and NAD^+. The rate of oxidation of NADH is monitored by reflectance spectrophotometry. The rate of change in reflection density is proportional to enzyme activity.

1) $alanine + \alpha\text{-}ketogluterate \xrightarrow{ALT,pyridoxal\text{-}5\text{-}phosphate} pyruvate + glutamate$

2) $pyruvate + NADH + H^+ \xrightarrow{lactate\ dehydrogenase} lactate + NAD^+$

Wavelength:

340 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

Lactate dehydrogenase, 0.12 U; L-alanine, 0.86 mg; sodium α-ketoglutarate, 54μg; nicotinamide adenine dinucleotide, reduced, 35 μg; and sodium pyridoxal-5-phosphate, 11 μg.

Other slide ingredients:

Pigment, binders, buffer, surfactants, cross-linking agent and stabiliser.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range (SI Units):

3 – 1000 $U.l^{-1}$.

The within assay CVa was 8.1 and 2.0% for 34 and 189 $U.l^{-1}$ respectively.

3.5.18 Total bilirubin

Total bilirubin was measured in serum using VITROS TBIL slides and the VITROS Chemistry Products Calibrator Kit 4 (Ortho-Clinical Diagnostics, Amersham, UK). The slide is a multi-layered, analytical element coated on a polyester support. The analysis is based on a modification of the diazo reaction (The reaction of diazotised sulfanilic acid with bilirubin to form azobilirubin, which forms the basis of quantifying the amount of bilirubin in biological fluids). A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. This layer provides a

reflective background for measuring diazo products of bilirubin and contains all reagents necessary to determine total bilirubin.

The method uses dyphylline to dissociate unconjugated bilirubin from albumin. Unconjugated bilirubin (Bu), conjugated bilirubin (Bc) and albumin-linked bilirubin (delta bilirubin, Bd) subsequently react with the diazonium salt 4-(*N*-carboxymethylsulfonyl) benezenediazonium hexafluorophosphate (4-(*N*-C)BH) to produce azobilirubin chromophores that have similar molar absorptiveities and absorbance maxima around 520 nm.

The concentration of total bilirubin is determined by measuring the azobilirubin chromophores at two wavelengths through the transparent support. The reflectance at 460 nm corrects for spectral interferences.

1) $total\ bilirubin(Bu, Bc, Bd) \xrightarrow{dyphylline\ [4\text{-}(N\text{-}C)BH]} azobilirubinchromophores$

Wavelength:

540 nm and 460 nm

Approximate incubation time and temperature:

5 mins, 37°C

Reactive slide ingredients per cm^2:

Dyphylline, 0.5 mg; and 4-(N-carboxymethylaminosulfonyl) benzene diazonium hexafluorophosphate, 57μg.

Other slide ingredients:

Pigment, binders, buffer, mordant, surfactants and stabiliser.

Calibration:

- When the slide lot number changes
- When critical system parts are replaced due to service or maintenance
- When government regulations require
- If quality control results are consistently outside acceptable range
- After certain service procedures have been performed.

Reportable range [SI Units (conventional units)]:

1.7 – 461.7 $\mu mol.l^{-1}$ (0.1 - 27.0 $mg.dl^{-1}$)

The within assay CVa was 2.6, 2.6 and 1.5% for 15, 17 and 265 $\mu mol.l^{-1}$ respectively.

3.6 Insulin resistance

Insulin resistance was assessed by homeostatic model assessment (HOMA-IR) (Matthews *et al.*, 1985) where:

$$HOMA\text{-}IR = \frac{(fasting\ plasma\ insulin \times fasting\ plasma\ glucose)}{22.5}$$

The model was subsequently updated (HOMA2) (Levy *et al.*, 1998) to account for variations in hepatic and peripheral glucose resistance, increases in the insulin secretion curve for plasma glucose concentrations >10 mmol/L and the contribution of circulating proinsulin (available at: http://www.dtu.ox.ac.uk/homacalculator/download.php).

3.7 Physical activity measurement

Physical activity was measured through self-reported, five-day recall questionnaire modified from a previously validated seven-day recall questionnaire (Riddoch, 1990) (Appendix 10).

3.8 Pubertal status assessment

Pubertal status was assessed through administration of a validated self-report questionnaire (Taylor *et al.*, 2001) (Appendix 12). The self assessment questionnaire was completed in private and based on the 5 Tanner stages of pubic hair growth (both sexes), breast development (girls) and penis development (boys). Similar to previous studies (Whincup *et al.*, 2005; Jeffery *et al.*, 2012), a mean score for both self-assessed Tanner measures (genital/breast and pubic hair development) was calculated. A mean score of 1.5 (e.g. genital stage 2, pubic hair stage 1) was treated as Tanner stage 2 (TS2), a score of 2.5 treated as TS3, etc.

3.9 Cardiorespiratory fitness measurement

Cardiorespiratory fitness was measured utilising the 20-metre multi-stage fitness test (MSFT) protocol (Léger *et al.*, 1988). For the 20 m shuttle run test, as described by Leger *et al.* (1988), subjects were required to run between two lines set 20 m apart, while keeping pace with audio signals emitted from a pre-recorded CD. The initial running velocity is 8.5 km h^{-1}, which is increased by 0.5 km h^{-1} min^{-1} (1 min equals one stage). Subjects were instructed to run in a straight line, to pivot on completing a shuttle, and to pace themselves in accordance with the audio signals. The test was finished when individual subjects failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped due to fatigue. All measurements were carried out under standardised conditions on an outdoor playing surface. The subjects were encouraged to keep running as long as possible throughout the course of the test. The last

completed stage or shuttle at which the subject dropped out was scored. A space large enough to mark out a 20 m track, a 20 m tape measure, a CD player and a CD with the audio signals recorded were used to perform the test. Shuttle run test performance was assessed as: the total number of shuttles performed; the level and stage attained; converted to predicted maximal oxygen uptake (VO_2max, ml kg^{-1} min^{-1}) using the original tables developed for the MSFT (Léger *et al.*, 1988).

3.10 Statistical analysis

Data was analysed using the statistical package SPSS/PASW (SPSS for Windows, Rel. 17.0.0. 2008; PASW Statistics, Rel. 18.0.0. 2009; Chicago: SPSS Inc.). Normal distribution of data was assessed by the Shapiro-Wilk test and the Kolmogorov-Smirnov test, in addition all variables were visually inspected via normal Q-Q plots and histograms. For the Shapiro-Wilk test and the Kolmogorov-Smirnov test, if $p > 0.05$ normal distribution was assumed, if $p \leq 0.05$ and the observed values on the normal Q-Q plots and histograms showed significant deviation from the expected values then non-normality was assumed. Where non-normal distribution was identified non-parametric equivalent statistical analyses were performed. Homogeneity of variance for group data was assessed using Levene's test.

Specific statistical analyses undertaken are described within each following study chapter.

4 Chapter Four: Prevalence and Agreement of Methods for the Determination of Overweight and Obesity.

4.1 Introduction

Obesity in childhood has been strongly suggested to cause hypertension, dyslipidaemia, chronic inflammation, an increased blood clotting tendency, endothelial dysfunction and hyperinsulinaemia. Moreover, it has been reported that the prevalence of childhood obesity has doubled within the last 20 years (Ebbeling *et al.*, 2002). For these reasons alone the early and accurate identification of childhood overweight and obesity is paramount. The methods used, and definitions of overweight and obesity employed, differ between countries and between epidemiological studies making comparisons difficult. More often, body mass index (BMI) is the preferred method for population surveillance (Cole *et al.*, 1995; Cole *et al.*, 2000; Kuczmarski *et al.*, 2000) although other methods have been employed including waist-to-hip ratio (Alberti and Zimmet, 1998; World Health Organisation, 1999b), waist circumference (Balkau and Charles, 1999; NCEP, 2001; Balkau *et al.*, 2002) and skinfold thickness (Raitakari *et al.*, 1994; Andersen *et al.*, 2003) amongst others.

Whilst there is no universally accepted criterion for identifying overweight and obesity in childhood and adolescence, standards that rely on BMI and WC predominate. Whilst studies have been conducted in England and internationally to evaluate BMI cut-off points to identify overweight and obesity, data in a Wales only cohort is absent. In England it has been reported (Jackson-Leach and Lobstein, 2006) that more than a third of children aged 11-15 years are overweight or obese (33% overweight, 19% obese) when using UK specific 85th and 95th percentile cut-off points. However, when international BMI cut-off criteria are applied these values decrease to 26% and 7%, respectively (Cole *et al.*, 2000). In Scotland more than a third (34.6%) of boys and almost a third (30%) of girls aged 2 - 15

are overweight or obese (Scottish Health Survey 2003, Scottish Health Survey, 2005). In a recent systematic review and comparison of the prevalence of childhood overweight and obesity using data from the 2001-2002 Health Behaviour in School-Aged Children Study (HBSC), conducted in 34 (mainly European and North American) countries, Welsh school children ranked third highest in terms of the total prevalence of overweight and obesity (21.5%; 16.7% overweight, 4.8% obese) (Janssen *et al.*, 2005). International age- and gender-specific child BMI cut-points were used to define overweight and obesity (Cole *et al.*, 2000). The more objective Welsh Health Survey 2009 reports that 34% of children were estimated to be overweight or obese with 19% classed as obese. Again, estimates using international cut-off points instead of the UK reference curves suggest that around 27% of children would be classified as overweight or obese, including 8% obese (Welsh Health Survey 2009, Welsh Health Survey, 2010). The limitation of the Wales Health Surveys are that they only obtain height and weight measurements for the calculation of BMI and no analysis of the sensitivity and specificity of this has been undertaken in Welsh schoolchildren. The current study collected data on waist circumference (WC), waist-to-height ratio (WHtR), waist-to-hip ratio (WHR) and triceps skinfolds thickness in addition to body mass index (BMI).

The purpose of this current project was to compare prevalence estimates of overweight and obesity, examine the sensitivity and specificity of cut-off points used in the UK National BMI percentile charts (UK 1990) (Cole *et al.*, 1995) and examine the sensitivity and specificity of several additionally proposed adiposity measures to identify overweight and obesity in a cohort of 228 children and adolescents in Wales, UK.

4.2 Methodology

Data collection was carried out as detailed in Chapter 3: General Methodology and consisted of measurement of height, weight, WC and hip circumference. BMI, WHtR and WHR were calculated. Overweight and obesity prevalence was calculated for:

BMI: using International Obesity Task Force (IOTF) age and gender specific criteria (Cole *et al.*, 2000) where reference curves have been smoothed to pass through BMI ≥25 and BMI ≥30 at 18 yrs to identify overweight and obesity, respectively; US (Centres for Disease Control and Prevention, CDC) age, gender and height specific criteria (Kuczmarski *et al.*, 2000) where subjects ≥85th percentile and ≥95th percentile are identified as overweight and obese, respectively; World Health Organisation (WHO) age and gender specific criteria (World Health Organisation, 2006b) where a subject ≥1 standard deviation score (SDS) and ≥2 SDS are identified as overweight or obese, respectively; and UK age and gender (controlled for height) specific criteria (Cole *et al.*, 1995) using cut-off criteria to identify overweight and obesity of ≥91st percentile and ≥98th percentile, respectively.

WC: using age and gender specific cut-off criteria to identify overweight and obesity of ≥91st percentile and ≥98th percentile, respectively (McCarthy *et al.*, 2001).

WHtR: using suggested child cut-offs of >0.5 and >0.6 to identify overweight and obesity, respectively (McCarthy and Ashwell, 2006).

4.3 Statistical analysis

Data were analysed using SPSS/PASW (versions 17.0 - 19.0). Differences between males and females were assessed by independent samples t-tests. Where data were found to be non-normally distributed the equivalent non-parametric tests (Mann-Whitney U) were performed. Differences with $p \leq 0.05$ were considered significant. Data was analysed by calculation of means ± standard deviation (SD) and simple prevalence's. Sensitivity and

specificity was calculated, where sensitivity measures the proportion of correctly identified true positives (i.e. subjects being correctly identified as overweight or obese) and specificity measures the proportion of correctly identified true negatives (i.e. subjects being correctly identified as normal weight). Sensitivity was calculated as:

$$Sensitivity = \frac{number\ of\ true\ positives}{number\ of\ true\ positives + number\ of\ false\ negatives}$$

Specificity was calculated as:

$$Specificity = \frac{number\ of\ true\ negatives}{number\ of\ true\ negatives + number\ of\ false\ positives}$$

[False positives are normal weight subjects incorrectly identified as overweight or obese, false negatives are overweight or obese subjects incorrectly identified as normal weight]

Sensitivity and specificity is reported with 95% confidence intervals.

Differences between pubertal stages were assessed by Bonferroni corrected ANOVA or by the non-parametric equivalent Kruskal-Wallis test.

4.4 Results

Selected subject descriptive characteristics are presented in Table 4.1. No significant differences were found between male and female subjects for age, height, weight, BMI, WHtR or pubertal ratings. Males had a higher WC and waist-to-hip ratio whereas females had higher hip circumference and triceps skinfolds. Overall, pubertal status data was obtained in 179 children (TS1 = 5; TS2 = 28; TS3 = 60; TS4 = 67 and TS5 = 19).

Unsurprisingly, significant positive linear associations ($p < 0.001$) were found with respect to pubertal status in that as age, height and weight increased so to did Tanner stage (TS). No differences between TS groups were found for any measures of adiposity.

Table 4.1: Subjects descriptive statistics

	N (M/F)	All	Males	Females	*P*
Age (years)	228 (122/106)	13.2 ± 1.0	13.2 ± 1.0	13.2 ± 1.0	*ns*
Height (cm)	227 (122/105)	157.4 ± 9.3	158.5 ± 10.2	156.1 ± 7.9	*ns*
Weight (kg)	227 (122/105)	51.2 ± 12.5	51.9 ± 12.9	50.4 ± 12.0	*ns*
BMI (kg/m^2)	227 (122/105)	20.5 ± 3.7	20.5 ± 3.8	20.5 ± 3.6	*ns*
WC (cm)	226 (122/104)	71.2 ± 9.9	72.6 ± 10.7	69.6 ± 8.7	*<0.05*
HC (cm)	150 (77/73)	85.3 ± 9.6	84.2 ± 10.2	86.6 ± 8.8	*≤0.05*
WHR	150 (77/73)	0.83 ± 0.07	0.85 ± 0.07	0.80 ± 0.06	*<0.0001*
WHtR	226 (122/104)	0.45 ± 0.06	0.46 ± 0.06	0.45 ± 0.05	*ns*
Triceps (mm)	227 (122/105)	15.4 ± 6.7	14.1 ± 6.8	16.9 ± 6.3	*<0.0001*
Pubertal rating:					
Breast/penis	182 (100/82)	3.2 ± 1.1	3.3 ± 1.2	3.1 ± 1.1	*ns*
Pubic hair	182 (100/82)	3.1 ± 1.0	3.2 ± 1.0	3.1 ± 1.0	*ns*

Data presented as means ± SD. HC, hip circumference; WC, waist circumference; WHR, waist-to-hip ratio; WHtR, waist-to-height ratio.

Prevalence estimates of overweight or obesity using different criteria are presented in Table 4.2 and Figure 4.1. Overall, WC identified more subjects as overweight or obese compared to all other estimates with females displaying a greater prevalence of elevated WC compared to males. Unsurprisingly, BMI based measures displayed differing prevalence with the least conservative estimates identifying greater numbers of subjects as overweight and obese (least conservative = 34.8%, most conservative = 24.7%) or obese alone (least conservative = 18%, most conservative = 8.2%). WHtR consistently produced the lowest estimates of overweight and obesity.

Sensitivity and specificity estimates are presented in Table 4.3. The IOTF classifications were used as comparison standards for all other overweight and obesity measures. Overall, all BMI measures showed good sensitivity (correctly identifying subjects as overweight or obese) and specificity (correctly identifying subjects as normal weight). Sensitivity and

specificity were highest when ≥91st (sensitivity = 0.98, CI 0.89 – 0.99; specificity = 0.98, CI 0.94 – 0.99) and ≥98th (sensitivity = 1, CI 0.73 - 1; specificity = 0.97, CI 0.94 – 0.99) BMI percentiles were used. WHtR >0.5 showed poor sensitivity (0.59, CI 0.45 – 0.72) for identifying overweight and obesity and for identifying obesity alone (0.43, CI 0.19 – 0.7).

Table 4.2: Prevalence estimates of overweight and obesity for males and females (n = 227; 122 male) aged 11-14 years in Wales, UK.

	Overweight and obesity (%)				Obesity (%)			
	Defining criteria	Overall	Males	Females	Defining criteria	Overall	Males	Females
IOTF	BMI ≥25 at 18 yrs	24.7	26.2	22.9	BMI ≥30 at 18 yrs	6.2	8.2	3.8
BMI	≥85th %ile	34.8	38.5	30.5	≥95th %ile	16.3	18.0	14.3
BMI	≥91st %ile	26.0	29.5	21.9	≥98th %ile	8.8	10.7	6.7
WC	≥91st %ile	37.2	33.6	41.3	≥98th %ile	18.6	15.6	22.1
WHtR	>0.5	15.9	18.0	13.5	>0.6	2.7	4.1	1.0
WHO	≥1 SDS	33.2	36.9	28.6	≥2 SDS	8.4	10.7	5.7

%ile, centile; BMI, body mass index (kg/m^2); IOTF, International Obesity Task Force; SDS, standard deviation score; ; WC, waist circumference; WHtR, waist circumference to height ratio

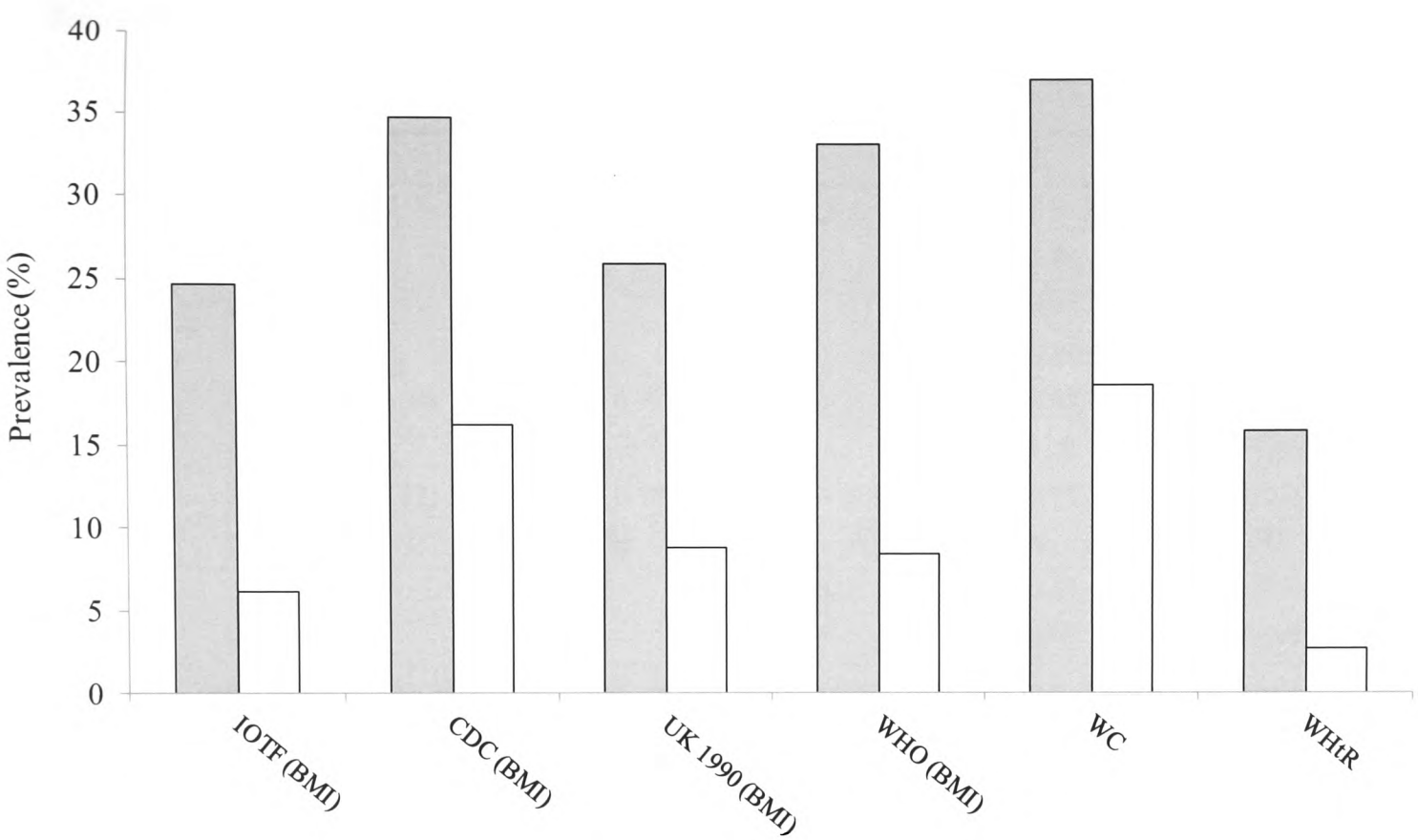

Figure 4.1: Prevalence of overweight and obesity (filled columns) and obesity alone (open columns) in children and adolescents. Overweight and obesity is defined as: IOTF, BMI ≥25 kg/m^2 at 18 yrs; ≥30 kg/m^2 at 18 yrs (Cole *et al.*, 2000); CDC, BMI ≥85th %ile; ≥95th %ile (Kuczmarski *et al.*, 2000); UK 1990, BMI ≥91st %ile; ≥98th %ile (Cole *et al.*, 1995); WHO, BMI ≥1 SDS; ≥2 SDS (World Health Organisation, 2006b); WC, ≥91st %ile; ≥98th %ile (McCarthy *et al.*, 2001); WHtR, >0.5; >0.6 (McCarthy and Ashwell, 2006).

Table 4.3: Sensitivity and specificity (95% confidence intervals) of obesity measurements using the IOTF classifications as the comparison standard for males and females (n = 227; 122 male) aged 11-14 years in Wales, UK.

		Sensitivity			Specificity		
		Overall	Males	Females	Overall	Males	Females
BMI	≥85th %ile	1 (0.92 – 1)	1 (0.87 – 1)	1 (0.83 – 1)	0.87 (0.8 - 0.91)	0.83 (0.74 – 0.9)	0.9 (0.81 – 0.95)
	≥95th %ile	1 (0.73 - 1)	1 (0.66 - 1)	1 (0.40 - 1)	0.89 (0.84 – 0.93)	0.89 (0.82 – 0.94)	0.89 (0.81 – 0.94)
BMI	≥91st %ile	0.98 (0.89 – 0.99)	1 (0.87 - 1)	0.96 (0.77 – 1)	0.98 (0.94 – 0.99)	0.96 (0.88 – 0.99)	1 (0.94 - 1)
	≥98th %ile	1 (0.73 - 1)	1 (0.66 - 1)	1 (0.40 - 1)	0.97 (0.94 – 0.99)	0.97 (0.92 – 0.99)	0.97 (0.91 – 0.99)
WC (cm)	≥91st %ile	0.95 (0.84 – 0.99)	0.94 (0.78 – 0.99)	0.96 (0.77 - 1)	0.82 (0.75 – 0.87)	0.88 (0.79 – 0.93)	0.75 (0.64 – 0.84)
	≥98th %ile	1 (0.73 - 1)	1 (0.66 - 1)	1 (0.4 - 1)	0.87 (0.81 – 0.91)	0.92 (0.85 – 0.96)	0.81 (0.72 – 0.88)
WHtR	>0.5	0.59 (0.45 – 0.72)	0.63 (0.44 – 0.78)	0.64 (0.45 – 0.8)	0.98 (0.95 - 1)	0.98 (0.91 - 1)	0.99 (0.92 – 1)
	>0.6	0.43 (0.19 – 0.7)	0.5 (0.2 – 0.8)	0.25 (0.01 – 0.78)	1 (0.98 - 1)	1 (0.96 - 1)	1 (0.95 - 1)
WHO	≥1 SDS	1 (0.92 - 1)	1 (0.87 - 1)	1 (0.83 - 1)	0.89 (0.83 – 0.93)	0.86 (0.76 – 0.92)	0.93 (0.84 – 0.97)
	≥2 SDS	1 (0.73 - 1)	1 (0.66 - 1)	1 (0.4 - 1)	0.98 (0.94 – 0.99)	0.97 (0.92 – 0.99)	0.98 (0.92 – 1)

95% CI in parentheses. %ile, centile; BMI, body mass index; SDS, standard deviation score; ; WC, waist circumference; WHtR, waist circumference-to-height ratio

4.5 Discussions

Prevalence of childhood overweight and obesity is increasing in western societies leading to increasing public health concern (Lobstein *et al.*, 2004; Wang and Lobstein, 2006). It has been identified that there is a need for a widely applicable growth reference for children and adolescents to allow international comparisons and to allow countries to assess the magnitude of the public health problem (de Onis *et al.*, 2007). There are now two suggested international reference data sets to assess BMI for age (Cole *et al.*, 2000; de Onis *et al.*, 2007) and many more national data sets are in use. The purpose of this study was to compare prevalence estimates of overweight and obesity in a small cohort of children and adolescents using international and UK specific BMI-based criteria and to examine the sensitivity and specificity of each in comparison to other anthropometry-based methods. This study has identified that the prevalence of overweight and obesity, and obesity alone in children and adolescents in Wales, UK is high yet varies greatly between both BMI-based measures (16 – 35%) and between different anthropometry-based measures (3 – 19%). BMI and WC measures produce broadly similar results whereas WHtR underestimates both overweight and obesity.

Male and female overweight and obesity prevalence in the current cohort is higher than that obtained by Cole *et al.* (2000) during the development of the IOTF BMI curves. They found a prevalence of overweight of 9.6% (males) and 11.7% (females) compared to our findings of 18.8% (males) and 15.2% (females). Prevalence of obesity was 0.9% and 1.2% in the IOTF study compared to 10.7% and 6.7% (males and females respectively) in the current study. In the HBSC study (Janssen *et al.*, 2005) the prevalence of overweight and obesity for males and females combined in Wales was 21.5%, much lower than in the current study (34.8%, $\geq 85^{th}$ percentile). However, it is likely that there is some room for error in the HBSC study given that data collection consisted of a questionnaire administered in a classroom setting. Moreover, height and body weight were based on self-

report measures. A recent study assessing the validity of self-reported weight, height and calculated body mass index in Chinese adolescents found good correlations between measured and self-reported height ($r = 0.94$), weight ($r = 0.91$) and BMI ($r = 0.81$) (Zhou *et al.*, 2010). However, the limits of agreement between self-reported and measured values of weight and height differed by more than one standard deviation whereas the calculated BMI differed by more than 2 standard deviations. Others have also found that children and adolescents tend to overestimate stature, underestimate weight and underestimate BMI (Himes and Story, 1992; Brener *et al.*, 2003; Himes *et al.*, 2005).

The results confirm that the identification of child and adolescent overweight and obesity based on BMI using the current National data sets (Cole *et al.*, 1995) is associated with high specificity and will lead to a low false-positive rate (i.e. incorrectly labelling children as overweight or obese) when compared to the IOTF comparison standard (Cole *et al.*, 2000). Sensitivity and specificity were highest when the 91st and 98th percentiles were used. The current data display similar results to those obtained from the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) (Reilly *et al.*, 2000). Using an obesity definition of the 95th percentile with body fat percentage obtained via bioelectrical impedance analysis as the comparison standard they reported a moderately high sensitivity of 0.88 and high specificity of 0.94, the current study obtained values of 0.89 and 1.0. However, they identified the optimum combination of sensitivity (0.92) and specificity (0.92) at a BMI cut-point of the 92nd percentile to identify obesity. In the current study the 91st percentile, clinically used to identify overweight in UK children and adolescents, displayed similar high sensitivity and specificity values (both 0.98). The IOTF criteria were chosen as the comparison standard because these are increasingly used and internationally recognised. In 2007, the WHO released their growth reference standards stating the "need to develop an appropriate single growth reference for the screening, surveillance and monitoring of school-aged children and adolescents"(de Onis *et al.*, 2007,

p. 660). They identified 115 data sets from 45 different countries, none of which made it in to the final analysis for development of the growth reference curves due to problems with heterogeneity in methods and data quality, sample size, age categories, socioeconomic status of participating children and various other factors critical to growth curve construction. Instead, the WHO growth reference standards for BMI are based on data collected only in the United States (Hamill *et al.*, 1977) thus limiting their international applicability. Despite this limitation of the WHO reference standards, it is not unusual that the criteria and supporting data sets from one country are used to estimate overweight and obesity prevalence in a differing country, and the US Centres for Disease Control (CDC) data which use $\geq 85^{th}$ percentile and $\geq 95^{th}$ percentile to identify overweight and obesity are also widely applied (Kuczmarski *et al.*, 2000). Interestingly, the same data used in the development of the CDC growth charts was also used in the development of the WHO growth reference standards. The Welsh National Child Measurement Programme regulation came in to force on 1st August 2011 (Welsh Assembly Government, 2011). This programme aims to measure the height and weight of every participating reception age (4 – 5 years) child in Wales with the remit to consider extension to children aged 8 and 9 years to contribute to the European Childhood Obesity Surveillance Initiative. Its minimum objectives are the standardisation of height and weight measurement and to allow reporting of prevalence trends of underweight, overweight and obesity that are comparable across Wales and with data produced internationally. To data there is no information on the measurement methods to be used or the criteria by which overweight and obesity will be defined. It is evident that further research is needed in Wales on the validity of BMI as a measure of excess adiposity in school-aged children.

In adults, waist circumference is a useful tool for assessing risk for obesity-related diseases such as cardiovascular disease (Lemieux *et al.*, 2000) and has been shown to correlate with intra-abdominal fat mass (Lean *et al.*, 1995). Moreover, WHtR in adults has been

suggested to be a better predictor of intra-abdominal fat than BMI or waist circumference (Ashwell *et al.*, 1996a). In the UK it has been reported that children's waist sizes have increased rapidly in recent years (McCarthy *et al.*, 2003; Rudolf *et al.*, 2004; McCarthy and Ashwell, 2006). WHtR has also been associated with increased cardiovascular risk and morbidity in children (Savva *et al.*, 2000; Hara *et al.*, 2002; Kahn *et al.*, 2005a). An elevated WHtR may, therefore, be an estimator of the visceral adipose tissue mass. Visceral adipose tissue is minimally present in newborns and is usually sparse among children (Goran *et al.*, 1997). Nevertheless, the emergence of visceral fat in children and adolescents could be interpreted as a specific marker of systemic lipid over-accumulation. In the current study the waist-to-height ratio proved to have low sensitivity for correctly identifying overweight or obese children.

Body composition changes during adolescence and includes changes in both total and percentage body fat. Specifically, in boys, fat-free mass tends to increase, and percentage body fat decreases. In girls, both fat and fat-free mass increase, and fat-free mass as a percentage of body weight decreases (Naumova *et al.*, 2001). In addition, patterns of body fat distribution change. These alterations differ for boys and girls and are, in part, mediated by hormonal differences. Pronounced centralisation of fat stores with increases in subcutaneous fat and visceral fat in the abdominal region occur in boys; this pattern is similar but less dramatic for girls (Morrison *et al.*, 1999a; Morrison *et al.*, 1999b). Childhood obesity persists in to adult life (Whitaker *et al.*, 1997), however, studies of adolescent obesity usually have not included measures earlier in childhood, making it impossible to distinguish between obesity present in adolescence and obesity with onset in adolescence (Daniels *et al.*, 2005). Obesity present in adolescence has been shown to be associated with increased overall mortality and with increased risk of CVD and diabetes in adult men and women (Must, 2003).

There were several limitations to the current study. In particular, the lack of an objective measure of total body fat as the comparison standard in the sensitivity and specificity analyses. If a comparison standard is imperfect any sensitivity and specificity values obtained will appear poorer and be misleading. For example, if the measure under examination is more sensitive than the comparison standard, additional cases identified would be considered to be false positives in relation to the comparison standard (Fletcher and Fletcher, 2005). However, the purpose of this study was to compare national obesity cut-offs with what has become an internationally recognised comparison standard for epidemiological studies and to identify the levels of agreement between different methods. Another limitation is the cross-sectional, convenience population design of the study which limits the application of these findings to the wider population. In addition, the study was predominantly conducted in Caucasian children and adolescents, with little representation from black, Asian, or individuals of other racial or ethnic backgrounds. As a result the conclusions may not be applicable to all populations. In particular, there are findings that show that cardiovascular outcomes occur in adult Asian populations at a lower waist circumference (Snehalatha *et al.*, 2003), thus suggesting also at a lower degree of visceral adiposity. There are also clear differences across ethnic populations in the relationship between overall adiposity, abdominal obesity and visceral fat accumulation (Després *et al.*, 2000; Lear *et al.*, 2003; Tan *et al.*, 2004). Therefore, it is possible that children and adolescents from different ethnic backgrounds may also have differing levels of visceral adiposity.

4.6 Conclusions

In summary this study has shown that the prevalence of overweight and obesity, and obesity alone in children and adolescents in Wales, UK is high yet varies greatly dependent on the measurement methods employed and the cut-off criteria applied. Body mass index and waist circumference measures produced broadly similar results whereas the waist-to-

height ratio underestimates both overweight and obesity. The data underlines the importance of assessing child and adolescent anthropometry using accurate and reproducible methods and, more importantly, identifying overweight and obesity using standards that allow both national and international comparisons to be made.

5 Chapter Five – Prevalence of Metabolic Syndrome in Children and Adolescents

5.1 Introduction

It has been estimated that some 1.8m children in the United Kingdom are overweight and a further 700 000 children are obese (Lobstein and Leach, 2004). More specifically, the Health Behaviour in School Children study conducted in 34 mainly European and North American countries identified Welsh school children as ranking third highest in terms of the total prevalence of overweight and obesity (21.5%; 16.7% overweight, 4.8% obese) (Janssen *et al.*, 2005). Obesity in young populations has previously been associated with multiple cardiovascular risk factors, including dyslipidaemia (Goran and Gower, 1998) and type 2 diabetes mellitus (Arslanian, 2002), has been cited as the most common cause of insulin resistance (Caprio, 2002) and is also associated with the development of atherosclerosis (Berenson *et al.*, 1998). Furthermore, obesity has been shown to track within childhood from the first year of life (Vogels *et al.*, 2006). Moreover, childhood obesity has also been shown to predict for the metabolic syndrome in adulthood (Vanhala *et al.*, 1999).

Several studies have reported on the prevalence of metabolic syndrome in child and adolescent populations using many differing criteria (Chapter Two, Table 2.2) and, as in adult populations, several different diagnostic criteria have been formally proposed (Cruz and Goran, 2004; de Ferranti *et al.*, 2004; Zimmet *et al.*, 2007). Overall prevalence of child and adolescent metabolic syndrome in varying populations has been reported between 2% (Freedman *et al.*, 1999a; Whincup *et al.*, 2005) and 16.5 – 18.2% (Katzmarzyk *et al.*, 2004) of subjects. In lean, overweight and obese individuals metabolic syndrome has been reported between 0% (Csabi *et al.*, 2000; da Silva *et al.*, 2005; Klein-Platat *et al.*, 2005; Platat *et al.*, 2006) and 1.2 – 4.2% (Morrison *et al.*, 1999a; Morrison *et al.*, 1999b); 0% (da

Silva *et al.*, 2005) and 48% (Golley *et al.*, 2006); and ~3% (Chu *et al.*, 1998) and 63% (Golley *et al.*, 2006), respectively. Much of the variation in prevalence between studies is likely to be due to differences between population ethnic groups studied (Morrison *et al.*, 1999a; Morrison *et al.*, 1999b). Much more of the variation is likely to be due to the diagnostic criteria applied. For example, a study of 99 overweight or mildly obese children aged 6 – 9 years, which applied multiple definitions, reported metabolic syndrome prevalence's of between 3 and 60% depending on the criteria employed (Golley *et al.*, 2006). Overall, metabolic syndrome occurs infrequently in lean individuals but is highly prevalent in the presence of overweight and obesity.

The prevalence of child and adolescent metabolic syndrome has previously been estimated in the UK (Viner *et al.*, 2005; Whincup *et al.*, 2005). These studies have predominantly been conducted in an English cohort and have identified an overall metabolic syndrome prevalence of 2% (Whincup *et al.*, 2005), while in obese children prevalence increases markedly (33%) (Viner *et al.*, 2005). In adults, cardiovascular disease rates are known to vary by region within individual countries (Scarborough *et al.*, 2010) and it is likely that cardiovascular disease risk factors in children are also likely to vary. Overall, this means that metabolic syndrome prevalence's estimated from an English child and adolescent population may not be representative of the Welsh population. This study sought to estimate the prevalence of metabolic syndrome and its components using 4 different metabolic syndrome definitions, and specifically the influence of overweight and obesity and insulin concentrations, in a cohort of 11-14 year old school children in South Wales.

5.2 Methodology

Data collection was carried out as detailed in Chapter 3: General Methodology and consisted of height and weight for the calculation of BMI, WC, SBP and DBP, insulin, glucose, calculation of HOMA-IR, TC, TG, HDL, LDL and pubertal rating. Metabolic

syndrome was identified using 4 differing proposed definitions as detailed in Table 5.1. Definition 1 (de Ferranti *et al.*, 2004) is a child and adolescent modification of the NCEP adult metabolic syndrome criteria (National Cholesterol Education Program, 2001b). Definition 2 is a modification of the paediatric criteria proposed by Cruz and Goran (2004) and was chosen because these criteria have been applied to a UK cohort of children (Whincup *et al.*, 2005). Definition 3 follows the same criteria as definition 2 with the exception that the criteria for elevated glucose have been lowered to a concentration >5.6 mmol/L in line with recent recommendations (Genuth *et al.*, 2003). Definition 4 is the worldwide child and adolescent criteria proposed by the International Diabetes Federation (IDF) (Zimmet *et al.*, 2007).

5.2.1 Statistical analysis

Data were analysed using SPSS/PASW (versions 17.0 - 19.0). Data are presented as means ± standard deviation unless specified otherwise. Differences between groups were determined by independent samples *t*-tests. Where data were found to be non-normally distributed corresponding non-parametric statistical tests were used. Pearson Product-Moment Correlation Coefficients and the non-parametric Spearman Rank-Sum Correlation were calculated to identify associations between metabolic syndrome components and related variables. Differences between prevalence estimates were assessed using the Chi squared test (χ^2). Differences between quartiles of fasting insulin between pubertal stages were each assessed by ANOVA (Kruskal-Wallis test) and Bonferroni corrected *t*-tests. A value of $p \leq 0.05$ was considered to represent statistical significance.

Table 5.1: Definitions of child and adolescent metabolic syndrome

	Definition 1 (de Ferranti *et al.*, 2004)	Definition 2 (Whincup *et al.*, 2005) Modification of (Cruz and Goran, 2004)	Definition 3 (Whincup *et al.*, 2005) Modification of (Cruz and Goran, 2004). Glucose lowered to 5.6 mmol/L	Definition 4 (Zimmet *et al.*, 2007)
	≥3 criteria	≥3 criteria	≥3 criteria	Obesity and ≥2 other criteria
Obesity (WC)	>75th percentile*	> 90th percentile* [a]	> 90th percentile* [a]	≥90th percentile
Blood pressure (SBP and/or DBP)	>90th percentile†	>90th percentile†	>90th percentile†	≥130 mmHg systolic or ≥85 mmHg diastolic
Triglyceride	≥1.1 mmol/L	>90th * [b]	>90th * [b]	≥1.7 mmol/L
HDL	<1.3 mmol/L	<10th * [b]	<10th * [b]	<1.03 mmol/L
Glucose	≥6.1 mmol/L	>6.1 mmol/L	>5.6 mmol/L	≥5·6 mmol/L

* For age and gender; † for age, gender and height. [a] using data tables (McCarthy *et al.*, 2001); [b] using data tables (Hickman *et al.*, 1998).

5.3 Results

Anthropometric and blood biochemistry data are shown in Table 5.2. Overall, male subjects were found to have significantly higher WC and systolic blood pressure and lower total cholesterol, triglycerides and LDL cholesterol than female subjects. There were no gender differences for age, height, weight, BMI, diastolic blood pressure, pubertal stage or other blood biochemistry. Overall, pubertal status data was obtained in 179 children (TS1 = 5; TS2 = 28; TS3 = 60; TS4 = 67 and TS5 = 19) for age, height, weight, BMI, WC and blood pressure and in 151 children for fasting glucose, TG and HDL. No differences between TS groups were found for WC, BMI, TG, HDL, DBP and fasting glucose. A significant linear trend (p = 0.02) was found for increased SBP with increases in maturational status.

Table 5.2: Subjects descriptive statistics

	N (M/F)	All	Males	Females	*P*
Age (years)	228 (122/106)	13.2 ± 1.0	13.2 ± 1.0	13.2 ± 1.0	*ns*
Height (cm)	227 (122/105)	157.4 ± 9.3	158.5 ± 10.2	156.1 ± 7.9	*ns*
Weight (kg)	227 (122/105)	51.2 ± 12.5	51.9 ± 12.9	50.4 ± 12.0	*ns*
BMI (kg/m^2)	227 (122/105)	20.5 ± 3.7	20.5 ± 3.8	20.5 ± 3.6	*ns*
WC (cm)	226 (122/104)	71.2 ± 9.9	72.6 ± 10.7	69.6 ± 8.7	*<0.05*
SBP (mmHg)	185 (102/83)	113.2 ± 9.6	115.9 ± 9.8	109.7 ± 8.0	*<0.0001*
DBP (mmHg)	185 (102/83)	70.2 ± 7.5	69.7 ± 7.7	70.9 ± 7.3	*ns*
Insulin (pmol/L)	155 (89/66)	51.8 ± 34.0	54.1 ± 39.6	48.8 ± 24.4	*ns*
Glucose (mmol/L)	156 (88/68)	4.7 ± 0.5	4.7 ± 0.5	4.7 ± 0.5	*ns*
HOMA-IR	156 (88/68)	1.75 ± 1.28	1.85 ± 1.51	1.61 ± 0.90	*ns*
TC (mmol/L)	160 (92/68)	4.31 ± 0.87	4.13 ± 0.77	4.55 ± 0.95	*<0.01*
TG (mmol/L)	156 (89/67)	0.78 ± 0.35	0.72 ± 0.33	0.85 ± 0.37	*<0.05*
HDL (mmol/L)	154 (88/66)	1.63 ± 0.27	1.60 ± 0.27	1.68 ± 0.26	*ns*
LDL (mmol/L)	152 (86/66)	2.33 ± 0.81	2.21 ± 0.68	2.49 ± 0.94	*<0.05*
Pubertal rating:					
Breast/penis	182 (100/82)	3.2 ± 1.1	3.3 ± 1.2	3.1 ± 1.1	*ns*
Pubic hair	182 (100/82)	3.1 ± 1.0	3.2 ± 1.0	3.1 ± 1.0	*ns*

Data presented as means ± SD. BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG triglyceride; WC, waist circumference.

Overall prevalence of metabolic syndrome as identified by the four proposed definitions are shown in Table 5.3. Definition 1 identified a greater prevalence of obesity as a result of the lower cut-off value applied (>75th percentile) compared to the other definitions. Elevated blood pressure was identified equally in definitions 1, 2 and 3 as a result of identical criteria (>90th percentile). Prevalence of elevated TG, decreased HDL and elevated glucose ranged from 1.3 – 8.8%, 0 – 5.7% and 0 – 2.6%, respectively. Metabolic syndrome in all subjects with complete data for all criteria was identified in 3.5%, 0.4%, and 0.9% of subjects when definitions 1, 2 and 3 were applied, respectively. When the IDF metabolic syndrome criteria for children aged 10 - <16 years were applied no individuals met the diagnostic criteria.

Prevalence of individual components of the metabolic syndrome for males and females are displayed in Figure 5.1. Prevalence estimates are presented based on percentile cut-offs derived from the sample data only (>90th for WC, SBP, DBP, TG and <10th for HDL). Female subjects had higher prevalence estimates for elevated WC, DBP and TG and males displayed higher prevalence of elevated SBP (p <0.01) and low HDL. BMI showed highly significant associations with all components of the metabolic syndrome (WC, r = 0.87, $p \leq$ 0.0001; SBP, r = 0.26, $p \leq$ 0.001; DBP, r = 0.27, $p \leq$ 0.001; HDL, r = -0.26, $p \leq$ 0.001;) with the exception of fasting plasma glucose. Moreover, all measures of adiposity (BMI, WC, WHtR, triceps skinfold thickness) showed significant associations with 2 or more components of the metabolic syndrome.

To investigate the influence of elevations in insulin on metabolic syndrome components subjects were subdivided in to quartiles of increasing insulin concentrations. Figure 5.2 displays BMI and metabolic syndrome components according to quartiles of fasting insulin levels. Significant trends for increasing insulin concentration were seen with increasing BMI (Panel A, p <0.01), WC (Panel B, $p \leq$0.001), TG (Panel E, p <0.01) and fasting

glucose (Panel G, p <0.001). Subjects in the highest quartile of fasting insulin had significantly higher BMI and WC (p <0.05) compared to subjects in the lowest quartile. Children in the top two quartiles had significantly higher fasting plasma glucose than children in the lowest quartile (p <0.05).

Table 5.3: Prevalence of the metabolic syndrome using previously proposed criteria.

							MS (number of components, %)				
		WC	BP	TG	HDL	Glucose	0	1	2	3	4
Definition 1 (de Ferranti *et al.*, 2004)	%	57.9	18.0	8.8	5.7	0	32.9	47.8	15.8	3.1	0.4
	n	132/226	41/184	20/156	13/154	0/156					
Definition 2 (Whincup *et al.*, 2005)	%	38.2	18.0	1.3	0	0	51.8	39.5	8.3	0.4	
	n	87/226	41/184	3/156	0/154	0/156					
Definition 3 (Whincup *et al.*, 2005, modified)	%	38.2	18.0	1.3	0	2.6	50.0	40.8	8.3	0.9	
		87/226	41/184	3/156	0/154	6/156					
Definition 4 (Zimmet *et al.*, 2007)	%	38.2	4.8	1.3	0	2.6	39.9	24.1	3.9		
	n	87/226	11/185	3/156	0/154	6/156					

Data presented as prevalence (%) of individual components among all subjects with measurements for each component (*n* / *n total*). Overall MS prevalence (%) calculated only using subjects with all measurements. BP, systolic and/or diastolic blood pressure; Glucose, fasting glucose; HDL, high-density lipoprotein; MS, metabolic syndrome; TG, triglycerides; WC, waist circumference.

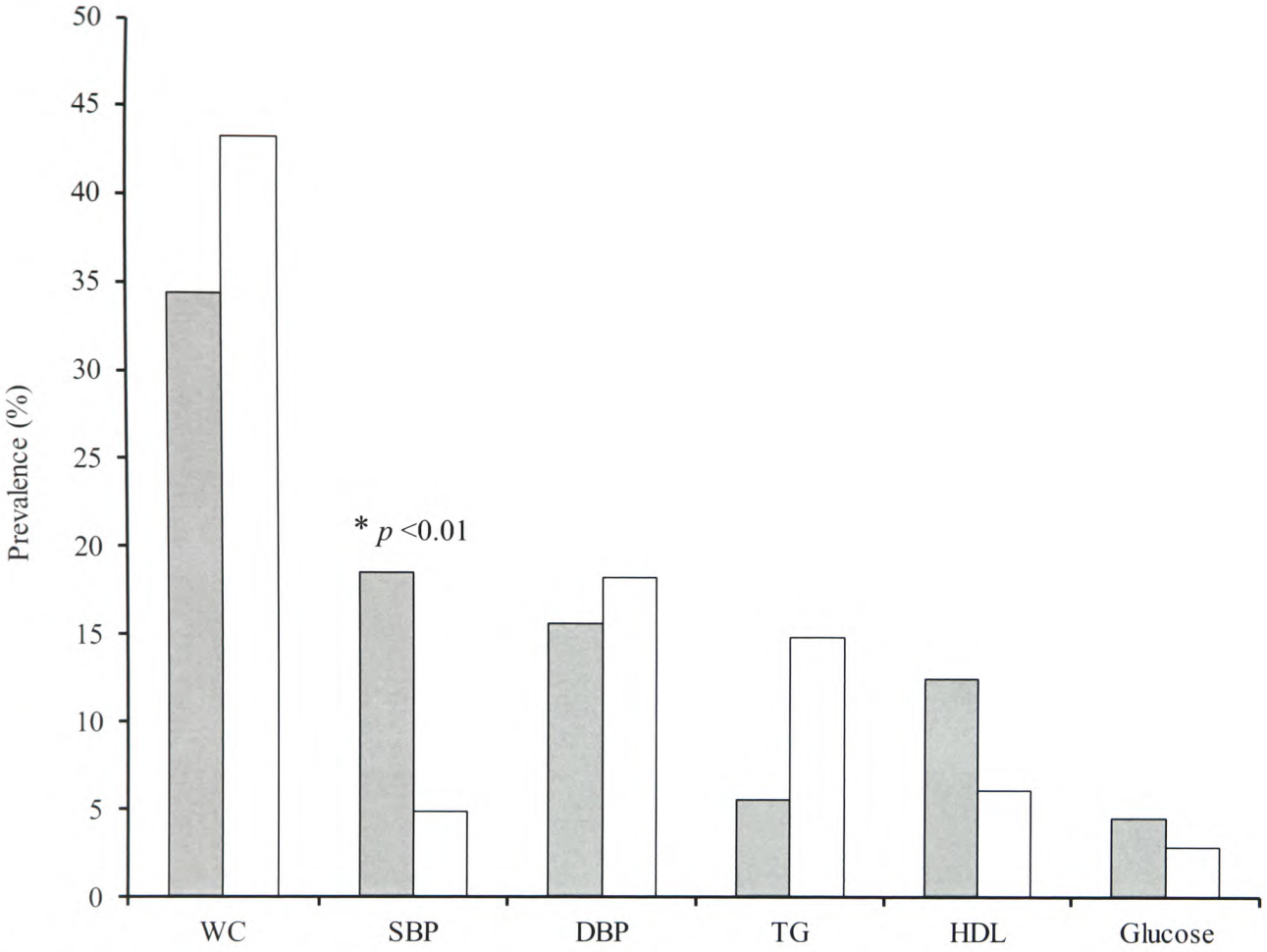

Figure 5.1: Prevalence estimates of individual components of the metabolic syndrome for males (filled bars) and females (open bars). Estimates are presented based on percentile cut-offs derived from the sample data only (>90th for WC, SBP, DBP, TG and <10th for HDL). * χ^2 test.

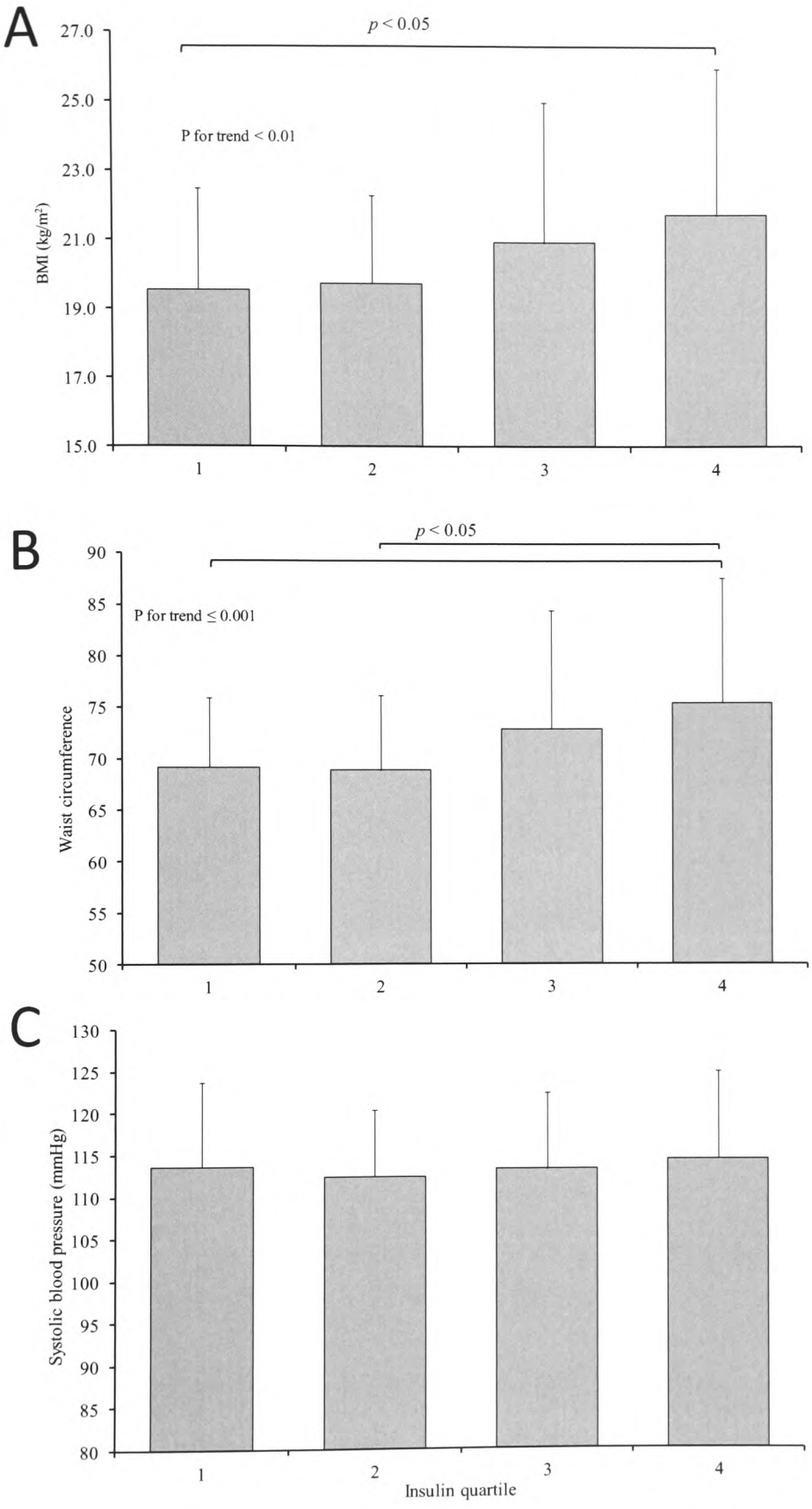

Figure 5.2 A-G: *continued.*

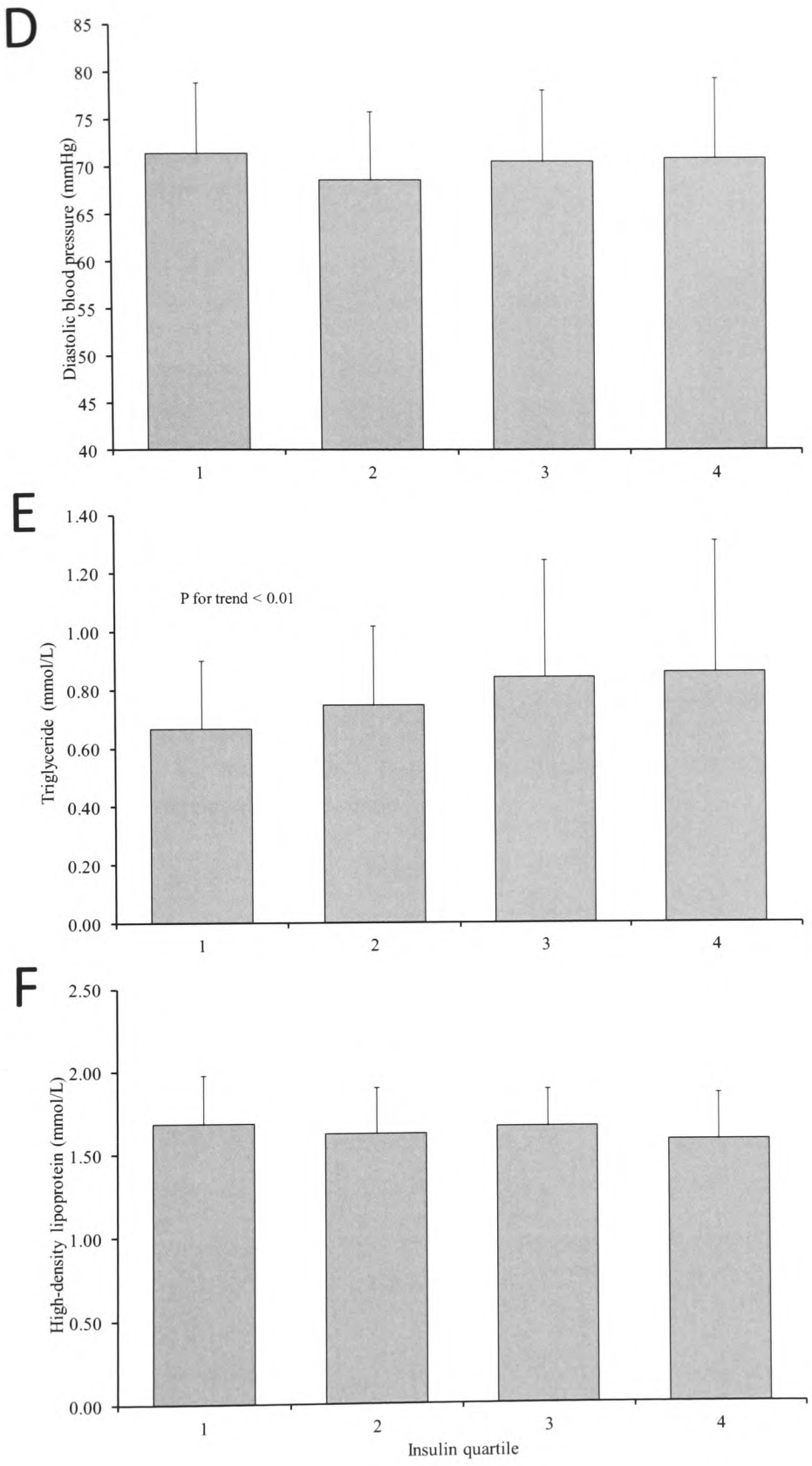

Figure 5.2 A-G: *continued.*

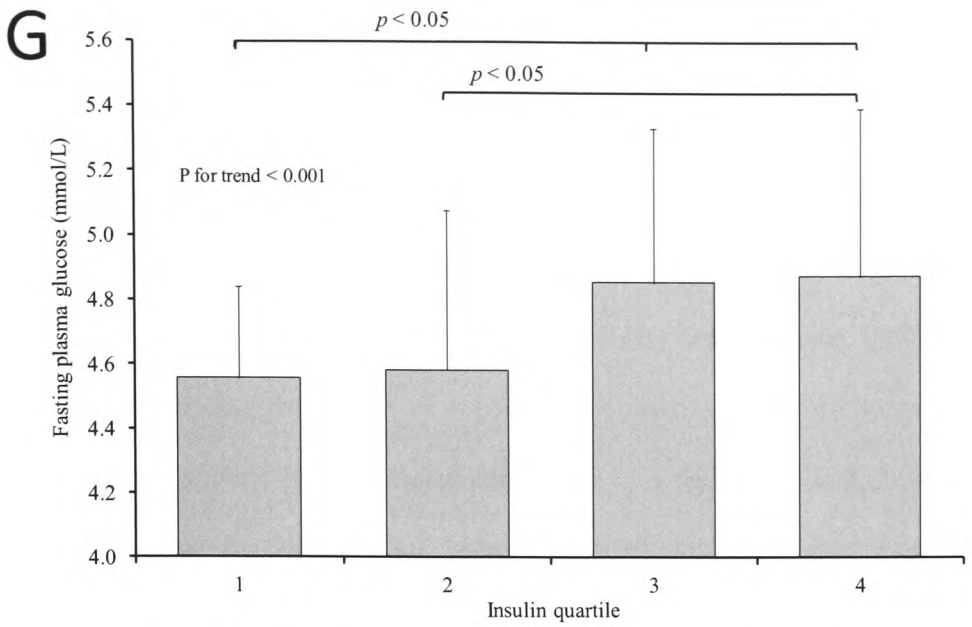

Figure 5.2 A-G: Relationship between BMI and metabolic syndrome components according to fasting insulin quartile (1, lowest quartile – 4, highest quartile). Panel A: BMI, p for trend <0.01; Panel B: WC, p for trend <0.001; Panel C; systolic blood pressure; Panel D: diastolic blood pressure; Panel E, triglyceride, p for trend <0.01; Panel G: fasting plasma glucose, p for trend <0.001. Data presented as means ± SD. Values of $p \leq 0.05$ were considered statistically significant.

5.4 Discussions

Metabolic syndrome occurs in children and adolescents and is particularly prevalent in the overweight and obese (Viner *et al.*, 2005; Golley *et al.*, 2006). Indeed, obesity in young populations is associated with multiple cardiovascular risk factors including lipid abnormalities (Goran and Gower, 1998) and type 2 diabetes mellitus (Arslanian, 2002) and has been cited as the most common cause of insulin resistance (Caprio, 2002). This study sought to estimate the prevalence of metabolic syndrome and its components, using 4 different definitions, in a cross-sectional cohort of 11-14 year old school children in South Wales. This study has shown that identification of metabolic syndrome is strongly dependent on the defining criteria employed with prevalence estimates ranging from 0% to 3.5%. Moreover, all measures of adiposity showed significant associations with 2 or more components of the metabolic syndrome. Similarly, insulin resistance was found to show significant positive associations with all measures of adiposity. Significant positive trends were also found between fasting insulin levels adiposity, TG and glucose levels.

This is the first study in which variations in metabolic syndrome prevalence, according to the definition utilised, have been described in an all-Wales cohort of children and adolescents. The finding that prevalence estimates differed between the four definitions employed is not unexpected and has been described previously in adult populations (Alberti *et al.*, 2009) and other child and adolescent populations (Goodman *et al.*, 2004). The need for a globally applicable metabolic syndrome definition has been repeatedly called for (Cook *et al.*, 2003; Jolliffe and Janssen, 2007; Zimmet *et al.*, 2007) to facilitate global comparisons and enable early detection and treatment. Rates of overweight and obesity in children are increasing (Flegal and Troiano, 2000) and it is likely that the prevalence of risk factor components associated with the metabolic syndrome is also increasing. Indeed, a US report on metabolic syndrome in adolescents based on an age-adjusted National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP

III) definition and using data from the National Health and Nutrition Examination Survey (NHANES) III (1988 – 1994) and NHANES 1999 – 2002, suggests that the prevalence of metabolic syndrome has risen from 4.2% in 1988-1992 to 6.4% in 1999 – 2000 (Duncan *et al.*, 2004). Similarly, a more recent analysis of the same data suggests that prevalence has increased from 4.7% to 7.6% using ATP III criteria, whereas using adolescent IDF criteria prevalence has increased significantly from 5.3% to 9.6% (Jolliffe and Janssen, 2007). Metabolic syndrome prevalence has been shown to increase directly with the degree of obesity. Moreover, each component of the syndrome worsens with increasing obesity independent of age, gender and pubertal stage (Weiss *et al.*, 2004b). This relationship would appear to be consistent across all child and adolescent metabolic syndrome studies (Table 2.2, Chapter 2). Interestingly, both the highest (Katzmarzyk *et al.*, 2004) and the lowest (Freedman *et al.*, 1999a) metabolic syndrome prevalence reported in all subjects both occur within the Bogalusa Heart Study group. The lowest prevalence occurred following an analysis of data from seven cross-sectional studies between 1973 and 1994, whereas the highest prevalence was found after analysis of only the three more recent years (1992 – 1994). This supports the findings from the NHANES studies (Duncan *et al.*, 2004; Jolliffe and Janssen, 2007) that metabolic syndrome prevalence is increasing.

Differences between diagnostic criteria utilised have led to differences in prevalence estimates. The IDF metabolic syndrome criteria were proposed and developed because an "accessible tool is needed to identify the metabolic syndrome in young people globally" (Zimmet *et al.*, 2007, pp.2059-60) and goes on to iterate the age and developmental differences that occur in relation to metabolic syndrome components. As a consequence, child and adolescent WC cut-off criteria are identified as $\geq 90^{th}$ percentile because children who meet this criteria are likely to have multiple risk factors for cardiovascular disease and, more pertinently, because other studies have used this same cut-off. However, citing an absence of contemporary data for blood pressure, triglyceride and fasting plasma

glucose, potential age and developmental influences are dismissed and adult criteria are applied. For HDL concentrations sex-specific differences are completely dismissed and a single adult male cut-off is applied. It has previously been identified that cholesterol levels, particularly HDL levels in males, are affected by puberty (Morrison *et al.*, 2002) and it is therefore likely that adult criteria will lead to misclassification of individuals. Data obtained in the current study also suggest that adult cut-off criteria may not be appropriate for younger populations. For example, the IDF criteria for SBP, DBP, TG and HDL are ≥130 mmHg, ≥85 mmHg, ≥1.7 mmol/L and ≤1.03 mmol/L, respectively, whereas the criteria of Cruz and Goran (2004) and Cruz *et al.* (2004) as applied by Whincup *et al.* (2005) apply criteria of >90th percentile for SBP, DBP and TG and < 10th percentile for HDL. When percentile cut-off points are identified for the current study SBP and DBP values >90th percentile were found to be equal to >125 mmHg and >80 mmHg, respectively; TG values > 90th percentile as equal to >1.2 mmol/L; and HDL values <10th percentile as equal to <1.33 mmol/L. Taken together these data suggest that child and adolescent cut-off points should be applied for all components otherwise definitions are likely to underestimate metabolic syndrome prevalence estimates.

In the present study insulin and insulin resistance were found to be strongly associated with all measures of adiposity and serum triglyceride concentrations. Many previous child and adolescent studies have included a measure of insulin (Freedman *et al.*, 1999a; Csabi *et al.*, 2000; Torok *et al.*, 2001; Andersen *et al.*, 2003; Goodman *et al.*, 2004; Katzmarzyk *et al.*, 2004; Lambert *et al.*, 2004; Ogawa *et al.*, 2005; Viner *et al.*, 2005; Atabek *et al.*, 2006; Golley *et al.*, 2006; Vikram *et al.*, 2006) or insulin resistance (Bavdekar *et al.*, 1999; Invitti *et al.*, 2003b; da Silva *et al.*, 2005; Druet *et al.*, 2006; Gilardini *et al.*, 2006; Invitti *et al.*, 2006) as a component of their metabolic syndrome criteria. Results from the Bogalusa Heart Study show that when insulin concentrations are increased in childhood they tend to remain elevated in adulthood, and those adults with consistently elevated insulin levels

tend also to have increased rates of obesity, hypertension and dyslipidaemia (Bao *et al.*, 1996). Better ways to define obesity in childhood have previously been called for (Kimm and Obarzanek, 2002), as has the development and use of a consensus definition of metabolic syndrome in children (Cook *et al.*, 2003; Jolliffe and Janssen, 2007). The present study's results suggest that adult cardiovascular risk factors may be easily identifiable in children from a young age and the importance of early identification in the management of coronary heart disease, cardiovascular disease and type 2 diabetes mellitus is self-evident (Zimmet *et al.*, 2007). It is worthy of note that long-term study of adult metabolic syndrome has enabled the link to be made with morbidity and mortality end-points (Lakka *et al.*, 2002; Malik *et al.*, 2004). Methodological complications and the timescales involved mean that, to date, no prospective studies have been able to confirm these findings in children. For this reason, it may be useful for metabolic syndrome, its components and other cardiovascular risk factors to be linked with known predictors of cardiovascular disease morbidity and mortality such as arterial stiffness.

In children, an increased waist circumference has been shown to correlate with elevated SBP and DBP and elevations in serum levels of TC, LDL, TG and insulin as well as lower concentrations of HDL (Freedman *et al.*, 1999b; Savva *et al.*, 2000; Maffeis *et al.*, 2001). The current study cannot fully confirm these findings although WC was found to be highly correlated with SBP, DBP, insulin, insulin resistance and HDL, but not with other lipid components. However, these studies predominantly used differing population groups (Savva *et al.*, 2000) and both younger (Maffeis *et al.*, 2001) and older (Freedman *et al.*, 1999b) age ranges than the present study. Taken together these differences between studies are likely to account for the small differences observed with this study's findings.

The finding of an increase in SBP with increasing maturational stage is in line with findings from other studies (Atabek *et al.*, 2006) and reflects the steep age-related increase

in SBP observed in both males and females between the ages of ~10 – 20 years (Roberts and Maurer, 1977).

This study had some limitations worthy of note. First, because the study sample was recruited from two local schools, had a relatively small sample size and consisted only of children aged 11 – 14 years, it cannot be claimed that this sample was nationally representative. In addition, parental and individual consent was required which allows for a degree of self-selection in the sample population and it therefore cannot be claimed that the sample was representative of the school population available. Nevertheless, our prevalence estimates are similar to a previous cross-sectional study that also included a number of Welsh school children among its subjects (Whincup *et al.*, 2005). The cross-sectional design of this study limits the ability to comment on causal relationships among the metabolic syndrome factors described. Despite this limitation, these findings are consistent with the findings of previous studies that have also followed a similar study design.

5.5 Conclusion

In summary, this study has identified that a diagnosis of the metabolic syndrome is highly dependent on the criteria applied and in the current cohort prevalence estimates ranged from 0%, using predominantly adult cut-off points, to 3.5% using less conservative criteria. This study also identified that all measures of adiposity were significantly associated with components of the metabolic syndrome. Similarly, insulin resistance was found to show significant positive associations with all measures of adiposity. Taken together, this study and others suggest that disturbances in insulin action and adiposity are associated with risk factor clustering and that this effect is identifiable from a young age.

6 Chapter Six – Arterial Stiffness, Metabolic Syndrome and Aerobic Fitness in Children and Adolescents

6.1 Introduction

The prevalence of overweight and obesity in children is increasing (Ogden *et al.*, 2002) and, as with adults, obesity in childhood is associated with the premature development of cardiovascular disease risk factors such as hypertriglyceridaemia, low HDL cholesterol, hypertension, (Thompson *et al.*, 2007), insulin resistance and impaired glucose metabolism (Freedman *et al.*, 1999a). In adults, risk factor clustering has been reported to be associated with atherosclerosis and increased risk for the development of cardiovascular disease. Moreover, there is emerging evidence that obesity related metabolic disease predicts the development of CVD in adulthood (Morrison *et al.*, 2007).

The large arteries play an important role in buffering the pulsatile ejection of blood from the heart. With arterial stiffening the buffering capacity of the central arteries is diminished leading to an increase in pulse pressure (Nichols and O'Rourke, 2005). Arterial stiffening occurs as a result of ageing (McEniery *et al.*, 2005a) and arteriosclerosis. Ageing results in the fatigue-fracture of elastin fibres leading to a gradual decline in elasticity. Arteriosclerosis is characterised by fibrosis (deposition of collagen) of the intima and calcification of the media. It is thought that a chronic increase in pulse pressure may accelerate the age-related fatigue-fracture of elastin fibres (Nichols and O'Rourke, 2005). Smooth muscle tone of the vasculature may be modified by vasoconstrictors and endothelial-derived vasodilators and factors which modulate vascular tone may also be important (Wilkinson and McEniery, 2004). Data from several large, prospective studies demonstrate that aortic pulse-wave velocity (PWV) predicts both cardiovascular and all-cause mortality in a number of patient populations (Blacher *et al.*, 1999b; Laurent *et al.*,

2001; Meaume *et al.*, 2001a; Cruickshank *et al.*, 2002; Sutton-Tyrrell *et al.*, 2005; Mattace-Raso *et al.*, 2006; Willum-Hansen *et al.*, 2006)

A recent systematic review examined the independent associations of arterial stiffness, specifically carotid-femoral (aortic) PWV, with cardiovascular risk factors in predominantly middle- to older aged adults (Cecelja and Chowienczyk, 2009). This review found that the contribution of risk factors other than age and blood pressure was small or insignificant. More recently, in a cohort of 825 men followed up for an average of 20 years it was confirmed that cardiovascular risk factors, other than blood pressure, had only a modest effect on both arterial stiffness and wave reflection (McEniery *et al.*, 2010). Due to methodological problems associated with epidemiological studies no similar study has been carried out in younger individuals yet obesity (Mimoun *et al.*, 2008), elevated BP (Urbina *et al.*, 2011a), dysglycaemia (Khan *et al.*, 2006) and dyslipidaemia (Leeson *et al.*, 2000b; Fang *et al.*, 2010) have been shown to have detrimental effects on arterial stiffness, structure and function in children and adolescents.

High levels of aerobic fitness are associated with reduced cardiovascular morbidity and mortality in adults (Blair *et al.*, 1996) and cross-sectional studies have shown that aerobic fitness is inversely associated with arterial stiffness (Vaitkevicius *et al.*, 1993; Ferreira *et al.*, 2002). It has also been shown that increases in aerobic capacity that occur from adolescence up to age 36 are associated with lower arterial stiffness (Ferreira *et al.*, 2003). There is limited information on the influence of aerobic fitness on arterial stiffness in children although one study has shown carotid-femoral PWV to be negatively correlated with cardiorespiratory fitness (Sakuragi *et al.*, 2009). In the present study, we aimed to evaluate the relationship between arterial stiffness, metabolic syndrome risk factors and physical fitness in an apparently healthy cohort of children and adolescents.

6.2 Methodology

Data collection was carried out as detailed in Chapter Three: General Methodology and consisted of: height and weight for the calculation of body mass index (BMI), waist circumference (WC), brachial systolic blood pressure (SBP), diastolic BP (DBP), pulse pressure (PP) and mean arterial pressure (MAP), heart rate (HR), maturational status, central blood pressures from both the radial (using the Sphygmocor generalised transfer function) and carotid arteries (without generalised transfer function), augmentation index (AIx), AIx corrected to a heart rate of 75 beats per minute (AIx_{75}), carotid-radial (brachial) pulse wave velocity (c-r PWV), carotid-femoral (aortic) PWV (c-f PWV), cardiac output (CO) and stroke volume (SV).

6.2.1 Statistical analysis

Association between arterial stiffness indices and cardiovascular risk factors was assessed by Pearson Product-Moment Correlation Coefficient and the non-parametric Spearman Correlation Coefficient for normally distributed data and non-normally distributed data, respectively. Analysis of relationships between pubertal status, quintiles of aerobic fitness and IOTF defined normal weight, overweight and obesity were assessed by non-parametric equivalent of ANOVA (Kruskal-Wallis test) with Bonferroni corrected *post hoc* Mann-Whitney tests. Significant trends were assessed by the non-parametric Jonckheere-Terpstra test. Two-way ANOVA was used to assess examine the effect of gender and self-assessed maturational stage on measures of arterial stiffness. Homogeneity of variance between groups was assessed by Levene's test.

6.3 Results

Descriptive data for all subjects is presented in Table 6.1. Overall, there were no differences between male and female subjects for age, height, weight, BMI or maturational status. Males displayed greater aerobic fitness had higher brachial SBP and PP. Brachial DBP and MAP and HR did not differ between genders.

Table 6.1: Demographic characteristics grouped by gender

	All $n = 184$	Males $n = 102$	Females $n = 82$	*P*
Age (years)	13.4 ± 1.0	13.4 ± 1.0	13.3 ± 1.0	*ns*
Height (cm)	157.8 ± 9.6	158.9 ± 10.4	156.4 ± 8.3	*ns*
Weight (kg)	51.1 ± 12.4	51.7 ± 12.8	50.4 ± 12.0	*ns*
BMI (kg/m^2)	20.3 ± 3.5	20.3 ± 3.6	20.4 ± 3.4	*ns*
Pubertal rating:				
Breast/Penis	3.2 ± 1.1	3.3 ± 1.2	3.0 ± 1.1	*ns*
Pubic hair	3.1 ± 1.0	3.2 ± 1.0	3.1 ± 1.0	*ns*
Aerobic fitness (shuttle)	60.6 ± 27.4	74.2 ± 24.7	40.1 ± 16.4	<0.001
Brachial SBP (mmHg)	113.1 ± 9.6	115.9 ± 9.8	109.7 ± 8.0	<0.001
Brachial DBP (mmHg)	70.3 ± 7.5	69.7 ± 7.7	71.0 ± 7.3	*ns*
Brachial PP (mmHg)	42.9 ± 9.6	46.3 ± 9.0	38.7 ± 8.6	<0.001
MAP (mmHg)	84.6 ± 7.0	85.1 ± 7.3	83.9 ± 6.4	*ns*
HR (bpm)	79.5 ± 12.5	79.3 ± 11.9	79.8 ± 13.3	*ns*

Data are presented as means ± SD. BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; shuttle, number of shuttles performed on the multi-stage fitness test.

Central blood pressure indices and arterial stiffness results are presented in Table 6.2. When central pressures were assessed by pulse wave analysis at the radial artery, using the generalised transfer function of the Sphygmocor system, males were found to have significantly greater cPP and significantly lower cDBP, AP, AIx and AIx_{75} than female subjects, the later findings of lower augmentation index represents a reduction in wave reflection for males in comparison to female subjects. Subsequent analysis of central pressures and augmentation assessed following carotid artery tonometry, where central pressures are derived independent of the generalised transfer function, confirmed these

findings. Carotid and radial cSBP was lower than brachial SBP and carotid and radial cPP was higher than brachial PP representing the amplification of the pressure wave from central to peripheral arteries. Male subjects also had significantly lower carotid-radial PWV and aortic PWV compared to females.

Table 6.2: Central pressure and arterial stiffness grouped by gender.

	All *n* = 177	Males *n* = 96	Females *n* = 81	*P*
Radial:				
cSBP (mmHg)	93 ± 7	93 ± 7	93 ± 7	*ns*
cDBP (mmHg)	67 ± 7	66 ± 7	69 ± 6	0.004
cMAP (mmHg)	79 ± 6	79 ± 6	80 ± 6	*ns*
cPP (mmHg)	26 ± 5	27 ± 5	24 ± 5	<0.0001
AIx (%)	-2.9 ± 11.9	-5.0 ± 11.9	-0.4 ± 11.5	0.01
AIx_{75} (%)	-2.4 ± 12.1	-4.6 ± 12.3	0.2 ± 11.5	0.008
AP (mmHg)	-0.9 ± 3.4	-1.5 ± 3.7	-0.1 ± 2.8	0.006
Carotid:				
cSBP (mmHg)	98 ± 8	99 ± 8	98 ± 8	*ns*
cDBP (mmHg)	67 ± 7	65 ± 7	69 ± 6	0.003
cMAP (mmHg)	79 ± 6	79 ± 6	80 ± 6	*ns*
cPP (mmHg)	31 ± 7	33 ± 6	29 ± 7	0.0001
AIx (%)	-3.2 ± 12.4	-5.5 ± 12.5	-0.4 ± 11.7	0.005
AIx_{75} (%)	-2.9 ± 11.7	-5.1 ± 11.8	-0.2 ± 11.1	0.005
AP (mmHg)	-1.1 ± 3.4	-1.7 ± 3.6	-0.3 ± 3.1	0.004
c-r PWV (m/s)	6.9 ± 1.1	6.8 ± 1.0	7.1 ± 1.1	0.03
c-f PWV (m/s)	4.6 ± 0.7	4.5 ± 0.7	4.7 ± 0.6	0.02
CO (L/min)	6.9 ± 1.6	7.1 ± 1.8	6.5 ± 1.4	0.01
SV (ml)	76.3 ± 17.2	79.9 ± 17.6	71.5 ± 15.5	0.001

Data are presented as means ± SD. AIx, augmentation index; AIx_{75}, augmentation index normalised to a heart rate of 75 bpm; AP, augmentation pressure; cDBP, central diastolic blood pressure; c-f PWV, carotid-femoral pulse wave velocity; cMAP, central mean arterial pressure; CO, cardiac output; cPP, central pulse pressure; c-r PWV, carotid-radial pulse wave velocity; cSBP, central systolic blood pressure; SV, stroke volume.

Overall, pubertal status data was obtained in 179 children (TS, Tanner stage; TS1 = 5; TS2 = 28; TS3 = 60; TS4 = 67 and TS5 = 19) of which 171 had full measures of arterial stiffness (TS1 = 5; TS2 = 28; TS3 = 55; TS4 = 64 and TS5 = 19). Due to low and uneven

numbers of boys and girls in TS1 this group was excluded from subsequent gender specific analyses. There were no differences in seated radial AIx, AIx75 and AP, carotid-femoral PWV or cardiac index (cardiac output corrected for body size) between maturational stages. However, significant differences ($p < 0.05$) were found for supine radial AIx and AIx_{75}, supine carotid AIx, AIx_{75} and AP, carotid-radial PWV, CO and SV. To further explore these differences the effect of gender and self-assessed maturational stage on indices of arterial stiffness were examined. There were no significant interaction effects between gender and maturational stage for any measure of arterial stiffness. In other words, the pattern of change in arterial stiffness during the assessed period of adolescence did not differ between boys and girls. Main effects for maturational status were seen with supine radial AIx and AIx_{75} displaying significant inverse linear trends ($p \leq 0.01$). These effects were matched by concomitant, significant positive linear associations for supine radial AIx and AIx_{75} with increasing height ($p < 0.001$). Main effects for both gender and maturational status were seen for supine carotid AIx, AIx_{75} and AP, and carotid-radial PWV displaying significant inverse linear trends ($p \leq 0.01$; carotid-radial PWV, $p < 0.05$) and with males generally displaying lower values than females (Figure 6.1, A-D). Main effects were also seen for CO and SV displaying significant positive linear trends ($p \leq 0.01$) and with males displaying higher values than females (Figure 6.1, E and F).

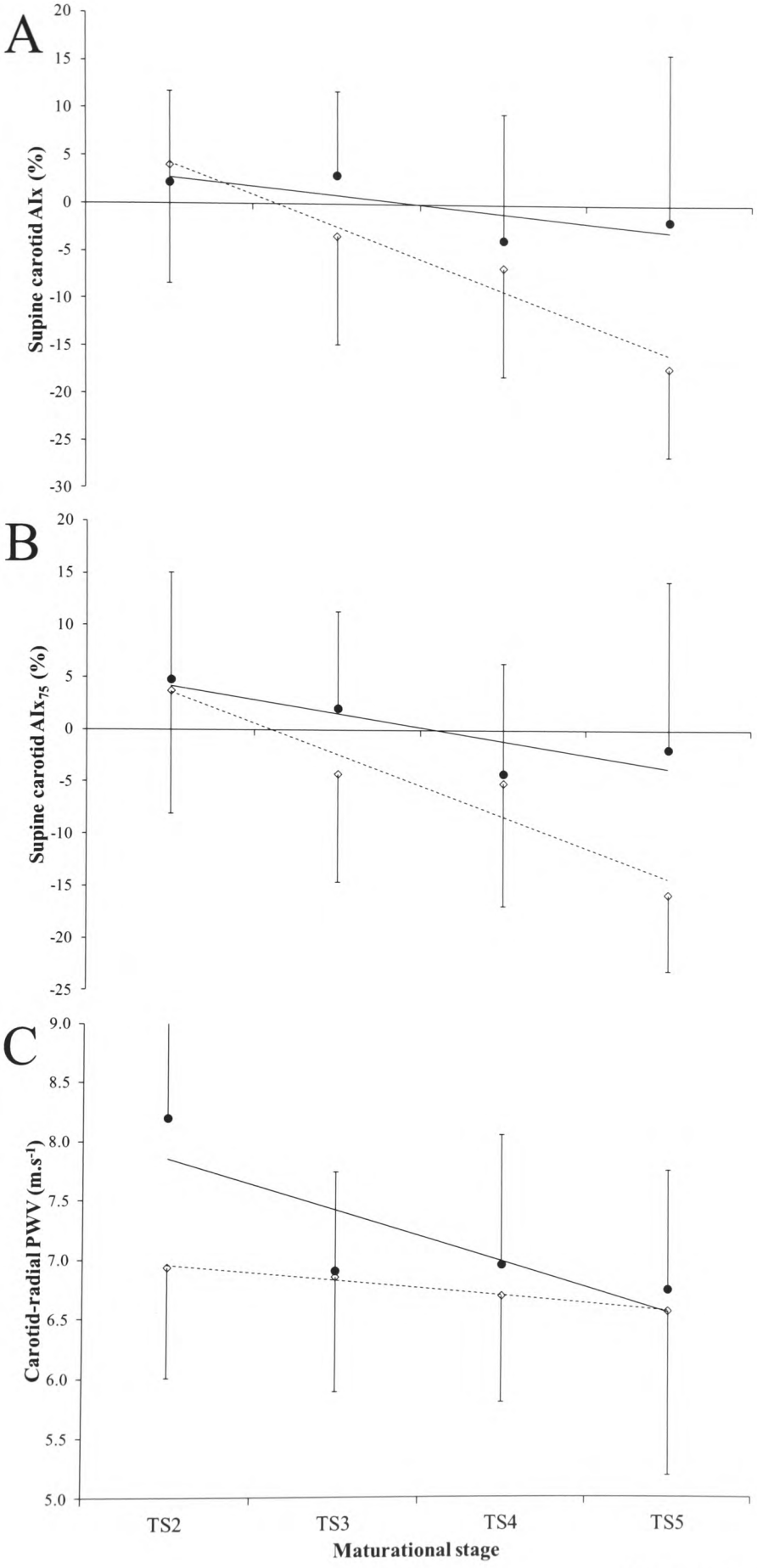

Figure 6.1, A-E: *continued.*

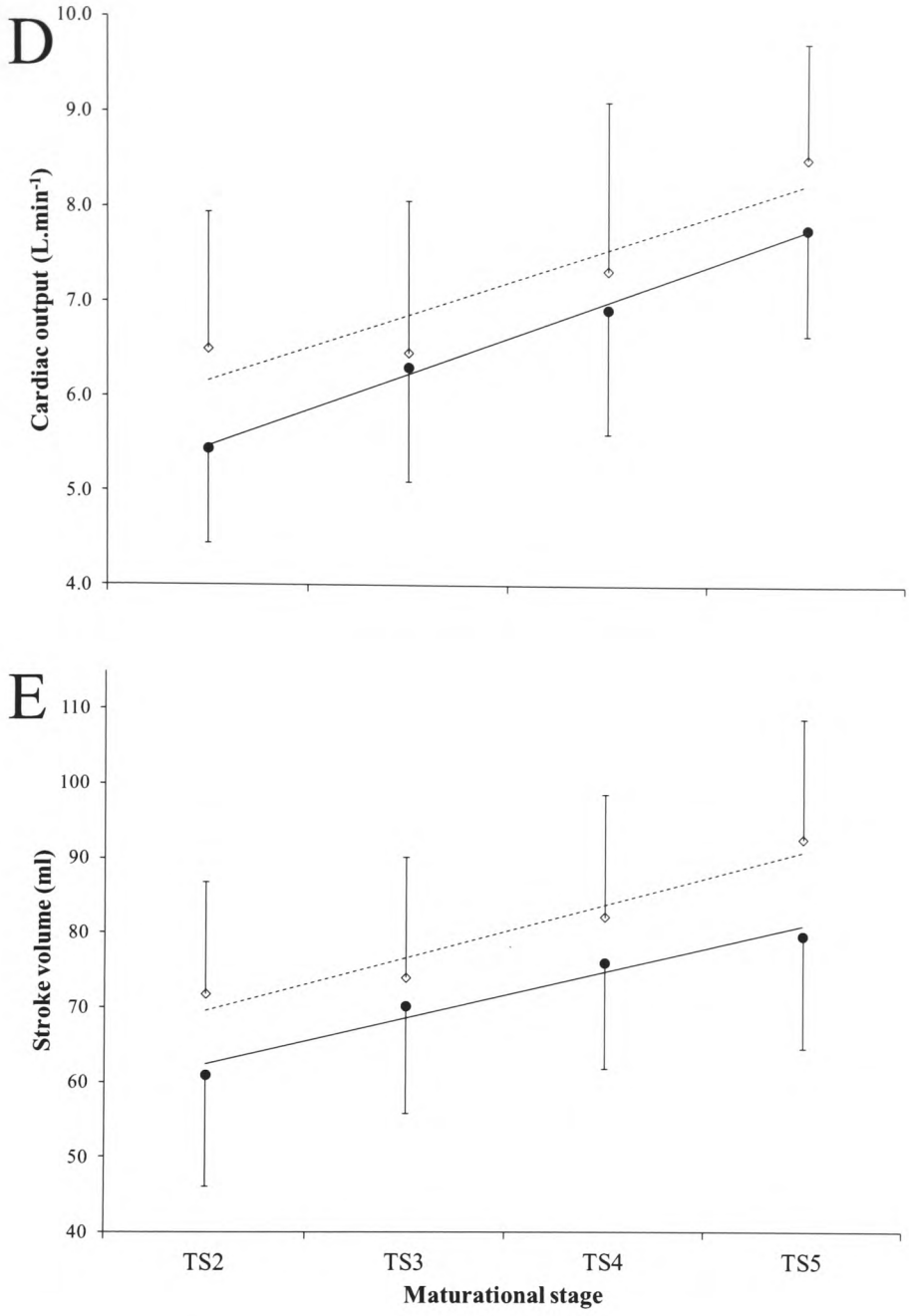

Figure 6.1, A-E: Relationship between indices of arterial stiffness, gender and maturation. Males = open diamonds, dashed lines; females = closed circles, solid lines. Panel A: supine carotid augmentation index (AIx) (%), p for trend <0.01; Panel B: supine carotid AIx at a heart rate of 75 bpm (AIx_{75}) (%), p for trend <0.001; Panel C: carotid-radial PWV (c-r PWV) ($m.s^{-1}$) , p for trend <0.05; Panel D: Cardiac output ($L.min^{-1}$) , p for trend <0.001; Panel E: stroke volume (ml) , p for trend <0.001. TS, Tanner stage. Data presented as means ± SD. Values of p <0.05 were considered statistically significant.

The association between arterial stiffness and selected cardiovascular risk factors was assessed by correlational analysis. BMI and WC were found to be inversely associated with brachial PWV (BMI, r = -0.25, p < 0.001; WC, r = -0.22, p < 0.01) and positively

associated with aortic PWV (BMI, $r = 0.21$, $p < 0.01$, Figure 6.2; WC, $r = 0.15$, $p < 0.05$). When subjects were stratified according to International Obesity Task Force definitions of normal weight, overweight and obesity normal weight subjects were found to have significantly lower aortic PWV than overweight subjects ($p = 0.02$) and overweight or obese subjects ($p = 0.008$) (Figure 6.3). A significant trend was also identified for increasing aortic PWV with increasing BMI status. When males and females were examined separately (Figure 6.4) normal weight males displayed significantly lower aortic stiffness than normal weight and overweight and obese females. Female subjects displayed a significant trend for increased aortic stiffness with increasing BMI status ($p < 0.01$). Strong inverse correlations were also found between all measures of AIx and measures of maturation.

When subjects were stratified to identify normotensive, pre-hypertensive and hypertensive individuals using national age and gender specific cut-off points normotensive individuals display significantly lower aortic PWV (Figure 6.5; $p < 0.001$) and brachial PWV (Figure 6.6; $p < 0.001$) than hypertensive subjects. Significant trends were seen for increases in both aortic and brachial PWV with increasing BP status ($p < 0.001$).

Aerobic fitness was found to have significant inverse correlations with both brachial PWV ($r = -0.21$, $p < 0.05$) and aortic PWV ($r = -0.26$, $p < 0.01$). When subjects were divided in to quintiles of aerobic fitness significant trends for decreasing arterial stiffness with increasing aerobic fitness were found for both aortic PWV (Figure 6.7; $p < 0.05$) and brachial PWV (Figure 6.8; $p < 0.05$). Despite female subjects having significantly lower levels of aerobic fitness (74.2 ± 24.7 shuttles vs. 40.1 ± 16.4 shuttles; $p < 0.001$) when males and females were separately divided into aerobic fitness quintiles this trend was still evident but was no longer significant (Figure 6.9).

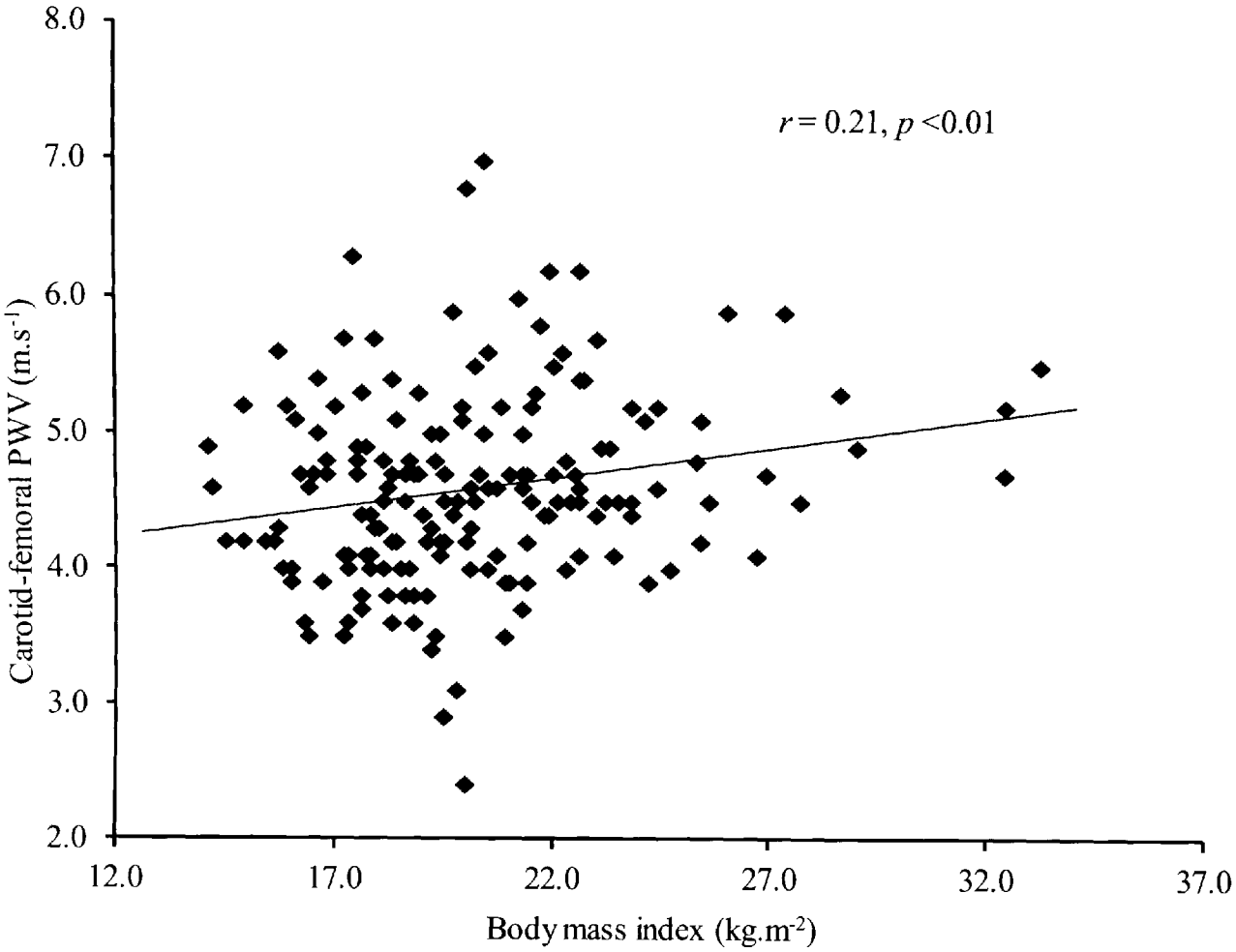

Figure 6.2: Association between carotid-femoral PWV and body mass index ($r = 0.21, p< 0.01$).

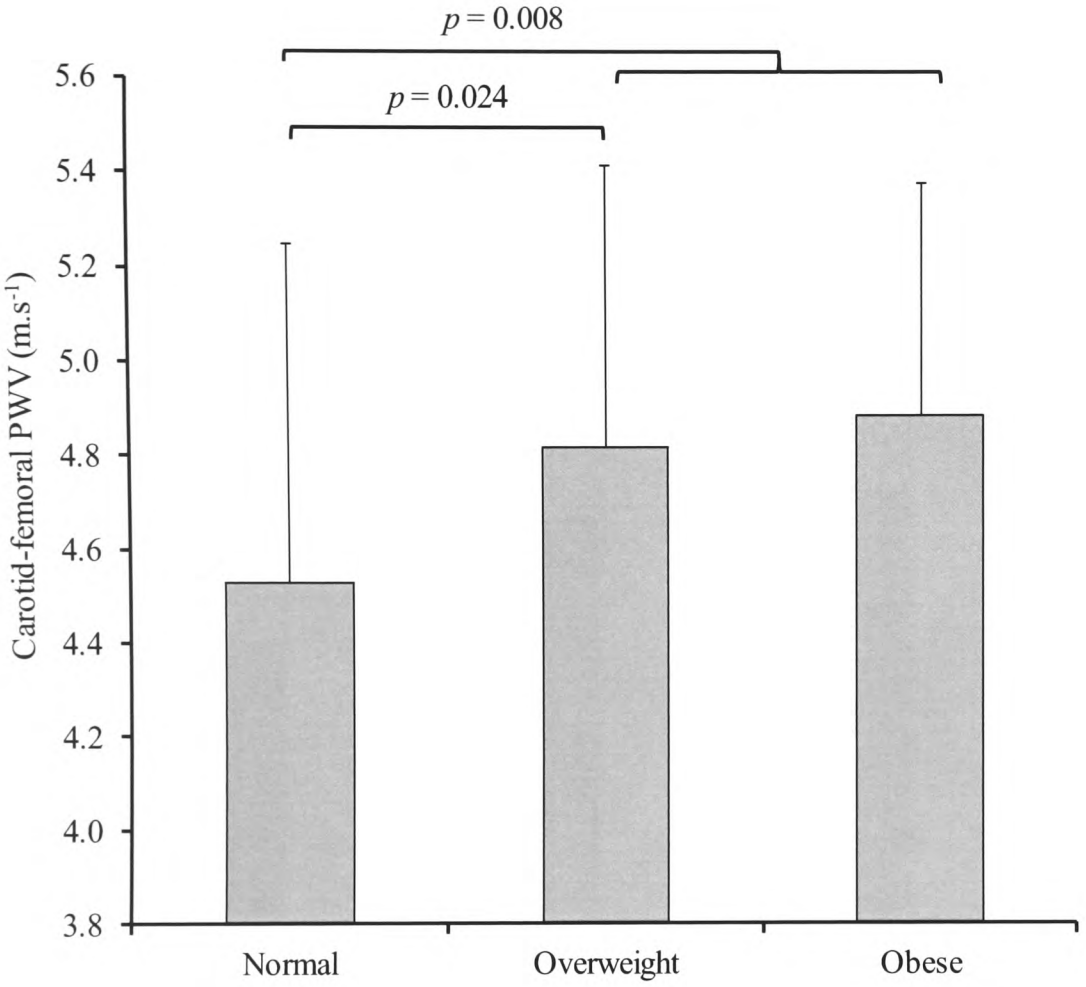

Figure 6.3: Association between carotid-femoral PWV and IOTF defined overweight and obesity (p for trend <0.01)

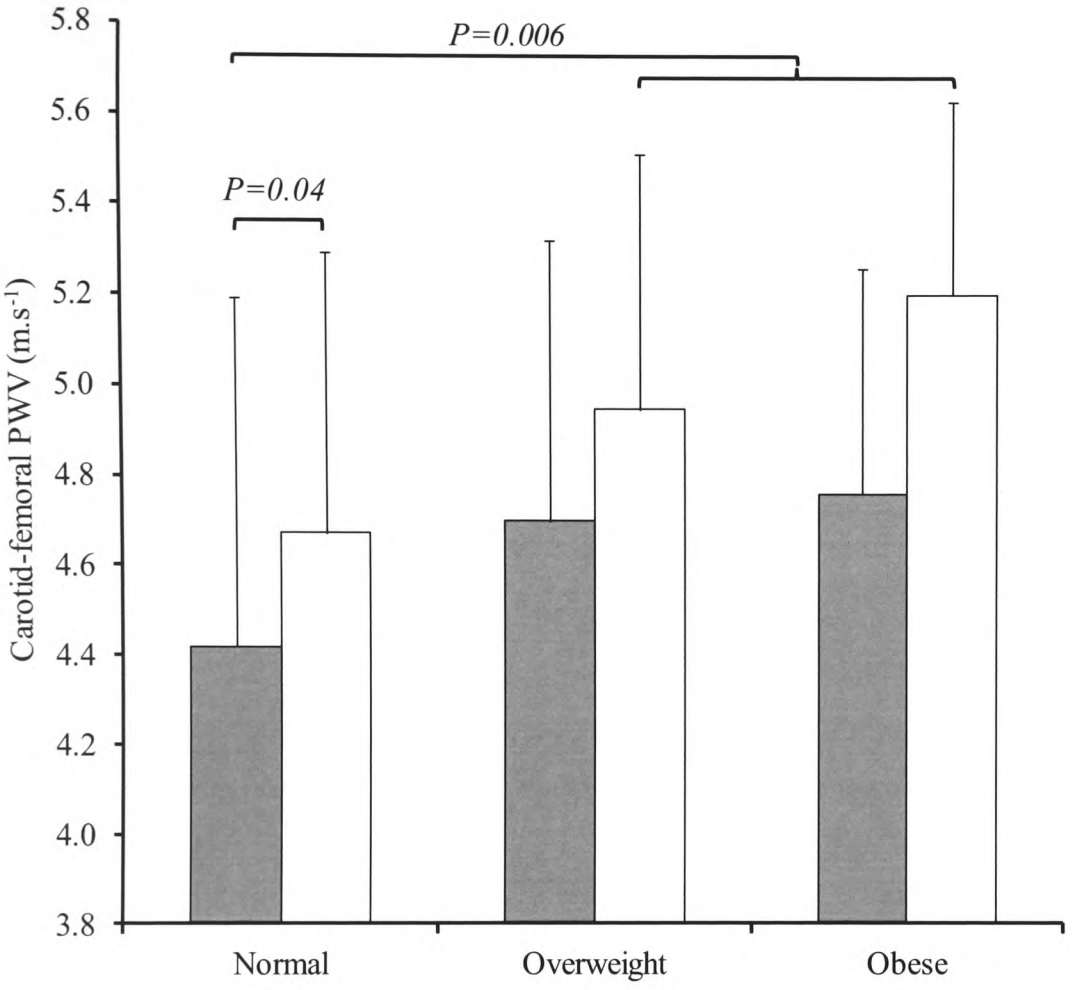

Figure 6.4: Association between carotid-femoral PWV and IOTF defined overweight and obesity in males (filled bars) and females (open bars).

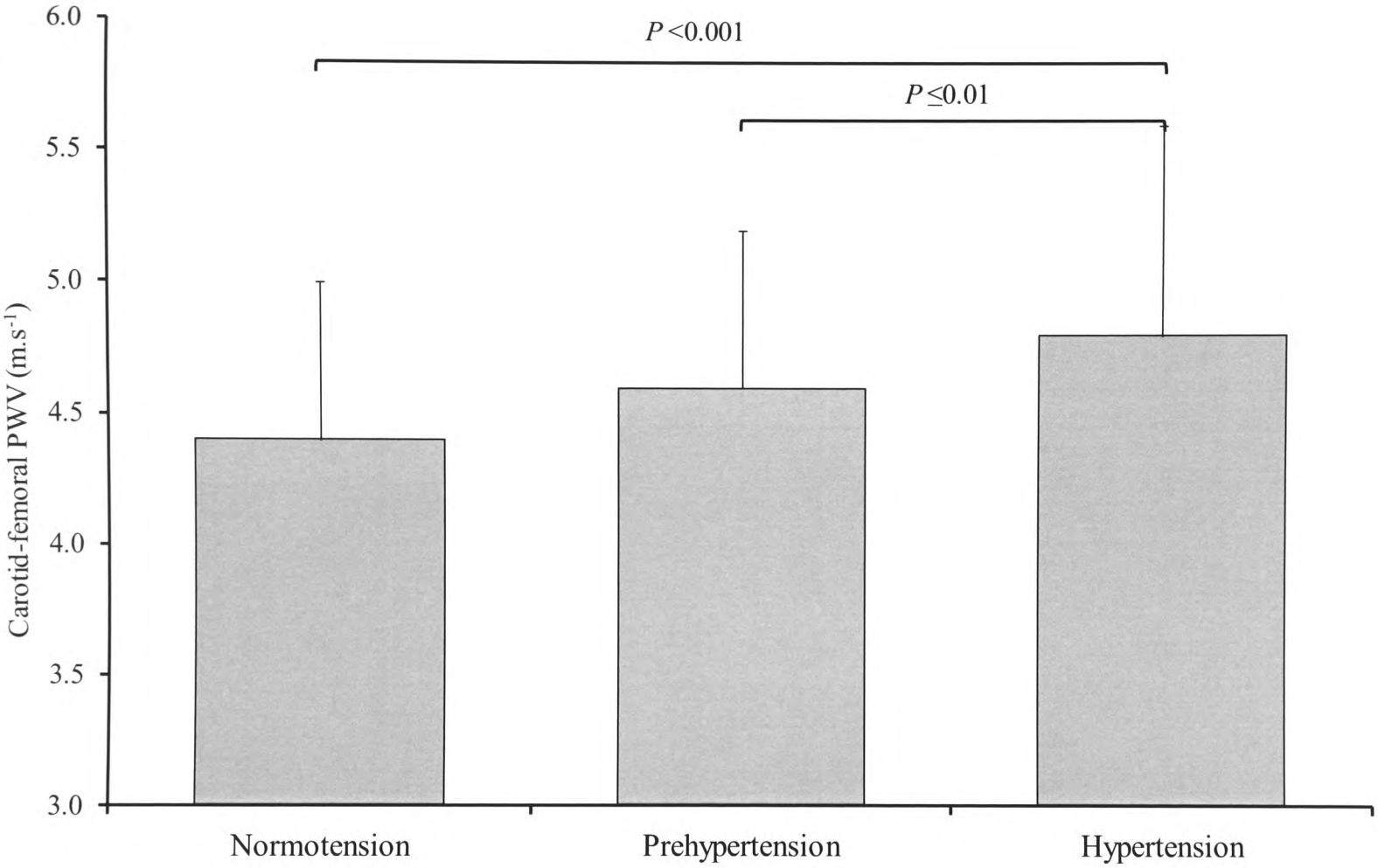

Figure 6.5: Association between carotid-femoral PWV and hypertension status (p for trend <0.001)

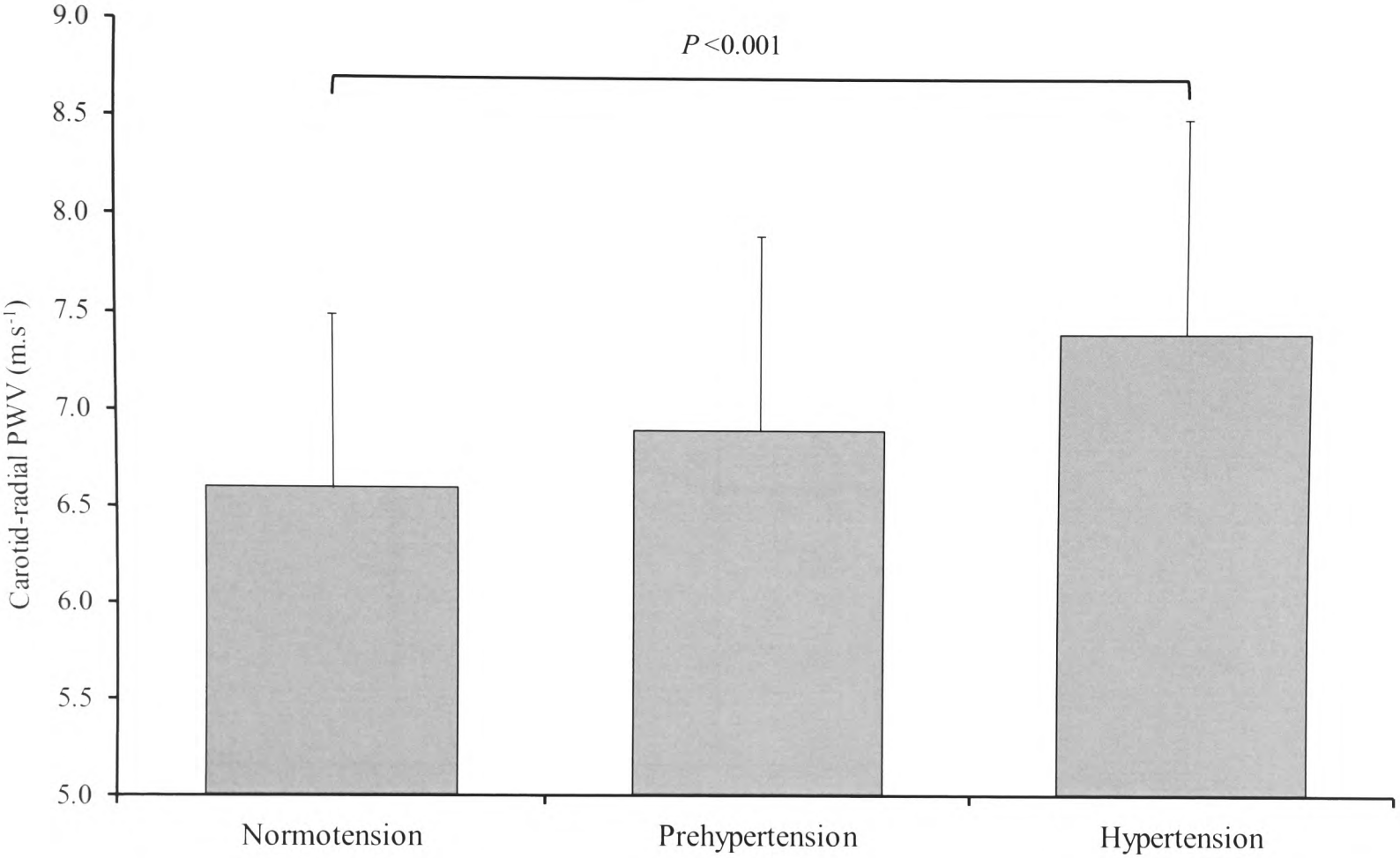

Figure 6.6: Association between carotid-radial PWV and hypertension status (*P* for trend <0.001).

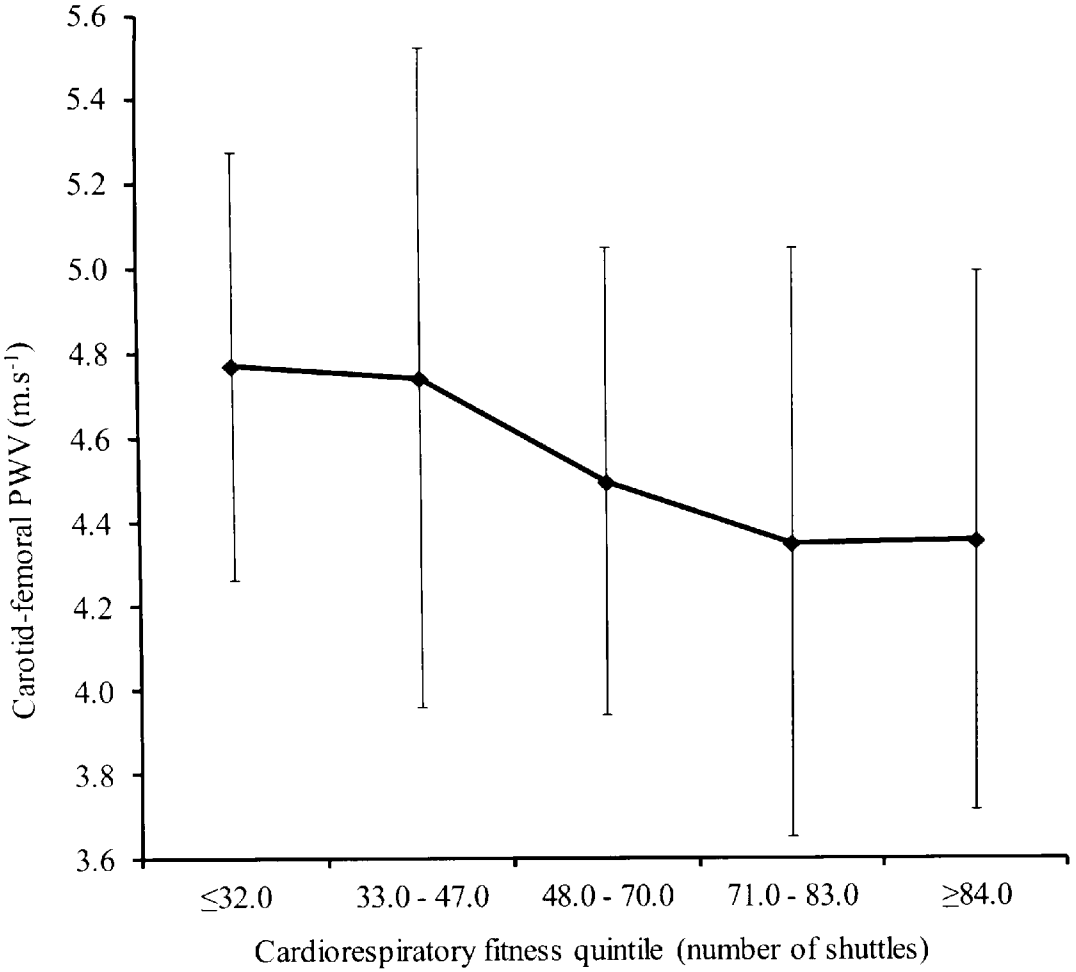

Figure 6.7: Association between carotid-femoral PWV and aerobic fitness quintile (p for trend <0.05).

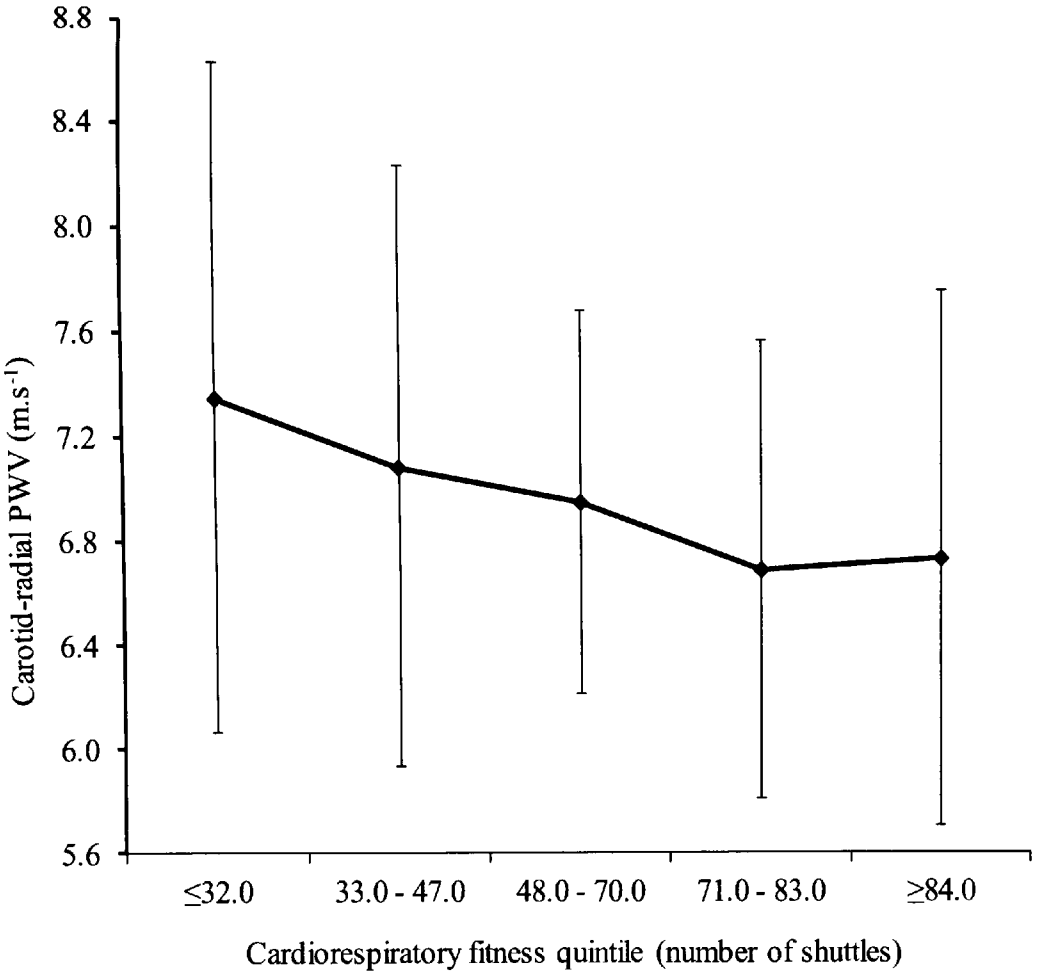

Figure 6.8: Association between carotid-radial PWV and aerobic fitness quintile (p for trend <0.05).

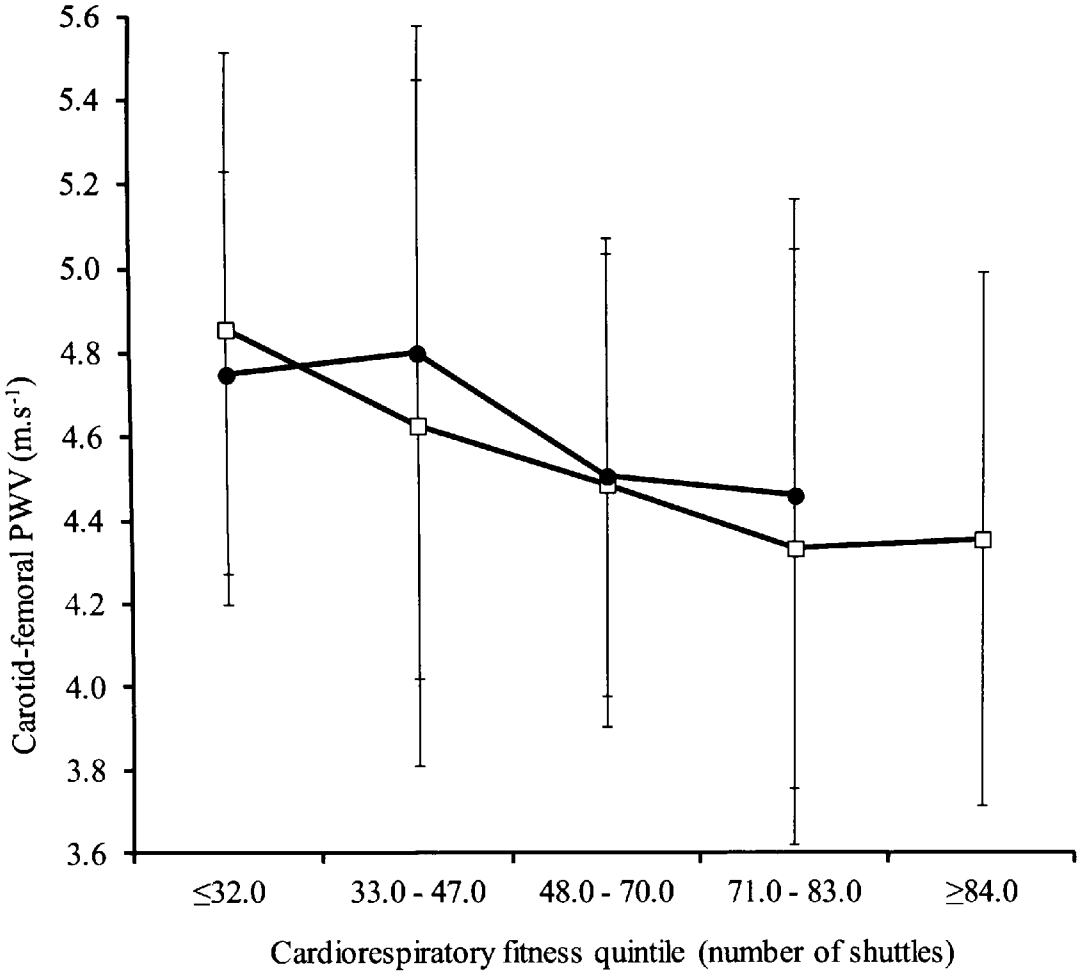

Figure 6.9: Association between carotid-radial PWV and aerobic fitness quintile for males (open squares) and females (closed circles).

6.4 Discussions

The finding that overweight and obesity was associated with increased arterial stiffness and changes in arterial structure and function has been shown in previous studies (Tounian *et al.*, 2001; Levent *et al.*, 2002; Whincup *et al.*, 2005; Iannuzzi *et al.*, 2006; Mimoun *et al.*, 2008; Polat *et al.*, 2008). However, the majority of these studies were conducted using obese populations (Tounian *et al.*, 2001; Levent *et al.*, 2002; Iannuzzi *et al.*, 2006; Mimoun *et al.*, 2008; Polat *et al.*, 2008) and all except one (Levent *et al.*, 2002) used arterial stiffness measurements other than aortic PWV. In a U.K. study, Whincup *et al.* (2005) reported strong inverse, graded associations between all adiposity measures (BMI, body fat, WC and sum of 4 skinfolds) and arterial distensibility. Zebekakis *et al.* (2005) recruited 1306 subjects aged 10 – 86 years and assessed carotid, brachial and aortic stiffness using ultrasound techniques. Arterial diameter increased in all three arterial segments before and after adjustment for potential confounding variables. Similarly, arterial distensibility consistently decreased with increasing BMI. Moreover, after adjustment, aortic PWV increased with BMI in female subjects only. The mechanisms by which obesity may be related to arterial stiffness remain to be resolved. It has been suggested (Zebekakis *et al.*, 2005, p.1843) that in humans, weight gain leads to expansion of the extracellular volume and increases cardiac output and regional blood flow to adipose as well as non-adipose tissues (Oren *et al.*, 1996). The increased cardiac output and regional blood flow are likely to result in increased blood pressure and shear stress and stimulation of neural and hormonal mechanisms. Visceral fat accumulation has shown a correlation with fat deposition in liver and skeletal muscle (Bjorntorp, 1991) and visceral adipocytes secrete a number of vasoactive cytokines including interleukin-6 and tumour necrosis factor-α (Ahima and Flier, 2000) which may play a role in the alteration of vascular tone and intima-medial remodelling.

In a study of obese children and adolescents, metabolic syndrome did not correlate with endothelial dysfunction or abnormalities in arterial mechanical properties and metabolic factors did not act synergistically on vascular measures (Mimoun *et al.*, 2008). Similarly, Tounian *et al.* (2001) found no relation between increased arterial stiffness and metabolic variables. In contrast, Whincup *et al.* (2005) did report that the number of metabolic syndrome variables present were inversely related to arterial distensibility. However, although the relationship of total cholesterol, LDL cholesterol and diastolic blood pressure with distensibility was present in 9 – 11 year old children, significant relationships with adiposity and insulin resistance only became apparent in 13 – 15 year old children and adolescents. Females were found to have lower cPP, CO and SV and increased AIx, AIx75 and both carotid-radial PWV and carotid-femoral PWV. Gender differences in arterial stiffness have not always been found in other studies. In their development of reference values for child and adolescent PWV Reusz *et al.* (2010) found no difference in PWV between age and height matched girls and boys. Their explanation for this finding was because the girls matched to the boys were taller than the average of their age group and, along with MAP, age and height were the major determinants of PWV in multiple regression analysis. However, they did note that the growth pattern and final height of the sexes differed significantly and that PWV differed in the third and fourth age quartiles (i.e. after puberty). The accuracy of PWV is critically dependent on the accuracy of measurement of the travel distance of the arterial pulse wave (Weber *et al.*, 2009) and variability between estimates of PWV have been shown to largely depend on inconsistencies in the measured travel distance (Rajzer *et al.*, 2008). Travel distance has been shown to be proportional to body height (Vermeersch *et al.*, 2009) which explains the inverse association between carotid-radial PWV and body height. However, no similar associations were observed for aortic PWV. Significant inverse associations were observed for augmentation and maturational stage which can be easily explained by the faster return of the reflected wave observed in shorter individuals (Nichols and O'Rourke, 2005).

Therefore, the observed decreases in arterial stiffness indices may be explained by changes in body height that occur during adolescence.

The present study found no difference in the pattern of change in arterial stiffness across maturational stages between boys and girls. Similarly, Whincup *et al.* (2005) found adjustment for pubertal status had little effect on the associations with distensibility. Conversely, Ahimastos *et al.* (2003) found pre-pubertal girls to have increased central PWV and peripheral (femoral to dorsal pedis) PWV compared to pre-pubertal boys. Post-puberty there was no difference in central PWV between genders although peripheral PWV was now lower than for males. Ahimastos *et al.* (2003) demonstrated that both males and females experience an increase in the capacitance of their large arteries post-puberty with females developing more distensible large arteries and males developing stiffer large arteries. The finding that several arterial stiffness measures produced negative correlations with maturational status confirms the findings of other studies. There was no difference in maturational status between males and females in the current study. However, the suggestion is that gender differences in large artery stiffness are intrinsic and likely to be modified by male and female sex steroids.

Few studies have investigated the influence of aerobic fitness on arterial stiffness in children. An Australian study of 9 – 10 year old, generally prepubescent children shows a clear positive correlation between carotid-femoral PWV and degree of body fat, and an inverse correlation with aerobic fitness (Sakuragi *et al.*, 2009). The study has been cited for its weaknesses including its use of sequential rather than simultaneous measurement of PWV at the carotid and femoral sites and using only 8 cardiac cycles and its use of a 20 metre shuttle run as the measure of aerobic fitness (Cruikshank *et al.*, 2009). However, despite these weaknesses, and that only 20% of the variance in PWV was related to any of the variables measured, significant associations with PWV were still observed. Moreover,

the influence of aerobic fitness on aortic PWV was attenuated after adjustment for adiposity although the authors suggest that this finding may be due to external factors such as compliance and motivation of the children to complete the aerobic fitness assessment. In another Australian study the association between vascular function, assessed by flow-mediated dilatation, and cardiorespiratory fitness, assessed during an incremental treadmill exercise test, was examined in 10 year old children (Hopkins *et al.*, 2009). Children in the lowest tertile for vascular function displayed significantly lower aerobic fitness than children in the highest tertile.

Most (Vaitkevicius *et al.*, 1993; Kingwell, 2002; Gates *et al.*, 2003), but not all (Schmitz *et al.*, 2001) studies in adults have found that higher levels of aerobic fitness and/or physical activity are associated with lower arterial stiffness and improved arterial function. The finding by Schmitz *et al.* (2001) was based on a very large study of 10,644 men and women aged 45 – 64 years and could not confirm that habitual physical activity had a strong, consistent positive effect on arterial distensibility. In the general population cardiorespiratory fitness levels may vary longitudinally due to changes in lifestyle and one Dutch study sought to evaluate the impact of changes in cardiorespiratory fitness on large artery properties (Ferreira *et al.*, 2003). Analysis of changes in aerobic fitness was investigated in 154 subjects tracked from adolescence (13 – 16 years of age) through to adulthood (36 years of age). Aerobic fitness (VO_{2max}) was measured with a maximal running treadmill test. The study found that changes in VO_{2max} were inversely and significantly associated with large artery stiffness. However, the authors do note that this relationship may be partially dependent on, or mediated by, changes in other risk factors. In a German study (Meyer *et al.*, 2006), 67 obese children aged 11 – 16 years were randomly assigned to either a control group or a 6 month exercise protocol consisting of 60 – 90 minutes of exercise (swimming, sports games or walking) 3 days per week. Arterial structure and function were assessed by intima-media thickness and flow-mediated

dilatation. The exercise groups showed significant improvement in intima-media thickness and flow-mediated dilatation and these improvements were associated with decreases in BMI standard deviation scores, fat mass, waist-to-hip ratio, SBP, fasting insulin, triglycerides, LDL/HDL ratio and C-reactive protein. In the Northern Ireland Young Hearts Project, an ongoing longitudinal study of 405 young adults, cardiorespiratory fitness, estimated with a submaximal cycle test of physical work capacity, was found to be inversely and significantly associated with PWV of both the elastic aortoiliac segment and the aortodorsalis pedis segment. These associations were only slightly stronger with the muscular segment and were independent of lifestyle variables, body fat and physical activity (Boreham *et al.*, 2004).

6.5 Limitations

There are several limitations to the current study. The use of the generalised transfer function to generate central blood pressure values and measure wave reflection has not been validated in child and adolescent populations although the system has been previously used in children and adolescents (Lurbe *et al.*, 2003; Haller *et al.*, 2004; Covic *et al.*, 2006; Donald *et al.*, 2006; Khan *et al.*, 2006). To overcome this limitation in the study carotid artery waveforms were generated without the use of the transfer function. This method computes mean arterial pressure from the area of the wave in the corresponding heart period (Laurent *et al.*, 2006). Carotid MAP is then set equal to brachial MAP. Carotid PP is then calculated from DBP and the position of MAP on the carotid pressure wave. Carotid SBP is obtained by adding PP to DBP (Kelly and Fitchett, 1992; Verbeke *et al.*, 2005). The overall results for radial central BP values were confirmed by the carotid central BP values. Another potential weakness of the study is the use of the multi-stage fitness test to assess aerobic fitness. It is possible that compliance and low motivation may affect these results. Although it is known that use of the high-dose oral contraceptive pill is associated with a small rise in BP this study did not require female subjects to report their use, or

method of contraception. However, it has only recently been established that use of the oral contraceptive pill is associated with elevated PP and SV and a small increase in aortic PWV (Hickson *et al.*, 2011). It should be noted that the modern low-dose pills produce less of an effect and only in a small number of users.

6.6 Conclusions

In summary, this study has shown that aortic stiffness increases in children and adolescents with absolute increases in BMI and when subjects are stratified as normal weight, overweight or obese. Furthermore, arterial stiffness was also positively associated with blood pressure status and inversely associated with aerobic fitness. These observations emphasise the importance of population-wide strategies to address excess childhood adiposity and increase cardiorespiratory fitness levels through increases in physical activity at intensities likely to elicit beneficial physiological responses. Obesity related vascular dysfunction and arterial stiffness can be moderated with weight loss (Meyer *et al.*, 2006) and increased physical fitness (Ferreira *et al.*, 2003) and are likely to be important for the prevention and management of cardiovascular disease and type 2 diabetes mellitus as this population moves in to adulthood.

7 Chapter Seven – Pseudo-Systolic Hypertension of Youth

7.1 Introduction

Hypertension is a common disorder (Burt *et al.*, 1995; Colhoun *et al.*, 1998), is a component of the metabolic syndrome (World Health Organisation, 1999a; NCEP, 2001; Zimmet *et al.*, 2005; Alberti *et al.*, 2006) and contributes to the development of cardiovascular disease (Chobanian *et al.*, 2003) in the general population. The metabolic syndrome has been shown to occur in children and adolescents (e.g. de Ferranti *et al.*, 2004), is particularly prevalent in child and adolescent obesity (Weiss *et al.*, 2004b), has been shown to track into adulthood (Katzmarzyk *et al.*, 2001) and in adults is associated with the development of diabetes and adverse cardiovascular outcomes (Wilson *et al.*, 2005). It has been reported that the prevalence of hypertension, when measured at the brachial artery by traditional cuff sphygmomanometry, in young adults is 12% and the most common form of hypertension in this group is isolated systolic hypertension (ISH) (McEniery *et al.*, 2005b). Although the mechanisms of ISH in younger individuals are poorly understood it has been suggested that in most cases there is an exaggerated amplification of pulse pressure from the central arteries (aorta) to the peripheral arteries (brachial) and that central systolic pressures are normal (O'Rourke *et al.*, 2000).

In their original case report, O'Rourke *et al.* (2000) described brachial systolic hypertension in 6 male subjects aged 14-23 years in whom brachial systolic pressure was elevated in the presence of a normal diastolic pressure and normal aortic systolic pressure. With radial artery waveform analysis attained by applanation tonometry they identified that the elevation in systolic pressure was associated with, and due to, a high narrow peak of the pressure wave recorded at the radial artery. In addition, the synthesised aortic pressure wave was of normal appearance and with normal aortic systolic pressure (defined as <126 mmHg). From these results they hypothesised that ISH in these subjects was due to an "unusually high amplification of the initial pressure wave" (O'Rourke *et al.*, 2000,

p142), although they did not specifically measure or report the pulse pressure (PP) amplification. They explain the exaggerated amplification as being a consequence of a relatively late return of wave reflection from peripheral sites (dependant on attainment of full adult height), but with highly distensible arteries and a low aortic pulse wave velocity. However, aortic pulse wave velocity was not measured in this report. Consequently, O'Rourke *et al.* (2000) termed this condition "spurious systolic hypertension of youth".

Mahmud and Feely (2003) sought to identify spurious systolic hypertension and specifically examine the role of PP amplification in a cohort of 174 medical students aged 23 ± 0.5 years. 11 subjects met the criteria for ISH (≥140/<90 mmHg) by current definitions in adults (World Health Organisation, 1999c). All subjects were male, non-smokers and were physically active. The 11 subjects with ISH had significantly higher brachial SBP (147.3 ± 2 vs. 120.5 ± 1.3 mmHg), aortic SBP (115.9 ± 1 vs. 100 ± 1.5 mmHg) and absolute SBP amplification (31.4 ± 1.5 vs. 20.5 ± 1.4 mmHg) than normotensive controls. Supporting the findings of O'Rourke *et al.* (2000) the authors conclude that spurious systolic hypertension of youth is therefore due to extreme PP amplification in "fit young men with elastic arteries" (Mahmud and Feely, 2003, p229). Moreover, they refer to the condition in this study, and in two earlier abstracts (Mahmud and Feely, 2001a; Mahmud and Feely, 2001b), as pseudo-systolic hypertension of youth with the implication that the condition is benign. However, although the authors report increased PP amplification in the ISH subjects these are absolute values. When amplification is expressed more conventionally as the peripheral-to-central PP ratio, pressure amplification is actually lower in the ISH subjects when compared to gender-matched normotensive controls (1.68 vs. 1.75, respectively). Furthermore, central PP also appeared elevated in ISH subjects when compared to gender-matched normotensive controls (45.9 mmHg vs. 29 mmHg, respectively). Subsequent analysis of the data of

O'Rourke *et al.* (2000) also suggest an elevated central PP in the subjects studied (46.7 mmHg).

For these reasons not all agree that ISH in young individuals is benign (McEniery *et al.*, 2005b), rather that ISH results primarily from an elevated cardiac output and/or aortic stiffening whereas essential hypertension (EH), involving an elevation of SBP and DBP, or DBP alone, is characterised by increase peripheral vascular resistance (PVR). In a cross-sectional cohort of 1008 young subjects aged 17 – 27 years from the ENIGMA study, in addition to blood pressure, measurements were also made of aortic stiffness, cardiac output (CO), stroke volume (SV), PVR and physical activity levels (McEniery *et al.*, 2005b). This study reports an overall hypertension prevalence of 12% with an ISH prevalence of 8%. Moreover, they confirmed their hypothesis that ISH and EH result from different underlying haemodynamic mechanisms with ISH involving elevations of SV and/or aortic stiffness with a normal PP amplification, and EH involving an elevated PVR, decreased SV, normal aortic stiffness and reduced PP amplification. Furthermore, although ISH subjects had a normal PP amplification ratio, central SBP was higher than in normotensive individuals suggesting that ISH is not a condition of PP amplification and may not be benign, as previously thought. In addition, differences were found between groups for physical activity levels.

A later study also assessed the prevalence of spurious systolic hypertension (SSH) and attempted to identify its determinants in a population-based cohort of 750 adults aged 26-30 years (Hulsen *et al.*, 2006). The authors chose a modified definition for identifying SSH as brachial SBP of ≥140 mmHg, brachial DBP <90 mmHg and central SBP <124 mmHg for males and <120 mmHg for females. The implication being that central SBP ≥124 or ≥120 mmHg (90^{th} percentile score for the cohort) is abnormal, for males and females respectively. Their results confirm that SSH is predominantly found in males but, apart

from weight and BMI, no other cardiovascular risk factors differed significantly between subjects with SSH, essential hypertension or normotension. SSH subjects had significantly higher PP amplification than normotensive subjects but there were no differences in heart rate, aortic PWV and AIx. CO and SV were not measured. Again, no differences were found between groups for physical activity levels. In agreement with McEniery *et al.* (2005b) central SBP was found to raised in SSH compared to normotensive subjects and Framingham risk score calculations (Anderson *et al.*, 1991) based on brachial SBP show SSH individuals were more at risk than normotensive individuals. However, the authors note that brachial DBP is a better predictor of risk in young individuals than brachial SBP (Wilkinson *et al.*, 2001a). Although not statistically significant, they do tentatively suggest that SSH individuals were at intermediate risk of coronary heart disease based on brachial DBP, between normotensive and hypertensive subjects, based on Framingham risk scores.

Studies undertaken to date investigating the determinants of ISH have been predominantly carried out in adults and not all have conducted measurements to address all of the major determinants of PP amplification. The purpose of this study was to identify the prevalence of brachial hypertension and its subtypes in children and adolescents and to study the determinants of ISH through examination of PP amplification, arterial stiffness, aortic pulse wave velocity, cardiac output and an objective measure of aerobic fitness.

7.2 Methodology

Stature, body mass, BMI, pubertal status, aerobic fitness, arterial stiffness indices, BP, CO, SV, CI, HR and PVR were measured according to the methods stated in Chapter 3, General Methodology. Hypertension status was identified using age and gender specific criteria for UK children and adolescents (Jackson *et al.*, 2007) and using age, gender and height specific criteria (NHBPEP, 2004, 2005 (revised)).

7.2.1 Statistical analysis

Data were analysed using SPSS/PASW (versions 17.0 - 19.0). Data were analysed using one-way analysis of variance (ANOVA) and one-way analysis of co-variance (ANCOVA). Post-hoc analyses were conducted using Bonferroni correction. Stepwise linear regression was used to investigate independent determinants of haemodynamic variables. Independent variables were identified based on simple correlation analysis or those known or likely to be associated with the parameters under investigation. Two-way ANOVA was used to assess examine the effect of gender and self-assessed maturational stage on blood pressure. Homogeneity of variance between groups was assessed by Levene's test. All values are presented as mean ± SD and differences with $P \leq 0.05$ were considered significant.

7.3 Results

Table 7.1 displays the general characteristics and seated blood pressure values of the study population overall and by gender.

Table 7.1: Demographic characteristics grouped by gender

	All $n = 184$	Males $n = 102$	Females $n = 82$	*P*
Age (years)	13.4 ± 1.0	13.4 ± 1.0	13.3 ± 1.0	*ns*
Height (cm)	157.8 ± 9.6	158.9 ± 10.4	156.4 ± 8.3	*ns*
Weight (kg)	51.1 ± 12.4	51.7 ± 12.8	50.4 ± 12.0	*ns*
BMI (kg/m^2)	20.3 ± 3.5	20.3 ± 3.6	20.4 ± 3.4	*ns*
Pubertal rating:				
Breast/Penis	3.2 ± 1.1	3.3 ± 1.2	3.0 ± 1.1	*ns*
Pubic hair	3.1 ± 1.0	3.2 ± 1.0	3.1 ± 1.0	*ns*
Aerobic fitness (shuttle)	60.6 ± 27.4	74.2 ± 24.7	40.1 ± 16.4	<0.001
Brachial SBP (mmHg)	113.1 ± 9.6	115.9 ± 9.8	109.7 ± 8.0	<0.001
Brachial DBP (mmHg)	70.3 ± 7.5	69.7 ± 7.7	71.0 ± 7.3	*ns*
Brachial PP (mmHg)	42.9 ± 9.6	46.3 ± 9.0	38.7 ± 8.6	<0.001
MAP (mmHg)	84.6 ± 7.0	85.1 ± 7.3	83.9 ± 6.4	*ns*
HR (bpm)	79.5 ± 12.5	79.3 ± 11.9	79.8 ± 13.3	*ns*

Data are presented as means ± SD. Smoker, proportion who reported smoking ≥1 cigarette per day; BMI, body mass index; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; shuttle, number of shuttles performed on the multi-stage fitness test.

Overall, there was no significant difference between males and females for age, height, weight, BMI and pubertal status. A significant linear trend ($p < 0.01$) was found for increased SBP with increases in maturational status for both males and females combined (previously discussed in Chapter 5, section 5.5). No differences between TS groups were found for SBP, DBP, MAP or PP. In general, males were more aerobically fit and had higher peripheral SBP and PP ($P \leq 0.001$).

Table 7.2 shows the mean age and gender of all 184 subjects grouped according to seated BP. The overall prevalence of hypertension using UK age and gender specific criteria (UK1990) was 34%, by these criteria no subjects were identified with ISH. 82% of individuals classified with EH exhibited isolated diastolic hypertension, 17.7% had elevated or high normal SBP and elevated DBP. Using age, gender and height specific criteria the overall prevalence of hypertension, regardless of its form, was 9.8% (ISH 4.4%; and EH 5.4%).

Table 7.2: Blood pressure classification of subjects grouped according to seated blood pressure and defined according to UK 1990 age and gender specific criteria and US CDC age, gender and height specific criteria

Category	Males		Females	
	n (%)	Age (years)	*n* (%)	Age (years)
Normotensive				
UK 1990	68 (66.7)	13.3 ± 1.0	54 (65.9)	13.4 ± 1.0
US CDC	89 (87.3)	13.4 ± 1.0	77 (93.9)	13.3 ± 1.0
Essential Hypertension				
UK 1990	34 (33.3)	13.5 ± 1.0	28 (34.1)	13.2 ± 1.1
US CDC	6 (5.9)	13.3 ± 1.3	4 (4.9)	13.9 ± 1.1
Isolated systolic hypertension				
UK 1990	0 (0)		0 (0)	
US CDC	7 (6.9)	13.4 ± 1.3	1 (1.2)	14.3
Total	102 (100)	13.4 ± 1.0	82 (100)	13.4 ± 1.0

Normotensive indicates normal BP; essential hypertension indicates increased systolic and/or diastolic BP.

Detailed blood pressure measurements and subject demographics grouped by hypertension status, identified by age gender and height specific criteria are shown in Table 7.3. There were no differences in age, height, weight, BMI or aerobic fitness between groups. However, the ISH group did have a lower proportion of females compared to both the normotensive and EH groups. Compared with normotensive subjects, subjects with ISH had significantly higher peripheral SBP and PP and a significant trend to increased central SBP and PP. Compared with EH subjects, peripheral and central DBP were significantly lower in ISH individuals, but peripheral and central PP were significantly higher.

Table 7.3: Subject characteristics and supine blood pressure indices in normotensive, isolated systolic hypertension and essential hypertension subjects defined according to US CDC age, gender and height specific criteria.

	Normotensive	ISH	EH	*P* ANCOVA
Age (years)	13.3 ± 1.0	13.5 ± 1.3	13.5 ± 1.2	*ns*
Gender (male/female)	89/77	7/1	6/4	
Height (m)	157.6 ± 9.6	158.2 ± 13.1	161.1 ± 6.7	*ns*
Weight (kg)	50.6 ± 12.5	53.0 ± 11.9	58.4 ± 11.0	*ns*
BMI (kg/m^2)	20.2 ± 3.5	20.9 ± 1.9	22.5 ± 3.5	*ns*
Aerobic fitness (shuttle)	60.4 ± 27.8	67.6 ± 26.7	56.0 ± 20.8	*ns*
Brachial SBP (mmHg)	109.5 ± 9.0	117.6 ± 12.5*	116.2 ± 10.6	0.01
Brachial DBP (mmHg)	65.1 ± 6.1	64.8 ± 8.9	75.3 ± 7.0‡†	<0.001
Brachial PP (mmHg)	44.5 ± 9.5	52.9 ± 7.3*	40.9 ± 7.5†	<0.001
MAP (mmHg)	78.5 ± 6.2	80.1 ± 8.7	88.9 ± 8.2‡	<0.001
Radial:				
Central SBP (mmHg)	92.2 ± 6.4	96.4 ± 10.5	101.8 ± 9.6§	<0.001
Central DBP (mmHg)	66.7 ± 6.1	66.9 ± 9.2	77.3 ± 7.0‡†	<0.001
Central PP (mmHg)	25.8 ± 5.3	29.6 ± 3.4	24.8 ± 5.9	<0.001
Carotid:				
Central SBP (mmHg)	97.6 ± 7.3	103.4 ± 11.9	106.9 ± 9.7§	<0.001
Central DBP (mmHg)	66.6 ± 6.2	66.4 ± 9.0	77.3 ± 7.0‡†	<0.001
Central PP (mmHg)	31.3 ± 7.0	36.5 ± 5.3	29.7 ± 7.5	<0.001
HR (bpm)	75.0 ± 10.9	79.0 ± 7.5	77.0 ± 11.6	*ns*

Data are presented as means ± SD. Smoker, proportion who reported smoking ≥1 cigarette per day; shuttle, number of shuttles performed on the multi-stage fitness test. ISH, elevated and high-normal brachial SBP combined; EH, elevated and high-normal brachial SBP and/or DBP combined. Data were analysed using univariate ANCOVA with gender as covariate. Post hoc comparisons were made using Bonferroni correction. * P≤0.05, § P≤0.01, ‡ P≤0.001 vs. normotensive; † P≤0.05, EH vs. ISH.

Detailed haemodynamic variables are shown in Tables 7.4 and 7.5. Although ISH subjects had higher absolute PP amplification, CO and SV, and lower PVR than both normotensive and EH individuals these relationships could not be statistically confirmed due to low overall subject numbers in both the ISH and EH groups. Although non significant, the differences in CO and SV remained after correction for body surface area. Indices of arterial stiffness appeared higher in EH subjects compared to both ISH and normotensive. Following adjustment for the confounding variables of gender and MAP (Table 7.5), PP amplification still appeared higher in ISH than both EH and normotensive groups.

Table 7.4: Haemodynamic variables in normotensive, ISH and EH subjects

	Normotensive	ISH	EH	*P*
	n = 159	*n* = 8	*n* = 9	*ANOVA*
PP amplification	1.73 ± 0.12	1.80 ± 0.11	1.72 ± 0.15	*ns*
AIx (%)	-2.7 ± 12.2	-7.4 ± 7.3	-2.0 ± 14.4	*ns*
AIx_{75} (%)	-2.5± 12.6	-4.9 ± 7.2	-0.4 ± 13.6	*ns*
AP (mmHg)	-0.9 ± 3.6	-2.5 ± 2.6	-0.1 ± 4.0	*ns*
Brachial PWV (m/s)	6.9 ± 1.1	7.0 ± 1.0	7.5 ± 0.7	*ns*
Aortic PWV (m/s)	4.6 ± 0.7	4.7 ± 0.9	5.0 ± 0.5	*ns*
CO (L/min)	6.8 ± 1.6	7.5 ± 1.8	7.2 ± 1.0	*ns*
SV (ml)	76.1 ± 17.5	80.0 ± 16.1	77.0 ± 13.9	*ns*
CI ($L/min/m^2$)	4.5 ± 0.8	5.0 ± 1.5	4.5 ± 0.5	*ns*
Carotid AIx	-2.9 ± 12.4	-10.5 ± 15.3	-0.8 ± 10.7	*ns*
Carotid AIx_{75}	-2.7 ± 11.9	-8.3 ± 14.8	0.2 ± 9.4	*ns*
Carotid AP	-1.0 ± 3.6	-3.9 ± 5.1	-0.1 ± 2.9	*ns*
PVR (PRU)	12.1 ± 3.3	11.1 ± 2.2	12.3 ± 1.9	*ns*

Data are presented as means ± SD. PP, pulse pressure; AIx, augmentation index; AIx75, augmentation index at heart rate of 75bpm; AP, augmentation pressure; PWv, pulse wave velocity; CO, cardiac output; SV, stroke volume; CI, cardiac index; PVR, peripheral vascular resistance (peripheral resistance units). *significantly different from

Table 7.5: Haemodynamic variables in normotensive, ISH and EH subjects after correction for MAP and gender

	Normotensive $n = 159$	ISH $n = 8$	EH $n = 9$	*P* ANCOVA
PP amplification	1.73 ± 0.01	1.79 ± 0.04	1.71 ± 0.04	*ns*
AIx (%)	-2.5 ± 0.94	-6.6 ± 4.20	-5.8 ± 4.15	*ns*
AIx_{75} (%)	-2.1 ± 0.92	-4.4 ± 4.13	-6.8 ± 4.08	*ns*
AP (mmHg)	-0.8 ± 0.28	-2. ± 1.23	-1.3 ± 1.22	*ns*
Brachial PWV (m/s)	6.9 ± 0.09	7.1 ± 0.38	7.3 ± 0.39	*ns*
Aortic PWV (m/s)	4.6 ± 0.05	4.8 ± 0.24	4.7 ± 0.27	*ns*
CO (L/min)	6.9 ± 0.13	7.2 ± 0.56	7.0 ± 0.56	*ns*
SV (ml)	76.3 ± 1.38	77.2 ± 5.99	80.0 ± 5.94	*ns*
CI ($L/min/m^2$)	4.6 ± 0.07	4.9 ± 0.29	4.4 ± 0.28	*ns*
Carotid AIx	-3.0 ± 0.98	-8.7 ± 4.38	-0.25 ± 4.33	*ns*
Carotid AIx_{75}	-2.6 ± 0.92	-7.0 ± 4.14	-2.1 ± 4.10	*ns*
Carotid AP	-1.0 ± 0.28	-3.4 ± 1.28	0.0 ± 1.26	*ns*
PVR (PRU)	12.1 ± 0.25	11.4 ± 1.10	11.2 ± 1.09	*ns*

Data are presented as means ± SE.. PP, pulse pressure; AIx, augmentation index; AIx75, augmentation index at heart rate of 75bpm; AP, augmentation pressure; PWv, pulse wave velocity; CO, cardiac output; SV, stroke volume; CI, cardiac index; PVR, peripheral vascular resistance (peripheral resistance units). *significantly different from

There was no significant difference in PP amplification between males and females (1.74 ± 0.11 vs. 1.72 ± 0.13, respectively). Mean PP amplification ratio was consistent with the apparent relationship obtained in previous studies (Figure 7.1).

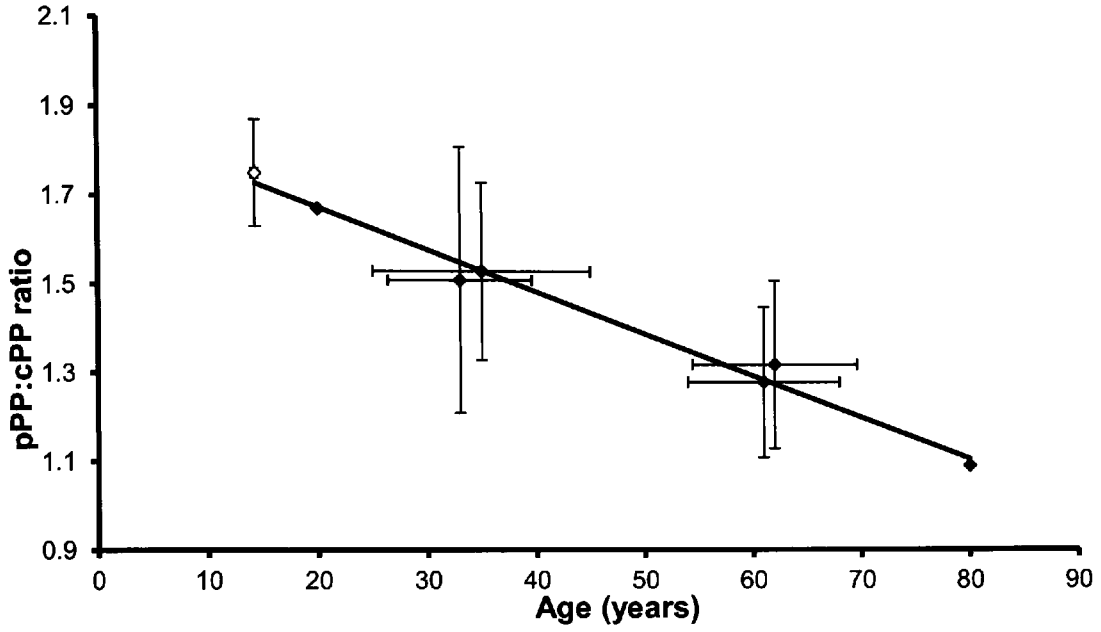

Figure 7.1: Relationship between PP amplification and age across studies. Data point to the far left (open diamond) is from the current study. Adapted from (Wilkinson *et al.*, 2001a; Wilkinson *et al.*, 2002b).

Stepwise multiple regression models were constructed to determine factors influencing peripheral and central PP, aortic PWV and SV (Table 7.6). Aerobic fitness emerged as the most important determinant of peripheral PP and was an important contributor to central PP, aortic PWV and SV. Brachial PWV emerged as a modest predictor of PP.

Table 7.6: Stepwise regression analyses

Model	Regression coefficient	SE	β	*P*	R^2 change (%)
Peripheral Pulse Pressure $R^2 = 0.28$, $P<0.001$					
Aerobic fitness	0.11	0.03	0.30	0.001	19
Brachial PWV	-1.96	0.72	-0.22	0.007	4
Carotid AIx_{75}	-0.18	0.07	-0.22	0.01	4
Central Pulse Pressure $R^2 = 0.31$, $P<0.001$					
Height	0.22	0.04	0.39	<0.001	21
Aerobic fitness	0.05	0.02	0.24	0.003	7
Brachial PWV	-0.84	0.39	-0.17	0.03	3
Aortic PWV $R^2 = 0.15$, $P<0.01$					
MAP	0.02	0.009	0.17	0.05	7
CO	0.11	0.04	0.24	0.006	4
Aerobic fitness	-0.01	0.002	-0.22	0.01	5
Stroke Volume $R^2 = 0.55$, $P=0.01$					
Height	0.85	0.12	0.47	<0.001	40
BMI	1.49	0.34	0.31	<0.001	5
Aerobic fitness	0.17	0.04	0.27	<0.001	8
HR	-0.26	0.10	-0.16	0.01	3

Stepwise linear regression analyses using 184 subjects. The regression coefficient provides the slope of the regression line. β provides a measure of the relative strength of the association, the number of standard deviations (SD) that the dependent variable will change as a result of 1SD change in the predictor variable. Dependent variables are shown in bold.

Subjects were stratified in to quartiles of SV and aortic PWV to investigate their influence on PP (Figure 7.2). There was a significant increase in peripheral and central PP from the lowest to the highest quartiles of SV ($P<0.001$). However, there was no significant difference in peripheral or central PP between the lowest to the highest quartile of aortic PWV (Figures 7.2 and 7.3). Both peripheral PP and central PP appeared to decrease in the lowest quartile of SV with increasing aortic PWV. However, this was not a significant change

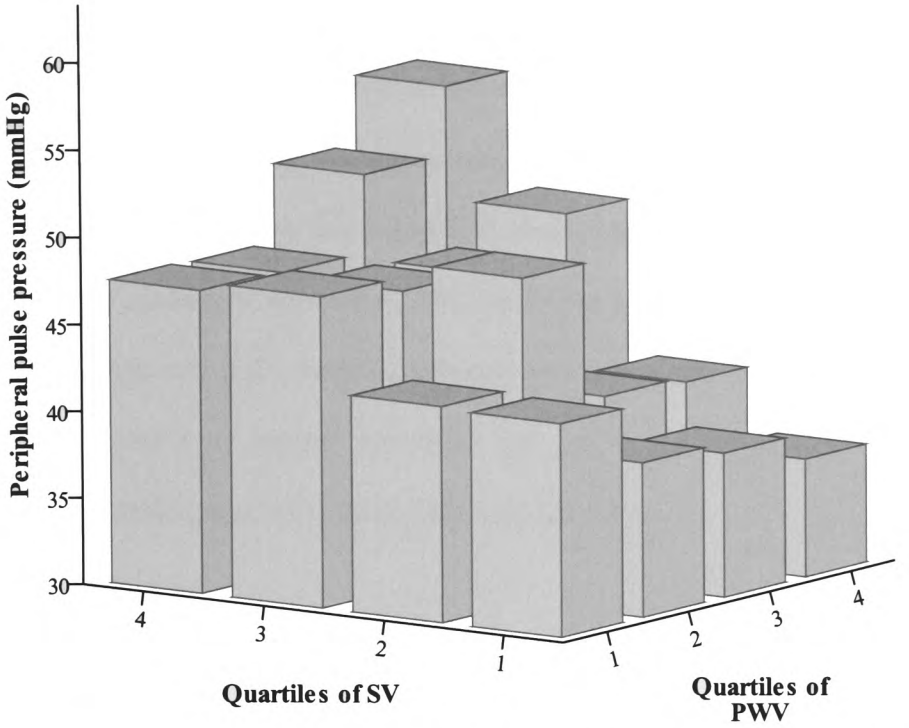

Figure 7.2: Influence of stroke volume and aortic PWV on peripheral pulse pressure. 1 = lowest quartile, 4 = highest quartile.

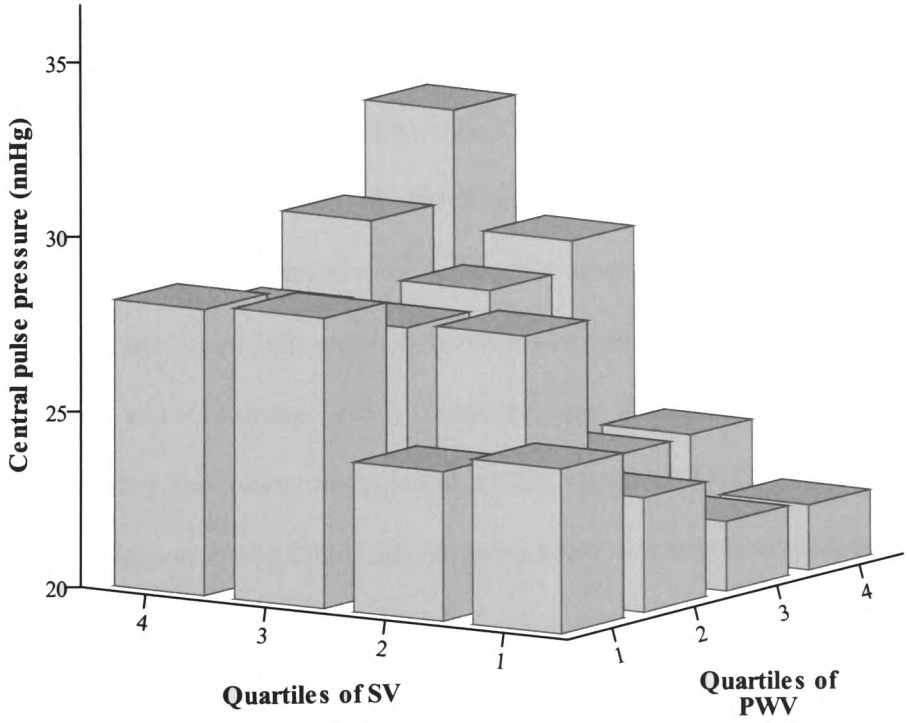

Figure 7.3: Influence of stroke volume and aortic PWV on central pulse pressure. 1 = lowest quartile, 4 = highest quartile.

7.4 Discussion

The aim of the current study was to investigate the prevalence of brachial hypertension and its subtypes in children and adolescents and, in particular, to study the determinants of ISH. To our knowledge this is the first attempt to directly address this issue solely in a child and adolescent population. This study has identified that the overall prevalence of hypertension was 34%, with no subjects presenting with ISH when UK age and gender specific criteria are applied. However, age, gender and height specific criteria returns an overall prevalence of 9.8% (ISH 4.4%; and EH 5.4%).

A recent review of hypertension in children taken from 11 population studies from different countries (Feber and Ahmed, 2010) highlighted the prevalence of abnormal BP to be between 1% and 17.3%. The authors note that an exact prevalence is difficult to obtain because results differ significantly depending on age, selection of subjects, measurements methods, number of BP readings, ethnicity differences and differing defining criteria. The prevalence of ISH in our study cohort is lower than those reported previously, 6.3% (Mahmud and Feely, 2003), 8% (McEniery *et al.*, 2005b) and 7.6% (Hulsen *et al.*, 2006), although this discrepancy may be as a result of the difference in the age ranges studied. It is known that child hypertension is often associated with an accelerated growth velocity (Nichols and O'Rourke, 2005; Genovesi and Pieruzzi, 2006). The previous studies investigating this phenomenon used an age range of 17-30 years. A study in a French cohort (Mallion *et al.*, 2003) also found a lower prevalence of ISH in male subjects aged 15-19 years compared to aged 20-24 years and 20-29 years (5.8%, 9.3% and 6.8%, respectively). In a large US study of 2460 children and adolescents aged 12-16 years, Sorof *et al.* (2002) found an ISH prevalence of 14.8%. However, this was in an ethnically diverse cohort predominantly composed of Hispanic and Black children. A multi-centre collaborative study pooled data from 8 large studies conducted between 1978-1991 in different regions of the United States (Rosner *et al.*, 2000). Data was collected for SBP on

47,196 children from 68,556 visits and DBP on 38,184 children from 52,053 visits. After pooling the data, the overall prevalence of SBP hypertension was 4.4% and DBP hypertension was 3.2%. Moreover, further analysis by Sorof (2002) shows that the prevalence of SBP hypertension was higher for virtually every grouping of subjects by race, gender, and age. It should be noted that most studies utilised different criteria for identifying elevated systolic and diastolic blood pressures which further confounds direct comparison of prevalence rates for ISH. Previous studies in young adults and adolescents show a greater prevalence of ISH than EH (Sorof *et al.*, 2002; Mallion *et al.*, 2003), this finding could not be confirmed in the current study.

In similarity with earlier studies (Mahmud and Feely, 2003; Mallion *et al.*, 2003; McEniery *et al.*, 2005b; Hulsen *et al.*, 2006) the current study confirms that ISH is found predominantly in males. O'Rourke *et al.* (2000) have hypothesised that this finding, at least in adolescents, is due to an exaggerated amplification of the brachial pressure wave as a consequence as a relatively late return of wave reflections from peripheral sites. This late return of the reflected wave is dependent on the length of the arterial pathway (height) and highly distensible arteries. In our study there was no significant difference in height between males and females and there was no difference in PP amplification, although males displayed a significantly higher peripheral and central PP.

A significant proportion of EH individuals displayed isolated diastolic hypertension when assessed using UK age and gender specific criteria. It has been well established that in young individuals DBP is a better predictor of CV risk than SBP, whereas at older age the reverse is true (Franklin *et al.*, 1999; Wilkinson *et al.*, 2001a). Several studies have identified that even moderate elevations in BP are associated with organ damage in children and adolescents. A study of 88 children with mild-to-moderate hypertension (either SBP or DBP greater than the 90th percentile) for evidence of target organ injury

found evidence of retinopathy in 50% of patients, glomerular hyperfiltration in 49%, and left ventricular hypertrophy in 36% (Daniels *et al.*, 1990). Similar changes to LV geometry in hypertensive children have been found in other studies (see Sorof (2001) for review). Moreover, data from the Bogalusa Heart Study in 654 healthy, normotensive subjects aged 7-22 years found that LV wall thickness increased throughout the SBP distribution indicating that higher SBP is associated with increased LV size (Burke *et al.*, 1987). Taken together the evidence suggests that target organ injury is found to occur in children with even moderate BP elevation.

Increased peripheral PP (Benetos *et al.*, 1998; van Trijp *et al.*, 2005) has been found to be a significant determinant of morbidity and mortality in adults. The significance in children is unknown, but increased pPP may be an indicator of early arterial disease, as has been found in young adults with type 1 diabetes (Schram *et al.*, 2003). We found a significant increase in peripheral and central PP from the lowest to the highest quartiles of SV, a finding consistent with a previous study (Alfie *et al.*, 2004). There is very little discussion of PP or PP amplification in children and adolescents in the literature. While producing blood pressure reference curves for children in the UK, Jackson *et al.* (2007) found that pPP peaks at the end of puberty in both sexes, before falling again in young adult life in contrast with systolic, diastolic and mean arterial pressures, which rise progressively with age. Data from the Framingham Heart Study (Franklin *et al.*, 1997) suggests that PP tracks throughout the lifespan and, specifically, that individuals aged 30-35 years with a high PP are more likely to develop ISH in later life. ISH subjects had higher absolute PP amplification, CO and SV, and lower PVR than both normotensive and EH individuals although these differences were not significant. The finding of a higher PP amplification in our ISH subjects would be consistent with other studies (Hulsen *et al.*, 2006) but contradicts others (Mahmud and Feely, 2003; McEniery *et al.*, 2005b), although our results were not statistically significant. Of more interest is our finding of an elevated central PP

in ISH subjects compared to normotensive subjects consistent with all previous reports (Mahmud and Feely, 2003; McEniery *et al.*, 2005b; Hulsen *et al.*, 2006). Although the relationship could not be identified in our study, it has been suggested that in the early stages of essential hypertension CO and HR are both raised before an increase in peripheral vascular resistance is seen (Cockcroft *et al.*, 2003).

Aerobic fitness emerged as the most important determinant of peripheral PP and was an important contributor to central PP, aortic PWV and SV. Males were found to have significantly greater aerobic fitness than females. However, despite a greater absolute fitness score in ISH individuals we found no significant differences in aerobic fitness between groups even after controlling for gender. Interestingly, a study of 684 female twins found that regular physical activity reduced the phenotypic expression of genetic susceptibility to increased arterial stiffness, measured by augmentation index (Greenfield *et al.*, 2003). This suggests the possibility that AIx, or an attenuated wave reflection, is the means by which physical activity decreases coronary disease risk, at least in genetically susceptible individuals.

7.5 Limitations

Although the overall prevalence of overall hypertension in our cohort was ≈9.8 - 34%, dependent on the criteria applied, data from population studies suggest a prevalence of ≈11% (Feber and Ahmed, 2010). It is likely that any discrepancy is due to differences in methodology. BP readings were taken during a single visit, albeit in duplicate (triplicate where first and second readings differed) and after 15 minutes of seated rest. With repeated measures and repeat visits regression to the mean and a reduction in white coat hypertension may have seen a reduced prevalence estimate. Indeed, in a multi-racial schools study Moore *et al.* (2009) report prevalence's of 13.8% at first visit, reducing to 4.5% and 2.3% at second and third visit, respectively. An additional limitation is related to

overall subject numbers. Although we were able to find statistical differences between the three groups for both peripheral and central BP variables we were unable to replicate this for measures of arterial stiffness, CO, PVR and PP amplification ratio. The effect of maturational stage also warrants further investigation in a larger cohort. Nevertheless, we were able to determine factors influencing peripheral and central PP, aortic PWV and SV with aerobic fitness emerging as an important determinant of peripheral PP and contributor to central PP, aortic PWV and SV.

7.6 Conclusions

In summary, this study aimed to investigate the prevalence of hypertension in children and adolescents and, in particular, to study the determinants of ISH. It is believed that this is the first attempt to directly address this issue in a child and adolescent population. The current study has demonstrated that hypertension is highly prevalent during single screening when using current child and adolescent definitions. We have been unable to conclusively demonstrate that isolated systolic hypertension in children and adolescents results from an increased stroke volume and/or aortic stiffness. However, this study was able to demonstrate the important influence of physical fitness as a determinant of both peripheral and central pulse pressure and aortic stiffness. These data indicate the restrictive nature of simply recording peripheral blood pressure which may not provide an accurate assessment of central haemodynamics.

8 Chapter Eight: Association Between Alanine Aminotransferase and Metabolic Risk Factors in Children and Adolescents

8.1 Introduction

Unexplained elevations in aminotransferase concentrations have been strongly associated with adiposity and thus may represent non-alcoholic fatty liver disease (NAFLD) (Clark *et al.*, 2003). Alanine aminotransferase (ALT) is the liver enzyme with the closest association with liver fat accumulation (Schindhelm *et al.*, 2006) and consequently has been used as a circulating marker of NAFLD. Moreover, liver fat accumulation predicts the development of metabolic syndrome, type 2 diabetes and cardiovascular disease (Kotronen and Yki-Jarvinen, 2008). In adults, NAFLD is considered to be the hepatic manifestation of the metabolic syndrome and is associated with insulin resistance (Marchesini *et al.*, 1999; Hanley *et al.*, 2007) and reductions in insulin sensitivity (Marchesini *et al.*, 2001). Furthermore, elevated ALT predicts future incidence of type 2 diabetes (Sattar *et al.*, 2004), metabolic syndrome (Hanley *et al.*, 2005), atherothrombotic cardiovascular disease (Marchesini *et al.*, 2001), risk of mortality from liver diseases (Kim *et al.*, 2004) and predicts coronary heart disease events (Schindhelm *et al.*, 2007).

The prevalence of NAFLD in adults has been estimated to range from 3% to 24% (Clark, 2006). In children there have been few population based studies identifying the prevalence of NAFLD although it has been reported to be 2.6% in Japanese children (Tominaga *et al.*, 1995) and 3% in American children (Strauss *et al.*, 2000). Autopsy findings of children and adolescents adjusted for age, gender and ethnicity have found a prevalence of 9.6% increasing with age (Schwimmer *et al.*, 2006). The prevalence of elevated ALT in adults has been reported between 2.8% (Ruhl and Everhart, 2003) and 11.1% (Kariv *et al.*, 2006), depending on the definition employed while in children and adolescents it has been reported to range from 7.4% to 11.5%, depending on ethnicity, with a greater prevalence

among males than females (Fraser *et al.*, 2007). The identification of elevated ALT concentrations in children depends on the cut-off criteria utilised and studies have used values of >30 U/L (Strauss *et al.*, 2000), >40U/L (Park *et al.*, 2005a), >75th percentile of the sample population (Patel *et al.*, 2011) or ≥95th percentile (England *et al.*, 2009). Recently, using data from the National Health and Nutrition Examination Survey (NHANES), it has been suggested that cut-off values are set too high for the reliable detection of childhood liver disease and 95th percentile values of >25 U/L and >22 U/L should be applied for males and females aged 12-17 years, respectively (Schwimmer *et al.*, 2010). Insulin resistance (Kahn *et al.*, 2005b) and adiposity (Zimmet *et al.*, 2005; Alberti *et al.*, 2006) are believed to play important roles in the pathogenesis of the metabolic syndrome. The rising prevalence of the metabolic syndrome and its adverse effects has prompted growing concern about this condition not only in adults but also in adolescents (Weiss *et al.*, 2004a). Moreover, elevations in ALT (Park *et al.*, 2005a; Patel *et al.*, 2011) and NAFLD in overweight and obese children (Schwimmer *et al.*, 2008) are adversely associated with all of the major components of the metabolic syndrome. Physical activity has also been found to be inversely associated with metabolic risk, independent of potential confounders, and this relationship may be stronger in children with low cardiorespiratory fitness (Brage *et al.*, 2004).

Although NAFLD, and elevations in its circulating marker ALT, are identifiable in children, data on the association of liver enzyme abnormalities with insulin resistance and physical fitness are lacking. This study examined the association of elevated ALT with insulin resistance, body composition and aerobic fitness in a cohort of apparently healthy children and adolescents from Wales, UK.

8.2 Methodology

Stature, body mass, BMI, waist circumference, pubertal rating, aerobic fitness, insulin, glucose, HOMA-IR, total cholesterol, triglyceride, HDL, LDL, ALT, ALK, SBP and DBP were measured according to the methods stated in Chapter 3, General Methodology. Metabolic syndrome was identified using the NCEP ATPIII criteria (Okubo *et al.*, 2001) modified for use in children (Whincup *et al.*, 2005). Metabolic syndrome was defined as the presence of 3 or more of the following components WC, TG, SBP and/or DBP >90th percentile, HDL < 10th percentile and fasting plasma glucose > 5.6mmol/L.

8.2.1 Statistical analysis

Data were analysed using SPSS/PASW (versions 17.0 - 19.0). Differences between males and females and elevated ALT versus normal ALT groups were assessed by independent samples t-tests. Correlations were conducted using Pearson Product-Moment Correlation Coefficients. Where data were found to be non-normally distributed the equivalent non-parametric tests (Mann-Whitney U; Spearman Rho test) were performed. Performing log transformations of non-normally distributed data and using parametric statistical tests did not significantly alter the associations. Two-way ANOVA was conducted that examined the effect of gender and maturational stage on ALT. Homogeneity of variance between groups was assessed by Levene's test. Stepwise linear regression was used to investigate independent determinants of haemodynamic variables. Independent variables were identified based on simple correlation analysis or those known or likely to be associated with the parameters under investigation. Trend analysis was performed using the non-parametric Kruskal–Wallis test with Jonckheere-Terpstra test, differences between means was assessed by Bonferonni corrected Mann-Whitney U tests. All values are presented as mean ± SD and differences with $p \leq 0.05$ were considered significant.

8.3 Results

Descriptive characteristics are displayed in Table 8.1. Overall there were no significant differences between male and female subjects for anthropometric measurements, insulin or glucose levels, insulin resistance, diastolic blood pressure or serum ALT. Males were found to be more aerobically fit, had higher serum alkaline phosphatase and higher systolic blood pressure but lower values for all blood lipids.

Table 8.1: Descriptive characteristics of the study population

	All $n = 158$	Males $n = 90$	Females $n = 68$	*P*
Age (years)	13.4 ± 1.0	13.4 ± 1.0	13.4 ± 1.0	*ns*
Height (cm)	158.4 ± 9.4	159.2 ± 10.5	157.3 ± 9.4	*ns*
Weight (kg)	51.8 ± 12.0	52.1 ± 12.4	51.4 ± 11.6	*ns*
BMI (kg/m^2)	20.5 ± 3.4	20.4 ± 3.5	20.6 ± 3.3	*ns*
Waist circumference (cm)	71.3 ± 9.6	72.4 ± 10.0	69.8 ± 8.8	*ns*
Pubertal rating:				
Breast/Penis	3.3 ± 1.1	3.4 ± 1.2	3.2 ± 1.1	*ns*
Pubic hair	3.2 ± 1.0	3.2 ± 1.0	3.2 ± 0.9	*ns*
Aerobic fitness (shuttle) (n=70)	62.9 ± 27.4	75.0 ± 24.9	41.0 ± 15.6	<0.001
Insulin (pmol/L)	51.7 ± 34.4	53.8 ± 40.2	49.0 ± 24.6	*ns*
Glucose (mmol/L)	4.7 ± 0.5	4.7 ± 0.5	4.7 ± 0.5	*ns*
HOMA-IR	1.76 ± 1.29	1.87 ± 1.51	1.62 ± 0.91	*ns*
Total cholesterol (mmol/L)	4.31 ± 0.88	4.12 ± 0.77	4.56 ± 0.95	0.002
Triglyceride (mmol/L)	0.78 ± 0.35	0.72 ± 0.33	0.85 ± 0.37	0.01
HDL (mmol/L)	1.63 ± 0.27	1.60 ± 0.28	1.68 ± 0.27	0.05
LDL (mmol/L)	2.34 ± 0.81	2.21 ± 0.68	2.50 ± 0.94	0.04
ALT (U/L)	24.4 ± 6.6	24.7 ± 7.0	23.9 ± 5.9	*ns*
ALK (U/L)	223.8 ± 90.9	262.4 ± 79.8	172.7 ± 79.2	<0.001
Brachial SBP (mmHg)	113.5 ± 9.6	115.8 ± 10.2	110.4 ± 7.7	<0.001
Brachial DBP (mmHg)	70.2 ± 7.5	69.6 ± 7.7	71.1 ± 7.3	*ns*

Data are presented as means ± SD.

Table 8.2 displays subject characteristics according to gender and ALT levels. The overall prevalence of elevated ALT levels was 12% (cut-off >30 U/L) with males displaying a higher prevalence than females (13.3% and 10.3%, respectively). However, when applying the recently proposed lower cut-off points for 12-17 year olds of ≥25 U/L for males and

≥22 U/L for females, the prevalence increased to 36.7% in males and 52.9% in females. Males with ALT levels <25 U/L were significantly older, had higher pubertal ratings and were significantly fitter aerobically compared to males with elevated ALT. Females with elevated ALT levels (≥22 U/L) had significantly increased insulin levels and were more insulin resistant. All subjects with elevated ALT had significantly higher fasting insulin levels (P <0.01) and significantly higher insulin resistance ($P \leq 0.01$).

Table 8.2: Descriptive characteristics according to ALT levels

	Males		Females	
	≤25 U/L	>25 U/L	≤22 U/L	>22 U/L
n	57	33	32	36
Age (years)	13.6 ± 1.0	13.1 ± 1.0*	13.7 ± 0.9	13.3 ± 1.1
Height (cm)	159.9 ± 10.6	157.9 ± 10.3	158.0 ± 6.9	156.7 ± 8.5
Weight (kg)	51.3 ± 11.8	53.5 ± 13.6	50.8 ± 8.8	52.0 ± 13.7
BMI (kg/m^2)	19.9 ± 3.0	21.2 ± 4.3	20.3 ± 2.8	20.9 ± 3.8
Waist circumference (cm)	71.1 ± 9.3	74.5 ± 10.9	67.9 ± 7.2	71.5 ± 9.8
Pubertal rating:				
Breast/Penis	3.6 ± 1.2	3.0 ± 1.1*	3.2 ± 1.0	3.2 ± 1.2
Pubic hair	3.5 ± 1.0	2.8 ± 1.0†	3.3 ± 0.9	3.2 ± 1.0
Aerobic fitness (shuttle) (n=70)	81.4 ± 20.8	63.6 ± 27.8†	43.7 ± 15.1	39.0 ± 16.1
Insulin (pmol/L)	48.3 ± 36.0	63.0 ± 45.5	37.7 ± 12.5	58.4 ± 28.2‡
Glucose (mmol/L)	4.7 ± 0.5	4.8 ± 0.4	4.8 ± 0.4	4.6 ± 0.6
HOMA-IR	1.62 ± 1.32	2.31 ± 1.72	1.22 ± 0.62	2.01 ± 0.97‡
Total cholesterol (mmol/L)	4.16 ± 0.69	4.05 ± 0.90	4.69 ± 1.01	4.44 ± 0.88
Triglyceride (mmol/L)	0.71 ± 0.29	0.74 ± 0.38	0.82 ± 0.31	0.87 ± 0.43
HDL (mmol/L)	1.62 ± 0.28	1.56 ± 0.28	1.68 ± 0.24	1.68 ± 0.30
LDL (mmol/L)	2.22 ± 0.60	2.20 ± 0.80	2.60 ± 1.01	2.41 ± 0.88
ALT (U/L)	20.7 ± 3.8	31.6 ± 6.0‡	19.0 ± 2.5	28.3 ± 4.5‡
ALK (U/L)	250.1 ± 71.2	282.2 ± 90.4	164.4 ± 82.0	180.1 ± 77.0
Brachial SBP (mmHg)	115.6 ± 10.0	116.2 ± 10.6	110.8 ± 6.6	110.2 ± 8.7
Brachial DBP (mmHg)	69.2 ± 8.6	70.2 ± 5.7	71.4 ± 5.9	70.8 ± 8.4

Data are presented as means ± SD. *$P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$.

The effect of gender and maturational stage on log transformed ALT (log ALT) was examined. Analysis of untransformed ALT values produced broadly similar results. There was a significant interaction between the effects of gender and maturational stage on log ALT (p = 0.029). Simple main effects analysis showed that males had significantly lower

ALT than females at TS5 (p = 0.027) but there were no differences between gender at any other Tanner stage (Figure 8.1).

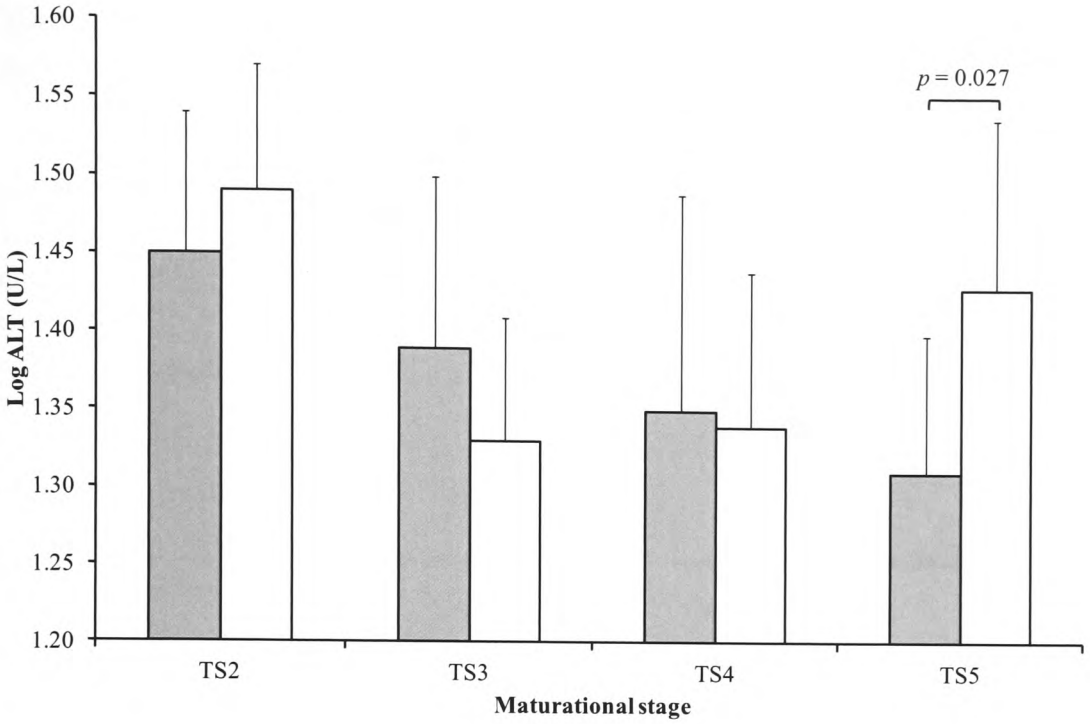

Figure 8.1: Effect of gender and maturational stage on log transformed alanine aminotransferase (ALT) concentrations. Males, filled bars; females, open bars.

Significant correlates of ALT and selected metabolic risk factor variables which are common components of the metabolic syndrome are summarised in Table 8.3. Among all subjects, ALT correlated positively with waist circumference and insulin resistance. In addition, females showed a positive association of ALT with insulin levels. In males, pubertal rating and aerobic fitness showed negative correlations with ALT. In all subjects age was negatively correlated with ALT concentrations.

Table 8.3: correlation analysis between ALT levels and clinical variables

	All	Males	Females
n	158	90	68
Age (years)	-0.34‡	-0.34‡	-0.34†
BMI (kg/m^2)	0.15	0.20	0.10
Waist circumference (cm)	0.24†	0.24*	0.25*
Pubertal rating:			
Breast/Penis	-0.19*	-0.31†	-0.06
Pubic hair	-0.25†	-0.34‡	-0.10
Aerobic fitness (shuttle) (*n*=70)	-0.29†	-0.40‡	-0.20
Insulin (pmol/L)	0.28‡	0.2	0.45‡
Glucose (mmol/L)	-0.003	0.10	-0.14
HOMA-IR	0.30‡	0.24*	0.42‡
Triglyceride (mmol/L)	-0.01	0.004	0.008
HDL (mmol/L)	-0.1	-0.14	0.004
ALK (U/L)	0.19	0.16	0.19
Brachial SBP (mmHg)	0.034	0.06	-0.08
Brachial DBP (mmHg)	0.1	0.1	0.12

*$P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$.

Although BMI in itself did not correlate significantly with ALT concentrations (r = 0.15; Figure 8.2), when subjects were classified as normal weight, overweight or obese by the International Obesity Task Force (IOTF) criteria for children and adolescents (Cole *et al.*, 2000) there was a significant trend for increased ALT with increasing BMI status (r = 0.31, $p < 0.001$; Figure 8.3). In addition, IOTF defined obesity category was also significantly associated with an increase in insulin resistance among subjects ($r = 0.29, p < 0.001$).

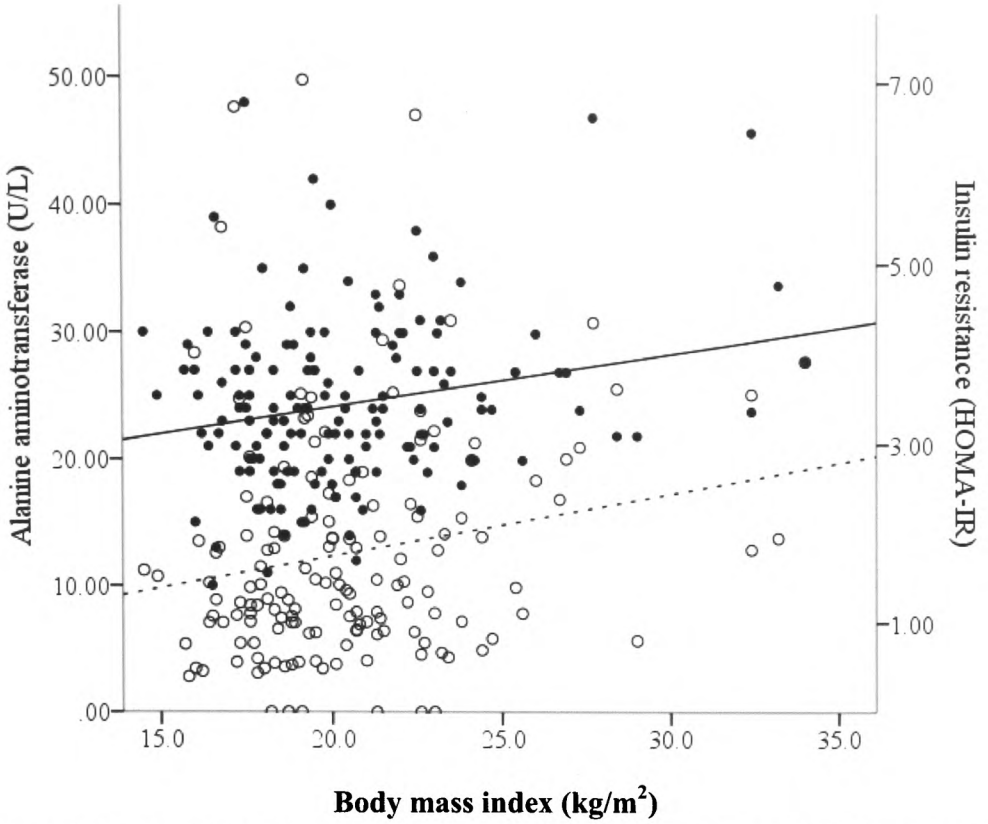

Figure 8.2: Correlation between body mass index and ALT and insulin resistance. ALT, closed circles and solid line; insulin resistance, open circles dotted line.

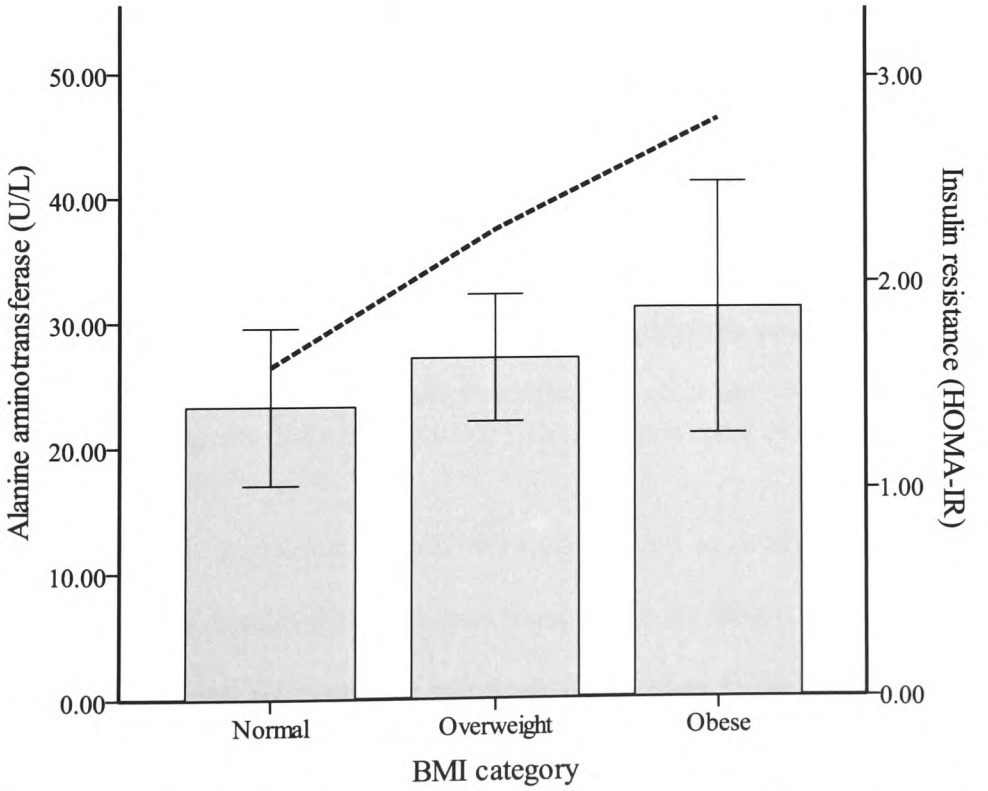

Figure 8.3: Association between BMI categories defined according to IOTF criteria and ALT (shaded bars; $R = 0.35$, $P = 0.001$) and insulin resistance (dotted line; $r = 0.36$, $p = 0.001$). Data are presented as means ± SD.

With the exception of WC, ALT did not exhibit any significant relationships with any metabolic syndrome risk factors (Table 8.3). The relationship between ALT and the clustering of components of the metabolic syndrome are shown in Figure 8.4. ALT concentrations showed a significant linear increase (P <0.001) according to the number of metabolic syndrome components.

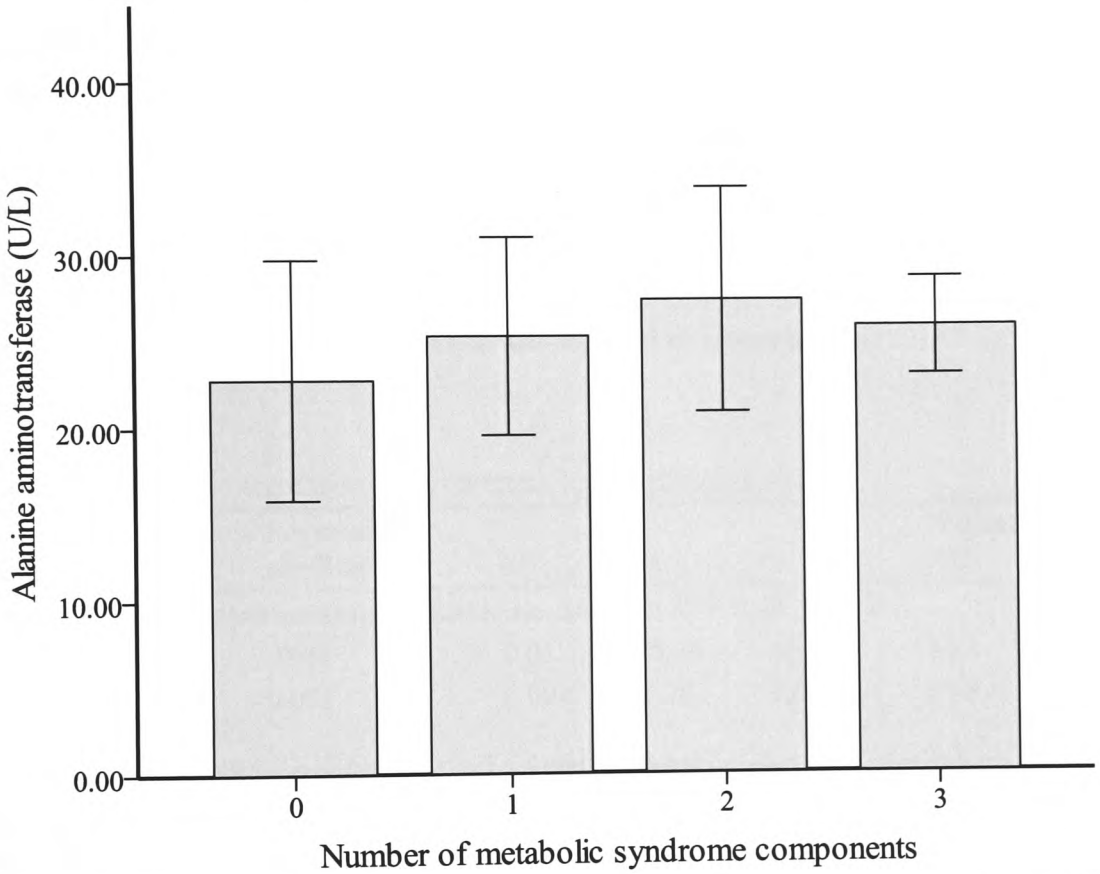

Figure 8.4: Alanine aminotransferase concentrations according to the number of metabolic syndrome components (p for trend <0.001). Data are presented as means ± SD.

Stepwise multiple regression models were constructed to determine factors influencing ALT (Table 8.4). Insulin resistance was found to be the major independent predictor of ALT levels. Age and WC were also independent predictors of ALT.

Table 8.4: Stepwise regression analyses

Model	Regression coefficient	SE	β	*p*	R^2 change (%)
Alanine aminotransferase, adjusted $R^2 = 0.24$, $P<0.001$					
Insulin resistance	1.36	0.45	0.26	0.003	13
Age	-2.06	0.57	-0.31	<0.001	8
Waist circumference	-0.17	0.06	-0.25	0.0051	6

Stepwise linear regression analyses using 158 subjects. The regression coefficient provides the slope of the regression line. β provides a measure of the relative strength of the association, the number of standard deviations (SD) that the dependent variable will change as a result of 1SD change in the predictor variable. Dependent variables are shown in bold.

Stepwise regression analysis was also conducted on log transformed ALT values because ALT was not normally distributed (Table 8.5). In this model insulin resistance no longer remained as an independent predictor and was replaced by serum insulin.

Table 8.5: Stepwise regression analyses using log transformed ALT

Model	Regression coefficient	SE	β	*p*	R^2 change (%)
Log transformed alanine aminotransferase, adjusted $R^2 = 0.25$, $P<0.001$					
Age	-0.04	0.01	-0.36	<0.001	10.8
Waist circumference	0.003	0.001	0.28	0.002	10.8
Insulin	0.001	<0.001	0.24	0.008	5.2

Stepwise linear regression analyses using 158 subjects. The regression coefficient provides the slope of the regression line. β provides a measure of the relative strength of the association, the number of standard deviations (SD) that the dependent variable will change as a result of 1SD change in the predictor variable. Dependent variables are shown in bold.

Significant correlations were found between ALT and aerobic fitness amongst all subjects ($r = -0.29$, $p = \leq 0.01$) and in particular in males ($r = -0.40$, $p \leq 0.001$). When aerobic fitness was expressed as quintiles, a significant trend was observed for decreasing ALT levels with increasing aerobic fitness ($p < 0.01$; Figure 8.5). More specifically, subjects in the two lowest quintiles of fitness exhibited significantly higher ALT levels than subjects in the

highest fitness quintile. The relationship was found to be stronger in males than in females (Table 8.3, Figure 8.6). Significant inverse relationships were also found between aerobic fitness and fasting insulin (r =-0.3, p <0.01) and HOMA-IR (r =-0.3, p <0.01).

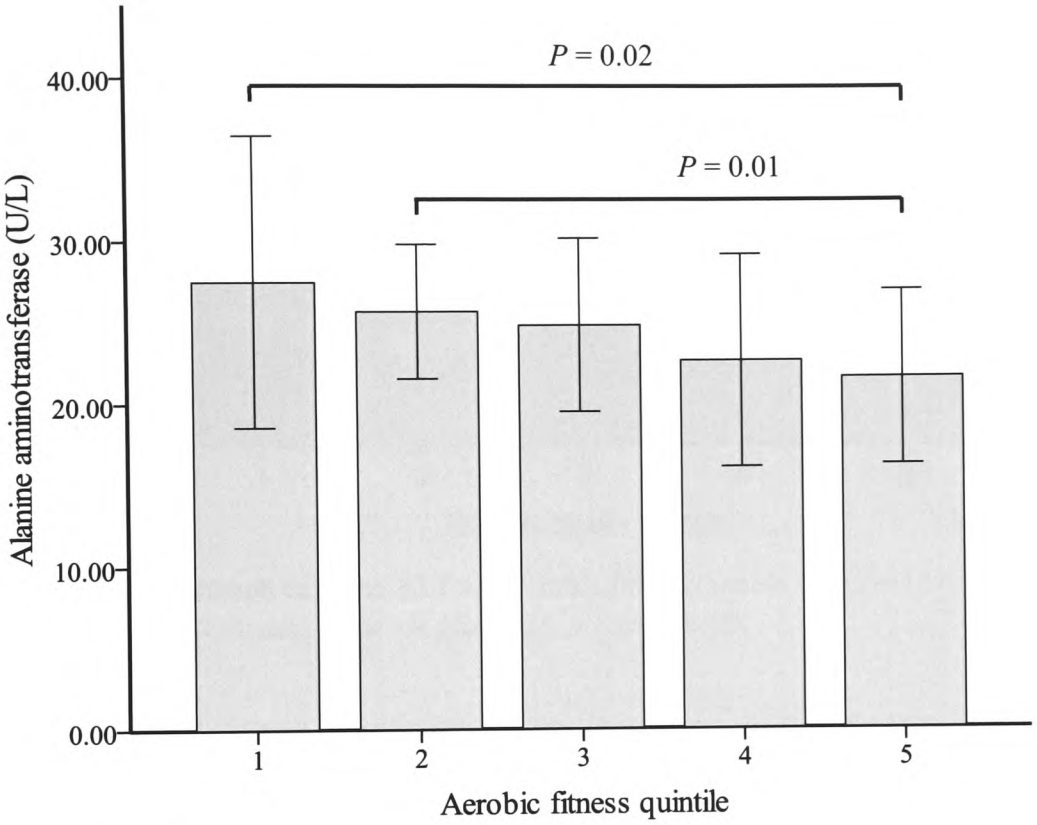

Figure 8.5: Association between ALT and aerobic fitness quintile in all subjects (1 = lowest fitness, 5 = highest fitness). Data are presented as means ± SD.

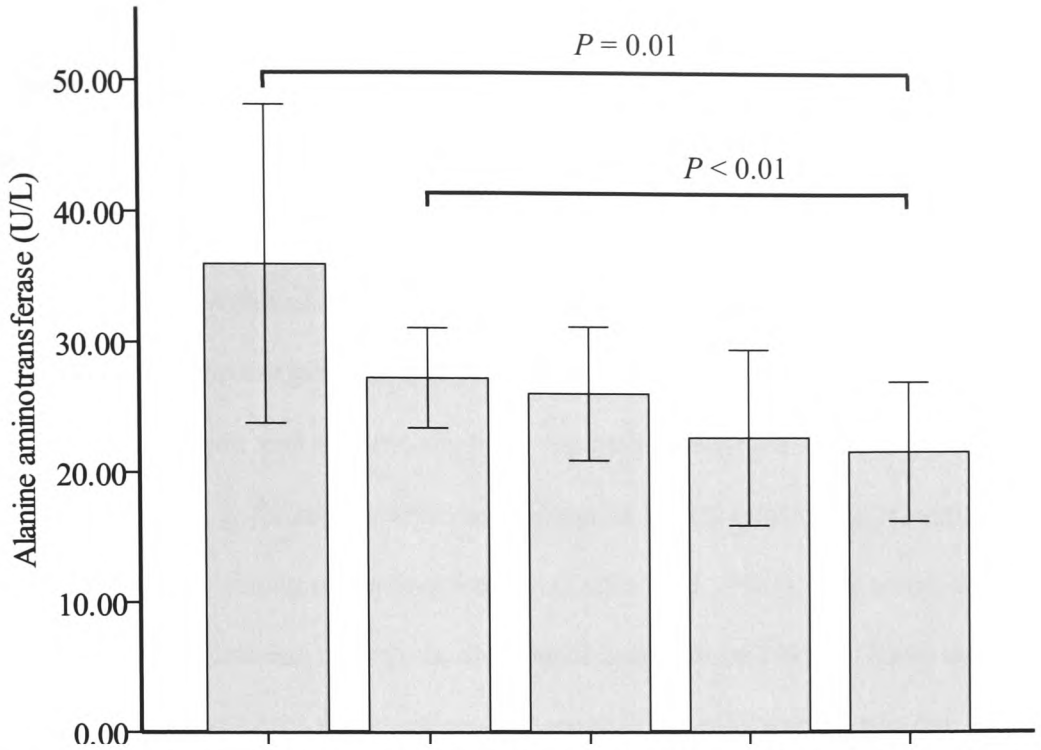

Figure 8.6: Association between ALT and aerobic fitness quintile in males (1 = lowest fitness, 5 = highest fitness). Data are presented as means ± SD.

8.4 Discussions

In adults, elevations in liver enzymes, particularly ALT, are associated with adiposity and insulin resistance (Hanley *et al.*, 2007), predict type 2 diabetes (Sattar *et al.*, 2004), metabolic risk factor clustering (Hanley *et al.*, 2005) and may be a marker for fatty liver disease (Clark *et al.*, 2003). ALT has been measured in children (Fraser *et al.*, 2007) but cut-off criteria used to identify elevated levels vary between studies. The aim of the current study was to examine the association of elevated ALT with insulin resistance, body composition and aerobic fitness in a cohort of apparently healthy children and adolescents. The major findings in this study were that elevations in ALT are highly prevalent in children and adolescents even when the more conservative cut-off points are applied; ALT was found to be positively associated with insulin resistance, body composition measures and aerobic fitness; and insulin resistance was found to be a predictor of elevated ALT

levels in children and adolescents. These findings suggest that a strong relationship exists between insulin resistance, insulin levels and ALT and that this relationship may be mediated by abdominal adiposity and low physical fitness.

8.4.1 Prevalence

Previous studies investigating the occurrence of elevated ALT, a surrogate measure of NAFLD, in children and adolescents have reported prevalence's ranging from 6.0% in black adolescents, 7.4% among white adolescents to 11.5% in Mexican Americans, with a greater prevalence among males than females (Fraser *et al.*, 2007). This study, based on the NHANES cross sectional surveys in the United States from 1999 to 2004, used data on 5586 children aged 12-17 years and applied an ALT cut-off value of >30 U/L. When this threshold was applied to the current study a prevalence of 12% was found with males displaying a higher prevalence than females (13.3% and 10.3%, respectively). It has recently been suggested that ALT cut-off values are set too high for the reliable detection of chronic liver disease in children and adolescents and that 95th percentile values of >25 U/L for males and >22 U/L for females aged 12-17 years should be applied (Schwimmer *et al.*, 2010). Unsurprisingly, application of these values yielded significantly greater prevalence, conversely with a greater prevalence in females than males. When applying the less conservative cut-off criteria the lower prevalence in males is likely to be due to the different cut-off values applied for males and females. If a universal cut-off value of 25 U/L is applied for both males and females the prevalence in females falls to 36.8%, almost identical to males. Interestingly, in the current study males with high ALT were significantly less mature than males with low ALT. Supporting this finding data from the Bogalusa Heart Study (Patel *et al.*, 2011) found that male adolescents (12-18 years) had higher ALT than females adolescents whereas the reverse was true in preadolescent children (4-11 years) suggesting a difference in the effect of sex hormones on ALT between the genders at different stages of maturation. In contrast, an Italian study of 358

obese children aged 6 – 16 years found a non-significant weak positive correlation between ALT and Tanner stage in boys ($r = 0.12$) but a significant positive correlation in girls ($r = 0.19$, $p < 0.05$) (Di Bonito *et al.*, 2009). Others have found pubertal status to have a modifying effect on the association of ALT with insulin resistance, fasting insulin and triglycerides (van Vliet *et al.*, 2009) although with pubertal boys displaying a higher prevalence of elevated ALT compared to pubertal girls. The authors acknowledged that contradictory findings have been previously reported and that the role of pubertal stage in the development of fatty liver remains to be resolved. It is possible that pubertal insulin resistance may be responsible for the observation that males with elevated ALT had lower pubertal stage ratings. Goran and Gower (1999) observed a 32% reduction in insulin sensitivity in children moving between Tanner stage I and III with similar changes in both boys and girls. The observation that girls did not display this same relationship between pubertal stage and ALT levels may be indicative of differing interactions between the sex hormones. Previous studies have consistently reported higher ALT levels in males compared to females (Prati *et al.*, 2002; Kariv *et al.*, 2006; Fraser *et al.*, 2007; Yoo *et al.*, 2008; Di Bonito *et al.*, 2009; Saito *et al.*, 2009; van Vliet *et al.*, 2009). The finding that ALT levels in girls were higher than boys at TS5 may have occurred as a result of uneven numbers of males and females within this Tanner stage group (16 boys, 6 girls). Moreover, it was not possible to assess prepubertal influences on ALT as a result of only 2 children rating TS1 with a measure of ALT. Although the current study has found a higher prevalence of elevated ALT than in previous studies, at present prevalence comparisons are difficult because of the use of predominantly overweight or obese subjects (Strauss *et al.*, 2000; Schwimmer *et al.*, 2008; Yoo *et al.*, 2008; Di Bonito *et al.*, 2009; van Vliet *et al.*, 2009), differing cut-off criteria (Park *et al.*, 2005a; Yoo *et al.*, 2008; Di Bonito *et al.*, 2009), and differing ethnicities (Strauss *et al.*, 2000; Park *et al.*, 2005a; Fraser *et al.*, 2007; Schwimmer *et al.*, 2008; Yoo *et al.*, 2008; Patel *et al.*, 2011). However, it is believed that

the current study is the first to estimate the prevalence of elevated ALT in a UK child and adolescent population.

8.4.2 Insulin resistance

In the current study, all subjects exhibiting elevations in ALT also displayed significant elevations in fasting serum insulin and insulin resistance. In adults and children, insulin resistance and hyperinsulinaemia are thought to be critical factors in NAFLD (Patton *et al.*, 2006) and several studies in children and adolescents have reported similar findings. A multi-ethnic study of 443 overweight or obese children aged 3 – 18 years reported significantly increased fasting plasma insulin and insulin resistance in subjects with ALT levels >30 U/L (van Vliet *et al.*, 2009). Interestingly, overweight and obese subjects with elevated ALT also had increased waist circumference compared to overweight and obese subjects with normal ALT. A study of obese children aged 6 – 16 years in Italy (Di Bonito *et al.*, 2009) found significant elevations in fasting insulin and insulin resistance assessed by HOMA-IR in boys with elevated ALT but not in girls. Further examination shows that the obese boys with elevated ALT had significantly higher BMI and WC than obese boys with normal ALT. Similar findings occur in non-overweight and obese children. Patel *et al.* (2011) found BMI to be the major independent predictor of ALT levels in the multi ethnic Bogalusa Heart Study; HOMA-IR was also found to be a predictor. Fraser *et al.* (2007) found that adolescents aged 12 – 19 years from the NHANES studies with raised ALT had higher WC and BMI than normal ALT subjects. Similarly, a study of 1594 Korean child and adolescent subjects aged 10 – 19 years found significantly increased WC and BMI in subjects with raised ALT, although fasting insulin or insulin resistance was not measured in this study (Park *et al.*, 2005a). The current study has shown that increasing overweight and obesity status is positively associated with elevations in ALT. NAFLD is strongly associated with reductions in whole body, hepatic and adipose tissue insulin sensitivity (see Utzschneider and Kahn, 2006 for review). It has been suggested that this strong

association may be explained by a decrease of hepatic insulin clearance in subjects with increased hepatic fat content (Goto *et al.*, 1995; Kotronen *et al.*, 2007).

8.4.3 Obesity/abdominal adiposity

Waist circumference, an indicator of abdominal adiposity, is a better predictor of cardiovascular disease risk than obesity (Alberti *et al.*, 2006) and has been associated with NAFLD using various invasive and imaging techniques (Kotronen and Yki-Jarvinen, 2008). In adults, some (Targher *et al.*, 2006) but not all studies (Church *et al.*, 2006) have confirmed the association of WC with NAFLD after adjustment for BMI. Accumulation of intra-abdominal adipose tissue has been positively correlated with liver fat in adults (Kelley *et al.*, 2003; Nguyen-Duy *et al.*, 2003) and in a case-control study of overweight children with or without NAFLD, patients were found to have significantly elevated ALT but no significant difference in WC (Schwimmer *et al.*, 2008). The present study in children and adolescents found a significant positive relationship between WC and ALT. However, it has been suggested that only 30 – 40% of the variation in liver fat can be explained by variation in the size of the intra-abdominal adipose tissue depot (Kotronen and Yki-Jarvinen, 2008) suggesting that factors other than central adiposity may regulate liver fat. Moreover, lean men with increased liver fat were found to have both hepatic and adipose tissue insulin resistance compared to well matched controls with low levels of liver fat, suggesting that liver fat accumulation may be associated with insulin resistance independent of intra-abdominal fat (Seppala-Lindroos *et al.*, 2002).

8.4.4 Metabolic syndrome

NAFLD has been proposed as an additional feature of the metabolic syndrome (Cortez-Pinto *et al.*, 1999; Marchesini *et al.*, 2001) and elevated ALT is regarded as a marker of liver fat accumulation and NAFLD (Clark *et al.*, 2003; Schindhelm *et al.*, 2006). Indeed, increased ALT has been found to be associated with metabolic syndrome related features

including obesity and insulin resistance (Saito *et al.*, 2009) and predicts future development of metabolic syndrome in adults (Hanley *et al.*, 2005). Kotronen *et al.* (2007) found that all components of the metabolic syndrome in adults correlated significantly with liver fat content, although the association was diminished or disappeared when adjusted for age and BMI. The current study could not confirm this finding in children and adolescents because only WC displayed a significant association with ALT. However, when individuals where categorised by the number of components of the metabolic syndrome a significant trend was evident of increasing concentration of ALT with increasing number of components. Similar relationships have been found in normal weight Korean adolescents (Park *et al.*, 2005a) and African American children (Patel *et al.*, 2011), more so in overweight and obese children (Schwimmer *et al.*, 2008; Di Bonito *et al.*, 2009; van Vliet *et al.*, 2009). Furthermore, more recent research has shown that these relationships are evident in prepubertal obese children (Calcaterra *et al.*, 2011).

8.4.5 Aerobic fitness

In adult males cardiorespiratory fitness has been found to be inversely associated with NALFD (Church *et al.*, 2006; Krasnoff *et al.*, 2008) but very little information is available in children and adolescents. A study of 100 children were divided into four groups; either obese, obese with three or more MetSyn components, normal weight or normal weight but with at least one MetSyn risk factor (Kelishadi *et al.*, 2009). Across all groups, the authors found that cardiorespiratory fitness was inversely correlated with the upper quartile for both ALT and HOMA-IR irrespective of obesity or metabolic abnormalities. Insulin resistance and fasting insulin are associated with adiposity in children, however, it has been suggested that the risk associated with increased adiposity in children could be attenuated by having high levels of aerobic fitness (Ruiz *et al.*, 2007). We found significant inverse association between aerobic fitness and insulin resistance and fasting insulin consistent with other studies (Kelishadi *et al.*, 2009) but this is not confirmed by others

who have shown that this association may be mediated, at least in part, through adiposity (Lee *et al.*, 2006b). It has been suggested that the differences between results might be due to ethnic differences in body composition (Goran and Gower, 1999) and in the age of the children studied. Ruiz *et al.* (2007) studied Estonian and Swedish children mean age 9.6 ± 0.4 years, Lee *et al.* (2006b) studied African American and white youth aged 8 – 17 years, whereas Kelishadi *et al.* (2009) studied Iranian children aged 12 – 18 years. Moreover, evidence in adults and children suggest that increasing physical activity or aerobic fitness may be beneficial, independent of changes in weight. Four weeks of aerobic cycling exercise in 19 sedentary obese men and women significantly reduced mean hepatic triglyceride concentration by 21%, along with a 12% reduction in visceral adipose tissue volume and a 14% reduction in plasma free fatty acids. Importantly, no change in weight or dietary intake was noted (Johnson *et al.*, 2009). A three-month resistance exercise program in 12 obese adolescents showed significant increases in strength and lean body mass, increased hepatic insulin sensitivity and decreased glucose production rate with no change in total, visceral, hepatic, and intramyocellular fat contents measured by magnetic resonance imaging and magnetic resonance spectroscopy (Van Der Heijden *et al.*, 2010). Taken together, these findings suggest that improving fitness may be of use in reducing insulin resistance independent of changes in body fatness.

8.4.6 Limitations

There are several limitations in the current study that should be noted. The cross-sectional nature of the study allowed only association to be determined rather than causation. Moreover, this study does not allow determination of the timing of puberty and its influence on increases in ALT in children. There was also no direct measure of body fat mass and distribution although WC is used as an indicator of abdominal adiposity (Alberti *et al.*, 2006) and BMI is widely used as a measure of overweight and obesity in children (Cole *et al.*, 2000). Insulin resistance was assessed by HOMA-IR rather than *in vivo*

measurement of insulin action frequently used in clinical studies. Finally, the definition of metabolic syndrome used in this study differs from that used in many other studies for the reason that there is still no single accepted definition of metabolic syndrome variables and cut-points in children, or indeed in adults. The cut-off criteria employed, however, have been used in previous studies in children in the UK (Whincup *et al.*, 2005) and worldwide.

8.5 Conclusion

In summary this study has shown that elevations in ALT are highly prevalent in apparently healthy Welsh schoolchildren and were strongly associated with insulin resistance, fasting insulin, body composition, clustering of metabolic risk factors and inversely related to aerobic fitness. This study has shown that traditional and newer metabolic risk factors are present, and identifiable, in children and adolescents thus underscoring the need for early preventative strategies against obesity and strategies for promoting increased physical activity and fitness.

9 Chapter Nine – Overall Conclusions

9.1 Summary of results

The purpose of the program of research was to collect anthropometric, haematologic and physiologic data from a large number of apparently healthy children and adolescents with the purpose of examining central and peripheral haemodynamic indices of arterial stiffness and their association with risk factors for cardiovascular disease in. In addition it sought to examine the prevalence of overweight and obesity, the prevalence of metabolic syndrome and other associated cardiovascular risk factors and examine the relationships with emerging risk factors. Between June 2004 and February 2005 data was collected in two local secondary schools. The study population consisted of 231 apparently healthy children and adolescents aged 11 – 14 years who volunteered to take part in the program of research.

The review of literature in Chapter Two presented evidence that overweight and obesity in children and adolescents is increasing in several worldwide populations including the United Kingdom, but that prevalence estimates are dependent on the definition of overweight and obesity employed. Metabolic syndrome describes a clustering of cardiovascular disease risk factors greater than would be expected by chance alone. In similarity with overweight and obesity, metabolic syndrome in children is reported to be highly prevalent and again these estimates are dependent on the diagnostic criteria utilised and the population group studied. Arterial stiffness and hypertension in adults is a risk factor for cardiovascular disease including coronary heart disease and stroke and have been shown to predict morbidity and mortality in several disease populations. Elevated arterial stiffness has been described in children and adolescents but this has largely occurred in obese populations and in children with identified underlying pathophysiologies. There is scant data on apparently healthy children and adolescents, particularly in a U.K. cohort,

that has utilised the gold standard measurement of large artery stiffness, aortic pulse wave velocity.

Therefore, the first study (Chapter Four) was undertaken to compare prevalence estimates of overweight and obesity, examine the sensitivity and specificity of cut-off points used in the U.K. and examine the sensitivity and specificity of several additionally proposed adiposity measures to identify overweight and obesity. We found that the prevalence of overweight and obesity, and obesity alone in children and adolescents in a Welsh cohort is high yet varies greatly between both body mass index-based measures (16 – 35%) and between different anthropometry-based measures (3 – 19%). Body mass index and waist circumference measures produced broadly similar results whereas the waist-to-height ratio underestimates both overweight and obesity.

As with adults, cardiovascular risk factors are apparent and tend to cluster in younger individuals. To investigate the prevalence of this clustering in a uniquely Welsh cohort we sought to estimate the prevalence of metabolic syndrome and its components, using internationally applied criteria in Chapter Five. This study found that identification of metabolic syndrome is strongly dependent on the defining criteria employed with prevalence estimates ranging from 0% to 3.5%. Moreover, adiposity showed significant associations with 2 or more components of metabolic syndrome. Similarly, insulin resistance was found to show significant positive associations with all measures of adiposity. Significant positive trends were also found between fasting insulin levels, adiposity, TG and glucose levels. Taken together, this study suggests that disturbances in insulin action and adiposity are associated with risk factor clustering and that this effect is identifiable from a young age.

To further investigate the burden of cardiovascular risk factors identifiable in children the next study (Chapter Six) sought to identify associations between aortic pulse wave velocity and other measures of arterial stiffness and traditional metabolic syndrome risk factors. There is scant information describing the association of aerobic fitness on aortic pulse wave velocity so, in addition, we sought to describe the effect of aerobic fitness on arterial stiffness. The results showed that aortic stiffness increases in children and adolescents with increases in body mass index. Arterial stiffness was also positively associated with blood pressure status and inversely associated with aerobic fitness but in similarity with studies that have used different methods to measure arterial stiffness little association was found with other metabolic syndrome variables.

Invasive studies have shown that there is significant variance in systolic blood pressure (BP) throughout the vascular tree, and peripheral BP is generally higher than the central arteries due to pressure amplification. Chapter Seven sought to further describe the association of central and peripheral measures of blood pressure and in particular the occurrence of isolated systolic hypertension (ISH) in children. Original case reports described characterised this form of hypertension being due to an exaggerated amplification of the initial pressure wave in the presence of highly elastic arteries, particularly so in fit young men. The purpose of this study was to identify the prevalence of brachial hypertension and its subtypes in children and adolescents and to study the determinants of ISH through examination of PP amplification, arterial stiffness, aortic pulse wave velocity, cardiac output and an objective measure of aerobic fitness. This study was unable to conclusively demonstrate that ISH in children and adolescents results from an increased stroke volume and/or aortic stiffness. However, it was able to demonstrate the important influence of physical fitness as a determinant of both peripheral and central pulse pressure and aortic stiffness. These data indicate the restrictive nature of simply

recording peripheral blood pressure which may not provide an accurate assessment of central haemodynamics.

The metabolic syndrome as a construct has been subject to much modification in terms of the variables included and the cut-off criteria applied. The purpose of all such modification is to better identify individuals at increased risk for the development of cardiovascular disease and type 2 diabetes. Elevations in alanine aminotransferase (ALT) concentrations have been strongly associated with adiposity and thus may represent non-alcoholic fatty liver disease (NAFLD). Liver fat accumulation, and its marker ALT, predicts the development of metabolic syndrome, type 2 diabetes and cardiovascular disease. The study presented in Chapter Eight describes the association between alanine aminotransferase and metabolic risk factors. In this study ALT was found to be positively associated with insulin resistance, body composition measures and aerobic fitness; and insulin resistance was found to be a predictor of elevated ALT levels in children and adolescents. These findings suggest that a strong relationship exists between insulin resistance, insulin levels and ALT and that this relationship may be mediated by abdominal adiposity and low physical fitness.

9.2 Future directions

It is hoped that the studies outlined in this thesis will significantly contribute to describing and understanding the measurement of overweight and obesity, the prevalence of cardiovascular risk factors, the characterisation of hypertension and the interrelationships between isolated systolic hypertension and arterial stiffness in apparently healthy young people. However, more studies are required to address the limitations of the current studies and satisfy some of the questions raised. For example:

1. The accurate identification of overweight and obesity in children in Wales is particularly important following the Welsh Assembly Governments announcement of the national Child Measurement Programme following the 2009 pilot study. Measures of overweight and obesity must include standardised measures that may be used for national and international monitoring.

2. The central haemodynamic response to acute and chronic exercise has been investigated in healthy adults and in populations at increased risk for cardiovascular disease. This needs to be examined in a child and adolescent population to determine if the response is similar to the adult response. Confirmation of this point may add weight to the general call for prescription of increased physical activity and physical fitness in children. Furthermore, the childhood response to the type of exercise needs clarification. Aerobic endurance exercise in adults leads to decreases in arterial stiffness whereas strength training has been shown to increase arterial stiffness (Kingwell, 2002).

3. Our confirmation that peripheral measures of blood pressure overestimate central blood pressure in children and that it has been argued that the pressure the heart "sees" is of greater importance (Wilkinson *et al.*, 2001a) may have implications for the interpretation of nationally applied cut-off values for hypertension. A number of children may undergo unnecessary therapeutic or pharmacological treatment unnecessarily. This research program was unable to confirm whether isolated systolic hypertension identified in children was due to an increased cardiac output, arterial stiffness or both.

4. The current study found that alanine aminotransferase (ALT) was elevated in a significant number of children and adolescents. The next step would be to confirm

whether the proposed lower cut-off point for identification of elevated ALT corresponds with an increase in hepatic fat deposition. In addition, it should be investigated whether the inclusion of ALT in to a definition of metabolic syndrome identified more children at risk for cardiovascular disease or type 2 diabetes.

5. Adipocyte derived cytokines have the potential to affect arterial structure and function. It is necessary to examine these in children at rest, following exercise interventions and following high fat meals (and balanced diets) in order to elucidate the time course of their appearance.

10 Appendices

Appendix 1: Supporting references for Table 2.2: Summary of studies identifying criteria (and sources of evidence) used for the identification of the metabolic syndrome and CVD risk factor clustering in children and adolescents.

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Appendix 2: Supporting references for Table 2.6: Summary of studies of arterial stiffness in children and adolescents and Table 2.7: Summary of studies measuring pulse wave velocity and augmentation index in children and adolescents.

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Appendix 3: Parents information letter, part 1

Coleg Meddygaeth Cymru

Wales College Of Medicine

DEPARTMENT OF CARDIOLOGY, WALES HEART RESEARCH INSTITUTE
CARDIFF UNIVERSITY, HEATH PARK, CARDIFF CF14 4XN
TEL: (029) 20 742348 FAX: (029) 20 743500
PROFESSOR J R COCKCROFT
E-MAIL: COCKCROFTJR@CARDIFF.AC.UK

HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

A PROJECT UNDERTAKEN BY THE UNIVERSITY OF GLAMORGAN

Dear parent or guardian,

A team from the University of Glamorgan, Pontypridd, the University of Wales College of Medicine, Cardiff and Addenbrookes Hospital, Cambridge is undertaking a survey of the health and fitness levels of Welsh schoolchildren, a project that has been approved by the South East Wales Local Research Ethics Committee. One aim of the project is to help schools educate children about healthy living.

A number of health problems related to coronary heart disease originate during childhood, and many of these problems e.g. high blood pressure, high cholesterol and obesity, are amenable to change. It would greatly benefit the present and future health status of Welsh children if we were able to propose preventive strategies that could be undertaken during school time. Health-related exercise units within the Physical Education programme would be ideally suited to this purpose.

For this to happen we need to conduct a series of health and fitness measurements on schoolchildren. Hawthorn School has agreed to participate in this important study. Since pupils in Years 7-13 are needed for this survey we have enclosed for your attention, a consent form detailing the intended tests. All measurements will be carried out in a safe environment and by qualified personnel.

We sincerely hope that you will allow your child to participate in this survey if they wish, but please ensure that they too are happy to be included. The testing promises to be enjoyable and educational for each individual pupil.

Yours faithfully,

Professor John Cockroft (Department of Cardiology, University of Wales College of Medicine, Cardiff)

For any further information please contact Christopher Retallick on 01443 482282 or cjretall@glam.ac.uk, or Dr Simon Williams on O1443 482290 or swilliam@glam.ac.uk, or Professor John Cockroft on 02920 743489.

Appendix 4: Parents information letter, part 2

Coleg Meddygaeth Cymru

Wales College Of Medicine

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For this to happen we need to conduct a series of health and fitness measurements on schoolchildren. Hawthorn School has agreed to participate in this important study. Since pupils in Years 7-13 are needed for this survey we have enclosed for your attention, a consent form detailing the intended tests. All measurements will be carried out in a safe environment and by qualified personnel. Blood sampling will be carried out by trained personnel, and will involve taking a 40ml sample of blood, about 8 teaspoonfuls.

We sincerely hope that you will allow your child to participate in this survey if they wish, but please ensure that they too are happy to be included. The testing promises to be enjoyable and educational for each individual pupil.

Yours faithfully,

Professor John Cockroft (Department of Cardiology, University of Wales College of Medicine, Cardiff)

For any further information please contact Christopher Retallick on 01443 482282 or cjretall@glam.ac.uk, or Dr Simon Williams on O1443 482290 or swilliam@glam.ac.uk, or Professor John Cockroft on 02920 743489.

Appendix 5: Parents information sheet, part 1

Coleg Meddygaeth Cymru

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PARENT/GUARDIAN INFORMATION SHEET

Health and fitness survey of Welsh Schoolchildren

Your son/daughter is being invited to take part in a research study. Before you decide whether or not they should participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled Medical Research and You. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, P O Box 1365, LONDON N16 0BW. Copies may also be provided on request. Thank you for reading this.

Why are we doing this study?
A number of health problems related to coronary heart disease originate during childhood, and many of these problems e.g. high blood pressure, high cholesterol and obesity, are amenable to change. It would greatly benefit the present and future health status of Welsh children if we were able to propose preventive strategies that could be undertaken during school time. Health-related exercise within the Physical Education programme would be ideally suited to this purpose.

Do I have to take part?
It is up to you, and your child, to decide whether or not you wish your son/daughter to take part. If they do take part you will be asked to sign a consent form. However, you and your son/daughter are free to withdraw from the study at any time without giving a reason.

What will happen to me if I take part?
The study involves participating in a brief screening visit (~10 minutes), which will take place at School. Your son/daughter will then have their height, weight, arm skinfold (an indicator of body fatness) and waist measured.

Will there be any side-effects?
All of the measurements are quick, simple and painless, therefore your son/daughter will not experience any discomfort or side effects from any of the measurements.

What about my expenses?
The study will be taking place during normal school hours; therefore we do not anticipate you incurring any additional out-of-pocket expenses.

Confidentiality – who will have access to the data?
If you consent to your son/daughter taking part in the research, we will ask you to consent to the collection, processing, disclosure and transfer of their personal data for medical research purposes. Their name will not be disclosed outside the clinical unit.

Will my GP be informed?
There will be no need to inform your GP of you son's/daughter's participation.

What will happen to the study results?
The results of the study may be published a few months after completing the study. Neither you nor your son/daughter will be identified in any report or publication.

Are there any compensation arrangements if something goes wrong?
The doctor involved has suitable indemnity insurance for this.

Who is organising this study?
This study is being organised by the University of Glamorgan, Pontypridd; University of Wales College of Medicine, Cardiff; and Addenbrookes Hospital, Cambridge, and the doctor in charge is Professor John Cockcroft.

Remember – Your son/daughter is under no obligation to participate in this study. If they wish to leave the study at any point they may do so for any reason. Please take as much time to read this leaflet as require; do not feel that you have to make a decision quickly. Researchers will be available to answer any questions you may have. All the data collected will be confidential and is only for the purposes of research.

The South East Wales Local Ethics Research Committee has approved this study. If you require more general information then please ask one of the people running the study. Alternatively, you may wish to contact the principal investigator:-

Professor John Cockcroft
Department of Cardiology
Cardiff University
Wales Heart Research Institute
Heath Park
Cardiff CF14 4XN

Appendix 6: Parents information sheet, part 2

Coleg Meddygaeth Cymru

Wales College of Medicine

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PARENT/GUARDIAN INFORMATION SHEET

Health and fitness survey of Welsh Schoolchildren – Part 2

Your son/daughter is being invited to take part in a research study. Before you decide whether or not they should participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Consumers for Ethics in Research (CERES) publish a leaflet entitled Medical Research and You. This leaflet gives more information about medical research and looks at some questions you may want to ask. A copy may be obtained from CERES, P O Box 1365, LONDON N16 0BW. Copies may also be provided on request. Thank you for reading this.

Why are we doing this study?

A number of health problems related to coronary heart disease originate during childhood, and many of these problems e.g. high blood pressure, high cholesterol and obesity, are amenable to change. It would greatly benefit the present and future health status of Welsh children if we were able to propose preventive strategies that could be undertaken during school time. Health-related exercise within the Physical Education programme would be ideally suited to this purpose.

High blood pressure is a condition that places us at an increased risk of heart disease and stroke. While we can treat high blood pressure with drugs, we cannot cure it. Therefore, once drug therapy has started, it must be continued throughout life. Although high blood pressure usually develops when we get older, we believe that in healthy young adults, certain factors such as how much blood the heart pumps (cardiac output) and how stiff the arteries are, may *predict* whether or not high blood pressure will develop later in life. We now have several simple methods, performed on the surface of the skin (i.e. do not require puncturing the skin) to measure cardiac output and arterial stiffness and we wish to use these methods to study a large number of healthy children and young adults now and also at various intervals over the next 30 years. If we find that increased cardiac output and/or arterial stiffness predicts the development of high blood pressure, then we may be able to target early treatment towards 'at risk' individuals thus providing a 'cure' for this condition. Furthermore, sometimes even healthy young adults are told that they have high blood pressure and we wish to learn more about why blood pressure might be raised at this early stage and whether drug treatment is warranted.

Do I have to take part?
It is up to you, and your child, to decide whether or not you wish your son/daughter to take part. If they do take part you will be asked to sign a consent form. However, you and your son/daughter are free to withdraw from the study at any time without giving a reason.

What will happen to me if I take part?
The study involves participating in a brief screening visit (30-40 minutes), which will take place at School. After sitting down for 5 minutes, your son/daughter will have their blood pressure measured, just like at your GP. We will then place a small pencil-like probe on the artery at their wrist. This records the pressure in the artery and uses this information to calculate how stiff the arteries are. These measurements are completely pain free and do not involve any needles.

Your son/daughter will then, after lying down for 15 minutes, have their blood pressure taken. We will also ask them to breathe some air and harmless gas in and out of a tube – this is a non-invasive and widely used method of measuring how much blood the heart pumps. We will then place the pencil-like probe on the artery at their wrist, neck and upper leg. If, through this screening visit, we find that your son/daughter has high blood pressure or an abnormal blood test result, then we will inform both you and your GP.

Your son/daughter will also be asked to complete an activity questionnaire, a lifestyle questionnaire, a dietary questionnaire and a pubertal stage self-assessment form; this will tell us their stage of physical maturation. You will be asked to complete a health and fitness questionnaire for your son/daughter telling us their Doctor's name and address.

Your son/daughter will also be asked to give a small blood sample of approximately 40 ml. This blood sample is used to measure various levels of naturally occurring substances in the blood, (e.g. cholesterol, glucose). Some blood will also be taken and stored for up to 30 years – this blood will be sent for genetic analysis to identify components that may have an influence on the stiffness of arteries.

We plan to track the participating pupils over the next 30 years via their NHS number. In the first place, we plan to write to the GP with whom your son/daughter is registered (or was registered with at the time of inclusion in the study), to check that the individual is still registered at that practice. If so, we then plan to write to you and your son/daughter to ask if they wish to continue their participation in the study.

We also plan to register each pupil with the Office of National Statistics (ONS) which means they will be flagged with the NHS central registry. If your son/daughter is no longer registered with the same GP as when they were included in the study, then we can find their current GP via the ONS. We will then write to their new GP to check that our details are correct and then to you and your son/daughter. Each time we re-visit the cohort, the study participants will be given a choice as to whether they wish to continue to participate in the study.

Will there be any side-effects?
Your son/daughter may experience some very minor discomfort from having a blood sample taken. However, the study does not involve taking any drugs, injections, or harmful measurements. Put simply, the measurements are just like having your blood pressure checked, but using some different methods.

What about my expenses?
The study will be taking place during normal school hours; therefore we do not anticipate you incurring any additional out-of-pocket expenses.

Confidentiality – who will have access to the data?
If you consent to your son/daughter taking part in the research, we will ask you to consent to the collection, processing, disclosure and transfer of their personal data for medical research purposes. Their name will not be disclosed outside the clinical unit.

Will my GP be informed?
The doctor in charge of the study will inform your GP of your son's/daughter's participation in the study. In addition, if we find that your son/daughter has high blood pressure or an abnormal blood test result we will inform your GP, and your son/daughter will be followed up by Professor John Cockcroft from the Department of Cardiology, University of Wales College of Medicine or referred to an appropriate clinician.

What will happen to the study results?
The results of the study may be published a few months after completing the study. Neither you nor your son/daughter will be identified in any report or publication.

Are there any compensation arrangements if something goes wrong?
The doctor involved has suitable indemnity insurance for this.

Who is organising this study?
This study is being organised by the University of Glamorgan, Pontypridd; Cardiff University College of Medicine, Cardiff; and Addenbrookes Hospital, Cambridge, and the doctor in charge is Professor John Cockcroft.

Remember – Your son/daughter is under no obligation to participate in this study. If they wish to leave the study at any point they may do so for any reason. Please take as much time to read this leaflet as required; do not feel that you have to make a decision quickly. Researchers will be available to answer any questions you may have. All the data collected will be confidential and is only for the purposes of research.

The South East Wales Local Ethics Research Committee has approved this study. If you require more general information then please ask one of the people running the study. Alternatively, you may wish to contact the principal investigator:-

Professor John Cockcroft
Department of Cardiology
Cardiff University College of Medicine
Cardiff CF14 4XN
Tel 02920 743 489

Appendix 7: Parental consent form, part 1

Coleg Meddygaeth Cymru

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HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

CONSENT FORM

To be completed by parent or guardian

1. I confirm that I have read and understand the information sheet dated 7th April 2004 (version 5) for the above study and have had the opportunity to ask questions.
2. I understand that my son/daughters participation is voluntary and that they are free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected
3. I understand that sections of any of my son's/daughter's medical notes may be looked at by responsible individuals from the study team or from regulatory authorities where it is relevant to my son's/daughter's taking part in the research. I give permission for these individuals to have access to my son's/daughter's records.
4. I understand that my child will participate in the following tests and that all test results will remain confidential at the participating institutions.

   Weight and height
   Skinfold thickness (body fat)
   Waist measurement

5. Please **circle** as appropriate.

   I **AGREE / DO NOT** agree to my child
   .....(name) taking part in the health and fitness survey, the nature of which has been explained to me in the accompanying letter and information sheet.

Signature.....(parent)

Signature.....(researcher)

PLEASE ENSURE THAT YOU HAVE ANSWERED **ALL** QUESTIONS.

Thank you.

Appendix 8: Parental consent form, part 2

Coleg Meddygaeth Cymru

Wales College Of Medicine

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HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

CONSENT FORM
To be completed by parent or guardian

Name of Child:_____ Date of Birth _____

1. I confirm that I have read and understand the information sheet dated 7th April 2004 (version 5) for the above study and have had the opportunity to ask questions.
2. I understand that my son/daughters participation is voluntary and that they are free to withdraw at any time, without giving any reason, without their medical care or legal rights being affected
3. I understand that sections of any of my son's/daughter's medical notes may be looked at by responsible individuals from the study team or from regulatory authorities where it is relevant to my son's/daughter's taking part in the research. I give permission for these individuals to have access to my son's/daughter's records.
4. I understand that my child will participate in the following tests and that all test results will remain confidential at the participating institutions.

20metre shuttle run (Bleep test) (fitness)	Activity Questionnaire
Blood pressure	Lifestyle Questionnaire
Blood analysis	Dietary Questionnaire
Cardiac Output	Weight and height
Pulse Wave Analysis	Skinfold thickness (body fat)
Pulse Wave Velocity	Waist measurement
Pubertal Stage Self-Assessment	

5. Please **circle** as appropriate.

   I **AGREE / DO NOT** agree to my child .....(name) taking part in the health and fitness survey, the nature of which has been explained to me in the accompanying letter and information sheet.

Signature.....(parent)

Signature.....(researcher)

PLEASE ENSURE THAT YOU HAVE ANSWERED **ALL** QUESTIONS.

Thank you

Appendix 9: Health and fitness questionnaire

Coleg Meddygaeth Prifysgol Cymru

University Of Wales College Of Medicine

DEPARTMENT OF CARDIOLOGY, WALES HEART RESEARCH INSTITUTE
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HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

HEALTH AND FITNESS QUESTIONNAIRE

To be completed by parent or guardian.
CONFIDENTIAL

Pupil's name: _____

Pupil's date of birth: _____

Pupil's school: _____

Name of pupil's GP: _____

GP's Practice Address: _____

_____

_____

_____

Pupils NHS Number: _____

Please answer the following questions if you agree to your child taking part in the survey.
For each question **circle** the appropriate answer.

1. Has your child ever suffered from any illness or disease that may affect his or her ability to take part in exercise?

YES / NO

If "YES", please give details.

2. Has your child ever complained of chest pain, wheeziness, headaches or dizziness during or after exercise?

YES / NO

If "YES", please give details.

3. Are you aware of any complaint (e.g. joint soreness) which may prevent your child taking part in normal exercise?

YES / NO

If "YES", please give details.

4. Is your child receiving any medication or medical treatment at present?

YES / NO

If "YES", please give details

5 Has your child ever been in hospital?

YES / NO

If "YES", please give details.

6. Is your child recovering from a viral complaint (such as 'flu') at present?

YES / NO

If "YES", please give details.

7. Has any member of your child's immediate family been treated for, or suspected to have had, any of these conditions? Please identify their relationship to your child (father, mother, grandparent etc).

Tick as appropriate.

a) heart disease
b) diabetes
c) stroke
d) high blood pressure
e) high cholesterol

8. What was your child's birth weight? You may give this in pounds and ounces or kilograms and grams.

.....pounds .....ounces. .....kilograms .....grams.

9. Was your child born prematurely?

YES / NO

If "YES", how many weeks premature were they born?

Signature.....(parent)

PLEASE RETURN THIS QUESTIONNAIRE TO THE PHYSICAL EDUCATION DEPARTMENT BY FRIDAY 11TH JUNE 2004. TESTING CANNOT BEGIN UNTIL ALL FORMS HAVE BEEN RETURNED.

PLEASE ENSURE THAT YOU HAVE ANSWERED **ALL** QUESTIONS.

Thank you.

Appendix 10: Physical Activity questionnaire

Coleg Meddygaeth Cymru
Wales College Of Medicine

DEPARTMENT OF CARDIOLOGY, WALES HEART RESEARCH INSTITUTE
CARDIFF UNIVERSITY, HEATH PARK, CARDIFF CF14 4XN
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PROFESSOR J R COCKCROFT
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HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

PHYSICAL ACTIVITY QUESTIONNAIRE

The main aim of this questionnaire is to discover how much physical activity you tend to do in one week. The information you provide is confidential but it is important that you answer each question as honestly as possible.

FULL NAME: _____ ***DATE OF BIRTH:*** _____

CLASS / YEAR: _____

TICK ONE:

BOY ☐

GIRL ☐

Tick (✓) ONE answer only. ✓

1.	How do you normally travel to school?	
	1. Bus, car, train etc	
	2. Bicycle	
	3. Walk	

2.	How do you normally travel home from school?	
	1. Bus, car, train etc	
	2. Bicycle	
	3. Walk	

3.	How long does it normally take you to travel to school after leaving your home?	
	1. Less than 5 minutes	
	2. 5-15 minutes	
	3. 15-30 minutes	
	4. 30 minutes – 1hour	
	5. More than 1 hour	

4.	During your PE and games lessons, how often do you get out of breath?	
	1. I don't do PE or games	
	2. Hardly ever	
	3. Occasionally	
	4. Quite often	
	5. Always	

5.	What do you normally do at morning break?	
	1. Sit down (talking, reading, doing schoolwork)	
	2. Stand or walk around	
	3. Run around playing games	

6.	What do you normally do at lunch time?	
	1. Sit down (talking, reading, doing schoolwork)	
	2. Stand or walk around	
	3. Run around playing games	

7.	How many days per week do you stay behind at school for sports?	
	1. None	
	2. Once or twice a week	
	3. 3-4 times a week	
	4. 5 times a week	

8.	How many evenings per week do you take part in sports or other physical activities?	
	1. None	
	2. Once or twice a week	
	3. 3-5 times a week	
	4. 6 or 7 times a week	

9.	At the moment, do you take part in sports or other physical activities at the weekend?	
	1. Yes	
	2. No	

10.	During school holidays, how active are you, compared to during term time?	
	1. Less active	
	2. About the same	
	3. More active	

Tick (✓) ONE answer only. ✓

11.	Is your answer to the last question typical of your exercise pattern at this time of year?	
	1. I am usually less active	
	2. About the same	
	3. I am usually more active	

12.	Generally speaking, do you enjoy physical activity?	
	1. Yes	
	2. No	

If 'YES' say why you like it:

.....

If 'NO' say why you do not like it:

.....

13.	Generally speaking, do you enjoy physical education and games lessons?	
	1. Yes	
	2. No	

If 'YES' say why you like it:

.....

If 'NO' say why you do not like it:

.....

14. Which ONE of the following statements describes you best?

1. All or most of my free time is spent doing things that involve little physical effort (eg watching TV, reading, talking to friends)	

OR

2. I occasionally (once or twice a week) do things in my free time that involve physical effort (eg swimming, cycling, jogging)	

OR

3. I quite often (4-6 times a week) do things in my free time that involve some physical effort	

OR

4. I very often (7 or more times a week) do things in my free time that involve some physical effort	

15. Below is a list of people, we want to know whether they normally take regular exercise.

Tick (✓) alongside if that person exercises at least twice a week.

1. Father	
2. Mother	
3. Elder sister	
4. Elder brother	
5. Best friend	

16. We want to know about all the exercise you have taken that made you get out of breath in the last 7 days

In the table below, fill in each section as accurately as you can. If you took no exercise in any of the sections, do not write in those boxes, go straight on to the next section.

	How Often? How many times in the last 7 days?	How Long? How many hours and minutes did each session last? Hours Minutes	How Hard? Did you get out of breath? Never 1 A little 2 or A lot 3
Travel to and from school (walking)	.....	.....	.....
Travel to and from school (cycling)	.....	.....	.....
Morning break activities e.g. netball hockey football rugby others (name)			 ....
Lunch time activities (including clubs and practices) e.g. netball hockey football rugby gymnastics others (name)			 ....
PE and games lessons e.g. gymnastics games dance health and fitness others (name)			 ....

	How Often? How many times in the last 7 days?	How Long? How many hours and minutes did each session last? Hours Minutes	How Hard? Did you get out of breath? Never 1 A little 2 or A lot 3
After school clubs and practices e.g. netball hockey football rugby gymnastics others (name)			 ....
School matches (name)			 ....
Evening activities (name)			 ....
Weekend activities (name)			 ....
Any other activities (name)			 ....

Appendix 11: Lifestyle questionnaire

Coleg Meddygaeth Cymru

Wales College Of Medicine

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HEALTH AND FITNESS SURVEY OF WELSH SCHOOLCHILDREN

LIFESTYLE QUESTIONNAIRE

The answers that you give on this questionnaire are confidential, **your parents and teachers will not be given the answers you supply.**

Girl or Boy:.....

School:.....

Age:..... **Year:**.....

1. Have you ever smoked cigarettes? YES / NO

   (If you answered **NO** to Question 1, go to Question 4)

2. Do you smoke cigarettes? YES / NO

   Cigarettes .....a day

3. At what age did you start smoking? .....years

4. Have you ever drunk alcohol? YES / NO

   (If you answered **NO** to Question 4, go to Question 7)

5. Do you drink presently? YES / NO

6. At what age did you start drinking? .....years

7. Have you ever taken illegal drugs? YES / NO
(e.g. cannabis, marijuana, etc.)

(If you answered **NO** to Question 7, **do not** answer any more questions)

8. Do you use illegal drugs presently YES / NO

9. At what age did you first start using illegal drugs? .....years

10. What illegal drugs have you taken? .....
.....

Thank you for completing this questionnaire.

Appendix 12: Pubertal Status Questionnaire

Study Subject No:

BOYS

School Heart Health Study

Dr. Peter Whincup
(Project Director)

Department of Primary Care
and Population Sciences,

Royal Free and University College
Medical School,
Rowland Hill Street,
London NW3 2PF.

UCL

BOYS

Self Assessment

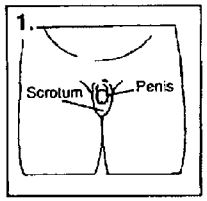
Self Assessment

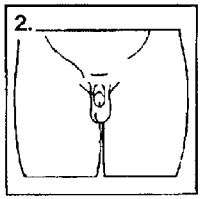
Study Subject No:

BOYS

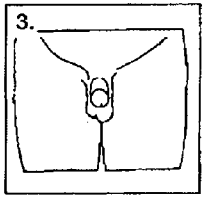
- Please look at the **Penis** and **Scrotum** only in these pictures.
- Please put a tick in the box that looks most like you now.

Scrotum and Penis same size as when you were younger.

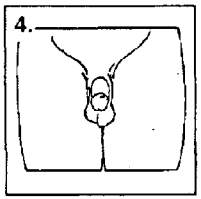




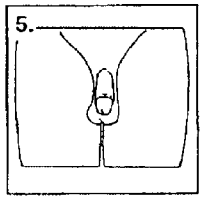
The Scrotum has lowered a bit and the Penis is a little larger.



The Penis is longer the Scrotum is larger.



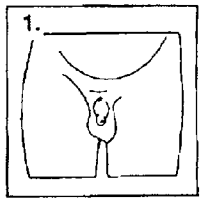
The Penis is longer and wider the Scrotum is darker and bigger than before

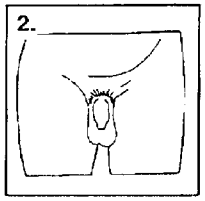


The Penis and Scrotum are the size and shape of an adult.

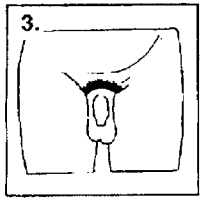
- Please look at the **Pubic Hair** only in these pictures.
- Please put a tick in the box that looks most like you now.

No hairs

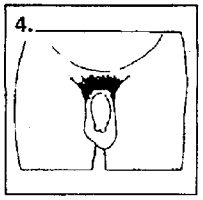




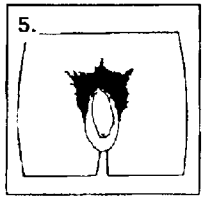
Very little hair



Quite a lot of hair



The hair has not spread over the thighs



The hair has spread over the thighs

Self Assessment

Self Assessment

School Heart Health Study

Dr. Peter Whincup
(Project Director)

Department of Primary Care
and Population Sciences,

Royal Free and University College
Medical School,
Rowland Hill Street,
London NW3 2PF.

G-I-R-L-S

Self Assessment

Study Subject No:

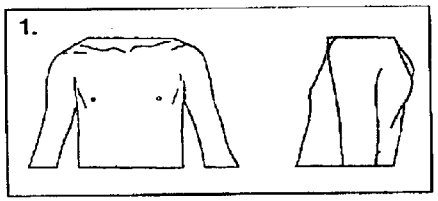
GIRLS

Self Assessment

Study Subject No:

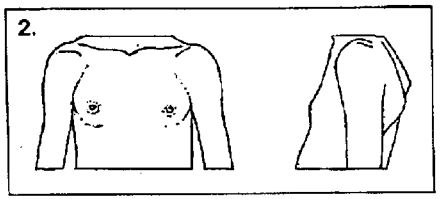
- Please put a tick in the box that looks most like you now....

1.



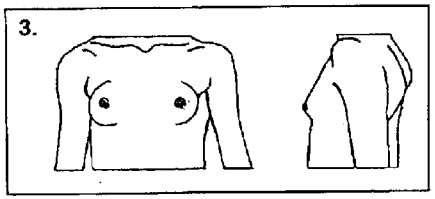
The Breasts are flat.

2.



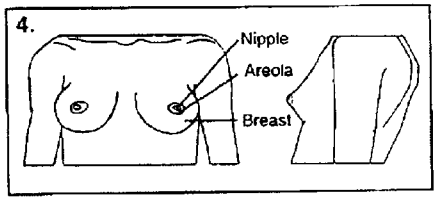
The Breasts form small mounds.

3.



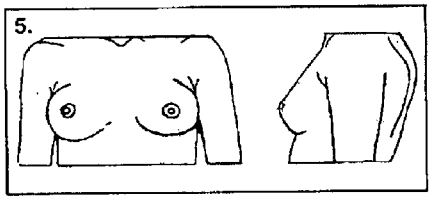
The breasts form larger mounds than in 2.

4.



The nipple and the surrounding part *(the Areola)* make up a mound that sticks up above the breast.

5.

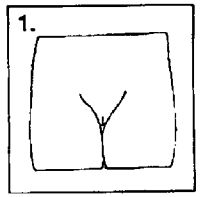


Only the nipple sticks out beyond the breast.

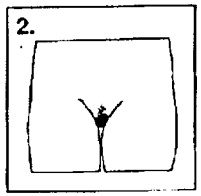
- Please put a tick in the box that looks most like you now....

1.

No hairs

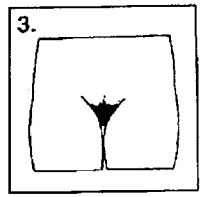


2.



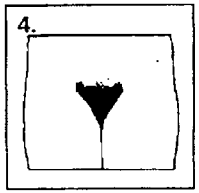
Very little hair

3.



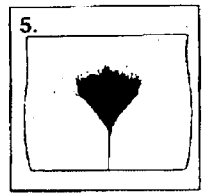
Quite a lot of hair

4.



The hair has not spread over the thighs

5.



The hair has spread over the thighs

Self Assessment

Self Assessment

Appendix 13: Data collection sheet, part 1

Anglo-Cardiff Collaborative Trial Hawthorn Screening Part 1	I.D. Number: Date:

Name:

_____

Consent Forms: PARENT ☐

Gender: MALE ☐ FEMALE ☐ **D.O.B:** _____/_____/19____

Age:_____

Height: _____cm **Weight:** _____Kg

Waist: _____cm **Hip:** _____cm **Triceps Skinfold:** _____mm

Ethnic Group: _____

Operator:	

NOTES:

Appendix 14: Data collection sheet, part 2

Anglo-Cardiff Collaborative Trial Hawthorn Screening Part 2	**I.D. Number:** **Date:**

Name: _____

Consent Forms: PARENT ☐

Gender: MALE ☐ FEMALE ☐ **D.O.B:** _____/_____/19____ **Age:**_____

Height: _____cm **Weight:** _____Kg

Waist: _____cm **Hip:** _____cm **Triceps Skinfold:** _____mm

Ethnic Group: _____

CARDIOVASCULAR MEASUREMENTS:

Seated BP (Omron) _____ _____ _____ **Avg:** _____

Supine BP (Omron) _____ _____ _____ **Avg:** _____

Standing BP (Omron) _____ _____ _____ **Avg:** _____

Cardiac Output (Gas) _____ **Stroke Volume** _____ **CI** _____

AUGMENTATION INDEX (%):

Radial (seated): 1._____ 2._____ 3._____ Avg: _____

Radial (supine): 1._____ 2._____ 3._____ Avg: _____

Carotid (supine): 1._____ 2._____ 3._____ Avg: _____

PATH LENGTH MEASUREMENTS (mm):

Notch to Carotid: _____ Notch to Radial: _____

Notch to Femoral: _____ Notch to Umbilicus: _____

PULSE WAVE VELOCITY (m/s)

Carotid to Radial: 1._____ 2._____ 3._____ Avg: _____

Carotid to Femoral: 1._____ 2._____ 3._____ Avg: _____

Blood Sample Taken? YES NO

Operator:	**Database I.D.: HAW**

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