Evaluation of Early Metabolic and Vascular Risk in Children of Parents with Early Ischaemic Heart Disease

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Declaration

I hereby declare that the work contained in this thesis is my own, and was completed with counsel of my supervisor Professor Colum Dunne and co-supervisors Professor Walter Cullen and Dr Michael O’Neill. This work has not been submitted to any other University or higher education institution, or for any other academic award in this University.

Where the work of others has been reported, it has been fully acknowledged and referenced.

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Alan Macken
Abstract

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Atherosclerotic cardiovascular disease is the leading cause of death in Ireland and throughout the developed world. Where a parent has had an early cardiac event, their offspring are at risk of developing heart problems in the future. In this study, we assessed children aged between 8-14 years, whose fathers had premature cardiac (or heart) disease defined as early heart attacks, heart bypass surgery, or angioplasty and evaluated these children [subjects, n=38] for early evidence of atherosclerosis and metabolic abnormalities and compared them to children whose parents do not have known early heart disease [controls, n=25].

The following evaluations were performed: anthropometrics; medical history; family history; fasting lipids; oral glucose tolerance testing; glycosylated haemoglobin (HbA1c); fibrinogen; Carotid Intima Media Thickness measurement; assessment of endothelial function by peripheral applanation tonometry; 24-hour ambulatory blood pressure recordings; blood pressure indices for gender and height [BPI(height)] as well as gender and age [BPI(age)] were calculated.

Subjects compared to controls were found to have: higher BMIs (18.7 vs 17.3kg/m2, p=0.01); similar BMI-standard deviation scores; higher waist/height ratios (0.47 vs 0.43, p=0.05); similar waist/hip ratios; similar fasting glucose and insulin sensitivity; similar fasting lipid profiles; lower alanine aminotransferase (ALT) level (16.96 vs
20.67IU/L, p=0.009); similar carotid intima media thickness measurements; lower reactive hyperemia peripheral arterial tonometry (RH-PAT) scores (1.64 vs 2.00, p=0.01), indicating relative endothelial dysfunction; similar mean systolic ambulatory blood pressure; higher mean diastolic ambulatory blood pressure (68.6 vs 65.4mmHg, p=0.02); higher mean diastolic blood pressure index for height (BPI [height]) (0.90 vs 0.86, p=0.03); higher mean diastolic blood pressure index for age (BPI [age]) (0.91 vs 0.87, p=0.02); higher mean arterial blood pressure index [age] (0.93 vs 0.90, p=0.03). Differences in mean ambulatory blood pressure (84.1 vs 81.3mmHg, p=0.06) and mean arterial pressure BPI [height] (0.93 vs 0.90mmHg, p=0.09) did not reach statistical significance.

Metabolic tests did not demonstrate differences between children with paternal history of premature cardiovascular disease and controls. We did not find evidence to support targeted lipid screening based on a paternal history of premature cardiovascular disease. This is interesting, as the current guidelines suggest using lipid screening to monitor for risk in our subject population.

Our vascular testing results suggest early impaired vascular health relative to controls in healthy children whose fathers have premature cardiovascular disease. This is the first study to evaluate endothelial function and 24 hour ambulatory blood pressure measurements in children with a parental history of premature cardiovascular disease.
Dedication

This thesis is dedicated with love to my wife Clodagh, our children Aidan, Keela and Cian, and my parents Pat and Ann.
Acknowledgements

Nature and extent of collaborative work:

There are few significant pieces of research work which are carried out exclusively by a single person. I owe a debt of gratitude to my supervisors, Prof Colum Dunne, Prof Walter Cullen and Dr Michael O’Neill and to my research collaborators, Prof Clodagh O’Gorman, Prof Ailish Hannigan, Dr Brendan Meany, Dr Ophelia Blake, Ms Catriona Ahearn, Ms Ann Breen and Ms Joan Egan.

Prof Colum Dunne is the Foundation Chair and Director of Research at Graduate Entry Medical School, University of Limerick. Prof Dunne has experience as: Programme Manager at Ireland’s National Food Biotechnology Centre with responsibility for coordination of probiotic- and prebiotic-related scientific projects and completion of multi-centre clinical trials; General Manager of what was then Ireland's only research centre focused solely on cancer prevention and therapy, investigating the therapeutic potential of functional foods; company Director to Glanbia with responsibility for Research and Development; Director of Westgate Biological, a biotechnology company commercialising a novel patented broad spectrum antimicrobial agent. Prof O’Gorman and Prof Dunne are the Principal Co-Investigators on this project. Prof Dunne is the supervisor of this MD thesis.

Prof Walter Cullen is Professor of Urban General Practice, School of Medicine and Medical Science, University College Dublin and a General Practitioner. Prof Walter Cullen’s research interests include: youth mental health, chronic illness (especially deprivation-related e.g., problem drug / alcohol use) and undergraduate education in general practice / primary care. This research, always collaborative, involves qualitative and quantitative methodologies which have described emerging issues in primary care, developed and evaluated new models of care. He advised on recruitment of patients and has been involved in the study design. He participates in data interpretation with regular research meetings. Prof Cullen is a co-supervisor of this MD thesis.
Dr Michael O’Neill is the Clinical Director of Mayo General Hospital, Castlebar and a Consultant Paediatrician. He advised on recruitment of patients and has been involved in the study design. He participates in data interpretation with regular research meetings. Dr O’Neill is a co-supervisor of this MD thesis.

Prof Clodagh O’Gorman is Professor of Paediatrics at Graduate Entry Medical School, University of Limerick, and Consultant Paediatrician / Paediatric Endocrinologist at University Hospital Limerick (UHL). She has worked extensively with paediatric obesity and paediatric cardiometabolic risk, in both clinical and research settings. Her published research on girls with Turner syndrome employs some similar methods to this research project, including assessments of insulin sensitivity, carotid intima media thickness and peripheral arterial tonometry. She has also used similar techniques in studies of adolescents with hypothalamic obesity (see publications list for references). She has MSc in research methodology from University of Oxford. She has also co-authored a systematic review on statins for hypercholesterolaemia in children, and is undertaking a Cochrane systematic review on a similar topic. Prof O’Gorman was the instigator of this research project and is a Principal Co-Investigator with Prof Dunne.

Prof Ailish Hanningan is Associate Professor of Biomedical Statistics at the Graduate Entry Medical School (GEMS), University of Limerick. Prof Hannigan provides all statistical support, advice and analysis for this project.

Dr Brendan Meaney is Consultant Adult Cardiologist at University Hospital Limerick, who runs a programme for cardiac rehabilitation of patients following myocardial infarction and/or coronary artery bypass grafting. From the clients attending this program, suitable families for inclusion in this study were identified.

Dr Ophelia Blake is a Consultant Biochemist at University Hospital. Dr Blake arranged blood sample processing at UHL, frozen serum storage and dispatch to external laboratories.
Ms Ann Breen is the acting Chief of Biochemistry Laboratory, UHL. Ms Breen arranged for and performed the laboratory reception, separation, processing and storage of research samples at the weekends of testing.

Ms Catriona Ahern is a Clinical Nurse Specialist in Cardiac Rehabilitation at UHL. Ms Ahern helped me identify potential subject families by retrospectively identifying men who had been inpatients in the coronary care unit at UHL and then contacting those men to determine if they had children that would fulfill our study criteria and then obtaining consent to contact these families about this study.

Ms Joan Egan is a vascular technician at St Camillus’ Hospital Limerick and formerly of University Hospital Limerick. Ms Egan performed all cIMT measurements and assisted in running the clinical testing days at UHL.

The National Children’s Research Centre, Crumlin, Dublin 12 provided a full grant to support me as a clinical MD research fellow and funded this research in its entirety. Professor Carlos Blanco and Dr Jacinta Kelly gave unwavering support to this research.
There were many challenges associated with establishing clinical research in paediatrics in Limerick. The research interface in paediatrics between the University and University Hospital was not previously established. At all points of testing the Lead Clinical Investigator, Prof Clodagh O’Gorman, and I proceeded with a process of problem solving and negotiation which succeeded in establishing new informal research mechanisms involving the services at University Hospital Limerick. I am very grateful to the clinical and laboratory staff who facilitated the work, in particular Ms Ann Breen and Dr Ophelia Blake of the Department of Clinical Biochemistry. This process required a considerable investment of time and resources which I hope will benefit future clinical researchers and children in Limerick.
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Lay Summary

Acquired heart disease is common in Ireland and throughout the developed world. In adults, it causes heart attacks and angina. Recognised risk factors for acquired heart disease include family history of acquired heart disease, smoking, overweight, decreased physical fitness, high blood pressure and high cholesterol. There are some studies examining the heart and vessels of children and these suggest that even young children can have evidence of acquired heart disease. The only risk factor that many of these children have is a family history of heart disease. It is possible that they may have more risk factors in the future, but by then, the irreversible processes of vessel changes may have started.

We were interested in identifying these changes of early acquired heart disease in young children. The results of this study might lead to significant future studies. For example, if it would prove possible to identify these changes, then we could also follow these children for progression of heart disease. And, at some stage, treatments to alter the process of acquired heart disease in these children may be considered.

The aim of this study was to evaluate children, aged between 8-14 years, of fathers who had early heart attacks or heart bypass surgery, or angioplasty (the subjects). The results of tests on these subject children were to be compared to children whose fathers do not have known early heart disease (control children). The aim of this study was to evaluate for differences between the two groups in the risk of diabetes, cholesterol, blood pressure, weight and body mass index.

63 children were recruited and tested, including 23 male and 15 female subjects and 14 male and 11 female controls. Testing performed included history taking and physical examination, with measurements of height and weight and calculation of body mass index. Blood tests included oral glucose tolerance testing (with measurements of the risk for diabetes) and cholesterol profiles. Ultrasound was used to measure the thickness of a particular layer of the carotid artery, a layer called the carotid intima media, which is known to be associated with the risks of developing heart disease. Blood pressure was recorded over 24 hours. Peripheral applanation tonometry was determined; this method measures the change in blood flow to the fingers at rest and compares it to the increased blood flow that occurs after blood supply has been stopped for five minutes and then
restored; thereby generating a ratio which reflects the health of the inner lining of the blood vessels (endothelial function).

The study showed the following findings:

1. Subjects had higher body mass indices than controls (p=0.01) although there were no differences in BMI Z-score (p=0.24).

2. Subjects had higher waist/height ratios than controls (p=0.05).

3. Waist/hip ratios were not different between subjects and controls (p=0.35).

4. Fasting glucose measurements were not different between subjects and controls (p=0.19).

5. Insulin sensitivity was not different between subjects and controls (p=0.85).

6. Fasting lipids were not different between subjects and controls.

7. Serum alanine aminotransferase (ALT) levels (a measure of liver function) were lower in subjects than controls (p=0.009).

8. There were no differences in c-IMT between the subjects and controls (p=0.25).

9. Peripheral arterial tonometry scores were lower in subjects than in controls (p=0.01) indicating disease or dysfunction of the endothelium in subjects.

10. Mean systolic ABPMs were similar between subjects and controls (p=0.20), as were Mean Systolic BPI[age] (p=0.09) and Mean Systolic BPI[height] (p=0.11).

11. Mean diastolic ABPMs were higher in subjects than in controls (p=0.02).

12. Mean Arterial Blood Pressure (MAP) was not different between subjects and controls (p=0.06) but the age-adjusted MAP blood pressure index [BPI(age)] was higher in subjects than in controls (p=0.03).
Conclusion

Healthy children with a paternal history of premature cardiovascular disease have similar insulin sensitivity to healthy matched controls. Subjects and controls have similar fasting lipid profiles. Subjects had lower ALTs than controls. Subjects and controls had similar results for other liver function tests. Subjects had lower peripheral arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower peripheral arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower ALTs than controls. Subjects and controls had similar results for other liver function tests. Subjects had lower peripheral arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower peripheral arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower peripheral arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction. Subjects had lower central arterial tonometry scores than controls, indicating that subjects had relative endothelial dysfunction.

Fasting lipid profiling did not identify the differences between children with paternal history of premature cardiovascular disease and controls. Thus, this study does not provide evidence to support targeted lipid screening based on a paternal history of premature cardiovascular disease.

Further studies are required but these data suggest early impaired vascular health in well, young children whose fathers have premature cardiovascular disease.

To our knowledge, this is the first study to evaluate endothelial function and 24 hour ambulatory blood pressure measurements in children with a parental history of premature cardiovascular disease.

These results suggest that the blood vessels of healthy children whose fathers have early heart disease are different from those of other children and that these differences can be measured.
Rationale and Overview.
This study was conducted during my time as a Clinical Research Fellow with the National Children’s Research Centre, Crumlin, Dublin Ireland while the research was carried out at The Children’s Ark, University Hospital Limerick, affiliated with the University of Limerick, Graduate Entry Medical School.

This study involved conducting an age- and body mass index (BMI)-matched case-controlled cross-sectional study of healthy children whose fathers have premature cardiovascular disease (PCD) (subjects) and age-matched children whose fathers are well (controls). This study evaluated children’s insulin sensitivity and also evaluated them for features of the metabolic syndrome, and looked for correlations between these variables and measures of vascular function. The underlying a priori basic hypothesis was that healthy children whose fathers had PCD would have lower insulin sensitivity, more features of the metabolic syndrome and evidence of abnormalities of both vascular function and metabolism, compared with healthy controls.

Our hypothesis was that the metabolic and vascular profiles of children whose fathers had premature cardiovascular disease are measurably different when compared to an age-matched healthy cohort, and that these differences place the children whose fathers had premature cardiovascular disease at increased vascular and metabolic risk.

The research question underpinning this project was:

“Are the metabolic and vascular profiles of children whose fathers had premature cardiovascular disease measurably different when compared with an age-matched healthy cohort?”

The aims of this study were:

(1) to evaluate cardio-metabolic profiles in a cohort of children known to be at increased risk of cardiovascular disease as young adults, i.e. the children whose fathers had premature cardiovascular disease; and

(2) to compare the cardio-metabolic profiles of these children to a cohort of age-, gender-, and BMI-matched healthy children, who do not have a significant family history of premature cardiovascular disease
The specific objectives of this research study were:

1. to conduct assessments and inter-group comparisons of risk factors, including novel assessments for metabolic and vascular risk, to the knowledge of the study group, not previously performed in this cohort of children
2. to evaluate the subjects for risk factors, in addition to premature cardiovascular disease, which may place them at further increased risk of development of early atherosclerosis.

The results of this study may influence the evaluation and management of healthy children whose fathers have premature cardiovascular disease.

**Research Objectives**

In a population of children whose fathers have early heart disease and healthy controls, to evaluate and compare:

(i) insulin sensitivity, cholesterol parameters and features of the metabolic syndrome;
(ii) vascular function and blood pressure measures;

AND:
(iii) to explore the relationships between these variables.

**Research Hypotheses**

**Hypothesis No. 1**

- That subjects will have lower insulin sensitivity, more dyslipidaemia and more features of the metabolic syndrome, compared to controls.

**Methods No. 1**

- Insulin sensitivity will be evaluated using a multiple sampled oral glucose tolerance test.
- Fasting lipid profiles will be sampled.
**Hypothesis No. 2**

- That subjects will have relatively impaired endothelial function and higher blood pressure measures compared with controls.
- That relatively impaired endothelial function and higher blood pressure will correlate with decreased insulin sensitivity and presence of MS.

**Methods No. 2**

- Carotid intima-media thickness will be measured using vascular ultrasound.
- Arterial stiffness indices will be measured using peripheral applanation tonometry.
- Ambulatory blood pressure recordings will be recorded.

**Hypotheses No. 3: Combined hypotheses**

- That amongst children whose father have early heart disease, insulin sensitivity will be positively correlated with measures of vascular dysfunction
- That amongst children whose fathers have early heart disease, presence of features of the metabolic syndrome will be negatively correlated with measures of vascular dysfunction
Chapter 1  Background & Literature Review
What is atherosclerosis?

Atherosclerosis is a disease of the large arteries. Its clinical manifestations depend on which of the large arteries are affected. Atherosclerosis of the coronary arteries leads to cardiovascular disease, manifesting as, for example, angina pectoris and myocardial infarctions. Atherosclerosis of the cerebral arteries leads to cerebrovascular disease, manifesting as transient ischaemic attacks (TIAs) and cerebrovascular accidents (CVAs) also known as strokes. Atherosclerosis of the renal arteries leads to chronic kidney disease (CKD) and hypertension. Atherosclerosis of the peripheral or limb arteries leads to peripheral arterial vascular disease. In westernized societies, atherosclerosis is thought to be the leading cause of cardiovascular diseases and cerebrovascular diseases and atherosclerosis is thought to be the underlying cause of about 50% of all deaths (1). The aetiology of atherosclerosis is complex and multifactorial. The risk factors, predisposing to vascular risk and metabolic risk, are multiple, inter-related and complex.

The process of atherosclerosis is complex, involving a fibroproliferative inflammatory process including events in the cardiovascular system, the inflammatory and immune systems, lipid and cholesterol handling mechanisms and blood clotting. Cells such as endothelial cells, T-lymphocytes, monocytes and macrophages, vascular smooth muscle cells and platelets have key roles in the atherosclerotic process. Vasodilators such as nitric oxide and prostacyclins, vaso-constrictors such as angiotensin, cytokines such as IL-1 and TNF-α, and growth factors such as fibroblast growth factor and epidermal growth factor, as well as cell adhesion molecules such as integrin, VCAM, ICAM and selectins all contribute to the development and progression of atherosclerosis (2).

The figure below outlines the structure of the normal artery (figure 1 part a) and illustrates graphically how the flow of blood through the artery decreases consequent upon the atherosclerotic process, with deposition of an atherosclerotic plaque in the arterial intimal layer (figure 1 part b). The clinical manifestations of atherosclerosis occur when the blood supply to the target organ is reduced, through either the
deposition of an ever-increasing sized atherosclerotic plaque in the arterial intimal layer, or through occlusion of the artery by a thrombus (which is often a plaque which has broken free and travelled to a vessel with a smaller lumen), or, commonly a combination of a thrombotic occlusion of an artery affected and narrowed by an atherosclerotic plaque.

**Figure 1:** Diagram of Normal and Atherosclerotic Artery

The PDAY (Pathobiological Determinants of Atherosclerosis in the Young) study, published in 1999, demonstrated that the atherosclerotic process progresses with age
(see figure 2) (3). Of relevance to paediatric medicine, the PDAY study also identified pathological evidence of atherosclerosis, albeit early atherosclerosis, in children as young as 15 years, which was the lower age limit for study inclusion (3). This supported an earlier editorial perspective that atherosclerosis begins in the paediatric age range (4). The PDAY study included data on a significant number, more than 3000, of subjects. These were all aged between 15 and 34 years at the time of death, and death was from external causes, for example accidents, homicides, suicides. In the initial study, there were more data on young, white males collected than on any other ethnic or gender group. A follow up study supported by the National Institutes of Health, called the Risk Factors in Early Atherogenesis, was performed to increase the number of study cases, and the number of female subjects in particular (5). The same protocols were used throughout, and the data were combined to form the PDAY dataset. The PDAY study showed that the coronary heart disease risk factors (gender, age, serum lipoprotein concentrations, smoking, hypertension, obesity and hyperglycaemia) are associated with both the early and advanced lesions of atherosclerosis in adolescence and young adulthood, decades before the occurrence of coronary heart disease. From the perspective of the study that this thesis reports, the PDAY data form a very valuable dataset, but it refers to a significantly older population than a paediatric age range population. Notwithstanding, if the PDAY identified gross pathological evidence of cardiovascular disease in children/adolescents as young as 15 years, then clearly the process of atherosclerosis began prior to this age, i.e. during the years of childhood.

It is also noteworthy that atherosclerosis is particularly prevalent in Irish populations. For example, the Irish National Audit of Stroke Care estimates that 10,000 people are admitted to Irish hospitals annually with “stroke” as their primary diagnosis (6). In 2005, CVD was the principal cause of death in Ireland with 5,064 (19%) cases of heart disease, 2,029 (7%) cases of heart disease, and 2,891 (10%) cases of other heart disease or diseases of the circulatory system (7). The worldwide prevalence of these diseases is expected to rise in high-income countries such as Ireland (8). Evidence about risk factors for disease is lacking in paediatric populations. The high prevalence in Irish populations, however, implies that addressing atherosclerosis risk factors is of particular importance in an Irish population.
It is now understood that the progressive nature of atherosclerosis includes some earlier steps in the process that are, importantly, reversible. Reduced bioavailability of endothelial nitric oxide (NO) produced from endothelial NO synthase (eNOS) play a crucial role in the development and progression of the atherosclerotic process (9). This reduced bioavailability of endothelial NO is referred to as endothelial dysfunction, and this is a reversible step in the atherosclerotic process (10). Much progress has been made in understanding the mechanisms of decreased endothelial NO bioavailability at the levels of regulation of eNOS gene expression (10), eNOS enzymatic activity and NO inactivation (11). Significant work has also focused on methods to identify atherosclerosis at this early and potentially reversible stage. The clinical implications of identifying atherosclerosis at a reversible stage and the potential to introduce therapies to alter the process and the outcomes of atherosclerosis are obvious and very appealing.

**Figure 2:** Illustration of prevalence of fatty streaks and prevalence of raised lesions in the right coronary artery at different ages. Data from the PDAY Study (3)

Following on from endothelial dysfunction, the subsequent, but still reversible, step in the atherosclerotic process is the build-up of lipid in the intimal layer of blood vessels. With progression of the atherosclerotic process, a core of extracellular lipid with a fibromuscular covering develops; this can cause thrombosis, vascular rupture or
ischaemia. Figure 3 shows a schematic representation of the atherosclerotic process, including some of the molecular factors involved in the process (2)

Figure 3: Schematic design depicting the involvement of oxidized low density lipoprotein (oxLDL), injury of endothelial cells and proliferation of vascular smooth muscle cells (SMC) in the development of atherosclerotic plaque. MAP Mitogen-activated protein (2)

The potential to reverse endothelial dysfunction is an attractive theory, currently being explored in clinical research settings (12, 13). However, it is unclear at or after which
point (or points) in the development of atherosclerosis, is endothelial dysfunction no longer reversible. If reversibility of early atherosclerosis and endothelial dysfunction is achieved, it might be possible to prevent or delay or reverse atherosclerosis development. In established atherosclerosis, reversibility of endothelial dysfunction may still be beneficial, as it is thought that endothelial dysfunction on a background of advanced atherosclerosis, can lead to arterial occlusion through vasoconstriction or thrombosis (14). Regardless, it has been suggested to be intuitive that any endothelial dysfunction or early atherosclerosis that might exist in paediatric populations is more likely to be reversible than in adult patients. Further research is required but this is an attractive theory for clinical paediatric researchers and clinical paediatricians (15). Assessments of endothelial function and impaired endothelial function can be difficult in clinical practice, but the use of various surrogate measures of endothelial function is becoming accepted in a widespread manner (14).

The mortality rate associated with atherosclerotic cardiovascular disease is high, particularly in the Irish population. In 2005, cardiovascular disease was the principal cause of death in Ireland with 5,064 (19%) cases of coronary heart disease, and 2,891 (10%) cases of other heart disease or diseases of the circulatory system (7). There was an additional 2,029 (7%) cases of mortality from stroke, which is also largely attributable to the atherosclerotic process (7). The worldwide prevalence of these diseases is expected to rise in high income countries such as Ireland (8). Atherosclerosis of other major arteries also leads to significant morbidity and mortality in Irish populations. For example with reference to atherosclerosis of the cerebrovasculature, the Irish National Audit of Stroke Care estimates that 10,000 people are admitted to Irish hospitals annually with “Stroke” as their primary diagnosis (6).

Although the diagram below is based on a data from the UK, specifically a report commissioned by Foresight (www.foresight.gov.uk)(16), it is certainly possible that there are similarities with the Irish situation. This chart illustrates that the life
expectancy of a 65 years old man with cardiovascular disease (here referred to as coronary artery disease) compared to a 65 years old man without cardiovascular disease is reduced by almost 3.5 years. For 65 years old women, the reduction in life expectancy due to having cardiovascular disease is just under 3 years.

**Figure 4:** Difference in life expectancy (LE), years free of any disability (Any DFLE) and years free of moderate or severe disability (Mod+DFLE) at age 65 between those without and with specific diseases, men and women (source: Cognitive Function and Ageing Study (16))

Historically, atherosclerosis was regarded as an inevitable effect of ageing. It is now understood that atherosclerosis is a chronic inflammatory condition that can deteriorate quickly into an acute clinical event, due to the rupture and thrombosis of an arterial atherosclerotic plaque. In keeping with this paradigm shift in the understanding of the aetiology of atherosclerosis, there has been a parallel paradigm shift in the approach to managing atherosclerosis, with increasing emphasis on identifying the early stages of atherosclerosis, targeting specifically the early and reversible stages of the process.

Metabolic risk and vascular risk are terms used to summarise the multitude of risk factors which predispose to the development of atherosclerosis. These risk factors can include diseases that increase the risk of atherosclerosis, for example diabetes mellitus,
hypertension, dyslipidaemia. Identification of metabolic risk and vascular risk are key to identifying early and potentially reversible stages of atherosclerosis.

**Premature Cardiovascular Disease**

Premature cardiovascular disease in adults is likely to be a familial trait, inherited from parents but equally passed on to children (17, 18). It is common in paediatric clinical practice to include information on a family history of any/all conditions when taking a paediatric medical history. A child who has a family history of cardiovascular disease has therefore 1 readily identifiable non-modifiable risk factor for cardiovascular disease and atherosclerosis themselves, namely their positive family history – of course, they may have other risk factors, additionally. However, when the age of onset of atherosclerosis in the affected family member (or members) is taken into consideration, this non-modifiable risk factor becomes more interesting; in brief, if the atherosclerotic event in the family member occurs at a younger age, the larger the likely risk factor for the child. It is noteworthy that there is no quantum for assessing the magnitude of this risk and further there are no studies describing if this risk changes with differing ages of onset of parental premature cardiovascular disease.

The term family history of premature cardiovascular disease was defined by the American Academy of Pediatrics (AAP) in the 1998 Guidelines for Cholesterol in Childhood (19) as

“children and adolescents, whose parents or grandparents, at 55 years of age or younger, had undergone diagnostic coronary arteriography and were found to have coronary atherosclerosis…..includes parents or grandparents who have undergone angioplasty or coronary artery bypass surgery or have suffered a documented myocardial infarction, angina pectoris, peripheral cardiovascular disease, or sudden cardiac death”.

This definition was consistent with the American Heart Association (AHA) 1992 guidelines on integrated cardiovascular health promotion in childhood (20).
The updated 2008 AAP guidelines changed the age defining parental or grandparental history of premature cardiovascular disease to ≤55 years of age for men and ≤65 years of age for women(21). The 2012 National Heart Blood Lung Institute (NHBLI, at the National Institutes for Health, NIH) also used these same age criteria to define the term “family history of premature cardiovascular disease” (22) but these data were not available at the start of this study and thesis. Nonetheless, it is pertinent to mention here as it gave the study team an opportunity to explore additional study findings. Further information on this paper is given below and another study which is equally relevant to discuss here despite its publication after the start of this thesis.

In 2008, the AAP recommended targeted cholesterol screening in children with parental history of premature cardiovascular disease (21). In 2012, the NHBLI published the Expert Panel on Integrated Guidelines for Cardiovascular Risk Reduction in Children and Adolescents (23). These guidelines are being implemented as national policy across the United States, and include the introduction of non-fasting lipid screening for all children from ages 9 to 11 years, and again from 17 to 21 years, along with targeted dyslipidaemia screening for high-risk groups at other ages (22). The following study is noteworthy: a recent Irish study evaluated cholesterol profiles in young adults who had a positive family history of premature cardiovascular disease and identified high cholesterol levels in these young adults compared to healthy controls (24). This Irish study described young adults, who were older than the age of the children and adolescents described in this research thesis.

Children and adolescents who have a family history of premature cardiovascular disease are at increased risk of having high blood cholesterol levels as adults and increased risk of coronary heart disease (22). Such children are recommended for selective lipid screening in the context of regular health care (22). There are currently no other screening tests recommended for this population of children, only lipid screening.

The 2012 NHLBI Expert Panel on Integrated Guidelines for Cardiovascular Risk Reduction in Children and Adolescents (22) asserts that there is moderately strong evidence (grade B evidence) from observational studies to warrant strongly supporting the “inclusion of a positive family history of early coronary heart disease in identifying
children at risk for accelerated atherosclerosis and for the presence of an abnormal risk profile”. Furthermore, the NHBLI Expert Panel recommend updating family history information at intervals (22).

It has long been recognised that a parental history of premature cardiovascular disease is associated with increased risk of coronary artery disease in their children (25). More recently, surrogate makers of atherosclerosis have also identified increased risk in children of parents with premature cardiovascular disease, for example these children have been demonstrated to have increased carotid intima media thickness (cIMT) (26). Additionally, young adults with a parental history of premature cardiovascular disease have evidence of impaired endothelial function (27) and higher cholesterol than controls (24). Below is an excerpt from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, which focuses specifically on the importance of family history information in 2 different age categories: up to 7 years old and 8-21 years old (23).
Table 1: Evidence-based recommendations for use of family history in cardiovascular health promotion, adapted from 2012 Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (22)
There have not been many published studies evaluating children and adolescents of parents with premature cardiovascular disease to date (28-31). There are some studies evaluating adult patients, but these are largely database studies or evaluate adult patients’ own risks of having established atherosclerosis. These are discussed in the discussion section of this thesis. The available literature is summarized below.

**Atherosclerosis begins in childhood**

Atherosclerosis has been described as “a pediatric problem” (4), and a “pediatric nutrition problem” (4), by which it was meant that atherosclerosis is associated typically with adults, but begins during the childhood years. Post-mortem studies of young soldiers during the Korean war (32) and the Vietnam war (33) provide evidence for these claims. These studies examined youth thought to be healthy before they suffered sudden death during these respective wars, and identified post-mortem evidence of atherosclerosis in these apparently healthy youth. For example, in the studies of 105 healthy deceased young soldiers during the Vietnam war, who died at a mean age of 22 years, 50% had gross evidence of coronary atherosclerosis: 45% had gross evidence of some coronary atherosclerosis; and 5% had gross evidence of severe coronary atherosclerosis (33); it is noteworthy that the subjects studied in this paper, were likely to be in conditions of very good physical fitness, as they were trained soldiers on active combat duty. Despite this, they had very high prevalence of gross coronary atherosclerosis.

Subsequently, the landmark P-Day Study (the previously mentioned Pathobiological Determinants of Atherosclerosis in Youth Study) was published (34). This multi-center post-mortem study in young people aged between 15 and 34 years, who died due to trauma, homicide or suicide identified the presence or absence of antemortem traditional cardiovascular risk factors in the first 48 hours immediately following death. The results of this PDAY study are charged with demonstrating that antemortem cardiovascular risk factors such as hypercholesterolemia and hypertension are directly correlated with the postmortem size of arterial fatty streaks and fibrous plaques in youth (34, 35). These emergent concepts and results were explored further in the Bogalusa Heart Study, which
included both epidemiological and opportunistic pathological studies, and concluded that both post-mortem coronary artery fatty streaks and aortic fatty streaks were positively associated with LDL cholesterol (LDLc) and negatively associated with HDL cholesterol (HDLc) measured during life (36). In its pathological study, the Bogalusa investigators found that the prevalence of fatty streaks and fibrous plaques were 50% and 8% respectively during childhood, and 69% and 85% respectively during young adulthood (37). Fatty streaks, the earliest precursor of atherosclerosis, were found in the coronary arteries of children as young as two years of age (37). Thus, the evidence had begun to accumulate that, as asserted in 1961 (4), atherosclerosis begins in childhood.

While atherosclerotic cardiovascular disease is the leading cause of death in Ireland (38) and throughout the developed world (39) and while it is now clear that atherosclerotic cardiovascular disease begins in childhood, it is not usually clinically apparent in childhood in “normal” children. The use of the term “normal” here, is intended to exclude children with diseases known to cause or include acceleration of the “normal” atherosclerotic processes. For example, children with inherited disorders of cholesterol metabolism do not have a normal atherosclerotic process and, in fact, these children have occasionally been reported to have end stage atherosclerotic events in childhood (40). Homozygous familial hypercholesterolaemia is one such diagnosis, which confers an accelerated atherosclerotic process. While rare, with an estimated incidence of 1 per 1 million populations, like any inherited disease, it occurs more commonly in some ethnic groups, such as the French Canadians. Due to its rarity, cohort studies are small. One such cohort study, however describes the increased incidence of end stage cardiovascular disease in 39 patients with the condition, including 22 who were aged less than 16 years (41). Clinical evidence of cardiovascular disease was identified in 15/17 subjects aged >16 years and in 2/22 of those <= 16 years. Fifteen children less than 16 years old had echocardiographic evidence of cardiovascular disease, even though they had no clinical evidence of disease; 7/15 had angiographically confirmed mild coronary artery disease and 8/15 had mild to moderate aortic regurgitation using echocardiography. During follow-up, 7 children developed progression of coronary and/or aortic valvular disease during their teenage years and 4 required surgical interventions (41).
There are other groups of children who may also have abnormalities of cholesterol or other vascular risk and metabolic risk, which predispose to accelerated atherosclerosis. While accelerated compared to “normal” populations, the acceleration is not as pronounced as in the example of homozygous familial hypercholesterolaemia above. Examples include children with obesity (42) and girls with Turner syndrome (43). Children of parents with premature cardiovascular disease are another group that could be at risk, but the literature around this question will be discussed later in this chapter.

In recent years there has been a dramatic rise in secondary dyslipidemia related to childhood obesity. In US epidemiological studies, childhood obesity affects up to 20% (44) of the population. The dyslipidemia associated with obesity is characterised by hypertriglyceridemia (44-46). There is a strong association between childhood obesity and the metabolic syndrome (dyslipidemia, hypertension and insulin resistance), all of which lead to atherosclerosis (44). It is established that childhood obesity, and indeed excess weight that is still in the normal range, is associated with the risk of cardiovascular disease in adulthood (47).

Turner syndrome occurs when all or part of one of the two X chromosomes is deleted or defective – thus it affects girls exclusively. Turner syndrome has a wide range of clinical characteristics, but one of the most common is congenital heart disease. In recent years, accelerated atherosclerosis, manifesting as early, acquired (as opposed to congenital) heart disease and early cerebrovascular disease, have been increasingly recognized in girls with Turner syndrome. The atherosclerotic process in Turner syndrome starts early (48) and the epidemiological data suggest that there is 3-fold increased risk of mortality from cardiovascular and cerebrovascular disease (49). Epidemiological data suggest that adult women with Turner syndrome are twice as likely to develop coronary artery disease, compared to the general population (50). Several studies have evaluated the metabolic risk and vascular risk that may predispose to the increased and accelerated atherosclerotic process in Turner syndrome. These studies describe higher total cholesterol, higher triglycerides and higher HDLc (48) as well as higher systolic, diastolic and mean blood pressure (BP) and resting heart rate (51) with a loss of the normal circadian rhythm of diastolic BP (52) in cohorts of
paediatric patients with Turner syndrome compared to non-Turner syndrome healthy controls.

Homozygous familial hypercholesterolaemia, paediatric obesity and Turner syndrome are three examples of well-studied models of early atherosclerosis and metabolic risk and vascular risk, each in a paediatric population. But in these studies, because the subjects have either homozygous familial hypercholesterolaemia, an intrinsic defect of cholesterol metabolism, or have obesity or Turner Syndrome, which can include many different clinical features, the subjects are by definition not “normal” children and have readily identifiable abnormalities on routine, accepted clinical examination or laboratory tests. To evaluate children who have no such readily identifiable abnormalities on routine, accepted clinical examination or laboratory tests, but who are known to be at risk of atherosclerotic processes as younger than average adults, as opposed to as paediatric patients, would be interesting. Otherwise healthy children of parents, or a parent, who have had early evidence of atherosclerosis or premature cardiovascular disease is one such healthy and “normal” but at risk child population.

In “normal” adults, cardiovascular disease causes clinically apparent diseases, such as myocardial infarctions, stroke, peripheral arterial disease and aortic aneurysm rupture as long-term endpoints to the atherosclerotic process. By contrast, atherosclerosis rarely causes morbidity in “normal” childhood. This implies that detecting atherosclerosis in children is more difficult. It can be identified by looking for risk factors or by using proxy measures of end-stage, symptomatic atherosclerosis. But in “normal” populations of children, it is not possible to define the child groups as having end stage atherosclerosis or not. This has implications for the methodology and investigations used by both clinicians in diagnosing and treating the evolving diseases and by researchers, in pushing the boundaries of science to identify early markers of diseases and to treat and monitor for changes in outcomes.
The Barker Hypothesis and the Thrifty Genotype

While the previous discussion of the relevant literature review supports the concept that atherosclerosis begins in childhood, increasingly, there is also a compelling argument that the origins of atherosclerosis and metabolic risk and vascular risk, including obesity, hypertension and dyslipidaemia, actually have their origins before childhood – in the antenatal milieu, in the health of the mother and even in the health of the father before birth. This is the concept of foetal programming, or metabolic programming. The Barker Hypothesis is the original theory underpinning the current concepts and understanding of foetal programming. Barker’s original hypothesis led to his design and conduct of an epidemiological study in Hertfordshire, an affluent region in the United Kingdom; this study traced the causes of death of more than 5000 men born between 1911 and 1930. As predicted by the hypothesis, men with the lowest birth weights had the highest death rates from cardiovascular disease (53).

The Barker hypothesis does not predict obesity risk in later life based on birth weight, but this hypothesis does propose the concept of the thrifty genotype. The thrifty genotype concept suggests that later health, including weight, of a baby is dependent in part on the intra-uterine milieu in which it develops, and also in turn to maternal health antenatally and even pre-pregnancy. For example, maternal insulin resistance (also known as risk for diabetes) during pregnancy is associated with increased infant weight gain and adiposity over the first year of life, independent of maternal glucose levels (54). Furthermore, babies’ birth weights during the Dutch famine from 1944-1945 demonstrated the adverse effects on babies’ birth weights of maternal undernutrition during pregnancy, and furthermore that under-nutrition in different antenatal trimesters impacts birth weights differently (55). By way of illustration, a poorly nourished pregnant mother is more likely to give birth to an underweight baby who will, in later life, have rapid weight gain in a nutrient-rich environment. An appropriately nourished mother is more likely to give birth to a normal weight baby, who will gain weight appropriately in a nutrient-rich environment in later life. This nutrient-rich antenatal uterine environment has been termed “obesogenic”, in that it is an environment which encourages inappropriate weight gain. The Barker Hypothesis also led to the concept of lifelong ageing (56), which suggests that the ageing process begins or is programmed during, or even before, the neonatal period. Thus, the intrauterine antenatal milieu and
birth weight are examples of vascular and metabolic risk factors for an individual’s future risk of atherosclerosis, as well as obesity and hypertension and diabetes risk, and these risks are non-modifiable.

The literature in this area (57, 58) leaves many unanswered questions. Does antenatal weight loss or even weight loss pre-pregnancy affect or prevent the thrifty genotype? Does paternal weight have an effect on the thrifty genotype? Weight and BMI are crude markers of health – what is the effect of body composition on the thrifty genotype? Does exercise modulate the thrifty genotype, even at the same weight? Does weight loss in women that occurs before pregnancy prevent the thrifty genotype and later obesity risk in offspring? However, this literature does suggest strongly that atherosclerosis begins even before childhood, in the antenatal period or in foetal programming, based on parental health or ill-health.

Any discussion about risk factors for cardiovascular disease includes references to risk factors which are either “modifiable” or “non-modifiable”. Clear and simple examples of modifiable risk factors are smoking, diet and exercise – these can all be changed by the subject. On the other hand, one’s family history, birth weight and maternal health prior to and during pregnancy are non-modifiable. Across generations, non-modifiable risk factors may be modified, for example, with public health interventions, maternal antenatal health and diet might be improved. But when faced with assessment of a child with risk factors for early cardiovascular disease, for example a parental history of early cardiovascular disease, this child’s maternal antenatal history cannot be changed. At this point, there is no system for quantifying the relative importance or cumulative effect on total risk of a child’s individual risk factors. That is, there is no equivalent in paediatric clinical medicine to scores such as the Framingham Score (59, 60) in adult medicine. The interactions between risk factors are likely to be very complex, for example separating the effect of a paternal history of early cardiovascular disease and a maternal history of poor antenatal health, will be very complex, and likely impossible at this point in time. Another very important nuance, is that, as a child ages, it is possible that his risk factors will change. For example, a child whose father is 30 when he is born and whose father then develops premature cardiovascular disease at 45 years old, is a child
who did not have evidence of a positive parental history of premature cardiovascular disease for the first 15 years of his life, but this risk factor only became apparent when the father had an acute event. Thus, the discussion of risk factors in children has several nuances which affect it significantly.

Risk factor assessment for atherosclerosis

Information on risk factors for cardiovascular disease or for metabolic risk and vascular risk are important factors in any clinical assessment for the presence of or the development of atherosclerosis, hypertension, obesity, dyslipidaemia and type 2 diabetes mellitus. It is noteworthy that these risk factors do not often exist in isolation, but rather one risk factor might lead to or co-exist with another risk factors, and both then contribute to the development of an additional factor. For example, hypertension, obesity, dyslipidaemia and type 2 diabetes mellitus are all risk factors for atherosclerosis (61), and indeed obesity is a risk factor for each of dyslipidaemia, hypertension and type 2 diabetes mellitus (62).

When several of these risk factors occur together, it can give rise to what is known as the metabolic syndrome. Metabolic Syndrome is a clustering of risk factors for diabetes and heart disease, including insulin resistance (63), abdominal obesity, hypertension, and dyslipidemia. Non-alcoholic fatty liver disease is now also recognized as an increasing clinical problem in children with risk factors for the metabolic syndrome, and may progress to hepatic cirrhosis, and end-stage liver disease in predisposed individuals(64). Using various metabolic syndrome definitions, the prevalence of the metabolic syndrome varies between 39-60% in overweight clinic-based studies and 3.8-12.5% in population-based studies (65-68). A compelling strategy to evaluate risk factors for the metabolic syndrome and vascular risk and metabolic risk, is to study the pathophysiological mechanisms that lead to their development in childhood.

Until recently there were no cardiovascular risk assessment calculators for use in children. In 2007, McMahan et al (69) published a predictive equation derived using multiple traditional risk factors that they claim accurately predicts post-mortem atherosclerosis in young adults. An expert group has suggested that this approach is
similar to the risk stratification equations in adults and may become clinically
meaningful when assessing vascular risk in youth (70). However, it is not currently in
wide use. Notwithstanding, there are clear guidelines for risk stratification and risk
reduction for some paediatric metabolic and vascular risk factors. For example, the US
Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in
Children and Adolescent released guidelines regarding childhood dyslipidemia in 2012
(22). These guidelines refer to primordial prevention (the prevention of risk factors
developing) and primary prevention (recognition and management of those children in
whom identified risk factors are already present) (22).

Recognised risk factors for atherosclerosis in children include family history of acquired
heart disease, especially positive family history of premature cardiovascular disease.
Other risk factors for atherosclerosis in children include smoking, dyslipidaemia,
hypertension, hyperglycaemia increased BMI, poor physical fitness and insufficient
active time (71). Established atherosclerosis in a young person is more likely to be
found against the backdrop of increased total cholesterol, increased LDLc, increased
BMI, and hypertension affecting both the systolic and diastolic BP (37, 72). However,
children in whom the atherosclerotic process has already begun are usually
asymptomatic (73). Increased total cholesterol levels in childhood are associated with
increased cIMT in adults aged 33-42 years – data from The Muscatine Study (74).
Furthermore, measuring the thickness of the intimal medial layer is a surrogate marker
of the atherosclerotic process.

There are an ever-increasing number of risk factors that are linked to and/or implicated
in causation of cardiovascular disease, mostly in adult medicine, and to a lesser extent
in paediatric medicine. A comprehensive, rigorous, scientific assessment of all of these
factors would be unwieldy. Instead, in most studies, risk factors are selected a priori. It
has been claimed that only 50% of the risk of atherosclerosis can be explained by the
classic, established risk factors (75, 76). But the evidence does not appear to support
this claim (77). In one study, 92% of cardiovascular heart disease deaths were explained
by poor control of dyslipidaemia, smoking, BP and BMI in a cohort study, which
followed 356,222 people screened for primary prevention of cardiovascular disease in
the USA. This trial was called Multiple Risk Factor Intervention Trial (acronym
MRFIT)(78). Additionally, the 1998 set of risk calculator equations from the
Framingham study (59, 60) includes the following risk factors: dyslipidaemia, smoking, BP and BMI; and has been suggested to explain approximately 75% to 77% of cardiovascular disease (79). Several authors suggest that even these figures are conservative underestimates and that the real effect of these 4 factors on cardiovascular disease is even higher (75, 80, 81). Thus, any assessment of vascular and metabolic risk, and risk for atherosclerosis should contain assessments of these 4 variables; namely, dyslipidaemia, smoking, BP and BMI. And, hopefully, in any child population, smoking will not be very prevalent. With reference to child populations, the data on estimated individual or cumulative contributions of various risk factors for cardiovascular disease in paediatrics are lacking.
Hypertension and atherosclerosis and metabolic and vascular risk

In the United States, one third of adults over 20 years old have hypertension (44). National Health and Nutrition Examination Survey (NHANES) data demonstrate a 2.3% increase in pre-hypertension and a 1% increase in hypertension in the 10 years from 1988 to 1999 (82). However, adjustment of the NHANES data for BMI attenuates the increase in hypertension, suggesting that obesity has a complex role to play in the increase in hypertension (83). The Bogalusa Heart Study and the PDAY study have both demonstrated increased atherosclerosis at postmortem examination in young people with antemortem hypertension (34, 37).

In 2008 (84) and again in an update in 2014 (85), the AHA published guidelines on monitoring for hypertension in children, using 24 hour ambulatory blood pressure monitoring (24ABPM), as opposed to intermittent BP evaluations. The caveat to the 2014 update, as referred to by the authors of the report, is that

“because no outcome studies are yet available relating ABPM levels in children to outcomes such as myocardial infarction or stroke, these guidelines are largely driven by expert opinion, although they are also informed by available pediatric data on ABPM [ambulatory blood pressure monitoring] and surrogate markers of cardiovascular disease.”(85)

The importance of this statement, referring to the lack of long-term outcome studies relating blood pressure to atherosclerotic outcomes in paediatrics cannot be understated. Unfortunately, while this is true for blood pressure and atherosclerosis outcomes in paediatric medicine, it is equally true for other metabolic and vascular risk factors and atherosclerosis outcomes in paediatric medicine. And it has significant implications for the tests that can be used in research studies to identify outcomes – ie, the end stages of atherosclerosis itself cannot be identified in paediatrics, and so surrogate markers of cardiovascular disease must be evaluated.
Dyslipidaemia and atherosclerosis and metabolic and vascular risk

Dyslipidaemia is a feature of the metabolic syndrome and the term implies any abnormality of the cholesterol profile. The typical atherogenic cholesterol profile includes high levels of LDLc, low levels of HDLc, high triglycerides and high total cholesterol. Hypertriglyceridemia is the dyslipidemia primarily associated with obesity.

The prevalence of dyslipidaemia is increasing. A study evaluating data on 10,000 healthy children from the NHANES 1999-2006 cohort study, identified that 5.2-6.6% of adolescents had abnormal LDLc levels (86). Studies evaluating at risk children also identify high rates of dyslipidaemia (87). A previous study of children in Ireland with a positive family history of premature cardiac disease (defined in this study as myocardial infarction at less than 55 years old) identified prevalences of 7/82 (8.5%) and 17/82 (20.7%) for high (>3.35 mmol/L, or >130 mg/dL) and borderline-high (>2.85 mmol/L, or >130 mg/dL) LDLc respectively (24).

Whether screening for dyslipidaemia in paediatrics should be universal or targeted remains the subject of debate. The AHA 2007 (88) position statement endorses targeted screening for dyslipidemia, citing that children with hyperlipidemia, who do not have a family history of early cardiovascular disease, are considered to be at low risk, and therefore should probably not start medications for hyperlipidemia until at least adulthood (88). Therefore, targeted screening was recommended for children with any of:

1. “A family history of parental or grand-parental vascular disease at less than 55 years of age. Vascular diseases cited include coronary artery disease, peripheral vascular disease, cerebrovascular disease, a coronary artery procedure, myocardial infarction or sudden cardiac death;

2. A family history of increased total cholesterol (>6.2 mmol/L). The recommendation is that these children should be screened with fasting total cholesterol measurements;
3. Overweight or obesity. If hyperlipidemia is identified, these children should then also screen for other elements of the metabolic syndrome.”

The AAP recommendations on screening for pediatric hyperlipidemia published in 2008 (21) go on to further develop some areas of the AHA guidelines, without deviating from these guidelines – for example, they provide guidance on managing the child whose family history is not known (70). The 2008 AAP guidelines recommend targeted screening for paediatric dyslipidemia, by testing a complete fasting lipid profile every 3-5 years, from the age of 2 years onwards, and interpreting these profiles using age- and gender-specific norms. The triggers for initiating targeted screening, taking into consideration other risk factors individual to the child, should include (21):

1. A known positive family history of early atherosclerosis;
2. A known positive family history of high cholesterol;
3. An unknown family history.

**Insulin resistance and atherosclerosis and metabolic and vascular risk**

Obesity beginning in childhood often precedes the hyperinsulinaemic state (89). Other components of the insulin resistance syndrome are also present in children and adolescents (90-92). There is an increasing amount of data showing that being overweight during childhood and adolescence is significantly associated with insulin resistance, dyslipidaemia, and elevated BP in young adulthood. Similar to the discussion under BP and atherosclerosis above, the expert group report on insulin resistance and cardiovascular risk in paediatrics suggests that the best available science and literature is still missing large pieces of information which would inform the “specifics of the transition from risk factors in childhood to diabetes and cardiovascular disease” (91). However, there is “compelling evidence [which] points to their association with overt disease in adults. On the basis of current knowledge and extrapolation from studies in adults, it is reasonable to suggest that lifestyle
modification and weight control in childhood could reduce the risk of developing the insulin resistance syndrome, type 2 diabetes mellitus, and cardiovascular disease.”(91)

**Obesity and atherosclerosis and metabolic and vascular risk**

Obesity is a confounding variable which accelerates metabolic ill-health. Obesity is one risk factor that is predictive of atherosclerosis in adults, and it is increasingly recognised to be predictive in children also. Obesity is a major risk factor for chronic diseases such as diabetes and heart disease (93, 94). Obesity evolves during childhood and tracks to adulthood (95, 96). Some studies would suggest that the number of metabolic risk factors, including obesity, has a bigger impact on cardiovascular risk in those with a significant family history of coronary artery disease, than in those without a family history of significant coronary artery disease (26). Obesity is one of the major public health threats of our time. Irish estimates from 2001-2002 are that 23% of boys and 28% of girls are overweight or obese (97).

**Smoking and atherosclerosis and metabolic and vascular risk**

With reference to the 2012 Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (22), the expert panel states that: “The quality of the evidence regarding the harm of smoking and the benefits of avoiding passive smoke exposure, smoking prevention and smoking cessation is uniformly Grade A. The reason that evidence grades in the recommendations are less than Grade A reflects the lack of existing evidence on interventions impacting smoking behaviors in specific pediatric age groups as opposed to the collective evidence.” Again, in this instance, high quality data are extrapolated to paediatric populations and paediatric age range specific data are less robust than adult data. Regardless, these guidelines recommend regular counseling to avoid and decrease both active and passive smoke exposure.

The link between smoking, both active and passive tobacco exposure, and atherosclerosis in adults is well-described (98-103). In children, information on tobacco exposure in history taking is not frequently collected; one study cited rates of
documentation of assessment and counseling for second-hand smoke exposure occurs as low as 4% of pediatric health supervision visits (104). However, at least one study has documented improved counseling rates on exposure and reducing exposure to tobacco, following a primary care intervention (105).

Non-Invasive Assessment of Atherosclerosis in Childhood

Endothelial dysfunction is associated with smoking (106) and tobacco exposure (107) as well as obesity (108). Improvement in endothelial dysfunction has been demonstrated in obese children after regular exercise (109). The metabolic syndrome is a clustering of risk factors for diabetes and heart disease, including insulin resistance (defined as the diminished ability of target cells to respond to insulin action to promote glucose transport, abdominal obesity, hypertension, dyslipidemia (63). Many questions remain about the evolution of the metabolic syndrome, insulin resistance, pancreatic beta cell dysfunction and their relationship to the development of cardiovascular disease and metabolic and vascular risk. Novel techniques for the assessment for vascular dysfunction, may help us to detect early abnormalities that could potentially contribute to risk for later disease.

Methods to assess subclinical atherosclerosis have developed in recent years (110). These include:

- coronary artery calcification scoring by computerized tomography. This is expensive and involves significant radiation exposure. This was not an option for this paediatric study and so this is not further discussed.
- measurement of the carotid artery intima media thickness by ultrasound (cIMT)
- endothelial dysfunction, manifest by reduced arterial dilatation measurement by ultrasound (e.g., Brachial Artery Reactivity [BAR]) or novel methods (e.g., peripheral applanation tonometry [PAT] as used in this study), and increased left ventricular mass on echocardiography.
Raised coronary calcium levels, increased cIMT and impaired endothelial function have been demonstrated in adolescents with familial heterozygous hypercholesterolaemia (41, 111, 112).

Increased cIMT and left ventricular mass have been demonstrated in children with hypertension (113).

Abnormal endothelial function has been demonstrated in children with type 1 diabetes mellitus (114-116).

**Ambulatory blood pressure assessment in paediatrics**

High BP is the leading risk factor–related cause of death throughout the world, accounting for 12.8% of all deaths, 51% of stroke deaths, 45% of coronary artery disease deaths (WHO: A global brief on Hypertension 2013). Hypertension in children is particularly concerning, as with any chronic disease that presents in childhood, because it exposes the child to risks over their lifetime. For example, at transition to adult medical care aged 18 years, a child diagnosed with hypertension will have had hypertension for 8 years already. The post-mortem studies, the Bogalusa Heart Study and the PDAY study have demonstrated clearly that atherosclerosis is more advanced in children and young adults with hypertension (117, 118). Hypertension and prehypertension are associated with endothelial dysfunction (119), an early step in the atherosclerotic process in children and adolescents.

It is noteworthy, however, that there are no good quality studies evaluating long-term outcomes of management of hypertension in these children. Current data suggest that ABPM is better than intermittent BP measurement in predicting cardiovascular morbidity and mortality in adults (120). There are no published studies to date evaluating ABPM in children whose parents have premature cardiovascular disease.
Non-invasive assessment of atherosclerosis in children with family history of premature cardiovascular diseases

The 2012 Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report by the NHBLI guidelines (22) address the utility of non-invasive vascular assessment in children with a family history of cardiovascular disease. The following statement is included in Chapter 2:

“Children and young adults with a family history of myocardial infarction have increased cIMT, higher prevalence of coronary calcium, and endothelial dysfunction (22).”

The full text of the NHBLI guidelines gives 4 citations in support of this statement (27, 121-123). A very brief summary of each study is given below. Additionally, there is a fifth paper which looks at cIMT in this high risk population. There are 2 published papers evaluating blood cholesterol measurements in this population (24) – although one of these refers to young adults, so older than the population to which this research study refers. Additionally there is a published study which describes decreased glomerular filtration rate in children with a parental history of premature cardiovascular disease (124), and this is not discussed further in this chapter.


This study included 114 young people (aged 5 to 30 years) as subjects with a parental history of myocardial infarction at less than 60 years old, and compared their data with data from 114 age- and sex-matched controls. Siblings were included in both groups. The study group included 2 age groups: 108 children and adolescents (aged 5 to 18 years) and 120 young adults (aged 19 to 30 years).

In both age groups, compared with controls, subjects with a parental history of premature myocardial infarction had increased intima media thickness of common carotid arteries (p=0.007).

This study included 40 healthy young people (mean age 19 years) of parents who had myocardial before 61 years of age and 40 age- and gender-matched control subjects. Siblings were included in both groups.

Subjects had lower flow-mediated reactivity of the brachial arteries (5.7 +/- 5.0% vs 10.2 +/- 6.6%; P=0.004). In subjects, an inverse relationship was found between brachial artery reactivity and carotid intima media thickness (r=-0.46, P=0.003). In a conditional logistic-regression analysis, both BAR and cIMT were significantly and independently correlated with parental history of myocardial infarction at age less than 60 years old.


Two generations of Framingham Heart Study subjects had multidetector computerized tomography measurements of coronary artery calcification and abdominal aortic calcification. Subjects included 797 Framingham Offspring (mean age 63 years) and 1238 third generation (Gen3) participants (mean age 46 years) free of CVD. Parental premature cardiovascular disease was associated with coronary artery calcification among Gen3 (odds ratio= 2.17 [1.41 to 3.33]). Parental premature cardiovascular disease was not associated with abdominal aortic calcification in either cohort. Parental premature cardiovascular disease was associated with abdominal aortic calcification among Gen3.


In this study of 50 young adults, with a mean age of 25 (± 8) years who were first-degree relatives of either (1) 17 men aged less than 46 years or (2) of 12 women aged less than 55 years with proven coronary artery disease, compared with 50 age- and sex-matched healthy controls. Many subjects were siblings. Endothelial function was assessed by measuring BAR. Flow mediated dilatation (FMD) was impaired in the subjects compared with controls (4.9+/-4.6% versus 8.3+/-3.5%, P< 0.005). Total
cholesterol levels were significantly higher in the subject group (4.9 ±1.0 mmol/L vs 4.5 ±0.9 p<0.05).

5. Barra S et al Heart 2009 (31)
This study included 26 healthy children (9 males and 17 females; 5–12 years, mean age 9.1 years) with parents who had a myocardial infarction under 50 years and 26 age-matched, sex-matched and BMI-matched controls, both groups included siblings. Compared to controls, subjects had increased carotid intima media thickness.

On careful critique of these 5 published studies, all have flaws (eg all include siblings) and none seem to entirely support the statement in the NHLBI guidelines by investigating the relationship between endothelial function and parental premature cardiac disease in the paediatric age group. Clinical research frequently has scientific flaws. And most of the expert group recommendations on topics related to this area include caveats that expert consensus is given, where the literature is lacking. That is not stated in the NHLBI guidelines for the recommendation to use endothelial function testing to investigate children of parents with premature cardiovascular disease, however. Further research in this area is clearly warranted to answer these questions in a scientific, rigorous manner, perhaps by addressing only 1 child per family and by including only paediatric age range subjects and controls.
Assessment of Endothelial Function in children

As previously discussed, an early and potentially reversible step in the process of atherosclerosis is impaired endothelial function, also referred to as endothelial dysfunction (125). The potential for reversibility if atherosclerosis is detected at this stage, is what makes the quest for diagnosing endothelial function so important. Impaired endothelial function and its measurement/quantification can be viewed as a surrogate marker of cardiovascular and metabolic risk assessments (126-129).

In specialised vascular laboratories, with specially trained paediatric vascular technicians, there are several non-invasive methodologies for measuring endothelial function. These include ultrasound of the brachial artery with calculation of flow-mediated dilatation before and after administration of glyceryl trinitrate, and measurement of the stiffness of the artery by calculating the augmentation index measurement (130). There are aspects of these tests which makes these tests difficult to perform, and particularly so when trying to perform them on young children (129). For example, the child is required to attend fasting, lie quietly and without moving in a darkened room, is not permitted to talk, and a sphygmomanometer cuff is inflated to approximately 20 mm Hg above systolic blood pressure and maintained at this pressure while various parts of the tests are performed. Further, the glyceryl trinitrate can make the child feel nauseous and anxious. For the technician also, there are technical elements to the tests which make it harder for the technician, and which imply that subspecialist paediatric training must be pursued, especially for dealing with young children. The cumulative effect of all of this, is that, historically, endothelial function has traditionally been only measured in the sub-specialised and expensive environment of the paediatric vascular research laboratory.

Two vascular laboratory methodologies of particular interest are discussed below: flow-mediated dilatation and intima media thickness measurement.

Flow-mediated dilatation on brachial artery reactivity testing

Flow-mediated dilatation is the gold standard assessment of endothelial function in children (FMD) (131). The brachial artery diameter is measured before inflation of an ipsilaterally placed blood pressure cuff to 20 mm Hg above systolic BP (baseline
diameter), measured again following a period of cuff inflation (ischaemic diameter) and measured a third time following release of the BP cuff (reperfusion diameter). Dilatation of the vessel occurs in response to release of NO from the arterial wall, following BP cuff release. NO is also released from glyceryl trinitrate so flow-mediated dilatation can also be measured following administration of this medication. By performing both tests, responsiveness of the vessel wall to endogenous and exogenous nitric oxide can be compared (132). In children, brachial artery reactivity is reproducible and is a good surrogate measure of atherosclerosis (133).

**Arterial Intima-Media Thickness (IMT)**

Ultrasound of the artery is used to measure the thickness of the intima media layer of the vessel wall, at different sites of the arterial tree, most frequently at or proximal to the carotid bulb. In several large studies, intima media thickness correlates well with traditional cardiovascular and metabolic risk factors including BP, cholesterol profile and smoking (or not) and it is predictive of future end-stage atherosclerosis, including myocardial infarction and cerebrovascular accident (134, 135). Ongoing research is required in paediatric IMT measurements. Understanding of disease progression in paediatrics, and relating exactly thickness measurements in childhood with cardiovascular outcomes in adulthood are lacking.

IMT measurements in children are more technically difficult than in adults, owing to the small size of the vessels and also to the fact that paediatric IMT measurements change little within a much smaller range, along the spectrum from normal to not normal (136). This has been somewhat mitigated with recent software improvements reducing variability of this test (137, 138). Regardless, it is important to make the test results clinically meaningful by focusing on clinical differences between and to define subjects and controls.

Newer technologies now allow for point-of-care non-invasive testing for endothelial function, without the need for a fully trained paediatric vascular technician. Peripheral arterial tonometry is one such technology. This is a simple, user-friendly test, conducted at the point of care. Peripheral arterial tonometry (PAT) has been endorsed by the Food
and Drug Administration in the USA for the assessment of endothelial function (139). This test seems to be increasing in popularity, with increasing numbers of published papers and it has been used to measure endothelial function in adult (140) and paediatric populations (114, 141).

Clinical Studies Measuring endothelial function with Digital PAT

Studies have examined the relation of PAT hyperaemia ratio (RH-PAT) to traditional and novel cardiovascular risk factors and to traditional and novel measures of endothelial function (142). Changes in RH-PAT after short-term interventions have also been measured.

In patients undergoing coronary angiography, lower RH-PAT correlated with the presence of coronary endothelial dysfunction measured by acetylcholine response (126). Two studies reported a modest relation between RH-PAT and brachial flow-mediated dilatation (128, 143). Thus, in small studies, digital vascular function appears to be associated with endothelial function in the brachial artery. However, the RH-PAT response largely reflects vasodilator responses in digital microvessels, whereas brachial artery flow-mediated dilation measures conduit artery vasodilation. Microvascular function as assessed by RH-PAT has the potential to evaluate a distinct vascular response from conduit vessel flow-mediated dilation.

Several studies have examined the relation of clinical cardiovascular risk factors with the RH-PAT hyperaemic response. Some authors reported that RH-PAT is progressively low (ie increasingly abnormal) with increasing numbers of cardiovascular risk factors (128). In patients without obstructive coronary artery disease undergoing coronary angiography, increasing BMI and decreasing high-density lipoprotein (HDL) were associated with lower RH-PAT in unadjusted analyses (126). Adolescents with type 1 diabetes mellitus also had lower RH-PAT responses compared to their healthy peers (114, 128).

The presence of clinical cardiovascular disease is associated with impaired digital vascular function. Individuals with evidence of ischemia on cardiac stress testing had lower PAT hyperemic responses than those individuals without ischemia(128). In an ambulatory setting, patients with coronary artery disease had lower RH-PAT than individuals without established coronary disease(144).
It is important to note that PAT in children is not equivalent to PAT in adults. There are some data indicating that PAT changes as children mature during adolescence (145). Furthermore, RH-PAT scores of 1.35 or less in adults detect coronary endothelial dysfunction (with 80% sensitivity and 85% specificity) (126). In healthy children, particularly in pre-pubertal children, it is common to find RH-PAT scores that are as low as in adults with cardiovascular disease (145).

Notwithstanding its limitations, and its novelty, PAT remains an exciting tool for measuring endothelial function and dysfunction in children.

Structured care for the secondary prevention of cardiovascular disease is now standard practice in primary care of adult patients

In recent years, the secondary prevention of cardiovascular disease has been identified as a population health priority in Ireland (146). ‘Heartwatch’ has demonstrated the feasibility of structured chronic illness management programmes in facilitating secondary prevention of cardiovascular disease and has led to the establishment of disease registers in primary care to enable the identification of patients with confirmed diagnoses of ischaemic heart disease (38). In addition, complex interventions to improve the care of patients with ischaemic heart disease attending primary care have demonstrated health gain (147). As a result of these initiatives, identification of patients with established ischaemic heart disease to enable screening for related morbidity is now feasible (148).

It is noteworthy that, at this point, broadly-speaking, with the exception of screening for children with certain diseases, eg type 1 diabetes mellitus and Turner Syndrome, there are no structured guidelines in place in Ireland for screening for early vascular and metabolic risk or early atherosclerosis in children in Ireland. With regards to children whose parents have premature cardiovascular disease, there is scant literature regarding their risks and there are no Irish guidelines to screen or monitor these children. They do appear to have metabolic and vascular risk. Further work is required.
Summary of Literature Review

Atherosclerosis is a disease of the large arteries. In westernized societies, atherosclerosis is thought to be the leading cause of cardiovascular diseases and cerebrovascular diseases and atherosclerosis is thought to be the underlying cause of about 50% of all deaths (1). In 2005, CVD was the principal cause of death in Ireland with 5,064 (19%) cases of heart disease, 2,029 (7%) cases of heart disease, and 2,891 (10%) cases of other heart disease or diseases of the circulatory system (7).

The PDAY (Pathobiological Determinants of Atherosclerosis in the Young) study, published in 1999, demonstrated that the atherosclerotic process progresses with age (see figure 2) (3). Of relevance to paediatric medicine, the PDAY study also identified pathological evidence of atherosclerosis, albeit early atherosclerosis, in children as young as 15 years, which was the lower age limit for study inclusion (3).

It is now understood that the progressive nature of atherosclerosis includes some earlier steps in the process that are, importantly, reversible. Reduced bioavailability of endothelial nitric oxide (NO) produced from endothelial NO synthase (eNOS) play a crucial role in the development and progression of the atherosclerotic process (9). This reduced bioavailability of endothelial NO is referred to as endothelial dysfunction, and this is a reversible step in the atherosclerotic process (10). Following on from endothelial dysfunction, the subsequent, but still reversible, step in the atherosclerotic process is the build-up of lipid in the intimal layer of blood vessels. If reversibility of early atherosclerosis and endothelial dysfunction is achieved, it might be possible to prevent or delay or reverse atherosclerosis development.

Assessments of endothelial function and impaired endothelial function can be difficult in clinical practice, but the use of various surrogate measures of endothelial function is becoming accepted in a widespread manner (14).

It is now understood that atherosclerosis is a chronic inflammatory condition that can deteriorate quickly into an acute clinical event, due to the rupture and thrombosis of an arterial atherosclerotic plaque. In keeping with this paradigm shift in the understanding of the aetiology of atherosclerosis, there has been a parallel paradigm shift in the approach to managing atherosclerosis, with increasing emphasis on identifying the early
stages of atherosclerosis, targeting specifically the early and reversible stages of the process. Metabolic risk and vascular risk are terms used to summarise the multitude of risk factors which predispose to the development of atherosclerosis.

Premature cardiovascular disease in adults is likely to be a familial trait, inherited from parents but equally passed on to children (17, 18). If an atherosclerotic event in the family member occurs at a younger age, the larger the likely risk factor for the child.

The updated 2008 AAP guidelines for cholesterol screening in children used the age defining parental or grandparental history of premature cardiovascular disease to ≤55 years of age for men and ≤65 years of age for women. Children and adolescents who have a family history of premature cardiovascular disease are at increased risk of having high blood cholesterol levels as adults and increased risk of coronary heart disease (22). Such children are recommended for selective lipid screening in the context of regular health care (22). There are currently no other screening tests recommended for this population of children, only lipid screening. Recently, surrogate makers of atherosclerosis have also identified increased risk in children of parents with premature cardiovascular disease, for example these children have been demonstrated to have increased carotid intima media thickness (cIMT) (26). Additionally, young adults with a parental history of premature cardiovascular disease have evidence of impaired endothelial function (27) and higher cholesterol than controls (24).

There have not been many published studies evaluating children and adolescents of parents with premature cardiovascular disease to date (28-31).

Atherosclerosis has been described as “a pediatric problem” (4), and a “pediatric nutrition problem” (4), by which it was meant that atherosclerosis is associated typically with adults, but begins during the childhood years. Post-mortem studies of young soldiers during the Korean war (32) and the Vietnam war (33) provide evidence for these claims. These studies examined youth thought to be healthy before they suffered sudden death during these respective wars, and identified post-mortem evidence of atherosclerosis in these apparently healthy youth. Subsequently, the PDAY study are charged with demonstrating that antemortem cardiovascular risk factors such as hypercholesterolemia and hypertension are directly correlated with the postmortem size of arterial fatty streaks and fibrous plaques in youth (34, 35). The Bogalusa Heart Study,
which included both epidemiological and opportunistic pathological studies, and concluded that both post-mortem coronary artery fatty streaks and aortic fatty streaks were positively associated with LDL cholesterol (LDLc) and negatively associated with HDL cholesterol (HDLc) measured during life (36). Thus, the evidence had begun to accumulate that atherosclerosis begins in childhood.

Atherosclerotic cardiovascular disease is not usually clinically apparent in childhood in “normal” children. Some children have diseases known to accelerate the “normal” atherosclerotic process: e.g., homozygous familial hypercholesterolaemia; obesity; Turner syndrome. Children whose parents have premature cardiovascular disease are another group that could be at risk.

In recent years there has been a dramatic rise in childhood obesity and related the secondary dyslipidemia (characterised by hypertriglyceridaemia) (44-46). There is a strong association between childhood obesity and the metabolic syndrome (dyslipidemia, hypertension and insulin resistance), all of which lead to atherosclerosis (44). It is established that childhood obesity, and indeed excess weight that is still in the normal range, is associated with the risk of cardiovascular disease in adulthood (47).

Several studies describe higher total cholesterol, higher triglycerides and higher HDLc (48) as well as higher systolic, diastolic and mean blood pressure (BP) and resting heart rate (51) with a loss of the normal circadian rhythm of diastolic BP (52) in cohorts of paediatric patients with Turner syndrome compared to non-Turner syndrome healthy controls.

Atherosclerosis rarely causes morbidity in “normal” childhood. This implies that detecting atherosclerosis in children is more difficult. It can be identified by looking for risk factors or by using proxy measures of end-stage, symptomatic atherosclerosis.

There is also a compelling argument that the origins of atherosclerosis and metabolic risk and vascular risk, including obesity, hypertension and dyslipidaemia, actually have their origins before childhood – in the antenatal milieu, in the health of the mother and even in the health of the father before birth. This is the concept of foetal programming, or metabolic programming.
The thrifty genotype concept suggests that later health, including weight, of a baby is dependent in part on the intra-uterine milieu in which it develops, and also in turn to maternal health antenatally and even pre-pregnancy. The intrauterine antenatal milieu and birth weight are examples of vascular and metabolic risk factors for an individual’s future risk of atherosclerosis, as well as obesity and hypertension and diabetes risk, and these risks are non-modifiable. The literature does suggest strongly that atherosclerosis begins even before childhood, in the antenatal period or in foetal programming, based on parental health or ill-health.

Risk factors for cardiovascular disease includes risk factors which are either “modifiable” or “non-modifiable”. Examples of modifiable risk factors are smoking, diet and exercise – these can all be changed by the subject. On the other hand, one’s family history, birth weight and maternal health prior to and during pregnancy are non-modifiable. There is no equivalent in paediatric clinical medicine to scores such as the Framingham Score (59, 60) in adult medicine.

Metabolic Syndrome is a clustering of risk factors for diabetes and heart disease, including insulin resistance (63), abdominal obesity, hypertension, and dyslipidemia. Non-alcoholic fatty liver disease is now also recognized as an increasing clinical problem in children with risk factors for the metabolic syndrome, and may progress to hepatic cirrhosis, and end-stage liver disease in predisposed individuals(64). The US Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescent released guidelines regarding childhood dyslipidemia in 2012 (22). These guidelines refer to primordial prevention (the prevention of risk factors developing) and primary prevention (recognition and management of those children in whom identified risk factors are already present) (22).

Recognised risk factors for atherosclerosis in children include family history of acquired heart disease, especially positive family history of premature cardiovascular disease. Other risk factors for atherosclerosis in children include smoking, dyslipidaemia, hypertension, hyperglycaemia increased BMI, poor physical fitness and insufficient active time (71).

The Bogalusa Heart Study and the PDAY study have both demonstrated increased atherosclerosis at postmortem examination in young people with antemortem
hypertension (34, 37). In 2008 (84) and again in an update in 2014 (85), the AHA published guidelines on monitoring for hypertension in children, using 24 hour ambulatory blood pressure monitoring (24ABPM), as opposed to intermittent BP evaluations. The end stages of atherosclerosis itself cannot be identified in paediatrics, and so surrogate markers of cardiovascular disease must be evaluated.

Dyslipidaemia is a feature of the metabolic syndrome and the term implies any abnormality of the cholesterol profile. The typical atherogenic cholesterol profile includes high levels of LDLc, low levels of HDLc, high triglycerides and high total cholesterol. Hypertriglycerideridemia is the dyslipidemia primarily associated with obesity. The prevalence of dyslipidaemia is increasing. A study evaluating data on 10,000 healthy children from the NHANES 1999-2006 cohort study, identified that 5.2-6.6% of adolescents had abnormal LDLc levels (86). The 2008 AAP guidelines recommend targeted screening for paediatric dyslipidemia, by testing a complete fasting lipid profile every 3-5 years, from the age of 2 years onwards, and interpreting these profiles using age- and gender-specific norms.

There is an increasing amount of data showing that being overweight during childhood and adolescence is significantly associated with insulin resistance, dyslipidaemia, and elevated BP in young adulthood. On the basis of current knowledge and extrapolation from studies in adults, it is reasonable to suggest that lifestyle modification and weight control in childhood could reduce the risk of developing the insulin resistance syndrome, type 2 diabetes mellitus, and cardiovascular disease.(91)

Obesity is a confounding variable which accelerates metabolic ill-health. Obesity is one risk factor that is predictive of atherosclerosis in adults, and it is increasingly recognised to be predictive in children also. Obesity is a major risk factor for chronic diseases such as diabetes and heart disease (93, 94). Obesity evolves during childhood and tracks to adulthood (95, 96). Irish estimates from 2001-2002 are that 23% of boys and 28% of girls are overweight or obese (97).

The link between smoking, both active and passive tobacco exposure, and atherosclerosis in adults is well-described (98-103). In children, information on tobacco exposure in history taking is not frequently collected; one study cited rates of
documentation of assessment and counseling for second-hand smoke exposure occurs as low as 4% of pediatric health supervision visits (104).

Endothelial dysfunction is associated with smoking (106) and tobacco exposure (107) as well as obesity (108). Improvement in endothelial dysfunction has been demonstrated in obese children after regular exercise (109). Novel techniques for the assessment for vascular dysfunction, may help us to detect early abnormalities that could potentially contribute to risk for later disease.

Methods to assess subclinical atherosclerosis have developed in recent years (110), these include: coronary artery calcification scoring by computerized tomography; measurement of the carotid artery intima media thickness by ultrasound (cIMT); endothelial dysfunction, manifest by reduced arterial dilatation measurement by ultrasound (e.g., Brachial Artery Reactivity [BAR]) or novel methods (e.g., peripheral applanation tonometry [PAT] as used in this study); increased left ventricular mass on echocardiography.

High BP is the leading risk factor–related cause of death throughout the world, accounting for 12.8% of all deaths, 51% of stroke deaths, 45% of coronary artery disease deaths (WHO: A global brief on Hypertension 2013). Hypertension in children is particularly concerning, as with any chronic disease that presents in childhood, because it exposes the child to risks over their lifetime. Hypertension and prehypertension are associated with endothelial dysfunction (119), an early step in the atherosclerotic process in children and adolescents. There are no published studies to date evaluating ABPM in children whose parents have premature cardiovascular disease.

The 2012 Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report by the NHBLI guidelines (22) address the utility of non-invasive vascular assessment in children with a family history of cardiovascular disease. The following statement is included in Chapter 2:

“Children and young adults with a family history of myocardial infarction have increased cIMT, higher prevalence of coronary calcium, and endothelial dysfunction (22).”
This statement is supported by reference to 5 published studies; all 5 have flaws (eg all include siblings) and none seem to entirely support the statement in the NHBLI guidelines by investigating the relationship between endothelial function and parental premature cardiac disease in the paediatric age group. Further research in this area is clearly warranted to assess this assertion in a scientific, rigorous manner, perhaps by addressing only 1 child per family and by including only paediatric age range subjects and controls.

Impaired endothelial function and its measurement/quantification can be viewed as a surrogate marker of cardiovascular and metabolic risk assessments (126-129).

Flow-mediated dilatation is the gold standard assessment of endothelial function in children (FMD) (131). In children, brachial artery reactivity is reproducible and is a good surrogate measure of atherosclerosis (133).

Ultrasound of the artery is used to measure the thickness of the intima media layer of the vessel wall, at different sites of the arterial tree, most frequently at or proximal to the carotid bulb. In several large studies, intima media thickness correlates well with traditional cardiovascular and metabolic risk factors including BP, cholesterol profile and smoking (or not) and it is predictive of future end-stage atherosclerosis, including myocardial infarction and cerebrovascular accident (134, 135). Understanding of disease progression in paediatrics, and relating exactly thickness measurements in childhood with cardiovascular outcomes in adulthood are lacking.

Peripheral arterial tonometry is a new technology that allows for point-of-care non-invasive testing for endothelial function, without the need for a fully trained paediatric vascular technician. In small studies, digital vascular function appears to be associated with endothelial function in the brachial artery. However, the RH-PAT response largely reflects vasodilator responses in digital microvessels, whereas brachial artery flow-mediated dilation measures conduit artery vasodilation. Microvascular function as assessed by RH-PAT has the potential to evaluate a distinct vascular response from conduit vessel flow-mediated dilation. Some studies reported that RH-PAT is progressively low (ie increasingly abnormal) with increasing numbers of cardiovascular risk factors (128). PAT in children is not equivalent to PAT in adults. In healthy children, particularly in pre-
pubertal children, it is common to find RH-PAT scores that are as low as in adults with cardiovascular disease (145).

With the exception of screening for children with certain diseases, eg type 1 diabetes mellitus and Turner Syndrome, there are no structured guidelines in place in Ireland for screening for early vascular and metabolic risk or early atherosclerosis in children in Ireland.
The aims of this study:

Our hypothesis was that the metabolic and vascular profiles of children whose fathers had premature cardiovascular disease are measurably different when compared to an age-matched healthy cohort, and that these differences place the children whose fathers had premature cardiovascular disease at increased vascular and metabolic risk.

The research question underpinning this project was:

“Are the metabolic and vascular profiles of children whose fathers had premature cardiovascular disease measurably different when compared with an age-matched healthy cohort?”

The aims of this study were:

1. to evaluate cardio-metabolic profiles in a cohort of children known to be at increased risk of cardiovascular disease as young adults, i.e. the children whose fathers had premature cardiovascular disease; and
2. to compare the cardio-metabolic profiles of these children to a cohort of age-, gender-, and BMI-matched healthy children, who do not have a significant family history of premature cardiovascular disease.

The specific objectives of this research study were:

a. to conduct assessments and inter-group comparisons of risk factors, including novel assessments for metabolic and vascular risk, to the knowledge of the study group, not previously performed in this cohort of children
b. to evaluate the subjects for risk factors, in addition to premature cardiovascular disease, which may place them at further increased risk of development of early atherosclerosis.

The results of this study may influence the evaluation and management of healthy children whose fathers have premature cardiovascular disease.
Chapter 2  Methods
Overview

This is a cross-sectional study comparing healthy children whose fathers have had premature cardiovascular disease, defined as either myocardial infarction or coronary artery bypass grafting, before aged 56 years [subjects, n=38 included and tested] and children of healthy parents, free from clinical cardiovascular disease [controls, n=25 included and tested]. This is an exploratory pilot study, designed to explore physiological differences between the two populations, and to generate hypotheses as the groundwork for future studies.

The research was conducted on campus at the Children’s Ark, Department of Paediatrics at University Hospital, Limerick and was funded and supported by the National Children’s Research Centre, Crumlin, Dublin 12. There was no dedicated clinical research space in this facility, and so the paediatric day ward clinical space was used for testing. This implied that the research subjects could only attend when a bed space was not required for a patient. To achieve this, the majority of research visits were scheduled over the weekends.

This research was a collaboration between The Children’s Ark, Department of Paediatrics at University Hospital Limerick and the Graduate Entry Medical School, University of Limerick.

Study population

Definition of subjects and controls

For the purposes of our study, subjects were identified and defined as they had a paternal history of premature cardiovascular disease, where the fathers had a myocardial infarction or had an angioplasty or coronary artery bypass graft performed before 56 years of age. The subjects themselves had no known significant illnesses at the time of their study participation. Thus, subjects were identified based on the health of their parents. Adult parents with premature cardiovascular disease were identified by examining the records of the cardiac rehabilitation team at University Hospital Limerick, by identifying those who: 1. had a coronary event; 2. had survived; 3. were within the age range for premature cardiovascular disease at the time of their event; and
4. had children who met the age limits for inclusion in our study. Due to the age requirements of both parent and child, we found that there were no appropriately aged mothers in the cardiac rehabilitation program. Thus, our inclusion criteria were refined to only include the children of fathers who had premature cardiovascular disease.

Controls were identified as the children were healthy, and the parents (specifically, the fathers) had no known, clinically-apparent cardiovascular disease at the time of participation in the study. There was no age limit placed on the fathers. In practice, controls were volunteers who presented for the research study. Most controls were friends of the subjects, recruited by the subject children’s parents.

**Inclusion Criteria for Subjects**

- Male or Female
- Where the subjects father had myocardial infarction or required angioplasty or coronary artery bypass grafting before the age of 56 years.
- Age 8-14 years (minimum 8 years in order to comply with study protocol).
- Ability to speak English and to comply with study protocol.
- Only 1 sibling per family was eligible for inclusion.

**Exclusion Criteria for Subjects**

- Past medical history of cardiovascular diseases.
- Known disorders of insulin, glucose or cholesterol in a child.
- A history of type 1 diabetes mellitus or type 2 diabetes mellitus or familial hypercholesterolemia in the affected parent that pre-dated the cardiac presentation.
- Known other chronic diseases in either parent or child. (Possible exceptions to this criterion included mild bronchial asthma, or other disorders that are common in
childhood and are not thought to increase the lifetime risk of cardiometabolic disorders.)

- Use of medications that may interfere with metabolism of insulin, glucose or cholesterol.
- Pregnancy or hormone-based contraception
- Alcohol and/or illicit drug use.
- A sibling already included in the study.

**Controls**

Controls were age-matched to the study subjects.

**Inclusion criteria for controls:**

- Male or Female
- Age 8-14 years (minimum 8 years in order to comply with study protocol).
- Ability to speak English and to comply with study protocol.
- Only 1 sibling per family was eligible for inclusion.

**Exclusion criteria for controls:**

- A biological parent who has a history of early ischaemic heart disease (defined as < age 56 years in father or < age 66 years in mother).
- Other exclusion criteria as per for subjects including only 1 sibling per family included.
Sample Size & Power Calculations

Insulin sensitivity was the primary outcome measure. Estimates of whole body insulin sensitivity index measured by oral glucose tolerance testing (OGTT) in children and adolescents vary according to pubertal status, age, gender and BMI. Reports of insulin sensitivity in moderately obese youth, using the same technique as described in this proposal, vary from 1.96 to 2.25 with standard deviations 0.15 to 1.25 (149),(150). Assuming α 0.05, power 0.80 and mean 2.0 and SD 0.7, recruitment of 17 patients in each group would be required to detect approximately 25% difference in Si between groups (ie insulin sensitivity difference of approximately 0.5). Therefore, we aimed to recruit 40 patients, 20 in each group, to allow for participant attrition.

Further analysis suggested that this sample size would also allow the study to be adequately powered to detect differences that might exist between groups for RH-PAT score (based on the EndoPAT test for endothelial dysfunction). This is based on the following studies (114, 151) which identified RH-PAT scores of 2.06-2.08 with standard deviations 0.32-0.4 in healthy controls and 1.64-1.78 and standard deviations 0.34-0.4 in subject populations. Using these data, and assuming α 0.05, power 0.80, 16 children in each of the control group and the subject group will provide power to detect a difference of 0.3 in RH-PAT between subjects and controls. This appears to be a clinically meaningful difference in PAT, based on the published literature(114, 141, 151).

Variables measured

There was a detailed schedule of variables to be measured during each child patient’s visit. These variables included: information in the medical history; details of the physical examination including anthropometrics; blood tests including oral glucose tolerance test; ultrasound including carotid intima media thickness; testing for endothelial function with EndoPAT; and 24 hour ambulatory blood pressure monitoring.
A detailed proforma was completed for each subject and control and a blank sample copy of this is provided in the appendix.

Variables:

Anthropometrics, medical history, family history, exercise habits, cIMT were recorded.

Fasting lipids, lipoproteins, glycosylated haemoglobin (HbA1c) and fibrinogen were measured. OGTT was performed, measures of insulin sensitivity were derived.

Carotid Intima Media Thickness was measured. Endothelial function was assessed by peripheral applanation tonometry and RH-PAT recordings were generated.

24-hour ambulatory blood pressure recordings were performed. Blood pressure indices were calculated to control for gender and height [BPI(height)] as well as gender and age [BPI(age)].
Description of Clinical Visit, Metabolic & Vascular Studies

1) History:

This was obtained from parent and subject, and supplemented by review of medical chart if necessary. Required data included: age, past medical history (e.g. hypothyroidism, coeliac disease, liver dysfunction, cardiac disease [exclusion criterion for all subjects and controls]), medication history, family history of cardiovascular disease (inclusion criterion for subjects and exclusion criterion for controls), type 2 diabetes mellitus, lipid disorders. Where needed, confirmation of premature cardiovascular disease was obtained from the cardiac rehabilitation team.

2) Physical Exam:

Systolic and diastolic blood pressure were recorded as the mean of 3 readings taken 1 minute apart in the left arm in the seated position using a Dinamapp device and appropriately sized cuff. Values were interpreted using appropriate age and gender normative curves(152) and blood pressure indices to correct for age and gender were calculated. Puberty was assessed using the staging method of Tanner (153, 154).

3) Anthropometric and body fat assessment:

A standard, calibrated scale and wall-mounted stadiometer will be used to measure weight and height, and BMI [calculated as wt in kg/(ht in metres)²]; waist and hip circumferences (measured in duplicate to the nearest mm using a plastic tape). These techniques predict adiposity in children and adolescents (155, 156)
4) Glucose and Insulin Dynamics:

Following overnight fast, an OGTT (1.75 mg/kg (max 75 g) was performed and venous blood obtained at 0, 30, 60, 90 and 120 minutes to screen for impaired glucose tolerance and type 2 diabetes(157). Insulin sensitivity (Si) will be measured by the Matsuda Model where $Si = \frac{10,000}{\sqrt{\text{fasting glucose} \times \text{fasting insulin}}} \times \text{mean glucose} \times \text{mean insulin during OGTT})$ (158). This is well validated against gold-standard measures of insulin sensitivity in adults and in children with ($r = 0.67 - 0.78$)(158, 159). Insulin secretion was evaluated at each time point and acute insulin secretion, a measure of β-cell function will be calculated from the insulinogenic index ($\frac{\text{30 min insulin} - \text{fasting insulin}}{\text{30 min glucose} - \text{fasting glucose}}$), which correlates in adults and children with hyperglycemic clamps and predicts development of type 2 diabetes mellitus in adults (160, 161).

5) Carotid Intima-Media Thickness:

Using antero-oblique insonation, B-mode imaging of the far-wall was optimized and end-diastolic images recorded in the right and left common carotid artery (20 to 60 mm proximal to the flow divider), in the carotid bifurcation (0 to 20 mm proximal to the flow divider) and in the internal carotid artery (0 to 20 mm distal to the flow divider). Carotid intima-media thickness was then measured off-line using semi-automated software (QLab IMT quantification, Philips Medical Systems, Andover, MA), ensuring a success rate >95% and a standard deviation of < 10% for the detection algorithm.

6) Peripheral Applanation Tonometry (PAT):

PAT is a non-invasive test which uses pneumatic probes, similar in shape to a thimble, which cover the fingertip and apply a uniform pressure field which allows for the measurement of the pulsatile oscillations of the digital vascular bed microcirculation. Minute changes, as detected by PAT, are correlated with the gold standard of coronary endothelial function. Higher PAT-HR indicates normal endothelial function, but lower PAT-HR scores are indicative of greater endothelial dysfunction. PAT-HR shows a strong sensitivity and specificity for the detection of coronary endothelial function, which has
been shown to predict cardiovascular events in adult patients (126, 129). PAT has been used previously as a surrogate marker of early endothelial function in paediatric populations (114, 141). PAT probes are placed on the index finger of each hand. After a 5 minute equilibration period, a blood pressure cuff is inflated on the study arm 40 mmHg above systolic for 5 minutes. The cuff is then deflated and tonometric recording is completed for an additional 5 minutes. The PAT-HR was determined for each patient and each recording was analyzed individually.

The EndoPAT™ device (Itamar Medical, Israel) consists of a hardware device with two probes placed on the finger tips of the subject. These thimble-like probes include a system of inflatable neoprene membranes within a rigid external case. These internal membranes are set to inflate to 10 mm Hg below diastolic pressure or 70 mm Hg (whichever value is lower.) When inflated these membranes apply a uniform pressure field to the finger tips, and measure the pulse waves in the microcirculation of the finger tips. A blood pressure cuff is placed on one upper arm (study arm), while the contralateral arm serves as a control (control arm). PAT records, non-invasively and at point-of-care, the changes in amplitude of the pulse wave form in the finger tips during 3 phases: 1) baseline; 2) occlusion (of the pulse wave by inflation of the blood pressure cuff); and 3) hyperaemia (period of vascular dilatation which occurs following release of the blood pressure cuff). Recordings are taken simultaneously from both fingers throughout the study. The control finger is that from which only baseline measurements are taken, i.e., the blood pressure cuff is not inflated or deflated on the control arm (see Table 1: The EndoPAT procedure). The pulse waves recorded in the control finger can be used to adjust for systemic effects (e.g., temperature, distraction, movement artefact). After the baseline phase, a blood pressure cuff is inflated on the test arm to 10 mm Hg above systolic blood pressures for 5 minutes. During this phase of occlusion, signals are absent from the test finger but continue from the control finger. The blood pressure cuff is then released rapidly, and pulse amplitude increases in the hyperaemetic finger; this is the phase of hyperaemia. The pulse wave amplitude recordings are analyzed automatically by copyrighted calculations. The average pulse amplitude is calculated over 30-seconds intervals for up to 5 minutes prior to and after cuff occlusion.

The PAT hyperaemia ratio (RH-PAT) score calculated automatically by the software, is the mathematical ratio of of the post-to-pre occlusion PAT amplitude of the study arm,
divided by the post-to-pre-occlusion ratio of the control arm. RH-PAT is therefore dependent on recordings from both the baseline and blood pressure cuff inflation phases of the test.

The augmentation index is also automatically calculated as an average from multiple pulses using the following formula:

\[
\frac{(P2-P1)}{P1} \times 100
\]

where \( P1 \) = the peak pressure of the recorded pulse wave and

\( P2 \) = the pressure of the inflection point corresponding to the arrival of the reflected waves.

Small changes in RH-PAT correlate well with the gold standard of coronary endothelial function (133).

PAT continues to be tested and validated in various patient populations (both adult and paediatric). In more than 2,000 eligible participants in the Framingham Heart Study, the rate of technically interpretable studies was over 90% (140). The major reasons for technical inadequacy in the other (<10%) studies included were incomplete cuff occlusion (3%), noisy signal quality (3%) and incomplete data acquisition (3%). There are some patient-specific factors which limit test performance, including Raynaud's disease, a finger size that does not match the fingertip probe, and a very long fingernail. Small finger size, intolerance to minor discomfort and inability to lie still for 15 minutes, may be reasons for inability to conduct PAT in young children.

Owing to computerized analysis approach, inter-observer measurement variability is minimized. Published data remain limited but the automated calculation of RH-PAT appears to be reproducible. In two small studies of healthy adults, there was evidence supporting the reproducibility of the PAT hyperemic ratio measured on two days (127) (162). In 44 children with type I diabetes mellitus, the mean coefficient of variation for RH-PAT measured 4 weeks apart was 14.8% (141) and in 15 healthy children the coefficient of variation measured 10 weeks apart was 14% (163).
7) **Ambulatory Blood Pressure Monitoring:**

The Spacelabs ambulatory blood pressure monitor 90207 was used, to measure and record 24 hour ambulatory blood pressure monitoring on our subjects and controls. The recordings were then downloaded onto a secure research laptop and analysed. The 2014 American Heart Association Update in Ambulatory Blood Pressure Monitoring in Children was followed to calculate Blood Pressure Indices for height [BPI (height)] and age [BPI (age)].

8) **Other labs:**

Fasting triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were measured at the time of the start of the oral glucose tolerance test. HbA1c (glycosylated haemoglobin), fibrinogen and alanine aminotransferase (ALT) levels were also measured.
Protocols

Fasting triglycerides (TG), total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and apolipoproteins were measured by standard enzymatic methods. Non-HDL cholesterol was calculated using the formula:

\[
\text{Non-HDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol}
\]

General venepuncture information

A total of 10.6 mL of blood was drawn (8.5 mL for the oral glucose tolerance test and 2.1 mL for the other assays). This is within the approval by the Research Ethics Board. Study participants subjects were counselled that they might experience a small amount of bleeding when blood was taken from a vein and that they might have slight discomfort and bruising or redness that would most likely disappear within a few days. Although each participant had already given informed consent (or parental consent & child assent), participants were given the opportunity to decline venepuncture.

Blood collection times and volumes

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose</strong></td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td><strong>Cholesterol assays</strong></td>
<td>0.6 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Research Questions

1. Do children whose fathers have premature cardiovascular disease (subjects) have impaired insulin sensitivity compared with controls? (Primary Question)
   
a. Does insulin sensitivity correlate with carotid intima media thickness / PAT abnormalities in either / both groups of children?

   (Study was powered to answer this question. The remaining questions were designed to be hypothesis-generating.)

2. Do subjects have increased carotid intima media thickness compared with controls?
   
a. Does this alter with the age or BMI?

3. Do subjects have increased prevalence or increased number of features of metabolic syndrome compared with controls?

4. Do subjects have evidence of altered RH-PAT scores compared with controls?
   
a. Do RH-PAT scores correlate with increased prevalence or increased number of features of metabolic syndrome in either / both groups of children?
Statistics

Summary statistics were used to compare baseline characteristics between subjects and controls. Continuous variables (such as insulin sensitivity) were compared between groups using the paired t-test and discrete variables will be compared by Fisher’s exact test and chi-squared analysis. Univariate linear regression analyses evaluated the significance of variables (eg age, BMI, gender, LDLc, adiponectin, carotid intima media thickness, etc) to the outcomes of interest (ie Si). Due to the large number of potential variables, and relatively small patient population, significance will be accepted at a level of p <0.05. Multivariate linear regression was performed to evaluate the potential contribution of variables found to be significant in univariate regression analyses, along with other variables known to contribute to Si. Secondary outcomes including lipid profiles, were analyzed by paired t-test or Wilcoxon Signed Rank test.

Statistical Methods

Counts (percentages) are presented for categorical data. Numeric variables were tested for normality and are summarised as mean (standard deviation) for normally distributed variables or geometric mean (95% confidence interval) for log-transformed normally distributed variables. Median (IQR) is presented for skewed distributions. An independent samples t test was used to compare means across groups (controls, subjects) and a Mann-Whitney test was used to compare medians. A Chi-square test or Fisher’s exact test was used to compare proportions across groups. A 5% level of significance was used for all statistical tests and no adjustments were made for multiple testing. A general linear model was fitted to the data to predict outcomes using group (controls, subjects) as a predictor variable after adjusting for BMI-SDS (BMI standard deviation score). R squared was used as a measure of goodness of fit.

Statistical analysis was carried out using SPSS Version 21 for Windows and SAS Version 9.2.
Potential Risks and Benefits

Potential risks
Blood work involved a pinch/poke which may cause transient pain/discomfort.
PAT caused some transient pain/discomfort during blood pressure cuff inflation.
Each subject and control needed to be fasting until after the oral glucose tolerance tests and this can cause some discomfort and irritability.

The total length of the visit is long, approximately 5 hours for 1 subject and 6.5 hours for 2 subjects. This often led to the children being tired.

There is always a risk in a clinical research study that an unexpected abnormality will be detected. A plan was made to refer any child with an abnormality for further investigations and management, as required.

Potential benefits
We anticipated that children who are at risk of ischaemic heart disease may have be identified as having an additional risk factor, ie evidence of early physiological changes of atherosclerosis, or insulin resistance or dyslipidaemia. We anticipated that in these early stages, these changes may be reversible. However, as this is a novel area of research, longitudinal studies may be required to establish whether these changes are indeed reversible.

It is difficult to interpret the results of PAT studies at this stage, as it is a novel test. But results were communicated to the best of our interpretation / knowledge.
Chapter 3 Results
Subjects had higher BMIs than controls (p=0.01) although differences in BMI-SDS did not reach statistical significance (p=0.24). The differences in waist/height ratios were significant (p=0.05) whilst waist/hip ratio differences were not (p=0.35). We used our statistical analysis to adjust for BMI-SDS [with the exceptions of the BPI(height) and BPI(age) analyses where we adjusted for waist/hip ratio instead].

There was no difference in insulin sensitivity (p=0.85) between subject and control groups. Differences in fasting glucose (p=0.19) did not reach statistical significance. Fasting lipids and lipoproteins were similar.

Serum alanine aminotransferase levels were lower in subjects than in controls (p=0.009).

Compared with controls, study subjects have lower RH-PAT scores (p=0.01) indicating relative endothelial dysfunction, and they have higher mean diastolic blood pressures (p=0.02). The Mean Arterial Blood Pressure (MAP) difference did not reach statistical significance (p=0.06) but the age-adjusted MAP blood pressure index [BPI (age)] (p=0.03) did. Differences in mean systolic blood pressure (p=0.20) and c-IMT (p=0.25) did not reach statistical significance.

Sixty-three families were recruited. Where more than 1 child per eligible family was recruited and tested, an individual child per family was selected, based on the availability of the majority of their blood samples. Outcomes by group (n=25 controls, n=38 subjects) are summarised below.
Demographics & Anthropometrics

Sixty-three children in total were tested, including 38 subjects and 25 controls. There was no significant difference in age or gender distribution of subjects and controls. There was a higher proportion of boys in the subject group (23 out of 38, or 60.5%) than controls (14 out of 25, or 56%).

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of Male subjects</th>
<th>Number of Female subjects</th>
<th>Number of Male controls</th>
<th>Number of Female controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 years old</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>9 years old</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>10 years old</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>11 years old</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12 years old</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>13 years old</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2: Ages and genders of subjects and controls

The subject and control groups were of similar heights (p=0.97), weights (p=0.92) and waist/hip ratios (p=0.35). Subjects’ waist/height ratios were significantly higher than controls (0.47 ± 0.10 vs 0.43 ± 0.05, p=0.05). BMI was significantly higher in subjects (18.7kg/m2 ± 5.04 vs 17.3kg/m2 ± 2.45, p=0.01) but mean BMI in each group was within the normal range. BMI-SDS was not different between groups (0.42 ± 0.99 vs 0.13 ± 0.89, p=0.24).

These anthropometrics describe 2 populations that appear to be healthy, not overweight, acceptable normal BMI-SDS and waist/height ratios less than the required 0.5.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.3 (1.84)</td>
<td>11.2 (1.70)</td>
<td>0.84</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (56.0%)</td>
<td>23 (60.5%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Female</td>
<td>11 (44.0%)</td>
<td>15 (39.5%)</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.47 (0.13)</td>
<td>1.47 (0.11)</td>
<td>0.97</td>
</tr>
<tr>
<td>Weight (kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.2 (16.70)</td>
<td>39.6 (18.46)</td>
<td>0.92</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.88 (0.07)</td>
<td>0.91 (0.09)</td>
<td>0.35</td>
</tr>
<tr>
<td>Waist/height ratio</td>
<td>0.43 (0.05)</td>
<td>0.47 (0.10)</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3 (2.45)</td>
<td>18.7 (5.04)</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI-SDS</td>
<td>0.13 (0.89)</td>
<td>0.42 (0.99)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 3:
Comparison of demographic and anthropometric variables by group. Data are presented as mean (SD) or n (%). Statistical analysis was used to adjust for BMI-SDS.
**Glucose and Insulin Sensitivity**

There were no differences in fasting glucose, fasting insulin, HbA1c or multiple measures of insulin sensitivity (If/Gf CA, If/Gf US, HOMA, Matsuda ISI3, IGI 303, WBISI (Matsuda) S.I. 3, ISSI-2, AUCins3, AUCglu3) between subjects and controls. These measures are not statistically different between groups, nor are there any clear clinically meaningful differences between groups. The fasting glucose and HbA1c mean and standard deviations showed normal values for all subjects and controls.

No subject or control was diagnosed with impaired fasting glucose or impaired glucose tolerance on oral glucose tolerance testing.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.72 (0.36)</td>
<td>4.87 (0.32)</td>
<td>0.10</td>
<td>0.19</td>
</tr>
<tr>
<td>Fasting Insulin (pmol/L)b</td>
<td>50.18 (39.55, 63.65)</td>
<td>54.06 (45.92, 63.65)</td>
<td>0.59</td>
<td>0.99</td>
</tr>
<tr>
<td>If/Gf CA&lt;b</td>
<td>10.66 (8.53, 13.32)</td>
<td>11.13 (9.48, 13.07)</td>
<td>0.74</td>
<td>0.84</td>
</tr>
<tr>
<td>If/Gf US&lt;b</td>
<td>0.08 (0.07, 0.10)</td>
<td>0.09 (0.07, 0.10)</td>
<td>0.74</td>
<td>0.84</td>
</tr>
<tr>
<td>HOMA&lt;b</td>
<td>1.46 (1.13, 1.89)</td>
<td>1.63 (1.37, 1.93)</td>
<td>0.47</td>
<td>0.85</td>
</tr>
<tr>
<td>HbA1c (IFCC units)</td>
<td>35.29 (1.99)</td>
<td>35.84 (2.85)</td>
<td>0.42</td>
<td>0.36</td>
</tr>
<tr>
<td>Matsuda ISI3</td>
<td>7.06 (5.68, 8.76)</td>
<td>6.24 (5.42, 7.19)</td>
<td>0.32</td>
<td>0.71</td>
</tr>
<tr>
<td>IGI 303</td>
<td>19.99 (14.04, 28.46)</td>
<td>21.20 (15.85, 28.37)</td>
<td>0.79</td>
<td>0.97</td>
</tr>
<tr>
<td>WBISI (Matsuda) S.I. 3</td>
<td>11.13 (8.98, 13.78)</td>
<td>9.89 (8.59, 11.39)</td>
<td>0.33</td>
<td>0.73</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>332.61 (109.58)</td>
<td>307.79 (96.78)</td>
<td>0.37</td>
<td>0.56</td>
</tr>
<tr>
<td>AUCins3</td>
<td>1073.49 (861.39, 1337.52)</td>
<td>1175.17 (1011.35, 1365.53)</td>
<td>0.47</td>
<td>0.87</td>
</tr>
<tr>
<td>AUCglu3</td>
<td>23.98 (22.47, 25.59)</td>
<td>24.90 (23.38, 26.52)</td>
<td>0.42</td>
<td>0.52</td>
</tr>
</tbody>
</table>

**Table 4:** Comparison of measures of insulin secretion, insulin sensitivity, glucose excursions and diabetes risk between subject and control groups. Statistical analysis was used to adjust for BMI-SDS (which is specific for age, gender, height and weight). Data are given as mean (standard deviation) or median (IQR).
Fasting Cholesterol and Lipid Profiles

Statistical analysis was used to adjust for BMI-SDS (which is specific for age, gender, height and weight). We found no differences in any of our fasting lipid indices (Cholesterol, HDLc, LDLc, Non-HDLc, Triglycerides, Non-HDLc/HDLc ratio, Triglycerides/HDLc ratio) between subjects and controls after this adjustment.

Fasting measures did not identify fasting hypercholesterolaemia in any individual subject or control; that is, there was no clinically meaningful abnormal cholesterol results detected.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Cholesterol (mmol/L)</td>
<td>4.03 (0.55)</td>
<td>4.15 (0.56)</td>
<td>0.40</td>
<td>0.45</td>
</tr>
<tr>
<td>Fasting HDLc (mmol/L)</td>
<td>1.43 (0.31)</td>
<td>1.35 (0.31)</td>
<td>0.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Fasting LDLc (mmol/L)</td>
<td>2.21 (0.50)</td>
<td>2.26 (0.69)</td>
<td>0.79</td>
<td>0.96</td>
</tr>
<tr>
<td>Fasting Non-HDLc (mmol/L)</td>
<td>2.60 (0.54)</td>
<td>2.80 (0.63)</td>
<td>0.20</td>
<td>0.29</td>
</tr>
<tr>
<td>Triglycerides&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51 (0.41, 0.64)</td>
<td>0.52 (0.43, 0.63)</td>
<td>0.93</td>
<td>0.72</td>
</tr>
<tr>
<td>Non-HDLc/HDLc ratio&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82 (1.57, 2.10)</td>
<td>2.07 (1.83, 2.34)</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
<td>Trig/HDLc ratio&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37 (0.28, 0.49)</td>
<td>0.39 (0.31, 0.49)</td>
<td>0.70</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Table 5: Fasting cholesterol and lipid profiles. Data are presented as mean (standard deviation) for fasting cholesterol, fasting HDLc, fasting LDLc, fasting non-HDLc. Data are presented as geometric mean (95% confidence interval for the mean) for triglycerides, non-HDLc/HDLc ratio, trig/HDLc ratio.
**Fibrinogen and Liver function Tests**

Fibrinogen levels were similar. The geometric mean alanine aminotransferase (ALT) levels were lower in subjects than controls (16.96 vs 20.67 IU/L, p=0.009) whilst other liver function tests (ALP, GGT & AST) were similar. All liver function test results, including ALT, were within the normal range.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for BMI Z score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.72 (0.42)</td>
<td>2.93 (0.54)</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>ALT (IU/L)(^b)</td>
<td>20.67 (17.97, 23.78)</td>
<td>16.96 (15.66, 18.36)</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>185.4 (59.44)</td>
<td>196.5 (58.94)</td>
<td>0.48</td>
<td>0.41</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>10.5 (2.06)</td>
<td>11.2 (2.72)</td>
<td>0.32</td>
<td>0.34</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>31.1 (8.23)</td>
<td>30.6 (5.88)</td>
<td>0.78</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^b\) geometric mean (95% confidence interval for the mean)

**Table 6:** Comparison of fibrinogen, ALT and other LFTs between subjects and controls. Statistical analysis was used to adjust these variables for BMI-SDS (which is specific for age, gender, height and weight). Data are presented as mean (standard deviation) other than ALT which is presented as geometric mean (95% confidence interval for the mean).
Figure 5: Boxplots of ALT by group
cIMT and RH-PAT

c-IMT levels, measured to the 0.1 millimeter, were similar (p=0.25). While there are no accepted normal values for c-IMT in paediatrics, these cIMT values are similar to values published in the literature for normal, healthy children.

RH-PAT scores were significantly lower in subjects compared with controls (1.64 ± 0.51 vs 2.00 ± 0.53, p=0.01) indicating relative endothelial dysfunction. Again, there are no accepted normal values for RH-PAT in the literature but these values are similar to published literature in normal, healthy children. In other studies, however, a difference in RH-PAT of this magnitude in children is considered meaningful.

Statistical analysis was used to adjust for BMI-SDS (which is specific for age, gender, height and weight).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value Adjusted for BMI Z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-IMT (cm)</td>
<td>0.05 (0)</td>
<td>0.05 (0.02)</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>RH-PAT</td>
<td>2.00 (0.63)</td>
<td>1.64 (0.51)</td>
<td><strong>0.02</strong></td>
<td><strong>0.01</strong></td>
</tr>
</tbody>
</table>

**Table 7:** Comparison of cIMT and RH-PAT between controls and subjects. Data are presented as mean (standard deviation).
Figure 6: Boxplots of RH-PAT by group
Ambulatory Blood Pressures Measurements

Statistical analysis was used to adjust for BMI-SDS (which is specific for age, gender, height and weight). Mean diastolic ambulatory BP measurements were significantly higher in subjects compared with controls (68.6mmHg ± 4.68 vs 65.4mmHg ± 4.90, p=0.02). Differences in mean systolic ambulatory blood pressure (115.1mmHg ± 7.74 vs 112.0mmHg ± 7.60, p=0.20) and ambulatory MAP (84.1mmHg ± 4.80 vs 81.3mmHg ± 5.21, p=0.06) did not reach statistical significance.

The mean BP in the subject and control groups was normal. One subject had high blood pressure for age, gender and height and was referred for tertiary paediatric follow-up. It is difficult to say if a blood pressure increase of 3 mm Hg in each of systolic and diastolic blood pressure in subjects compared with controls is clinically meaningful. Long-term studies would be required to evaluate for this.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Systolic BP (mmHg)</td>
<td>112.0 (7.60)</td>
<td>115.1 (7.74)</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean diastolic BP (mmHg)</td>
<td>65.4 (4.90)</td>
<td>68.6 (4.68)</td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81.3 (5.21)</td>
<td>84.1 (4.80)</td>
<td><strong>0.04</strong></td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 8: Ambulatory blood pressure monitoring measures. Data are presented as mean (Standard deviation).
Ambulatory Blood Pressure Indices[height]

The calculation of blood pressure indices[height] adjusts for gender and height so these results were not further adjusted for BMI Z-score (which also adjusts for gender and height). Instead, statistical analysis was used to adjust for age and waist/hip ratios. Mean diastolic ambulatory BPI[height] measurements were significantly higher in subjects compared with controls (0.90 ± 0.06 vs 0.86 ± 0.07, p=0.03). Differences in mean systolic ambulatory BPI[height] (0.94 ± 0.06 vs 0.92 ± 0.06, p=0.11) and ambulatory MAP BPI[height] (0.93 ± 0.06 vs 0.90 ± 0.06, p=0.09) did not reach statistical significance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value Adjusted for age &amp; waist/hip ratios</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Systolic BPI[height]</td>
<td>0.92 (0.06)</td>
<td>0.94 (0.06)</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Mean Diastolic BPI[height]</td>
<td>0.86 (0.07)</td>
<td>0.90 (0.06)</td>
<td><strong>0.03</strong></td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>MAP BPI[height]</td>
<td>0.90 (0.06)</td>
<td>0.93 (0.06)</td>
<td>0.09</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Table 9:** Comparison of Blood Pressure Indices for height (BPI[height]) between subjects and controls. Data are presented as mean (standard deviation).
Ambulatory Blood Pressure Indices [age]

The calculation of blood pressure indices [age] adjusts for gender and age so these results were not further adjusted for BMI-SDS (which also adjusts for gender and age), instead statistical analysis was used to adjust for waist/hip ratios. Mean diastolic ambulatory BPI[age] measurements were significantly higher in subjects compared with controls (0.91 ± 0.06 vs 0.87 ± 0.06, p=0.02). Ambulatory MAP BPI[age] measurements were also significantly higher in subjects compared with controls (0.93 ± 0.06 vs 0.90± 0.06, p=0.03). Differences in mean systolic ambulatory BPI[age] (0.93 ± 0.06 vs 0.90 ± 0.06, p=0.09) did not reach statistical significance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=25)</th>
<th>Subjects (n=38)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Systolic BPI[age]</td>
<td>0.90 (0.06)</td>
<td>0.93 (0.06)</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean Diastolic BPI[age]</td>
<td>0.87 (0.06)</td>
<td>0.91 (0.06)</td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>MAP BPI[age]</td>
<td>0.90 (0.06)</td>
<td>0.93 (0.06)</td>
<td><strong>0.04</strong></td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>

Table 10: Comparison of Blood Pressure Indices for age (BPI[age]) between subjects and controls. Data are presented as mean (standard deviation).
**Figure 3** Boxplots of mean diastolic BP by group

**Figure 7:** Boxplots of MAP by group
Chapter 4    Discussion
This was an exploratory, pilot study comparing anthropometric, physiological and early metabolic and vascular risk in subject children whose fathers had premature cardiovascular disease and control children (limited to 1 included child per family) of healthy parents, without known cardiovascular disease. The study protocol developed for this study utilized a combination of accepted markers of cardiovascular risk (including carotid IMT, blood pressure and insulin sensitivity) as well as emerging markers (including PAT). This allows for robust interpretation of the results, with emerging markers being interpreted in the context of established markers. The following discusses some of the results of this research project and thesis, in the context of the published literature, under the subheadings: anthropometrics; diabetes and metabolic risk tests; cardiovascular risk tests.

**Anthropometrics**

Subjects and controls were well-matched for age, height, weight, waist-to-hip ratio, and BMI-SDS. Waist-to-height ratio was statistically significantly higher in subjects (p=0.05) and BMI was statistically significantly higher in subjects (p=0.01). To our knowledge, these data have not previously been presented in children in this age group.

One small Polish study assessed dietary intake in 54 young adults (males aged 21-38 years, mean age 25.8 years and females aged 19-39 years, mean age 28.2 years) whose parents had premature ischaemic stroke, identified that these offspring, compared to controls, ate an insufficient, unbalanced and monotonous diet, which the authors felt would contribute to their own risk of atherosclerosis (164). In our study, we did not measure dietary intake but this would be an interesting avenue for future related research. There have been no previous studies to our knowledge measuring diet in children of parents with premature cardiovascular disease.
**Diabetes and metabolic risk tests**

Subjects compared to controls had similar fasting glucose and insulin sensitivity; similar fasting lipid profiles; lower ALTs ($p=0.009$).

Metabolic tests did not demonstrate differences between children with paternal history of premature cardiovascular disease and controls. Literature searching has not yielded any previous published results on comparing insulin and glucose or ALT measures between children with and without a parental history of premature cardiovascular disease.

We did not find evidence to support targeted lipid screening based on a paternal history of premature cardiovascular disease. This is interesting, as the current guidelines suggest using lipid screening to monitor for risk in our subject population (22, 23).

Cholesterol measures have been evaluated in similar groups previously. Firstly, a recent Irish study evaluated cholesterol profiles in young adults who had a positive family history of premature cardiovascular disease and identified high cholesterol levels in these young adults compared to healthy controls (24). Secondly, a study based in India also identified dyslipidaemia in children with a family history of premature cardiovascular disease compared with healthy controls (165). Thirdly, in one older study, higher LDLc levels were associated with a family history of premature cardiovascular disease, but lipoprotein(a) levels were not associated with this history (166). This was a study of laboratory levels of cholesterol measures, and the numbers were reasonably small ($N=27$ children with a positive family history, $N=42$ with a negative family history of premature cardiovascular disease). Perhaps the laboratory-based population sample explains the reason that this study identified differences
between cohorts, while our sample did not. Also, this study does not comment on inclusion or exclusion of sibling pairs.

Finally, interpreting ALT values in paediatric populations is difficult, particularly when trying to interpret to evaluate high normal ALTs for non-alcoholic fatty liver disease and metabolic risk. One study (167) evaluated for upper limit of normal ALTs in children and adolescents, all of whom were healthy without known risk factors (i.e. not known to have a family history of premature cardiovascular disease, for example). This study found a linear relationship between age and ALT in females (p<0.001) but not in males. By multiple logistic regression, independent predictors of an elevated ALT included the BMI, waist hip ratio and levels of serum total cholesterol. With respect to our study, the mean ALT values were all within the normal reference range, and comparison between subjects and controls included controlling for BMI-SDS (which therefore controls for age, gender, height and weight). So the implication in interpreting ALT in our study is that, given that ALT was lower in subjects, they are unlikely to have NAFLD, and thus do not have this feature of the metabolic syndrome and this cannot therefore be a contributor to any other differences between groups. Put briefly, it eliminates NAFLD as an aetiology in the impairments of cardiovascular tests in subjects compared with controls.

**Cardiovascular risk tests**

Subjects compared to controls had similar cIMTs; lower RH-PAT scores (p=0.01), indicating relative endothelial dysfunction; similar mean systolic AMBP; higher mean diastolic AMBP (p=0.02); higher mean diastolic BPI [height] (p=0.03); higher mean
diastolic BPI [age] (p=0.02); higher MAP blood pressure index [age] (p=0.03).
Differences in mean AMBP (p=0.06) and MAP BPI [height] (p=0.09) did not reach statistical significance.

Cardiovascular risk testing results suggest early, impaired vascular health relative to controls in healthy children whose fathers have premature cardiovascular disease; specifically, there were significant abnormalities in PAT testing and blood pressure recordings. To the best of our knowledge, this is the first study to evaluate endothelial function by PAT and 24 hour ambulatory blood pressure measurements in children with a parental history of premature cardiovascular disease.

Some noteworthy aspects of the results include that we did not identify differences in fasting cholesterol measures, even though professional groups had suggested using fasting cholesterol as a screening tool for investigating kids whose parents had premature cardiovascular disease. Furthermore, the professional groups describe differences in cIMT in kids whose parents have premature cardiovascular disease but this study does not support that these differences exist, and it is the only study we are aware of which includes only 1 child per family. We believe that these results may form the initial evidence which will call for a new paradigm for screening children of parents with premature cardiovascular disease.

PAT has been used as a surrogate marker of early impaired endothelial function in paediatric populations (114, 141) and, in adolescents it has been validated in a repeated measures study (168). Inter- and intra-observer variability studies have shown the technique of PAT to be simple and reproducible (169). When performed at point-of-care, compared with tests performed in a vascular laboratory, it is less likely to cause anxiety to young children. It is not operator-dependent, which can also minimise
anxiety, as it can be performed by a professional known to the child. Both of these factors contribute to relatively lower costs of this test compared with vascular tests, which require a dedicated laboratory and highly-skilled vascular personnel. This technique has been used extensively in paediatric age range populations and is well tolerated (114, 141).

Children and adolescents who have a family history of premature cardiovascular disease are at increased risk of having high blood cholesterol levels as adults and increased risk of coronary heart disease (22). Such children are recommended for selective lipid screening in the context of regular health care (22). This is the rationale for targeted cholesterol screening in childhood on the basis of a family history of premature cardiovascular disease. There are currently no other screening tests recommended for this population of children, only lipid screening.

The 2012 publication from the NIH NHLBI Expert Panel on Integrated Guidelines for Cardiovascular Risk Reduction in Children and Adolescents (22) asserts that there is moderately strong evidence (grade B evidence) from observational studies to warrant strongly supporting the “inclusion of a positive family history of early coronary heart disease in identifying children at risk for accelerated atherosclerosis and for the presence of an abnormal risk profile”. Furthermore, the recommendations include updating family history information at intervals, as a child ages, and their family history information may change (22).

Previous studies using high-resolution (measured to one thousandth of a centimetre) B-mode ultrasonographic determination of carotid intima media thickness had demonstrated statistically significantly higher measurements in subjects versus controls (31, 121, 170). This study used standard B-mode cIMT measurements (measured to one
hundredth of a centimetre) and results were similar in subjects and controls (p=0.25). It would have been interesting to have had high-resolution B-mode cIMT measurements to compare. With hindsight it would have been very interesting to further interrogate and compare the vascular functions of our subjects and controls. Brachial artery reactivity (BAR) determination is considered the “gold-standard” for assessing endothelial function. We could have explored this in more detail if we had had the ability to measure BAR in our subjects and controls and compare it to our RH-PAT data. The results support further assessment of vascular function and blood pressure in similar populations. It would also be exciting to reassess these children as they approach adulthood.

**Other factors evaluated in the literature, which may be of relevance to this population.**

A recent study evaluated estimated glomerular filtration rate in adults whose parents had a history of premature cardiovascular disease (defined as before the age of 50 years) and identified that parental history of premature cardiovascular disease was associated with an estimated glomerular filtration rate in the offspring (adults) which was: (1) lower at baseline; (2) higher odds of decreasing further; and (3) a faster rate of decline(124). We did not measure the same parameters, but the authors of this study postulate that the glomerular filtration rate may be of help in future screening programs for this vulnerable cohort. Furthermore, another study in adults with a family history of premature cardiovascular disease have higher C-reactive protein and greater carotid intima media thickness than adults without this family history(171).
Adult patients with psoriasis are themselves at increased risk of cardiovascular disease and they have a higher prevalence of premature parental cardiovascular disease; in a recent study, in particular, adults with psoriasis had a higher prevalence of premature maternal myocardial infarctions and a higher prevalence of paternal stroke (172).

In the Framingham Generation 3 cohort, premature parental cardiovascular disease is associated with measures of coronary artery calcification, and with abdominal aortic calcification, particularly in young middle-aged adults (123). The magnitudes of these risks are bigger for paternal than for maternal premature cardiovascular disease. In this study, 797 Framingham offspring were studied using computed tomography to detect coronary and aortic calcification. Conversely, Sundquist identified increased risks to offspring for cardiovascular disease for maternal history of disease, rather than for paternal history of disease(173). Importantly, this study did not refer to premature parental cardiovascular diseases, except to highlight that the highest standardized incidence ratios for cardiovascular disease occurred in the youngest subjects, among those with both parents affected by cardiovascular disease or those with a single parent with premature cardiovascular disease. This is explicit in quantifying higher risks related to even 1 parent with premature disease.

While intima media thickness of the carotid artery is most commonly performed, in one large recent study, children with type 1 diabetes mellitus had thicker aortic intima media thickness than controls, without any differences in carotid intima media thickness (174).

One study has tried to quantify risks to family members of developing any of cardiovascular heart disease, ischaemic stroke, peripheral arterial disease and aortic disease, when a proband family member develops one of these diseases (175). This study identified that across all four disease entities, the risks of any of the four diseases
were increased for each of unaffected siblings, offspring and spouses. With respect to offspring, the highest risk category was the risk of premature cardiovascular disease in the offspring, if the parent had premature cardiovascular disease. But the offspring had increased standardized incidence ratios for each of cardiovascular heart disease, ischaemic stroke, peripheral arterial disease and aortic disease. Thus, this study identified end-stage increased risk of premature cardiovascular disease in an adult population whose own parents had premature cardiovascular disease; in effect, these are the adult versions of the children in our study & we certainly seem to have identified early evidence of these risks. This study was conducted on a large patient registry, with 140,708 cases of cardiovascular disease, and the study definition of premature cardiovascular disease was consistent with ours, but this study also included premature maternal cardiovascular disease (<55 years for males and <65 years for females). This large registry database study, when interpreted with the findings of our small pilot exploratory study, would certainly suggest that further research is justified and that these studies are and should change the way that we look at, measure, interpret and intervene in cardiovascular risk in young people.

The expert group on hypertension consensus statement maintains that addressing hypertension is key to addressing these cardiovascular risks in the future for growing children who have cardiovascular risk: accurate diagnosis, treatment and prevention of hypertension in children thus appear to be key to primary prevention of cardiovascular as these children become adults (22).

Our data reported in this thesis, demonstrates evidence of increased BP using various different measures, and in particular using 24ABPM which appears to give reliable BP measurements, certainly supports the approach of diagnosing and treating BP. While our
study did not address the results of reductions on BP and this certainly needs to be performed and evaluated, our study does support definite increased BP in children and adolescents with a paternal history of premature cardiovascular disease. Our study refutes the relevance of using fasting cholesterol, as we did not find any differences in fasting cholesterol between subject and control groups, but we did find increased higher BP in subjects than in controls. This underpins the importance of further exploration of BP evaluation and management in this population.

Another recent study in an adult population has examined, using a yearly questionnaire, whether self-reported healthy behaviours (healthy weight, regular exercise, moderate alcohol consumption, abstaining from smoking) can decrease the risks of heart failure in adults whose parents had a history of premature cardiovascular disease (176). The authors identified that compared to subjects with good lifestyle scores and no parental history of premature cardiovascular disease, multivariate adjusted hazard ratios for heart failure with antecedent myocardial infarction were 3.21 (95% CI 1.74-5.91) for subjects with good lifestyle scores and a positive parental history of premature cardiovascular disease, were 1.52 (95% CI 1.12-2.07) for subjects with poor lifestyle score and no parental history of premature cardiovascular disease, and 4.60 (95% CI 2.55-8.30) for subjects with poor lifestyle scores with positive parental history of premature cardiovascular disease. Put differently, compared to the “healthiest” group with good lifestyle and no family history of note, a poor lifestyle and no family history was the next healthiest group. The groups with a positive family history of premature cardiovascular disease were of the poorest health, with improved hazard ratios in the group which had better lifestyle scores. Thus, even a good lifestyle does not negate the risks implicit in having a parent with premature cardiovascular disease. Thus, much
work remains to be done. But this study suggests that future interventions will have to focus on factors that include but are not limited to healthy lifestyle. Such factors might include revised and earlier introduction of statins or antihypertensives or other pharmaceutical agents, for example.

Limitations

This study has limitations. The power calculations are based on the best available data to calculate numbers needed to investigate, based on data for insulin sensitivity and for PAT testing. Data required for power calculations in children, to power for insulin sensitivity and PAT testing as well as carotid IMT and cholesterol assays in children are limited at best. Notwithstanding, the power calculations are as robust as it was possible to conduct, using the best-available evidence. This study will provide data upon which future power calculations for future studies can be calculated.

We recruited and tested sufficient male subjects and controls to meet our power calculation requirements. Unfortunately, we were underpowered for female subjects, and thus the absence of statistical significance in female subjects compared to male subjects might reflect insufficient numbers tested. However, the initial protocol did not differentiate between male and female subjects or controls and thus the fact that we recruited and tested sufficient subjects and controls to analyse gender separately strengthens this study and its conclusions significantly.

siblings were included in this study, were recruited and tested based on their father having had or not having had premature cardiovascular disease. This can pose a problem in statistical analysis, in that siblings are not random, and statistics can over-
emphasise a factor which is related to the family, but not necessarily to the exposure and outcome of interest. To mitigate this, no siblings were included in the final statistical analyses. It is unfortunate, however, that siblings had already been recruited and tested prior to inclusion. Thus it was expensive and perhaps questionably unfair to involve and test subjects who were later excluded.

A large number of tests were performed on a relatively small number of subjects and controls. Thus, it is possible that some statistically significant differences were identified by chance. However, statistical corrections with Bonferroni were not made for multiple tests. It was chosen to not perform corrections for multiple tests as this is an exploratory pilot study, with very few studies similar to it in the population we studied. In fact, to our knowledge, this is the first study of its kind where only 1 sibling from sibling multiples were included as subjects or controls. Thus, we anticipate that further studies will be performed, both in our research unit and elsewhere, which will seek to replicate and further investigate the results identified in this study.

The aetiology of cardiovascular risk is multifactorial and it is likely that there are many contributing factors which are not yet elucidated. This study does not establish causality. But we are hopeful that it does focus the scientific microscope on some clinically significant variables which may be measurable and may even be ameliorable in paediatric age patients, and which may lead eventually to cardiovascular risk reduction in adults. The investigations which we chose to perform are not exhaustive in the multifactorial aetiology of cardiovascular risk, but they were chosen based on the best available evidence, as well as feasibility and acceptability in a paediatric age study population. Examples of factors which were not explored include parental education level, socioeconomic status.
A significant limitation of this study, and one which might only be addressed by performing a follow-up study, is that it is possible that the parents of some children in the control groups may actually develop apparent cardiovascular disease prior to the age of eligibility for inclusion as subjects, rather than controls. There is very little that could be done to prevent or ameliorate this limitation. It is unreasonable and would be unethical to suggest that asymptomatic parents would have an angiogram done prior to including their children as controls. A follow up study could re-evaluate these parents in due course and re-evaluate the results in the context of any control parents becoming subject parents.

Additionally, there are other differences potentially between the parents in the subject and control groups. But it is beyond the scope of this study to characterize these differences – indeed, this would be for adult physicians as opposed to paediatric physicians to perform. A further limitation of this study, but one that could only be mitigated by continuing this study as a prospective longitudinal cohort study or repeating a cross-sectional cohort study after some years, is that the fathers in the control group could go on to have an acute coronary event before the age of 45 years, thus implying that a child originally classed as a control, would be reclassified as a subject.

Another limitation is that birth history data in this study is incomplete. It would have been interesting to collect and analyse data on parental health, fitness, weight and diet at the time of conception (or its close colleague, the first antenatal visit) and at the time of birth. There are some data now that these have a correlation with health measures in the child. For example, a longitudinal epidemiological study in the United Kingdom, the weight of a boy at the age of 6 years was most strongly predicted by the weight of his
father at the time of the child’s birth (177). It has been acknowledged that collecting
birth information in young children is difficult and flawed (178). The optimum way to
collect accurate birth information would be a prospective longitudinal study, following
a birth cohort, but that was beyond the scope of this research study and thesis.

**Strengths**

To the best of our knowledge, this appears to be the first study of its kind in this study
population, where only 1 sibling in any family (either control or subject) was eligible
for inclusion. Very few studies have been performed in this population. Other previous
studies include undetermined numbers of siblings, and inclusion of siblings can lead to
statistical over-emphasis of findings. Thus, our study is likely to provide more robust
results than similar previous studies, of which there are few.

This is a clinically very important area of research, and one of the first studies to
evaluate any conditions in the emerging field of paediatric preventive cardiology.
Ireland is well-placed as a setting for studies in paediatric preventive cardiology, given
the high rates of cardiovascular disease in Irish adults.

All testing was performed by a single investigator. The MD candidate, Alan Macken,
recruited all patients, performed all clinical examinations, took detailed histories and
performed all the clinical tests on all patients. Ms Joan Egan, an experienced echo
technician performed all CIMT measurements and assisted AM in most of the patient
testing days.

The findings of this study are novel, noteworthy, some are statistically significant and I
believe that those that are statistically significant are also potentially clinically
significant. Further research will be required to confirm these findings. Regardless, the research team thinks that these results might be an initial step towards further investigating the novel paediatric origins and potentially paediatric interventions for this important condition.

**Comparison with data from other paediatric populations**

Turner Syndrome occurs when all or part of one of the two X chromosomes is deleted or defective – thus it affects girls exclusively. Turner syndrome has a wide range of clinical characteristics, but one of the most common is congenital heart disease. In recent years, accelerated atherosclerosis, manifesting as early, acquired (as opposed to congenital) heart disease and early cerebrovascular disease, have been increasingly recognized in girls with Turner syndrome. The atherosclerotic process in Turner syndrome starts early (48) and the epidemiological data suggests that there is 3-fold increased risk of mortality from cardiovascular and cerebrovascular disease (49). Epidemiological data suggest that adult women with Turner syndrome are twice as likely to develop coronary artery disease, compared to the general population (50). Several studies have evaluated the metabolic risk and vascular risk that may predispose to the increased and accelerated atherosclerotic process in Turner syndrome. These studies describe higher total cholesterol, higher triglycerides and higher HDLc (48, 179, 180) as well as higher systolic, diastolic and mean BP and resting heart rate (51) with a loss of the normal circadian rhythm of diastolic BP (52) in cohorts of paediatric patients with Turner syndrome compared to non-Turner syndrome healthy controls.
Publication bias is a factor worth considering in critiquing the literature on this topic. When the data are not available, consensus statements are produced. There are several consensus statements that are now available, which, to varying degrees, affect the population being studied in this research (84, 85, 88), of which some were published after this thesis work was commenced. Consensus statements are often required because the evidence is limited. This is a problem in this field of work. Additionally, various studies of medications, notably statins, are supported by pharmaceutical industry sponsorship and the financial transactions to support clinicians’ research can be evidenced in the disclosure statements of studies, including these consensus statements. Of course, we rely on clinicians’ and researchers’ own codes of ethics in reporting their work, but in a discussion of publication bias in this field, the possible distracting effects of pharmaceutical support must be noted. Finally in publication bias, the literature review for this research and thesis yielded few negative published studies. It would be interesting to conduct a thorough and comprehensive systematic review in this area, to identify potentially missing, unreported studies.

The use of BMI centiles and standard deviation scores (SDS, or also known as Z-scores) is common in paediatric clinical practice. BMI as an absolute number has limited use in paediatrics, as it is expected that BMI will change as the child grows and gains weight. Instead, BMI should be interpreted in the context of centiles and/or standard deviation scores. In this study, BMI was converted to BMI-SDS. With respect to the data in this research study, Wang describes 3 reasons why BMI-SDS is better than BMI (see table 10, reproduced from Wang et al, 2012 (181)). Firstly, SDS-scores are calculated based on the distribution of the reference population (both the mean and the standard deviation [SD]); and thus, they reflect the reference distribution. Secondly, given that they are
standardized measures, SDS-scores are comparable across age and gender. Thirdly, a
group of SDS-scores can be subject to summary statistics such as mean and SD and can
be studied as a continuous variable. There are other arguments for using BMI-SDS in
place of BMI. Firstly, SDS-scores can quantify the growth status of children outside of
the percentile ranges. Secondly, the BMI-SDS is better to track & interpret changes, in
an individual child or in a population of children, in BMI over time, as the BMI itself is
supposed to change as a child ages – i.e., there are not simple BMI cutoffs for
overweight and obesity in children, as exist in adults. Notwithstanding, BMI-SDS-
scores are difficult to conceptualise and to explain to the public. The World Health
Organisation and US Centers for Disease Control and Prevention have developed
statistical software to help researchers to calculate BMI-SDS.
Table 10: Adapted from Wang et al, 2012(181). Comparison of centiles and Z-scores in anthropology in paediatrics.
Chapter 5  Conclusions
Healthy children with a paternal history of premature cardiovascular disease have similar insulin sensitivity to healthy matched controls. Subjects and controls have similar fasting lipid profiles. Subjects ALTs were lower than controls, other liver function tests were similar. Subjects RH-PAT scores were lower than controls indicating relative endothelial dysfunction. Subjects diastolic ambulatory blood pressure measurements were higher compared with matched controls.

Fasting lipid profiling did not identify the differences between children with paternal history of premature cardiovascular disease and controls. Contrary to the 2011 NHBLI guidelines and the 2008 AAP recommendations, this study does not provide evidence to support targeted lipid screening based on a paternal history of premature cardiovascular disease.

Further studies are required but these data suggest early impaired vascular health and raised diastolic blood pressures in well young children whose fathers have premature cardiovascular disease.

To our knowledge, this study was the first of its kind, to evaluate risk in children whose parents who had early heart disease.
Future direction of related research, health policy and clinical practice

This work forms a novel part of the new subspecialty of paediatric preventive cardiology. There is increasing understanding that cardiovascular illness is caused by risk factors and behaviours that originate early in life, and even are related to parental health, a form of inter-generational health which is difficult to ameliorate. In adult medicine, preventive cardiology and obesity strategies are the key in tackling this epidemic in heart disease and stroke. National initiatives such as the Royal College of Physicians of Ireland Policy Group on Obesity and the recent launch of The National Institute for Preventive Cardiology are taking on the challenges that these risk factors represent to the health of the Irish population.

Paediatric preventive cardiology is an emerging field, based on the imperative to address the epidemic of cardiovascular disease risk factors in children. It is well-recognized that cardiovascular risk factors and health behaviours have their origins during early childhood, and have been directly related to an acceleration of the atherosclerotic process predicted to lead to early manifestation of cardiovascular disease in adults. Paediatric programs in North America are developing preventive cardiology programs, which serve both the general paediatric population, but also those children with congenital heart disease and other chronic high risk conditions. The field encompasses risk factors (lipids, blood pressure and obesity), risk conditions (diabetes, inflammatory diseases, survivors of childhood cancer, Kawasaki disease patients) and risk behaviours (smoking, nutrition, physical inactivity and sleep disorders).
The 2012 NHBLI Expert Panel on Integrated Guidelines for Cardiovascular Risk Reduction in Children and Adolescents guidelines are being implemented as national policies across the USA, including the introduction of non-fasting lipid screening for all children from ages 9 to 11, and again from 17 to 21, along with targeted lipid in high-risk groups at other ages. The prevalence of atherosclerotic heart disease in adults in Ireland is high and this places an imperative on Irish medicine and Irish clinical research to address this risk, and part of addressing this risk, is to develop the subspecialty of preventive cardiology to assess and address risks in Irish children. The increasing burden of cardiovascular risk factors in Irish children and adolescents must be addressed, with a national concerted multi-faceted approach, including public health, paediatrics and cardiology responses, incorporating the best elements of the North American experience and applying these to the Irish population.
References
References:


7. office Cs. Mortality from cardiovascular disease (CVD) i.e. from coronary heart disease, stroke, and other disease of the circulation. 2006.


## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
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<tr>
<td>ABPM</td>
<td>Ambulatory blood pressure measures</td>
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<td>AHA</td>
<td>American Heart Association</td>
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<td>ALT</td>
<td>Alanine aminotransferase</td>
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<td>BAR</td>
<td>Brachial Artery Reactivity</td>
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<td>BMI-SDS</td>
<td>BMI standard deviation score</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>BPI</td>
<td>Blood Pressure Index</td>
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<td>BSA</td>
<td>Body Surface Area</td>
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<tr>
<td>CHD</td>
<td>Congenital Heart Disease</td>
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<td>cIMT</td>
<td>Carotid Intima Media Thickness</td>
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<td>CKD</td>
<td>Chronic kidney disease</td>
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<td>CVA</td>
<td>Cerebrovascular accidents</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<tr>
<td>FMD</td>
<td>Flow-mediated dilatation</td>
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<td>HbA1c</td>
<td>Glycosylated haemoglobin</td>
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<td>HDLc</td>
<td>High density lipoprotein cholesterol</td>
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<tr>
<td>hsCRP</td>
<td>highly sensitive C-Reactive Protein</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
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<tr>
<td>IR</td>
<td>Insulin Resistance</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>LDLc</td>
<td>Low density lipoprotein cholesterol</td>
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<td>MAP</td>
<td>Mean arterial pressure</td>
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<td>MI</td>
<td>Myocardial Infarction</td>
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<td>MS</td>
<td>Metabolic Syndrome</td>
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<td>NAFLD</td>
<td>Non-Alcoholic Fatty Liver Disease</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NHBLI</td>
<td>National Heart Blood Lung Institute</td>
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<tr>
<td>NIH</td>
<td>National Institutes for Health</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthetase</td>
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<tr>
<td>PAT</td>
<td>Peripheral Applanation Tonometry</td>
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<tr>
<td>PCD</td>
<td>Premature Cardiovascular Disease</td>
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<tr>
<td>PDAY</td>
<td>Pathobiological Determinants of Atherosclerosis in the Young</td>
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<tr>
<td>Si</td>
<td>Insulin Sensitivity</td>
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<tr>
<td>TIA</td>
<td>Transient ischaemic attack</td>
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<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<td>SBP</td>
<td>Systolic Blood Pressure</td>
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Study Protocol: Description of the day when the subject/ control attended for testing

09:00 Arrival at the hospital, having been fasting for the previous 12 hours.

Meet study co-ordinator.

Re-read and sign consent (parent and/or older child) and assent (child) forms.

Check height and weight, calculate BMI (kg/m²).

Estimated time required: 30 minutes

09:30 Vascular testing with measurement of: carotid IMT.

PAT (peripheral applanation tonometry) testing performed.

Estimated time required: 30 minutes

10:00 Intravenous line insertion.

Fasting bloodwork drawn: cholesterol profile, insulin, glucose, HbA1c, ALT, Fibrinogen.

Oral glucose tolerance test with lozozade, with insulin and glucose assays drawn at 0, 30, 60, 90, 120 minutes.

Also during the time taken to perform the OGTT, subjects will undergo a full history and physical examination, including measurement of waist and hip circumferences, and measurement of skin-fold thickness using calipers.

Estimated time required: 150 minutes

12:30 24 hours blood pressure recording devices given to parents & use explained. These were posted back / otherwise returned by parents subsequently.

13:00 Lunchtime & home.

Thank you letter and thank you token given to child and parents.
Consent Form for Children Participating in Research

**Title of Research Project:**

Evaluation of early metabolic and vascular risk in children of parents with early ischaemic heart disease.

**Principle Investigators:**

Prof. Clodagh O’Gorman, Chair of Paediatrics, UL  
Prof. Colum Dunne, Director of Research, UL

**Co-investigators:**

Dr. Alan Macken  Research Fellow in Paediatrics, UL  
Prof. Walter Cullen  Chair of General Practice, UL  
Dr. Michael O’Neill  Consultant Paediatrician, Mayo General Hospital  
Dr. Brendan Meaney  Consultant Cardiologist, MWRH Limerick  
Ms Catriona Aherne  CNS in Cardiac Rehabilitation, MWRH, Limerick

**Purpose of the Research:**

Acquired heart disease is common in Ireland. In adults, it causes heart attacks and angina. Recognised risk factors for acquired heart disease include family history of acquired heart disease, smoking, overweight, decreased physical fitness, high blood pressure and high cholesterol. There are some studies examining the heart and vessels of children and these suggest that even young children can have evidence of acquired heart disease. The only risk factor that many of these children is a family history of heart disease. It is possible that they may have more risk factors in the future, but by then, the irreversible processes of vessel changes may have started. We are interested in trying to identify these changes of early acquired heart disease in young children. If we can identify these changes, we can follow these children for progression of heart disease. And, at some stage, we may consider treatments to alter the process of acquired heart disease in these children.

We aim to evaluate children, aged between 8-13 years, of parents who had early heart attacks or heart bypass surgery. We will compare the results to children whose parents do not have known early heart disease. We will look for differences between the two groups in the risk of diabetes, cholesterol, blood pressure, weight and physical fitness. This study will be the first of its kind, to evaluate risk in children whose parents who had early heart disease. It will evaluate whether the children of these parents are at risk during childhood.

**Description of the Research:**

This study will be conducted over one visit. For this study visit, you should arrive fasting. This means nothing to eat or drink, with the exception of water, on the morning of the test.

**Visit**

On this visit you will arrive in the morning to the Mid-Western Regional Hospital, Limerick. You will be weighed and measured and a brief list of questions will be asked (past problems with heart conditions, medications, family history, dietary history). You
will then be brought to the vascular studies laboratory, for ultrasound measurements of
the heart and blood vessels. This will not hurt. Some cold gel will be applied to your
chest, neck and arm and a small probe, that looks like a torch, will be pressed against
your skin. This will not be sore.

Following this, we will bring you to the paediatric day ward. There, we will do another
short heart test. This will take 15 minutes and it will not hurt. We will put a small probe,
like a thimble on your finger, and we will measure your heart beat in your finger. We
will also inflate a blood pressure cuff for about 5 minutes, to see how your heart beat
responds to this. Sometimes, this can be a little uncomfortable. After this test, an
intravenous cannula (IV) will be inserted and a blood sample of approximately 15ml (3
teaspoons) will be taken. EMLA cream, a special cream that numbs the skin for the
needle poke can be applied by the nurse if you wish. Then you will be asked to drink a
cup of sugary drink. This is the test for diabetes. We will then take small amounts of
blood, about 5 mls (1 teaspoon) every 30 minutes for 2 hours through the IV cannula
(this will not hurt). The IV cannula will then be removed and no further blood tests will
be required during this visit.

After these tests, you will be free to have some lunch and take a short break (about 1
hour).

Consent for retention and use of leftover blood samples
I agree to allow any leftover blood samples from my blood samples to be frozen and
used for special tests to measure factors in the blood related to insulin, metabolic,
vascular or muscle disorders.

Yes, with all identifying information (name, hospital no., etc) on leftover blood samples
___________

OR

Yes, if all identifying information (name, hospital no., etc.) is removed___________

OR

No______

Potential Harms, discomforts or inconvenience:

There may be a small amount of bleeding when blood is taken from a vein or when an
IV is inserted into a vein and there may be slight discomfort and bruising or redness that
will usually disappear in a few days. The amount of blood drawn should not cause any
harm. If you would like to use the EMLA cream, this will help minimize any
discomfort. Occasionally, some people report dizziness for a short time after taking
GTN spray. We will monitor you for this.
The risk of early knowledge of the potential for future disease includes additional potential for anxiety and depression. This will be carefully considered and discussed with families when consent is obtained.

Traveling to the hospital for these visits is an inconvenience. We will try to schedule the visit (or visits) to make it (or them) as convenient as possible for your schedule.

**Potential Benefits:**

**To individual subjects:**
After you complete this study, we will be able to provide you with detailed information regarding your risk for diabetes and vascular function. It is possible that we will diagnose a cardiac or insulin or metabolic disorder in some children who may not yet have any symptoms. Earlier detection of disease would allow for treatment, such as dietary and exercise counselling, or in some cases specific medications, such as cholesterol-lowering medications, medications to lower blood pressure or medications for diabetes. We will refer you to whichever specialist is necessary (cardiologist for heart or cholesterol, or to our own endocrinology clinic for diabetes) to discuss this further if needed. Earlier detection of risk for disease will allow for regular monitoring in the endocrine clinic, so that therapy can be instituted in a timely fashion.

**To society:**
This study may help us understand whether children whose parents have had heart disease at a young age show metabolic or vascular differences compared with children whose parents do not have heart disease. If there are differences it may be possible to provide advice or treatment at an early stage which might reduce a child’s risk of having heart disease as an adult.

The results from this study may be presented at medical conferences and published in medical journals to share our findings with others.

**Confidentiality:**
We will respect your privacy. No information about who you are will be given to anyone or be published without your permission, unless the law makes us do this.

For example, the law could make us give information about you:
- If someone has been abused
- If you have an illness that could spread to others
- If you or someone else talks about suicide (killing themselves), or
- If the court orders us to give them the study papers

The data produced from this study will be stored in a secure, locked location. Only members of the research team will have access to the data. The data will then be destroyed 5 years after the project has ended.

**Reimbursement:**
We will reimburse you for all reasonable expenses incurred (meals, travel, parking) as a result of your child’s participation in this study. We will also give your child a small
thank you gift for taking part in the study. Your child will also receive a letter indicating her time spent as a volunteer participating in research.

**Participation:**
Participation in research is voluntary. It is your choice for you to take part in this study. You can stop at any time. New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study. During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you any of this money now or in the future because you took part in this study.

We will give you a copy of this consent form for your records.

In some situations, the study doctor or the company paying for the study may decide to stop the study. If this happens, the study doctor will talk to you about what will happen next. If you become ill or are harmed because you took part in this study, we will treat you for free. Your signing this consent form does not interfere with your legal rights in any way. The staff of the study, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

**Sponsorship:**
The sponsor of this research is the National Children’s Research Centre.

**Questions:**
If you have any questions about this program please contact Dr. Alan Macken at alanmacken@gmail.com.
Consent:

By signing this form, I agree that:

1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of taking part in this study. I understand that I have the right not to take part in the study and the right to stop at any time. My decision about taking part in the study will not affect my health care.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my medical records will be kept private. You will give no one information about me, unless the law requires you to.
6) I understand that no information about who I am will be given to anyone or be published without first asking my permission.
7) I have read and understood pages 1 to 5 of this consent form. I agree, or consent, to take part in this study.

__________________________________
Printed Name of Subject

__________________________________  ______________________________
Printed Name of Parent or Guardian  Parent or Guardian’s Signature & date

__________________________________  ______________________________
Name of person who explained consent  Signature & date
Assent Form for Children Participating in Research

Title of Research Project:

Evaluation of early metabolic and vascular risk in children of parents with early ischaemic heart disease.

Principle Investigators:  
Prof. Clodagh O’Gorman, Chair of Paediatrics, UL  
Prof. Colum Dunne, Director of Research, UL

Co-investigators:  
Dr. Alan Macken  Research Fellow in Paediatrics, UL  
Prof. Walter Cullen  Chair of General Practice, UL  
Dr. Michael O’Neill  Consultant Paediatrician, Mayo General Hospital  
Dr. Brendan Meaney  Consultant Cardiologist, MWRH Limerick  
Ms Catriona Aherne  CNS in Cardiac Rehabilitation, MWRH, Limerick

Why are we doing this study?

We are conducting a study to check if children whose parents have had heart problems at a young age show different test results compared with children whose parents have not had heart problems.

What will happen during the study?
You will have one visit to the hospital.

For this visit, you will be asked to come in the morning. You will also be asked not to eat or drink anything except water until you come to the hospital. First, we will do some tests to look at your blood vessels. These are ultrasound tests, so they will not hurt. Some cold gel will be applied to your chest, neck and arm and a small probe, that looks like a flashlight, will be pressed against your skin. This will not be sore. Next, we will bring you to another room to take a test to check for diabetes. You will get a small poke from a needle when we take a sample of blood and put in a small straw into your vein (IV), but you can have a special cream to numb the arm beforehand. Then, from the same IV, we will be able to take blood samples a few times over 2 hours, so we will not have to do any extra pokes to get this blood. This should not feel sore. Just before the test for diabetes, we will do another short heart test. This will take 15 minutes and it will not hurt. We will put a small probe, like a thimble on your finger, and we will measure your heart beat in your finger. Then we will put a blood pressure cuff filled with air around your arm, and leave it there for a few minutes. This is sometimes uncomfortable. We could do this test on a different day, when you come to clinic, if you are very hungry and don't want to wait. Then, we will bring you for some lunch.

**Are there good things and bad things about the study?**

It may hurt or pinch a bit when the blood test is taken. If you want, we can help to numb the skin where the needle goes in with a special cream.

You may feel hungry because you have to wait a little longer to eat breakfast on the day of your test.
The good things about this study are that we will be able to see how your blood vessels are working and do a check for diabetes. If these tests are not normal, we will be able to send you for treatment.

**Who will know about what I did in the study?**

Doctors and nurses will know what you did in the study. Your parents will sign a form agreeing to be in this study and this form will be kept in your chart. The doctors and nurses will not tell anyone that you were in this study unless you want them to. However, if we feel that your health is in danger, we may have to report your results to your doctor.

**Can I decide if I want to be in the study?**

You do not have to be in this study if you don’t want to be. Nobody will be angry or upset if you do not want to be in the study. We are discussing this study with your parents and you should talk to them about it too.

**Assent:**

"I was present when ________________________________ read this form and gave his/her verbal assent."

Name of person who obtained assent

__________________________________________

Signature

__________________________________________

DATE