Monitoring of vascular health in children at risk for atherosclerosis

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<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>aIMT</td>
<td>Aortic intima media thickness</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CAH</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>CGMS</td>
<td>Continuous glucose monitoring system</td>
</tr>
<tr>
<td>cIMT</td>
<td>Carotid intima media thickness</td>
</tr>
<tr>
<td>CONGA</td>
<td>Continuous overall net glycaemic action</td>
</tr>
<tr>
<td>CSII</td>
<td>Continuous subcutaneous insulin infusion</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EDIC</td>
<td>Epidemiology of Diabetes Interventions and Complications Trial</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl trinitrate induced dilatation</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis model of assessment of insulin resistance</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>High sensitive C-reactive protein</td>
</tr>
<tr>
<td>ICC</td>
<td>Intra-class correlation coefficient</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LGBI</td>
<td>Low glucose blood index</td>
</tr>
<tr>
<td>MAGE</td>
<td>Mean of glycaemic excursions</td>
</tr>
<tr>
<td>Abbr.</td>
<td>Description</td>
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<tr>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>MDI</td>
<td>Multiple daily injections</td>
</tr>
<tr>
<td>MODD</td>
<td>Mean of daily difference</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovarian syndrome</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>T1D</td>
<td>Type 1 diabetes</td>
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<tr>
<td>T2D</td>
<td>Type 2 diabetes</td>
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ABSTRACT

Adult cardiovascular disease has its origins in childhood, and adolescence is a critical period in determining lifetime risk. Early changes in arterial structure and function measured non-invasively have prognostic significance. Assessment of vascular structure and function provide an opportunity to test intervention strategies at an age when vascular damage is potentially reversible. Understanding the relative sensitivity of these markers of vascular damage is essential in identifying children at risk and enabling evaluation of clinical and public health interventions.

In a cross-sectional study, aortic and carotid intima media thickness were assessed in 66 children with type 1 diabetes and 32 healthy children. Aortic intima media thickness (aIMT) was significantly greater in the children with type 1 diabetes and related to age, glycosolated haemoglobin and low-density lipoprotein cholesterol concentrations. In contrast, there was no significant difference in carotid intima media thickness between groups, suggesting that aIMT is an earlier marker of subclinical atherosclerosis in children with type 1 diabetes.

An interventional trial of 22 children with type 1 diabetes was performed to evaluate whether reduction in glucose variability with initiation of continuous subcutaneous insulin infusion (CSII) therapy would improve vascular function. At 3 weeks post commencement of CSII, vascular function improved associated with a reduction in glucose variability; however the effects on vascular function over 6 to 12 months were not sustained, with deterioration of glycaemic control.

Finally, in a cross-sectional study, vascular function and structure was assessed in 14 children with congenital adrenal hyperplasia (CAH), a relatively novel
patient population, whose risk for atherosclerosis has not been previously investigated. The results from the children with CAH were compared to 28 obese and 53 healthy controls. The children with CAH had evidence of vascular dysfunction, comparable to the obese cohort, despite having a lower body mass index.

It was concluded that use of non-invasive ultrasound markers of preclinical atherosclerosis can allow early detection of changes in vascular structure in children at known risk for future atherosclerosis, identify novel groups of children with medical conditions not previously recognized to have future cardiovascular risk, and is a valuable tool that can be used to test interventions in a timely manner.
DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree of diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree of diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Jennifer Harrington Date

6/10/2014
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Roger Gent (Pediatric ultrasonographer) who has volunteered countless early mornings to perform the ultrasound studies involved in this thesis, and continues to always have a smile on his face.

The children, adolescents and families who so generously participated in the studies, without whose time and commitment, the research would not have been possible.

Finally to my family, who continue to provide me with the love and support for me to pursue my aspirations.
Chapter 1: Introduction
1.1 Problem Statement

Ischaemic heart disease and cerebrovascular disease are the two leading causes of mortality and morbidity in Australia, accounting for 24.8% of all deaths in 2011[1]. The term ischaemic heart disease includes angina and myocardial infarction, and cerebrovascular disease includes cerebral infarction, hemorrhage and stroke [2]. Atherosclerosis is a progressive disease characterized by the accumulation of lipid and fibrous elements in arteries [3], and is the principle contributor to the pathogenesis of ischaemic heart and cerebrovascular disease [4]. For this thesis I will use the term atherosclerosis as the disease of all of the arteries in the body, but specifically focusing upon atherosclerosis affecting the coronary arteries that causes ischaemic heart disease.

Mortality rates from ischaemic heart disease have significantly reduced over the past forty years [5-8]. In Australia from 1975 to 2006, the age-adjusted mortality rate declined by 73% in men and 70% in women [8]. Population based autopsy studies have demonstrated that the extent and severity of atherosclerosis has also declined over this period [9]. This decrease in atherosclerotic related cardiovascular disease has been attributed, in part, to a reduction in risk factors [5, 10]. Modifiable risk factors for cardiovascular disease and atherosclerosis include cigarette smoking, hypertension, abnormal lipid profile, increased body weight, physical inactivity and diabetes [5]. While risk factor control in adults reduces ischaemic heart disease, there has been, over the past decade, an attenuation in the decline in ischaemic heart disease mortality [8].
The earliest stages of atherosclerosis start in childhood [4], and thus childhood and adolescence are potential early points of intervention to further reduce future cardiovascular disease. Autopsy studies have demonstrated the presence of fatty streaks, the first stage of atherosclerosis, in the arteries of children and adolescents [11, 12], and that the severity of this atherosclerosis is associated with ante-mortem vascular risk factors [11, 13, 14]. Vascular risk factors measured in children and early childhood are associated with the degree of coronary calcification in young adults [15].

Given that progression in atherosclerosis occurs over decades, epidemiological studies and intervention trials with clinical end points require long term follow up [16]. The need to have early, robust surrogate markers of vascular disease is therefore critical to be able to identify children at increased risk of atherosclerosis and to assess interventions to slow the progression of the disease. The first signs of atherosclerosis, arterial vascular dysfunction and arterial wall thickening, can be reliably measured non-invasively using high-resolution ultrasound [17-20].

Atherosclerosis begins as deposits of cholesterol and its esters, referred to as fatty streaks, in the inner lining (the intima) of large muscular arteries [21]. Ultrasound visualisation of the intima media thickness allows assessment of this process. In adults, intima media thickness of the carotid arteries (cIMT) is related to incident and prevalent ischaemic heart disease and stroke [22-24], as well as known cardiovascular risk factors such as lipids and blood pressure [25, 26]. Atherosclerotic changes in the abdominal aorta precede those seen in the carotid arteries [27], and therefore aortic intima media thickness (aIMT) may be an earlier and more sensitive marker of the first structural change of atherosclerosis.
Vascular dysfunction, as measured by endothelial and smooth muscle function, can be assessed non-invasively using ultrasound of the brachial artery [17, 18, 28]. Endothelial function can be measured using flow mediated dilatation (FMD), which assesses the change in brachial artery diameter in response to alterations in blood flow. The brachial artery diameter change is related to the amount of nitric oxide released by the endothelial cells. Abnormal FMD is associated with abnormal coronary angiography in adults [29, 30]. Vascular smooth muscle dysfunction is also an independent risk factor for atherosclerosis in adults [31, 32]. Glyceryl tri-nitrate is an exogenous nitric oxide donor that dilates the vessel wall independent of the endothelium, and therefore can be used to assess vascular smooth muscle responses (glyceryl trinitrate induced dilatation [GTN]).

The use of these surrogate markers of early atherosclerosis is particularly important in the assessment of cohorts of children at increased risk of future cardiovascular disease. In this thesis I will be primarily evaluating the vascular health of children with two different medical conditions; type 1 diabetes and congenital adrenal hyperplasia.

Type 1 diabetes is the most common type of diabetes in childhood [33], with an incidence in Australia among 1 to 14 years old children of 22 per 100,000 person years [34]. It results from autoimmune destruction of the insulin-producing beta cells in the islets of Langerhans within the pancreas [35]. Despite improvements in treatment and life-expectancy, adults with type 1 diabetes continue to have higher mortality rates compared to the general population, predominantly related to cardiovascular disease [36-40]. Reductions in mean glycaemia, as measured by HbA1c, through intensive management of type 1 diabetes, has been shown to improve
morbidity as seen in the Diabetes Control and Complications Trial (DCCT) [41], and its follow up study, the Epidemiology of Diabetes Interventions and Complications Trial (EDIC) [42, 43]. Whether interventions to reduce glucose variability, a potential independent vascular risk factor, lead to improved vascular health has not been assessed in children with type 1 diabetes.

Assessment of vascular health is not only important in patients with medical conditions established to cause accelerated atherosclerosis, but also can help identify additional patient populations who may be at future risk. Congenital adrenal hyperplasia (CAH), due to 21-hydroxylase enzyme deficiency, is an autosomal recessive condition that is caused by mutations in the gene CYP21A2 [44]. It is characterized by glucocorticoid and mineralocorticoid deficiency and androgen excess. There is increasing evidence that both adults and children with CAH have multiple vascular risk factors such as hypertension and elevated body mass index [45-49]. Adults with CAH have been shown to have increased cIMT [50], but there has been no assessment of vascular structure and function in children with CAH to determine whether these changes occur during childhood.

1.2 Hypothesis

Given the points outlined in the problem statement as well as in the background discussed in Chapter 2, I generated the following hypotheses:

1. Aortic intima media thickness (aIMT) is a more sensitive marker of early atherosclerosis in children with type 1 diabetes than carotid intima media
thickness (cIMT).

2. Commencing children with type I diabetes on a continuous subcutaneous insulin infusion improves vascular function and structure due to reduced glucose variability.

3. Children with congenital adrenal hyperplasia (CAH) secondary to 21 hydroxylase deficiency have reduced vascular function and changes in vascular structure when compared with healthy controls.

### 1.3 Aims

The objectives of this thesis were to examine aspects of vascular health in children by firstly exploring the sensitivity of a relatively new surrogate marker of early atherosclerosis (aortic intima media thickness) in children with known accelerated atherosclerosis (type 1 diabetes); secondly undertaking an intervention study to assess the effect of initiation of continuous subcutaneous insulin infusion (insulin pump therapy) on vascular function and structure in children with type 1 diabetes; and thirdly assessing vascular health in a novel population (children with congenital adrenal hyperplasia), where there is no previous paediatric data.

The specific aims of the thesis were

1. To measure carotid and aortic intima media thickness in children with type 1 diabetes and healthy children and assess their respective determinants.
2. To assess the utility of aIMT compared to cIMT to assess subclinical atherosclerosis in children with type 1 diabetes.

3. To assess whether in children with type 1 diabetes, glucose variability is an independent predictor for vascular dysfunction, independent of HbA1c.

4. To determine whether reducing glucose variability in children with type 1 diabetes, by the use of continuous subcutaneous insulin infusion, improves vascular function and structure.

5. To compare vascular function (endothelial and smooth muscle function) and vascular structure (carotid and aortic intima media thickness) in children with congenital adrenal hyperplasia, to children with obesity and healthy children.

6. To evaluate the determinants of vascular function and structure in children with congenital adrenal hyperplasia.

1.4 Research Strategy

A literature review of vascular structure (cIMT and aIMT) and vascular function (FMD and GTN) in general as well as specifically in type 1 diabetes and congenital adrenal hyperplasia was conducted. The role of glycaemic variability in patients with type 1 diabetes and its effects on vascular function was also reviewed. This explanatory background is included in Chapter 2.
In Chapter 3 I detail the methods that were used to assess vascular function and structure in the subsequent studies. Potential factors that can confound measures of vascular function and structure are also discussed.

In Chapter 4, I investigate and compare cIMT and aIMT in children with type 1 diabetes and healthy children in a cross sectional study. In addition in this Chapter, I include determinants of vascular structure identified in the literature review such as lipid concentrations, blood pressure, inflammatory markers and body size. Chapter 4 relates to hypothesis one and specific aims one and two.

Chapter 5 assesses the association between glycaemic variability and vascular function in children with type 1 diabetes, and whether changing glycaemic variability by the use of a continuous subcutaneous insulin infusion alters vascular function in a prospective interventional trial. Chapter 5 relates to hypothesis 2 and specific aims three and four.

Chapter 6 investigates vascular function and structure in children with congenital adrenal hyperplasia as compared to children with obesity and healthy children in a cross sectional study. Again in this chapter I include determinants of vascular function and structure identified in the literature review. Chapter 6 relates to hypothesis 3 and specific aims five and six.

In Chapter 7 the findings of all of the studies are discussed in the context of both research validity and recently published data. Future research questions will be addressed as well as the implications of the results for children with type 1 diabetes and children with congenital adrenal hyperplasia.
In Chapter 8 the main findings of this thesis are summarised with a final conclusion.
2 Chapter 2: Literature review
In this chapter I will briefly review normal vascular function and structure, followed by the changes seen in atherosclerosis. Next, I will review the various techniques available to assess vascular function and structure in clinical trials. Following this I discuss vascular health in adults and children with T1D, focusing on the pathogenesis of atherosclerosis, the evidence assessing vascular function and structure and the interventions that have trialed to improve vascular health. Finally I review the literature around vascular health in adults and children with congenital adrenal hyperplasia.

2.1 Effects of atherosclerosis on vascular function and structure

The wall of an artery is composed of three layers. The innermost layer, or the intima, is composed of endothelial cells, and is in direct contact with the blood within the lumen of the artery. The middle layer, or the media, contains bundles of smooth muscle cells, along with elastic fibers. The adventitia makes up the outer layer and is composed of fibroblast cells and connective tissue [51].

2.1.1 Endothelial function

While initially regarded as an inert barrier between the circulating blood and vessel wall, since the 1980’s the vascular endothelium has been recognised as important regulator of vascular homeostasis [52]. The endothelium participates in vascular tone, cellular and nutrient trafficking, inflammation, thrombosis and coagulation, as well as angiogenesis [53]. These processes occur in response to either mechanical stimuli (such as stretch, shear or pressure), chemical factors (such as...
glucose, homocysteine, reactive oxygen species [ROS]), humoral agents (such as bradykinin, thromboxane, angiotensin II) or endothelial derived mediators [54-57]. Amongst the endothelial derived mediators, nitric oxide (NO) is the best characterized [58]. I will focus my discussion upon this mediator.

NO is a short lived molecule that is synthesized from the oxidation of L-arginine by nitric oxide synthetase (NOS) [52, 59]. Of the three NOS isoforms, neuronal NOS, inducible NOS and endothelial NOS (eNOS), it is eNOS that is involved primarily with endothelial-derived NO [60]. NO diffuses locally within endothelial cells, both to the luminal surface as well as into the smooth muscle cells in the media, where it signals through numerous downstream pathways to cause vasodilatation [61]. In addition to its effects on vascular tone, NO decreases endothelial expression of adhesion molecules and pro-inflammatory cytokines [62, 63]. Multiple other endothelial derived factors have also been identified that are involved in the regulation of vascular tone, coagulation and inflammation [64-68]. Changes in the bioavailability of these molecules can create an environment favorable for thrombosis and the development of atherosclerosis [69].

2.1.2 Smooth muscle function

Differentiated vascular smooth muscle cells regulate vessel tone, diameter and blood pressure by contraction and relaxation in response to stimuli [70]. Vascular smooth muscle cells exhibit plasticity, switching between differentiated and dedifferentiated phenotypes in response to changes in the local environment [71]. This phenotypic modulation is an important process in embryogenesis, vascular remodeling and repair [72], however it can also augment the progression of vascular
disease. Dedifferentiated smooth muscle cells exhibit increased secretory capacity [72], proliferation and migration [73], as well as disorganization of actin fibers [74], leading to decreased contractility [71]. Dedifferentiation of smooth muscle cells from a contractile to a synthetic phenotype is an early event in cardiovascular disease, including atherosclerosis [72] and restenosis of arteries [75].

2.1.3 Alterations in vascular function and structure in atherosclerosis

Atherosclerosis is a progressive disease characterized by the initial presence of endothelial and smooth muscle dysfunction, to the progressive development of intima-media thickening, fatty streaks, early fibroatheromas to complex fibrous plaques [4, 76]. The underlying pathogenesis involves an imbalanced lipid metabolism and maladaptive inflammatory response to various forms of insults to the endothelium [77]. The progression is accelerated in the presence of certain risk factors, but lesion regression can occur if the injurious agents are removed.

2.1.3.1 Endothelial dysfunction in atherosclerosis

Endothelial injury leading to endothelial dysfunction was first described by Ross et al in the 1970s as the precipitating factor in atherosclerosis [78]. Sources of injury to the endothelium include, but are not limited to, exposure to increased low-density lipoproteins (LDL) which can diffuse into and accumulate within the sub-endothelial space, hyperglycemia, mechanical forces and viruses [3, 4]. Accumulation of LDL which becomes oxidized within the sub-endothelium, has been recognized as an important triggering event [79] and has been shown to antagonize the production of NO [80]. Exposure to oxidized LDL leads to changes in endothelial cellular signaling and endothelial derived substances. There is increased leucocyte adhesion
and permeability (e.g. through secretion of vascular or intracellular adhesion molecule-1[VCAM-1 and ICAM1], and monocyte chemoattractant protein-1), platelet activation and production of growth factors (e.g. platelet-derived growth factor, insulin-like growth factor, transforming growth factor-β) and pro-inflammatory cytokines (e.g. interleukin-1, tumour necrosis factor α) [77, 81-85].

Oxidized LDL promotes the expression of adhesion molecules and chemotactic proteins to increase entry of monocytes and lymphocytes into the artery wall [86]. Oxidized LDL, is in turn, taken up by monocyte-derived macrophages leading to foam cell formation [3]. These macrophages are not only important as scavenger cells, but potentiate the atherosclerotic process by their capacity to secrete numerous growth factors, in particular platelet derived growth factor [4] and cytokines such as interleukin-1 and interleukin-6 [85].

2.1.3.2 Vascular smooth muscle dysfunction in atherosclerosis

In atherosclerosis there is proliferation of vascular smooth muscle cells. This occurs due to dedifferentiation of the smooth muscle cells with a change from the contractile to synthetic phenotype [87]. Molecular regulation of the smooth muscle phenotype is influenced by multiple factors, including platelet derived growth factor [88]. The synthetic smooth muscle cells secrete matrix proteins which, when accumulated over time, contribute to the development of an atherosclerotic plaque [89].
2.1.3.3 Changes in vascular structure in atherosclerosis

The first structural lesion of atherosclerosis that can be observed microscopically is the appearance of lipid-engorged foam cells within the intima of arteries [90]. This process can occur from early infancy [91]. Atherosclerosis can then progress to the development of fatty streaks, visible on gross examination at autopsy, on the luminal surface of arteries [92], and is seen from childhood. Preatheromas, atheromas and fibroatheromas are characterized by a progressive accumulation of extracellular lipid, which is circumscribed by a fibrous core composed of smooth muscle cells and extracellular matrix [93]. There is a significant increase in the presence of fatty streaks and atheromas around the time of puberty [11, 91]. Plaques can become increasingly complex with calcification, ulceration at the luminal surface and hemorrhage from small blood vessels [3].

The Bogalusa Study [11] and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) [94], two large cohort autopsy studies of children and young adults, demonstrated that atherosclerosis begins in childhood. Atherosclerotic lesions occur especially in areas of hemodynamic or mechanical stress [91]. The earliest changes occur in the dorsal wall of the aortic aorta, with a significantly greater percentage of the aortic intimal surface covered with fatty streaks in adolescents compared to the coronary artery [11, 95]. The extent and progression of the vascular structural changes, relates to the presence and number of antemortem cardiovascular risk factors [11, 96]. In both the Bogalusa and PDAY studies, the cardiovascular risk factors found to be predictive of the severity of atherosclerotic lesions included increasing age, increased body mass index (BMI), increased LDL cholesterol and decreased high density lipoprotein (HDL) cholesterol, hypertension
and smoking [11, 96]. In the PDAY study hyperglycemia was also found to be an independent predictor [97].

2.2 Assessment of vascular function and structure

2.2.1 Assessment of vascular function

Endothelial function can be evaluated by measuring circulating biomarkers or by the use of functional testing. Reduced concentrations of NO metabolites (nitrites and nitrates) and increased endothelin-1 (an endothelial derived vasoconstrictor) have been found in patients with coronary artery disease [98, 99]. These measurements however can be confounded by dietary influences, assay difficulties and do not always reflect the tissue activity of the compound [100-102]. Functional testing of the endothelium assesses the change in arterial diameter or blood flow in response to various stimuli. Methods of assessment include coronary angiography [103], plethysmography measuring forearm blood flow [104], brachial artery flow mediated dilatation (FMD) and reactive hyperemia peripheral arterial tonometry (PAT)[105]. Of these methods, FMD and PAT are non-invasive. FMD is the most widely used technique and has been shown to have better reliability for within and between day measurements compared to PAT [106].

FMD assesses the endothelial cells’ ability to release NO and other endothelium-derived vasodilators in response to mechanical stress [17]. Using high resolution B mode ultrasound, the change in brachial arterial diameter is measured in response to increased blood flow induced by a period of ischaemia to the distal
circulatory bed [107, 108]. FMD is expressed as a change in the post-stimulus diameter as a percentage of the baseline diameter [17]. Guidelines standardizing the technique for FMD have been published [108, 109], and large population assessments of FMD have demonstrated good inter and intra-observer reproducibility of measurements [110, 111]. FMD has been shown to correlate with coronary endothelial function [29] and to the extent of coronary artery disease on coronary angiography [30]. A meta-analysis of observational studies demonstrated that impairment of FMD is associated with increased risk of future cardiovascular events [112].

Smooth muscle function can be measured using invasive methods such as coronary angiography using nitroglycerin [113], or plethysmography using sodium nitroprusside [114]. Glyceryl trinitrate induced dilatation (GTN) provides a non-invasive method of assessing smooth muscle function [31]. Exogenous administration of NO in the form of glyceryl trinitrate, acts at the level of the smooth muscle cells, producing an endothelial-independent dilatation of the brachial artery which can be measured using ultrasound. Decreased GTN is independently associated with atherosclerosis seen on coronary angiography [32].

Decreased vascular function in adults has been associated with cardiovascular risk factors such as cigarette smoking [115], elevated serum LDL concentrations [17], hypertension [116], diabetes [117] and obesity [118]. The presence of risk factors in childhood has also been shown to be a predictor for vascular dysfunction in adulthood. Elevated cholesterol concentrations, blood pressure and adiposity at 9 years of age is associated with decreased FMD in early adulthood, independent of adult cardiovascular risk factor exposure [119-121].
In pediatric cohorts, vascular dysfunction has been identified in children with medical conditions that put them at risk for accelerated atherosclerosis. Endothelial and smooth muscle dysfunction occurs in children with familial hypercholesterolemia [122], type 1 diabetes [123, 124], obesity [125] HIV infection [126] and low birth weight [127]. Endothelial dysfunction has also been demonstrated in children with chronic renal failure [128], systemic lupus erythematosus [129] and Kawasaki syndrome [130].

2.2.2 Assessment of vascular structure

Methods to assess vascular structure and the presence of atherosclerotic lesions include invasive imaging in the form of coronary angiography and intravascular ultrasound [131], as well as non-invasive modalities such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) and scintigraphic techniques (e.g. positron emission tomography [PET]). CT imaging can assess the degree of coronary calcification. Significant coronary calcification has been demonstrated in children with Kawasaki disease [132] and is associated with the degree of coronary artery dilation [133, 134] as well as in children with chronic renal failure on haemodialysis [135]. The use of CT is limited however because of the radiation exposure. Atherosclerotic plaques have been imaged in adults using MRI or PET scans [136-138], but there has been no data of the applicability of these methods in children. Ultrasound assessment of the intima media thickness (IMT) is the most commonly utilized and most feasible method in this age group.

IMT is assessed using high-frequency B mode ultrasound to measure the combined thickness of the intima and media, when the vessel is perpendicular to the
plane of sound from the transducer [109]. IMT of carotid, aortic and femoral arteries have been used as markers of atherosclerosis. Carotid IMT (cIMT) has been the most extensively studied.

Carotid IMT is associated with atherosclerotic plaque thickness on autopsy [139]. In adults, increased cIMT is associated with incident coronary artery disease and is predictive of future cardiovascular events [140-143]. The cardiovascular risk factor profile of adolescents, including elevated LDL cholesterol, systolic blood pressure, body mass index (BMI) and smoking, predicts cIMT in young adulthood [144]. Intervention trials in adults assessing the impact of antihypertensive and lipid-lowering medication on cardiovascular risk have demonstrated improvement in cIMT in the treated subjects compared to placebo groups [145-147].

Carotid IMT has also been studied extensively in children, and normative age related values have been published for children and adolescents [148, 149]. Elevated LDL cholesterol, blood pressure and BMI are associated with increased cIMT [150]. Increased cIMT in comparison to healthy children is seen in pediatric patients with familial hypercholesterolemia [151-153], obesity [154-157], chronic renal failure [158-160] hypertension [161-163], systemic lupus erythematosis [164] and HIV infection [165]. In children with type 1 diabetes there have been conflicting cIMT data, with some studies demonstrating increased cIMT compared to healthy controls, while others have not. I will review the literature of cIMT in children with type 1 diabetes in more detail in section 2.3.2.1 of this thesis.

There are difficulties when comparing studies, because of considerable variability in the method and site of measurement chosen to measure cIMT. Carotid
IMT can be measured at the distal carotid, carotid bifurcation or internal carotid artery. Different protocols have been proposed using a combination of these sites of measurements [140, 166]. In addition some investigators have measured the cIMT from the near or anterior carotid artery wall rather than the distal posterior wall. Distal wall cIMT measurements have been demonstrated to have excellent correlation with histological and intravascular ultrasound analysis, compared to comparatively poor correlation seen with near wall cIMT measurement [167].

Increased femoral IMT has also been demonstrated to be associated with coronary heart disease [168] and the presence of cardiovascular risk factors [169]. There have not been studies assessing its use in pediatric populations.

In spite of the evidence that the first atherosclerotic lesions occur more commonly in the aorta, there is limited data assessing aortic IMT (aIMT). IMT of the ascending aorta in adults using transesophageal ultrasound imaging is increased in patients with coronary artery disease [170] and familial hypercholesterolemia [171]. Distal aIMT measured just proximal to the aortic bifurcation, can be assessed non-invasively using transcutaneous ultrasound. The majority of studies of distal aIMT (here to referred as simply aIMT in this thesis) have been performed in pediatric populations, as increased abdominal circumference and adiposity can limit the quality of the images obtained in adulthood.

The Muscatine Offspring Study assessed the association between cardiovascular risk factors and aIMT in 220 adolescents aged 11 to 18 years [150]. Increased aIMT associated with increased LDL cholesterol, blood pressure, BMI and wait to hip ratio. The association of these risk factors was stronger to aIMT than
concurrently measured cIMT. Exposure to tobacco smoke was also associated with increased aIMT [172].

Aortic IMT has been demonstrated to be increased in children with medical conditions that put them at risk for accelerated atherosclerosis. (Table 1 – *this table includes both studies published prior and after the trial performed as part of this thesis*). The majority of studies have been in neonates. There has been only one previous study assessing aIMT in children with type 1 diabetes [152].
**Table 1. Trials assessing aIMT in children at risk of accelerated atherosclerosis compared to healthy controls**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Medical Condition</th>
<th>No. of subjects, mean age(years)</th>
<th>Mean aIMT-mm (*p value &lt;0.05 compared to controls)</th>
<th>Associations with increased aIMT within subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>[173]</td>
<td>Macrosomic neonates of diabetic mother (MN-DM)</td>
<td>30 MN-DM, 30 MN-HM, 30 healthy neonates</td>
<td>MD-DM: 0.49±0.02* MN-HM: 0.47±0.02* Controls: 0.38±0.02</td>
<td>Increased birth weight Increased birth length</td>
</tr>
<tr>
<td></td>
<td>Macrosomic neonates of healthy mother (MN-HM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[174]</td>
<td>Neonates with intrauterine growth retardation (IUGR)</td>
<td>25 IUGR neonates, 25 control neonates (appropriate for gestational age)</td>
<td>IUGR: 0.56±0.06 Controls: 0.53±0.58 There was a significant difference in maximal aIMT between groups</td>
<td>Decreased birth weight Decreased head circumference</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[175]</td>
<td>Neonates with IUGR</td>
<td>40 IUGR neonates, 40 control neonates (appropriate for gestational age)</td>
<td>IUGR: 0.52±0.03* Controls: 0.40±0.03</td>
<td>Decreased birth weight Increased head circumference Increased serum triglycerides</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[176]</td>
<td>Neonates with IUGR</td>
<td>40 IUGR neonates, 40 control neonates (appropriate for gestational age)</td>
<td>IUGR: 0.45±0.03* Controls: 0.39±0.04</td>
<td>Decreased IGF1 Increased gestational age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[177]</td>
<td>Children born preterm (gestational age &lt;37 weeks)</td>
<td>26 Preterm, 11±0.5 yrs, 11 Controls(born at ≥37 weeks), 5±0.5 yrs</td>
<td>Preterm:0.58 (0.52-0.6)* Control: 0.52 (0.44-0.54)</td>
<td>Not assessed</td>
</tr>
<tr>
<td>[152]</td>
<td>Familial hypercholesterolemia (FH) Type 1 Diabetes (T1D)</td>
<td>16 FH, 11±1 yrs, 44 T1D, 11±2 yrs, 28 controls, 11±3 yrs</td>
<td>FH: 0.53±0.1* T1D: 0.50±0.09* Controls: 0.44±0.05</td>
<td>Increasing Age Increased diastolic blood pressure</td>
</tr>
<tr>
<td>[178]</td>
<td>Children with persistent <em>Chlamydia pneumonia</em> seropositivity</td>
<td>34 Persistent seropositivity: 11.1±0.1yrs, 24 Transient seropositivity, 11.1±0.1yrs, 77 Seronegative controls, 11.1±0.1</td>
<td>Persistent: 0.53±0.09* Transient: 0.49±0.06 Controls: 0.50±0.05</td>
<td>No significant univariate associations</td>
</tr>
</tbody>
</table>
2.3 Type 1 diabetes mellitus and vascular health

Adults with type 1 diabetes (T1D) have both an increased incidence of and mortality rates associated with cerebrovascular and ischaemic heart disease [38, 179-181]. Cardiovascular disease occurs at an earlier age. The comparative relative risk for cardiovascular disease in adults with T1D is highest in adults aged 35 to 45 years when compared to age-matched young adults without T1D [182]. The protective effect of female gender is also lost, with a 10-15 fold relative risk for cardiovascular disease in women with T1D between 35 to 55 years of age [182].

In this section I will firstly describe the underlying pathogenesis that leads to vascular dysfunction and atherosclerosis in patients with type 1 diabetes. Next I will review the literature assessing ultrasound measures of vascular function (FMD and GTN) and vascular structure (cIMT and aIMT) in T1D, focusing on pediatric studies. Finally I will describe studies that have assessed interventions to improve vascular health in patients with T1D.

2.3.1 General pathogenesis of atherosclerosis in T1D

Factors associated with the development of atherosclerosis in T1D include hyperglycemia, inflammation, abnormal lipid profile, pro-coagulatory state, insulin resistance and obesity. Gender, age, tobacco exposure, genetics, ethnicity and blood pressure are other risk factors, but as they are not specifically caused by T1D, will not be discussed in this thesis.
2.3.1.1 Hyperglycemia

Hyperglycemia was proposed as the unifying mechanism leading to endothelial dysfunction in individuals with diabetes by Michael Brownlee in 2001[183]. Endothelial cells are prone to damage caused by hyperglycemia because, unlike other cell types, they are unable to reduce the intracellular transport of glucose when exposed to extracellular hyperglycemia [184]. The molecular mechanisms by which chronic hyperglycemia leads to endothelial dysfunction include increased shunting of glucose to the aldose reductase pathway, intracellular production of advanced glycation end (AGE) products, activation of protein kinase C and increased hexosamine pathway activity [183]. These pathways lead to an overproduction of reactive oxygen species (ROS) by the mitochondrial electron transplant chain, increasing oxidative stress [183]. ROS have been demonstrated to promote the expression of pro-inflammatory cytokines by endothelial cells [185], decrease eNOS activity and hence reduce NO production [186] and promote the formation of pro-atherogenic oxidized LDL [187].

In addition to detrimental effects on the endothelium, hyperglycemia has been demonstrated to alter smooth muscle function through promoting proliferation of smooth muscle cells via down-regulation of protein kinase C [188, 189]. The production of AGEs activates specific receptors on smooth muscle cells, stimulating the production of inflammatory cytokines and pro-fibrotic growth factors [190].

There is conflicting data from population cohort studies of adults with T1D about the association between mean glucose levels, as measured by HbA1c, to cardiovascular events. The Pittsburgh Epidemiology of Diabetes Complications Study
[191] and the cross-sectional EURODIAB Prospective Complications Study [192] found that HbA1c was not associated with ischaemic heart disease after adjustment for other risk factors. In contrast, a 20-year follow up study of 891 adults with T1D in Wisconsin, who were free of cardiovascular disease at baseline, demonstrated an association between elevated HbA1c and increased cardiovascular mortality [193].

Not only does chronic hyperglycemia have adverse effects on endothelial and smooth muscle function, there is increasing evidence to support the role of fluctuating glucose levels or glucose variability as independent causative factor of vascular disease. Oscillating glucose levels lead to increased endothelial apoptosis [194] through the increased production of ROS [195, 196]. Fluctuations of hyperglycemia also result in significantly greater monocyte and macrophage endothelial adhesion as compared with sustained hyperglycemia [197, 198], and proliferation of vascular smooth muscle cells [199]. In the FinnDiane study, a prospective multi-centre study of 2107 adults with T1D, increased HbA1c variability, but not mean HbA1c, was associated with increased relative risk for a cardiovascular event [200].

2.3.1.2 Inflammation

Propagation of inflammation with the production of cytokines and leucocyte recruitment is a key step in the atherosclerosis process [77]. Individuals with T1D have evidence of increased circulating acute inflammatory proteins [201]. C-reactive protein, interleukin 6, tumour necrosis factor α and E-selectin have been independently associated with cardiovascular events in T1D [202, 203]. Toll-like receptors (TLR), an important first line of defense to exogenous and endogenous stimuli, when unregulated can induce increased leucocyte activation with subsequent
tissue damage [204]. Activation of TLR2 and TLR4 has been demonstrated in mice-models to be involved in the accelerated inflammatory response seen in T1D [205-207]. Inflammation is also accentuated by the effects of hyperglycemia and dyslipidaemia.

2.3.1.3. Abnormal lipid profile

Increased plasma lipoproteins represent one of the most important metabolic components involved with the onset and progression of atherosclerosis in patients with T1D [208]. There are several mechanisms by which lipid metabolism is altered in T1D. Insulin deficiency leads to a reduction in lipoprotein lipase activity with a subsequent decrease in clearance of very-low-density lipoprotein cholesterol [209]. Hyperglycemia induces increased glycation and oxidation of subendothelial LDL [210]. In cohort studies 15 to 20% of adolescents with T1D have been demonstrated to have dyslipidemia [211, 212].

Hyperlipidemia can lead to endothelial and smooth muscle dysfunction, independent of glucose concentrations [213]. Increased circulating concentrations of cholesterol bound to apolipoprotein B bind to proteoglycans, leading to retention of LDL particles within the intima [77]. Similar to hyperglycemia-dependent toxicity, lipotoxicity from elevated free fatty acids leads to increased production of ROS, activation of protein kinase C and decreased NO synthesis [214-216]. Elevated cholesterol concentrations impair vascular smooth muscle function by modulating smooth muscle cell proliferation, migration and apoptosis [217, 218].
2.3.1.4. Pro-coagulatory state

Hyperglycemia through multiple mechanisms can lead to a pro-coagulatory state. Increased serum glucose leads to increased factor VII clotting activity [219] and inhibits fibrinolysis by increasing plasminogen activator inhibitor-1 levels [220]. Increased AGEs dose-dependently increase pro-coagulant activity [221]. Hyperglycemia can also directly influence the vulnerability of the vascular endothelium by affecting the protective layer of proteoglycans covering the vessel wall, resulting in enhanced platelet-endothelial cell adhesion [222]. Children with T1D have increased serum coagulation markers such as elevated tissue plasminogen activator factor and von Willebrand factor [223].

2.3.1.5 Obesity and insulin resistance

Children with T1D have an increased prevalence of being overweight, as measured by BMI, compared to non-diabetic youth [224]. This increase in BMI can result from relative supraphysiological insulin concentrations and excessive caloric and fat dietary intake [225, 226]. Increase in adipose tissue, particularly visceral adiposity, leads to endothelial dysfunction through the production of inflammatory cytokines, increased ROS and pro-coagulatory factors [227-229]. While often related to obesity, patients with T1D have been shown to have evidence of insulin resistance as measured using a euglycaemic hyperinsulinemic clamp compared to non-diabetic adults, controlling for BMI [230]. In the Pittsburgh Epidemiology of Diabetes Complications Study insulin resistance was shown to be an independent risk factor for cardiovascular events [191].
2.3.2 Assessment of vascular function and structure in T1D

2.3.2.1 Assessment of vascular function (FMD and GTN) in T1D

Adults with T1D have evidence of both endothelial and smooth muscle dysfunction before the onset of clinical micro- or macrovascular disease [231, 232]. Impairment in endothelial function, as measured by FMD, has similarly been demonstrated in pediatric T1D cohorts at risk of atherosclerosis [123-125, 233-238]. Decreased FMD is associated with elevated LDL cholesterol [125, 233, 234, 238], low folate status [239] and decreased activity levels [235]. In pediatric cohorts, decreased FMD has not been associated with increased HbA1c concentrations [123, 125, 233, 238].

There have been only a few studies in children and adolescents with T1D assessing vascular smooth muscle dysfunction, as measured by GTN induced dilatation. Decreased vascular smooth muscle function has been demonstrated in children with T1D compared to healthy controls in most [125, 240, 241], but not all of trials [233]. BMI, but not HbA1c, is an important determinant of GTN [125, 240].

2.3.2.2 Assessment of vascular structure (cIMT and aIMT) in T1D

Carotid intima media thickness

A recent large trial (the SEARCH CVD study) which assessed 402 adolescents and young adults with T1D, with a mean age of 18.8 years, demonstrated significantly greater IMT of the carotid artery bulb in the individuals with T1D compared to age matched controls [242]. Of note, there was no significant difference in IMT of the distal common carotid artery between groups, the site assessed for cIMT.
measurements in most other studies. While this study provides strong evidence that cIMT in young adults and older adolescents with T1D is increased, studies of younger children and adolescents show differing results (Table 2). The heterogeneity of the results may in part reflect the younger age of the children included, sample sizes of the cohorts, the different ultrasound protocols used and sites imaged, plus a possible publication bias for positive trials.
Table 2. cIMT in children and adolescents with T1D compared to healthy controls

<table>
<thead>
<tr>
<th>Ref</th>
<th>No. of subjects, mean age (years)</th>
<th>Mean cIMT (mm)</th>
<th>P value TID vs. controls</th>
<th>Associations with increased cIMT within TID subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>[236]</td>
<td>21 T1D, 8.3 ± 0.3 15 controls, 7.6 ± 0.3</td>
<td>0.48 ± 0.01 0.48 ± 0.02</td>
<td>p = NS</td>
<td>No significant correlations</td>
</tr>
<tr>
<td>[243]</td>
<td>5 T1D, &lt; 10 yrs 23 controls, &lt; 10 yrs 12 T1D, 10 to 19 68 controls, 10 to 19</td>
<td>0.38 ± 0.06 0.45 ± 0.08 0.53 ± 0.12 0.44 ± 0.06</td>
<td>&lt;10 yrs: p = NS 10-19 yrs: p = 0.01</td>
<td>↑ duration of diabetes ↑ age</td>
</tr>
<tr>
<td>[244]</td>
<td>50 T1D, 11 ± 2 35 controls, 11 ± 1</td>
<td>0.47 ± 0.04 0.42 ± 0.04</td>
<td>p &lt; 0.001</td>
<td>↑ LDL cholesterol ↑ systolic BP</td>
</tr>
<tr>
<td>[235]</td>
<td>32 T1D, 11.5 (10.2-12.8) 42 controls, 10.7 (9.6-11.8)</td>
<td>0.50 (0.48-0.52) 0.48 (0.47-0.49)</td>
<td>p = 0.02</td>
<td></td>
</tr>
<tr>
<td>[245]</td>
<td>52 T1D, 11.6 ± 3.8 43 controls, 11.3 ± 3.3</td>
<td>0.42 ± 0.06 0.40 ± 0.04</td>
<td>p = NS</td>
<td>↑ age</td>
</tr>
<tr>
<td>[246]</td>
<td>52 T1D, 11.8 ± 3.1 47 controls, 11.8 ± 2.7</td>
<td>0.46 ± 0.04 0.44 ± 0.04</td>
<td>p = 0.001</td>
<td>↑ LDL cholesterol ↑ systolic BP</td>
</tr>
<tr>
<td>[247]</td>
<td>20 T1D, 11.9 ± 3.6 20 controls, 12.1 ± 13.4</td>
<td>0.60 ± 0.02 0.60 ± 0.03</td>
<td>p = NS</td>
<td></td>
</tr>
<tr>
<td>[248]</td>
<td>14 T1D, 13 ± 2 14 controls, 13 ± 2</td>
<td>0.36 ± 0.04 0.31 ± 0.02</td>
<td>p = 0.002</td>
<td></td>
</tr>
<tr>
<td>[249]</td>
<td>30 T1D, 13.1 ± 3.6 20 controls, 13.2 ± 3.9</td>
<td>0.45 ± 0.06 0.42 ± 0.05</td>
<td>p = 0.005</td>
<td>↑ HbA1c</td>
</tr>
<tr>
<td>[250]</td>
<td>314 T1D, 13.8 ± 2.8 118 controls, 13.2 ± 2.6</td>
<td>0.45 ± 0.05 0.44 ± 0.05</td>
<td>p = NS</td>
<td></td>
</tr>
<tr>
<td>[251]</td>
<td>150 T1D, 13.9 ± 2.8 58 controls, 14.1 ± 3.1</td>
<td>0.46 ± 0.03 0.42 ± 0.01</td>
<td>p &lt; 0.001</td>
<td>Younger age of diabetes onset, ↑ systolic BP, ↑ total cholesterol</td>
</tr>
<tr>
<td>[237]</td>
<td>52 T1D, 14.5 ± 2.4 36 controls, 15.1 ± 2.7</td>
<td>0.50 ± 0.07 0.44 ± 0.06</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>[252]</td>
<td>45 T1D, 14.8 ± 2.5 33 controls, 14.1 ± 1.9</td>
<td>0.48 ± 0.06 0.33 ± 0.07</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>[253]</td>
<td>35 T1D, 14.8 ± 2.6 37 controls, 14.5 ± 3.0</td>
<td>0.50 ± 0.06 0.48 ± 0.04</td>
<td>p = NS</td>
<td></td>
</tr>
<tr>
<td>[254]</td>
<td>31 T1D, 15.2 ± 2.4 35 controls, 15.7 ± 2.7</td>
<td>0.33 ± 0.05 0.32 ± 0.08</td>
<td>p = NS</td>
<td></td>
</tr>
<tr>
<td>[255]</td>
<td>33 T1D, 15.8 ± 1.3 16 controls, 17.4 ± 1.7</td>
<td>0.61 ± 0.13 0.60 ± 0.14</td>
<td>p = NS</td>
<td></td>
</tr>
<tr>
<td>[256]</td>
<td>142 T1D, 16.0 ± 2.6 87 controls, 18.8 ± 3.1</td>
<td>0.56 ± 0.06 0.54 ± 0.06</td>
<td>p= 0.002</td>
<td>↓ HDL cholesterol in male subjects</td>
</tr>
<tr>
<td>[257]</td>
<td>28 T1D, 17.6 ± 1.4 11 controls, 16.8 ± 0.9</td>
<td>0.49 ± 0.06 0.42 ± 0.03</td>
<td>p = 0.001</td>
<td>Presence of retinopathy</td>
</tr>
<tr>
<td>[242]</td>
<td>402 T1D, 18.8 ± 3.3 206 controls, 19.2 ± 3.3</td>
<td>0.46 ± 0.07 0.45 ± 0.07</td>
<td>p = 0.01</td>
<td>male sex, ↑ age, ↑ systolic BP, ↑ BMI</td>
</tr>
</tbody>
</table>
Aortic intima media thickness

Prior to the publication of the study associated with this thesis, there had been only one previous trial assessing aIMT in children with T1D. Jarvisalo et al. demonstrated that in 44 children with T1D, with a mean age of 11 ± 2 years, aIMT was significantly greater than in an aged matched healthy control group [152]. Significant associations with aIMT within the children with T1D included systolic and diastolic blood pressure.

2.3.3 Interventions to improve vascular health in T1D

2.3.3.1 Intensive glycaemic control

The DCCT demonstrated the importance of glycaemic control and reduction in HbA1c in preventing or delaying microvascular complications associated with T1D [41]. Improvement in macrovascular complications was not initially demonstrated in the DCCT due to the low prevalence of macrovascular disease in the relatively young cohort [258]. Subsequent follow-up of this cohort (the EDIC trial) has demonstrated the benefit of glycaemic control for delaying the development of atherosclerosis, with decreased cIMT progression [43], coronary artery calcification measured on CT [259] and lower risk for cardiovascular events [260] in the intensive treatment arm participants. This sustained benefit was seen despite both treatment arms of the study having the same mean HbA1c after the trial completed, highlighting the importance of glycaemic control in adolescence in determining future cardiovascular risk. The improvement in cIMT and coronary artery calcification scores was however attenuated in those patients who with intensive glycaemic control had the most weight gain [261]. Unlike population cohort studies, which have, as previously described in
this thesis, shown conflicting results regarding an association between long term glycaemic control and cardiovascular disease, a meta-analysis of 8 interventional randomized controlled trials involving 1800 patients with T1D demonstrated a clear association [262].

While the use of adjunct non-insulin therapies in patients with T1D such as metformin, alpha glucosidase inhibitors, amylin analogues, glucagon like peptide-1 agonists and DPP4 inhibitors have been shown to reduce mean HbA1c [263-267], to date there have been no studies assessing their effect on vascular function or structure. Neither have there been any intervention studies in patients with diabetes to determine whether independent of HbA1c, reduction in glycaemic variability reduces cardiovascular disease.

2.3.3.2 Lipid lowering agents

The use of hydroxymethylglutaryl-CoA reductase inhibitors (statins) in adults with T1D leads to improvement in endothelial function as measured by FMD [268-270]. Improvements in FMD with statin use are associated with younger age, lower lipid concentrations and lower blood pressure [271], suggesting earlier intervention may be beneficial. A meta-analysis evaluating the results of fourteen randomized trials of statin use in 10686 adults with both type 2 and type 1 diabetes determined that the incidence of major cardiovascular events was reduced by 20% for every mmol/L reduction in LDL cholesterol [272]. There have not been any completed interventional lipid lowering studies in children with T1D to date. In children with familial hypercholesterolemia, statin use has been associated with decreased cIMT [273] and improvement in FMD [274].
2.3.3.3 Antioxidants

Vitamin C has been demonstrated to alter endothelial function by increasing NO availability [275]. In adults [276] and children [277] with T1D, administration of an infusion of vitamin C improves endothelial function.

Vitamin E has beneficial effects on the endothelium [278], and epidemiological studies have demonstrated an inverse relationship between cardiovascular disease and vitamin E consumption [279, 280]. Administration of vitamin E improves endothelial [281, 282], but not smooth muscle function [282] in adults with T1D. While high-dose vitamin E supplementation decreases urinary markers of oxidative stress in children with T1D [283], there have been no studies assessing the effect on vascular function in pediatric cohorts.

The beneficial effects of folic acid on the endothelium are not only by decreasing ROS [284, 285], but by increasing NO bioavailability [286] and improving coagulation abnormalities associated with increased homocysteine concentrations [287]. Folic acid improves FMD but not GTN in children with T1D [238, 288], but the effect is limited to children who do not receive routine folate fortification from food [289]. The effect of folate supplementation has been disappointing in adults with T1D and established cardiovascular disease [290].

2.3.3.4 Increased physical activity

Physical exercise improves vascular function and decreases cardiovascular events [291]. In patients with T1D, exercise training has been demonstrated to improve FMD in adults [292] and in children [293], but not decrease cIMT [293].
Further work is still needed to identify effective, durable and tolerable interventions to reduce the future risk of cardiovascular disease in children with T1D.

2.4 Congenital adrenal hyperplasia and vascular health

Not only is it important to investigate vascular health in children at recognized increased risk, but to identify novel populations of children, who from mechanisms related to their underlying condition, or from a clustering of cardiovascular risk factors, are at potential risk for accelerated atherosclerosis.

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterized by impaired biosynthesis of cortisol. The most common cause of CAH, accounting for more than 90% of cases, is a deficiency of the 21-hydroxylase enzyme encoded by the CYP21A2 gene [294, 295]. This leads to a failure of glucocorticoid and often mineralocorticoid secretion, subsequent increased pituitary adrenocorticotropic hormone release with resultant androgen excess. CAH secondary to 21-hydroxylase deficiency is clinically classified into three different subtypes based upon the genotype and degree of mineralocorticoid deficiency; the classical salt-wasting, classical simple-virilizing and non-classical forms [296]. Based upon newborn screening data the prevalence of classical CAH is 1 in 15,000 live births [297], although the prevalence varies according to ethnicity [295, 298, 299]. From here on in this thesis the term CAH will refer specifically to classical congenital adrenal hyperplasia secondary to 21-hydroxylase deficiency (both salt-wasting and simple-virilizing forms) unless otherwise specified.
Glucocorticoid and mineralocorticoid replacement in CAH both aims to replace the deficient hormones and decrease the androgen excess. The balance between overtreatment and under-treatment can be difficult to achieve, especially as the available glucocorticoid formulations do not replicate the circadian rhythm of cortisol secretion [300, 301]. A large cohort study of 203 adults with CAH demonstrated that only 36% of the patients had appropriate serum androgen concentrations and 38% had evidence of glucocorticoid overtreatment [302].

2.4.1 Vascular risk factors in CAH

It has been increasingly recognized that compared to the general population, patients with CAH have increased incidence of cardiovascular risk factors that may predispose them to accelerated atherosclerosis and future cardiovascular disease.

2.4.1.1 Obesity

Elevated BMI compared to age matched controls has been described in several cross-sectional cohort studies of adults with CAH [48, 303, 304]. Increase in BMI is related to an increase in fat mass as measured by dual X-ray absorptiometry (DXA) [48, 303], but does not relate to the hydrocortisone dose or concentrations of circulating androgens (17 hydroxyprogesterone).

Children with CAH also have an increased BMI Z score compared to aged matched controls [305]. The increase in BMI occurs early in life, by five years of age [306]. While children with classical CAH have increased fat mass, this is not seen in age-matched children with non-classical CAH [307]. There does not appear to be a difference in BMI between children with salt-wasting and simple virilizing CAH [46].
In children with CAH, increase in BMI has been shown to be associated in one study to higher glucocorticoid doses, advanced bone age maturation and increased parental BMI [46]. Leptin resistance has also been implicated in the development of the obesity seen in CAH [308]. Leptin, a hormone produced by adipocytes, plays a role in the regulation of body weight, with increased concentrations leading to suppression of the appetite [309]. Changes in the leptin axis, with the development of “leptin resistance”, is thought to be involved in the development of obesity [310]. Glucocorticoids and increased insulin secretion increases leptin concentrations [311]. In patients with CAH, leptin concentrations have been shown to be increased, and relate to BMI and insulin sensitivity [312, 313].

2.4.1.2 Blood pressure

While standard blood pressure measurements have in some studies shown to be normal [304] and others increased [314], 24-hour ambulatory blood pressure measurements are higher both in children and adults with CAH [47, 315, 316]. Over-treatment with mineralocorticoids, with suppression of renin levels, can cause hypertension. In the studies assessing blood pressure in children with CAH, correlations between renin concentrations and blood pressure however have not been found to be significant [47, 314, 316]. Elevated systolic blood pressure in children with CAH is associated with increased BMI [47, 316] and increased serum insulin concentrations [47].

2.4.1.3 Insulin Resistance

Studies of insulin sensitivity in patients with CAH have mostly utilized the homeostasis assessment method (HOMA-IR) [HOMA-IR = insulin (microunits per
milliliter) X glucose (millimoles per liter)/22.5] or oral glucose tolerance test. There have not been any studies that have utilized more sensitive measures such as a euglycaemic hyperinsulinemic clamps. Both adults and children with CAH have been shown to have evidence of insulin resistance, even when corrected for BMI [50, 308, 313, 317, 318]. Increased HOMA-IR is associated with increased serum androgen concentrations in adults with CAH [319] and to hydrocortisone dose in children [318].

2.4.1.4 Dyslipidemia

There is less convincing evidence that dyslipidemia is present in patients with CAH, with several adult cohort studies, demonstrating no significant difference in lipid concentrations compared to healthy controls [50, 304, 315]. In children, Zimmermann et al. demonstrated increased LDL concentrations, but similar triglyceride and HDL concentrations in twenty-seven 13 year old patients with CAH compared to age-matched healthy controls [318]. Further confirmatory research is needed to assess whether the risk for dyslipidemia is indeed increased in children with CAH, given this risk factor is not seen in adult CAH populations.

The combination of obesity, insulin resistance, hypertension, along with intermittent exposure to hypercortisolism from glucocorticoid over-treatment and hyperandrogenism from under-treatment, creates a complex interplay of metabolic abnormalities in patients with CAH that can predispose them towards accelerated atherosclerosis (Figure 1).
2.4.2 Assessment of vascular function and structure in CAH

Despite the presence of these cardiovascular risk factors, prior to the trial undertaken as part of this thesis, there had been only one study directly assessing vascular health in adults with CAH, and none in children. Sartorato et al. demonstrated that young adults with CAH at a mean age of 28 ± 3.5 years, have increased carotid, aortic and femoral IMT compared to age-matched healthy controls. Increased carotid and femoral IMT was significantly associated with increased BMI, but no significant correlations were seen with glucocorticoid dose, 17 hydroxyprogesterone concentration, HOMA-IR or lipids. Subsequent to the publication of my study, a further trial demonstrated increased aIMT and cIMT in a total of eighteen adolescents with classical and non-classical CAH [320].

The increased incidence of cardiovascular events, vascular dysfunction and increased IMT in women with polycystic ovarian syndrome (PCOS) provides indirect supportive evidence that CAH is a condition of accelerated atherosclerosis. PCOS is a
common reproductive disorder characterized by clinical or biochemical hyperandrogenism, irregular anovulatory menstrual periods and polycystic ovaries on ultrasound [321]. Women with PCOS also have metabolic features such as obesity, hyperandrogenism and insulin resistance, similar to what is seen in patients with CAH.

There is a two-fold increased risk for coronary heart disease and stroke in women with PCOS [322]. This increase in cardiovascular risk is present, even when controlling for BMI. Two recent meta-analyses of published observational studies demonstrated that women with PCOS have decreased FMD [323] and increased cIMT [324]. The vascular dysfunction and increased IMT in women with PCOS is only partially explained by cardiovascular risk factors such as increased BMI, insulin resistance and dyslipidemia [325]. Androgen excess has been demonstrated in some studies to be an independent risk factor [326-328]. This is a potentially important distinction, given androgen concentrations can be more significantly raised in undertreated patients with CAH.

2.5 Conclusions

Atherosclerosis is characterized by initial endothelial and smooth muscle dysfunction that can progress to vascular structural changes, including increased intima media thickness, arterial streaks and plaques. Aetiopathogenic factors such as hyperglycemia, inflammation, elevated lipids, obesity and insulin resistance have been implicated in the development and progression of atherosclerosis. Early changes in arterial structure and function measured non-invasively with methods such as
FMD, GTN, cIMT and aIMT, have prognostic significance and provide the opportunity to test early intervention strategies, at an age when vascular damage is reversible. Type 1 diabetes and CAH are examples of two medical conditions, which are associated with multiple vascular risk factors in childhood. The study of vascular function and structure in children with these conditions allows better characterisation of potential future cardiovascular risk, and the ability to investigate mechanisms by which this risk could be reduced.
Chapter 3: Methods
In this chapter I describe the methods used to assess vascular function and structure in the studies outlined in the following chapters. The specific laboratory and statistical methods are discussed in each individual chapter.

3.1 Flow mediated and glyceryl trinitrate induced dilatation

Endothelial and smooth muscle function were measured using the recommended techniques in the published guidelines by the American College of Cardiology [108]. Endothelium-dependent FMD is a measure of the endothelium’s endogenous ability to produce NO in response to a change in blood flow or shear stress [17]. In comparison, GTN induced dilatation is an endothelium-independent measure, and is used to assess vascular smooth muscle response to exogenous NO [31].

3.1.1 Acquisition of the images for FMD and GTN

The equipment used to assess FMD and GTN in all subjects included a high resolution B mode ultrasound with two-dimensional imaging, colour and spectral doppler (Phillips iU22, Bothel, Washington, USA). The target artery was measured using a 17.0 MHz linear array transducer. An electrocardiogram (ECG) was recorded continuously with the ultrasonic images. All scans were recorded on a high quality videotape-based cassette. The studies were performed by one of three experienced ultrasonographers who were unaware of the subject’s clinical characteristics.

After the subject was rested supine in a quiet, temperature controlled room, a suitable site on the right brachial artery, 2 to 15 centimeters above the elbow was
selected. In order to be suitable for accurate measurements, there needed to be clear visualisation of the vessel’s anterior and posterior lumen-arterial wall interfaces in the longitudinal axis. Depth and gain settings were set to optimise image clarity. When the best imaging site was determined, the position was marked on the skin and the arm remained in the same position throughout the study.

For each subject four scans were performed. The initial or baseline scan of the brachial artery was measured with the subject at rest (figure 2).

*Figure 2: Ultrasound of the brachial artery at rest*

Following the resting scan, an adult sized sphygmomanometer was placed on the right arm above the elbow and inflated to 250 mmHg for four minutes, occluding arterial blood flow. After deflating the cuff, the increase in blood flow, creating a shear stress, led to endothelium-dependent arterial dilatation [107]. The second scan was taken between 45 and 75 seconds after cuff deflation to capture maximal arterial vasodilatation.
Ten to fifteen minutes was allowed for vessel recovery, after which a third scan was taken at rest to re-establish the baseline conditions. Sublingual glyceryl trinitrate spray (400 micrograms) was administered as an exogenous NO donor to elicit endothelium-independent vasodilatation (GTN dilatation). The final scan was performed four minutes after administration of the spray to ascertain maximum obtainable vasodilatation response.

3.1.2 Analysis of the images for FMD and GTN

All of the images were recorded on high quality video-cassette tape and analysed later by one of two independent blinded observers. The diameter of the brachial artery from each of the four scans was measured from images in which both the anterior and posterior lumen-intima interface as well as the media-adventia interface were clearly seen (the “double line” sign). A clear “double line” sign ensures that the image had been taken perpendicular to the vessel wall [109].

The vessel diameter was measured using ultrasonic calipers to assess the distance between the anterior and posterior intima-lumen interfaces. For each scan, measurements were taken incident with the R wave on the ECG over four consecutive cardiac cycles. Onset of the R wave is a tool used to identify end diastole. During systole, blood vessel diameter increases to accommodate changes in blood volume generated by the left ventricle contraction. These functional vessel diameter changes may confound the change that is seen secondary to endogenous or exogenous NO exposure alone [108]. Hence measurements for FMD and GTN were taken during end diastole to minimize these effects. The four measurements for each scan were averaged. FMD and GTN were then expressed as a percentage of the vessel diameter.
post reactive hyperemia and post glyceryl tri-nitrate compared to the resting baseline measurements.

3.1.3 Variability and confounders in the measurement of FMD and GTN

The technique used to measure FMD in our studies involved placing the sphygmomanometer cuff above the elbow. Other studies have, in contrast, placed the cuff distal to the elbow, on the forearm. Cuff placement on the upper arm has been shown to elicit a greater change in vessel diameter [329, 330] although can be more uncomfortable and technically challenging, as acquisition of the image can be distorted by collapse of the brachial artery [108, 331].

There are also external and patient related factors that can influence vascular function. Consumption of high fat foods has been shown to induce transient acute endothelial dysfunction [332]. As such, all of our subjects were fasting at the time of the assessment of vascular function. Other substances such as caffeine, vitamin C and medications that interact with the vasculature, such as anti-hypertensives and aspirin, need to be held for at least 12 hours prior to the assessment. All of the children in our studies were asked to refrain from exercise in the 12 hours prior to the assessment of vascular function, as this has also been shown to influence FMD results [333]. In females, the phase of the menstrual cycle can affect endothelial function, with enhanced vasodilation occurring during the end follicular phase [334, 335].

As discussed in chapter 2, chronic hyperglycemia is thought to be a critical mechanism by which endothelial dysfunction results in individuals with diabetes [336]. The acute role of glucose concentration at the time of assessment on endothelial function appears to differ between patient populations. Vascular function
relates to serum glucose levels within the normal range in individuals without diabetes [125, 337-339]. In children with type 1 diabetes, with glucose levels extending outside the normal range, there does not appear to be a direct relationship between serum glucose and vascular function [125, 236]. Within an individual with type I diabetes, however, acutely changing blood glucose has been shown to lead to a change in endothelial function [340]. Routine insulin doses were given after vascular function assessment in our subjects, to try and avoid rapid changes in blood glucose concentrations during the study period.

### 3.2 Carotid and aortic intima media thickness

#### 3.2.1 Acquisition of the images for cIMT and aIMT

Similar to assessment of vascular function, images were obtained using B mode ultrasound with a 17 MHz linear array transducer, with a continuous ECG tracing. With the child lying in a supine position, with the neck slightly extended and the head turned 45 degrees towards the opposite side, both left and right common carotid arteries were imaged, just proximal to the carotid bulb. A minimum of four images were captured of each side, incident with the R wave, for later analysis.

Carotid IMT has been demonstrated to vary by approximately 5% during the cardiac cycle [341], and thus standardization of timing of measurements is critical. For aIMT, a minimum of four images were taken of a straight, non-branched segment of the abdominal aorta, just proximal to the bifurcation. Similar to cIMT, images were taken incident with the R wave.
3.2.2 Analysis of the images for cIMT and aIMT

The three best quality images, where the distal wall was most clearly seen, were measured by one of two blinded independent readers. The distance between the lumen-intima interface and media-adventia interface was measured using a semi-automated edge detection and measurement software package (figure 3). The mean and maximum carotid and aortic IMT of the three images were then averaged for each subject.

Figure 3: Measurement of carotid and aortic intima media thickness

Carotid intima media thickness

Aortic intima media thickness
3.2.3 Variability and confounders in the measurement of cIMT and aIMT

Unlike vascular function, which is a dynamic measurement, acute variations in patient and environmental factors do not appear to have a significant effect on the measurement of IMT. The most significant factor that contributes to variability in cIMT is, as previously mentioned in chapter 2, differences in image acquisition methodology. Several adult research protocols have advocated for the average measurement of three arterial segments on each side; the common carotid, the carotid bulb and the internal carotid arterial segments [166, 342, 343]. In adults, while common carotid IMT has been shown to predict future cardiac events [141], other groups have found a stronger relationship between cardiac risk factors and carotid bulb IMT [344]. Due to this variability, measurement of all three segments has been proposed [109]. Most pediatric cohorts, in contrast, have used only one segment, although the studies have varied in which segment has been used.

Carotid IMT images should be obtained where the artery is perpendicular to the plane of sound. The ability to obtain cIMT images consistently can be sonographer dependent, with 70 to 80% of cIMT measurement variability due to differences in sonographer technique [345]. The use of an externally applied measuring angle device, such as a Meyer’s arc, can assist in ensuring consistency with the angle of the transducer used [346].

Given its relative limited use to date, there is little known about the variability in measurement of aIMT. Abdominal adiposity and its effect on the ability to obtain clear images, appears to be the most significant limitation in the use of aIMT.
Chapter 4: Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes

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STATEMENT OF AUTHORSHIP

Aortic intima media thickness is an early marker of atherosclerosis in children with type 1 diabetes. *J Pediatrics* 2010; 156(2): 237-41

**Harrington, J (Candidate)**
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Signed...... Date......2/1/14

Peña, A

Involved in helping with acquisition and interpretation of data and manuscript evaluation
Signed. Date......3/4/14

Gent, R

Performed the ultrasounds, assisted data interpretation and manuscript evaluation
Signed.... Date......3/4/14

Hirte, C

Performed advanced statistical analysis, help with interpretation of the data and manuscript evaluation
Signed..... Date......8/1/16

Couper, J

Supervised conception and design, interpretation of data and provided manuscript evaluation
Signed..... Date......3/1/16
The paper presented in this chapter demonstrates that aortic intima media thickness is an earlier and more sensitive marker of atherosclerosis in children with T1D than carotid intima media thickness. The relationship between cardiovascular risk factors and IMT is discussed.

The impact factor of the journal where this paper has been published is 4.035.

This article has been cited 23 times in peer-reviewed literature.

Data from this paper has been presented at the following national and international meetings:

- European Society of Paediatric Endocrinology Annual Scientific Meeting, Istanbul, Turkey, September 2008 (oral presentation: FC6-98)
- Australasian Paediatric Endocrine Group Annual Scientific Meeting, Canberra, Australia, November 2008 (oral presentation: OP05)
The specific components of this publication that I was responsible for include:

- Being involved in the study design and coordination of submission of the project to the Research Ethics Board

- Recruitment of the majority of the study subjects

- Collection of data: including administration of study questionnaire, anthropometric measurements, phlebotomy collection, measurements of intima media thickness, FMD and GTN from ultrasound images

- In collaboration with our statistician, involved with statistical analysis of data

- Wrote the initial version of the manuscript and was involved in the editing process for publication

- Acted as corresponding author for the published manuscript
4.1 Abstract

Objective: To compare aortic intima media thickness (aIMT) to carotid intima media thickness (cIMT) as a marker of early atherosclerosis in children with type 1 diabetes, and to examine the associations of aIMT to known cardiovascular risk factors.

Study Design: 66 children with type 1 diabetes (14.1 ± 2.5 years, 37 males) and 32 healthy controls (14.2 ± 3 years, 15 males) had assessment of vascular structure (cIMT and aIMT) and vascular function (flow mediated dilatation: FMD and glyceryl trinitrate induced dilatation: GTN). Fasting bloods were taken for HbA1c, high sensitive C reactive protein, total homocyst(e)ine, serum folate, red cell folate and lipids.

Results: aIMT, but not cIMT, was significantly greater in the children with type 1 diabetes than in controls (p<0.001). In children with type 1 diabetes, aIMT correlated with HbA1c (r=0.31, p=0.01) and was independently associated with age (β=0.38, p=0.001) and LDL cholesterol (β=0.38, p=0.001). Vascular function (GTN) was worse in those children with type 1 diabetes who had an aIMT greater than the 95th percentile, as defined by the controls.

Conclusions: aIMT is an earlier marker than cIMT of preclinical atherosclerosis in children with type 1 diabetes and relates to known cardiovascular risk factors and metabolic control.
4.2 Introduction

The earliest changes of atherosclerosis begin in childhood, and these changes are accelerated in high risk pediatric populations [4]. The scientific statement from the American Heart Association Expert Panel on Population and Prevention Science identified children with T1D as being in the highest risk tier for early cardiovascular disease, along with children with homozygous familial hypercholesterolemia and end-stage renal disease [347]. The extent of atherosclerotic vascular change has been shown in children to relate to both the number and intensity of risk factors, and a reduction in these risk factors is associated with an improvement in vascular abnormalities [347]. The need to have early, robust surrogate markers of vascular disease is therefore critical to be able to assess interventions to slow progression of accelerated atherosclerosis in childhood.

High-resolution ultrasound is a reliable and non-invasive method to measure vascular structure. Atherosclerosis begins as deposits of cholesterol and its esters, referred to as fatty streaks, in the intima of large muscular arteries [21]. In adults, cIMT is related to incident and prevalent coronary heart disease and stroke [22, 142], as well as known cardiovascular risk factors such as lipids and blood pressure [144]. In pediatric cohorts however, the data has been inconsistent. Some studies have shown that children with T1D have a significantly greater cIMT than healthy controls whilst others have not [244, 251, 254].

Post-mortem studies show that the earliest changes in vascular structure first occur in the dorsal wall of the distal abdominal aorta, and the progression from fatty
streaks to raised plaques is accelerated in high-risk subjects [27]. Aortic IMT may therefore be an earlier marker than cIMT of these vascular changes.

One study of children with T1D or familial hypercholesterolemia showed significantly greater aIMT when compared with healthy controls [152].

In this study we aimed to determine whether aIMT is an earlier marker of altered vascular structure than cIMT in children with T1D, and its association to cardiovascular risk factors.

4.3 Methods

4.3.1 Subjects

A total of 100 children [52 males,] were studied. Sixty-eight children with T1D [age range, 8.5-18.1 years] were recruited consecutively from the diabetes clinic at the Women’s and Children’s Hospital (Adelaide, Australia). No subjects with diabetes had diabetic retinopathy, microalbuminuria or neuropathy as determined by dilated fundoscopy by an ophthalmologist, early morning urinary albumin/creatinine (reference range <30 µg/mg) and clinical examination. 32 healthy aged matched controls [age range 8.9–19.9 years] were recruited from friends or siblings of the subjects with diabetes participating in the study. Exclusion criteria were diabetes duration of less than 6 months and use of anti-hypertensive or lipid lowering medication. None of the participants in the study were smokers as determined by history. The study was approved by the Adelaide Children, Youth and Women’s Health Service Human Research Committee. Written informed consent was obtained
from the parents / guardians of the subjects and the subject if he / she was more than 16 years old.

Height was measured with a wall-mounted stadiometer to the nearest 0.1cm. Weight with minimal clothing was taken on an electric digital scale to the nearest 0.1kg. BMI \[\text{weight (kg)} / \text{height (m)}^2\] and BMI z-score were calculated using EpiInfo database version 3.2.2 and Centres for Disease Control 2000 standardised reference charts. Waist circumference was measured three times to confirm the measurement, at the midpoint between the lower edge of the ribs in the midaxillary line and the top of the iliac crest, at minimal respiration. Pubertal development was assessed by self report using Tanner stage illustrations, and categorized as group 1: Tanner 1 (pre-pubertal), group 2: Tanner 2 and 3 (early to mid-puberty) and group 3, Tanner 4 and 5 (late puberty).

4.3.2 Ultrasound assessment of vascular structure and function

Carotid and aortic intima media thickness, and endothelial and smooth muscle function (Flow mediated dilatation: FMD and glyceryl trinitrate induced dilatation: GTN) were assessed using B mode ultrasound (Philips iU22, Bothel, Washington, USA) with a 17MHz linear array transducer after an overnight fast in a quiet and stable temperature environment. The ultrasound studies were performed by one of 3 experienced ultrasonographers who were unaware of the clinical characteristics of the subject. Blood pressure was measured with an appropriately sized cuff three times after 10 minutes rest in the supine position, and the mean of the measurements was recorded. Endothelial vasomotor function was assessed using FMD and GTN as
previously described [108, 123]. Our coefficient of variation between 20 subjects is 3.9% for FMD and 4.0% for GTN [123].

For cIMT, the left and right common carotid arteries were imaged in a standardised magnification (2 x 2cm) using images of the posterior wall of the distal 10 mm of the common carotid artery, just proximal to the carotid bulb. The posterior wall of a straight, non branched 1cm longitudinal segment of the distal abdominal aorta was imaged just proximal to the aortic bifurcation for aIMT. Images were captured when both the anterior and posterior wall margins were clearly seen to ensure the images were taken perpendicular to the vessel. A minimum of 4 images of each of the common carotid arteries and the aorta were taken. All images were taken at end-diastole, incident with the R-wave on a continuously recorded ECG and then digitally stored for later analysis. The three best quality images for each of the carotid arteries and the two best for the aorta were selected and analysed by 2 independent readers who were blinded to the subjects’ clinical details and diagnosis. Best quality was defined by those images that produced the most number of points for analysis. For each image the greatest distance between the lumen-intima interface and media-adventitia interface (intima media thickness - IMT) was measured at a minimum of 100 points using a semi-automated edge detection and measurement computer software package (Brian Bailey, Royal Prince Alfred Hospital, Sydney, Australia). The mean and maximum IMT of each image were then averaged to give the final result for each subject.

Two children, both girls with type I diabetes, were excluded from the study as inadequate images were obtained of the aorta due to the amount of visceral adiposity.
4.3.3 Laboratory tests

Overnight fasting venous blood samples were collected. High sensitive C reactive protein (hsCRP) was measured using a near infrared particle immunoassay method using IMMAGE Immunochemistry Systems Reagent (Beckman Coulter Inc., Fullerton, CA). Triglycerides, total cholesterol, and high-density lipoprotein (HDL) were measured using enzyme-based assays on the Beckman Coulter Synchron CX5 analyzer. LDL cholesterol was calculated using the Friedewald equation. Apolipoprotein A1 and B were measured using rate immuno-nephelometry using the Beckman Array System (Beckman Instruments, Fullerton, CA). Glycosylated hemoglobin (HbA1c) was measured using a latex immunoagglutination inhibition methodology (DCA 2000 Hemoglobin A1c Reagent Kit; Bayer, Toronto, Ontario; cross-referenced to Diabetes Control and Complications Trial control standards). In addition the median 2 year HbA1c was calculated from HbA1c values taken at the previous three monthly visits over the preceding 2 years for the children with type 1 diabetes. Glucose was measured by hexokinase spectrophotometry method (Synchron cx5ce system; Beckman Coulter).

Serum folate and red cell folate (RCF) were measured using Ion Capture technology (Abbott IMx analyzer; Abbott Laboratories, Oslo, Norway). Total plasma homocyst(e)ine (tHcy) was measured by a fluorescence polarization immunoassay using the commercial IMx Homocysteine assay (Abbott Diagnostic Division). The interassay coefficient variation in the measurements for folate, RCF and tHcy in our labs are 6.6%, 15% and 3.9% respectively.
4.3.4 Statistical analysis

The data were analyzed using SPSS software version 15.0. Differences in measurements between groups were assessed with independent sample t-tests for normally distributed data and Mann-Whitney test for data that were not normally distributed. Associations between cIMT or aIMT, and their determinants were evaluated with Pearson and Spearman rank correlations. Multiple linear regression analysis was undertaken to determine independent predictors of IMT. Predictor variables with a P value of less than 0.05 in their univariable test of IMT were included in the multiple linear regression. A forward selection method was used to estimate the final linear regression. Statistical significance was inferred with a P value less than 0.05.

4.4 Results

The characteristics of the study groups are shown in Table 3. There were no significant differences between the groups with respect to age, gender, pubertal stage, body size or lipids. The children with T1D had a significantly higher systolic blood pressure, hsCRP, RCF and a lower tHcy than the controls. The mean T1D duration was 5.4 ± 3.8 years and insulin dose was 0.96 ± 0.3 units / Kg / day. Median HbA1c for the group with T1D was 8.6% (range, 6 – 14%) at the time of the study and 8.2% (range, 5.8 – 14%) for the 2 year median. These results were not significantly different from the median HbA1c for the entire diabetes clinic of 8 to 18 year old children. 34 of the children with T1D were on basal bolus insulin regimen, 23 on twice daily insulin and 9 on continuous subcutaneous insulin infusion.
### Table 3. Results of vascular structure and function studies and other variables by group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control subjects</th>
<th>Subjects with T1D</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>15/17</td>
<td>37/29</td>
<td>0.40†</td>
</tr>
<tr>
<td>Age (years)</td>
<td>14.2 (3.0)</td>
<td>14.1 (2.5)</td>
<td>0.85</td>
</tr>
<tr>
<td>Pubertal status (group 1:2:3)</td>
<td>9:12:11</td>
<td>15:30:21</td>
<td>0.73†</td>
</tr>
<tr>
<td>BMI Z score</td>
<td>0.11 (0.92)</td>
<td>0.41 (0.74)</td>
<td>0.09</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>69.4 (8.1)</td>
<td>71.6 (8.9)</td>
<td>0.24</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>109.1 (7.9)</td>
<td>113.3 (10.0)</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>60.0 (6.5)</td>
<td>62.4 (6.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>hs-CRP (mg/L)*</td>
<td>0.32 (0.1-2.6)</td>
<td>0.66 (0.1-2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum folate (ng/ml)</td>
<td>18.6 (7.5)</td>
<td>25.2 (9.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Red cell folate (ng/ml)</td>
<td>341 (133)</td>
<td>487 (202)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy (µmol/L)</td>
<td>9.0 (2.6)</td>
<td>7.0 (2.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)*</td>
<td>62.4 (31.9-116.9)</td>
<td>64.7 (39.9-434)</td>
<td>0.55</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>156.8 (28.5)</td>
<td>169.1 (36)</td>
<td>0.15</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>60.8 (119)</td>
<td>61.8 (12.8)</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>82.4 (20.8)</td>
<td>90.2 (25.6)</td>
<td>0.16</td>
</tr>
<tr>
<td>Apolipoprotein A1 (mg/dL)</td>
<td>58.6 (8.4)</td>
<td>61.3 (10.3)</td>
<td>0.24</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>25.1 (6.6)</td>
<td>28.1 (9.0)</td>
<td>0.13</td>
</tr>
<tr>
<td>Apolipoprotein B / Apolipoprotein A1</td>
<td>0.42 (0.09)</td>
<td>0.46 (0.14)</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>83.3 (5.4)</td>
<td>200 (81.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.2 (4.6-5.5)</td>
<td>8.6 (6-14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>7.1 (5.0)</td>
<td>5.2 (4.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>GTN (%)</td>
<td>26.7 (9.6)</td>
<td>22.9 (7.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.42 (0.05)</td>
<td>0.43 (0.05)</td>
<td>0.35</td>
</tr>
<tr>
<td>Max cIMT (mm)</td>
<td>0.50 (0.06)</td>
<td>0.51 (0.06)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean aIMT (mm)</td>
<td>0.50 (0.07)</td>
<td>0.57 (0.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Max aIMT (mm)</td>
<td>0.61 (0.09)</td>
<td>0.69 (0.14)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are mean (SD) and independent sample t tests used to compare groups unless otherwise stated.

* Median (range) and Mann-Whitney test used to compare groups,
† Chi-squared test used to compare gender and puberty staging.
The children with T1D had a significantly greater aIMT than controls (0.57 ± 0.11mm versus 0.5 ± 0.07mm; \( p=0.002 \)), but there was no difference in cIMT between the two groups (0.43 ± 0.05mm versus 0.42 ± 0.05mm; \( p=NS \)), (figure 2).

**Figure 4. Mean carotid and aortic IMT in the subjects with T1D and controls**

The horizontal line is the median, the edges of the box represent the 25\textsuperscript{th} and 75\textsuperscript{th} centiles, the bars represent the values within 1.5X the interquartile range and outliers (*) are shown.

In control subjects, cIMT and aIMT showed no univariate associations with measured risk factors. In the children with T1D, cIMT correlated negatively with diastolic blood pressure, but positively to brachial pulse pressure and total cholesterol level. Univariate associations for aIMT included age, waist circumference, total cholesterol, LDL cholesterol, apolipoprotein B, HbA1c and 2 year median HbA1c (table 4). aIMT increased through puberty in children with T1D (one way ANOVA \( p = 0.003 \)), but no change was seen with cIMT (\( p = 0.65 \)). In addition, aIMT was greater in those children with T1D when compared to the control group in pubertal group 2 (\( p=0.05 \)) and group 3 (\( p=0.005 \)), but no significant difference was seen between the
groups in those subjects in puberty group 1 (pre-pubertal). There was no correlation with systolic blood pressure, or gender to aIMT. In multivariate regression models for aIMT, in children with T1D, significant associations were age (β=0.38, p=0.001) and LDL cholesterol (β=0.38, p=0.001) and for cIMT vessel diameter (β=0.35, p=0.005) and diastolic blood pressure (β=-0.32, p=0.008).

**Table 4. Pearson’s correlation for IMT in the children with T1D**

<table>
<thead>
<tr>
<th></th>
<th>Mean cIMT</th>
<th>Mean aIMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.1 (p = 0.45)</td>
<td>0.45 (p = 0.001)</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.11 (p = 0.37)</td>
<td>0.25 (p = 0.05)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.38 (p=0.002)</td>
<td>0.12 (p=0.35)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.26 (p = 0.04)</td>
<td>0.38 (p = 0.02)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.19 (p = 0.13)</td>
<td>0.42 (p = 0.001)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>-0.03 (p = 0.80)</td>
<td>0.42 (p = 0.001)</td>
</tr>
<tr>
<td>Ratio Apolipoprotein B/Apolipoprotein A1</td>
<td>-0.02 (p = 0.99)</td>
<td>0.37 (p = 0.004)</td>
</tr>
<tr>
<td>HbA1c*</td>
<td>0.04 (p = 0.77)</td>
<td>0.26 (p = 0.03)</td>
</tr>
<tr>
<td>2 year median HbA1c*</td>
<td>-0.13 (p = 0.3)</td>
<td>0.31 (p = 0.01)</td>
</tr>
</tbody>
</table>

Data are Pearson’s correlation r, (p values) unless otherwise stated. *Spearman’s correlation

Although there was no continuous association with markers of vascular dysfunction (FMD or GTN) and aIMT, those children with T1D who had an aIMT greater than the 95\(^{th}\) percentile (n=18/66), as defined by the controls, had a significantly worse GTN (19% ± 3.6 vs. 24% ± 8.1, p = 0.03).

To test the reproducibility of IMT measurements, the inter-observer and intra-observer coefficient for 20 randomly chosen subjects (both children with T1D and controls) were calculated. The inter-observer intraclass coefficient for cIMT
measurements was 0.99 (coefficient of variation [CV] 1.2%) and for aIMT 0.99 (CV 1.6%). To assess intra-observer repeatability the same observer repeated the measurements on the 20 scans on two different occasions, and was blinded from the initial measurements. The intra-observer intraclass coefficient for cIMT measurements was 0.97 (CV 2.4%) and for aIMT 0.99 (CV 1.2%).

4.5 Discussion

This study demonstrates that aIMT is an earlier marker than cIMT for detecting the changes of vascular structure that may be an indicator of early atherosclerosis. Children with T1D had a significantly greater aIMT but not cIMT than age matched healthy children. This is consistent with the findings of Järvisalo et al in children with T1D or familial hypercholesterolemia (15). We extended these findings to show for the first time that aIMT, but not cIMT, related to known cardiovascular risk factors: LDL cholesterol, age and pubertal stage. These associations with aIMT have not been shown previously in children with T1D or any other cause of accelerated atherosclerosis. A further original finding was the association between vascular smooth muscle function and aIMT when aIMT was greater than the 95th centile as defined by controls.

Elevated plasma levels of LDL cholesterol accelerate the development of juvenile fatty streaks and the progression into fibrous plaques [21]. Maternal dyslipidemias are associated with the increased aIMT seen in neonates with intra-uterine growth retardation and macrosomia [173, 175]. There is an increase in aIMT in children with familial hypercholesterolemia comparable to children with T1D.
aIMT may therefore be a useful outcome measure in lipid lowering trials in children with T1D or other conditions of increased vascular risk.

We also showed for the first time an association between HbA1c and aIMT. The 2 year median HbA1c was more closely associated with aIMT than the HbA1c taken on the day of the study, consistent with aIMT being a slowly changing variable.

Autopsy studies have shown that the distal aorta is the site in subjects under the age of 20 years that is most likely to have fatty streaks, and fatty streaks in the distal aorta develop and progress most rapidly to raised lesions [27]. This progression is accelerated in subjects at higher cardiovascular risk. In adult studies, an increase in the IMT measured with B mode ultrasound scanning of aortic autopsy specimens correlates with histological findings of either an accumulation of lipid in the intima or fibroplastic intimal thickening, thought to be an early stage in plaque progression [348, 349]. Whether the increase in IMT in the children with T1D in our study represents sub-clinical atherosclerosis or instead a change in the vascular wall that predisposes to atherosclerosis is unclear, because correlation between histological and ultrasound findings is still needed in pediatric subjects.

Markers used to measure endothelial dysfunction (FMD and GTN), thought to be the first crucial step in the process to atherosclerosis, are variable and change rapidly to both environmental conditions and interventions. We have shown that administration of oral folate, for example, improves FMD within two hours of administration [238]. This variability may explain why we were not able to detect a continuous association between FMD or GTN to aIMT. We did however show that those children with T1D who had an aIMT greater than the 95th percentile as defined
by the healthy controls, had significantly worse smooth muscle function. Other studies have found that the presence of endothelial dysfunction strengthens the association between risk factors and IMT [350].

In contrast to Järvisalo et al [152], we did not find any significant differences between the two groups with respect to cIMT. There have been conflicting cIMT results in paediatric cohorts [251, 254]. A high resolution 17 MHZ linear array probe was used in this study, whilst other studies have only used medium resolution probes up to 8 MHZ. We were also able to demonstrate excellent intra and inter observer reliability with respect to our IMT measurement. Our group had a median HbA1c of 8.2% (interquartile range, 7.5 – 9%), compared to a mean of 8.8 % ± 1.4 in the group studied by Järvisalo et al [152]. The lack of significant difference in cIMT between the two groups compared to the increased aIMT in the group with T1D may relate to the need for a larger sample size of longer duration and /or poorer metabolic control to detect a difference in cIMT. In addition, the common carotid alone was imaged, whilst other studies have imaged the internal carotid arteries or carotid bulb where changes may precede those of the common carotid.

Interestingly cIMT in the children with T1D, was inversely associated with diastolic blood pressure. Pulse pressure (the difference between systolic and diastolic blood pressure), has been shown to be an independent risk factor for cardiovascular disease [351]. A larger pulse pressure in adolescence relates to increased cIMT in adulthood [352]. Pulse pressure positively correlated with cIMT in the children with T1D (r = 0.36, p = 0.003), and was inversely associated with diastolic blood pressure (r = -0.38, p = 0.002), potentially explaining the relationship between cIMT and diastolic blood pressure that was seen.
One limitation of aIMT is that it can be technically more demanding than cIMT. It requires high resolution probes to obtain adequate images to calculate the intima media thickness and an experienced ultrasonographer. In addition the amount of abdominal adiposity can be a limiting factor in obtaining images. Despite this only 2 of 100 children were excluded as adequate images could not be obtained, both of whom had a waist circumference greater than the 90th percentile for age [353]. The main limitation of our study was that this was a cross sectional cohort and longitudinal data is now required to establish the natural progression of aIMT over time.

Adolescence has been identified as a critical time in determining risk of future vascular complications in T1D [42]. Interventions to prevent these complications will be most effective if implemented at a young age. There is therefore a need for robust surrogate markers of vascular disease at this age to test interventions. Our data supports the use of aortic IMT as an outcome measure for trials of interventions to reduce macrovascular disease.
Chapter 5: Vascular function and glucose variability improve transiently following initiation of continuous subcutaneous insulin infusion in children with type 1 diabetes

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Pediatric Diabetes. 2013, 14 (7), 504-511
STATEMENT OF AUTHORSHIP

Vascular function and glucose variability improve transiently following initiation of subcutaneous insulin infusion in children with type 1 diabetes. Pediatric Diabetes. 2013, 14(7), 504-511

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Involved in conception and design, acquisition of data, analysis and interpretation of data, wrote the manuscript and acted as corresponding author
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Performed advanced statistical analysis, help with interpretation of the data and manuscript evaluation
Signed... Date...........2/01/14
Baghurst, P

Performed advanced statistical analysis, help with interpretation of the data and manuscript evaluation
Signed.... .............. Date.............3/1/14

Couper, J

Supervised conception and design, interpretation of data and provided manuscript evaluation
Signed.... .............. Date.............3/1/14
The paper presented in this chapter is the first paper looking at the effects of commencing continuous subcutaneous insulin infusion on vascular function and structure.

The impact factor of the journal where this paper has been published is 2.077.

This article has been cited once in peer-reviewed literature.

Data from this paper has been presented at the following national and international meetings:

- Australian Diabetes Association Annual Scientific Meeting, Adelaide, Australia, August 2009 (oral presentation: AO-11)

- 8th Joint Meeting of the Lawson-Wilkins Paediatric Endocrine Society and European Society of Paediatric Endocrinology, New York, USA, September 2009 (poster presentation PO2-348)

- Australasian Paediatric Endocrine Group Annual Scientific Meeting, Perth, Australia, August 2011 (oral presentation: O20)
The specific components of this publication that I was responsible for include

- Being involved in the study design and coordination of submission of the project to the Research Ethics Board
- Recruitment of the study subjects
- Collection of data: including administration of study questionnaire, anthropometric measurements, phlebotomy collection, measurements of intima media thickness, FMD and GTN from ultrasound images, downloading and organizing of the CGMS data
- In collaboration with our statistician, involved with statistical analysis of data
- Wrote the initial version of the manuscript and was involved in the editing process for publication
- Acted as corresponding author for the published manuscript
5.1 Abstract

Objective: The effect of continuous subcutaneous insulin infusion (CSII) and glucose variability on vascular health in T1D is not known. We aimed to determine whether initiation of CSII improves vascular function and reduces glucose variability, independent of changes in HbA1c.

Methods: 22 children with T1D (12.5±2.9 years) were reviewed immediately prior, 3 weeks and 12 months after initiation of CSII. Vascular function (FMD and GTN), glucose variability (mean of daily differences [MODD], mean amplitude of glycaemic excursions [MAGE] and continuous overlapping net glycaemic action [CONGA]), clinical and biochemical data were measured at each visit. Results for the first two visits were compared to a previously studied cohort of 31 children with T1D who remained on multiple daily injections (MDI).

Results: FMD, GTN, blood pressure, HbA1c, fructosamine and glucose variability significantly improved 3 weeks after CSII commencement (all p<0.05), but there was no change in the MDI control group. At 3 weeks, vascular function related to glucose variability ([FMD: MODD r=-0.62, p=0.002] and [GTN: MAGE r=-0.59, p=0.004, CONGA-4 r=-0.51, p=0.01, MODD r=-0.62, p=0.002]) but not to blood pressure, HbA1c, or fructosamine. At 12 months, FMD, GTN, blood pressure and glucose variability returned to baseline levels, while HbA1c deteriorated. Carotid intima media thickness was unchanged over 12 months.

Conclusions: Initiation of CSII rapidly improves vascular function in association with decreased glucose variability; however the effects are not sustained with deterioration of metabolic control and glucose variability.
5.2 Introduction

The risk of developing future micro and macro-vascular complications in T1D closely relates to an individual’s mean level of glycaemia, as measured by HbA1c [41, 183]. The role of acute glucose fluctuations or glucose variability as an independent risk factor remains controversial, with contrasting results in published studies [199, 354-357].

Intermittent high glucose exposure in-vitro enhances endothelial cell apoptosis and proliferation of vascular smooth muscle cells through mitochondrial superoxide overproduction [196, 199]. While in adults with type 2 diabetes (T2D), oscillating blood glucose levels and greater glucose variability relates to worse endothelial dysfunction [355, 356], the evidence in T1D is less clear. In adults with T1D, glucose variability, as determined from seven daily isolated blood glucose levels, may be a predictor for the incidence of peripheral neuropathy [358], but is not an additional risk factor for the development of retinopathy or nephropathy [354] or arterial stiffness [357]. In children with T1D, hypoglycemia, but not glucose variability related to vascular dysfunction in our cross sectional study [359].

The earliest changes of atherosclerosis begin in childhood [4] and children with T1D have abnormal vascular function and structure compared to healthy age-matched controls [123, 240]. Endothelial and smooth muscle dysfunction, as measured by FMD and GTN respectively, and cIMT are early surrogate markers of atherosclerosis [23, 360] and correlate with abnormal coronary angiography in adults [30].
The use of continuous subcutaneous insulin infusion (CSII) in clinical practice is increasing. CSII lowers glucose variability compared to multiple daily injections (MDI), both in children and adults [361-363]. The reduction in glucose fluctuations has been postulated to allow a lower HbA1c without increasing the risk of hypoglycemia [363]. However it is not known whether the reduction in glucose variability and hypoglycaemia with CSII is also associated with an improvement in vascular function. This potential effect could have particular importance for children with T1D, as they have higher blood glucose variability than adults with type 1 and type 2 diabetes [364].

We aimed, therefore, in a real-life clinical setting, to assess glucose variability using continuous glucose monitoring system (CGMS) data and vascular function (FMD and GTN), as the primary outcome measures, in children with T1D, referred for CSII, prior to, early after, and 1 year following changing from MDI to CSII therapy. We hypothesized that reduction in glucose variability with CSII initiation would be associated with an improvement in vascular function, independent of the change in HbA1c.

5.3 Methods

5.3.1 Subjects: CSII

Subjects aged between 8 and 18 years with T1D, were recruited consecutively from children referred for commencement on CSII at the diabetes clinics at the Women’s and Children’s Hospital (Adelaide, Australia). Exclusion criteria were
Monitoring of vascular health in children at risk for atherosclerosis

...diabetes duration of less than 6 months, retinopathy on direct fundoscopy, microalbuminuria as measured by early morning urinary albumin / creatinine (normal <30 µg/mg) and use of anti-hypertensive or lipid lowering medication. Subjects had assessment of vascular function, glucose variability, clinical and biochemical indices immediately prior (visit 1), 3 weeks (visit 2) and 6 to 24 (visit 3) months after initiation on CSII.

5.3.2 Subjects: MDI controls

The results from the visit 1 and 2 assessments in children who commenced CSII, were compared to data from a previously studied control group of 31 children with T1D who remained on MDI during the study [238]. The controls were the placebo arm of a randomised controlled intervention trial of folic acid and vitamin B6 supplementation. The controls had assessment of vascular function and clinical and biochemical indices at baseline and 4 weeks later.

5.3.3 Clinical and biochemical measurements

Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight with minimal clothing was taken on an electric digital scale to the nearest 0.1 kg. BMI [weight (kg) / height (m)^2] and BMI z-score were calculated using EpiInfo database version 3.2.2 and Centres for Disease Control 2000 standardised reference charts. Waist circumference was measured at the midpoint between the lower edge of the ribs in the mid axillary line and the top of the iliac crest, at minimal respiration. Blood pressure was measured with an appropriately sized cuff three times after 10 minutes rest in the supine position, and the mean of the measurements was recorded.
Fasting venous blood samples were collected. HbA1c was measured using a latex immunoagglutination inhibition methodology (DCA 2000 Hemoglobin A1c Reagent Kit; Bayer, Toronto, Ontario). Fructosamine was measured using Cobras Integras system (normal range 190–285 μmol/l; Roche Diagnostics). Glucose, lipid profile and high-sensitivity C-reactive protein (hs-CRP) were measured as previously described [365].

5.3.4 Glucose variability assessment

Glucose variability was evaluated using data downloaded from a CGMS (Medtronic Minimed, Northbridge, CA, USA) that was worn for 72 hours prior to each study visit. For the assessment immediately prior to CSII start (visit 1), the subjects were asked to carry the insulin pump (Medtronic Paradigm 722), but not insert the insulin delivery set, to allow communication of the information from the glucose sensor to the pump. The CGMS was inserted by a diabetes educator (LW) on day 0 and children and families were instructed to perform at least four blood glucose measurements a day and to enter them into the insulin pump for calibration of the system. While the families were instructed to respond as they normally would to the capillary blood glucose levels, they were blinded to the data from the CGMS. Data from the CGMS was downloaded on day 3. Glucose measurements on day 0 and day 3 were not included in the calculations to avoid bias related to anxiety associated with sensor insertion or removal, and to ensure a consistent 48 hour for all subjects (from 12 a.m. on Day 1 to 11:59 p.m. on Day 2).

Glucose measurements obtained from day 1 and 2 were exported into an excel database and entered into a computer algorithm developed by PB as previously
described [366, 367]. This computer algorithm allows calculations of the following measurements of glucose variability: mean amplitude of glycaemic excursions (MAGE), mean blood glucose (MBG) and its standard deviation (SD), continuous overall net glycaemic action (CONGA) and mean of daily differences (MODD). MAGE was calculated by an automated algorithm designed to locate all the peaks and nadirs in each CGMS data set (and its subsets) according to the rules defined by Service et al [368]. The standard deviation required to determine whether a glycaemic excursion was eligible to be included in MAGE was estimated from each subject’s entire CGMS (not recalculated for each 24 hour period); and only the magnitudes of upward excursions were averaged. CONGA evaluates intraday glycaemic variation. It was calculated after different hour intervals of observations called n (n=1, 2, 3, 4, 5, 6, 7 and 8). For each observation or glucose value after n hours of observations, the difference between the current observation and the observation n hours previously was calculated [369]. MODD was calculated from the absolute differences between paired sensor glucose values during two successive 24 h periods of CGMS [370].

The degree of hypoglycaemia was evaluated by calculating GRADE and Low Blood Glucose Index (LGBI) from the CGMS data as previously described [359]. GRADE scores are an empirical representation of the “risk” (on a scale of 0 to 50) associated with a specified glucose concentration [371]. A GRADE score is assigned to each glucose observation in an individual’s CGMS profile according to the formula

$$\text{GRADE score} = 425(\log \log \text{glucose}) + 0.16)^2,$$

and the scores are averaged over the entire CGMS profile. The relative contributions (as a percentage) to the overall GRADE score from the glucose observations in the range < 3.9 form the
hypoglycaemic GRADE score [371]. LGBI was calculated and adapted for CGMS as described by McCall et al [372]. Duration of hypoglycaemia was calculated as percentage of time with glucose levels under 3.5 mmol/L using the data obtained from CGMS.

5.3.5 Vascular function and structure assessment

Vascular function (FMD and GTN) evaluation was performed while the subjects were fasting on day 3 of wearing the CGMS before the download for each of the visits, and was assessed as previously described [123]. Briefly, brachial artery diameter was measured in a longitudinal section 2-15 cm above the elbow using B mode ultrasound with a 17MHz linear array transducer (Philips iU22, Bothel, Washington, USA). An electrocardiogram (ECG) was recorded simultaneously with the ultrasound images. Each study included 4 scans: (1) resting scan; subsequently, reactive hyperaemia was induced by occluding arterial blood flow using a sphygmomanometer inflated to 250 mmHg for 4 minutes; (2) FMD scan recorded between 45-75 seconds after cuff deflation; (3) re-control scan 10-15 minutes later; and (4) last scan, taken 4 minutes after the sublingual administration of the GTN spray (400 µg, Nitrolingual Pumpspray, Sanofi Aventis). Images were recorded onto VHS videotape and analysed by a blinded observer. For each scan, measurements were made over 4 consecutive cardiac cycles, incident with the R wave on the ECG using ultrasonic callipers. Measurements were averaged and expressed as percentages of the resting scan. Our coefficient of variation in 20 subjects is 3.9% for FMD and 4.0% for GTN [123].
Carotid IMT was measured at visit 1 and 3 as previously described [365]. The left and right common carotid arteries were imaged in a standardized magnification (2 x 2 cm) using images of the posterior wall of the distal 10 mm of the common carotid artery, just proximal to the carotid bulb. The greatest distance between the lumen-intima interface and media-adventitia interface (intima media thickness - IMT) was measured at a minimum of 100 points using a semi-automated edge detection and measurement computer software package (Brian Bailey, Royal Prince Alfred Hospital, Sydney, Australia). Three best quality images were selected and analyzed for mean and maximum IMT of the right and left carotid arteries. The mean of the readings was recorded to give the final result for each subject. The coefficient of variation in 20 subjects is 1.2% [365].

The study was approved by the Children, Youth and Women’s Health Service Human Research Ethics Committee. Written informed consent was obtained from the parents / guardians of the subjects and the subject if he / she was more than 16 years old. The study was registered under the Australian and New Zealand Trial Registry, trial number ACTRN 12608000189325.

5.3.6 Statistical analysis

The data were analysed using Stata version 10.1. Analyses followed the ‘intention to treat’ principle. Comparison of baseline characteristics between the CSII and MDI control group were assessed with ANOVA for normally distributed data and Kruskal-Wallis for data that were not normally distributed. Data were transformed as appropriate to meet normality. Differences in vascular function (FMD and GTN), glucose variability and clinical and biochemical variables across the three visits were determined using ANOVA or Skillings-Mack tests as appropriate. Pearson’s and
Spearman’s correlations were used to compare the associations between FMD or GTN and clinical variables at each visit. No adjustment for multiple comparisons was made. The associations between the change in glucose variability and vascular function across visit 1 and 2 were determined using a linear mixed model. The change in HbA1c and fructosamine were included in the model to control for their effect on glucose variability. P values less than 0.05 were considered significant. A sample size of 18 with two repeated measurements for each subject provide a power of 70% at a significance of 0.05 to detect an association (r=0.6) between vascular function and glucose variability.

5.4 Results

Thirty four children with T1D, aged between 8 and 18 years, who were on the waiting list to commence CSII treatment were consecutively approached for participation in the study. Twelve declined to be involved due to either having difficulty in attending the study visits, or not wanting to participate. Twenty two children (9 males, mean age 12.5±2.9 years, mean diabetes duration 3.4±3.0 years) participated in the study. Average insulin dose prior to changing to CSII was 0.86±0.33 units/kg/day. At visit 1, one subject had CGMS sensor failure and at visit 2 all data was collected and analysed from the 22 subjects. At visit 3 (mean time from CSII commencement 1.2 years) 2 subjects withdrew from the study due to moving interstate. The remaining 20 subjects at visit 3 had assessment of clinical, biochemical and vascular function, but 5 of the 20 did not have assessment of glycaemic variability (2 CGMS sensor failures, 3 subjects declined CGMS assessment).
5.4.1 Early effects (3 weeks) after initiation of CSII on vascular function and glucose variability

Three weeks after initiation of CSII there was a significant improvement in vascular function (FMD p=0.04 and GTN p=0.03) and significant decrease in systolic and diastolic blood pressure, HbA1c and fructosamine (Table 5). Lipids, fasting glucose and hs-CRP remained unchanged across the two visits (all p>0.05).

Table 5: Change at 3 weeks in vascular function, blood pressure and biochemistry by group

<table>
<thead>
<tr>
<th></th>
<th>CSII group (n=22)</th>
<th>MDI Control group (n=31)</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-CSII Baseline</td>
<td>3 weeks post CSII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD %</td>
<td>5.0 (3.9)</td>
<td>7.1 (2.6)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>GTN %</td>
<td>25.3 (6.9)</td>
<td>27.7 (5.7)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure mmHg</td>
<td>107.6 (10.7)</td>
<td>103.3 (10.9)</td>
<td>0.009</td>
<td>112.1 (11.4)</td>
</tr>
<tr>
<td>Diastolic blood pressure mmHg</td>
<td>61.2 (5.9)</td>
<td>58.0 (5.4)</td>
<td>&lt;0.001</td>
<td>61.7 (8.4)</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>8.6 (1.5)</td>
<td>8.0 (1.0)</td>
<td>0.001</td>
<td>8.8 (1.2)</td>
</tr>
<tr>
<td>Fructosamine µmol/L a</td>
<td>394 (282 to 882)</td>
<td>352 (278 to 424)</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (SD) and ANOVA unless otherwise specified. aMedians (range) and Skillings Mack tests

The change in vascular function, biochemical and clinical indices between 0 and 3 weeks was compared to previously collected data from 31 children (18 males)
with T1D who remained on multiple daily injections (MDI) (mean age 13.6 ± 2.8 years, mean diabetes duration 4.2±2.8 years, mean insulin dose 1.1 ± 0.4 units/kg/day). MDI control group and CSII study group were comparable with respect to age (p=0.16), gender (p=0.15), diabetes duration (p=0.39), insulin dose (p=0.10), blood pressure (systolic p=0.13, diastolic p=0.82), HbA1c (p=0.60) and baseline vascular function (FMD p=0.26 and GTN p=0.54). Fasting glucose was higher in the MDI group (13.9 ±4.5 vs. 10.8±5.9 mmol/L, p=0.04). In contrast to the CSII group, there were no changes in vascular function, HbA1c or blood pressure in the MDI (control) group between the two assessments (Table 5). The change in vascular function between visit 1 and 2 was significantly greater in the CSII subjects compared to the MDI group (change in FMD: 2.4 ± 4.4% vs. -1.5 ± 4.1%, p=0.002, change in GTN: 2.4 ± 4.9% vs.-2.0 ± 6.0%, p = 0.007).

After 3 weeks, the CSII subjects improved some of the markers of glucose variability (CONGA-1 p<0.01, CONGA-4 p=0.04, MAGE p=0.06, SD of MBG p=0.09, MODD p=0.14). There was no significant change in measurements assessing glucose at the time of vascular function (p=0.68), the degree of hypoglycaemia (GRADE hypo (p=0.83) and LGBI [p=0.92]), nor the percentage of time with a blood glucose <3.5 mmol/L during the CGMS between visit 1 and 2.

There was no significant association between vascular function and glucose variability at baseline. Improved vascular function was related to reduced glucose variability at 3 weeks but not to HbA1c, blood pressure, insulin dose, BMI Z score, hs-CRP or lipids (Table 6). Change in FMD between visit 1 and 2 was associated with the change in MODD (p=0.005), and change in GTN was associated with the change in multiple glucose variability measures (MAGE p=0.01, SD of MBG p=0.003,
CONGA-8 \( p=0.02, \) MODD \( p=0.001 \). These associations remained significant after controlling for changes in HbA1c and fructosamine.

**Table 6: Univariate associations with endothelial function (FMD) and smooth muscle function (GTN) 3 weeks post CSII commencement**

<table>
<thead>
<tr>
<th></th>
<th>FMD</th>
<th></th>
<th>GTN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( p ) value</td>
<td>( r )</td>
<td>( p ) value</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.07</td>
<td>0.75</td>
<td>-0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Fructosamine</td>
<td>0.18</td>
<td>0.42</td>
<td>-0.29</td>
<td>0.21</td>
</tr>
<tr>
<td>SD of MBG</td>
<td>-0.49</td>
<td>0.02</td>
<td>-0.65</td>
<td>0.001</td>
</tr>
<tr>
<td>MAGE</td>
<td>-0.37</td>
<td>0.09</td>
<td>-0.59</td>
<td>0.004</td>
</tr>
<tr>
<td>CONGA-1</td>
<td>-0.35</td>
<td>0.11</td>
<td>-0.43</td>
<td>0.05</td>
</tr>
<tr>
<td>CONGA-4</td>
<td>-0.34</td>
<td>0.12</td>
<td>-0.51</td>
<td>0.01</td>
</tr>
<tr>
<td>MODD</td>
<td>-0.62</td>
<td>0.002</td>
<td>-0.62</td>
<td>0.002</td>
</tr>
<tr>
<td>GRADE – hypoglycaemic ( a )</td>
<td>-0.07</td>
<td>0.74</td>
<td>-0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>LGBI</td>
<td>0.09</td>
<td>0.69</td>
<td>0.23</td>
<td>0.30</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.40</td>
<td>0.07</td>
<td>-0.31</td>
<td>0.16</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.38</td>
<td>0.08</td>
<td>-0.08</td>
<td>0.71</td>
</tr>
<tr>
<td>Insulin dose (units/kg/day)</td>
<td>-0.21</td>
<td>0.35</td>
<td>-0.36</td>
<td>0.10</td>
</tr>
<tr>
<td>BMI z score</td>
<td>-0.02</td>
<td>0.93</td>
<td>-0.36</td>
<td>0.10</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>-0.37</td>
<td>0.09</td>
<td>0.07</td>
<td>0.76</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.31</td>
<td>0.16</td>
<td>0.03</td>
<td>0.90</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.13</td>
<td>0.58</td>
<td>0.27</td>
<td>0.22</td>
</tr>
<tr>
<td>Triglycerides ( a )</td>
<td>-0.23</td>
<td>0.31</td>
<td>-0.27</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data are Pearson’s correlation unless otherwise indicated. \( a \)Spearman’s correlation
5.4.2 Effects of initiation of CSII on vascular function, structure and glucose variability after 12 months

The initial improvement at 3 weeks in vascular function (FMD and GTN), systolic and diastolic blood pressure and HbA1c was not sustained by the end of study period (Table 7). The early decrease in glucose variability was also not sustained (Table 3). There was no significant difference in percentage of time with a blood glucose <3.5 mmol/L across the three visits (p=0.17). The deterioration in vascular function did not relate to changes in glucose variability, HbA1c, lipids or blood pressure. After 12 months of CSII, there was no significant change in vascular structure (p=0.78 for mean cIMT and p=0.82 for max cIMT) or BMI z score (p=0.47).
**Table 7: Change in variables across the three visits**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)</td>
<td>5.0 (3.9)</td>
<td>7.1 (2.6)</td>
<td>6.4 (4.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>GTN (%)</td>
<td>25.3 (6.9)</td>
<td>27.7 (5.7)</td>
<td>24.9 (7.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin dose (units/kg/day)</td>
<td>0.86 (0.3)</td>
<td>0.70 (0.3)</td>
<td>0.72 (0.2)</td>
<td>0.008*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>107.6 (10.7)</td>
<td>103.3 (10.9)</td>
<td>109.0 (9.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>61.2 (5.9)</td>
<td>58.0 (5.4)</td>
<td>60.8 (4.5)</td>
<td>0.007</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.6 (1.5)</td>
<td>8.0 (1.0)</td>
<td>9.2 (1.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>10.8 (5.9)</td>
<td>10.1 (4.2)</td>
<td>10.6 (4.2)</td>
<td>0.88</td>
</tr>
<tr>
<td>SD of MBG (mmol/L)</td>
<td>3.6 (1.2)</td>
<td>3.2 (1.2)</td>
<td>3.6 (0.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>MAGE (mmol/L)</td>
<td>8.7 (2.9)</td>
<td>7.7 (3.1)</td>
<td>8.6 (2.9)</td>
<td>0.20</td>
</tr>
<tr>
<td>CONGA-1 (mmol/L)</td>
<td>2.5 (0.6)</td>
<td>2.1 (0.6)</td>
<td>2.5 (0.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>CONGA-4 (mmol/L)</td>
<td>4.8 (1.4)</td>
<td>4.0 (1.5)</td>
<td>4.7 (1.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>MODD (mmol/L)</td>
<td>4.1 (1.4)</td>
<td>3.5 (1.7)</td>
<td>4.1 (1.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>1.8 (2.3)</td>
<td>1.8 (3.5)</td>
<td>1.6 (2.3)</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.3 (0.6)</td>
<td>2.2 (0.5)</td>
<td>2.3 (0.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.6 (0.4)</td>
<td>1.6 (0.3)</td>
<td>1.6 (0.3)</td>
<td>0.87</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 (1.0)</td>
<td>0.8 (0.4)</td>
<td>0.9 (0.5)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are mean (SD) and ANOVA comparison across the three visits
Triglycerides and hs-CRP log transformed, GTN square root transformed
*p < 0.05 for visit 1 vs. visit 3

5.5 Discussion

Initiation of CSII caused a rapid improvement in vascular function, blood pressure, and metabolic control and reduced glucose variability in children with T1D. The transient improvement in vascular function was independent of improvement in HbA1c, fructosamine or blood pressure, but related to a reduction in glucose variability. No improvement was seen in a MDI control group of children with T1D. Early improvements in vascular function, blood pressure and metabolic control, and
the reduction in glucose variability (as measured by CONGA) with CSII initiation were not sustained over time, with a return to baseline vascular function, blood pressure and glucose variability, with deterioration in HbA1c. This is the first study, to our knowledge, to show that CSII improves vascular function in relation to a fall in glucose variability in T1D.

Three weeks post CSII initiation, vascular function strongly related to several measures of glucose variability. Similarly, other groups have demonstrated that glucose variability measured during a hyperglycaemic–euglycaemic clamp or during CGMS relates to abnormal vascular function in adults with T2D [355, 356]. In T1D, fluctuations in glucose concentrations following hypoglycaemia have been linked to endothelial dysfunction [373]. In addition the administration of an intravenous insulin infusion to stabilise both blood glucose levels and glucose variability has been shown to decrease oxidative stress and improve endothelial function [374]. The association between glucose variability and smooth muscle function has also been demonstrated, with enhanced vascular smooth muscle cell proliferation seen after exposure to intermittent hyperglycaemia [199]. Alterations to vascular smooth muscle may explain the results demonstrated in this study, given both endothelial dependent (FMD) and non-endothelial dependent (GTN) vascular function improved transiently following CSII initiation. These results are in contrast our previous published findings which showed no significant association between vascular function and glucose variability in a cross-sectional study of 52 children with T1D [359]. Subjects in that cross-sectional study were mainly on MDI and had a higher HbA1c and glucose variability than the children in this study, which potentially could affect the relationship between vascular function and glucose variability.
It has been hypothesised that oxidative stress is the link between glucose variability and vascular dysfunction. Both in vitro and animal models have demonstrated evidence of vascular inflammation and endothelial cell apoptosis following fluctuations in blood glucose levels, via the production of reactive oxygen species [196, 197, 373]. Oxidative stress is also associated with hypertension, and has been proposed as a potential causal factor [375]. Three weeks post CSII initiation our subjects, in addition to improvements in glucose variability, had a significant decrease in blood pressure. Blood pressure improvement was not sustained overtime as glucose variability and metabolic control deteriorated.

Our findings of deterioration in metabolic control over the longer study period are common to other paediatric cohorts. Early improvement in metabolic control is not sustained in other studies of CSII. Only 25% of teenagers on CSII for 24 months improved HbA1c by at least 1% in a large multi centre Swedish study [376], and in a national study in Denmark, HbA1c increased on CSII with increasing duration of diabetes [377]. Poor adherence to CSII-related tasks, such as insulin bolusing for meals, is frequently seen in adolescents and is related to glycaemic outcome [378]. The subjects in our study were not specifically chosen prior to enrolment to ensure good treatment adherence, but were consecutively recruited from patients who had been referred for commencement on CSII by their regular diabetes doctor. While their response is representative of the real-life clinic, it limits our ability to extrapolate these findings to patients who are able to achieve sustained improvement in glycaemic control.

In considering potential confounders of the demonstrated relationship between vascular function and glucose variability, we considered the effect of ambient glucose
at the time of vascular function assessment. Vascular function relates to serum glucose concentrations in individuals without diabetes [125, 337-339]. However, this relationship has not been demonstrated when glucose levels are outside the normal range in individuals with type 1 diabetes [125, 236]. In our cohort, ambient CGMS glucose at the time of FMD and GTN, did not relate to vascular function, nor varied significantly across the three visits. It therefore does not appear to be a significant confounder for our reported outcomes.

A limitation of the study is that 5 subjects did not have CGMS data at visit 3 that could be analysed, because of either sensor failure or subjects declining to have a repeat CGMS insertion. We had a sensor failure rate of 5% in our study (3 out of 60 insertions). The subsequent generation of CGMS that have been introduced in Australia since this study was conducted, have improved reliability and longer duration [379]. Our patients were blinded from their sensor readings, and only wore the CGMS for 72 hours prior to each visit. Real-time CGMS has been shown to lead to sustained reduction in MAGE and hypoglycaemia [380, 381]. While it would be interesting to postulate whether real-time CGMS could therefore also lead to sustained improvements in vascular function, benefits from sensor augmented pumps relate to frequency of sensor use [380, 381]. This underpins the importance of developing strategies to increase adherence in adolescents, if any potential advantages of these new technologies can be translated to clinical practice.

A further limitation of this study was the use of historical rather than contemporary controls and the lack of glucose variability measurements in these controls. However the MDI control group had similar age, duration of T1D, vascular function, blood pressure, HbA1c and insulin dose at baseline. They also received
similar hours of health professional contact apart from the explicit education for pump initiation.

In conclusion, we have shown that children with T1D have early improvements in vascular function, blood pressure and metabolic control following CSII initiation. This improvement in vascular function is associated with reduced glucose variability. The beneficial effects are, however, not sustained over time with deterioration in glucose variability and metabolic control.
Chapter 5: Adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency have vascular dysfunction

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Clin Endocrinol (Oxf). 2012; 76(6), 837-42
STATEMENT OF AUTHORSHIP

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**Harrington, J (Candidate)**
Involved in conception and design, acquisition of data, analysis and interpretation of data, wrote the manuscript and acted as corresponding author
Signed. 
Date. 3/1/1/2014

**Peña, A**
Involved in helping with acquisition and interpretation of data and manuscript evaluation
Signed. 
Date. 3/1/1/14

**Gent, R**
Performed the ultrasounds, assisted data interpretation and manuscript evaluation
Signed. 
Date. 3/1/1/14

**Hirte, C**
Performed advanced statistical analysis, help with interpretation of the data and manuscript evaluation
Signed. 
Date. 3/1/1/14

**Couper, J**
Supervised conception and design, interpretation of data and provided manuscript evaluation
Signed. 
Date. 3/1/1/14
The paper presented in this chapter demonstrates that a relatively novel group of children at potential risk for accelerated atherosclerosis, children with congenital adrenal hyperplasia, have evidence of vascular dysfunction.

The impact factor of the journal where this paper has been published is 3.396

This article has been cited three times in peer-reviewed literature.

Data from this paper has been presented at the following national meeting:

- Australasian Paediatric Endocrine Group Annual Scientific Meeting, Adelaide, Australia, August 2010 (oral presentation)
The specific components of this publication that I was responsible for include:

- Being involved in the study design and coordination of submission of the project to the Research Ethics Board

- Recruitment of all of the study subjects

- Collection of data: including administration of study questionnaire, anthropometric measurements, phlebotomy collection, measurements of intima media thickness, FMD and GTN from ultrasound images

- In collaboration with our statistician, involved with statistical analysis of data

- Wrote the initial version of the manuscript and was involved in the editing process for publication

- Acted as corresponding author for the published manuscript
6.1 Abstract

Context: Patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency have multiple vascular risk factors. Young adults with CAH have increased intima media thickness, but there have been no studies of vascular function and structure in children CAH.

Objective: To establish whether children with CAH have reduced vascular function and increased cIMT when compared to healthy and obese children.

Design and Patients: Cross-sectional study of 14 patients (14.8 years ± 3.2, 7 males) with CAH secondary to 21-hydroxylase deficiency compared to 28 obese and 53 healthy controls.

Measurements: All subjects had assessment of endothelial function (FMD), smooth muscle function (GTN) and cIMT. Anthropometric data, resting blood pressure and biochemical variables were also measured.

Results: CAH subjects had significantly reduced FMD (4.5 ± 3.0% vs. 7.5 ± 5.2%; p = 0.04) and GTN (17.2 ± 1.6% vs. 28.4 ± 8.4%; p < 0.001) when compared to controls and the impairment was comparable to the obese cohort. There was no significant difference in cIMT between groups. CAH subjects had increased insulin resistance (HOMA-IR 2.5 [0.2 to 2.9] vs. 1.8 [0.5 to 4.2]; p = 0.04), waist-to-height ratio (0.47 ± 0.05 vs. 0.44 ± 0.04; p = 0.02) and higher systolic blood pressure Z score (0.29 ± 0.9 vs. -0.24 ± 0.64, p = 0.01) compared to healthy controls but not when compared to obese controls.
Conclusions: Subjects with CAH have evidence of vascular dysfunction by adolescence.

6.2 Introduction

Congenital adrenal hyperplasia (CAH), due to 21-hydroxylase enzyme deficiency, is an autosomal recessive condition that is caused by mutations in CYP21A2. It is characterized by glucocorticoid and mineralocorticoid deficiency and androgen excess. The goal of treatment is to reduce adrenal androgens by replacing the deficient hormones, and by doing so preventing adrenal crisis and allowing normal growth and development [44]. The balance between overtreatment leading to hypercortisolism and under treatment leading to hyperandrogenism can be difficult to manage.

There has been increasing evidence that patients with CAH have multiple vascular risk factors that may put them at increased risk of cardiovascular disease in adulthood. Adults with CAH have an increased risk of elevated body mass index (BMI) [48], fat mass [49] and insulin resistance [319, 382]. Vascular health may be particularly pertinent to children with CAH, as the earliest stages of atherosclerosis first appear in childhood and are accelerated in high risk populations [17]. Children and adolescents with CAH are at a greater risk of obesity as measured by having a higher BMI and truncal fat mass [46], increased fasting serum insulin levels and insulin resistance [308], and elevated ambulatory 24-hour blood pressure measurements [47]. The role of hyperandrogenism as an independent risk factor is still not known.
Monitoring of vascular health in children at risk for atherosclerosis

Endothelial and smooth muscle dysfunction as measured by FMD and GTN respectively, as well as cIMT have been used as early surrogate markers of atherosclerosis[360, 383]. Abnormal endothelial function as measured by FMD correlates with abnormal coronary angiography in adults [30]. Vascular dysfunction and increased cIMT occur in children with accelerated atherosclerosis as in obesity, type 1 diabetes and hypercholesterolemia [125, 244].

Adults with CAH were found to have increased cIMT in one study [384], but there is no data on early markers such as vascular function. Vascular dysfunction occurs early and is independently associated with the progression of cIMT over time [385]. Our aim was therefore to determine whether there are early changes in vascular function and structure in children and adolescents with CAH secondary to 21-hydroxylase deficiency.

6.3 Subjects and Methods

6.3.1 Subjects and Controls

A total of 95 children [42 males, mean age 15.1(3.0) years] were studied. 14 children [7 males] with CAH secondary to 21-hydroxylase deficiency were recruited consecutively from the endocrinology clinic at the Women’s and Children’s Hospital (Adelaide, Australia), 3 with simple virilizing and the remainder with salt wasting CAH. Exclusion criteria were subjects younger than 8 years to ensure cooperation with ultrasound tests. They represented 14/24 of patients with CAH aged 9 - 20 years in South Australia. The diagnosis had been previously made by the presence of elevated 17-hydroxyprogesterone. All of the salt wasting patients were diagnosed
through the presence of virilization of the genitalia in girls at birth and raised renin levels or with acute salt loss in the boys. All subjects with CAH were being treated with hydrocortisone [mean dose 13.3 (4.1) mg/m$^2$/day] and fludrocortisone [mean dose 108.3 (19.5) µg/day]. Treatment had commenced within the first 2 and ½ weeks of life in all subjects with salt wasting CAH, and at 6 months and 4 years of age in the three patients with simple virilizing. Therapy was monitored by regular assessment of clinical and laboratory data in accordance with current guidelines [44]. Reasons for not entering the study were disinterest in the study, travelling distance from the study centre, and refusal to have additional blood testing.

28 children [14 males] with mild to moderate obesity [BMI Z score 1.5-3.3] and 53 healthy controls [21 males] were used as comparison groups. The obese children were recruited consecutively from pediatric outpatient clinics at the Women’s and Children’s Hospital (Adelaide, Australia). Diabetes, syndromal obesity and/or endocrinological causes of obesity were exclusion criteria and one subject with diabetes was excluded on this basis. The 53 healthy aged matched controls were recruited from friends or siblings of subjects who had participated in a previous study [386].

The study was approved by the Children, Youth and Women’s Health Service Human Research Committee. Written informed consent was obtained from the parents / guardians of the subjects and the subject if he / she was more than 16 years old.

Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight with minimal clothing was taken on an electric digital scale to the nearest 0.1 kg. BMI [weight (kg) / height (m)$^2$] and BMI z-score were calculated using EpiInfo
Monitoring of vascular health in children at risk for atherosclerosis

Database version 3.2.2 and Centers for Disease Control 2000 standardized reference charts. Waist circumference was measured at the midpoint between the lower edge of the ribs in the mid-axillary line and the top of the iliac crest, at minimal respiration. Blood pressure was measured using an automated oscillometric device with an appropriately sized cuff three times and the mean of the measurements was recorded. Systolic and diastolic blood pressure Z scores were calculated as per the National High Blood Pressure Working Group on high blood pressure in children and adolescents guidelines [387].

6.3.2 Ultrasound assessment of vascular function and structure

Vascular endothelial and smooth muscle function and cIMT were assessed using B mode ultrasound (Philips iU22, Bothel, Washington, USA) with a 17MHz linear array transducer after an overnight fast in a quiet and stable temperature environment. The ultrasound studies were performed by experienced ultrasonographers who were unaware of the clinical characteristics of the subject.

Vascular function was assessed using FMD and GTN induced dilatation as has been previously described using ultrasound of the brachial artery to evaluate changes in blood vessel diameter [123, 288]. Brachial artery diameter (2-15 cm above the elbow) was measured in a longitudinal section from two-dimensional ultrasound images. Each study included four scans. The first scan was taken at rest. Reactive hyperemia was then induced by occluding arterial blood flow for four minutes using a sphygmomanometer inflated to 250 mmHg. The second scan (reactive hyperemia or FMD) was recorded 30-90 seconds after cuff deflation, with measurements between 45 and 75 seconds after deflation. Ten to fifteen minutes were allowed for vessel recovery and then the third (re-control) scan was taken. The final scan was taken four
minutes after sublingual administration of glycercyl trinitrate spray (400µg, Nitrolingual Spray, G. Pohl-Boskamp, Hohenlockstedet, Germany).

Images were recorded onto VHS videotape and analyzed subsequently by a blinded observer. For each scan, measurements were made incident with the electrocardiogram R-wave (i.e. at end diastole) over four cardiac cycles using ultrasonic calipers. Then, the measurements were averaged and expressed as percentages of the first control (resting) scan. There were four final measurements in total: resting, FMD, recontrol, and GTN. Reactive hyperemia was calculated as the flow in the first 15 s after cuff deflation divided by the flow during the resting scan. The coefficient of variation between 20 subjects is 3.9% for FMD and 4.0% for GTN[123].

For cIMT, the left and right common carotid arteries were imaged in a standardized magnification (2 x 2 cm) using images of the posterior wall of the distal 10 mm of the common carotid artery, just proximal to the carotid bulb. A minimum of 4 images of each of the common carotid arteries were taken. All images were taken at end-diastole, incident with the R-wave on a continuously recorded ECG and then digitally stored for later analysis. The images were later analyzed by 2 independent readers who were blinded to the subjects’ clinical details. The greatest distance between the lumen-intima interface and media-adventitia interface (intima media thickness - IMT) was measured at a minimum of 100 points using a semi-automated edge detection and measurement computer software package (Brian Bailey, Royal Prince Alfred Hospital, Sydney, Australia). Three best quality images were selected and analyzed for mean and maximum IMT of the right and left carotid arteries. The
mean of the readings was recorded to give the final result for each subject. The coefficient of variation between 20 subjects is 1.2% [386].

6.3.3 Laboratory tests

Overnight fasting venous blood samples were taken after the subjects’ morning medication. High sensitive C reactive protein (hsCRP) was measured using a near infrared particle immunoassay method using IMMAGE Immunochemistry Systems Reagent (Beckman Coulter Inc., Fullerton, CA). Triglycerides, total cholesterol, high-density lipoprotein (HDL), glucose and insulin were measured using routine methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. 17-hydroxyprogesterone and androstenedione were measured using radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA) and direct renin by chemoluminescent immunoassay (Liaison, Vercelli, Italy). Insulin resistance was estimated using the homeostasis model of assessment (HOMA-IR) method according to the formula HOMA-IR = insulin (microunits per milliliter) X glucose (millimoles per liter)/22.5.

6.3.4 Statistical analysis

The data were analyzed using SPSS software version 15.0. Comparisons of variables across the three groups were made using a one-way ANOVA and comparisons between two groups were made using independent samples t-tests. A log transformation was used in the analysis for HsCRP and HOMA-IR as these variables were not normally distributed. The strength of the linear association between a number of variables with FMD and GTN were assessed using Spearman’s correlations. Statistical significance was inferred with a P value less than 0.05.
6.4 Results

The characteristics of the study groups are shown in Table 8 and Table 9.
There was no significant difference with respect to age, BMI Z score, waist hip ratio, or lipids between the group with CAH and healthy controls. Nor was there any significant difference with respect to pubertal distribution across all three groups, with 28% of the subjects in both the CAH and obese group being in early to mid puberty compared to 26% in the control group.

Table 8: Anthropometric data across three groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=53)</th>
<th>Obese (n=28)</th>
<th>CAH (n=14)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.7 ± 3.1</td>
<td>16.2 ± 2.3</td>
<td>14.8 ± 3.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Height Z score**</td>
<td>0.8 ± 1.0</td>
<td>0.75 ± 1.0</td>
<td>-0.7 ± 1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Weight Z score</td>
<td>0.5 ± 0.8</td>
<td>2.6 ± 0.6</td>
<td>0.3 ± 1.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg / m²)</td>
<td>20.6 ± 3.4</td>
<td>36.5 ± 6.9</td>
<td>23.0 ± 5.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI Z score</td>
<td>0.2 ± 0.9</td>
<td>2.3 ± 0.4</td>
<td>0.6 ± 1.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>70.8 ± 8.6</td>
<td>103.8 ± 14.6</td>
<td>73.6 ± 10.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.87 ± 0.05</td>
<td>0.9 ± 0.06</td>
<td>0.87 ± 0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Waist height ratio*</td>
<td>0.44 ± 0.04</td>
<td>0.68 ± 0.1</td>
<td>0.47 ± 0.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>108.8 ± 8.1</td>
<td>122.2 ± 10.5</td>
<td>112.0 ± 8.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure Z score*</td>
<td>-0.24 ± 0.64</td>
<td>0.63 ± 0.9</td>
<td>0.29 ± 0.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>60.0 ± 6.4</td>
<td>62.8 ± 6.6</td>
<td>61.4 ± 5.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Diastolic blood pressure Z score</td>
<td>-0.52 ± 0.58</td>
<td>-0.42 ± 0.63</td>
<td>-0.27 ± 0.63</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. CAH vs. Controls: p<0.05*, p < 0.001 **
Values are given as mean ± SD, a geometric mean (range). CAH vs. Controls: p<0.05*, p < 0.001 **
Table 9: Vascular function and structure and metabolic data across three groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=53)</th>
<th>Obese (n=28)</th>
<th>CAH (n=14)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)*</td>
<td>7.5 ± 5.2</td>
<td>5.8 ± 4.1</td>
<td>4.5 ± 3.0</td>
<td>0.06</td>
</tr>
<tr>
<td>GTN (%)**</td>
<td>28.4 ± 8.4</td>
<td>19.1 ± 7.5</td>
<td>17.2 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.42 ± 0.05</td>
<td>0.44 ± 0.06</td>
<td>0.41 ± 0.06</td>
<td>0.27</td>
</tr>
<tr>
<td>HsCRP (mg/L)*</td>
<td>0.5 (0.1-7.8)</td>
<td>4.6 (0.6-30)</td>
<td>1.0 (0.2-3.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.7 ± 0.3</td>
<td>1.3 ± 0.6</td>
<td>0.8 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± 0.9</td>
<td>4.1 ± 0.7</td>
<td>0.51</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.5 ± 0.9</td>
<td>2.5 ± 0.7</td>
<td>2.3 ± 0.5</td>
<td>0.69</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR **</td>
<td>1.8 (0.5-4.2)</td>
<td>6.8 (1.9-32.4)</td>
<td>2.5 (0.2-2.9)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD, *geometric mean (range)
CAH vs. Controls: p<0.05*, p < 0.001 **

In comparison to the healthy control group, the CAH group had a decreased height Z score (-0.7 ± 1.4 vs. 0.8 ± 1.0, p<0.001) and greater waist to height ratio (0.47 ± 0.05 vs. 0.44 ± 0.04, p = 0.02). In addition the CAH group had a higher insulin resistance compared to the healthy controls (2.5 [0.2 to 2.0] vs. 1.8 [0.5 to 4.2], p = 0.04), but significantly less insulin resistance (2.5 [0.2 to 2.9] versus 6.8 [1.9 to 32.4], p < 0.001) and waist to height ratio (0.47 ± 0.05 vs. 0.68 ± 0.14, p <0.001) when compared to obese controls. The CAH group had a significantly greater systolic blood pressure Z score corrected for age, gender and height (0.29 ± 0.9 vs. -0.24 ± 0.64, p = 0.01).
Both endothelial (FMD) (4.5% ± 3.0 vs. 7.5% ± 5.2, p = 0.04) and smooth muscle function (GTN) (17.2% ± 6.0 vs. 28.4% ± 8.4, p < 0.001) were significantly decreased in the CAH cohort (Figure 3). This impairment was similar to that in the obese group (FMD p = 0.5, GTN p = 0.4). We did not however find any difference with respect to cIMT across all three groups (p = 0.27), nor any difference in cIMT in the obese versus the CAH group (p=0.17).

Figure 5: FMD (%) and GTN (%) in healthy controls, obese and children with CAH.

In the group with CAH mean early am (0800-1000) hormone levels were 17-hydroxyprogesterone 17.4 ± 12.8 nmol/L, androstenedione 9.0 ± 12.3 nmol/L, and renin 7.4 ± 12.9 ng/L. There were no significant correlations between FMD or GTN with BMI Z score, blood pressure, lipids, 17-hydroxyprogesterone, androstenedione, renin, hydrocortisone dose, duration of treatment or HOMA-IR. There was a positive trend between waist to height ratio and HOMA-IR (r = 0.52, p = 0.05). Neither
systolic blood pressure Z score \( r = -0.19, p = 0.51 \), or diastolic blood pressure Z score \( r = 0.01, p = 0.97 \) related to renin levels.

The calculated power at the 5% significance level to detect the observed effect size for GTN and FMD between CAH and healthy controls was 99.6% and 52.9% respectively.

### 6.5 Discussion

This study demonstrated that children with CAH have significant vascular endothelial and smooth muscle dysfunction when compared to healthy controls, as measured by FMD and GTN. The vascular dysfunction was comparable to the subjects with mild to moderate obesity. Both the healthy control and obese children in this study had comparable FMD and GTN measurements to previous published data in healthy and obese children of similar age [28, 388]. This is the first study to our knowledge detecting early changes of atherosclerosis in adolescents with CAH. This has important implications as adolescence is a critical time in determining future vascular risk [17].

Possible risk factors causing vascular dysfunction in CAH are overweight/obesity, insulin resistance, hypertension, dyslipidemia, hypercortisolism and hyperandrogenism. The impairment in vascular function in our CAH subjects could not be explained by any difference from controls in BMI Z score or fasting lipids. However, the CAH subjects had a significantly lower height Z score as has been described [389] and a greater waist to height ratio than the healthy controls. Waist to height ratio is a comparable if not better marker of cardiovascular risk than
BMI Z score [390]. It is strongly associated with visceral adiposity as measured by dual X-ray absorptiometry in children and correlates with insulin resistance [391], in accordance with our findings.

Children and adolescents with CAH have elevated 24-hour ambulatory systolic blood pressure [47]. Despite a limitation of our study being that blood pressure was the mean of three measurements on a single occasion, rather than a 24-hour profile, we were still able to demonstrate a significant increase in systolic blood pressure Z score in the group with CAH. Only one of the subjects with CAH had an elevated systolic blood pressure above the 95th percentile for age. Higher blood pressures even in the normal range however relate to early markers of atherosclerosis such as cIMT in healthy children [150, 392]. Whilst mineralocorticoid excess can elevate blood pressure [393], we did not demonstrate an association between blood pressure and renin levels in the CAH group consistent with other studies [47, 316].

The CAH subjects also had greater insulin resistance, as measured by HOMA-IR index, in comparison to the controls as previously described in pediatric and adult cohorts [308, 319, 382]. Most studies, like ours, have used HOMA-IR to assess insulin resistance in patients with CAH, rather than the gold standard euglycaemic clamp. Insulin resistance as measured by clamp methods has been shown to correlate strongly with HOMA-IR in children, and as such is a good approximation [394]. One explanation as to the cause of insulin resistance in CAH is its association with long standing adrenomedullary hypofunction, with decreased catecholamines leading to a loss of inhibition of insulin secretion[308]. In addition periods of iatrogenic hypercortisolism may further contribute to the insulin resistance [395]. In our CAH
subjects, hydrocortisone dose range was within recommended guidelines [44] and comparable to other reported cohorts [46, 47].

The reduction in vascular function in our CAH subjects was comparable to the obese group who had a significantly higher BMI, waist to height ratio, systolic blood pressure Z score and insulin resistance. This suggests that there may be other factors specific to CAH that contribute to the vascular dysfunction, including hyperandrogenism and hypercortisolism. We were not able to demonstrate a direct association between androgen levels and vascular function in CAH, but our study was not powered to do this.

The close association between excess androgens and insulin resistance in women is well known[396]; and insulin resistance is a well established independent cardiovascular risk factor [397]. However the role of hyperandrogenism as an independent cardiovascular risk factor remains uncertain. Women with polycystic ovarian syndrome (PCOS) have endothelial and smooth muscle dysfunction [398]. Several studies have demonstrated that the vascular dysfunction is independent of insulin resistance, weight and lipids [325, 398]. cIMT is associated with testosterone and androstenedione levels in young women with PCOS [326]. Whether there is a direct association between androgen levels and vascular function is still unresolved, as others have not found any independent effect of androgens on vascular function in PCOS [399].

A limitation of our study was that the sample was not large enough to determine associations between vascular function and cardiovascular risk factors, but we had adequate power to detect differences between groups. The CAH subjects were representative of our South Australia’s population of children under 18 years
with CAH, in terms of their hydrocortisone and fludrocortisone medication doses which were within published guidelines [44]. In addition, their androstenedione and 17-hydroxyprogesterone levels were comparable with other published cohorts [47].

We did not find any difference with respect to cIMT between the group with CAH and healthy controls, unlike Sartorato et al [384]. Given that our cohort was over a decade younger, changes in vascular structure may not yet have developed.

Endothelial dysfunction is a first critical step in the development of atherosclerosis [17], and thus the results that were seen in this study may simply represent an earlier stage prior to any change in cIMT. Being able to identify the early changes in vascular function is important as endothelial dysfunction has been shown to be independently associated with the progression of cIMT over time [385].

CAH is a lifelong condition that is associated with multiple vascular risk factors and we have shown for the first time that endothelial and smooth muscle dysfunction is present by adolescence. In addition to optimal hormonal treatment of CAH according to current guidelines [44], all children should have assessment of potential additional cardiovascular risk factors. Monitoring and intervention for central adiposity, hypertension and insulin resistance, as well as optimizing hormone replacement to use the minimum hydrocortisone dose to adequately suppress androgens in adolescence, may have an important role in decreasing future cardiovascular disease.

Addendum:

Since the publication of our trial, a subsequent study by Wasniewska et al [320], who assessed IMT in 18 adolescents with CAH compared to 16 healthy age
and BMI matched controls has been published. They reported increased cIMT and aIMT in the CAH group (p<0.01), in contrast to our findings of no significant difference in IMT between groups. Of note, their adolescents with CAH were older than our cohort (16.2 ± 2.2 vs. 14.8 ± 3.2 years) and were receiving higher hydrocortisone doses (17.1±2.9 vs. 13.3±4.1 mg/m$^2$/day) which may explain the different results between the two studies. In addition the mean aIMT values reported by Wasniewska et al, for the healthy control group (0.8 [0.7-0.90] mm) were above the 90$^{th}$ percentile for normative aIMT values as measured in a study of 228 healthy adolescents (90$^{th}$ % for 16 year adolescent: aIMT = 0.7 mm) [400]. The fact that the control group had a significantly increased aIMT from what has been previously reported, gives rise to question about the robustness of the technique used to obtain the aIMT values in the trial by Wasniewska et al.
7. Chapter 7: Discussion
In this chapter I will discuss the results of the research in the context of study design, how the findings contribute to current published evidence, the clinical implications of the results and what potential future research questions arise from this work.

7.1 Aortic intima media thickness is an early marker of atherosclerosis in children with Type 1 Diabetes Mellitus

7.1.1 Study design

In this study we assessed the sensitivity of aIMT to detect early changes of atherosclerosis in children and adolescents with T1D using a cross-sectional case-control design. When compared to a prospective cohort study, a cross-sectional design has the advantage of not having to wait for a long period of time for the outcome to occur. This makes cross-sectional studies relatively fast and inexpensive to perform, with no loss of subjects to follow up. A case-control design also allows a comparably high yield of information from relatively few subjects [401]. The use of a control group, allows the subjects and controls to be comparable with respect to major risk factors that are related to the disease, but not of interest to the investigator. In our study the controls were age matched to the children with T1D, given IMT increases with age during childhood and adolescence [148, 149]. The controls were recruited as either friends, or predominantly siblings of the children with T1D. The advantage of using siblings as controls subjects is that this controls for other potential confounding
variables, such as underlying genetic predisposition for atherosclerosis, as well as environmental factors such as socioeconomic status.

In looking at the potential weaknesses of case-control studies, a consideration is susceptibility to bias. Sampling bias can occur when the sample of subjects studied are unrepresentative with respect to the risk factor being analysed [401]. For example, sampling bias can occur if the more motivated families who are interested in participating in research, and who correspondingly may be more motivated about achieving better diabetes control, are enrolled. The children and adolescents with T1D in our study, however, had a mean HbA1c that was not statistically different from the entire diabetes clinic. Children with T1D who met the inclusion criteria were also approached for study recruitment consecutively within the clinic, which provides medical care to over 85% of the children with type 1 diabetes in South Australia. The children who were recruited for the control group were not selected as a random sample, but age-matched to the children with T1D.

A secondary important source of bias to consider, particularly in this study, is measurement bias in relation to IMT. To reduce this both the ultrasonographers performing the ultrasound studies and the readers of the images were blinded to the subjects’ clinical details and diagnosis.

Given that a measurement technique (i.e. IMT) was the main outcome measure, we also performed as part of the study measures of reliability. Scans from twenty random subjects, including both children with T1D and healthy controls, were read by two blinded observers to assess inter-observer reliability. In addition twenty scans were read on two separate occasions by the same observer to allow measurement of intra-observer reliability. Different methods to assess inter and intra-
observer reliability have been reported in the literature. The use of correlation coefficients, has significant limitations and is potentially misleading, and as such, was not used in our study. Factors such as the scale of the measurement and the range of values may produce high correlation that does not equate to a high level of agreement [402, 403].

Bland-Altman plots are a method of testing reproducibility where by the difference of a measured outcome (e.g. mean aIMT) between observers is plotted against their mean. For good agreement, the points should be randomly scattered about zero and predominantly within two standard deviations [404]. Figure 4 and 5 provide evidence to suggest good inter and intra observer agreement for measurement of cIMT and aIMT in our study

**Figure 6: Bland-Altman plot for Inter-Observer agreement for cIMT and aIMT**
Figure 7: Bland-Altman plot for Intra-Observer agreement for cIMT and aIMT
An alternate approach to assess reliability is to calculate an intra-class correlation coefficient (ICC) [405]. An ICC indicates the proportion of the variability in an outcome (such as mean aIMT) within a particular factor (such as between two observers) that is due only to variation among subjects. ICC results range from zero to one, with values closer to one representing higher reliability [406]. Details of the intra and inter-observer ICC in our study are outlined below.

**Table 10: ICC for absolute agreement between the two observers**

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>95% CI of ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cIMT</td>
<td>0.994</td>
<td>0.982 to 0.998</td>
</tr>
<tr>
<td>Mean aIMT</td>
<td>0.991</td>
<td>0.977 to 0.996</td>
</tr>
</tbody>
</table>

**Table 11: ICC for absolute agreement within one observer for repeated readings**

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>95% CI of ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cIMT</td>
<td>0.970</td>
<td>0.860 to 0.990</td>
</tr>
<tr>
<td>Mean aIMT</td>
<td>0.996</td>
<td>0.984 to 0.999</td>
</tr>
</tbody>
</table>
Our results for reproducibility of cIMT measurements is similar to previously published studies in children, which report ICC of between 0.95 to 0.99[250, 407]. Our reproducibility data for aIMT is also comparable to the other study assessing aIMT in children with T1D by Jarvisalo et al. who reported intra and inter-observer ICC of 0.86 [152].

71.2 Study results

The most significant finding of this study is that aIMT, but not cIMT, is significantly greater in children with T1D compared to age-matched healthy control children. The lack of difference in cIMT between groups from this study, adds to the currently published heterogeneity in cIMT results, as previously discussed in the literature review of this thesis. There is good evidence now since the publication of the SEARCH CVD study, to suggest that by young adulthood or late adolescence cIMT is significantly greater in T1D [242] and relates to cardiovascular risk factor exposure in childhood [150, 408]. The lack of consistency in the published literature regarding cIMT in younger populations may reflect that the increase in cIMT in high risk populations healthy occurs later in the carotid than the aortic vascular bed. It is also possible that the drivers and associated risk factors for atherosclerosis in different vascular beds are different. The Muscatine Offspring study, assessed cIMT and aIMT in 228 eleven to seventeen year old adolescents, as well as 407 eighteen to thirty-four year old young adults. In the adolescent cohort, aIMT was more strongly associated with cardiovascular risk factors than cIMT, a relationship that was not demonstrated in the older aged cohort [150]. This data as well as the results of our study would support the hypothesis that aIMT is an earlier marker than cIMT of atherosclerosis in children.
The second significant finding from our study is that aIMT in children with T1D relates to cardiovascular risk factors, in particular HbA1c and LDL cholesterol. This is the first time that this has been demonstrated. While cIMT correlated with HbA1c in the SEARCH CVD study [242], in most other cross-sectional pediatric studies, cIMT has not been demonstrated to relate to HbA1c [250, 251, 255]. The relationship between lower HbA1c and reduced atherosclerosis risk has however been demonstrated in interventional trials in adults with T1D, where reductions in HbA1c during adolescence, decreases cIMT [43] and cardiovascular disease [260] in adults. Aortic IMT has not, to date, been used as an outcome marker in interventional trials of patients with T1D. Given in our study, the association between aIMT with not only current but previous glycaemic control (i.e. median HbA1c from previous 2 years), the data from this study would support its use as an early surrogate marker of atherosclerosis for future intervention trials.

We also demonstrated that increased aIMT is associated with increased total and LDL cholesterol in adolescents with T1D. While cIMT is also associated with total cholesterol, the relationship is not as strong, again supportive evidence that aIMT is a more sensitive marker of early atherosclerosis. Increased cholesterol concentration, both total and LDL cholesterol, is a significant risk factor for future cardiovascular disease. In adults with T1D, every 1 mmol/L increase in cholesterol is associated with a 10% rise in mean coronary vessel stenosis [409]. LDL cholesterol correlates with the extent of aortic fatty streaks seen on autopsy [27].

The data from this study both confirms the work by Jarvisalo et al, that children with T1D have increased aIMT, but also expands current knowledge to importantly demonstrate associations between aIMT and cardiovascular risk factors.
7.1.3 Research implications and future research

The finding that aIMT is an earlier marker of atherosclerosis in children with T1D has implications for future research. Given that adolescence is a critical period of time in determining future cardiovascular risk [144, 258], the use of aIMT in clinical studies may both allow investigators to identify sub-populations of children at increased risk, recognise important new modifiable risk factors and test interventions in a timely manner. Higher urinary albumin excretion, before the development of microalbuminuria, for example, has been demonstrated, since the publication of the study, to be associated with increased aIMT in children with T1D (abstract from the 2012 International Society of Pediatric and Adolescent Diabetes Annual Meeting) [410]. Aortic IMT is also being used as a surrogate end-point in a sub-study of the AdDIT trial (Adolescent Type 1 Diabetes Cardio-renal Intervention Trial), an interventional trial of angiotensin converting enzyme inhibitors and statins in adolescents with T1D screened to be at higher cardiovascular risk by urinary albumin excretion rate [411].

In addition to research implications there are clinical implications of this research. The association between increased aIMT and poor glycaemic control and abnormal lipid profile reinforces the need for exploring means to reduce these risk factors in children with T1D. In children with T1D with elevated LDL cholesterol, improvements in lipid profile with the use of statins, could potentially lead to improvements in aIMT, as what has been demonstrated in children with familial hypercholesterolemia with cIMT [274]. Further research is still needed to answer this question.
Further research is still needed with respect to the use of aIMT as a marker of early atherosclerosis. Longitudinal assessment of progression of aIMT, particularly through adolescence and puberty, both in healthy controls as well as high-risk disease groups is needed. For long-term interventional studies, this data are needed to distinguish changes in aIMT related to natural history compared to an interventional effect. Longer prospective cohort data is needed to determine whether aIMT in childhood and adolescence does predict adult cardiovascular disease, and whether interventions that improve aIMT in childhood also lead to improved future cardiovascular morbidity and mortality.

7.2 Vascular function and glucose variability improve transiently following initiation of continuous subcutaneous insulin infusion in children with type 1 diabetes

7.2.1 Study design

A significant proportion of children with T1D are managed using CSII, with a 30-50% prevalence of use reported in the large T1D Exchange Registry of 14592 children and adolescents within the United States [412]. The ability of CSII to reduce glucose variability compared to multiple daily injections, independent of any difference in HbA1c [413], have led investigators to speculate whether this could equate to a reduction of diabetes associated cardiovascular complications.

A non-randomized within series design was used to assess whether commencing children with T1D on CSII would improve vascular function by
decreasing glucose variability. While a randomized trial is the optimum method to assess the effect of an intervention, given that the decision to commence a child or adolescent on CSII in our study was a clinical choice made by the family in conjunction with their routine diabetes doctor, randomization would not have been feasible. The advantage of within series design is that each participant in the study serves as their own control to evaluate the effect of treatment. Innate characteristics such as age, sex and genetic factors are therefore eliminated as confounding variables [401].

The disadvantage of a within-group design is the lack of concurrent control group, to distinguish the effects of the intervention from potential study effects (improvements seen from simply being involved in a clinical trial). To overcome this we compared our study subjects’ data with a historical control group, who had remained on insulin injections but had had similar hours of health professional contact. The historical control group had been studied 5 years prior to the current children involved in our study, but had comparable baseline data. In retrospect, a wait group with delayed CSII initiation would have been an alternative design for this study.

Recruitment occurred consecutively from children and adolescents with T1D referred for commencement on CSII to reduce sampling bias. Of the thirty-four eligible children, 22 were recruited for the study. The main reasons for not wanting to be involved in the trial were lack of interest (n=7), difficulty in attending research appointments (n=3) and the child not wanting to wear a CGMS (n=2).

An important variable in the study was the measurement of glucose variability. One of the difficulties of the trial is that there is not one established gold-standard
method of measuring glucose variability. The various measures assess different aspects of glucose variability and have associated advantages and disadvantages [414]. The different aspects of the measures of glucose variability utilized in our study are outlined below.
Table 12: Measurements of glucose variability calculated from CGMS

<table>
<thead>
<tr>
<th>Measure</th>
<th>Definition</th>
<th>Use and advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean glucose</td>
<td>Mean of all glucose values</td>
<td>Correlation with HbA1c, Ease of calculation</td>
<td>Does not directly measure glucose variability. No importance placed on hypo or hyperglycemia</td>
</tr>
<tr>
<td>Standard deviation of mean blood glucose (SD of MBG) [415]</td>
<td>Standard deviation of all glucose values</td>
<td>Ease of calculation</td>
<td>Does not address non-gaussian skewed distribution of glucose values or outliers [416]</td>
</tr>
<tr>
<td>Mean amplitude of glycemic action (MAGE) [368]</td>
<td>Average amplitude of glycemic excursions (either increases or decreases) greater than one SD</td>
<td>Attempts of describe major glucose fluctuations</td>
<td>Final value differs depending on whether increases or decreases are used. Only assesses impact of excursions at an arbitrary cut-off of 1 SD [417]</td>
</tr>
<tr>
<td>Continuous overall net glycemic action [369]</td>
<td>Mean difference between all glucose values from the sensor obtained n hours apart</td>
<td>Describes intra-day variability</td>
<td>CONGA values vary depending on n hour chosen. Unclear which n hour is of most clinical relevance</td>
</tr>
<tr>
<td>Mean of daily difference (MODD) [370]</td>
<td>Mean difference between glucose values obtained at the same time of day on 2 consecutive days</td>
<td>Describes between-day variability</td>
<td>Underlying assumption is that meals, activities and insulin therapy is similar on the two consecutive days [414]</td>
</tr>
<tr>
<td>GRADE-hypoglycemic [371]</td>
<td>GRADE scores are an empirical representation of the risk of a specified glucose concentration. GRADE-hypoglycemic is the percentage of GRADE scores attributable to a glucose below 3.9 mmol/L</td>
<td>Measures relative contribution of hypoglycemia</td>
<td>Does not measure glucose variability directly but a hypoglycemia frequency distribution. Limited use in clinical practice [416]</td>
</tr>
<tr>
<td>Low blood glucose index (LBGI) [372]</td>
<td>Mathematical formula to correct the skewness of glycemia through centralisation around a glucose of 6.2 mmol/L, by expanding the hypoglycemic range and reducing the hyperglycemic range</td>
<td>Larger values of LBGI indicate higher risk for hypoglycemia.</td>
<td>Obscure mathematical form with limited use in clinical practice.</td>
</tr>
</tbody>
</table>
7.2.3 **Study results**

There are two main findings of this study. The first is that vascular function rapidly improved with commencement on CSII, a change which was not seen in the control group of children who remained on insulin injections. This is the first time the effect of CSII initiation on vascular function has been assessed. The improvement in vascular function correlated with reduction in glucose variability, but not the change in HbA1c. While our data provides interesting insights into potential associations between glucose variability and vascular function, given the design of the trial it is important to recognise it does not provide evidence for causality.

The second main finding that the improvement in vascular function was not sustained over time with deterioration in metabolic control, is an important consideration. Our evidence would suggest that any effect of glucose variability on vascular function is mitigated when mean glycemic control significantly worsens. It also highlights the challenges in achieving optimal diabetes control in an adolescent cohort in a real life clinical setting.

7.2.3 **Research implications and future research**

The results from this trial support the argument that glucose variability is an independent risk factor for atherosclerosis, although this clearly remains a controversial issue [418, 419] and further research is still needed. In order to tease out the potential independent effects of both mean glycemia and glycemic variability on vascular function, larger populations, with both good glycemic and poor glycemic control, need to be analysed.
If indeed in subsequent trials there is ongoing evidence to support the role of glucose variability as an independent risk factor for subclinical atherosclerosis, this would have important clinical implications. In addition to targeting lower mean glycaemia, a reduction of glucose excursions would become an important clinical target. As well as the use of CSII, with and without CGMS, investigators have and are currently studying other methods to reduce glucose variability in children and adults with T1D. For example, Liraglutide, a glucagon like peptide-1 agonist, acts in a glucose dependent manner to augment insulin secretion. In a trial of 14 adults with well controlled T1D, glucose variability significantly improved with co-administration of Liraglutide with their routine insulin therapy after 1 week. This effect was sustained at 24 weeks [266]. Whether these changes equate to an improvement in vascular health and cardiovascular related complications have not been assessed, but are important questions to address.

7.3 Adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency have vascular dysfunction

7.3.1 Study design

A cross-sectional case-control study was used to assess vascular function and structure in children with CAH compared to both healthy children and children with obesity. This study design has potential strengths and weaknesses as discussed previously in the discussion section of this thesis. One of the challenges of the study given the relative rarity of CAH as a diagnosis is that there were only a limited
number of eligible patients which to recruit from. Of the 24 eligible patients, reasons for not entering the study were disinterest in participating in the study (n=5), distance needed to travel to participate (n=3), not wanting to have additional blood tests (n=2). The number of control children in the study was much greater than the CAH group. Inclusion of a relatively greater number of control subjects can allow a study to have adequate power when the number of cases is relatively small [401].

7.3.2 Study results

The main finding of this study is that children with CAH have reduced FMD and GTN when compared to healthy controls; their vascular dysfunction is comparable to an obese cohort. This is the first time that children with CAH have been demonstrated to be at risk for accelerated atherosclerosis. Interestingly we did not demonstrate a difference in vascular structure between the groups. Although not part of the published data, aIMT was measured in the CAH and healthy control group. Aortic IMT was not, however, measured in the obese group (increased abdominal adiposity limits the ability to visualize the aorta adequately) and as such was not reported in the study. We did not find any difference (p=0.3) in mean aIMT between the adolescents with CAH (0.53 ± 0.08 mm) compared to the healthy control group (0.50 ± 0.07 mm). Vascular dysfunction is the first step in the atherosclerosis process and precedes increases in IMT [17, 233]. Our data would suggest that while children with CAH have evidence of reductions in FMD and GTN, increases in IMT do not occur by adolescence. Our limited sample size, however, may be another explanation for the lack of difference in IMT seen.

We were also able to demonstrate in our study a more adverse cardiovascular risk profile in the adolescents with CAH compared to the healthy controls. Increased
insulin resistance and systolic blood pressure was seen in the adolescents with CAH, confirming what other groups have published [308, 316, 318]. While an increase in BMI has been reported in children with CAH [46], other groups have observed similar pattern to what was demonstrated in our study, increase in abdominal adiposity (e.g. as measured by waist to height ratio) without a significant increase in BMI [307]. We were not able to demonstrate associations between cardiovascular risk factors or with other clinical variables such as serum androgens, to vascular function. This is probably a reflection of the relatively small number of adolescents with CAH in the study.

7.3.3 Research implications and future research

The finding that children with CAH are at risk for accelerated atherosclerosis and that vascular dysfunction is present by adolescence is relevant to clinical practice. While there is emerging awareness of increased incidence of cardiovascular risk factors in adults with CAH [302], this is a relatively novel concept for the pediatric age range. Part of the management and counseling of adolescents with CAH should include ways to maintain a healthy lifestyle and increase physical activity. Clinicians should carefully monitor for evidence of elevated blood pressure and increased abdominal adiposity. To limit the adverse effects of glucocorticoids on body composition and insulin resistance, the lowest dose that adequately controls androgen concentrations should be prescribed. Ongoing research is looking at ways to give glucocorticoid replacement in extended and delayed formulations that achieve more optimal pharmacokinetic profiles in the management of CAH [301].

Further research is needed to tease out which clinical factors relate to the vascular dysfunction that is seen in adolescents with CAH. In particular, more
research is needed to evaluate whether novel risk factors such as serum androgen concentrations, are independently associated with vascular function. This will allow targeted management strategies to help reduce future cardiovascular risk. While there is recognition of increased incidence of cardiovascular risk factors in adults with CAH, there is no current reported data on cardiovascular related morbidity or mortality.
Chapter 8: Summary and final conclusions
The overall aims of this thesis were to evaluate vascular health in children at risk for accelerated atherosclerosis. Specifically I aimed to evaluate the sensitivity of aIMT, a relatively new surrogate marker of atherosclerosis in children with T1D; use assessment of vascular function as the primary outcome measure in an intervention study of CSII initiation in children with T1D; and finally evaluate vascular function and structure in children with CAH compared to healthy and obese children.

While it is recognized that atherosclerosis begins in childhood, and that childhood vascular risk factors are important determinants of adult cardiovascular health, from the literature review it was evident that there is still limited, and at times conflicting data, around the evaluation of vascular health in children using ultrasound surrogate markers of atherosclerosis. There is heterogeneity in published cIMT study results in children with T1D, and a paucity of evidence of the use of the potentially more sensitive marker aIMT. There were no studies on whether the use of CSII in children with T1D, a commonly used management approach, improves vascular function. Although children with CAH have a clustering of vascular risk factors, there had been no prior assessment whether this equated to impairment in vascular health.

The first part of this thesis demonstrated that aIMT is indeed an earlier maker of pre-clinical atherosclerosis in children with T1D. It correlates with age and known vascular risk factors such as HbA1c and LDL cholesterol. Aortic IMT therefore has a potential role as an outcome measure for trials in younger patients with T1D, to allow timely assessment of interventions.

The second part of the thesis further explored vascular health in children with T1D, by assessing the effect of reduction in glucose variability through initiation of
CSII on vascular function. We were able to provide evidence that reductions in glucose variability soon after starting CSII therapy relate to improvements in vascular function, independent of changes in HbA1c. In our cohort of patients these improvements in vascular function were not however sustained over time with deterioration in metabolic control. These results highlight the difficulty in achieving sustainable improvements, particularly in adolescent patients, where patients’ behaviors modify the effectiveness of interventions.

The final part of the thesis assessed vascular function and structure in subjects with CAH, demonstrating that there is evidence of vascular dysfunction by adolescence. The reductions in FMD and GTN were comparable to what is seen in obese adolescents, despite the CAH cohort having a lower BMI. This suggests a potential role of other cardiovascular risk factors such as blood pressure, insulin resistance and potentially elevated androgens in the pathogenesis of the vascular dysfunction seen in adolescents with CAH.

In conclusion, the use of non-invasive ultrasound assessment of both vascular function and structure allows timely assessment of vascular health in at risk pediatric populations. While further work is still needed to assess the natural progression of these surrogate markers of atherosclerosis through adolescence to adulthood, measurement of vascular function and IMT are feasible, reliable methods that can be utilized at present to both identify at risk groups and assess interventions.
Chapter 9: Appendices
9.1 Appendix A: Ethics and Drug and Therapeutic Committee approvals

2nd April 2008

Dr J Harrington
Endocrinology & Diabetes
CYWHS

Dear Jenny

Re: Does commencing a continuous subcutaneous insulin infusion reduce glucose variability and improve vascular dysfunction with type 1 diabetes? REC2032/2/11

I refer to your letter of 31st March 2008 in which you responded to my letter dated 20th March 2008. All matters have been resolved. I enclose herewith the CTN form duly signed. Although TGA advises that the study may proceed once the attached CTN form (and payment) has been forwarded, it also advises that if TGA has a problem with the CTN form, e.g. incomplete information, then the CTN form may be invalidated. Therefore, you should not proceed until you receive a letter from TGA acknowledging that it has received the CTN.

Additionally, you should sight Police Checks for any students or non-CYWHS employees involved in the project now, or in the future, and record that you have sighted all relevant Police checks.

I remind you approval is given subject to:
- immediate notification of any serious or unexpected adverse events to subjects;
- immediate notification of any unforeseen events that might affect continued ethical acceptability of the project;
- submission of any proposed changes to the original protocol. Changes must be approved by the Committee before they are implemented;
- immediate advice, giving reasons, if the protocol is discontinued before its completion;
- submission of an annual report on the progress of the study, and a final report when it is completed. Please note it is your responsibility to provide these reports – without reminder from the Ethics Committee.

Approval is given for three years only, and if the study is more prolonged than this, a new submission will be required. Please note the approval number above indicates the month and year in which approval expires and it should be used in any future communication.

If University of Adelaide personnel are involved in this project, you, as chief investigator must submit a Human Research Approval notification form (available at: http://www.adelaide.edu.au/research/ethics/human/guidelines/) within 14 days of receiving this ethical clearance to ensure compliance with University requirements and appropriate indemnification.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
CYWHS RESEARCH ETHICS COMMITTEE
28th March 2008

Dr J Harrington
Endocrinology & Diabetes
CYWHS

Dear Jenny

Re: Do children with congenital adrenal hyperplasia secondary to 21 hydroxylase deficiency have vascular dysfunction? (Dr J Harrington) REC2033/2/11

I refer to your letter of 13th March 2008 in which you responded to matters raised by the CYWHS Research Ethics Committee and DTC Clinical Trials Group at their February 2008 meetings. All matters have been resolved. I enclose herewith the CTN form duly signed. Although TGA advises that the study may proceed once the attached CTN form (and payment) has been forwarded, it also advises that if TGA has a problem with the CTN form, e.g. incomplete information, then the CTN form may be invalidated. Therefore, you should not proceed until you receive a letter from TGA acknowledging that it has received the CTN.

I remind you approval is given subject to:
• immediate notification of any serious or unexpected adverse events to subjects;
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If University of Adelaide personnel are involved in this project, you, as chief investigator must submit a Human Research Approval notification form (available at: http://www.adelaide.edu.au/research/ethics/human/guidelines/) within 14 days of receiving this ethical clearance to ensure compliance with University requirements and appropriate indemnification.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
CYWHS RESEARCH ETHICS COMMITTEE

Jennifer Harrington, January 2014

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Australian Government
Department of Health and Ageing
Therapeutic Goods Administration

File Number: 08/3773

Children Youth & Women's Health Service
72 King William Road

NORTH ADELAIDE  SA 5006
Attention: Brenda Penny

CTN Scheme (Drugs): Acknowledgement of New Trial

Your notification to conduct a clinical trial under the Clinical Trial Notification (CTN) Scheme, pursuant to Schedule 5A of Regulation 12 of the Therapeutics Goods Regulations, has been received by the Drug Safety and Evaluation Branch (DSEB).

Trial Number: 2008/220
Protocol Number: REC2032
Drug Name(s): glyceryl trinitrate

It is noted that:

i. the approval of the goods for this trial was given in accordance with Item 3 of Schedule 5A of the Therapeutic Goods Regulations by the body or organisation conducting the trial at each site.

ii. the representative of the Ethics Committee for each site has certified that the Committee is constituted and operates in accordance with the NH&MRC "National Statement on Ethical Conduct in Research Involving Humans", has considered this clinical trial, and has provided advice to the body or organisation conducting the trial.

The Therapeutic Goods Administration has not carried out an assessment of the quality, safety or efficacy of any drug product in relation to this notification.

Please note that, in the event that the Secretary of the Commonwealth Department of Health and Ageing becomes aware that to undertake or continue the clinical trial would be contrary to the public interest, the Secretary has the authority to direct that use of the drug product(s) for this clinical trial must cease.

A form for "CTN Scheme (Drugs): Trial Completion Advice" is enclosed. Please fill out and return this form after the Clinical Trial has completed.

Please direct enquiries to the Experimental Drugs Section on (02) 6232 8106 (phone) or (02) 6232 8112 (fax).

Michelle Butman
Experimental Drugs Section
Drug Safety & Evaluation Branch
05/05/08
19th February 2008

Dr Tamara Zutlevics
Chair
CYWHS Research Ethic: Committee

Dear Tamara

Re: Does commencing a continuous subcutaneous insulin infusion reduce glucose variability and improve vascular dysfunction with type 1 diabetes? REC2032

This CTN application was reviewed by the DTC Clinical Trials Group at its meeting on 14th February 2008. The study is recommended for approval subject to:

1. Children under the age of eight being excluded from the trial.
2. An Information Sheet being provided for child participants and the current Information Sheet being consistent with its use of you/your child.
3. In the Information Sheet –
   • Advise that the Glyceryl Trinitrate is a sublingual spray and placed under the tongue and delete any reference to it being administered by nasal spray.
   • In the footer, advise page number, version and date.
   • Typographical errors being corrected (as attached).
4. Divisional certification (3.3.1 of the application form) to include Pharmacy.

Yours sincerely

SEAN TURNER
CHAIR
DTC CLINICAL TRIALS GROUP

Enc. Cc: Dr J Harrington, Endocrinology & Diabetes, NB: Any correspondence relating to the above should be forwarded care of the Research Secretariat, 2nd Floor, Samuel Way Building.
9.2 Appendix B: Study information sheets

Research Study: Information Sheet

LAY TITLE
Blood vessel function changes in type 1 diabetes during adolescence

SCIENTIFIC TITLE
Peripubertal change in vascular function in children with type 1 diabetes

1) What is the study for and why is it being done?
Our endocrine unit has been studying how blood vessels work in children for several years. We have found that conditions such as diabetes and obesity affect the way the blood vessels work. The way the blood vessels work change rapidly during the growth spurt that occurs in puberty. We therefore want to find out what are the changes that occur during puberty in children with diabetes or obesity in comparison to healthy children who will be the controls. The importance of this is that very early changes in blood vessel function (as determined by an ultrasound test that we use) are related in the long term to how healthy the blood vessels are in adult life.

2) What would I be asked to do if I took part in this study?
Your child will be asked to have ultrasound studies. This is the way that we use to determine how the blood vessel works.

FIRST, we will assess the thickness of the blood vessel wall, which is Intima Media Thickness. We will use an ultrasound to examine 2 arteries in your neck, one on each side of the neck. These arteries are called the carotid arteries.

SECOND, we will assess the changes in the blood vessel diameter in response to changes in the blood flow within the blood vessel. This is the Flow Mediated Dilatation. We will use the ultrasound to examine an artery in your arm. This includes:
1. First, an ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it. An electrocardiogram recording is also taken. This is a measurement of the electrical activity of the heart using 3 electrodes placed on the trunk.
2. Second, a blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow are measured again with ultrasound, to check this difference. If the blood vessel is not working properly, it will not dilate as much.
3. Third, another ultrasound picture is taken after a period of 10-15 minutes rest, to allow the vessel to return to its initial size.
4. Fourth and lastly, an ultrasound picture is taken after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working. Blood pressure and heart rate will be measure immediately after the GTN spray and 10 minutes after.

The ultrasound measurements take about 1 minute each.
The whole visit takes about 45 minutes. Each visit includes the ultrasound studies, clinical examination that include checking at the back of the neck and in the arm pits for markers of high insulin levels and pubertal stage (I will show the children diagrams of different pubertal stages on the approved growth charts, known as Tanner, and they will point to the one it describes them best); weight, height, blood pressure, waist and hip circumference, and a blood test after being fasting (nothing to eat or drink except for water after 10pm the previous night).
The blood test will measure levels of glucose, lipids (cholesterol and triglycerides), folate acid in your body and levels of homocysteine which is an amino acid which is important in blood vessel function in children and adolescents. The amount of blood required for these tests on each visit is 10 ml. There will be a total of 3 blood samples collected (30ml). Taking blood causes brief discomfort or pain, much like a pinprick. This can be minimised by using a simple local anaesthetic cream (EMLA or AnGEL) and by comforting the child. Temporary bruising can occur and infection is possible but extremely rare. The local anaesthetic cream rarely causes any irritation of the skin.
There will be 3 visits in total over 2 years.
There will be no payment to my child for taking part in this study.
3) Are there any risks of side effects associated with Intima Media Thickness or Flow Mediated Dilatation?
The procedure is very safe and has been used in a large number of children and adults. The first part of the test, where the blood pressure cuff is left up can be uncomfortable, although most children have not been bothered by it. The medication, GTN, is very safe and remains active for only a very short period of time. It can cause brief headaches in some people, although in practice this has not been a problem when used as part of this ultrasound test in children. GTN can also cause a temporary drop in blood pressure, which we will be monitoring, light-headiness, facial flushing and a fast heart rate, although these have also all been very uncommon when used in children and adolescents.

4) What will be done with this information?
We will compare the results of the ultrasound and blood tests taken over the 2 years. We will try to look at changes in the way the blood vessels work while you are growing fast. We will also write articles about the study and publish these, or talk about the study at conferences. All of the above information will remain confidential.

5) Do I have to take part in the study?
No, not at all. You should only take part in this study if you want to be involved.

6) Can I change my mind later if I decide to participate?
Yes, you can choose to leave the study at any time.

7) Will the study benefit me in any way?
We can’t be certain that you will get any benefit from taking part. However by examining the blood vessel directly and its function, we hope that we will get better information that will help us understanding the changes in blood vessel function during growth. This can relate in the long term to how healthy the blood vessels are in adult life.

8) Do you have permission to do the study?
We have obtained permission from the Research Ethics Committee at the Children, Youth and Women’s Health Services to do this study. You can contact the Secretary of this Committee, Ms Brenda Penny on (08) 81616521.

9) What if I have other questions about the study?
Please contact Dr Jenny Harrington, Endocrine Fellow, at any time. She can be paged through the Children, Youth and Women’s Health Services on (08) 81617000, pager 4458 or (08) 81616402. You can also call Associate Professor Jenny Couper on (08) 81617000, pager 4127 or (08) 8161 6402.
Research Study: Information Sheet

Lay Title: Does starting children on an insulin pump improve how blood vessels work due to less variable blood glucose levels?

Scientific Title: Does commencing a continuous subcutaneous insulin infusion reduce glucose variability and improve vascular function in children with type 1 diabetes?

You / your child has been referred to the clinic for commencement of an insulin pump. We are currently approaching patients to see if they would be interested in participating in the following study. There is no obligation to take part in the study if you / your child does not wish to.

1) Why is this study being done?
Our endocrine unit has been studying how blood vessels work in children for several years. We already know that blood glucose levels relate to the long term vascular complications in type 1 diabetes. There have been other studies that suggest that blood glucose variability (i.e. how much the blood glucose levels vary through the day) may also be an important factor. The purpose of this study is to look at blood vessel function and glucose variability in children whilst they are on insulin injections compared with shortly after starting an insulin pump.

2) What would I be asked to do if I took part in the study?
You / your child will be asked to attend the WCH on four different occasions: four days before starting on the pump, on the day of starting the pump and then 3 weeks later.

You / your child would visit the WCH 4 days prior to the date of commencing on the insulin pump to have a continuous glucose monitor sensor (CGMS) inserted. Over the next 4 days we will get you / your child to fill out a diary which records your food intake, insulin and exercise. You / your child will also need to take at least 4 blood glucose measurements to ensure that the monitor is recording the blood glucose levels accurately. Inserting the CGMS will take approximately 45 minutes.

You / your child would then return to the WCH on the morning of pump commencement after fasting from 10pm the night before. At the visit you / your child would have the sensor removed, a blood test and an ultrasound test.
The blood test (around 10ml) will measure levels of glucose, lipids (cholesterol and triglycerides), folic acid in your body and levels of homocysteine, which is an amino acid which is important in blood vessel function. Taking blood causes brief pain or discomfort, much like a pinprick. This can be minimised by using a simple local anaesthetic cream (EMLA or AnGEL) and by comforting the child. Temporary bruising can occur and infection is possible but extremely rare. The local anaesthetic cream rarely causes some irritation of the skin.

The ultrasound test takes about 35 minutes of your time.

*Flow mediated dilatation* uses ultrasound to examine an artery in an arm and works as follows:

1. An ultrasound picture is taken of the artery in the upper part of the arm.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel normally gets larger (dilates). The vessel and blood flow are measured again with ultrasound, to check this difference in size. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a spray called Glyceryl trinitrate (GTN) which is sprayed beneath the tongue. It dilates the blood vessel. The change in blood vessel size in the first part of the procedure (1,2) is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

The whole procedure takes about 35 minutes altogether, although the ultrasound measurements only take about 1 minute each.

You / your child would then be asked to repeat the CGMS 3 weeks after the insulin pump was started (at your / your child’s regular 3 week review), with a repeat ultrasound and blood test 4 days later.

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In addition, we will contact you / your child, 6 to 12 months after commencement on the pump to see if you / your child would be happy to be involved in one final follow up visit. This would involve again repeating the CGMS, with a repeat ultrasound and blood test 4 days later. Participation in the follow up of this trial, as in all parts, is voluntary.

We will provide reimbursement in the form of either a car park ticket or public transport ticket for each of the four visits. There will be no money reimbursement or payment.
3) Are there any risks or side-effects associated with the CGMS or ultrasound measurements of Flow Mediated Dilatation?
The CGMS has been used before in many children with type 1 diabetes. The sensor, as it is inserted under the skin, can sometimes be a little uncomfortable, and so AnGEL or EMLA cream can be used to numb the skin.
The ultrasound that is used for Flow Mediated Dilatation is a safe mode of imaging often used in pregnant women. Both are safe and have been used in a large numbers of children and adults, including many adults and children with diabetes. The first part of the Flow Mediated Dilatation test, where the blood pressure cuff is blown up, can be uncomfortable; although most children tolerate it well. The medication, GTN, is safe and remains active for only a few minutes.

Adverse effects of GTN spray: GTN spray can cause brief headaches in some people, although in practice this has not been a common problem when used as part of this ultrasound test in children. GTN can also cause low blood pressure, which we will be monitoring, light-headedness, facial flushing and a fast heart rate, although these have also all been uncommon when used in children.

4) What will be done with this information?
We will compare the results of the CGMS and ultrasounds with each other and with your / your child’s blood measurements. We will publish articles about the study and present the study at conferences, so that other people will be helped by the information. All of the information, including the personal details and results, will remain confidential.

5) Do I have to take part in the study?
No, not at all. You / your child should only take part in this part of the study if you want to be involved.

6) Can I change my mind later if I decide to participate?
Yes, you / your child can choose to leave the study at any time.

7) Will the study benefit me / my child in any way?
We can’t be certain that you / your child will get any benefit from taking part. However, by examining the blood vessel function and blood glucose variability we hope that we will get better information that will help us have new ways to prevent blood vessel complications in adult life. In addition the CGMS will provide additional information to your regular diabetes doctor to help improve your insulin regimen.

8) Do you have approval to do the study?
We have approval from the Research Ethics Committee at the Women’s and Children's Hospital to do this study. To contact the Research Committee you can call Ms B Penny on 81616521.
9) What if I have other questions about the study?
Please contact Dr Jenny Harrington, endocrine fellow at any time. She can be paged through the Children Youth and Women's Health Service on 81617000 pager 4458, or 81616402.
You can also call Professor Jenny Couper on 81617000 pager 4127 or 81616402.
Research Study: Information Sheet

Lay Title: Do children with congenital adrenal hyperplasia have changes in how their blood vessels work?

Scientific Title: Do children with congenital adrenal hyperplasia secondary to 21 hydroxylase deficiency have vascular dysfunction?

We are currently approaching patients to see if they would be interested in participating in the following study. This research is being undertaken by some of the doctors at the Adelaide Women's and Children's Endocrine unit. The chief researcher is Dr Jenny Harrington, one of the senior registrars working in the department. There is no obligation to take part in the study if you / you child does not wish to.

1) Why is this study being done?
We know that children with certain medical conditions such as diabetes or high cholesterol can have altered blood vessel structure and function. In adults with congenital adrenal hyperplasia, research has shown changes in their blood vessels compared to adults without congenital adrenal hyperplasia. We think that these early changes may start in childhood. We would like to now find out whether these changes can be detected in ultrasound tests of the blood vessels in children with congenital adrenal hyperplasia.

2) What would I be asked to do if I took part in the study?
You / your child would visit the WCH for one morning in addition to your normal clinic review. The total length of the visit would be approximately 45 minutes. At the visit you/your child would have a blood test and 2 ultrasound tests.

The blood test (around 10ml) will measure levels of glucose, lipids (cholesterol and triglycerides) and other chemicals that are important in blood vessel function. In addition we will measure you / your child's adrenal hormone levels. Taking blood causes brief pain or discomfort, much like a pinprick. This can be minimised by using a simple local anaesthetic cream (EMLA or AnGEL) and by comforting the child. Temporary bruising can occur and infection is possible but extremely rare. The local anaesthetic cream rarely causes some irritation of the skin.

On that day we will also ask for you / your child to do a finger-prick blood test (17-hydroxy-progesterone profile) before each dose of hydrocortisone.

Providing you are willing and give consent, we will access your / your child's case notes to check the most recent bone age that has been done.
There are 2 ultrasound tests done one after another and taking about 35 minutes of your time altogether.

The test for **Intima Media Thickness** uses ultrasound to examine 2 arteries in your neck, one on each side of the neck, and the large artery in your abdomen. These arteries in your neck are called the carotid arteries. The artery in your abdomen is called the aorta. The ultrasound probe is placed on each side of your neck and your abdomen briefly. There can be some slight discomfort as the ultrasound probe is pressed onto the abdomen but generally it is a well tolerated procedure.

The test for **Flow mediated dilatation** uses ultrasound to examine an artery in an arm and works as follows:
1. An ultrasound picture is taken of the artery in the upper part of the arm.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel normally gets larger (dilates). The vessel and blood flow are measured again with ultrasound, to check this difference in size. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a spray called **Glyceryl trinitrate (GTN)** which is sprayed beneath the tongue. It dilates the blood vessel. The change in blood vessel size in the first part of the procedure (1,2) is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

The whole procedure takes about 35 minutes altogether, although the ultrasound measurements only take about 1 minute each.

We will also organise for you / your child to have a DEXA scan in the nuclear medicine department on Level 7 at the Royal Adelaide Hospital on a separate day. The scans take place between 9am and 5pm, Monday through to Friday. We will make an appointment time that is convenient for you. This is a scan looking at the composition of the body and the ratio of muscle to fat. You / your child will be asked to lie down on a table and remain still as possible as the pictures are being taken. The scan takes approximately 20 minutes and is painless.

**3) Are there any risks or side-effects associated with ultrasound or DEXA scan?**

The DEXA scan uses a very low amount of radiation (typically 5 hours of background radiation). It is a safe method of imaging that has been used in children before.

The ultrasound that is used for Intima Media Thickness and Flow Mediated Dilatation is a safe mode of imaging often used in pregnant women. Both are safe and have been used in a large numbers of children and adults, including many adults and children with
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Jennifer Harrington, January 2014
9.3 Appendix C: Study consent forms

CHILDEm, YOUTH & WOMEN'S HEALTH SERVICE (CYWHS)  
HUMAN RESEARCH ETHICS COMMITTEE (HREC)

CONSENT FORM

LAY TITLE
Blood vessel function changes in type 1 diabetes during adolescence

SCIENTIFIC TITLE
Peripubertal change in vascular function in children with type 1 diabetes

I __________________________________________________________________________

hereby consent to my child’s involvement in the research project entitled:

Peripubertal change in vascular function in children with type 1 diabetes and obesity

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to my child taking part.

2. I understand that my child may not directly benefit by taking part in this study.

3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.

4. I understand that while information gained in the study may be published, my child will not be identified and information will be kept confidential.

5. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my child’s relationship with this healthcare service.

6. I understand that there will be no payment to my child for taking part in this study.

7. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.

30/11/06 Version 2
9. a) I consent to a specimen of the following blood being taken from my child
and being used in the above project.

b) I do / do not consent to the blood samples being used in any other research
project, provided the project has the approval of the Women’s & Children’s
Hospital Research Ethics Committee.

10. I understand that information will be kept confidential except where there is a
requirement by law for it to be divulged.

Signed: ..............................................................

Relationship to Patient: ...........................................

Full name of patient: .............................................

Dated: .........................

--- Child assent to participate if mature enough ---

Signed: ..............................................................

Full name of patient: .............................................

Dated: .........................

I certify that I have explained the study to the parent and child) and consider that
he/she understands what is involved.

Signed: .............................................................. Title: ..............................................................

Dated: .................................
CONSENT FORM

LAY TITLE
Does starting children on an insulin pump improve how blood vessels work due to less variable blood glucose levels?

SCIENTIFIC TITLE
Does commencing children on a continuous subcutaneous insulin infusion improve vascular dysfunction due to reduced glucose variability?

I

hereby consent to my child's involvement in the research project entitled:

Does commencing a continuous subcutaneous insulin infusion reduce glucose variability and improve vascular dysfunction in children with type 1 diabetes?

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to my child taking part.

2. I understand that my child may not directly benefit by taking part in this study.

3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.

4. I understand that while information gained in the study may be published, my child will not be identified and information will be kept confidential.

5. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my child's relationship with this healthcare service.

6. I understand that there will be no payment to my child for taking part in this study. Travel reimbursement in the form of either car park ticket or public transport ticket will be provided for each of the four visits.

7. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.

05/03/08 Version 3
9. a) I consent to a specimen of the following blood being taken from my child and being used in the above project.

b) I do / do not consent to the blood samples being used in any other research project, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

10. I understand that information will be kept confidential except where there is a requirement by law for it to be divulged.

Signed: 

Relationship to Patient: 

Full name of patient: 

Dated:

Child assent to participate if mature enough

Signed: 

Full name of patient: 

Dated:

I certify that I have explained the study to the parent and child) and consider that he/she understands what is involved.

Signed: 

Title:

Dated:
CHILDREN, YOUTH & WOMEN’S HEALTH SERVICE (CYWHS)  
HUMAN RESEARCH ETHICS COMMITTEE (HREC)  

CONSENT FORM  

LAY TITLE  
Do children with congenital adrenal hyperplasia have changes in how their blood vessels work?  

SCIENTIFIC TITLE  
Do children with congenital adrenal hyperplasia secondary to 21 hydroxylase deficiency have vascular dysfunction?  

I hereby consent to my child’s involvement in the research project entitled:  

Do children with Congenital Adrenal Hyperplasia secondary to 21-hydroxylase deficiency have vascular dysfunction?  

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it and agree to my child taking part.  

2. I understand that my child may not directly benefit by taking part in this study.  

3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.  

4. I understand that while information gained in the study may be published, my child will not be identified and information will be kept confidential.  

5. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my child’s relationship with this healthcare service.  

6. I understand that there will be no payment to my child for taking part in this study.  

7. I have had the opportunity to discuss taking part in this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.  

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.  

13/03/08 Version 2
Monitoring of vascular health in children at risk for atherosclerosis

9. a) I consent to a specimen of the following blood being taken from my child and being used in the above project.

   b) I do / do not consent to the blood samples being used in any other research project, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

10. I agree/disagree to the accessing of my (my child’s) medical records to check the most recent bone age.

11. I understand that information will be kept confidential except where there is a requirement by law for it to be divulged.

Signed: ............................................................

Relationship to Patient: ........................................

Full name of patient: ............................................

Dated:.................................

Child assent to participate if mature enough

Signed: ............................................................

Full name of patient: ............................................

Dated:.................................

I certify that I have explained the study to the parent and child and consider that he/she understands what is involved.

Signed: ............................................................ Title: ..............................................................

Dated: .........................
10 Chapter 10: Bibliography


2. WHO. International Statistical Classification of Diseases and Related Health Problems 10th Revision. 2010; Available from:


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Monitoring of vascular health in children at risk for atherosclerosis


Monitoring of vascular health in children at risk for atherosclerosis


