Vascular health in children and adolescents with type 1 diabetes:

The influence of exercise, diet, and metformin

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This degree is submitted for the Degree of Philosophy.

At the University of Adelaide

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May 2021

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Citation list of published papers included in this thesis

1. Anderson J, Peña AS, Sullivan T, Gent R, D'Arcy B, Olds T, Coppin B, Couper J: **Does metformin improve vascular health in children with type 1 diabetes? Protocol for a one year, double blind, randomised, placebo controlled trial**. *BMC pediatrics* 2013, **13**:108.
2. Anderson J, Couper JJ, Mpundu-Kaambwa C, Giles LC, Gent R, Coppin B, Peña AS: **An Extra 1,000 Steps Per Day Relates to Improved Cardiovascular Health in Children With Type 1 Diabetes**. *Diabetes care* 2016, **39**(8):e108-109.
3. Anderson J, Couper JJ, Toome S, Mpundu-Kaambwa C, Giles LC, Gent R, Coppin B, Pena AS: **Dietary sodium intake relates to vascular health in children with type 1 diabetes**. *Pediatric diabetes* 2018, **19**(1):138-142.
4. Anderson JJA, Couper JJ, Giles LC, Leggett CE, Gent R, Coppin B, Pena AS: **Effect of Metformin on Vascular Function in Children With Type 1 Diabetes: A 12-Month Randomized Controlled Trial**. *The Journal of clinical endocrinology and metabolism* 2017, **102**(12):4448-4456.

List of abbreviations

ACAES – Australian child and adolescent eating survey

ACR – albumin creatinine ratio

ALT – alanine aminotransferase

aIMT – aortic intima media thickness

AMPK – 5′ adenosine monophosphate-activated protein kinase

BIA – bioelectrical impedance

BMI – body mass index

BP – blood pressure

CI – confidence interval

cIMT – carotid intima media thickness

CRF – cardiorespiratory fitness

CSII – continuous subcutaneous insulin infusion

CV – coefficient of variation

CVD – cardiovascular disease

DKA – diabetic ketoacidosis

DXA – dual energy X-ray absorptiometry

FMD – flow mediated dilatation

FSIGT – frequently sampled intravenous glucose tolerance test

GTN – glyceryl trinitrate mediated dilatation

HsCRP – high sensitivity C-reactive protein

HbA1c – glycosylated haemoglobin

HDL – high density lipoprotein

IMT – intima media thickness

IQR – interquartile range

IRR – incidence rate ratio

LDL – low density lipoprotein

MEMS - medication event monitoring system

MVPA – moderate to vigorous physical activity

RCT – randomised controlled trial

SD – standard deviation

SE – standard error

T1D – type 1 diabetes

T2D – type 2 diabetes

VLDL – very low-density lipoprotein

Abstract

The antecedents of clinical cardiovascular disease begin in childhood and accelerate during puberty. Early vascular changes are measurable in children and adolescents who are at increased risk of cardiovascular disease. Vascular health can be measured non invasively using ultrasound. Flow mediated dilatation (FMD) and Glyceryl Trinitrate mediated dilatation (GTN) are measures of endothelial and smooth muscle vascular function respectively. FMD and GTN correlate with coronary atherosclerosis and to traditional cardivovascualr risk factors. Aortic and carotid intima media thickness (aIMT/cIMT) are measures of vascular structure. Intima media thickness of the arteries is a surrogate marker for clinical cardiovascular disease and predicts future cardiovascular events.

Type 1 diabetes (T1D) confers a higher risk of cardiovascular morbidity and mortality, even with modern insulin regimens. The increased risk of cardiovascular disease in T1D is multifactorial. It is related to glycaemic control but also to increased insulin resistance. In addition, the population shift to a higher body mass index also affects those with T1D and increases their vascular risk.

Therefore I aimed to investigate lifestyle factors which may correlate with vascular health in youth with T1D and whether metformin, an insulin sensitising agent, would improve vascular health over 12 months in children and adolescents with T1D. The specific lifestyle factors investigated included measures of diet, based on a validated food frequency questionnaire, and exercise, using a wearable activity monitor (Senswear device). The metformin intervention was delivered in a randomised, double blind, placebo controlled trial over 12 months in 90 children and adolescents with T1D.

There were two relationships identified between lifestyle factors and vascular health. Daily step count, a surrogate marker for activity levels, related to aIMT in youth with T1D. Those

with a higher daily step count had better aIMT. Higher dietary sodium intake related to worse vascular smooth muscle function (GTN) in children with T1D. The total dietary sodium intake was also higher than the recommended daily intake with no children having dietary sodium within the recommended levels.

Increasing activity and reduction in dietary sodium are practical ways, which may improve vascular health in children and adolescents with T1D. Longitudinal studies are needed to further investigate the relationship between activity, diet and cardiovascular disease prevention in people with T1D.

I found that metformin had several measurable outcomes in children and adolescents with T1D. Firstly, it had no impact on FMD which was our primary outcome measure. It did improve GTN which indicates a modest effect on vascular function. There was no change in IMT over 12 months. There was also a reduction in insulin dose over 12 months, in estimated insulin resistance and a transient reduction in HbA1c.

These results add to the body of knowledge on using metformin as an adjunct therapy in children and adolescents with T1D. To date, studies have consistently shown a reduction in daily insulin dose and insulin sensitivity in treated individuals with mixed results in relation to vascular health and HbA1c. The benefits of metformin and its good safety profile warrant further consideration of its use during and beyond puberty.

Declaration

I, Jemma Jay Angela Anderson, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 in my name, for any other degree or 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. I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Acknowledgments

I would like to acknowledge the patients and their families who participated in this research. Their dedication to furthering knowledge in type 1 diabetes is a wonderful part of working in partnership with children and adolescents with type 1 diabetes and their families.

I also thank my supervisors, Professor Jenny Couper and Associate Professor Alexia Peña who are the most dedicated of researchers and are kind, generous, warm women who I can only hope to emulate in work and in life.

My co-authors who were generous with their time and expertise in assisting with the publications of my work. In particular, statistician Dr Lynne Giles whose expertise in translating the statistical to the clinical was invaluable.

I am also forever indebted to ultrasonographers Roger Gent, Lino Piotto and Melissa La Forgia, with whom I shared many early mornings in the ultrasound department of the Women's and Children's hospital.

The staff in the diabetes and endocrine department of the Women's and Children's hospital, in particular Dr Jan Fairchild, Dr Elaine Tham, Dr Oana Maftei, Dr Jess Harbison, Dr John Welch and Dr Priya Augustine, diabetes nurse educators Tania Kelly, Louise Wilson, Kate Williams and Marianne Lambert, diabetes dieticians Bronwyn D'Arcy, Sarah Toome and Stephanie Savio and our dedicated administrator Julie Temby for their support in being flexible when I needed to recruit patients from clinics, fit research appointments around other schedules, and the general support that comes from working in a great workplace.

My sincere thanks to the MS McLeod foundation who provided a generous PhD scholarship which supported me in doing my research and to the funding sources, Diabetes Australia Research Trust, Women's and Children's Foundation and an Australasian Paediatric Endocrine Care grant.

And finally, to my family. My parents and parents in law, who cared for my children while I was writing my thesis, to my partner Alethea who read a draft of my thesis and my most wonderful and delightful children, Rhys, Finn and Cleo, all of whom were born during my PhD degree. Although I had often wished I had done this study before I had my children, I wouldn't change them for the world.

1. Chapter 1: Introduction

In this chapter, I will introduce the thesis with a problem statement, hypothesis, overall objective and aims, and research strategy.

1.1 Problem statement

Cardiovascular disease is the leading cause of morbidity and mortality in adulthood and is a disease process which begins in childhood and accelerates during puberty[1]. Cardiovascular disease manifests most commonly as hypertension, ischaemic heart disease, stroke, renal impairment and peripheral vascular disease. The clinical manifestations of cardiovascular disease are preceded by measurable changes in the blood vessels of children and adolescents who have traditional cardiovascular risk factors such as obesity and T1D[2, 3]. Cardiovascular disease may be mitigated by physical activity levels and a healthy diet. Interventions which impact on modifiable risk factors during childhood and adolescence may reduce the disease burden of cardiovascular disease in adulthood.

The clinical manifestations of cardiovascular disease are preceded by atherosclerosis which is the process of hardening of arteries due to the formation of an atheromatous plaque. It begins with the development of fatty streaks, an accumulation in the intima (inner lining of the artery) of foam cells (lipid laden macrophages) and extracellular matrix. Over time, the fatty streaks transform into atherosclerotic plaques which narrow the blood vessel lumen size over time or may have accelerated plaque progression after intraplaque haemorrhage. Atherosclerosis leads to the clinical manifestations of cardiovascular disease. Fatty streaks are present in coronary arteries from adolescence and in the aorta from early childhood[4].

The process of atherosclerosis begins in childhood[5, 6] and the precursors of atherosclerosis are measurable in childhood. Carotid and aortic IMT are surrogate markers for clinical cardiovascular disease and predict future cardiovascular events[7-9]. This first sign of atherosclerosis can be measured with non-invasive ultrasound. It is increased in those with traditional cardiovascular risk factors such as T1D, smoking, hyperlipidaemia and obesity.

Vascular dysfunction, specifically endothelial and smooth muscle dysfunction are early and potentially reversible indicators of cardiovascular disease. Vascular function, which can measured by FMD and GTN of the brachial artery, is a non-invasive measure of endothelial function[3].

The endothelial layer lines the arteries and is a barrier between the laminar blood flow within the artery and the subendothelial tissues. Endothelial dysfunction is one of the first signs of vascular disease and is thought to be due to a loss of nitric oxide production and responsiveness in the endothelium.

Endothelial dysfunction, as measured by FMD, correlates with abnormal coronary angiography in adults[10] and with traditional cardiovascular risk factors[11] and can be improved with interventions for example, statin therapy[12], folate in T1D[13], and exercise training. This indicates the potential for initial reversibility of vascular disease. Abnormal GTN, a measure of vasodilation produced in response to exogenous glyceryl trinitrate, is a result of smooth muscle dysfunction which is correlated with coronary atherosclerosis in adults and adults at risk of atherosclerosis.

T1D is an autoimmune disease which leads to loss of beta cell function in the pancreas. The consequent insulin deficiency and hyperglycaemia requires life-long treatment with insulin. Despite significant advances in diabetes management in recent years with intensification of insulin regimens, cardiovascular disease remains the most common cause of morbidity and mortality in these patients[14]. The development of macrovascular complications has been less responsive than that of microvascular complications to the improvement improvements in glycaemic control over the past 20 years[14-16]. Females with T1D are disproportionally affected by cardiovascular disease that females without diabetes experience[17]. Further, children who develop T1D before 10 years are particularly at risk of cardiovascular disease in

adult life (with a lifetime loss of 16 years on average) and this phenomenon is more pronounced in females[18]. Individuals with T1D experience cardiovascular complications much earlier in life than those without T1D which results in a substantial annual health care cost of $570 million annually[19]. Even with optimal metabolic control in T1D, the risk of cardiovascular disease is increased indicating the opportunity for additional interventions in childhood while the vascular changes are still potentially reversible, to reduce the significant adult disease burden. Less than 25% of children, adolescents and young adults with T1D in Australia achieve glycaemic control targets that are required to prevent life threatening cardiovascular complications in adulthood[20].

Lifestyle factors, such as diet and exercise, are known to contribute to cardiovascular risk. Dietary risk factors include diets which are high in sodium, calories and processed foods and not meeting targets for fruit and vegetable intake. Exercise is an independent modifiable risk factor for cardiovascular disease[21-23]. Australian children and adolescents, including those with T1D are not meeting recommended targets for exercise. There is little data on the effect of exercise and diet on the cardiovascular health in children and adolescents with T1D.

Metformin is a biguanide traditionally used in type 2 diabetes (T2D) where it has a protective effect on cardiovascular risk and mortality[24]. Its mechanism of action is to improve insulin sensitivity by increasing glucose uptake in peripheral tissues and suppressing hepatic glucose output. It improves endothelial function (as measured by FMD) in adults with T1D[25]. It also improves body composition in children and adolescents with obesity[26]. Metformin has also been shown to reduce the total daily dose of insulin in children, adolescents and adults with T1D and induces a short-term improvement in HbA1c[27, 28]. At the time of commencement of this study, the effect of metformin on vascular function and structure in children and adolescents with type 1 diabetes was not known.

1.2 Hypothesis

Based on the problem statement and literature review, the following hypotheses were generated:

1. Children and adolescents with type 1 diabetes who are more active have better cardiovascular health compared with those who are less active.
2. Poor dietary composition relates to abnormal vascular health in children and adolescents with type 1 diabetes.
3. Metformin improves vascular function in children and adolescents with type 1 diabetes.

1.3 Overall objectives and aims.

The overall objectives of this thesis were to evaluate the vascular function and structure of children and adolescents with type 1 diabetes and its determinants and to measure the effect of metformin on these same measures over 12 months.

The specific aims of this thesis were:

1. To measure the activity levels of children and adolescents with type 1 diabetes and correlate with vascular function and structure measurements.
2. To evaluate the dietary intake of children and adolescents with type 1 diabetes and correlate with vascular function and structure measurements.
3. To evaluate the effect of metformin, in addition to insulin, on vascular function (endothelial and smooth muscle function) and structure in children and adolescents with type 1 diabetes over 12 months.

1.4 Research strategy

In chapter 2, I detail a literature review relating to the measurement of vascular function and structure in children and adolescents, the effect of diet and exercise on vascular health, and the effect of metformin on cardiovascular health.

In chapter 3, I describe the methodology of the thesis in detail.

Chapter 4 details the relationship between activity levels and vascular structure in children and adolescents with type 1 diabetes with specific reference to step count as a measure of activity levels. This relates to hypothesis 1 and specific aim 1.

Chapter 5 details the relationship between dietary composition and vascular function in children and adolescents with type 1 diabetes. This relates to hypothesis 2 and specific aim 2.

Chapter 6 investigates the effect of Metformin on vascular function, inflammatory markers, insulin dose and body composition in children and adolescents with type 1 diabetes. This relates to hypothesis 3 and specific aim 3.

In Chapter 7, I discuss the findings of all studies in view of clinical significance and relationships to recently published data. I will also articulate future research questions in the area.

In chapter 8, I will summarise the main findings of this thesis with a final conclusion.

2 Chapter 2: Literature Review

In this chapter, I will summarise the literature relating to the antecedents of cardiovascular disease in children and how they are measured, the modifiers of cardiovascular risk in children and adolescents including exercise and diet, and finally the use of metformin in type 1 diabetes including what is known about how this relates to cardiovascular protection.

2.1 Introduction

Cardiovascular disease is a significant cause of morbidity and mortality. Atherosclerosis is a disease process leading to cardiovascular disease. It is a process affecting large and medium sized arteries. The process begins with endothelial dysfunction related to reduced laminar flow and the subendothelial accumulation of LDL (low density lipoprotein) cholesterol which is oxidised and exposes adhesion molecules leading to intimal cellular infiltration. Subsequently, fatty streaks are formed which are infiltrations within the intimal layer of t-cells, monocytes and lipids. These are evident on autopsy studies in children from the age of 3[4]. Over time, fatty streaks develop into plaques which are a necrotic core, composed of apoptotic cells, debris and cholesterol crystals covered with a fibrous cap composed of collagen and smooth muscle cells. These caps then thin and the fibrous cap is replaced by macrophages which makes them prone to plaque rupture and clinically evident acute thrombotic events, e.g. acute coronary syndrome and cerebrovascular events.

The process of atherosclerosis begins in childhood and accelerates during puberty[6]. Therefore, interventions to improve cardiovascular morbidity and mortality in adulthood may be beneficial in those identified at risk in childhood.

2.2 Measurement of vascular health

Vascular structure and function can be measured non-invasively using ultrasound. Alterations in vascular structure and function are detectable prior to clinically evident cardiovascular disease and these markers also track along with traditional cardiovascular risk factors.

2.2.1 Preclinical vascular assessment in childhood

Atherosclerosis manifests in adulthood with clinical cardiovascular disease. The process begins in childhood with the development of fatty streaks in the intimal layer of the artery which progress over time, and exposure to cardiovascular risk factors, to fibro atheromatous

plaques which may result in stenosis of the artery or plaque rupture and acute vascular events. Fatty streaks which develop early in life may regress or go on to form atherosclerotic plaques. Acceleration of this process occurs in those with exposure to an adverse vascular environment including hypertension, hyperlipidaemia and diabetes mellitus. This process also hastens during puberty, even in those without exposure to risk factors, and is more pronounced in males.

2.2.2 Autopsy studies

Autopsy studies in children and adolescents who died from non-cardiovascular related causes have demonstrated early atherosclerotic changes in the aorta and coronary arteries[4, 29-31]. Fatty streaks, the accumulation of lipid laden macrophages in the intimal layer of the artery have appeared in the aorta in children as young as 3 years old[4].

Histological study of the coronary arteries of young people age 0-29 years who died of non-cardiovascular causes has demonstrated increasing prevalence of arterial lesions from puberty, which increase with time[29]. Advanced lesions (defined as preatheroma, atheroma and fibroatheromas) increased in prevalence from 8% at age 12-14 years to 34 % at age 27-29 years. Early changes (Isolated macrophage foam cells and fatty streaks) were present in 35% of 9-11 year olds which accelerated during puberty to 65% of 12-14 year olds.

In a longitudinal study of cardiovascular risk factors and atherosclerosis, the Bogalusa heart study examined the effect of risk factors and the progression of atherosclerosis in children. It showed that fatty streaks in the aorta and coronary arteries are associated with serum total and LDL cholesterol[5], that multiple risk factors (BMI (body mass index), systolic blood pressure (BP), serum triglycerides, serum LDL cholesterol) are associated with increasing severity of atherosclerotic changes and that fatty streak lesions were more prevalent in the coronary vessels of smokers than non-smokers[30].

A large multicentre study (the Pathobiological Determinants of Atherosclerosis in Youth – PDAY study) examined 300 sets of coronary arteries and aortas to assess the relationship between established cardiovascular risk factors and preclinical atherosclerotic changes in 15-34 year olds[31]. They found that there was a linear increase in the extent of fatty streaks in the right coronary artery over time which were increased by VLDL (very low-density lipoprotein)/LDL cholesterol and negatively associated with HDL (high density lipoprotein) cholesterol. Raised lesions were similarly associated with VLDL/LDL and HDL and also smoking (greater prevalence of lesions involving ≥5% of the intimal surface in the right coronary artery, 3-fold increase in the abdominal aorta) and hypertension and BMI in men. Increased HbA1c was associated with greater fatty streaks in the abdominal aorta and raised lesions in the coronary arteries (2-fold those 15-24 years, 8-fold in those 30-34 years). The results indicated that the traditional cardiovascular risk factors were associated with a more rapid progression from fatty streaks to raised lesions suggesting that risk factor mitigation in early life, prior to clinical vascular disease, may reduce eventual morbidity and mortality.

2.2.3 Vascular structure – Intima media thickness

The thickness of the intimal layer of the artery is a surrogate measure for pre-clinical and clinically apparent atherosclerosis. IMT can be measured using ultrasound at the carotid and aorta and is non-invasive. It is safe and reproducible measure of atherosclerosis.

In adults, cIMT is a well-established surrogate marker of pre-clinical atherosclerosis. cIMT relates to traditional cardiovascular risk factors including hypertension[32, 33], hyperlipidaemia[34, 35], smoking [36, 37], diabetes[38-40] and central adiposity [40]. It is also associated with asymptomatic cardiac ischaemia [41].

cIMT predicts future cardiovascular and cerebrovascular events[7, 42] independently of the aforementioned cardiovascular risk factors and adds a small but not clinically significant

improvement to the Framingham risk score[8]. The relative risk of stroke or myocardial infarction in older adults without a history of cardiovascular disease increases by 35% with an increase in cIMT of 1 standard deviation (SD), i.e., a relatively large clinical effect for a small change in cIMT[43]. cIMT is a useful research tool but not as useful for stratifying individual clinical risk above traditional risk factors. cIMT is frequently used as an endpoint in clinical trials to determine treatment efficacy[44, 45] and has been used as an outcome measure in landmark cardiovascular trials in adolescents and adults with T1D[46, 47]. The positive predictive value of using a cIMT trial as a basis for a larger cardiovascular morbidity and mortality trial is 96% (95% Confidence interval (CI) 80,99%) and the negative predictive value is 83% (95% C 64-93%)[48]. Systematic review demonstrates cIMT is a predictor of clinical cardiovascular events[9].

2.2.3.1 Carotid Intima media thickness in children and adolescents

IMT is a useful marker of pre-clinical atherosclerotic disease in children and adolescents given its ease of measurement, reliability, and reproducibility. cIMT is related to traditional cardiovascular risk factors in children and adolescents including dyslipidaemia[49], obesity[50], T1D[51], metabolic syndrome, hypertension[49], family history[52], smoking (active and passive), inflammatory disorders such as systemic lupus erythematosus [53], chronic kidney disease[54], familial hypercholesterolaemia[55], childhood cancer[56], HIV[57] and mental health [58].

Longitudinal studies have also demonstrated a consistent relationship between cardiovascular risk factors in childhood and IMT thickness in adulthood. The cardiovascular risk in young Finns study found that exposure to cardiovascular risk factors in childhood related to increased IMT in adulthood, especially waist circumference, LDL cholesterol and high insulin levels[59]. For some risk factors (physical activity and low fruit consumption) these related to adult IMT progression independently of adult risk factors[60].

LDL cholesterol measured in childhood, adulthood, and as a cumulative burden over time was associated with cIMT in adulthood in the Bogalusa heart study[61]. BMI in childhood also predicted cIMT in adulthood[61].

The Muscatine study demonstrated that childhood total cholesterol (measured as early as age 8-11 years) was a predictive of adult cIMT[62]. Childhood BMI was a predictor of cIMT in adult women.

The International Childhood Cardiovascular Cohort Consortium (i3C) conducted a meta-analysis of longitudinal studies the which included the aforementioned studies, in addition to the Childhood Determinants of Adult Health study, and determined that cardiovascular risk factors (total cholesterol, LDL cholesterol, systolic BP and BMI) measured from as young as 9 years of age in childhood predicted cIMT in adulthood[63].

2.2.3.2 Aortic Intima media thickness

Atherosclerosis begins in the abdominal aorta as evidenced by autopsy studies. The extent of fatty streaks in the abdominal aorta correlated with the streaks in the coronary arteries in the Bogalusa Heart study[5].

aIMT correlates with traditional cardiovascular risk factors. In a cross-sectional study of children at risk of atherosclerosis (T1D or hypercholesterolaemia) compared with healthy controls, aIMT was increased in those at risk[2]. aIMT demonstrated a greater increase than cIMT. Data from the Muscatine cohort showed that aIMT related to triglycerides, systolic BP, diastolic BP, BMI, and waist/hip ratio, after adjusting for age, gender, and height[64]. aIMT is also thicker in children with obesity [65, 66].

In children and adolescents with T1D, aIMT has been shown to be a reliably associated with CVD risk factors. In a study of children and adolescents with T1D, aIMT was greater than in

controls and correlated with HbA1c, age and LDL cholesterol[67]. aIMT has been shown to be a more sensitive marker than cIMT in adolescents with T1D[68].

Studies have also demonstrated that aIMT is inversely associated with diet[69] and fitness levels[70] in children born small for gestational age and healthy adolescents respectively.

Therefore, abdominal aIMT is a reproducible measure of preclinical atherosclerosis in children and adolescents and abdominal aIMT is the site with the earliest changes of atherosclerosis and strong associations with childhood cardiovascular risk factors. It is a useful surrogate marker of cardiovascular risk in children and adolescents.

2.2.4 Vascular function

The endothelium is the innermost layer of the arterial vasculature. It is a biologically active layer which is responsible for maintaining arterial tone, controls proliferation of the smooth muscle cell layer, regulates cell-cell interactions and is an inhibitor of atherogenesis. It is comprised of a single layer of cells and lines the entire vasculature. Atherosclerotic risk factors are known to predispose to loss of the usual protective endothelial responses. Loss of endothelial function is one of the first detectable events in the process of atherosclerosis and can be reversible. Vascular function can be assessed by measuring endothelial responses to shear stress (flow mediated dilatation) and exogenous administration of glyceryl trinitrate.

2.2.4.1 Vascular endothelial function

Endothelial dysfunction in the coronary arteries correlates with atherosclerosis in adults[71-74] and coronary endothelial dysfunction precedes clinically evident atherosclerosis in adults with cardiovascular risk factors[75], including those with known mild coronary artery atherosclerosis[76]. Endothelial function can be assessed invasively by intravenous infusion of acetylcholine. This direct measurement of endothelial function correlates well with non-invasive ultrasound assessment at the brachial artery[77] and coronary artery during cardiac

catheterisation[78]. Endothelial dysfunction in the coronary arteries correlates with flow mediated dilatation in the brachial artery making it a useful surrogate for coronary artery risk [78].

Brachial artery flow mediated dilatation relates to cIMT[52] and with coronary atherosclerosis on angiography[10, 79]. It also correlates with traditional cardiovascular risk factors in asymptomatic adults [11, 80] and children[81]. Endothelial function is an independent predictor of cardiovascular events in adults with cardiovascular risk factors including hypertension[82] and peripheral vascular disease[83]. It predicts worse outcomes in those with established atherosclerosis[84] and predicts incident cardiovascular disease within 5 years in those free of disease at baseline[85]. Meta-analysis of longitudinal studies affirms that FMD is a predictor of future cardiovascular events with a 1% decrease in FMD or 1sd decrease predicting a 8% or 22% increased chance of cardiovascular disease respectively [86].

FMD is impaired in young adults [87], adolescents and children with T1D[88, 89]. FMD is reduced in children and adolescents with obesity[50, 88] and this reduction is partially reversible with lifestyle modification[90]. In children and adolescents with normal cholesterol, FMD is inversely proportional to LDL cholesterol[91] and FMD is impaired in those with familial hypercholesterolaemia from early childhood[92]. The cardiovascular risk in young Finns study determined that Systolic BP in adolescence predicted FMD of the brachial artery in adulthood[93]. Intervention studies have demonstrated an improvement of FMD in adults [25, 94] and children [12] with reduced FMD.

When done in a specialised setting, FMD is a reliable, reproducible measure of endothelial function[3, 79, 95].

2.2.4.2 Vascular smooth muscle function

The vasodilator smooth muscle response to exogenous Glyceryl Trinitrate in the brachial artery has been reported to be relatively preserved in early endothelial dysfunction. Some studies have shown a trend toward reduction in GTN in those with impaired FMD which did not reach statistical significance[71, 72, 75]. GTN is impaired in young people with T1D, [87], current smokers[80, 96], people with peripheral vascular disease [97], children with familial hypercholesterolaemia[92] and is impaired in adults with coronary atherosclerosis[98]. GTN is independently associated with lower FMD, increased oxidised LDL and higher IMT [99]. This infers that there is abnormal function at the level of the smooth muscle cell in addition to the endothelial layer. Impaired brachial artery GTN infers pre-clinical atherosclerotic processes in the artery making it a useful and reproducible outcome measure in clinical studies.

2.3 Modifiers of vascular health: exercise

2.3.1 Exercise

The American Heart association has identified physical activity as one of the 7 ideal health behaviours, along with not smoking, diet, BMI, BP, blood glucose and total cholesterol, which can be used to monitor ideal cardiovascular health over time. Australian guidelines recommend children and adolescents spend at least 60 minutes per day in moderate to vigorous physical activity (MVPA).

The Australian activity guidelines for children and adolescents are, in the main, based on expert consensus. There is evidence that following the guidelines is associated with improvements in cardiovascular health, more favourable body composition and better cardiorespiratory fitness (CRF) however large randomised controlled trials are lacking. Australian 24-Hour movement guidelines for children and young people note the importance of considering the interaction between physical activity, sedentary behaviour and sleep

[100].The Canadian society for Exercise Physiology developed the original 24-hour movement guidelines which were utilised in the development of the Australian guidelines. Physical activity measurement can be divided into sedentary time, active time and time spent in MVPA. Each may confer a separate cardiovascular risk or protection as each will elicit different physiological mechanisms which can protect the cardiovascular system.

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Physical activity has well established cardioprotective benefits and the effect is in addition to the benefits on traditional cardiovascular risk factors. It is effective in both primary and secondary prevention of atherosclerotic disease[101]. Exercise modifies traditional cardiovascular risk factors which in one large study (27 000 subjects) accounted for than 59% of the risk reduction with exercise[102]. The remaining effect on cardiovascular risk reduction is thought due to a direct effect on vascular function. A systematic review of over 900000 individuals demonstrated a pooled risk reduction of 35% reduction in all-cause mortality with a 33% reduction in cardiovascular mortality [103]. This effect in lower mortality and major cardiovascular events occurs independently of other risk factors[21]. Meta-analysis of 7683 subjects participating in exercise based rehabilitation with coronary heart disease found a reduction in cardiac mortality of 31%[104] indicating the benefits of exercise on the cardiovasculature are beneficial even with clinical atherosclerotic change.

2.3.1.1 The effect of exercise on cardiovascular structure and function

Increased physical activity is associated with reduced IMT progression [60] and a composite cardiovascular child risk score comprising of multiple variables (LDL cholesterol, HDL cholesterol, BP, BMI, fruit consumption, lower quintile physical activity, smoking and diabetes)

showed that a more adverse composite score was associated with IMT progression into adulthood[60]. In the CARDIA (Coronary Artery Risk Development In young Adults) study, better cardiorespiratory fitness (CRF) was associated with lower cIMT in 413 healthy 11-12 year old children[105].Better CRF in young adults was also associated with lower coronary artery calcification 15 years later[106] indicating that CRF is an important risk factor for CVD risk which tracks from childhood. Exercise training is postulated to improve insulin sensitivity and reduce atherogenesis partly through an increase in insulin mediated, nitric oxide-induced dilatation[107].

Vascular function is associated with physical activity levels. In moderately active, healthy, 5 to 10 year old children, those with higher physical activity levels had more favourable endothelial function as measured by FMD of the brachial artery[108]. Healthy children with low levels of physical activity were in the lowest tertile of FMD[109]. For the children with the lowest tertile of FMD, those who engaged in MVPA were more likely to have a higher FMD within their tertile.

Arterial stiffness is associated with lower CRF, lower MVPA and higher sedentary time[110-112]. An increase in MVPA from childhood to adulthood is associated with a reduced in arterial stiffness[111].

Physical activity may mitigate cardiovascular risk in children and adolescents with traditional cardiovascular risk factors. In obese adolescents, lower cIMT correlates with more time spent in moderate physical activity [113] and higher physical activity, measured using accelerometry, correlated with lower systolic and diastolic BP [114]. In children with impaired endothelial function, high intensity physical activity correlated with better endothelial function, indicating that the type of physical activity may be important in cardiovascular protection[109].

Intervention studies have demonstrated improvements in cardiovascular function with exercise regimens. In adults with traditional cardiovascular risk factors (including stable coronary artery disease, hypercholesterolaemia and T2D), exercise training regimens improve FMD [115-117]. Exercise intervention programs in children and adolescents demonstrate improvements in vascular structure and function. In obese adolescents, longer duration (6- 10 month exercise regimens) improved cIMT and FMD [118], reduced BMI, improved lipid profile, improved exercise capacity lowered systolic BP and improved markers of endothelial function (increased endothelial progenitor cells and reduced endothelial microparticles)[119]. In obese prepubertal children, longer exercise regimens improve FMD, stabilise IMT and reduce systolic/diastolic BP and CRF (measured by maximal oxygen consumption - VO2 max)[120]. Meta-analysis of exercise intervention studies in children and adolescents demonstrated improved vascular function (FMD) and CRF (measured by (peak oxygen consumption - VO2 peak) with little change in body composition [121].

Shorter duration programs improve FMD in obese children[90, 122] and adolescents[123]. A 14-day diet and exercise intervention improved lipids, reduced Matrix metallopeptidase 9 (a marker of vascular plaque destabilisation), reduced markers of endothelial cell activation and reduced High sensitivity C-reactive protein (HsCRP), in a small group of obese children[124].

In Australian adolescents with T2D, a 12-week supervised exercise program improved FMD without any change to BMI, CRF or insulin resistance[125].

A meta-analysis of 12 intervention studies in 2003 demonstrated that exercise regimens do not reduce BP in children and adolescents [126]. A subsequent study demonstrated that higher levels of physical activity (measured by accelerometer), particularly of longer duration, is associated with lower systolic and diastolic BP in children[114].

2.3.1.2 Physical activity and Type 1 diabetes

Children and adolescents with T1D are recommended to exercise in accordance with standard national and international guidelines. The intended benefits of exercise are for improved insulin resistance, cardiovascular health and BMI and the anticipated reduction in cardiovascular events in later life. This is offset by the risk of hypoglycaemia, both during and after exercise.

Children and adolescents with T1D may not be as active as their age matched peers. Some studies suggest that children and adolescents have lower physical activity levels than their peers[127, 128] however another small study showed that children and adolescents with T1D spend as much time as peers without T1D in physical activity and met 2005 guidelines for physical activity as measured by 24 hour heart rate via holter monitor [129]. Adolescents with T1D have reduced exercise capacity than healthy controls [130-132]. Reduced exercise capacity in adolescents with T1D has been associated with poorer renal health[132]. Conversely, physical fitness is associated with reduced HbA1c, a favourable lipid profile, improved athletic competence and health perception in youth with T1D[133]. Increasing metabolic equivalents improves surrogate measures of cardiovascular autonomic function in adolescents with T1D [134] and FMD is more favourable in children and adolescents with T1D who met health guidelines for physical activity (at least 60min/day of MVPA) which was comparable to inactive peers without T1D.

The Hvidoere study group on childhood diabetes investigated the associations between physical activity and metabolic control in adolescents with T1D[135]. They found that physical activity was associated with a positive health perception but not HbA1c. There was an association between higher HbA1c, and more time spent on the computer and less time spent doing homework. In addition, older or female participants reported less physical activity.

In adults with T1D, physical activity is associated with less microalbuminuria and retinopathy, lower BMI in women, less obesity in both sexes and lower HbA1c[136]. In children and adolescents with T1D, regular physical activity is associated with lower HbA1c [137-139] and with a favourable lipid profile and reduced diastolic BP[140]. Sedentary time is associated with smaller retinal vascular calibre in children and adolescents with T1D [141]. Meta-analysis of physical activity interventions in children and adolescents with T1D demonstrated modest but favourable effects on HbA1c, BMI and lipid profile[142].

FMD was improved in a small group of children with T1D who were exposed to a twice weekly supervised exercise programme for 18 weeks with no effect on cIMT[143]. Given the short duration of the study it was unlikely that structural arterial changes would have been seen within this time frame. Improvements in FMD have been seen in adult exercise intervention studies of similar duration[144].

2.3.1.3 Measuring physical activity in children and adolescents

Accelerometers are widely used to estimate physical activity and sedentary time in children and adolescents. The data obtained from accelerometers is more objective and reliable than self-report questionnaires[145]. Accelerometers obtain directional information which is then analysed using proprietary algorithms to estimate energy expenditure, time spent in physical activity, amplitude of physical activity, sedentary time, sleep time and step count, all of which may be factors which influence cardiovascular health.

Step count is a surrogate measure for levels of physical activity. Although it does not indicate intensity of activity (i.e., whether the activity is moderate or vigorous) it does provide information about how generally active a person is. Step counts of around 12000 steps per day are likely to correlate with meeting current activity guidelines for children[146]. Step count has demonstrated an inverse association with obesity[147], all-cause mortality in

adults[148], cardiovascular disease event rate[149] and cardiometabolic risk factors including waist circumference, BMI and insulin levels[150].

The Senswear device is an accelerometer and galvanic skin response monitor which uses proprietary algorithms to calculate energy expenditure, step count, sedentary time, sleep duration and time spent in moderate or vigorous physical activity. The Senswear device has reasonable accuracy in measuring energy expenditure and step count during treadmill walking in adults [151]. With current algorithms, it is accurate at estimating energy expenditure in children and adolescents [152] and adequately approximates step count, although may underestimate step count at low walking speed, similar to other body movement devices[153].

2.4 Modifiers of vascular function: diet

There is little known about the influence of dietary factors and cardiovascular risk in children and adolescents with T1D, aside from the relationship with HbA1c. Dietary recommendations for children and adolescents with T1D are the same as those recommended for the general population[154]. Nutritional management aids optimal glycaemic control and aims to reduce rates of obesity both of which impact on cardiovascular health[155]. Dietary management with respect to carbohydrate and insulin matching is essential for optimal glycaemic control and should be guided by healthy eating standards and tailored for the individual child. Less well studied is the impact of other macro and micronutrients on the cardiovascular risk factor profile of children and adolescents with T1D.

Although dietary recommendations for children and adolescents with T1D are the same as population recommendations, additional factors impact on the dietary needs of those with T1D. Regular meals, carbohydrate counting, and parental supervision have all been found to impact on dietary adherence in this population. It has been reported that T1D children and

adolescents do not meet current nutritional targets [156] which may impact cardiovascular health.

Results from the Diabetes Control and Complications Trial (DCCT) identified 4 diet related behaviours in T1D individuals treated with intensive insulin treatment (multiple daily injections or continuous subcutaneous insulin infusion) that related to better glycaemic control: adherence to diet, prompt treatment of hyperglycaemia, not overtreating hypoglycaemia and not having extra snacks[157].

In adults, there has been no specific macronutrient dietary content which has been identified as better for glycaemic control and therefore current recommendations are to support national standard guidelines for macronutrient content[158].

BMI increases faster in children with T1D during adolescence than in age matched peers[159] which is likely to contribute to cardiovascular health. Glycaemic control, the most significant risk factor for micro and macrovascular disease is impacted not only by carbohydrate intake but also protein, long chain fatty acids and dietary leucine intake[160].

With regard to specific interventions to support cardiovascular health, there is evidence that low glycaemic index diets improve metabolic control in individuals with T1D[161]and this meta-analysis included 2 paediatric studies, one of which reported HbA1c[162].

In adolescents, self-reported intake of diet beverages is associated with higher cardiovascular risk factors (lipid profile and HbA1c) which is postulated to be that diet beverage intake is a surrogate marker for other adverse dietary or lifestyle factors[163]. Meta-analysis in adults did not find any relationship between non-nutritive sweeteners and glycaemic control[158]. High self-reported sweetened beverage intake is similarly associated with poor lipid profile but the association was lost with adjustment for saturated fat and fibre intake[163].

Dietary sodium intake is a modifiable risk factor for cardiovascular disease. Excess sodium intake influences BP and may also have a direct effect on endothelial function. Dietary sodium reduction has been shown to reduce cardiovascular events by 25%[164] and a Cochrane review of dietary sodium reduction has demonstrated a reduction in BP associated with reduced sodium intake[165]. In hypertensive middle age and older adults, lower sodium intake is associated with higher FMD[166] and a reduction in daily sodium intake improves endothelial function in normotensive overweight and obese adults [167].

In children and adolescents, there is an association between urinary sodium excretion (as a surrogate measure of sodium intake) and BP[168]. Meta-analysis of dietary salt reduction intervention trials in children and adolescents has demonstrated that a modest reduction in salt equates to a small but significant reduction in BP[169].

Rodent models suggest that dietary sodium intake has an effect on nitric oxide availability in the endothelium. This occurs via inhibition of a co factor required for nitric oxide production and suppression of the antioxidant enzyme superoxide dismutase[170-173].

2.5 Metformin as a modifier of cardiovascular risk

Metformin is a biguanide which is derived from the plant Galega officinalis. It is an insulin sensitising agent, used since the 1950s in adults with T2D. 70% of an oral dose of metformin is absorbed and is excreted via the renal system unchanged. Metformin is transported into cells via several types of organic cation transporters. Genetic variations in organic cation transporters are thought to be responsible for the variation in serum levels of metformin in different individuals. Metformin has an antihyperglycaemic effect without causing hypoglycaemia. It is first line treatment in people with T2D and has been used in adults and children and adolescents with other conditions which relate to increased insulin resistance including polycystic ovarian syndrome, obesity and T1D.

Aside from the effect on insulin resistance, metformin may also have an impact on traditional cardiovascular risk factors. Furthermore, it may have a direct impact on vascular function and structure independent of the glucose lowering effect. Several pathways have been identified by which metformin could directly impact on vascular health. Metformin has a direct effect in vitro on smooth muscle by stimulating nitric oxide synthesis via activation of AMPK (5' adenosine monophosphate-activated protein kinase) in vascular smooth muscle.[174] The activation of metformin activates AMPK in vascular tissues which inhibits monocyte to macrophage differentiation[175]. Metformin also inhibits the formation of advanced glycation end products[176] which would normally act to accelerate atherosclerosis in the arteries in those with dysglycaemia.

2.5.1 Metformin and Type 2 Diabetes

The UK Prospective Diabetes Study reported evidence of cardiovascular benefits in obese adults with T2D who were taking metformin with a 36% reduction in all-cause mortality[24]. There was a reduction in macrovascular disease (myocardial infarction, sudden death, angina, stroke and peripheral vascular disease) of 30% in the group treated with metformin compared to the conventional group (primarily diet only treatment) which was comparable to the group treated intensively (chlorpropamide, glibenclamide or insulin). Subsequent meta-analysis of additional studies looking at cardiovascular end points has been less convincing of cardiovascular benefits however these compare small studies with low event rates [177]. Outcomes are generally in favour of metformin but do not reach statistical significance. Metformin has also been shown to improve body composition and glycaemic control in obese T2D women[178]. Metformin in people with impaired glucose tolerance also led to a reduction in progression to T2D although less than a lifestyle intervention [179].

2.5.2 Metformin in children and adolescents without Type 1 diabetes

Metformin has a positive effect on BMI in children and adolescents who are hyperinsulinaemic and obese with a meta-analysis demonstrating a mean BMI reduction of 1.42kg/m2 (95% CI 0.83-2.02)[26] and an additional benefit on insulin resistance.

2.5.3 Metformin in Type 1 diabetes

Several studies have examined the use of metformin as an adjunct medication in children, adolescents and adults with T1D (Table 1). Of note, of the studies included in Table 1, four paediatric studies[28, 180-182] and four adult studies[25, 183-185] were published after the commencement of this study. Table 1 does not include studies published after our study was published in 2017. The majority of metformin studies used HbA1c as the primary outcome measure. Two adult studies published after the commencement of this study looked at vascular outcome measures[25, 185]. One of these studies, the REMOVAL trial, is the largest and longest metformin study published to this date [185]. Studies evaluating metformin had variable selection criteria including age, diabetes duration, diabetes control and BMI. They also used different metformin doses (1000-2250 mg/day) and the duration of the studies ranged from less than a month to 36 months (Table 1). [25, 185].

Table 1. Baseline characteristics of metformin studies in type 1 diabetes

Author Year Reference	Selection criteria	Exclusion criteria	Metformin dose (mg/day)	Primary outcome	Follow up (months)	Metformin Control	Age (years)	Diabetes duration (years)	HbA1c (%)	Daily insulin dose (U/kg/day)	BMI (kg/m2) or Weight (kg)
Gin 1985 [186]	Regular Blood glucose testing (12 per week)	Ketosis Renal or liver impairment	1700	Insulin sensitivity (EHC)	0.25	10 (randomised cross over)	40.8 (4)	10.3 (2.2)	9.95 (2.75)	70.3 (4.6)[b]	62 (3)[a]
Meyer 2002 [187]	HbA1c < 9% CSII ≥ 12 months	Non-stable retinopathy, any disease affecting blood glucose control, pregnancy, renal/cardiac or hepatic impairment	1700	Insulin resistance as measured by insulin dose	6	31	39.9 (12.9)	16.9 (8.9)	7.58 (0.84)	0.72 (0.21)	25.8 (3.6)
						31	41.1 (9.8)	21.6 (10.2)	7.57 (0.76)	0.73 (0.22)	26.4 (4.6)
Hamilton 2003 [188]	Age < 18 y HbA1c 8-11% T1D duration > 3 y Insulin dose > 1.0u/kg/day Tanner stage 2-5	Nephropathy, Proliferative retinopathy Recurrent DKA or severe hypoglycaemia, hepatic or renal impairment, known eating disorder, serious medical	< 50kg – 1000 50-75kg 1500 >75kg 2000	Change in insulin sensitivity as measured by FSIGT	3	14	15.9 (1.9)	9.9 (4.4)	9.3 (1.4)	1.21 (0.3)	22.8 (4.2)
						13	16 (1.7)	7 (3.8)	8.6 (0.8)	1.28 (0.19)	25.7 (2.9)

		illness, or risk of pregnancy									
Särnblad 2003 [189]	Age 14-20 y(girls) 16-20 y(boys) HbA1c>8% Insulin dose > 0.9u/kg/day		2000	HbA1c	3	16	17.2 (1.7)	9.1 (5.0)	9.3 (1.1)	1.2 (0.4)	26.2 (18.6-35.4)
						14	16.9 (1.4)	7.1 (3.0)	9.3 (1.4)	1.2 (0.2)	23.9 (17.0-29.2)
Khan 2006 [190]	HbA1c > 6.1% T1D duration ≥ 1 y BMI > 27kg/m2	Other medical conditions Diabetes complications	2550	HbA1c	4	15 (randomised cross over)	48 (12)	19 (10)	8.6 (1.4)	60[b] (14)	31.3 (2.6)
Lund 2008 [191] Lund 2009 [192]	Age over 18 y HbA1c ≥ 8.5% T1D duration ≥ 5 y	Renal, hepatic or cardiac impairment Serious co-morbidities Pregnancy Hypoglycaemic unawareness AOD abuse	2000	HbA1c	12	49	46.1	30 (5 to 51)	9.48 (0.99)	0.74 (0.26)	26.2 (3.4)
						51	44.9	26 (6 to 56)	9.60 (0.86)	0.75 (0.22)	25.8 (4.3)
Jacobsen 2009 [193]	Age 18-60 y HbA1c ≥ 8% T1D duration > 1 y BMI ≥ 25kg/m2	Pregnancy Impaired vision Impaired renal or hepatic function Cardiac disease Uncontrolled hypertension Hypoglycaemic unawareness	2000	HbA1c	6	12	43.5 (13.1)	17.8 (10.3)	8.85 (0.10)	62.7[b] (3.1)	29.5 (2.7)
						12	37.3 (9.6)	20.3 (10.2)	9.34 (0.94)	73.5[b] (20.8)	29.2 (2.8)

Codner 2013 [180]	Age < 21 y Clinical or biochemical hyper-androgenism	T2D T1D in honeymoon period T1D < 1.5y Abnormal TFTs Raised creatinine On other medications Other chronic conditions	1700	Serum testosterone	9	13	17.7 (1.6)	9.3 (5.1)	10.3 (2.3)	1.2 (0.4)	23.7 (3.0)
						11	16.7 (1.7)	5.5 (3.1)	9.6 (1.5)	1.0 (0.4)	26.2 (5.5)
Pitocco 2013 [25]	Age ≥ 18 y HbA1c < 10% T1D duration ≥ 5 y Basal bolus insulin	HbA1c ≥ 10% Renal or liver impairment Comorbidities Pregnancy Current or previous alcohol or smoking Other than insulin medications	2550	Not defined	6	21	46 (8)	9.2 (0.7)	7.24 (0.90)	0.61 (0.22)	28.7 (2.1)
						21	41 (10)	8.8 (0.8)	7.73 (0.42)	0.63 (0.15)	27.3 (2.0)
Burchardt 2013 (open label study) [184]	Age 18-60 y HbA1c> 7.5% T1D duration > 5 y	Poor diabetes control Hypoglycaemic unawareness Recurrent severe hypoglycaemia Recurrent DKA	Overweight: 500-1500 Obese: 1000-2550 Mean dose 1124.1 ±523.1	Not defined	6	33	35.3 (11.2)	15.9 (7.8)	9.0 (1.9)	Not reported	29.5 (3.2)
						19	30.5 (10.6)	15.89 (7.7)	8.3 (1.0)	Not reported	27.1 (2.4)

		Pregnancy Renal or liver impairment									
Libman 2015 [28] (T1D Exchange trial)	Age 12-20 y HbA1c 7.5-9.9% T1D duration > 1 y Insulin dose ≥0.8u/kg/day BMI ≥ 85th%		2000	HbA1c	6	71	15.4 (1.7)	7.5 (3.6)	8.8 (0.8)	1.1 (0.2)	93.7 (4.1)[a]
						69	15.1 (1.8)	6.4 (3.0)	8.8 (0.7)	1.1 (0.2)	94.3 (3.8)[a]
Nadeau 2015 [181]	Age 13-20 y HbA1c > 8.5% T1D duration ≥ 1y Tanner stage 4	Renal impairment Pregnancy ≥ 2 DKA in previous 12m Hypertension ≥ stage 2 retinopathy Significant non-compliance in previous 12m Severe psychiatric diagnosis	1000	HbA1c	6	40	15.9 (1.7)	6.7 (3.6)	9.5 (1.3)	1.2 (0.24)	23.5 (3.0)
						40	16.0 (1.6)	6.3 (3.5)	9.4 (1.1)	1.1 (0.34)	24.3 (4.1)
Nwosu 2015 [182]	Age 10-20 y HbA1c 8-14% T1D duration > 1y BMI > 85th%	Pregnant, breastfeeding On weight altering therapies Recurrent hypoglycaemia Systemic illness	1000	HbA1c at 9 months	9	15	15.0 (2.5)	5.7 (4.4)	9.3 (1.5)	1.1 (0.2)	28.2 (6.6)
						13	14.5 (3.1)	5.7 (5.0)	8.7 (0.4)	1.44 (0.5)	27.5 (3.7)

		≥ 2 episodes of DKA in previous 12m									
Burchardt 2016 (Open label study) [183]	Age 18-60 y HbA1c> 7.5% T1D duration > 5 y	Poor diabetes control Hypoglycaemic unawareness Recurrent severe hypoglycaemia Recurrent DKA Pregnancy Renal or liver impairment	1000±500	Not defined	6	45	35.2 (11)	Not reported	8.6 (1.7)	0.6 (0.19)	90 (15)[a]
						68	31.2 (11.7)	Not reported	8.5 (1.7)	0.56 (0.13)	80.9 (9.7)[a]
Petrie 2017 [185] REMOVAL trial	Age ≥ 40y T1D duration ≥ 5 y HbA1c> 8.0%, 3/10 CVD risk factors[c]		2000	Average mean cIMT	36	219	55.2 (8.5)	33.4 (11.0)	8.08 (0.86)	0.63 (0.26)	28.4 (4.5)
						209	55.8 (8.8)	34.3 (10.5)	8.02 (0.78)	0.68 (0.30)	28. 5(4.1)

[a] Weight

[b] Units per day

[c] CVD risk factors: (BMI> 27kg/m2, CVD, strong family hx CVD, current smoker, microalbuminuria, eGFR <90ml/min per 1.73m2, hypertension, dyslipidaemia, diabetes duration > 20y

Data presented as mean (SD) or median (range)

Paediatric studies highlighted in orange.

Abbreviations: AOD – alcohol and other drug, BMI, Body mass index; cIMT, carotid intima media thickness; CSII, Continuous subcutaneous insulin infusion; CVD, cardiovascular disease; DKA, diabetic ketoacidosis; eGFR, estimated glomerular filtration rate; EHC, euglycaemic hyperinsulinaemic clamp; FSIGT, frequently sampled intravenous glucose tolerance test, HbA1c, glycosylated haemoglobin; TFTs, thyroid function tests, T1D, type 1 diabetes; T2D, type 2 diabetes

A meta-analysis of metformin use in T1D published in 2010 identified that metformin reduces insulin requirements in T1D of 6.6 units per day [27]. Table 2 includes a summary of metformin studies published up to 2017 that evaluated insulin requirements with most studies showing reduction in insulin requirements. The REMOVAL trial in adults did not show an overall reduction in average insulin dose over 3 years but showed a treatment by visit interaction which highlighted that although there was no difference in the first 6 months, there was as small reduction in insulin dose in the metformin treated group thereafter[185]. The reduction in insulin dose in the largest study in overweight/obese adolescents (T1D Exchange trial) was 0.1 unit/kg/day (Table 2)[28]. At 6 months, 23% of those in the metformin group had a reduction in insulin dose of 25% compared to 1% of those in the placebo group[28]. A small study in adolescents found a reduction in HbA1c with metformin treatment which was greatest in those with a higher insulin resistance at the commencement of the study [189]. This same study also found no change in BMI to account for the reduction in peripheral insulin resistance.

Table 2. Results of Metformin studies in type 1 diabetes - insulin dose

Author Year Reference	Months		Δ Insulin dose from baseline (u/kg/day)	Δ Insulin dose from baseline U/day	EOT insulin dose (u/kg/day)	EOT Insulin dose U/day
Meyer 2002[187]	6	M		-4.3(9.9)[b]	0.65 (0.17)	
		C		1.7 (8.3)	0.74 (0.24)	
Hamilton 2003 [188]	3	M	-0.14 (0.1)[b]			
		C	0.02 (0.2)			
Särnblad 2003 [189]	3	M			1.1 (0.3)	
		C			1.3 (0.2)	
Khan 2006 [190]	4	M				50(13)[ac]
		C				58(12)
Lund 2008 [191]	12	M	-0.03 (-0.06 to -0.01)[b]	-3.2 (-5.2 to -1.2)[b]	0.71 (0.25)	56.8 (24.3)
Lund 2009 [192]		C	0.03 (-0.01to 0.06)	2.5 (0.6 to 4.5)	0.77 (0.24)	60.9 (20.0)
		M vs C	-0.06 (-0.10 to -0.02)[c]	- 5.7 (-8.6 to -2.9)[c]		
Jacobsen 2009 [193]	6	M		-5.9 (2.2)[b]		56.8 (2.9)
		C		2.9 (1.7)		70.6 (30.9)
		M vs C		-8.8(2.8)[b] (95% CI -14.62 to -3.04]		
Codner 2013 [180]	9	M			1.2(0.5)	
		C			1.1(0.2)	

Pitocco 2013 [25]	6	MvC	-0.027 (-0.10 to 0.51)			
Libman 2015 [28]	3	M	**-0.1 (-0.1 to -0.1)[a]**		1.0 (0.9 to 1.0)	
		C	0.0 (-0.1 to 0.0)		1.1 (1.0 to 1.1)	
		MvC	**-0.1 (-0.2 to 0.0)[b]**			
	6	M	-0.1 (-0.2 to -0.1)		0.9 (0.9 to 1.0)	
		C	0.0 (-0.1 to 0.0)		1.1 (1.0 to 1.1)	
		MvC	**-0.1 (-0.2 to 0.0)[b]**			
Nadeau 2015 [181]	3	M			**1.17(0.27)[a]**	**75.9 (20.8)[a]**
		C			1.16(0.32)	**78.1 (24.9)[a]**
	6	M			**1.12(0.21)[a]**	**74.2 (18.5)[a]**
		C			1.16(0.34)	**79.1 (26.2)[a]**
Nwosu 2015 [182]	9	M			1.42 (1.13 to 1.71)	
		C			1.73 (1.44 to 2.02)	
Petrie 2017 [185] REMOVAL trial	36	M			0.62 (0.26)	
		C			0.67 (0.30)	
		M vs C	-0.005 (-0.022 to 0.012) **-0.023 (-0.045 to -0.0005)[d]**			

[a] P < 0.05 when comparing within group values with baseline

[b] P < 0.05 when comparing change in metformin with change in control from baseline

[c] P < 0.05 when comparing final metformin group dose to final control group dose

[d] P < 0.05 for interaction between treatment and visit – post hoc analysis

Data presented as mean (SD) or mean (95% CI). Data included is what was reported for individual studies. If the outcome was not reported it was not included in the table.

Abbreviations: C, Control; EOT, end of treatment; M, metformin

A meta-analysis of metformin studies in T1D published in 2010 showed inconsistent findings related to HbA1c with no statistically significant reduction in those treated with metformin with a reduction in HbA1c of 0.10 % (95%CI -0.36 to 0.15, p=0.42)[27]. Based on current evidence, metformin has a transient effect on HbA1c which is not sustained in the longer term (Table 3). In adults, the REMOVAL trial showed an effect on HbA1c over the 36 months of the trial which was accounted for by a reduction at 3 months which was not sustained thereafter[185]. This was also shown in adolescents in the T1D exchange trial with a reduction at 13 weeks and a reversion to baseline HbA1c at 26 weeks[28].

Table 3. Results of Metformin studies in type 1 diabetes – HbA1c

Author Year Reference	Timepoint Months		ΔHbA1c (%) from baseline	HbA1c (%) at timepoint in months
Meyer 2002 [187]	6	M		7.45 (0.78)
		C		7.46 (0.6)
Hamilton 2003 [188]	3	M	- 0.3 (0.7) [ab]	
		C	0.3 (0.7) [a]	
Särnblad 2003 [189]	3	M		8.7 (1.5)[a]
		C		9.2(1.3)
Khan 2006 [190]	4	M		7.8 (1.1)[ab]
		C		8.6(1.2)
Lund 2008 [191] Lund 2009 [192]	12	M	-0.10 (-0.32 to 0.12)	9.25(0.94)
		C	-0.23 (-0.45 to -0.01)	9.12 (0.86)
Jacobsen 2009 [193]	6	M	-0.48 (0.26)	8.37(0.20)
		C	-0.17 (0.17)	9.17 (1.13)
Codner 2013 [180]	9	M		10.4(2.6)
		C		9.6(1.4)
Pitocco 2013 [25]	6	M vs C	0.17 (-0.36 to 0.72)	
Burchardt 2013 [184]	6	M		7.7 (1.2)
		C		8.1 (1.4)
Libman 2015 [28]	3	M	-0.2 (0.4 to 0)	8.7 (8.4 to 8.9)
		C	0.1 (-0.1 to 0.3)	8.9 (8.6 to 9.1)
		M vs C	-0.3 (-0.6 to 0.0)[a]	
	6	M	0.2 (0.0-0.4)	9.0 (8.8 to 9.2)
		C	0.2 (-0.1-0.4)	8.9 (8.7 to 9.2)
		M vs C	0.0 (-0.3 to 0.3)	
Nadeau 2015 [181]	3	M		9.4(1.7)
		C		9.5(1.2)
	6	M		9.2(1.2)
		C		9.6(1.2)
Nwosu 2015 [182]	3	M	0.03 (0.94)	
		C	-0.03 (0.67)	
	6	M	-0.26 (1.58)	
		C	-0.06 (0.74)	
	9	M	-0.72 (2.06)	9.46 (8.47 to 10.46)

		C	-0.45 (1.22)	9.85 (8,82 to 10.88)
Burchardt 2016 [183]	6	M		7.6 (1.2)[a]
Petrie 2017 [185] REMOVAL trial	36	M		8.1 (0.9)
		C		8.1 (0.8)
		M vs C	-0.13 (-0.22 to -0.04) [a]	

[a] P < 0.05 when comparing within group values with baseline
[b] P < 0.05 when comparing metformin group values with control group values at the same timepoint
Data presented as mean (SD) or mean (95% CI). Data included is what was reported for individual studies. If the outcome was not reported it was not included in the table.
Abbreviations: C, control; M, Metformin.

Three small studies have specifically investigated insulin sensitivity using 2 different methods (Table 4). Metformin improved insulin sensitivity measured by hyperinsulinaemic euglycaemic clamp studies in adults[186] and adolescents[189]. A single study used frequently sampled intravenous glucose tolerance test in a trial with adolescents with T1D randomised to metformin or placebo[188]. This trial did not show a significant difference in insulin sensitivity between the two groups.

Table 4. Results of Metformin studies in type 1 diabetes – Measures of insulin sensitivity

Author Year Reference	Months	Insulin sensitivity measures		EOT value
Gin 1985 [186]	0.5	Euglycaemic/hyperinsulinaemic glucose clamp. Mean glucose infusion at steady state (mg/kg/min) Mean (SEM)	M	3.71 (1.05)[b]
			C	3.4 (1.05)
Hamilton 2003 [188]	3	Frequently sampled intravenous glucose tolerance test $\Delta S_1 = X10^{-4}\ min^{-1}.\mu U^{-1}.ml^{-1}$ Geometric mean (95% CI)	M	2.6 (1.0 to 4.1)
			C	2.5 (1.9 to 2.9)
Särnblad 2003 [189]	3	Euglycaemic/hyperinsulinaemic glucose clamp Mean glucose/mean insulin (M/I) at steady state (mg/m2/min x µU/ml) Median (range)	M	2.2 (1.0 to 3.8)[a]
			C	2.3 (0.6 to 4.2)

[a] P < 0.05 within group change
[b] P < 0.05 for metformin vs control
Data included is what was reported for individual studies. If the outcome was not reported it was not included in the table.
Abbreviations: C, control; EOT, End of treatment; M, metformin.

Table 5 includes results of 14 metformin studies evaluating weight and BMI. Metformin has a reported beneficial effect on body weight and/or BMI in 5 adult studies[25, 183, 185, 191, 193] and 2 paediatric studies[28, 181]

The longest sustained benefit in weight was shown in the largest study to date (REMOVAL trial), the metformin group had a mean reduction in body weight of 1.17kg, sustained over 3 years[185]. This was similar to the effect seen in at previous smaller study over 12 months[191] (Table 5).

In the largest study of metformin treatment in adolescents with T1D who were overweight or obese at the commencement of the study (T1D exchange trial), metformin was beneficial in reducing the BMI trajectory with less weight gain occurring in the treatment group (0kg vs 2kg weight gain in the placebo group, 95% CI -3 to -1, p=0.003) over 6 months [28]. A smaller study without BMI selection criteria also showed a benefit in BMI over 6 months, even in those with

a normal weight[181]. Other paediatric studies have not demonstrated a benefit; however they are much smaller studies.

Table 5. Results of Metformin studies in type 1 diabetes – body mass index and body composition measures

Author Year Reference	Months		EOT BMI kg/m2	Change in BMI (kg/m2)	EOT BMI z score	Change in BMI z-score	EOT Weight (kg)	Change in weight from baseline (kg)
Meyer 2002 [187]	6	M vs C	NS					
Hamilton 2003 [188]	3	M		-0.05 (1.0)				
		C		0.2 (0.5)				
Särnblad 2003 [189]	3	M	23.3 (18.4-34.4)				67 (58 to 86.9)	
		C	23.3 (17.7-29.4)				66 (55.4 to 89.5)	
Khan 2006 [190]	4	M					89(11)	
		C					90(12)	
Lund 2008 [191] Lund 2009 [192]	12	M	25.61(3.39)	-0.37 (-0.72 to -0.02)[a]			78.78 (12.7)	-1.21 (-2.31 to -0.12)[a]
		C	25.85(4.87)	0.19 (-0.18 to 0.55)			79.16 (16.63)	0.53 (-0.60 to 1.66)
		M vs C		-0.56 (-1.06 to -0.05)[b]				-1.74 (-3.32 to -0.17)[b]
Jacobsen 2009 [193]	6	M					84.6(3.2)	-3.0(1.0)[a]
		C					92.9(2.6)	0.8(1.1)
		M vs C						-3.9(1.5) [-7.01 to -0.71][b]
Codner 2013[180]	9	M	23.7(2.8)					
		C	26.3(5.2)					
Pitocco 2013[25]	6	M v C		-0.97 (-1.75 to - 0.18)[b]				-2.27 (-3.99 to 0.54)[b]
Burchardt 2013	6	M	28.9(3.2)					

[184]		C	27.3(2.9)					
Libman 2015 [28]	3	M			1.6 (1.5 to 1.7)	0.0 (-0.1 to 0.0)	77 (74 to 79)	
		C			1.7 (1.6 to 1.8)	0.1 (0.0 to 0.1)	77 (75 to 80)	
		M vs C				-0.1 (-0.1 to 0.0)[b]		-2 (-3 to -1)[b]
	6	M			1.6 (1.5 to 1.7)	0.0 (-0.1 to 0.0)	78 (75-80)	
		C			1.7 (1.7 to 1.8)	0.1 (0.0 to 0.1)	78 (76 to 81)	
		M vs C				-0.1 (-0.2 to -0.1)[b]		-2 (-3 to -1)[b]
Nadeau 2015 [181]	3	M	23.2 (3.0)		0.64 (0.71)[a]		65.6 (12.1)	
		C	24.3(3.9)		0.78 (0.77)		67.5 (12.5)	
		M vs C			NS			
	6	M	23.5(2.4)		0.70 (0.55)[a]		66.2 (11.2)	
		C	24.7(3.7)		0.88 (0.69)		68.5 (11.7)	
		M vs C			NS			
Nwosu 2015 [182]	3	M	27.6 (5.1)				69.9 (15.7)	
		C	28.0 (3.64)				74.3 (16.5)	
	6	M	27.8 (5.4)				71.3 (18.0)	
		C	28.7 (3.4)				76.6 (16.5)	
	9	M	28.6 (5.1)				74.2 (16.8)	
		C	28.8 (3.1)				77.3 (15.6)	
Burchardt 2016 [183]	6	M						-1.7 (4)[a]
		C						0.4 (10.4)

Petrie 2017 [185] REMOVAL trial	36	M					82.0(15.4)	
		C					83.2(13.8)	
		M vs C						-1.17 (-1.66 to 0.69)[a]

[a] P < 0.05 for within group change

[b] P < 0.05 for metformin vs control

Data presented as mean (SD) or mean (95% CI). Data included is what was reported for individual studies including when data was stated as not significant without a specific value. If the outcome was not reported it was not included in the table.

Abbreviations: BMI, body mass index; C, control; EOT, end of treatment; M, Metformin; NS, Not significant

Several trials have reported a reduction in cholesterol levels in adults with T1D treated with metformin (Table 6). Total and LDL cholesterol reduced by 0.3mmol/L in a trial of 100 adults with T1D randomised to metformin or placebo[192]. In the REMOVAL trial, metformin treatment resulted in a reduction of LDL cholesterol of 0.13mmol/L which was sustained for the 3-year trial duration[185]. There was a significant reduction in total, LDL and HDL cholesterol in adults with T1D on CSII treated with metformin for 6 months[187] within the metformin treated group however there was no statistically significant difference between the metformin and placebo groups at the end of the trial. There were no changes in cholesterol observed in any of the studies in children or adolescents.

Table 6. Results of Metformin studies in type 1 diabetes – Lipids

Author Year Reference	Months		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	Triglycerides (mmol/L)
Meyer 2002 [187]	6	M	**4.79(0.67)[a]**	**1.45(0.47)[a]**	**3.1(0.6)[a]**	1.01(0.54)
		C	NS	NS	NS	NS
Hamilton 2003 [188]	3		NS	NS	NS	NS
Särnblad 2003 [189]	3		NS	NS	NS	NS
Khan 2006 [190]	4	M	4.8(1.0)	1.3(0.3)	2.9(0.9)	1.4(1.1)
		C	5.1(1.2)	1.3(0.3)	3.1(0.9)	2.0(3.0)
Lund 2009 [192]	12	M	4.37(0.91)	1.57 (0.39)	2.26(0.73)	0.97(0.36)
		C	4.80 (0.92)	1.68(0.48)	2.66(0.89)	0.91(0.45-2.71)
		M vs C	**-0.37 (-0.67 to -0.06)[b]**	-0.06(-0.16 to 0.04)	**-0.33 (0.61 to -0.06)[b]**	3 (-11 to 20)
Jacobsen 2009 [193]	6	ΔM	-0.09 (0.18)	-0.03 (0.03)	-0.23 (0.16)	0.28 (0.11)
		ΔC	0.03 (0.13)	0.02 (0.05)	-0.10 (0.11)	0.09 (0.13)
Pitocco 2013 [25]	6	M v C	2.25 (-16.58 to 12.08)	3.56 (-1.90 to 9.02)	-3.71(-16.88 to 9.46)	-1.26 (-24.13 to 21.61)
Libman 2015 [28] T1D Exchange trial	6	M	4.42(4.19 to 4.63)	1.31 (1.27 to 1.40)	2.59 (2.4 to 2.77)	1.06(0.92 to 1.21)
		C	4.42 (4.22 to 4.63)	1.32 (1.24 to 1.40)	2.59 (2.4 to 2.74)	1.30 (0.93 to 1.32)
Nadeau 2015 [181]	6	M	4.59 (0.94)	1.35 (0.25)		
		C	4.58 (0.93)	1.30 (0.39)		
Petrie 2017 [185] REMOVAL trial	12	M vs C			**-0.13 (-0.24 to -0.03)[b]**	

[a] P < 0.05 for within group change

[b] P < 0.05 for metformin vs control

Data presented as mean (SD) or Mean (95% CI). Data included is what was reported for individual studies including when data was stated as not significant without a specific value. If the outcome was not reported it was not included in the table.

Abbreviations: C, control; HDL, high density lipoprotein; LDL, low density lipoprotein; M, metformin; NS, not significant – value not reported.

Three studies published after our study started examined the effect of metformin in T1D on vascular outcome measures (Table 7). In one pilot study in 42 adults with T1D, metformin improved endothelial function as measured by FMD[25]. In one randomised open label study, cIMT improved in adults with T1D who had excess body fat and were treated with metformin in addition to insulin[183]. The REMOVAL trial, which is the largest and longest study published to this date, demonstrated that metformin treatment in adults with T1D slowed averaged maximal cIMT progression over 3 years without an attendant sustained decreased in HbA1c indicating that the effect may be a direct effect of metformin on the vasculature[185]. Metformin has been shown to simulate nitric oxide synthesis in vitro in endothelium and smooth muscle[174] which may contribute to its mechanism of action for improving vascular function and structure.

Table 7. Results of Metformin studies in type 1 diabetes – Vascular structure and function

Author Year Reference	Months		FMD	GTN	cIMT (averaged mean far wall) progression mm/yr	cIMT (maximal far wall) progression mm/yr
Pitocco 2013 [25]	6	M vs C	1.32 (0.30 to 2.43)[b]	-0.45 (-0.96 to 0.07)		
Burchardt 2016 [183]	6	M				0.003 (0.0056)[b]
		C				0.0042 (0.0124)
Petrie 2017 [185] REMOVAL trial	36	M vs C			-0.005 (-0.012 to 0.002)	-0.013[a] (-0.024 to -0.003)

[a] P < 0.05 for within group change
[b] P < 0.05 for metformin vs control
Data presented as mean (SD) or mean (95% CI). Data included is what was reported for individual studies. If the outcome was not reported it was not included in the table.
Abbreviations: cIMT, carotid intima media thickness; C, control; FMD, flow mediated dilatation; GTN, GTN mediated dilatation; M, metformin.

At the time of commencement of this study there have been no studies looking at the effect of metformin on vascular function or structure in children or adolescents with T1D.

2.6 Summary

Despite improvements in glycaemic control with intensive insulin regimens and rapidly evolving insulin delivery and adjustment technology, cardiovascular morbidity and mortality are significant in people with T1D. For children who develop T1D prior to 10 years of age, there is an average lifetime loss of 16 years. In addition to these lost years, the annual health care cost for T1D in Australia is substantial. Lifestyle education incorporating physical activity and a healthful diet may moderate cardiovascular risk. Intervention with metformin may also mitigate the effect of T1D on cardiovascular structure and function in children and adolescents with T1D.

3. Chapter 3: Methods

Does Metformin improve vascular heath in children with type 1 diabetes? Protocol for a one-year, double blind, randomised, placebo controlled trial

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BMC Pediatrics. 2013; **13**: 108

Statement of Authorship

Title of Paper	**Does metformin improve vascular health in children with type 1 diabetes? Protocol for a one year, double blind, randomised, placebo controlled trial**
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	*BMC Pediatrics* **13,** 108 (2013) https://doi.org/10.1186/1471-2431-13-108 Accepted 11 July 2013 Published 16 July 2013

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Contribution to the Paper	Conceived of the study, codesigned the study, wrote the first draft of the manuscript. Critically reviewed and corrected the manuscript; and approved final version. Acted as corresponding author
Overall percentage (%)	75
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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The paper presented in this chapter demonstrates the detailed methods for the investigation into the vascular effects of metformin in children and adolescents with type 1 diabetes. It also provides the protocol for the investigation of the effect of dietary components and activity levels on vascular health in type 1 diabetes.

Some variables listed in the methods, including sex hormones (Testosterone, free androgen index, Sex Hormone-Binding Globulin, Dehydroepiandrosterone, Anti-Mullerian Hormone, 17-hydroxy progesterone, prolactin, Luteinizing hormone, Follicle Stimulating Hormone, progesterone, oestradiol) and retinal photographs are not part of this thesis Sex hormones and retinal photograph evaluation were part of an ancillary studies.

The impact factor of this journal is 2.083

This article has been cited 24 times in peer reviewed literature.

3.1 Abstract

Background: Cardiovascular disease is the leading cause of mortality in T1D. Vascular dysfunction is an early and critical event in the development of cardiovascular disease. Children with T1D have vascular dysfunction therefore early interventions to improve vascular health are essential to reduce cardiovascular mortality in T1D. Metformin is an insulin sensitising agent which is known to improve vascular health outcomes in T2D and other individuals with insulin resistance. It has been used safely in children and adolescents with T2D for over 10 years. This study aims to assess the effect of metformin on vascular health in children with T1D.

Methods/design: This study is a 12-month, double blind, randomised, placebo-controlled trial of determine the effect of metformin on vascular health in children (age 8-18) with T1D. The sample size is 76 with 38 children in the metformin group and 38 children in the placebo group. Vascular health and biochemical markers will be measured at baseline, 3, 6 and 12 months. Vascular function will be measured using FMD and GTN of the brachial artery and vascular structure will be measured with carotid and aortic IMT using standardised protocols.

Discussion: This study will be the first to investigate the effect of metformin on vascular health in children with T1D. It will provide important information on a potential intervention to improve cardiovascular morbidity and mortality in this population at high risk from cardiovascular disease.

Trial registration: Australian New Zealand Clinical Trials Registry ACTRN12611000148976

3.2 Background

3.2.1 Vascular dysfunction and cardiovascular disease in type 1 diabetes

Cardiovascular disease remains the leading cause of mortality in T1D despite significant developments in management over the past 20 years[194, 195]. Children with T1D have evidence of cardiovascular abnormalities such as vascular endothelial and smooth muscle dysfunction and increased IMT [2, 67, 88]. Vascular dysfunction precedes clinically evident vascular disease[196] and is potentially reversible. Although vascular complications are rarely seen during childhood, their pathogenesis begins soon after diagnosis and accelerates during puberty[46]. Vascular dysfunction in T1D is also accelerated by poor glycaemic control, overweight, obesity, genetic predisposition and insulin resistance. Early interventions to improve cardiovascular health in T1D are essential to reduce the burden of cardiovascular morbidity and mortality.

The doubling in incidence of T1D in childhood in Australia over the last 20 years parallels the overweight/obesity epidemic in Western childhood populations. In addition, age of diagnosis of T1D has decreased over the last 20 years and younger age of T1D onset is associated in some populations with higher BMI at diagnosis [197, 198]. The T1D population is susceptible to the population shift in BMI, with 65% of adults in a US cohort with T1D in the overweight or obese BMI range[199]. Higher BMI is associated with higher IMT, BP, and dyslipidaemia[200].

The prevalence of overweight and obesity in children with T1D is higher than the normal population, at just over 38.5% in a Dutch cohort[201], 22.1% in a US cohort (compared with 16.1% in the normal population)[202] and 31% in our local South Australian cohort [203]. This is associated with other cardiovascular risk factors including hypertension, dyslipidaemia, and

metabolic syndrome. Overweight and obesity may have additional clinical consequences with the associated higher insulin resistance contributing to vascular disease[201, 204].

Adolescence is a critical time in determining risk of future vascular complications[202] and is a time when early vascular dysfunction is potentially reversible. Optimisation of diabetes control should be the initial strategy to improve vascular health in children and adolescents with T1D. However, HbA1c levels are higher than target levels recommended for prevention of complications[205, 206]. This is despite the advances in insulin delivery with insulin analogues and CSII [207], highlighting the need to identify additional vascular protective strategies to prevent cardiovascular disease at its inception. The aim of this study is to determine whether metformin improves vascular health in children with T1D.

3.3 Assessment of vascular health

3.3.1 Vascular function

The endothelium is a key regulator of vascular function [208] and endothelial dysfunction occurs early in the development of atherosclerosis [209]. Vascular dysfunction can be measured by FMD and GTN. These are early, non-invasive markers of atherosclerosis and predate the development of clinically evident disease[81, 210].

Ultrasound assesses brachial artery responses to increased blood flow (FMD). This induces nitric oxide release from the endothelium with a resultant increase in brachial artery diameter and is a measure of endothelial function. Exogenous administration of glyceryl trinitrate increases vessel diameter independent of the endothelium and is a measure of smooth muscle function (GTN). FMD and GTN have been proved to be accurate and reproducible methods for assessment of vascular function[211].

Endothelial dysfunction of the coronary arteries, as measured by coronary artery vasomotor responses, is associated with a higher incidence of cardiovascular events in adulthood. FMD

of the brachial artery relates to both coronary artery vasomotor responses and to the extent of coronary artery disease on coronary angiography findings[10]. Reduced FMD in adults relates to traditional cardiovascular risk factors[210]. FMD is abnormal in children and young adults at risk of atherosclerosis[81]. FMD and GTN will be outcome measures in this study.

3.3.2 Vascular structure

The thickness of the intima and media layer of the carotid (cIMT) and aortic (aIMT) walls are structural markers of atherosclerosis and can be evaluated by ultrasound.

cIMT is a well-established index of early atherosclerosis that correlates with prevalent and incident coronary heart disease and stroke[43]. Relative risk of stroke or myocardial infarction in older adults without a history of cardiovascular disease increases by 35% with an increase in cIMT of 1 SD, i.e., a relatively large clinical effect for a small change in cIMT[43]. Overweight and obese children have increased cIMT compared with age matched controls[50]. cIMT has been used as a primary outcome measure in landmark intervention trials [46, 47]. Diet and exercise improve endothelial dysfunction and reduce cIMT in obese children over 12 months[43]. In this study FMD improved early and was maintained and cIMT improved at 12 months [43]. Atherosclerosis begins in the abdominal aorta. We and others have shown that aIMT precedes changes in cIMT in children with accelerated atherosclerosis, including T1D[2, 67]. cIMT and aIMT will be used in this study as structural outcome measures.

3.3.3 Adiponectin

Adiponectin is an adipocytokine that is a regulator of nitrous oxide by activating endothelial nitric oxide synthase. It may provide a measurable link between visceral obesity, insulin resistance and vascular dysfunction. We have shown that adiponectin levels relate to vascular smooth muscle function in obesity in youth [212]. Adiponectin/leptin ratio is an emerging

measure of insulin resistance and is substantially higher in children with T2D compared with T1D[213, 214].

3.4 Metformin as a therapy to improve vascular health

3.4.1 Mechanism of action

Metformin is a biguanide that reduces glucose output from the liver and increases insulin sensitivity. Metformin activates the energy regulating AMPK, principally in muscle and liver. This is the major mechanism of metformin's action, to increase insulin stimulated glucose uptake in skeletal muscle and adipocytes, and reduce hepatic glucose output. Metformin also activates AMPK in the endothelium and smooth muscle. This is likely to explain the improvement in endothelium dependent and independent vascular responses with the administration of metformin in adults with T1D and polycystic ovarian syndrome[25, 174, 215]. It may also explain recognized benefits on cardiovascular risk independent of its glucose lowering effect. Metformin also improves adiponectin and leptin levels[215-217].

3.4.2 Type 2 diabetes

Metformin is the first line medication (with diet and exercise) in youth with T2D and/or with metabolic syndrome[218]. In T2D it improves endothelial function, decreases weight gain, triglycerides, LDL cholesterol and pro-inflammatory and pro-coagulation factors[219, 220]. Importantly, in two large, randomised trials metformin reduced the rate of myocardial infarction in adults with T2D by 33%. Metformin also improves BMI, body composition and fasting insulin in obese youth without diabetes[221, 222].

3.4.3 Type 1 diabetes

A 6 month pilot study reported improvements in vascular health, as measured by FMD, in 44 adults with T1D treated with metformin or placebo in addition to insulin[25]. Meta-analysis of randomised studies of metformin in T1D shows benefits in reducing daily insulin requirement

and reduced weight gain[27]. The potential benefit on improving LDL cholesterol is not confirmed and reductions in HbA1c are inconsistent.

There are only two studies in children using metformin in addition to insulin. They show that metformin lowered HbA1c and decreased insulin dose without weight gain in children with poor metabolic control[188, 189].

Therefore, metformin is a logical adjunct treatment for T1D in addition to insulin. There are no data on the effects of metformin on vascular health in children and adolescents with T1D. We have shown that children with T1D have severe vascular dysfunction which relates to BMI[203]. We will therefore determine the effect of metformin on vascular function and structure in children with T1D and above average BMI, in the absence of any published trials.

3.5 Aims and Hypotheses

The *primary objective* of this double blind, randomised, placebo-controlled trial is to determine whether metformin improves vascular function as measured by FMD in children aged 8-18 years who have T1D.

The *secondary objectives* are to determine the effect of metformin on vascular health as measured by GTN, cIMT and aIMT in addition to other variables including adiponectin/leptin ratio, waist circumference, BMI, body composition, insulin requirements, HbA1c and lipids.

We hypothesise that the intervention group (those that receive metformin) will have a significant improvement in vascular health compared to the placebo group. We also hypothesise that those in the intervention group will have improvements in adiponectin/leptin ratio, waist circumference, BMI, body composition, insulin requirements, HbA1c and lipids.

3.6 Methods and design

The study is a parallel, double blind, randomised placebo-controlled trial over 12 months in two paediatric diabetes centres in Adelaide, South Australia. The trial has been approved by the Women's and Children's Hospital Research Ethics Committee (HREC 2327/12/13) and Flinders Medical Centre Research Ethics Committee (HREC 443.12) and is prospectively registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12611000148976).

All children with T1D who are seen in the diabetes outpatient clinics of the Women's and Children's Hospital and Flinders Medical Centre paediatrics department will be approached consecutively to be screened for eligibility in the study.

A total sample of 76 children aged 8-18 years with T1D will be recruited. The treatment period is 12 months in duration. Written informed consent will be obtained from all parents of participants and written assent will be obtained from all participants.

3.6.1 Inclusion criteria

Each child must meet the following criteria to be involved in this study:

- diagnosed with T1D
- aged between 8 and 18 years.
- BMI > 50th centile for age and sex [Centers for Disease Control and Prevention 2000 standardized reference charts (www.ncdc.gov/epiinfo)].
- T1D duration greater than 1 year
- insulin requirements > 0.5 units /kg/day to exclude subjects in the remission phase of T1D

3.6.2 Exclusion criteria

Children are excluded from the study if they meet any of the following criteria:

- non T1D i.e., T2D or other forms of diabetes.
- severe hypoglycaemia episode in preceding 6 months defined as a loss of consciousness or convulsion associated with hypoglycaemia.
- recurrent diabetic ketoacidosis (more than 2 episodes in the preceding year).
- other serious co-morbidities but not including treated hypothyroidism or coeliac disease.
- contraindications to metformin therapy: hypersensitivity to metformin, renal or liver dysfunction, vitamin B12 deficiency, inability to abstain from alcohol.
- pregnancy or breast feeding.
- subjects taking metformin, statins, multivitamins, or anti-hypertensives.

3.6.3 Randomisation

The study has a planned sample size of 76 with 38 in each group. Randomisation was performed by an independent statistician using statistical software S-plus version 8.1 to provide equal representation of placebo and metformin. Allocation concealment was used to implement the random sequence allocation.

Participants will be recruited from paediatric outpatient clinics at Women's and Children's Hospital and Flinders Medical Centre by a single investigator (JA). Participants will be assigned randomisation numbers by the investigator in sequence and allocated to a treatment group by the pharmacist using the generated randomisation list (Figure 1). Treatment allocation will

be concealed from all study investigators, the distributing pharmacist and participants to minimise potential bias.

Analysis of the data will be performed by a statistician (TS) who was not involved in the initial randomisation.

3.6.4 Participant withdrawal

Any participant may terminate participation in the study without giving a reason and without any disadvantage or interference with the diabetes care provided to the participant at the treating hospital. An investigator can stop the participation of a subject after consideration of the benefit/risk ratio of participation in the trial. Reasons to stop participation include: serious adverse events, request by safety committee of early termination due to safety reasons and limitation to continue the trial such as a participant moving interstate.

3.6.5 Treatment arms and dosage of medication

The children will receive either metformin or placebo according to their randomly allocated group. Metformin 500 mg oral tablets will be supplied by GenericHealth (Camberwell, Victoria, Australia). The placebo will be also supplied by the same company and is identical in appearance and ingredients, aside from the active ingredient, metformin hydrochloride. If the participant's weight is less than 60 kg they will be assigned to a dosage of 500 mg twice a day (titrated up over 2 weeks). If the participant weighs greater than 60 kg they will be assigned to a dosage of 1 g twice a day (titrated up over 6 weeks). Dose titration will be limited to tolerance of the medication. If a participant experiences a reduction in insulin dose of greater than 20%, or if they have persistent gastrointestinal side effects, they will be prescribed the maximum tolerated dose.

3.7 Details of study procedure.

Figure 1. Study procedure details

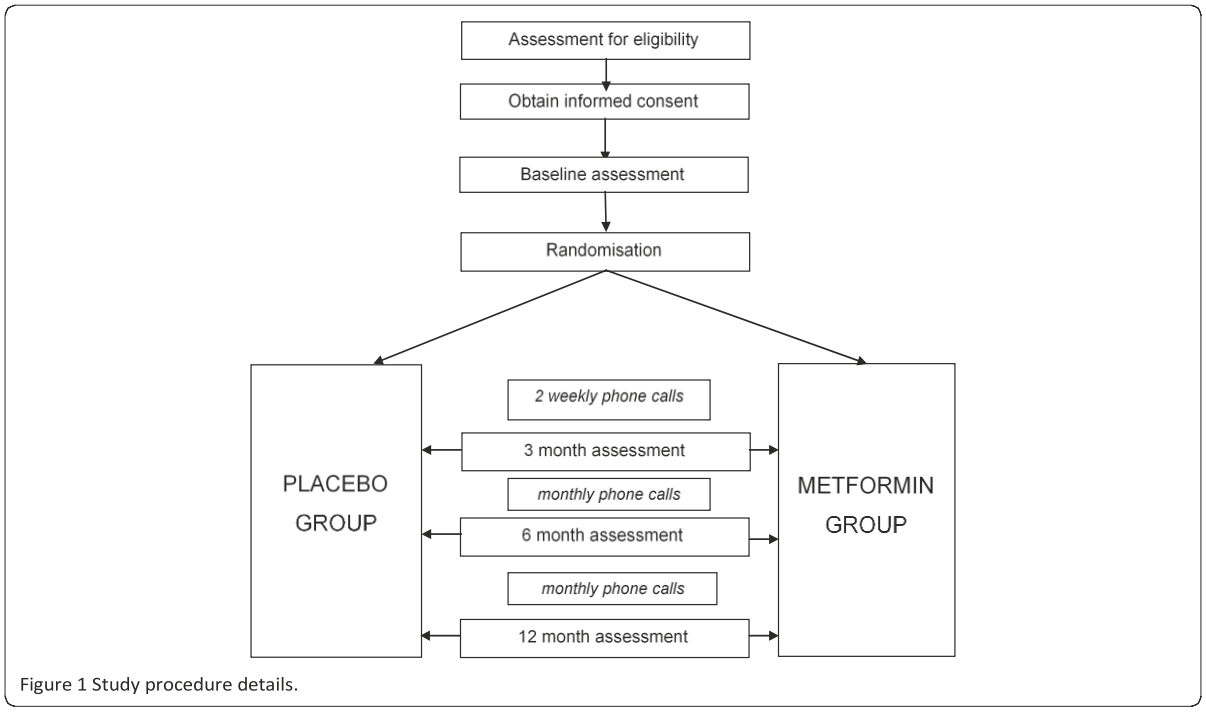

Figure 1 Study procedure details.

Both groups will also receive a dietary intervention at the commencement of the study and at 3 months. At the first visit, all subjects will receive standardised information regarding healthy diet, portion sizes, regular meals, and physical activity. This information will be delivered using a 'Type 1 Diabetes healthy eating' resource, developed by our dietician (BD) using current guidelines for healthy eating in T1D.

At the 3-month assessment, all participants will take part in a standardised interactive nutrition session developed by BD comparing the relative nutritional value of healthy snack foods to 'sometimes' and 'treat' snack foods.

3.8 Outcome measures

Table 8 shows the primary and secondary outcomes at different time points in the study. Table 9 shows additional information which will be collected at the assessments and Table 10 shows the additional information collected at each phone call (which occurs 2 weekly for the first 3 months and then monthly thereafter).

Table 8. Outcome measures

	Baseline	3 months	6 months	12 months
Primary outcome				
Vascular endothelial function (FMD)	*	*	*	*
Secondary outcomes				
Vascular smooth muscle function (GTN)	*	*	*	*
Vascular structure (cIMT and aIMT)	*			*
Clinical data – physical examination including anthropometric measurements (height, weight, BMI, waist circumference)	*	*	*	*
Frequency and severity of hypoglycaemia	*	*	*	*
Insulin dose (units/kg/day)	*	*	*	*
Glycosylated haemoglobin	*	*	*	*
Fasting glucose	*	*	*	*
Fasting total/HDL/LDL cholesterol/triglycerides	*	*	*	*
High sensitivity C-Reactive Protein (HsCRP)	*	*	*	*
Early morning urinary albumin/creatinine	*	*	*	*
Urinary prostaglandin F2α (PGF2 α)	*	*	*	*
Liver function, renal function tests and lactate	*	*	*	*
Total Plasma homocysteine	*	*	*	*
Girls only: Testosterone, free androgen index, Sex Hormone-Binding Globulin, Dehydroepiandrosterone, Anti-Mullerian Hormone, 17-hydroxy progesterone, prolactin, Luteinizing hormone, Follicle Stimulating Hormone, progesterone & oestradiol	*	*	*	*
Total Adiponectin	*	*	*	*
Leptin	*	*	*	*
Body composition (bio-electrical impedance analysis using Tanita body composition scales)	*	*	*	*
Retinal photograph	*			*
DXA scan	*			*

Abbreviations: aIMT, aortic intima media thickness; BMI, body mass index; cIMT, carotid intima media thickness; DXA, dual energy X-ray absorptiometry; FMD, flow mediated dilatation; GTN, Glyceryl trinitrate mediated dilatation; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 9. Additional information collected at assessments

	Baseline	3 months	6 months	12 months
ACAES questionnaire v1.2	*	*	*	*
Daily energy expenditure (SenseWear arm band)	*	*	*	*
Family history of premature cardiovascular disease	*			*
Pregnancy test in post menarchal girls	*	*	*	*
Serum cotinine	*	*	*	*
Adherence data using MEMS caps and manual tablet count	*	*	*	*
Serum Vitamin B12, Folate, red cell folate	*	*	*	*

Abbreviations: ACAES, Australian Child and Adolescent Eating Survey; MEMS, medication event monitoring system

Table 10. Additional information collected with each phone call

	0-3 months	3-12 months
Side effects - hypoglycaemia (number, severity) - gastrointestinal symptoms (nausea, vomiting, diarrhoea, anorexia) - rash - other side effects Insulin requirements	2 weekly phone calls	Monthly phone calls

3.8.1 Ultrasound assessment of vascular function and structure

Experienced and blinded sonographers (trained and led by RG) will perform all B mode ultrasound examinations with a 17-5 MHz linear array transducer (iU22; Phillips, Bothel, Washington, USA) in a temperature-controlled room (22-24 ºC).

3.8.2 Flow mediated dilatation and glyceryl trinitrate mediated dilatation

FMD and GTN will be assessed as previously described [13, 67, 89, 203]. Ultrasound images of the brachial artery in longitudinal section, 2-15cm above the elbow will allow measurements of the arterial diameter. Each study will include 4 scans: (1) resting scan; subsequently, reactive hyperemia is 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 (allowing for vessel recovery); and (4) last scan, taken 4 minutes after the sublingual administration of the GTN spray (400 μg, Nitrolingual Pumpspray, manufactured by G.Pohl-Boskamp GmbH & co, Hohenlockstedt, Germany, distributed by Sanofi-Aventis, Macquarie park, NSW, Australia). For each scan, measurements will be made over 4 consecutive cardiac cycles, incident with the R wave on the ECG (i.e., at the end of diastole), by observers blinded to the intervention type, using ultrasonic callipers. Measurements will be averaged and expressed as percentages of the resting scan. Our coefficient of variation (CV) between 20 controls is 3.9% for FMD and 4.0% for GTN-mediated dilatation[89].

3.8.3 Carotid and aortic intima media thickness

cIMT and aIMT will be assessed as previously described [67, 223]. In brief, the left and right common carotid artery will be imaged in a standardized magnification (2 x 2cm) using images of the posterior (far) wall of the distal 10 mm of the common carotid artery, just proximal to the carotid bulb. Optimal images will be captured at the end of diastole, incident with the R-wave of the ECG, for later analysis. The greatest distance between the lumen-intima interface and media – adventitia interface (IMT) is measured using an automatic edge detection and measurement computer software package and mean and maximum IMT are recorded. The mean of 2 measurements for aIMT and 6 measurements (3 on each side) for cIMT will be calculated according to the current Gold Standard protocol for cIMT. Analysis will be by two blinded observers. Sonographers have been trained and have undergone an accreditation process to evaluate the quality of their scans for this study in 2009. Our inter-observer intra-class coefficient for cIMT measurements is 0.99 (CV 1.2%) and the intra-observer intra-class coefficient for cIMT measurements is 0.97 (CV 2.4%)[67].

3.8.4 Physical Examination

Weight and body composition will be measured in light clothing using BC-418 segmental body composition analyser (Tanita, Tokyo, Japan distributed by Wedderburn, Inglewood, NSW, Australia). Height will be measured on a wall-mounted stadiometer (to 0.1 cm). Waist and hip circumference will be measured with a tape (to 0.5 cm). Waist circumference will be measured at the midpoint between the lower edge of the ribs in the midaxillary line and the top of the iliac crest, at minimal respiration. Hip circumference will be measured at the maximum circumference of the buttocks with the tape parallel to the floor. BMI z score will be calculated using 3.2 EpiInfo database version.2 and Centers for Disease Control and Prevention 2000 standardized reference charts (www.ncdc.gov/epiinfo). BP will be measured using DINAMAP (Carescape V100 Vital signs monitor, GE Healthcare, Milwauke, WI) with appropriate-size cuff on the left arm after 10 minutes of rest in supine position. The mean of 3 consecutive measurements will be recorded.

3.8.5 Laboratory methods

Cholesterol (LDL and HDL) and triglycerides will be measured using commercial enzymatic assays on Roche/Hitachi cobas C systems. HsCRP will be measured using a near infrared particle immunoassay method using IMMAGE Immunochemistry Systems Reagent (Beckman Coulter Inc, Fullerton, California, USA). HBA1C will be measured using a latex immunoagglutination inhibition methodology (DCA 2000 Hemoglobin A1c Reagent Kit; Bayer, Toronto, Ontario). Serum cotinine will be measured using a cotinine micro-plate EIA (STC Technologies). Urinary albumin/creatinine will be measured by immunoturbidometric and enzymatic colorimetric methods in routine laboratory assays. Oxidative stress will be assessed by measuring urinary PGF2α using a competitive enzyme-linked immunoassay and values obtained will be corrected for urinary creatinine. Liver function and renal function tests will be measured by Roche/Hitachi cobas C systems.

Serum samples will be frozen at –80 degrees Celsius for later measurement of adiponectin, leptin and androgens. Total adiponectin and leptin will be analysed using an enzyme-linked immunoassay (ALPCO diagnostics, Salem, NH, USA). Serum androgens will be measured by liquid chromatography mass spectroscopy (CPR Pharma Services, Adelaide, South Australia).

3.8.6 Total body dual-energy X-ray absorptiometry scan

The ratio of total body fat (g) to lean body mass (g) will be measured using DXA (dual-energy X-ray absorptiometry) on a GE-Lunar Prodigy machine (GE LunarCorp, Maddison, WI) equipped with adult proprietary software, version 3.6, which is appropriate for body weight of subjects 8-18 years. Fast scan mode and standard subject positioning will be used for these measurements. Mean precision of our machine is 1.3% for soft tissue.

3.8.7 Assessment of Energy intake and diet quality

The Australian Child and Adolescent Eating Survey (ACAES – version 1.2) [224] is a food frequency questionnaire based on the previous 3 - 6 months of food intake across all food categories. It will be interviewer-administered by the same dietitian at each assessment. At baseline, the ACAES will calculate the dietary intake over the previous 6 months. At subsequent assessments (3, 6 and 12 months) the ACAES questionnaire will calculate the dietary intake since the previous assessment. The ACAES will determine total caloric intake and macro and micronutrient content.

3.8.8 Assessment of Energy Expenditure

Prior to investigations at 0, 3, 6 and 12 months, an assessment of daily energy expenditure will be obtained. This will be assessed with the use of a SenseWear MF (Mini Form factor) device (analysed with SenseWear Software Version 7.0. Temple Healthcare Pty Ltd, Bowral, Australia) which is an external armband worn in this study for 5 days before each assessment time point. Based on measurements of accelerometry, heat flux, galvanic skin response, skin

temperature, near-body temperature and patient characteristics (gender, age, height, weight) proprietary algorithms will calculate energy expenditure, number of steps taken and sleep duration.

3.9 Monitoring of safety and adverse events

3.9.1 Blood glucose management during study

All subjects will receive 2 weekly phone review of blood glucose control for the first 3 months and then monthly thereafter. They will also have access to a 24 hour phone hotline serviced by one of 4 paediatric endocrinologists for acute insulin dose adjustment.

3.9.2 Compliance assessment

Assessment of adherence to the study medication regimen will be assessed at each assessment time point (3 months, 6 months, 12 months). All unused study drugs will be returned to the investigator for counting. MEMS (Medication Event Monitoring System) caps (AARDEX group LTD, Sion Switzerland) will also be utilised to measure compliance. These caps record how many times the study medication bottle is opened between study visits and the data will be downloaded for review and analysis. Compliance will be encouraged at the 3 months and 6 month study visit using the downloaded data from the MEMS cap.

3.9.3 Monitoring of adverse events

Renal, liver function tests and lactate will be performed at 0, 3, 6 and 12 months. Metformin can decrease levels of vitamin B12, so children with vitamin B12 deficiency will be excluded and vitamin B12 will be monitored during the study. The risk of hypoglycaemia will be monitored, and insulin will be adjusted accordingly.

Metformin can cause nausea and diarrhoea. Any illness or need for additional medication in a subject will be recorded throughout the study. Letters to the subjects' endocrinologists and

general practitioner advising of entry into the trial will be sent, and the Women's and Children's Hospital and Flinders Medical Centre emergency departments will have a record of the subject's participation in the trial in the medical records.

All adverse events will be reported to the medication safety committee for the trial in a monthly monitoring update. All serious adverse events will be reported to the medication safety committee and the Women's and Children's Hospital human research ethics committee within 24 hours of the event occurring. A serious adverse event is defined as one which is fatal or life threatening or requires hospitalization.

3.10 Data analysis

All analyses will be performed under the intention to treat principle. The primary outcome, FMD, will be compared between metformin and placebo groups over 12 months using a linear mixed effects model. Linear mixed effects models will also be used to compare changes in secondary continuous outcomes (GTN, adiponectin/leptin ratio, lipids, HbA1c, insulin requirements, cIMT and aIMT) between treatment groups (metformin/placebo) over the four reviews. The linear mixed effects models will account for the within-subject correlations that are produced when repeating outcome measurements in individual subjects over time. Both unadjusted and adjusted analyses will be performed, with adjustment for important pre-specified baseline covariates including age, sex and BMI.

While no studies have looked at the effect of metformin on FMD in T1D in this age group, our previous work found an improvement in FMD of 3.1% with an SD of 4.3 in children with T1D receiving folic acid over 8 weeks[13, 89]. Assuming an equivalent improvement with metformin from baseline to 12 months, we would require 32 subjects per group to have 80% power to detect a difference in FMD changes of 3.1% between randomised groups (alpha = 0.05 two-sided). Our dropout rate in previous studies in this population with similar

intervention and investigation over 12 weeks has been 1 – 2%. We have conservatively factored in a dropout rate of 15 % giving a total recruitment required of 76 subjects (38 in each group).

NB. The sample size reported in this paper was expanded prior to commencement of the trial increasing the power of the study from 80 to 90% and to account for an increased expected attrition of subjects during the study. The sample size in the final study was 90 children rather than 76 children as reported in the paper including results[225].

3.11 Discussion

Cardiovascular disease is the commonest cause of morbidity and mortality in T1D. The origins of cardiovascular disease are in childhood. Good metabolic control is essential in the prevention of cardiovascular disease however targets for optimal glycaemic control are often not met in children and adolescents with T1D. It is crucial to investigate additional effective and early interventions to prevent or reduce vascular pathology in this population. Metformin is a good candidate for early prevention of cardiovascular disease as it has been prescribed in youth with T2D for over 10 years and reduces cardiovascular disease in T2D.

Metformin is generally well tolerated and easy to administer with a good safety profile. Vascular health outcomes will be measured using non-invasive, valid, and reliable measurements by experienced investigators. This is, to our knowledge, the first study to examine whether administration of metformin will improve vascular health in children with T1D. Determining the effect of metformin on vascular health in children with T1D will provide a potential early intervention for cardiovascular disease in this population.

4. Chapter 4: An extra one thousand steps per day relates to improved cardiovascular health in children with Type 1 Diabetes

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Received 9 March 2016 accepted 27 April 2016

Diabetes Care 2016;39:e108-e109 | DOI: 10.2337/dc16-0526

Statement of Authorship

Title of Paper	**An extra 1000 steps per day relates to improved cardiovascular health in children with Type 1 Diabetes**
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Diabetes care 2016;39e108-e109

Principal Author

Name of Principal Author (Candidate)	Jemma Jay Angela Anderson
Contribution to the Paper	Conception and design of the study, acquisition, analysis and interpretation of data. Wrote the first draft of the manuscript. Critically reviewed and corrected the manuscript; and approved final version.
Overall percentage (%)	70
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 06/04/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Jennifer Couper
Contribution to the Paper	Supervised conception and design of the study; and interpretation of data Obtained funding for the study Critically reviewed and corrected the manuscript; and approved final version. 5%
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Contribution to the Paper	Performed advanced statistical analysis and assisted with data interpretation. Critically reviewed and corrected the manuscript; and approved final version. 4%
Signature	Date 11/03/2021

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Signature		Date	16/3/21

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Contribution to the Paper	Performed vascular ultrasound assessment and assisted with data interpretation. Critically reviewed and corrected the manuscript; and approved final version. 4%		
Signature		Date	30/3/2021

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Signature		Date	30/3/2021

Name of Co-Author	Associate Professor Alexia S Peña		
Contribution to the Paper	Supervised conception and design of the study; and interpretation of data. Obtained funding for the study. Acted as corresponding author. Critically reviewed and corrected the manuscript; and approved final version. 10%		
Signature		Date	6/04/2021

The paper presented in this chapter is the first paper looking at the impact of activity levels on vascular health in children and adolescents with type 1 diabetes.

The impact factor of the journal where this paper is published is 16.02.

This article has been cited 8 times in peer reviewed literature.

Children with T1D report lower physical activity levels than those given in the current recommendations[226] and the effect of this on vascular health, measured objectively, is not well described. Children with T1D have vascular structural changes (increased aIMT and cIMT)[67, 68]. aIMT relates to cIMT and both relate to cardiovascular risk factors. aIMT has been shown to be an earlier marker of atherosclerosis in children[67, 68]. We aimed to determine the relationship between activity levels and IMT in children with T1D. We hypothesized that lower activity levels would relate to thicker IMT.

The study included 90 children with T1D (41 boys, aged 13.6 ± 3.5 years) and was approved by ethics committees[227]. Children had evaluation of cIMT and aIMT as described [67, 68, 227]. Inter and intra-observer coefficients of variation for cIMT were 1.2% and 2.4% and for aIMT were 1.6% and 1.2% respectively [67]. Activity levels (step count/day) were measured using a SenseWear MiniForm Factor Armband (Body Media Inc., Pittsburgh, USA) worn for a minimum of 5 consecutive days including one weekend day.

The mean ± SD BMI was 22.43±3.23 kg/m^2 and diabetes duration was 5.5±3.9 years, and median (interquartile range) HbA_{1c} was 8.7% (8.1-9.9) (72mmol/mol [65-85]mmol/mol). Eighty- eight of 90 (98%) children wore the arm band for 23.2±0.76 h/day. Forty-eight of 88 (55%) children took less than 10,000 steps a day (lower limit of recommended steps) [228].

Mean/maximum aIMT related to average steps taken per day (r = -0.30, p=0.005 and r=-0.29, p=0.007, respectively) (Figure 2). An increase in step count of 1000 steps/day related to a decrease in mean/ maximum aIMT of 0.0082mm and 0.0093 mm, (95%CI -0.014, -0.002; p=0.005, and 95%CI -0.016, -0.003, p=0.007, respectively). The association was independent of age, HbA1c, BMI-z score, BP, triglycerides, LDL, HDL and total cholesterol. Children with a daily step count lower than 10,000 steps/day had higher mean/maximum aIMT than those with higher step counts (0.56±0.09 vs 0.51±0.07, p=0.01 and 0.65±0.09 vs 0.60±0.09, p=0.02,

respectively). An increase in 1000 steps per day was related to lower cardiovascular risk: 1.6kg (95%CI -2.66, -0.54; p=0.004) reduction in weight; 1.02 mmHg (95%CI -1.55, -0.49, p <0.001) and 0.50 mmHg (95%CI[-0.85, -0.15], p=0.005) reduction in systolic and diastolic BP respectively; 0.03mmol/L (95%CI 0.003, 0.05, p=0.03) increase in HDL cholesterol; 0.02mmol/L (95%CI -0.04, -0.001, p=0.04) reduction in triglycerides. There were no associations with cIMT.

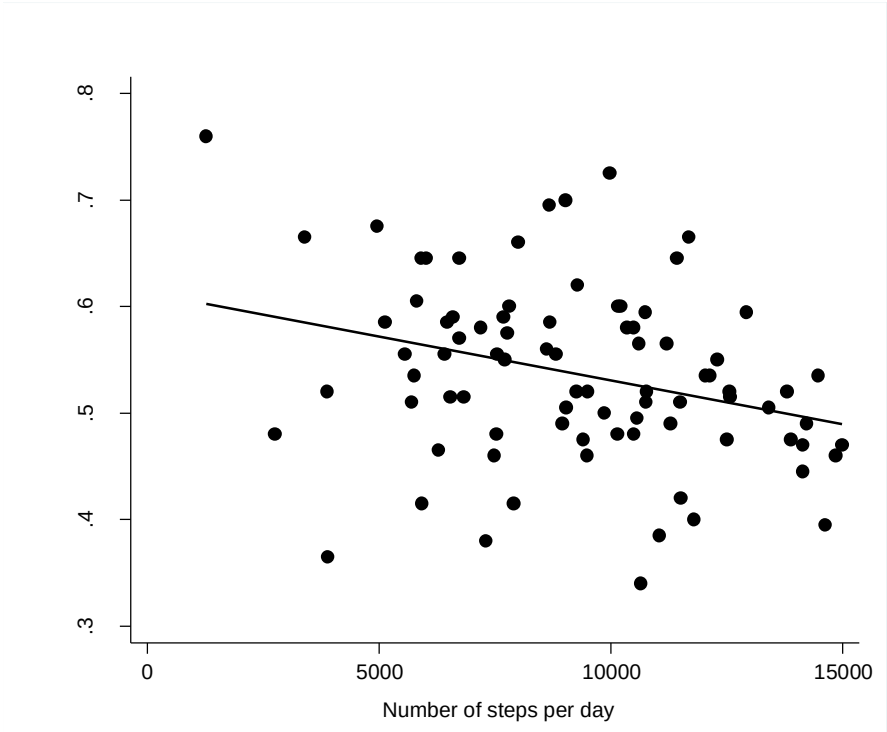

Figure 2. Mean aortic intima media thickness related to average steps taken per day (r = -0.30, p = 0.005) in children with type 1 diabetes.

We have demonstrated for the first time that the daily number of steps relates to early signs of atherosclerosis and adverse cardiovascular risk in children with T1D. An important clinical message is that even a small increase in activity relates to better vascular structure and risk factors. Limitations of the study are the small cohort size and the cross-sectional analysis preventing exploration of causal factors. However, we achieved power of 0.91 to detect the correlation of 0.31 between steps per day and aIMT (assuming $\alpha = 0.05$). Our findings emphasize the importance of including advice for the benefits of exercise in routine education for children with T1D.

5. Chapter 5: Dietary sodium intake relates to vascular health in children with Type 1 Diabetes

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Pediatric Diabetes. 2018;19:138-142

Statement of Authorship

Title of Paper	**Dietary sodium intake relates to vascular health in children with type 1 diabetes**
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Pediatric Diabetes 2018 Feb;19(1):138-142. doi: 10.1111/pedi.12537. Epub 2017 May 10.

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Signature	Date 06/04/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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Signature	Date 6/04/2021

The paper presented in this chapter demonstrates the relationship between dietary salt intake and vascular health in children with type 1 diabetes.

The impact factor of the journal where this paper is published is 4.267.

This article has been cited 8 times including in the ISPAD Clinical Practice Consensus Guidelines 2018: Nutritional management in children and adolescents with diabetes.

5.1 Abstract

Background and objective: Children with T1D have vascular dysfunction and frequently struggle to adhere to dietary recommendations. Limited data exist for the vascular consequences of poor diet quality in children. We aimed to evaluate the association between dietary components and vascular function in children with T1D.

Methods: Cross sectional study including ninety children (13.6 [3.5] years, 41 boys) with T1D. They had evaluation of dietary micro and macronutrients (ACAES), vascular endothelial and smooth muscle function (FMD and GTN), clinical and biochemical variables.

Results: Children had a sodium intake of 3.013 (0.76) [mean (SD)] g/day. Vascular smooth muscle dysfunction, as measured by GTN, related to higher daily sodium intake (r = - 0.31, P = 0.003), independent of the inverse relationships between GTN and total energy (r = - 0.30, P = 0.005) and fat intake (r = - 0.28, P = 0.007). Multi-regression model showed that an increase in 1g of daily sodium intake was independently associated with a deterioration of 3 percentage units in GTN (95% CI -4.3, -0.9, P = 0.003). There was an association between sodium intake and systolic BP after adjustment for age and gender (regression coefficient 2.4, 95% CI 0.5, 4.3, P = 0.01).

Conclusions: High dietary sodium intake in children with T1D is common and relates to vascular dysfunction, independently of other dietary intake, BP and glycaemic control.

5.2 Introduction

The leading cause of mortality in T1D is cardiovascular disease[229]. Vascular dysfunction is a critical event in its development and is detectable years before clinically evident atherosclerosis[87].

Vascular function can be evaluated by ultrasound measurement of brachial artery responses to increase in flow (FMD) and to Glyceryl trinitrate (GTN). FMD is dependent on endothelium

nitric-oxide release (endothelium-dependent response). Glyceryl trinitrate is a nitric-oxide donor that increases artery diameter independent of the endothelium and, therefore, assesses vascular smooth muscle response (endothelium-independent response)[88]. Abnormal vascular endothelial function and smooth muscle function, measured by FMD and GTN, correlate with abnormal coronary angiography and predict coronary artery disease in adults[10, 78, 98, 230, 231]. Prepubertal children with T1D of short duration have vascular dysfunction[89, 99, 232].

Glycaemic control is the major modifiable risk factor in the prevention of vascular complications in T1D. However, individuals with T1D and excellent glycaemic control still have almost three times the risk for cardiovascular causes of death[14]. Therefore, there is a need to address the non-glycaemic determinants of vascular disease.

Adherence to dietary recommendations is generally low in children with T1D [226, 233] but the impact on vascular health has not been evaluated. We therefore aimed to determine the relationship between diet, including macronutrient and micronutrient intake, and markers of vascular function in children with T1D participating in a cross-sectional study. We hypothesized that higher fat, and sodium intake would relate to worse vascular function.

5.3 Research Design and Methods

5.3.1 Subjects and study design

Cross sectional study including 90 children with T1D (mean (SD) age 13.6 (3.5) years, 87 Caucasian, 41 boys). They were recruited consecutively from paediatric diabetes clinics in Adelaide as part of a randomized controlled trial investigating the effect of metformin on vascular function (Registration number for clinical trials: ACTRN12611000148976)[227]. Baseline data, prior to intervention, of all children included in the study were utilized for this analysis. Inclusion criteria were T1D, age 8 to 18 years, BMI greater than 50th centile for age

and sex and T1D duration greater than 6 months. Exclusion criteria were a severe hypoglycaemic episode in the preceding 6 months, recurrent DKA (diabetic ketoacidosis), other serious co-morbidities and children taking metformin, statins, multivitamins, or anti-hypertensives[227]. The study was approved by the Women's and Children's Hospital Research Ethics Committee (HREC 2327/12/13) and Flinders Medical Centre Research Ethics Committee (HREC 443.12). Written informed consent was obtained from all parents of participants and written assent from all participants.

5.3.2 Clinical and biochemical assessments

Height was measured on a wall stadiometer and weight was measured in light clothing using BC-418 segmental body composition analyser (Tanita, Tokyo, Japan). BMI z score was calculated using 3.2 EpiInfo database version.2 (**www.ncdc.gov/epiinfo**). Body composition was measured using DXA on a GE-Lunar Prodigy machine (GE LunarCorp, Maddison, Wisconsin, USA). Puberty was assessed on self-report using pubertal staging images and the child categorized as pre-pubertal (Tanner 1), in early to mid-puberty (Tanner 2-3) or late puberty (Tanner 4-5). BP was measured after 10 minutes rest in the supine position and the average of three measurements was recorded.

Participants were well with no inter-current illness, fever, or ketosis. All participants were studied prior to breakfast following an overnight fast. Venous blood was taken to measure fasting lipids (cholesterol, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol and triglycerides) using commercial enzymatic assays on Roche/Hitachi cobas C systems. HbA1c was measured using a latex immunoagglutination inhibition methodology (DCA 2000 Hemoglobin A1c Reagent Kit; Bayer, Toronto, Ontario).

5.3.3 Ultrasound assessment of vascular function

Two experienced sonographers performed all B mode ultrasound examinations with a 17-5 MHz linear array transducer (iU22; Phillips, Bothel, Washington, USA) in a temperature-controlled room (22-24 ºC). FMD and GTN were assessed as previously described[13, 89, 227]. In brief, each study included 4 scans of the brachial artery: (1) baseline scan; (2) FMD scan 45-75 seconds after inducing reactive hyperemia by occluding arterial blood flow using a sphygmomanometer inflated to 250 mmHg for four minutes; (3) Re-control scan 10-15 minutes later; and (4) GTN scan, taken four minutes after the sublingual administration of Glyceryl Trinitrate spray (400 µg, Nitrolingual Pump spray, Hohenlockstedt, Germany). For each scan, measurements were made over 4 consecutive cardiac cycles, incident with the R wave on the electrocardiogram, using ultrasonic calipers. Measurements were averaged and expressed as percentage unit change from the resting scan. Our inter-observer coefficient of variation is 3.9% for FMD and 4.0% for GTN[89]. Pubertal girls were assessed in the follicular phase of the menstrual cycle.

5.3.4 Dietary Assessment

Dietary intake data were collected using the ACAES (Version 1.2) administered by the same dietician (ST). The ACAES is a semi-quantitative questionnaire which contains 120 food frequency questions divided into 8 areas: general diet, drinks, milk and dairy foods, bread and cereals, sweets and snacks, main meals, other foods and fruit and vegetables (seasonal and non-seasonal fruit). It measures dietary intake over the previous 6 months. Nutrient intakes responses from the ACAES were used to generate individual mean daily macro- and micronutrient intakes. ACAES has been evaluated for reliability and relative validity in Australian children and adolescents and has demonstrated high accuracy[224].

5.3.5 Statistical analysis

Demographic measures were summarized by their mean (SD) or median (interquartile range [IQR]) for continuous variables. The Shapiro Wilks test was used to assess for normality of the distribution of continuous variables. Correlations between variables were estimated using Pearson or Spearman correlation coefficients as appropriate.

Simple linear regression models were initially fit to assess the effect of each putative predictor variable (carbohydrates, fat, protein, energy intake, fibre and sodium) on the vascular health outcome (FMD and GTN). Predictor variables with an associated P value < 0.05 and other independent variables (age, gender, pubertal stage, insulin dose, BMI z score, HbA1c, BP, LDL cholesterol and insulin pump use) were entered into the multivariable model. Variables that were not significant were sequentially eliminated. Collinearity was assessed and variables with an associated variance inflation factor > 10 were removed from the model. HbA1c was added to the final model to control for any confounding effect. Statistical analyses were conducted using Stata version 13.0 (StataCorp LP, College Station, TX, USA).

5.4 Results

The study included 90 children with T1D (mean (SD) age 13.6 (3.5) years, 87 Caucasian, 41 boys). Children had T1D duration of 5.5 (3.9) years and median (IQR) HbA1c of 8.7%/72mmol/mol (8.1-9.9%/65-85mmol/mol). None of the children had microvascular complications of microalbuminuria or retinopathy. 25 children were prepubertal (Tanner 1), 16 were in early to mid-puberty (Tanner 2-3) and 49 were in late puberty (Tanner 4-5). Mean BMI z score was 0.90 (0.57), mean DXA fat mass was 18.2 (8.8) kg, and mean DXA lean fat mass was 39.3 (10.1) kg. FMD and GTN were measurable in all children. Children with T1D had lower FMD and GTN in comparison to 53 healthy children previously studied by us using the same methodology at the same imaging centre [FMD 6.2 ± 4.5vs 7.8 ± 4.6 percentage units, p=0.04

and GTN 24.5 ± 6.4 vs 27.7 ± 7.5, p=0.007][88]. All children/parents completed the ACAES questionnaire. Sodium intake was 3.013 (0.76) [mean (SD)] grams/day. Other daily dietary intake, vascular health and clinical variables are shown in Table 11.

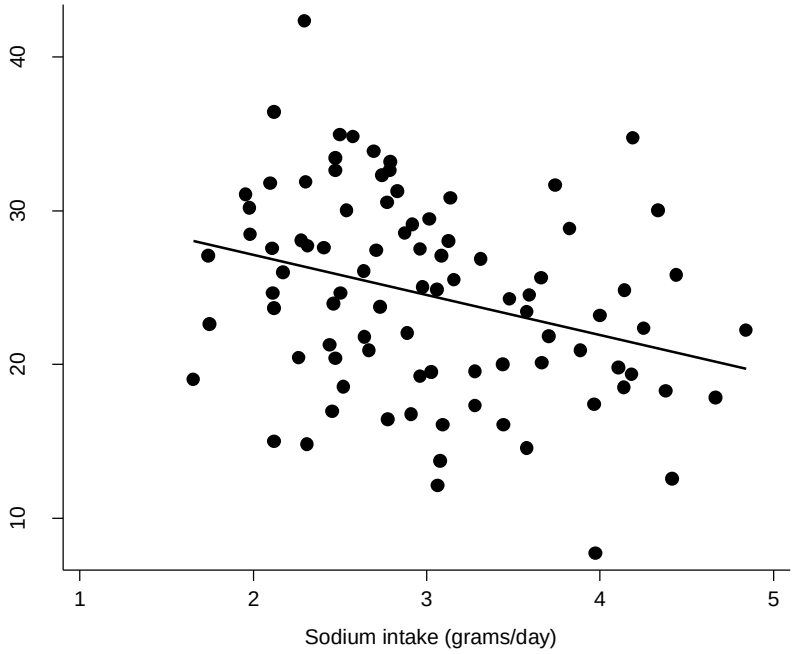
Table 11. Vascular health and diet variables in children with type 1 diabetes

Number of children	90
Systolic BP (mmHg)	112.3 (8.2)
Diastolic BP (mmHg)	63 (5.2)
FMD (percentage units)	6.2 (4.5)
GTN (percentage units)	24.5 (6.4)
Energy intake (kj/day)	10657 (2447)
Protein intake (grams/day)	111.65 (26.71)
Fat intake (grams/day)	86.52 (27.74)
Carbohydrate intake (grams/day)	317.52 (76.13)
Fibre intake (grams/day)	31.69 (8.64)
Sodium intake (grams/day)	3.013 (0.76)
Triglycerides (mmol/L)	0.77 (0.29)
Total Cholesterol (mmol/L)	4.37 (0.74)
HDL Cholesterol (mmol/L)	1.57 (0.35)
LDL Cholesterol (mmol/L)	2.45 (0.58)

Data presented as mean (SD)
Abbreviations: BP, blood pressure; FMD, flow mediated dilatation; GTN, GTN mediated dilatation; HDL, high density lipoprotein; LDL, low density lipoprotein.

Univariate analysis showed that poorer vascular smooth muscle function (GTN) was associated with higher daily sodium intake (r = - 0.31, P = 0.003, Figure 1). This association persisted after adjustment for the GTN associations with total energy intake (r = - 0.30, P = 0.005), fat intake (r = - 0.28, P = 0.007), carbohydrate intake (r = -0.23, P = 0.03) and protein intake (r = - 0.29, P = 0.006). GTN did not relate to fibre intake, or systolic and diastolic BP.

Figure 3. Glyceryl trinitrate mediated dilatation related to sodium intake (g/day) [r= -0.31, p=0.003] in children with type 1 diabetes



Higher sodium intake was associated with higher intake of take away food and processed snack foods (r = 0.34, P = 0.001 and r = 0.40, P = 0.0001, respectively). Sodium intake was higher in children who ate take away foods more than once a week (n = 36) in comparison to children who ate take away less than once a week (Mean [SD] sodium intake 3.258 [0.78] g/day, p=0.01 vs 2.849 [0.70] g/day]. There was a trend for the association between higher daily sodium intake and higher systolic BP (r = 0.18, P = 0.08), that became significant after adjustment for age and gender (regression coefficient 2.4 95% CI 0.5, 4.3, P = 0.01).

There were no significant associations between FMD and diet macro and micronutrients. FMD was not associated with sodium intake (r = 0.06, p=0.6).

Multivariate analysis showed that sodium intake was the only significant and independent predictor of GTN. An increase of 1 gram in daily sodium intake was associated with a 3 percentage units deterioration in GTN (95% CI [- 4.3, - 0.9], P = 0.003). This association was

also independent of age, gender and systolic BP (regression coefficient - 2.4; 95% CI - 4.3, -0.5; P = 0.01)

5.5 Discussion

We have demonstrated for the first time that dietary sodium intake relates to vascular dysfunction in children with T1D. The relationship was independent of other dietary components and traditional cardiovascular risk factors including diabetes control. Relatively small increases in daily sodium intake related to worse vascular function. We also confirmed that children with T1D, like the normal childhood population, have daily sodium intake well above recommendations and that sodium intake relates to systolic BP within the normal range.

Dietary sodium intake was calculated from a food frequency questionnaire validated in the Australian child and adolescent normal population[224]. None of our participants had a sodium intake in the recommended range of ≤1.5g/day[155]. Likewise, children and adolescents with T1D studied in the U.S. SEARCH cardiovascular disease cohort and healthy adolescents had sodium intake above the recommended range [226, 234]. Mean daily sodium intake, evaluated by survey and 24-hour urine sodium excretion, in healthy Australian children is considerably higher than recommendations and comparable with our T1D cohort, at 2.52 g in 9-13 year olds and 3.16 g in 14-16 year olds [235, 236]. Our participants' raised sodium intake was comparable with both the healthy US and Australian childhood populations and children with T1D in the United States.

Dietary sodium intake is a potentially modifiable risk factor for cardiovascular disease that generally does not receive a lot of emphasis in childhood T1D education. Strategies to reduce sodium intake in children include education on healthy unprocessed snacks and our results indicate that there is a detectable reduction in dietary salt intake when take away food is

eaten less than once per week. In healthy normotensive adolescents, higher sodium intake is mostly derived from snack food and relates to a small increase in BP[237, 238]. Meta-analysis also shows that reduced sodium intake decreases BP in healthy adolescents and high dietary sodium intake impairs vascular function in healthy adults[239, 240] . We have extended the relationship between sodium intake and BP in health to normotensive adolescents with T1D, noting that the relationship between sodium intake and vascular smooth muscle function was independent of systolic BP.

Sodium intake related to vascular smooth muscle function but not endothelial function. Smooth muscle function may be a more sensitive marker of early vessel disease in children as we have shown previously[88]. While most experimental models have investigated the established pressure-independent effect of dietary salt on endothelium-dependent dilation, high salt intake also can reduce the responsiveness of vascular smooth muscle to vasodilator stimuli[241].

Strengths of this study design are that all the participants were studied at the same centre, by the same investigators, and that we used detailed and validated measures for diet and vascular health evaluation which were completed by all study participants. Our study has some limitations. All children had BMI higher or equal than 50th centile as they were the baseline cohort for a metformin intervention trial. Their HbA1c was higher than our total clinic's average. However, it is noteworthy that the detrimental effects of a high salt diet were still able to be detected in this higher cardiovascular risk group and were independent of HbA1c. The other limitation is that we report cross sectional associations, so that causative links cannot be explored. The vascular effects of a high salt meal that is controlled for other nutrients should now be investigated to confirm a causative link between sodium intake and vascular function.

In conclusion, this study shows that dietary sodium intake relates to vascular health in children and adolescents with T1D, independent of glycaemic control and BP. Dietary sodium also relates to systolic BP within the normal range in these children. This modifiable exposure is of high interest during puberty when vascular changes are accelerating but are potentially reversible. Our findings emphasize the need to further investigate the detrimental effect of a high sodium diet to inform advice for appropriate dietary sodium intake in routine education for children with T1D and their families.

6. Chapter 6: Effect of Metformin on vascular function in children with Type 1 Diabetes: A 12 month randomized controlled trial

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J Clin Endocrinol Metab, December 2017, 102(12):4448-4456

Statement of Authorship

Title of Paper	Effect of Metformin on Vascular Function in Children With Type 1 Diabetes: A 12-Month Randomized Controlled Trial
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	*The Journal of Clinical Endocrinology & Metabolism*, Volume 102, Issue 12, 1 December 2017, Pages 4448–4456, https://doi.org/10.1210/jc.2017-00781 **Published:** 09 October 2017

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Contribution to the Paper	Conception and design of the study, acquisition, analysis and interpretation of data. Wrote the first draft of the manuscript. Critically reviewed and corrected the manuscript; and approved final version. Acted as corresponding author.
Overall percentage (%)	75
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 06/04/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Professor Jennifer Couper
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Signature	Date 31/3/2021

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Contribution to the Paper	Performed advanced statistical analysis and assisted with interpretation of the data. Critically reviewed and corrected the manuscript; and approved final version.

Signature	5%	Date	16/3/2021

Name of Co-Author	Ms Catherine Leggett
Contribution to the Paper	Assisted with data interpretation. Critically reviewed and corrected the manuscript; and approved final version. 2.5%
Signature	Date 30/3/2021

Name of Co-Author	Mr Roger Gent
Contribution to the Paper	Performed vascular ultrasound assessment and assisted with data interpretation. Critically reviewed and corrected the manuscript; and approved final version. 2.5%
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Contribution to the Paper	Involved with data acquisition. Critically reviewed and corrected the manuscript; and approved final version. 2.5%
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Name of Co-Author	Dr Alexia S Pena
Contribution to the Paper	Supervised conception and design of the study; and interpretation of data. Obtained funding for the study. Critically reviewed and corrected the manuscript; and approved final version. 7.5%
Signature	Date 6/04/2021

The paper presented in this chapter investigates the effect of metformin on vascular health in children and adolescents with T1D over 12 months.

The impact factor of the journal where this paper has been published is 5.605.

This article has been cited 37 times in peer reviewed literature and was also selected for the 2018 Yearbook Advanced Technologies and Treatments for Diabetes in the Diabetes Technology and Therapy in the Pediatric Age Group section.

This data has been presented in a poster presentation at the American Endocrine Society Annual Scientific Meeting, 2016.

6.1 Abstract

Context: Children with T1D have vascular dysfunction preceding atherosclerosis. Early interventions are needed to reduce cardiovascular disease.

Objective: To evaluate the effect of metformin on vascular function in children with T1D

Design: Twelve-month double-blind, randomized, placebo-controlled trial

Setting: Tertiary pediatric diabetes clinic

Participants: Ninety children (8-18 years), >50th percentile BMI with T1D

Intervention: Metformin (up to 1g twice a day) or placebo.

Main outcome measure: Vascular function measured by brachial artery ultrasound (FMD/GTN)

Results: Ninety participants were enrolled [41 boys, 13.6(2.5) years, 45 per group],10 discontinued intervention, and 1 was lost to follow up. On metformin, GTN improved, independent of HbA1c, by 3.3 percentage units (95% CI 0.3, 6.3, P=0.03) and insulin dose reduced by 0.2U/kg/day (95%CI 0.1, 0.3, P=0.001) during 12 months, with effects from 3 months. Metformin had a beneficial effect on HbA1c at 3 months (P=0.001) and adjusted difference in HbA1c between groups during 12 months was 1.0%; 95% CI 0.4, 1.5 (10.9mmol/mol; 95% CI 4.4,16.4), P=0.001 There were no effects on carotid/aortic IMT, BMI, lipids, BP, or other cardiovascular risk factors. Median (95%CI) adherence, evaluated by electronic monitoring, was 75.5% (65.7, 81.5), without group differences. More gastrointestinal side effects were reported on metformin (incidence rate ratio 1.65, 95% CI 1.08, 2.52, P=0.02), with no difference in hypoglycaemia or DKA.

Conclusions: Metformin improved vascular smooth muscle function and HbA1c, and lowered insulin dose in children with T1D. These benefits and good safety profile warrant further consideration of its use.

6.2 Introduction

Cardiovascular disease is the leading cause of mortality in T1D[229], and glycaemic control is the major modifiable risk factor for prevention of vascular complications. However, individuals with T1D and excellent glycaemic control still have three times the risk for cardiovascular causes of death as matched controls[14] , so that many patients need additional strategies to improve cardiovascular health.

Vascular dysfunction is a critical event in the development of cardiovascular disease and is detectable years before cardiovascular disease develops[242]. Vascular function can be assessed by ultrasound measurement of brachial artery responses to increase in flow (FMD) and to glyceryl trinitrate (GTN). FMD increases in artery diameter are dependent on endothelium nitric oxide release (endothelium-dependent response). Glyceryl trinitrate is a nitric oxide donor that increases the artery diameter independent of the endothelium and, therefore, assesses vascular smooth muscle response [88]. Vascular function, measured by FMD and GTN, correlates with coronary atherosclerosis on angiography [10, 98] and with cardiovascular risk factors [98, 243, 244].

We and others have shown that vascular function is impaired and IMT is increased in children at increased risk of atherosclerosis, including children with T1D [67, 68, 88, 89, 245]. Importantly, these early vascular changes in function and structure are potentially reversible.

Metformin reduces cardiovascular events and improves body composition and glycaemic control in adults with T2D [24, 178]. In adults with T1D, metformin has inconsistently improved HbA1c, BMI, and insulin dose [27, 246]. In one pilot study in adults with T1D,

metformin improved endothelial function [25]. In children with T1D, metformin can reduce HbA1c, insulin dose and BMI[28, 181, 188], but there are no data on its effect on vascular health. Metformin stimulates nitric oxide synthesis in vitro in endothelium and smooth muscle [174]. Therefore, we aimed to determine the effect of metformin on vascular health in children with T1D and above average weight. We hypothesised that metformin would improve vascular function, independent of other benefits on cardiovascular risk factors.

6.3 Research design and methods

6.3.1 Study design and setting

This parallel, randomized, placebo-controlled trial was conducted at a single site at Women's and Children's Hospital in Adelaide (SA, Australia). It was approved by 2 recruitment sites (Women's and Children's Hospital HREC 2327/12/13 and Flinders Medical Centre HREC 443.12) and prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611000148976). Participants were recruited from August 2011 and the trial was completed in June 2015. Written informed consent was obtained from all participants' parents and written informed assent obtained from all participants. Assessments were done at baseline, 3, 6 and 12 months. The study protocol was published [227] and is summarized later.

6.3.2 Study participants

Participants were eligible for the study if they were diagnosed with T1D at least 6 months prior, had an insulin requirement >0.5 U insulin/kg/d, aged 8-18 years and BMI > 50th centile for age and sex (Centers for Disease Control and Prevention 2000 standardized reference charts (http://wwwn.cdc.gov/epiinfo/)). They were excluded if they had a severe hypoglycaemic episode in 6 months prior to recruitment, more than 2 episodes of diabetes ketoacidosis in the previous 12 months, serious co-morbidities, contraindication to metformin therapy, or were already on metformin, statins, multivitamins, or anti-hypertensives.

Participants were recruited and enrolled by a single investigator (J.J.A.A) and assigned a code in sequence (1-90). Participants were allocated in a 1:1 ratio to metformin or placebo by the pharmacist using the randomization list generated by a statistician external to the study using statistical software S-PLUS version 8.1. Medication bottles were identical between groups aside from batch number which the pharmacist used to allocate participants to treatment group (labelled as A or B). Participants, their care providers and investigators were blinded to treatment group[227].

6.3.3 Intervention

Participants received up to 1 gram twice a day according to weight (≥ 60kg 1 gram twice per day; <60 kg 500mg twice per day). The dose was increased during 2 to 6 weeks up to the full dose, as tolerated. Metformin and placebo tablets (Generic Health, Melbourne, VIC, Australia) were identical in appearance and ingredients aside from the active ingredient, metformin hydrochloride. All participants received standardized T1D dietary advice for 60 minutes from the one dietitian at baseline and 3 months [227].

6.3.4 Vascular function outcomes: Flow mediated dilatation (primary outcome measure) and Glyceryl trinitrate mediated dilatation (secondary outcome measure)

FMD and GTN were assessed at each visit as we have previously described [13, 67, 88, 89, 227]. Experienced and blinded sonographers (trained by R.G.) performed B mode ultrasound examinations with a 17–5 MHz linear array transducer (iU22; Phillips, Bothel, Washington, USA). In brief, brachial arterial diameter was measured in 4 scans: (1) baseline artery diameter scan; (2) reactive hyperemia was induced by occluding arterial blood flow using a sphygmomanometer inflated to 250 mmHg for four minutes; FMD scan was recorded 45–75 seconds after cuff deflation; (3) re-control scan 10–15 minutes later; and (4) GTN scan, taken four minutes after sublingual administration of GTN spray (400 µg, Nitrolingual Pump

spray, G. Pohl-Boskamp GmbH &co. KG Hohenlockstedt, Germany). For each scan, measurements were made using ultrasonic calipers by observers blinded to intervention type over four consecutive cardiac cycles, incident with R wave on electrocardiogram (i.e., at end-diastole). Measurements were averaged and expressed as percentages of the baseline artery diameter scan. Our coefficient of variation between 20 controls was 3.9% for FMD and 4.0% for GTN[89, 227].

6.3.5 Secondary and other outcomes

HbA1c, insulin dose, BMI , body composition, waist circumference, mean of 3 consecutive BPs, fasting lipid profile , HsCRP, adiponectin, leptin and early morning urinary ACR were evaluated at all visits; as previously described[227]. Estimated insulin sensitivity was calculated using a validated equation as follows: 4.06154 - 0.01317 × waist (cm) - 1.09615 × insulin dose (units/kg/day) + 0.02027 × adiponectin (μg/ml) - 0.27168 × triglycerides (mmol/L) - 0.00733 × diastolic BP[247]. This surrogate marker of insulin sensitivity has been shown to correlate well with measured glucose infusion rate in euglycaemic hyperinsulinemic clamps in adolescents and adults with T1D[247].

Mean/maximum cIMT, aIMT and total body DXA scan were assessed at baseline and 12 months as previously described [67, 68, 227].

6.3.6 Safety outcomes and adherence assessment

Participants received fortnightly phone calls (J.J.A.A) for the first 3 months and monthly phone calls thereafter to complete a side effects questionnaire, titrate medication and adjust insulin doses. The single blinded investigator (J.J.A.A) adjusted insulin during the commencement of the study medication, as the dose was increased according to the protocol, and then throughout the study participants were able to phone J.J.A.A for adjustments and were requested to do so if there was any increased frequency of hypoglycaemia. Additionally all

participants continued to see their standard health professionals (paediatric endocrinologist and diabetes educator) at 3 monthly intervals at diabetes clinics according to routine care and aiming for a target HbA1c of < 7.5%.

The questionnaire included gastrointestinal and other side effects, as well as hypoglycaemic events (defined as moderate if required assistance and severe if the participant collapsed or had a seizure). Side effects were also recorded at study visits. Any hospital admissions were reported within 24 hours to an independent safety monitoring committee. Events were reported regardless of whether they were considered related to medication. Lactate, liver and renal function tests, vitamin B12 and folate status were measured at each visit[227]. Study medication adherence was assessed at each visit by pill count and MEMS cap download (AARDEX group LTD, Sion, Switzerland).

6.3.7 Statistical analysis

Demographic measures were summarized by mean and SD or median and interquartile range for continuous variables according to normality. Distributions of all variables were assessed for normality using histograms and Kolmogorov-Smirnov tests. Variables with skewed distributions were log transformed, as appropriate, and the transformed variables were analyzed; geometric means were then reported for these transformed variables. Statistical significance was set at $P < 0.05$ (two-sided) with no adjustment for multiple comparisons. All analyses followed a pre-specified analysis plan and used Stata version 14.1 (StataCorp LP, College Station, TX, USA).

Analyses were performed (L.C.G) on an intention to treat basis and blinded to which treatment group was metformin (i.e., analyses were conducted comparing groups A and B). Once the analyses were completed, un-blinding of groups occurred (A corresponded to placebo and B to metformin). Continuous outcomes were analysed with linear mixed effects models,

including treatment group, time, and their interaction in the models. Treatment effects were expressed as differences in means and 95% CI. Analyses were adjusted for age and HbA1c at each timepoint as well as sex.

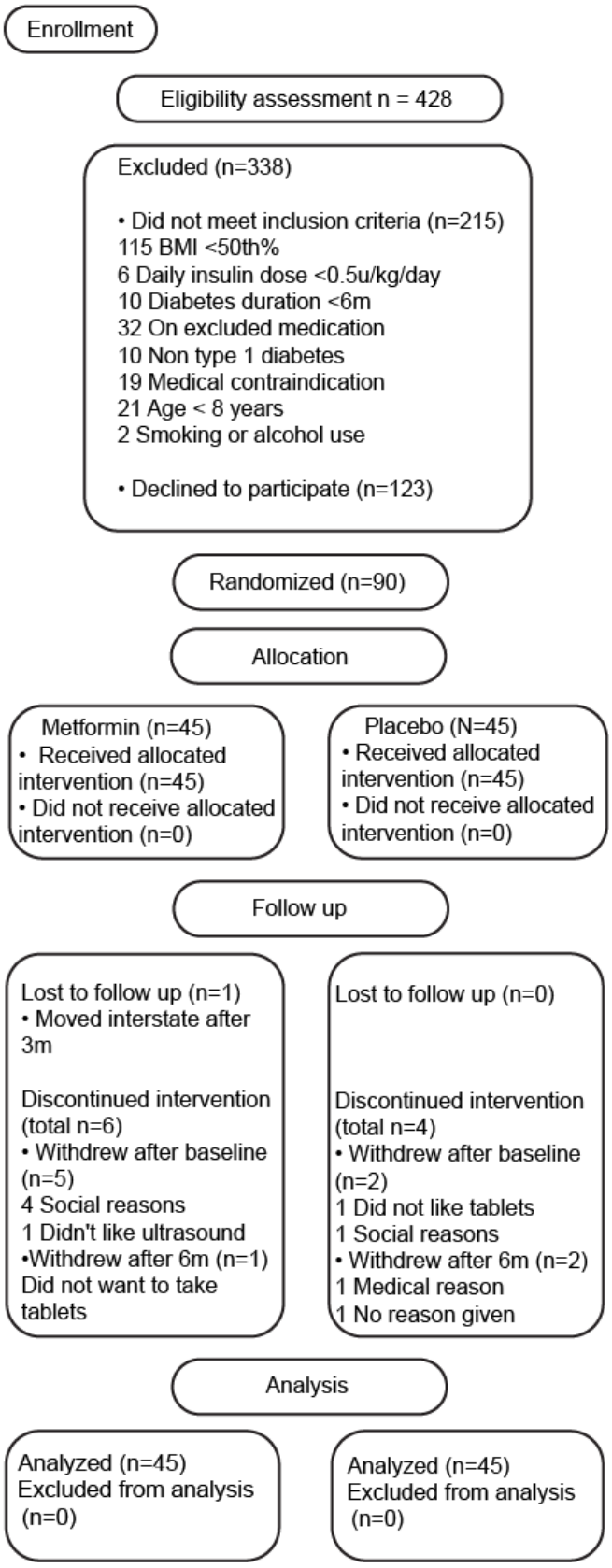
A random subject effect was included in each statistical model to allow for correlation between observations on the same subject at different time points. Multiple imputation was performed separately by treatment group using chained equations to create 100 complete datasets[248]. Sensitivity analyses with available data and different imputation models were performed to assess stability of results. The relative risk and associated 95% CI of any side effects for metformin vs placebo was calculated. Similarly, incidence rate ratios and 95% CI were calculated to compare total count of each side effect per participant between treatment groups.

Although no studies have looked at effect of metformin on vascular function (FMD or GTN) in T1D in this age group, our previous work found improvement in FMD of 3.1% (SD 4.3) in children with T1D receiving folic acid during 8 weeks [13]. Assuming equivalent improvement with metformin from baseline to 12 months, we estimated a total sample size of 90 participants would have 90% statistical power (with two-tailed α level 0.05) to detect an absolute mean difference in FMD of 3.1% (SD 4.3) in comparison of metformin and placebo groups, allowing for 10% attrition.

6.4 Results

Of 428 consecutive clinic patients, 215 were ineligible, 123 declined and 90 participants were randomized to metformin or placebo. One participant was lost to follow up; 10 discontinued intervention (Figure 4).

Figure 4. Consort flow diagram of participants.



Mean (SD) age of participants was 13.6 (3.5) years and 54% (n=49) were female. Median (interquartile range) HbA1c was 8.7% (8.1 to 9.9) / 72mmol/mol (65 to 85) and mean (SD) BMI-

z score was 0.89 (0.57). Fifty-four participants had normal BMI (BMI 50 to 84%), 25 were overweight (BMI 85 to 95%) and 11 were obese (BMI >95%). Eighty-eight (97%) participants were white, one was African, and one was Asian. Twenty-one of 45 (47%) placebo and 22 of 45 (49%) metformin participants were < 60kg. There were no statistically significant differences between groups at baseline in each measured variable with the exception of diastolic BP (Table 12).

Table 12. Baseline characteristics

Variable	Placebo n=45	Metformin n=45
Age (years)	13.3 (2.6)	14.0 (2.5)
Sex (M/F)	20/25	21/24
Diabetes duration (years)	5.8 (4.1)	5.2 (3.6)
Puberty (n) (pre/mid /post)	14/9/22	11/7/27
Insulin regimen (MDI/CSII)	23/22	22/23
Insulin dose (u/kg/day)	0.85 (0.21)	0.82 (0.22)
BMI z-score	0.9 (0.5)	0.9 (0.6)
Weight z-score	1.1 (0.7)	0.9 (0.7)
Waist circumference (cm)	71 (1)	72 (9)
Hip circumference (cm)	89 (1)	89 (9)
Waist/Hip ratio	0.8 (0.1)	0.8 (0.1)
DXA total lean mass (kg)	39 (11)	39 (9)
DXA fat percent (%)	31 (10)	31 (10)
BIA fat (%)	27.8 (7.1)	27.1 (6.8)
BIA fat mass (kg)	17.1 (8.1)	16.5 (6.7)
BIA fat free mass (kg)	42.5 (11.6)	43.2 (10.0)
Systolic BP (mm Hg)	112 (8)	112 (9)
Diastolic BP (mm Hg) [a]	62 (5)	64 (6)
Total cholesterol (mmol/L)	4.4 (0.8)	4.4 (0.7)
Triglycerides (mmol/L)	0.8 (0.3)	0.8 (0.3)
HDL cholesterol (mmol/L)	1.6 (0.3)	1.6 (0.4)
LDL cholesterol (mmol/L)	2.5 (0.7)	2.5 (0.5)
Glucose (mmol/L)	12 (5)	10 (4)
HbA1c (%) [b]	8.8 (8.2 - 9.9)	8.4 (7.8 - 9.7)
HbA1c (mmol/mol) [b]	73 (66 - 85)	68 (62 - 83)

hsCRP (mg/L)	2.7 (7.1)	2.3 (5.4)
Alanine transaminase (IU/L)	14.4 (3.9)	15.2 (5.5)
Urea (mmo/L)	4.8 (1.1)	4.3 (1.1)
Creatinine (µmol/L)	48.8 (10.8)	51.8 (11.6)
Lactate (mmol/L)	1.2 (0.7)	1.1 (0.4)
Homocysteine (µmol/L)	5.6 (1.4)	6.2 (1.9)
Adiponectin (mcg/mL)	13.8 (8.8)	12.2 (6.1)
Leptin (ng/mL)	12.6 (1.7)	11.7 (1.6)
Adiponectin/Leptin ratio (mcg/ng)	2.3 (3.7)	2.0 (2.2)
Vitamin B12 (pmol/L)	477 (181)	414 (168)
Urine albumin creatinine ratio (mg/mmol)	1.0 (1.8)	0.9 (1.6)
Mean aIMT (mm)	0.5 (0.1)	0.5 (0.1)
Mean cIMT (mm)	0.4 (0.1)	0.4 (0.1)
Maximum aIMT (mm)	0.6 (0.1)	0.6 (0.1)
Maximum cIMT (mm)	0.5 (0.1)	0.5 (0.1)
FMD (percentage units)	6.3 (4.5)	6.1 (4.5)
GTN (percentage units)	23.7 (6.7)	25.3 (6.2)
Brachial artery diameter (cm)	0.3 (0.04)	0.3 (0.04)

Data are mean (SD)
Abbreviations: BIA, bioelectrical impedance analysis; CSII, continuous subcutaneous insulin infusion; DXA, dual-energy X-ray absorptiometry; FMD, flow mediated dilatation; GTN, GTN mediated dilatation; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein, MDI, multiple daily injection
[a] P = 0.03
[b] Median (interquartile range)

Median (95% CI) adherence, evaluated by MEMS and pill count, was 75.5% (65.7, 81.5) and 76.6% (69.7, 80.5) respectively, during 12 months, with no differences between metformin and placebo groups. Adherence was highest at 3 months [MEMS median 88.9% (77.0, 91.6); pill count 87.1% (78.2, 90.5)] and decreased over time (time effect P < 0.001 for MEMS and pill count). All participants tolerated full dose according to the weight criteria, described previously, except for three participants < 60kg receiving metformin (two tolerated 250mg twice per day and one tolerated 250mg per day) and 3 participants > 60kg who tolerated 250 mg to 1.5 g per day. Side effects in these six patients included nausea (five of six), reduced

appetite (one of six), vomiting (four of six), and diarrhoea (two of six), All side effects resolved on the reduced metformin dose.

6.4.1 Cardiovascular health and risk factors

Vascular smooth muscle function (GTN) improved, independent of HbA1c, by 3.3 percentage units (95%CI 0.3, 6.3, P = 0.03) over the 12 months intervention in the metformin group compared with placebo (Table 13). The improvement in GTN was also independent of baseline pubertal status in the participants (coefficient 3.4 percentage units; 95% CI 0.4, 6.4, P = 0.03). Sensitivity analyses with available data and different imputation models showed the same results. GTN adjusted for covariates (age, sex and HbA1c) was highest in the metformin group at 3 months compared to placebo [25.0 percentage units (95% CI 22.9, 27.2] vs 21.7 percentage units (95% CI 19.6, 23.8), P = 0.03

Table 13. Primary, secondary, and safety measures

Variable	Adjusted mean (SE)						Adjusted Overall Treatment Effect (95% CI)[a]	P
	3 months		6 months		12 months			
	Placebo	Metformin	Placebo	Metformin	Placebo	Metformin		
Primary outcome measure								
FMD (percentage units)	5.5 (0.6)	6.5 (0.6)	5.7 (0.5)	6.0 (0.6)	6.8 (0.5)	5.9 (0.5)	1.1 (-0.7, 2.8)	0.2
Secondary outcome measures								
GTN (percentage units)	21.7 (1.1)	25.0 (1.1)	22.1 (1.0)	24.5 (1.1)	22.9 (1.1)	25.1 (1.2)	3.3 (0.3, 6.3)	0.03
Brachial artery diameter (cm)	0.28 (0.01)	0.29 (0.01)	0.28 (0.01)	0.29 (0.01)	0.28 (0.01)	0.29 (0.01)	-0.005 (-0.02, 0.01)	0.5
Insulin dose (u/kg/day)	0.9 (0.03)	0.7 (0.03)	0.9 (0.03)	0.7 (0.03)	0.9 (0.03)	0.7 (0.03)	-0.2 (-0.3, -0.1)	0.001
HbA1c (%) [b]	9.3 (0.2)	8.4 (0.2)	9.3 (0.2)	8.7 (0.2)	9.5 (0.2)	9.0 (0.3)	-1.0 (-1.5, -0.4)	0.001
HbA1c (mmol/mol) [b]	78 (17.5)	68(9.8)	78(17.5)	72(13.1)	80(18.6)	75(16.4)	-10.9(-16.4, -4.4)	0.001
Glucose (mmol/L)	11.5 (0.7)	11.1 (0.7)	11.1 (0.6)	11.5 (0.7)	11.0 (0.6)	11.3 (0.7)	-0.4 (-2.4, 1.6)	0.7
hsCRP (mg/L) [c]	1.3 (0.1)	1.0 (0.1)	1.3 (0.1)	1.0 (0.1)	1.8 (0.1)	1.2 (0.1)	-0.1 (-0.3, 0.1)	0.3
LDL (mmol/L)	2.5 (0.1)	2.3 (0.1)	2.5 (0.1)	2.4 (0.1)	2.3 (0.1)	2.2 (0.1)	-0.2 (-0.4, 0.1)	0.2
HDL (mmol/L)	1.6 (0.1)	1.5 (0.1)	1.6 (0.1)	1.5 (0.1)	1.6 (0.1)	1.6 (0.1)	-0.1 (-0.2, 0.1)	0.4
Total cholesterol (mmol/L)	4.4 (0.1)	4.2 (0.1)	4.4 (0.1)	4.3 (0.1)	4.3 (0.1)	4.2 (0.1)	-0.2 (-0.6, 0.2)	0.3
Triglycerides (mmol/L)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	-0.01 (-0.2, 0.2)	0.9
Adiponectin (mcg/mL)	11.3 (1.0)	10.1 (1.1)	11.4 (1.0)	8.8 (1.0)	11.2 (1.0)	9.2 (1.0)	-1.2 (-4.1, 1.6)	0.4
Leptin (ng/mL) [c]	13.2 (0.1)	8.6 (0.1)	8.4 (0.1)	13.3 (0.1)	12.7 (0.1)	9.5 (0.1)	-0.3 (-0.5, -0.1)	0.02
Adiponectin leptin ratio	2.4 (0.7)	2.6 (0.8)	2.5 (0.7)	2.1 (0.7)	2.1 (0.8)	1.9 (0.8)	0.2 (-1.9, 2.3)	0.9

Urea (mmol/L)	4.7 (0.2)	4.7 (0.2)	4.5 (0.2)	4.7 (0.2)	4.6 (0.2)	4.5 (0.2)	0.04 (-0.4, 0.5)	0.9
Creatinine (µmol/L)	52.6 (1.2)	52.6 (1.2)	52.1 (1.2)	51.7 (1.2)	52.0 (1.2)	52.4 (1.3)	0.02 (-3.4, 3.4)	1.0
ALT (IU/L)	14.7 (0.9)	14.5 (1.0)	15.8 (0.9)	14.9 (1.0)	14.2 (0.9)	14.0 (0.9)	-0.2 (-2.9, 2.5)	0.9
Homocysteine (µmol/L)	6.0 (0.2)	6.5 (0.3)	6.1 (0.3)	6.3 (0.3)	6.3 (0.3)	7.1 (0.3)	0.5 (-0.3, 1.1)	0.2
Urine ACR (mg/mmol)	0.9 (0.4)	0.9 (0.4)	0.8 (0.3)	1.0 (0.3)	0.6 (1.1)	1.5 (0.3)	0.5 (-0.5, 1.5)	1.0
Waist (cm) [b]	73.4 (1.2)	71.0 (1.2)	73.0 (1.1)	70.4 (1.2)	73.2 (1.1)	70.2 (1.2)	-2.4 (-5.8, 1.0)	0.2
Hip (cm) [b]	92.4 (1.0)	90.5 (1.0)	92.8 (1.0)	90.6 (1.0)	92.6 (1.1)	90.0 (1.2)	-1.9 (-4.6, 0.8)	0.2
Waist hip ratio [b]	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	0.8 (0.01)	-0.01 (-0.04, 0.01)	0.4
BIA fat % [b]	28.0 (0.8)	27.0 (0.9)	28.0 (0.8)	26.3 (0.9)	27.8 (0.9)	26.5 (0.9)	-1.0 (-3.3, 1.4)	0.4
BIA fat free mass (kg) [b]	46.3 (1.0)	43.8 (1.0)	46.1 (0.9)	43.9 (1.0)	46.0 (1.0)	43.2 (1.0)	-2.5 (-5.2, 0.2)	0.07
Systolic BP (mmHg) [b]	113.1 (1.1)	110.6 (1.1)	111.2 (1.1)	111.2 (1.1)	112.4 (1.1)	110.0 (1.1)	-2.5 (-5.6, 0.6)	0.1
Diastolic BP (mmHg) [b]	62.5 (0.7)	62.4 (0.7)	62.0 (0.7)	62.8 (0.7)	62.2 (0.7)	62.7 (0.8)	-0.1 (-2.0, 1.7)	0.9
Safety outcome measures								
Lactate (mmol/L)	1.3 (0.1)	1.4 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	0.1 (-0.2, 0.4)	0.5
VitaminB12 (pmol/L)	494 (21)	407 (22)	489 (24)	377 (25)	451 (33)	347 (34)	-87.3 (-148.4, -26.2)	0.01

[a] Adjusted for age, gender and HbA1c, unless otherwise indicated

[b] Adjusted for age and gender only

[c] Geometric means

Abbreviations: ACR, Albumin creatinine ratio; ALT, Alanine aminotransferase; BIA, Bioelectric Impedance Analysis; BP, Blood Pressure; HDL, high density lipoprotein; HbA1c, glycosylated haemoglobin; HsCRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; SE, standard error.

There was no significant effect of metformin on other measures of vascular health and cardiovascular risk factors including FMD, BP, BMI, waist and hip circumference, fat mass, lipid profile, high sensitivity C-reactive protein, adiponectin, adiponectin/leptin ratio and urine albumin creatinine ratio during 12 months There was a significant decrease in leptin in the metformin group (Table 13). Inclusion of study medication dose/kg/d and adherence, as measured by tablet count or MEMS made no significant difference in the results for FMD or GTN (data not shown).

There was no significant effect of metformin on mean/maximum aIMT (-0.02 mm; 95%CI -0.08, 0.03, P = 0.4/-0.04mm, 95%CI -0.1, 0.02, P = 0.2), mean/maximum cIMT (-0.01mm, 95%CI -0.04, 0.01, p=0.3 /-0.01mm, 95%CI -0.04, 0.02, p=0.5), DXA fat (-0.8 percentage, 95%CI -4.4, 2.7, p=0.7) or DXA fat mass (-1.7kg, 95%CI -5.1, 1.8, p=0.3) over 12 months. There was a difference in DXA lean mass at 12 months in the metformin group of 2.9kg (95%CI -5.4, -0.3, p=0.03) compared with placebo.

6.4.2 Glycaemic control and insulin dose

There was a significant benefit in adjusted (age, sex) HbA1c at 3 months for the metformin group (8.4%; 95% CI 8.0, 8.8) (68mmol/mol; 95% CI 64, 73) vs placebo group (9.3%; 95% CI 9.0, 9.7) (78mmol/mol; 95% CI 75, 83) (P=0.0001), and this was primarily responsible for the overall benefit of metformin compared with placebo during the study period. The adjusted overall difference in HbA1c between the groups during the intervention was 1.0% (95% CI 0.4, 1.5) 10.9 mmol/mol (95% CI 4.4, 16.4) P = 0.001 (Table 13) during 12 months. HbA1c was significantly related to study medication dose, measured in mg/kg/d, but the magnitude of the dose effect was very small (0.08%; 95% CI 0.04, 0.12) (0.9 mmol/mol; 95% CI 0.4, 1.3) (P < 0.001).

Total daily insulin dose was reduced by 0.2 U/kg/d during 12 months (95% CI 0.1, 0.3, P = 0.001) in the metformin group compared with placebo (Table 13). The adjusted insulin doses remained the same in each group from 3 to 12 months [metformin: 0.7 (95% CI 0.4, -1.0); placebo: 0.9 (95% CI 0.6, -1.2) U/kg/d, P = 0.001). Estimated insulin sensitivity as calculated previously remained 0.2 U higher during 12 months (95% CI 0.06, 0.34, P = 0.005) in the metformin group compared with placebo.

Those who discontinued intervention (n = 11, metformin = 7, placebo = 4) vs those who continued intervention for 12 months (n = 79) had higher baseline (mean ± SD) HbA1c (10.3% ± 2.2% vs 8.8% ± 1.2%, 89 ± 24 vs 73 ± 13.1 mmol/mol, P = 0.001), total cholesterol (4.8 ± 1.0 vs 4.1 ± 1.0 mmol/L, P = 0.01), low-density lipoprotein cholesterol (2.9 ± 0.7 vs 2.4 ± 0.5, P = 0.01) and total daily insulin dose (1.0 ± 0.2 vs 0.8 ± 0.2 U/kg/d, P = 0.003). There were no statistically significant differences in other baseline variables, including vascular function: FMD (6.3 ± 4.5 vs 6.1 ± 4.5 percentage units, P = 0.9) and GTN (23.7 ± 6.7 vs 25.3 ± 6.2 percentage units, P = 0.2).

6.4.3 Safety data

A total of 133 side effects were reported with more side effects in the metformin group compared with placebo [80 vs 53, relative risk 1.51 (95% CI 1.05, 2.18), P = 0.02]. Gastrointestinal side effects were more common in the metformin group compared to placebo (Table 3). There were no significant differences in non-gastrointestinal side effects, moderate hypoglycaemic events or DKA between groups (Table 14). There were no episodes of severe hypoglycaemia or lactic acidosis during 12 months.

Table 14. Summary of side effects

	Metformin (n=45)	Placebo (n=45)	Total (n=90)	RR/IRR (95% CI)	p-value
Gastrointestinal side effects					
No. affected n (%)	22 (49)	14 (31)	36 (40)	1.57 [a] (0.93 – 2.66)	0.09
No. per participant Median (IQR)	0 (0-2)	0 (0-1)	0 (0-1)	1.65 [b] (1.08 – 2.52)	0.02
Non gastrointestinal side effects					
No. affected n (%)	10 (22)	10 (22)	20 (22)	1.00 [a] (0.46 – 2.17)	1.00
No. per participant Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	1.17 [b] (0.54 – 2.52)	0.7
Moderate hypoglycaemic events					
No. affected n (%)	4 (9)	2 (4)	6 (7)	2.00 [a] (0.39 – 10.38)	0.4
No. per participant Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	4.00 [b] (0.85 – 18.84)	0.08
Diabetic ketoacidosis					
No. affected n (%)	2 (4)	2 (4)	4 (4)	1.00 [a] (0.15 – 6.79)	1.00
No. per participant Median (IQR)	0 (0,0)	0 (0,0)	0 (0,0)	1.00 [b] (0.14 – 7.10)	1.00

[a] Risk ratios reported for comparison of participants with side effects between metformin and placebo
[b] Incidence rate ratios for comparison of number of events per participant between metformin and placebo
Abbreviations: IQR, interquartile range; IRR, Incidence rate ratio; RR, Risk ratio

Vitamin B12 levels were significantly lower overall in the metformin group compared with placebo but were still within reported reference range (140 – 700 pmol/L) with no change in homocysteine levels (Table 13). There was no significant difference in lactate, liver function and renal function tests between metformin and placebo groups over 12 months (Table 13).

6.5 Conclusions

We report that metformin improved vascular smooth muscle function during 12 months in above average weight children with T1D. The effect was modest and independent of the improvement in HbA1c on metformin. Benefits for both vascular smooth function and HbA1c were greatest at 3 months, when the participants' adherence was at its highest. These benefits were seen in participants who were pre-pubertal or pubertal at baseline. There was no

significant benefit of metformin on measures of vascular structure, nor on traditional cardiovascular risk factors.

The study contributes important original findings to our knowledge of metformin use in children with T1D. First, it has shown an effect on vascular function. Second, children studied were above average weight, but most were not overweight, unlike other metformin studies in adolescents [28, 182]. Therefore, our results can be applied to patients of a wider weight range and different pubertal status. Third, the study was of longer duration than previous studies in childhood and as such was well placed to demonstrate a good safety profile during 12 months. Finally, adherence was measured objectively using prospective electronic monitoring.

The significance of vascular smooth muscle function as measured by GTN is as follows: it relates to preclinical carotid atherosclerosis in children[99] and in adults [249] with accelerated atherosclerosis. It is impaired in adults with coronary artery disease[98]. It can be impaired independently of endothelial dysfunction[243] and in some studies it is a better predictor than FMD of cardiovascular events and coronary artery calcification [250, 251]. Furthermore, the improvement in vascular function, independent of HbA1c, is consistent with metformin's direct effect in vitro on smooth muscle by stimulating nitric oxide synthesis via activation of AMP kinase in smooth muscle [174] which is reflected by improvement in GTN rather than FMD. The effect on GTN alone rather than both GTN and FMD does suggest a more modest benefit of metformin on vascular function.

The HbA1c improvement with a fall in insulin dose, and an increase in estimated insulin sensitivity during 12 months in the metformin group, provides a significant clinical benefit. Note that overall benefit of metformin was primarily explained by the improvement in HbA1c at 3 months which then waned over time. Most children with T1D in Australia do not achieve

target HbA1c levels [20, 252] and approximately a third of children with T1D in Australia are overweight [20]. Therefore, the combination of improved metabolic control and a lower insulin dose requirement is particularly relevant to this group. A similar improvement in HbA1c from a similar baseline level was not sustained beyond 13 weeks in a recent randomized controlled trial of metformin in adolescents with T1D [28]. There were several differences in this United States study in comparison with our study that may explain this: the adolescents were all overweight/obese, had a higher insulin dose, were older and mostly post-pubertal, and had a longer duration of T1D. Additionally, these adolescents were required to be adherent with blood glucose monitoring prior to study entry, and HbA1c level > 10% was an exclusion criteria. Additionally, the study duration was 6 months and was conducted in 26 centres, with a higher number of clinicians adjusting insulin doses, whereas our study was conducted in one centre with only four paediatric endocrinologists, in addition to the primary investigator, adjusting insulin doses throughout the study to aid consistency.

Metformin was well tolerated in our study, as expected from previous systematic reviews in children [253, 254]. The mild fall in vitamin B12 detected on metformin, without homocysteine changes, would not normally be regarded as clinically significant, unless baseline levels of vitamin B12 were already low. No participant experienced severe hypoglycaemia and moderate hypoglycaemia was not significantly increased in the metformin group. In addition to weekly phone calls, participants had 24-hour access to one of four clinicians if they experienced an increase in mild hypoglycaemic events and insulin dose was adjusted accordingly. This consistency in care may explain why we had no severe hypoglycaemia unlike some other studies [28, 182, 188].

We did not demonstrate any reduction in BMI, body fat percentage, waist circumference, or adiponectin/leptin ratio during 12 months. This is in contrast to other studies of metformin in overweight/obese children with T1D. The difference may be explained by our inclusion criteria

threshold of BMI above 50th percentile rather than overweight/obese. Meta-analysis supports this difference in study findings whereby BMI is reduced in overweight/obese, but not in above average weight, populations [253]. The loss in lean body mass in the metformin group was an unexpected finding of uncertain clinical significance.

The strengths of our study are that it was conducted at a single centre with the same experienced sonographer performing ultrasound studies for assessment of vascular function and conferring high fidelity on all measures. Adherence was accurately assessed with two objective measures (MEMS caps and pill count) and there was a high study retention rate over 12 months. Limitations were that the duration of 12 months only provided the opportunity to realistically detect change in vascular function. Change in vascular structure likely requires several years of follow up in T1D [194, 255, 256] and the study was powered to detect significant change in vascular dysfunction, not other vascular structural markers. Additionally, we relied on participant reports of hypoglycaemia so accurate assessment of severe, and moderate episodes, but not mild episodes of hypoglycaemia could be made.

In summary, the demonstrated benefit of metformin on vascular function, HbA1c, insulin dose and estimated insulin sensitivity over 12 months in children with T1Dwho are above average weight, and the good safety profile, warrants ongoing consideration of its use in this population.

7. Chapter: 7 Discussion

In this chapter, I will discuss the results from each study with reference to study design, how the results are seen in the context of the current published evidence, the limitations of the study, the clinical implications and potential future research.

7.1 Exercise and vascular health

7.1.1 Study results

The current study showed for the first time, that daily step count, which is a surrogate measure of physical activity, relates to early signs of atherosclerosis in children and adolescents with T1D. The relationship between step count and aIMT was linear with higher step count relating to a lower IMT. For every 1000 step increase per day there was a decrease in mean and maximum aIMT of 0.0082mm and 0.0093mm respectively which was independent of HbA1c, BMI-z, BP, triglycerides and lipids. aIMT has previously been shown to relate to LDL cholesterol and urine ACR (albumin creatinine ratio) in adolescents with T1D[68]. For every 1 unit increase in urine ACR there is a 0.013mm and 0.023mm increase in mean and maximum aIMT respectively. There was a similar effect size related to LDL cholesterol.

There was also a relationship between higher step count being associated with lower cardiovascular risk factors. For every 1000 step per day increment, there was an associated reduction in mean weight (1.6kg), systolic and diastolic BP (1.02 and 0.5 mmHg respectively), triglycerides (0.02mmol/L) and an increase in mean HDL cholesterol (0.03mmol/L).

Number of steps per day is an easily understood measure of activity by the community. With the advent of wearable activity technology by the public, step count can be measured and tracked on a daily basis. In the 2011/2012 Australian Health Survey, healthy children and adolescents averaged 9140 steps per day with males taking more daily steps than females[257]. This is similar to our study with an average step count of 9391 (SD 3089) across all participants. Current Australian guidelines do not make specific recommendations about daily step count, however do recommend 60 minutes of moderate-vigorous activity per day as well as several hours per day of light physical activities[100]. Some groups have recommended a daily step count for healthy children and adolescents in this age group of

around 12000 steps[258]. Sixty minutes of moderate-vigorous physical activity equates to around 10000-11700 steps per day in healthy children and adolescents although studies comparing MVPA to step count are limited[228].

Higher step counts relate to lower all-cause mortality in adults with those taking over 10 000 steps per day experiencing a 46% reduced risk of mortality[148]. A recent systematic review and meta-analysis of steps-per-day relating to arterial stiffness (measured by pulse wave velocity) demonstrated that an increase of 1000 steps per day equates to a reduction in pulse wave velocity[259]. Pulse wave velocity is an independent predictor of all-cause mortality and 1 SD increase in pulse wave velocity relates to a 30% increase in cardiovascular events [260].

Physical activity is one of the modifiable risk factors identified as one of the ideal cardiovascular health metrics by the American Heart Association [261]. The SEARCH CVD study of cardiovascular health in adolescents with T1D has previously reported that only 12.6% of the youth in their study met their modified criteria for activity and those who met the physical activity criteria had a reduced cIMT[226]. This study used a recall of activity over 3 days rather than an objective measure of physical activity like the one we used in our study.

For the first time, we have demonstrated that objectively measured step count relates to early atherosclerotic changes and an adverse cardiovascular risk profile in children and adolescents with T1D, who are at risk of early cardiovascular disease. A small incremental increase in activity related to a measurable change in arterial structure. Previous literature in children and adolescents with T1D reports that exercise interventions improve FMD and have a favourable effect on cardiovascular risk profile including HbA1c, BMI and lipids [142, 143] and that meeting physical activity guidelines based on 3 day physical activity recall questionnaire relates to cIMT[226]. Meta-analysis of previous exercise intervention studies in children and adolescents with T1D has shown an improvement in total cholesterol of 0.91mmol/L and a

reduction in triglycerides of 0.7mmol/L with activity[142]. This is consistent with our study that found that increments of 1000 steps a day was associated with better lipid profile (increase in HDL cholesterol of 0.03mmol/L and a decrease of 0.02mmol/L in triglycerides).

7.1.2 Strengths

The strength of the study is that aIMT is a robust measure of vascular structure in children and adolescents and is often a better predictor of early cardiovascular changes than cIMT [67, 68]. We used validated measures of cardiovascular function with experienced ultrasonographers at a single centre. Step count was objectively measured over at least 5 complete days including 1 weekend day by a special device (Sensewear) that records the time the device was worn using proprietary algorithms based on input from accelerometry and galvanic skin temperature. The Senswear device is accurate at estimating energy expenditure in children and adolescents [152]and adequately approximates step count, although may underestimate step count at low walking speed, similar to other body movement devices[153]. 98% of children and adolescents (88/90) wore the device for an average of 23.2±0.76 hours per day. Although step count is not the gold standard for measuring physical activity levels, it is a reliable and simple measure of physical activity and step count is a clinically translatable message for patients which is easy to comprehend and put in to action.

7.1.3 Limitations

There are important limitations to this study. Firstly, this is a small study with only 90 participants. However, we achieved a power of 0.91 to detect the correlation of 0.31 between steps per day and aIMT (assuming $\alpha = 0.05$). This is a cross-sectional study and so no statements about causation can be made. The study participants all had a BMI of greater than the 50th percentile with a higher than clinic average HbA1c and so findings may not be generalisable to the whole clinic population however we had 54 out of 90 children and

adolescents who had a normal BMI (50-84.9th percentile). Whilst step count was associated with vascular health, there was no association between time spent in MVPA, another marker of activity levels, and vascular function or structure in this study. This may have been due to multiple factors. It may be that incidental activity was more predictive of vascular health than the vigorousness of the activity, and that the step count was more reflective of general fitness. It is also possible that the Sensewear device used in this study measured step count more accurately than intensity of activity.

7.1.4 Clinical implications

This study supports the current exercise guidelines for children and adolescents with T1D [262]. The findings emphasise the importance of exercise as part of a wholistic approach to cardiovascular risk reduction. A small incremental increase in step count related to a measurable difference in vascular structure which is a simple message for children and adolescents with T1D.

7.1.5 Research implications

Physical activity is an important modifier of cardiovascular risk in the non T1D population. Further research in larger T1D cohorts is needed to extend the knowledge of the relationship between vascular health and activity, including intervention trials. This information would be helpful to inform best practice guidelines and advise young people with T1D on efficacious ways to reduce CV risk in addition to glycaemic control and dietary management.

7.2 Sodium and Vascular health

7.2.1 Study results

For the first time, we have shown that higher dietary sodium intake, calculated by validated dietary recall questionnaires, relates to vascular dysfunction in children and adolescents with T1D, independent of other dietary factors or diabetes control. Our population showed a similar high dietary sodium intake to other studies in children and adolescents with and without T1D. Our data also supports previous studies relating sodium intake to BP, within the normal range. In our study, vascular function, as measured by GTN, was worse with higher dietary sodium intake and this relationship was independent of BP.

Excessive sodium intake is related to cardiovascular disease. It is well established in the literature that there is a direct relationship between dietary sodium intake and BP[263] including in studies of children and adolescents [168]. Recent meta-analysis demonstrated that there is a dose response linear association between dietary sodium intake and risk of cardiovascular disease [264]. For every 1g increase in dietary sodium intake, there was an increase in cardiovascular disease risk of 6%. Dietary intervention studies have previously shown that high salt intake can reduce the responsiveness of the vascular smooth muscle to vasodilator stimuli without the presence of hypertension likely via a reduction in vascular nitric oxide bioavailability[241]. In salt resistant individuals, who do not have a BP difference when on a high salt compared with a low salt diet, there is a reduction in FMD when on a high salt diet implying that there is an independent effect of higher dietary salt on the vasculature[265]. Our finding of impaired GTN supports this hypothesis. We did not see an impairment in FMD, however smooth muscle function impairment may be a more sensitive marker of early vessel disease in children and adolescents as we have shown previously[88].

Diet is one of the modifiable risk factors identified as one of the ideal cardiovascular health metrics by the American Heart Association[261]. The SEARCH CVD study of 190 subjects investigated the relationship between ideal cardiovascular health metrics and arterial stiffness in youth with T1D. The study found that no participants met the dietary sodium target with only 19.5% meeting fruit and vegetable intake targets[226]. There was a relationship in this cohort between arterial stiffness and not meeting ideal cardiovascular health metrics[226]. In the context of children and adolescents with T1D with a significantly higher risk of cardiovascular disease, all risk reduction strategies should be considered. Current dietary advice for T1D supports the standard dietary guidelines for dietary content which includes low dietary sodium.

We evaluated dietary sodium content via a broader assessment of dietary macro and micronutrient content using the ACAES. This was delivered to all participants by the same, experienced dietician and measured intake over the previous 6 months. Dietary questionnaires are frequently used to estimate sodium intake in population studies. All dietary assessment tools are limited in their validity by issues regarding accurate recall. This dietary assessment measure has the benefit of having shown to be reliable and valid in the Australian population[224].

The dietary sodium content of the children and adolescent's diets in our study was comparable to other studies using 24-hour sodium excretion and dietary survey which found that the salt intake for 9-13 year olds was 2.52g and 3.16g in 14-16 year olds. Dietary sodium intake was double the recommendations for children and adolescents with none of the participants in the study having the recommended intake of sodium. Given what is currently known about sodium intake and cardiovascular disease, this may have implications for cardiovascular health in children and adolescents with T1D. The sodium intake of our cohort is similar to the healthy US and Australian childhood populations which are above

recommended daily intake. Targeting sodium reduction in public health campaigns for the general population may benefit the overall cardiovascular health in children and adolescents as well as children and adolescents with T1D. Sodium reduction is not usually emphasised in T1D dietary education but our current finding relating dietary sodium to vascular function provides additional evidence that dietary sodium reduction may be a worthy target for intervention. This could include advice around reducing take away and snack foods which are higher in sodium than foods prepared at home.

7.2.2 Strengths

We used detailed and validated measures of cardiovascular function with experienced ultrasonographers at a single centre. The ACAES questionnaire was delivered to participants by an experienced dietician in a standardised format. The questionnaire is specifically designed for the Australian child and adolescent population and has been validated in this group.

7.2.3 Limitations

This is a cross sectional study so no inference regarding causation can be made. The study participants all had a BMI of greater than the 50th percentile with a higher than clinic average HbA1c and so findings may not be generalisable to the whole clinic population however we had 54 out of 90 children and adolescents who had a normal BMI (50-84.9th percentile). Sodium related changes might have been affected by the fact that this group of children and adolescents had a higher cardiovascular risk profile.

Sodium intake is challenging to fully quantify using dietary measures alone. Dietary recall is known to have measurement error as it relies on memory. We standardised the tests in the current study as much as possible by using an experienced dietician to deliver the measurement tool. We also used a food frequency questionnaire which is specific to children

and adolescents in Australia and has been validated in our population [20]. Dietary sodium content can also be assessed using spot urine tests and 24-hour urine sodium excretion tests. Urine spot tests have less accuracy for measuring sodium intake. 24-hour urine sodium excretion studies, although more accurate, are a more burdensome collection tool for children and adolescents.

7.2.4 Clinical implications

This study has extended the relationship between dietary sodium intake and cardiovascular health inferring that sodium has a direct effect on the vasculature, independent of its known effect on BP. It supports the current dietary guidelines which advise a sodium intake of no more than 1.5 grams per day of dietary salt[155]. It also demonstrates that despite some nutritional education regarding dietary salt intake, the salt intake of children and adolescents with T1D is excessively high, much like the general population. The focus on carbohydrate content of food at diagnosis of T1D should not prevent the delivery of simple, established dietary advice in relation to salt, fibre and saturated fat intake.

Targeted sodium reduction dietary advice in children and adolescents with T1D may improve cardiovascular health in a population vulnerable to higher cardiovascular morbidity and mortality. Simple measures such as decreased take away or intake of pre-package foods can decrease dietary sodium intake.

7.2.5 Research implications

Given the elevated risk of cardiovascular disease in the T1D population, additional interventions are required to reduce cardiovascular risk. To further clarify the relationship between sodium intake and vascular function that is independent of established risk factors. Intervention studies assessing low sodium diets would be useful to explore causation. In

addition, larger cross-sectional studies to investigate whether there is a similar effect from dietary sodium in those with a BMI less than 50th percentile.

7.3 Metformin

7.3.1 Study results

7.3.1.1 Vascular outcomes

For the first time, this study reported an improvement in vascular smooth muscle function in youth with T1D when treated with metformin in addition to insulin over 12 months. This modest effect was independent of improvement in HbA1c. The effect was greatest at 3 months, when adherence to the intervention was highest. The effect was seen in both the pubertal and pre-pubertal age groups. Improvement in vascular smooth muscle function occurred without any improvement in FMD or aIMT/cIMT.

Metformin has an in vitro effect on smooth muscle by stimulation nitric oxide synthesis via activation of AMP kinase in smooth muscle which may explain why an improvement was seen in smooth muscle function without other changes in vascular endothelial function or structure[174].

There was no change in FMD which was our primary outcome measure. This is in contrast to a small pilot randomised controlled trial of metformin or placebo in adults with T1D which demonstrated an improvement in FMD with metformin without any significant change in GTN[25]. There are significant differences between the populations in these studies, the latter being older (mean age 46 years in the metformin group, 41 years in the placebo group), with a longer T1D duration (9.2 years in the metformin group and 8.8 years in the placebo group) and better diabetes control (HbA1c 7.24 % in the metformin group and 7.73 %in the placebo group).

The current study did not show any improvement in cIMT or aIMT over the 12 months. It was not powered to find a change in IMT, so this was not an unexpected finding. Other metformin intervention trials in adults[185] and adolescents with T1D [266] (the EMERALD trial, published after our study) have demonstrated changes in cIMT. There are several important differences to note:

In the REMOVAL trial, a 3-year randomised controlled trial (RCT) of metformin or placebo in adults with T1D found that averaged maximal cIMT (prespecified tertiary outcome) was reduced in the intervention group. There was no change in the primary outcome measure of averaged mean cIMT. Averaged maximal cIMT may be a better predictor of atherosclerosis progression as it includes measurement of more advanced stages of disease given that averaged mean cIMT excludes individual readings greater than 1.5mm and plaque. This measure is not as useful in children and adolescents who do not yet have any atherosclerotic plaque[185, 267]. The REMOVAL trial included participants with comorbidities and 12% had established cardiovascular disease. This is in contrast with our younger population with shorter diabetes duration and who did not have any comorbidities, established cardiovascular disease and/or atherosclerotic plaques. The baseline cIMT was therefore much higher, as would be expected, in the REMOVAL population. This finding of improved averaged maximal cIMT supports the current finding that a benefit of metformin is more readily detected in older individuals with more established cardiovascular disease. The cardiovascular benefit may be due to a direct effect of metformin or indirectly via reduced insulin resistance.

The EMERALD trial (published after our study) reported improvements in insulin resistance and vascular health in youth with T1D[266]. Forty-eight adolescents with T1D were randomised to either placebo or 2000mg of metformin per day for 12 weeks with average adherence rates of 93%. The primary outcome measure was insulin resistance, measured by hyperinsulinaemic euglycaemic clamp and vascular health was measured using aortic

magnetic resonance imaging (pulse wave velocity, relative area change, wall shear stress), carotid ultrasound (IMT/diameter/distension) and brachial distensibility. It showed a reduction in mean diastolic far wall cIMT as well as improvements in aortic health, insulin resistance, BMI, weight, fat mass and insulin dose. There was no improvement in brachial distensibility. The change in cIMT remained after correcting for BMI and SBP but was not significant after correcting for the change in insulin sensitivity (M/I per 1 lean kg).

There are several differences between the EMERALD trial and the current trial. The EMERALD study participants were older, in later stages of puberty or post pubertal, with longer T1D duration and higher cIMT at baseline. The duration of the study was also different, cIMT in the EMERALD trial was measured at 3 months whereas we measured cIMT at 12 months. We chose 12 months as usually changes in cIMT take time and it was thought that changes were more likely to be seen with a longer duration. Adherence rates had reduced by 12 months in the current study which could possibly have impacted on whether cIMT changes could have occurred. It is more likely that changes in cIMT were not seen as our population was younger with shorter T1D duration and therefore had less measurable baseline abnormalities in cIMT.

The EMERALD trial also reported improvements in aortic stiffness and wall shear stress with metformin treatment[266] which were not measured in our study.

7.3.1.2 HbA1c

In this study, metformin conferred a benefit to HbA1c which was greatest at 3 months (at the point of greatest adherence to metformin). The benefit to metformin was seen in both pre-pubertal and pubertal participants with T1D. A meta-analysis of RCTs of metformin in T1D youth published in 2017 did not demonstrate a beneficial effect of metformin on T1D[268]. There are several differences in the current study from those in the meta-analysis: These studies were of shorter duration, with older age participants and longer diabetes duration.

The T1D exchange trial contributed the largest number of participants to this meta-analysis with 140 overweight adolescents with T1D assigned to metformin or placebo for 6 months[28]. There was an improvement in HbA1c at 13 weeks (-0.3%), but not sustained at 26 weeks[28]. In this study compared to our study the participants were older (15.3[1.7] vs 13.6[3.5] years), pubertal or post pubertal (1% pre puberty/24% mid puberty/75%post puberty vs 27% pre puberty/18% mid puberty/ 55% post puberty), were excluded with an HbA1c> 10%, had a higher BMI z score (1.6[0.3] vs 0.89 [0.57]) and longer T1D duration (7.0[3.3] vs 5.5 [3.9] years). The two adolescent metformin intervention studies, published after our study, found no change in HbA1c with metformin treatment in adolescents. A cohort from the T1D exchange trial examining the effect of metformin on insulin sensitivity did not demonstrate a change in HbA1c at 3 months but was not powered to do so[269]. Similarly, the EMERALD trial group did not demonstrate a change in HbA1c over 3 months with metformin[266].

Our finding of an improvement in HbA1c may have been demonstrated as we did not exclude those with an HbA1c > 10% who had the biggest margin for improvement, we had high objectively measured adherence at 3 months to metformin (median adherence measured by MEMS was 88.9 [77.0-91.6]%) and we had consistency of approach to insulin adjustment (a single investigator with 4 paediatric endocrinologists adjusting insulin doses). It should also be noted that the HbA1c in the placebo group worsened during the study period, whereas the metformin group showed no significant change from baseline to 3 months. The REMOVAL trial also demonstrated a reduction in HbA1c (-0.13%) in the adult population with T1D over 3 years which was accounted for by a reduction at 3 month which was not sustained[185].

7.3.1.3 Insulin dose and insulin sensitivity

Insulin dose was reduced in this study which is consistent with the literature in children and adolescents with T1D to date. We demonstrated a reduction in insulin requirements of 0.2u/kg/day over 12 months. Meta-analysis previously showed that metformin conferred a favourable effect on insulin dose of a reduction of 0.16u/kg/day in children and adolescents with T1D[268]. Treatment in older adolescents and adults with T1D also supports this with meta-analysis demonstrating a reduction in insulin dose of 6.6 units per day[27]. The longest of these studies was 12 months. The 3-year REMOVAL trial demonstrated a more modest reduction in insulin dose of 0.023 u/kg/day in year 2 and 3 of the study[185]. However, this was an adult population who are more sensitive to insulin and require less daily insulin doses than the adolescents in our study and the US T1D exchange trial.

Adolescents with T1D have reduced adipose, hepatic, and peripheral insulin sensitivity[270]. Metformin likely reduces insulin dose by increasing muscle, liver, and adipose insulin sensitivity. This is supported by the two recent studies in adolescents with T1D which investigated the effect of metformin on gold standard insulin sensitivity measures of hyperinsulinaemic/euglycaemic clamp[266, 269]. Metformin improved whole body and peripheral insulin resistance in obese youth with T1D[269] and in overweight and lean adolescents with T1D [266]. This improvement remained after correction for a change in BMI. The largest studies of metformin in adolescents with T1D have previously shown a reduction in total daily insulin dose and BMI with no change in HbA1c indicating an improvement in insulin sensitivity.

Insulin resistance in T1D is incompletely understood and is different to the insulin resistance phenotype in T2D. Although hyperglycaemia is known to result in insulin resistance, this is less of a contributor with modern insulin delivery regimens. Higher insulin resistance in T1D is associated with more atherogenic cardiovascular risk factors in adolescents[271] , atherogenic

lipid profile[272], reduced exercise and cardiovascular dysfunction[130] and in adults predicts the extent of coronary artery calcification [273] and coronary artery disease end points including death[274]. It is likely to result from multiple factors including non-portal insulin delivery, reduced glucagon release, elevated free fatty acid levels and mitochondrial dysfunction in peripheral muscles [270, 275]

Better insulin sensitivity in adolescents with T1D is associated with a cardiovascular profile more similar to healthy adolescents[271]. Insulin sensitivity is reduced by adiposity however adolescents with T1D and a normal BMI have decreased insulin sensitivity. In the current study, the metformin group had higher estimated insulin sensitivity than the placebo group which remained throughout the 12 months as a surrogate marker for directly measured insulin sensitivity.

Metformin may confer cardiovascular protection, in part, by improving insulin sensitivity in T1D. The mechanism by which metformin improves hepatic insulin sensitivity is likely by reducing glucose output by the liver and inhibiting the effect of glucagon[276] by enhanced insulin suppression of endogenous glucose production and increased plasma glucose clearance[269].

7.3.1.4 Traditional cardiovascular risk factors – lipids and blood pressure

Our study showed no benefit on traditional cardiovascular risk factors. Prior studies in children and adolescents have not shown any significant improvement in lipids or BP[268]. Studies in adults with T1D have demonstrated a small but significant reduction in LDL-cholesterol [185, 192]. It is likely that this small effect is seen in adults more so than adolescents due to the higher prevalence of lipid abnormalities in the older population. Additionally, adult studies include a large number of subjects compared to adolescent studies.

7.3.1.5 Body composition and body mass index

In the recent meta-analysis of children and adolescents with T1D, there was a significant reduction in BMI with metformin treatment[268]. The baseline BMI in the studies that reported BMI was 22.8 to 28.2kg/m2. With metformin, there was a favourable effect on BMI of 1.46kg/m2 compared to the placebo group (95%CI -2.54 to -0.38), however this was not adjusted for age and gender. In those that reported BMI-z, there was a modest reduction in BMI z of -0.11 (95% CI -0.21 to -0.01).

In the recent study of obese youth with T1D treated with metformin over 3 months, there was a reduction in BMI-z score of 0.04kg/m2 in the metformin treated group[269]. This was a subset of a larger overweight/obese cohort with T1D which also demonstrated a reduction in BMI over 6 months[28]. Similarly, in the recent EMERALD trial of youth with T1D, there was a reduction in BMI, weight, and fat mass favouring metformin over placebo[266].

This current study did not demonstrate a reduction in BMI over 12 months treatment with metformin. The lack of effect in BMI might be related to lower baseline BMI-z (0.89(0.57)) compared to other studies and the fact that 54 participants had a normal BMI (percentile of 50-84), 25 were overweight and 11 were obese. This sample size did not allow for subgroup analysis of those who were overweight/obese and would have had most benefit from a reduction in BMI. These results contrast with other literature which report a reduction in weight, BMI and BMI z score in adolescents and adults with T1D, even those who were of normal weight [181, 268]. However, previous meta-analysis has demonstrated that the impact of metformin on weight and BMI is highest in those who are overweight and obese which may, in part, explain our findings given that most of our cohort were in the normal BMI range [246].

7.3.1.6 Adherence

A strength of the current study is that we objectively measured adherence using MEMS bottle caps and manual pill count. This gave accurate information on adherence, not only in terms of total dose received but also the daily adherence pattern [277]. Overall adherence to the intervention was good with a reduction over the 12 months, as seen in other similar studies. There was no relationship between adherence and the presence of adverse events in the current study. Medication dose related to change in HbA1c, but magnitude of dose effect was very small and not clinically relevant [0.077 (95% CI 0.038, 0.12; P<0.001)]. The study was not powered to assess the relationship between adherence to metformin and HbA1c and so this analysis was not performed.

7.3.1.7 Safety

Severe hypoglycaemic events are of concern with metformin treatment given the mechanism of action in increasing insulin sensitivity. There were no reported episodes of severe hypoglycaemia in the current study. Moderate hypoglycaemic events occurred in the same rate in each group in the current study. Pooled data from prior studies have shown a trend toward severe hypoglycaemic events which is not statistically significant (8 vs 1 events)[268]. Of note, studies reporting severe hypoglycaemic events have been mostly reported in the first 6 weeks of metformin treatment[268]. In the current study, a single investigator contacted participants weekly during the initial 3 months of the trial for insulin adjustments and participants also had access to diabetes doctors on call including 4 paediatric endocrinologists (usual diabetes care at our centre). This may have accounted for our low rate of severe hypoglycaemic episodes. Given the evidence that metformin improves insulin sensitivity[266, 269], care should be given when initiating metformin to avoid severe hypoglycaemic episodes.

Previous meta-analysis in youth with T1D demonstrated a trend toward more DKA events in participants treated with metformin however there was a very small number of events in these studies (5 vs 2 events). The current study reported 2 participants per group getting DKA. The reason for a higher overall rate of DKA in the current study was likely due to a larger number of adolescents in the trial, a longer study duration than previous studies and not excluding those with an HbA1c > 10% who are more at risk of DKA due to non-adherence to insulin. Although there was a higher overall rate of DKA in this study, there was no difference between the metformin and placebo groups.

There was a significant increase in reported gastrointestinal side effects in the metformin treated group in the current study similar to that of previous studies who used a similar metformin dose and had a similar frequency of participant contact to discuss symptoms[28].

Not all other studies in adolescents with T1D have reported an increase in gastrointestinal side effects in the metformin treated groups. This may be as they used a lower metformin dose [181] or that they did not elicit all the gastrointestinal symptoms as they contacted participants less frequently (monthly or longer, compared to 2 weekly or less in our study and the T1D exchange trial) [180, 188, 189].

The previous study using 'low dose' metformin (500mg BD for all participants) did not report any difference in gastrointestinal side effects between the two groups[181], supporting a dose relationship to gastrointestinal side effects.

Serum vitamin B12 levels were reduced in this study in the metformin group compared to the placebo group however no participant had level in the deficient range. Liver and renal function were maintained and there was no significant difference in lactate levels between the metformin and placebo group which is supported by the other paediatric literature. [268]

7.3.2 Strengths

This study was a double blind, randomised controlled trial. The benefit of this study design is that inferences about causation can be made. The study was conducted and reported in accordance with the CONSORT guidelines using a pre specified statistical analysis plan using intention to treat analysis. Missing data was addressed using multiple imputation analysis. The ultrasound outcome measures (FMD/GTN/IMT) were assessed by two blinded observers to assess for robust inter-observer reliability. The outcome assessors including the statistician were blinded to the allocation and unblinding occurred only after the results were analysed. The study therefore has a low risk of bias.

The current study had broad inclusion criteria which means the results are more applicable to our clinical population who have a wide range of diabetes control during adolescence. We included those with an HbA1c of greater than 10% which have been excluded from other T1D metformin intervention studies and our median HbA1c was 8.7% [IQR 8.1-9.9]. There was also inclusion of those with a BMI > 50th percentile rather than just those in the overweight or obese weight range. In fact, 54/90 of children and adolescents included in the study had a normal BMI range (50-84.9 percentile). The age range in the study was 8 to 18 years. This included children prior to the onset of early vascular disease, which had the advantage of aiming to prevent the onset of vascular changes in order to optimise the life-long impact of the intervention.

This study used metformin standard preparation which can be easily administered in lower doses at initiation of treatment and was well tolerated. Only 1 participant in the metformin group withdrew from the study due to not wanting to take tablets but not due to side effects perse. Six participants in the metformin group who completed the trial received a less than maximal dose of metformin according to the protocol due to side effects.

This study was conducted at a single centre with good inter and intra observer reporting of vascular function and structure studies. This gives the best evidence for reliable reporting of the vascular function and structure studies which are reliant on experienced ultrasonographers and measurements[3].

There was strong adherence reporting using two different measures. We used MEMS caps which gave accurate, objective recording of times medications were taken and also used manual pill count at clinic visits. We subsequently published adherence findings observing that adherence was reduced on weekends and school holidays[277]. We also reported a strong correlation between MEMS and pill count measured adherence (r=0.95) in our study.

7.3.3 Limitations

There are several characteristics of the participants in this study that limited accurate comparison with other work. The age of participants in this study was lower than in other RCTs as we included children and adolescents age 8 to 18 years. The mean age of the patients in this study is younger that in the other published literature in T1D and metformin. Our study participants also had a shorter diabetes duration. The effect of this is that the vasculature of our participants has had less exposure to the cardiovascular effects related to T1D (exposure to hyperglycaemia, increased insulin resistance and inflammation) and fewer participants had entered or completed puberty. Puberty is known to be a time of increased insulin resistance and worsening cardiovascular health and therefore less likely to improve from a better baseline cardiovasculature with a metformin intervention. This may be why we did not see an improvement in cIMT over 12 months, unlike the EMERALD trial group who saw a change at 3 months. In addition, the effect of metformin may be different in those who are pre puberty compared to puberty as one of the effects of metformin may be to improve vascular health by reducing insulin resistance, which is lower in pre puberty.

The FMD at baseline was higher across all patients in this study compared to our previous T1D cohorts [13, 89, 278]. This overall improvement in baseline FMD meant that it was less likely that we would see a change in FMD over time with our intervention given that our power calculation was based on FMD from previous FMD intervention studies at our centre.

The study duration of 12 months limited the ability to look at longer term changes in cardiovascular structure. Although the EMERALD cohort saw changes in cIMT at 3 months[266], structural changes usually take much longer to be observed[185, 194, 255, 256] especially in children and adolescents with a lower baseline cIMT and no atherosclerotic plaques.

We relied on participant reports of hypoglycaemia which is a limitation as we could not reliably estimate the frequency and duration of milder episodes of hypoglycaemia. Future studies using continuous glucose monitoring while giving metformin would be useful in assessing the rate of hypoglycaemia, especially in the initiation phase. Continuous glucose monitoring systems were not readily available at the time the study was conducted.

7.3.4 Clinical implications

This study adds to the current body of knowledge about metformin as an adjunct medication to improve cardiovascular health in youth with T1D. The novel finding of improving GTN in those treated with metformin infers that metformin demonstrates a modest improvement in cardiovascular function in children and adolescents with above average BMI. We also showed a transient improvement in HbA1c and improvement in insulin dose which is consistent with previous literature and supports the mechanism of action of metformin increasing insulin sensitivity.

This current study, in the context of the other paediatric literature, supports the reassuring safety profile of metformin. It recommends increased support when initiating metformin to

avoid hypoglycaemia and elicit important gastrointestinal side effects as these may reduce adherence if not addressed.

7.3.5 Research implications

Given the possible benefit on long term cardiovascular health and good safety profile, longer term studies using metformin as an adjunct medication in T1D warrant consideration in the paediatric population. A long-term follow-up of the children and adolescents that participated in the current study is being performed at our centre which aims to provide further insights into the cardiovascular outcomes and evaluate if the effects observed around puberty persisted.

8. Chapter 8: Summary and final conclusions

The overall aims of this thesis were to measure the vascular function and structure and their determinants in children and adolescents with T1D; and to measure the effect of metformin on these same measures over 12 months. Specifically, I aimed to evaluate the effect of metformin on non-invasive measures of pre-clinical atherosclerosis in children and adolescents with T1D using a primary outcome measure of FMD and the correlation between measures of diet and activity with vascular function and structure in the same population.

The origins of atherosclerosis are in childhood and accelerate during puberty. Even with new diabetes technology and improvements in glycaemic control with modern insulin regimens, people with T1D still have significantly higher cardiovascular morbidity and mortality that their non T1D counterparts. As such, other measures to improve cardiovascular health are needed. About 1/3 of Australian children and adolescents with T1D are overweight further emphasising the risk of cardiovascular disease, which remains the main contributor to premature death and morbidity in adults with T1D.

The biopsychosocial transitions which take place during adolescence may impact on the interpretation and translation of research in this age group. As well as being a period of acceleration of atherosclerosis, there are changes to physical activity, dietary autonomy and chronic illness self-management which may impact on vascular health variables, measures of glycaemic control and adherence. I attempted to account for some of these changes by directly measuring activity, dietary intake and adherence over the duration of the study.

The first part of this thesis demonstrated that physical activity, as measured by step count, is related to aIMT, which is a measure of pre-clinical atherosclerosis. Step count is a simple measure which is clinically translatable for patients. It supports current guidelines for physical

activity in children and adolescents with T1D and strengthens the recommendation regarding physical activity for cardiovascular risk prevention.

The second part of the thesis explored the relationship between dietary sodium intake and vascular function. It provided evidence that higher dietary sodium relates to vascular dysfunction in above-average weight children and adolescents with T1D. Reducing dietary sodium intake should be considered in T1D dietary guidelines and in targeted dietary clinical advice as a way of reducing cardiovascular risk.

The final part of the thesis assessed the impact of a metformin intervention in children and adolescents with T1D over 12 months. This demonstrated the key finding that metformin improved smooth muscle vascular function as well as HbA1c, insulin dose and estimated insulin sensitivity. This warrants further consideration in this population as an adjunct treatment for cardiovascular risk prevention, especially in those with above average BMI or clinical insulin resistance.

In conclusion, cardiovascular risk remains elevated in the population with T1D despite improved glycaemic control with modern insulin regimens. Optimisation of simple lifestyle measures including dietary sodium reduction and increasing activity, may mitigate some of the increased cardiovascular risk. Consideration of metformin, in addition to insulin, may be clinically warranted in some individuals with T1D and longer-term research is needed to further quantify the possible long term cardiovascular risk reduction from this safe and inexpensive medication.

9. Chapter 9: Appendices

Appendix A – Ethics approval

Women's & Children's Hospital

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18th December 2012

Dr J Anderson
Endocrine & Diabetes Dept
WCHN

Dear Jemma

Re: Does Metformin improve vascular function and structure in youth with type 1 diabetes? REC2327/12/13

I refer to your letter dated 6th November 2012 in which you responded to my letter dated 30th October 2012 and note approval of protocol amendment, version 5 (5/11/12) and accompanying documents subject to a recommendation of approval from the DTC Clinical Trials Group. I also refer to a letter dated 11th December 2012 from the Chair of the DTC Clinical Trials recommending approval of the amendment subject to modifications to the study protocol, namely:

1. *Paragraph three, page 4 under Methodology changing the sentence to read "We aim to use a dose of 2000 mg a day for > 60kg…."*

2. *Inclusion in the objectives on page 2 and table on page 8 the information on page 7 "if the subject's weight is < 60kg, the dose will be titrated up to 500 mg BD of Metformin hydrochloride or placebo tablet according to the randomised allocated group."*

I note in your letter dated 12th December 2012 that you have amended the protocol in line with the DTC CTG's recommendations and, in addition, have added in figure 2 to describe the study design for a patient who is under 60 kg. I advise approval of the following documents:

- Protocol version 6 (12/12/12)
- Flyer, version 1 (5/11/12)
- Parent/guardian consent form for healthy (control) children (version 2, 5/11/12)
- Child assent form for healthy (control) children (version 2, 5/11/12)
- Information sheet for healthy (control) children (version 2, 5/11/12)

Approval is conditional on:

- Parents/carers of children under the age of 18 years being approached about the possible involvement of their children in the study. Children under 18 years should be directly approached.
- The approval of the Public Relations and Communications Department being obtained before the flyer is displayed on notice boards in the hospital.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
WCHN HUMAN RESEARCH ETHICS COMMITTEE

Government of South Australia
SA Health

Appendix B – Patient information sheet

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

LAY TITLE

Does Metformin improve blood vessel health in youth with type 1 diabetes?

SCIENTIFIC TITLE

Does Metformin improve vascular function and structure in youth with type 1 diabetes?

This information sheet will inform you about a study looking at Metformin, a medication used to treat type 2 diabetes, and the effects on blood vessel function in children and adolescents with type 1 diabetes. Metformin will be used in addition to your child's usual insulin treatment.

1) What is the study for and why is it being done?

Our Diabetes Unit has been studying how blood vessels change in children with diabetes for several years. Very early changes in blood vessel function (narrowing and expanding of blood vessels) and blood vessel structure (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. Children with type 1 diabetes are more likely to have changes in blood vessel function from early age. These changes can be reversed with B vitamins or other medications.
Metformin is a medication used to treat children or adults with type 2 diabetes who are overweight. Previous studies have shown that Metformin in addition to insulin in type 1 diabetes improves the effects of insulin and weight. Metformin reduces the risk of cardiovascular disease in adults with type 2 diabetes. There are no studies looking at the effects of Metformin on early markers of cardiovascular disease in type 1 diabetes.
In this study we want to find out if Metformin in addition to usual insulin treatment improves blood vessel health in above average weight adolescents with type 1 diabetes.

2) What would I be asked to do if I took part in this study?

In this study your child will be asked to take 2 tablets by mouth twice a day for 12 months (if they weigh more than 60kg) or 1 tablet by mouth twice a day for 12 months (if they weigh less than 60kg) . These tablets could be either Metformin or a non active tablet, called a placebo. Your child will be randomly assigned to either Metformin or placebo which means that your child will have an equal chance of getting either Metformin or placebo. Both tablets look the same and neither your child nor you nor the investigators taking part in the study will know which tablet (Metformin or placebo) your child is taking until the end of the study. This is called a double blinded randomised clinical trial and allows us to differentiate the effect of Metformin on blood vessel health in comparison to the effect of the non active drug (placebo).
This study will also include four visits to the hospital (at the start of the trial and then 3, 6 and 12 months later). Each visit will take 2 hours and will include:

I. **Assessment of activity levels**
II. **Clinical examination**
III. **Urine test**
IV. **Ultrasound studies**, along with an electrocardiogram
V. **Blood sample** (20 ml blood in total, approximately 4 teaspoons)
VI. **Retinal photograph or picture of the back of your eye** (at first and last visit)
VII. Ingestion of **first tablet,** and
VIII. **Dietitian review**.

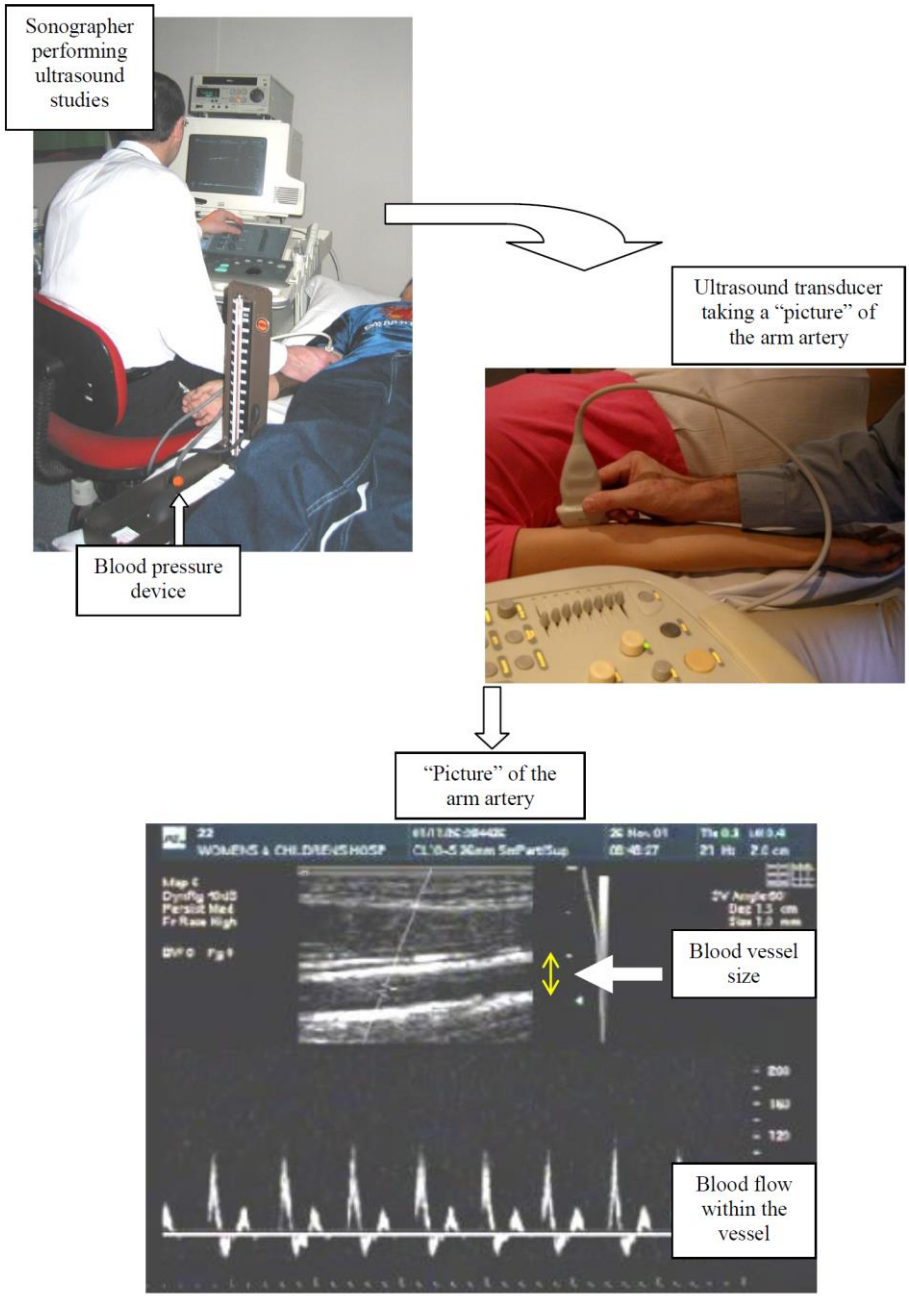
In between visits, you and your child will be contacted by one of the investigators to ensure glucose levels are under control. This will occur every 2 weeks during the first 6 weeks of the study and then monthly. Each phone call will take approximately 5-10 minutes.
Your child will be required not to take any multivitamins that might affect blood vessel function and your child will be asked not to consume alcohol while on the trial.

I. **Assessment of activity levels**. The amount of energy that you use every day will be calculated using a special arm band device called SenseWear. This is an arm band which is worn on the upper arm for the 5 days before your appointments.
II. **Clinical examination** will focus on measuring your child's height, weight, waist and hip circumferences, body composition, blood pressure and pubertal stage according to viewing images of pubertal staging.
III. **Urine test** will check proteins in the urine. It is a usual part of the annual test for diabetes complications. In addition we will measure in the urine prostaglandins (substances present in the body, that might change with Metformin and influence blood vessel function in children and adolescents).

Version 5 Updated on 17/11/2011

IV. The **ultrasound studies** will be performed to show us how your child's blood vessels work and how healthy the blood vessel walls are (see figure 1). At the same time with the ultrasound studies, an electrocardiogram recording will also be taken. This is a measurement of the electrical activity of the heart using three electrodes (like stickers) placed on the chest.

Figure 1.



The ultrasound studies will take about 1 minute each. These ultrasound studies will include:

A. Examination of an artery in the upper part of your child's right arm (known as brachial artery), in order to assess the blood vessel function. This will include:
   - an ultrasound picture of the brachial artery to measure how wide it is and how the blood flow is in it (ultrasound 1);
   - inflating a blood pressure cuff around the arm for 4 minutes;
   - releasing the air from the blood pressure cuff. Normally, the blood vessel gets wider (it dilates); this process is known as flow mediated dilatation (FMD);
   - a second ultrasound picture to measure the new size of the blood vessel and the blood flow in it (ultrasound 2) and compare them to the initial ultrasound measurements;
   - a third ultrasound picture taken after 10-15 minutes of rest, allowing the blood vessel to return to its initial size (ultrasound 3);
   - administration of Glyceryl trinitrate (GTN) sublingually (spray under the tongue). This medicine is used in people with angina. It makes the blood vessel dilate to a maximal degree. Blood pressure and heart rate will be measured immediately after the GTN spray and again 10 minutes after;
   - a last ultrasound picture to measure the new size of the blood vessel and the blood flow in it (ultrasound 4), and compare them to the initial ultrasound measurements.

   We are interested in the change of blood vessel size after deflating the blood pressure cuff, and after GTN, which gives the best measure of how well a blood vessel is working.

B. Examination of the two main neck arteries (known as carotid arteries), one on each side of the neck, as well as the main abdominal artery (known as aorta). We will measure the thickness of their walls or blood vessel structure, also called intima media thickness (IMT) at the first, 6 month and 12 month visit.

V. **Fasting blood test** (having nothing to eat or drink, except for water, after 10 pm the previous night) will measure levels of glucose control (glucose and HbA1c), lipids (cholesterol and triglycerides), folic acid, B vitamins, homocysteine and adiponectin/leptin (substances that indicate the amount of fat tissue in the body). 20 ml of blood will be collected at each visit which is approximately (4 teaspoons).
   If you agree, a part of this blood will be used in another research project, which has been approved by the Ethics committee. In this project, we will examine genes that affect the way your child's blood vessels work; these genes affect the amount of nitric oxide (substance that increases the size of the vessels) in the body.

VI. **Retinal photograph.** A picture of the retina (back of the eye) will be taken by an optician. This is to look at the blood vessels in the back of the eye. It does not require dilatation drops on your eyes, it is not painful and it is often done as part of routine yearly diabetes eye checks. It will be done during your firstvisit and your 12 month visit.

VII. Following the ultrasound studies and the fasting blood test, your child will be asked to take the first tablet and instructions will be given about how to take the tablets at home which includes one tablet a day for 2 weeks then a slow increase over the following 6 weeks up to 2 tablets twice a day(if your child weighs more than 60kg) or 1 tablet twice a day (if your child weights less than 60kg). The dose will be increased with careful monitoring of how you/your child is tolerating the tablets. Direction on dose increments will be provided at each 2 weekly phonecall. Breakfast will be provided afterwards.

VIII. **Dietitian review**. This will include completing a questionnaire and receive recommendations about healthy eating.

In the first and the last visit you will asked to have a DEXA scan in the nuclear medicine department on Level 7 at the Royal Adelaide Hospital. The scans take place between 9am and 5pm, Monday through to Friday. This is a scan looking at the composition of the body and the ratio of muscle to fat. 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.
Your child's case notes will be accessed to obtain information regarding diabetes diagnosis and treatment.

3) Are there any risks of side effects associated with ultrasound studies (Flow Mediated Dilatation or Glyceryl trinitrate mediated dilatation) or blood collection?

The ultrasound procedure is safe and has been used in a large number of peoples, including adults and adolescents with type 1 diabetes. 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, glyceryltrinitrate, remains active for only a very short period of time. Glyceryltrinitrate may cause lower blood pressure, which we will be checking, light-headiness, facial flushing, a fast heart rate and headaches, although these are all very uncommon when used in children.
Taking blood may cause brief discomfort or pain, similar to a pinprick. This can be minimised by using a simple local anaesthetic cream (EMLA or AnGEL) and by comforting the child. Temporary bruising and local infection may occur, but extremely rare. The local anaesthetic cream may rarely cause some skin irritation.

4) Are there any risks or side effects associated with Metformin?

Metformin is a widely used medication in adults with type 2 diabetes and has been used in pregnant women with gestational diabetes. Main common side effects of Metformin include nausea, vomiting, abdominal discomfort and diarrhoea. These side effects are mild and reduced with a slow increase of the dose. A lower dose will be considered if you child is unable to tolerate the maximum dose.Metformin can rarely lower your vitamin B12 so we will be monitoring your vitamin B 12 level. In people with kidney problems Metformin can rarely cause acidosis in the blood called "lactic acidosis". This has not been reported in children. Metformin should be stopped if your child has ketones as your child is more prone to have a different type of acidosis in your blood "diabetic ketoacidosis" due to lack of insulin. There is an increased risk of hypoglycaemia while taking Metformin and insulin. This will be minimized with the adjustments on the insulin dose during the phone calls every 2 weeks during the study. The insulin adjustments will be done in liaison with your own Doctor.
Allergies have been very rarely reported including a rash.
You or your child will be required to contact the hospital if your child experiences any of the listed side effects (nausea, vomiting, abdominal discomfort and diarrhoea) or should your child require surgery, as the Metformin dose may need to be changed or the medication stopped.

5) Are there any risks or side effects associated with DEXA scan?

This research study involves exposure to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives a dose of about 2 millisieverts (mSv) each year. The effective dose from this study is about 0.0008 mSv. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk from this level of radiation is believed to be negligible.

6) What will be done with this information?

We will compare the results of blood vessel function and other substances we measure in the blood and urine between children that receive Metformin and the children that receive the non active drug (placebo) at the end of the study. We will also write and publish articles about the study, or talk about the study at conferences, so that other people will be helped by the information.All of the information will remain confidential except in the case of a legal requirement to pass on personal information to authorised parties. This requirement is standard practice and applies to information collected both in research or non-research settings.

7) Do I have to take part in the study?

No, not at all. You and your child should only take part in this study if you want to be involved.

8) Can I change my mind later if I decide not to participate?

Yes, your child can choose to leave the study at any time. This will not affect in any way you or your child's relationship or treatment with your child's doctor or the hospital.

9) Will the study benefit me in any way?

We cannot be certain that your child will get any benefit from taking part in the study. We hope that this study will give information about the effects of Metformin on blood vessel function in addition to insulin. This will give us more information about early intervention for blood vessel changes in children and adolescents with type 1 diabetes.
There will be no reimbursement for taking part of the study.

10) Do you have permission to do the study?

We have permission from the Research Ethics Committee at the Children, Youth and Women's Health Service Human Research Ethics Committee to do this study. If you have any queries regarding ethics of this trial you can contact the Secretary of the Committee, Ms Brenda Penny on 81616521.

11) What if I have other questions about the study?

Please contact Dr Jemma Anderson, Clinical Fellow on 81618805 or Dr Alexia Peña, Paediatric Endocrinologist on 81616402.

12) What if I need to contact the hospital regarding blood glucose levels or ketones, ?

There is an increased risk of hypoglycaemia while taking insulin and metformin. If there are any issues regarding low blood glucose levels or ketones after hours please contact the hospital switchboard (81617000) and ask for Diabetes Consultant on call.

Appendix C – Parent consent form

CHILDREN, YOUTH & WOMEN'S HEALTH SERVICE (CYWHS)

HUMAN RESEARCH ETHICS COMMITTEE (HREC)

PARENT CONSENT FORM

LAY TITLE: Does Metformin improve blood vessel health in youth with type 1 diabetes?

SCIENTIFIC TITLE: Does Metformin improve vascular function and structure in youth with type 1 diabetes?

I_____

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

Does Metformin improve blood vessel health in youth 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 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.

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

6. 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.

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

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

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

9. I agree to the accessing of my child's medical records.

10. I understand that my child's information will be kept confidential as explained in the information sheet except where there is a requirement by law for it to be divulged.

Signed: .....

Relationship to Patient: .....

Full name of patient: .....

Dated:.....

Patient signature .....

Dated.....

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

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

Dated: .....

Appendix D – Child assent form

CHILDREN, YOUTH & WOMEN'S HEALTH SERVICE (CYWHS)

HUMAN RESEARCH ETHICS COMMITTEE (HREC)

CHILD ASSENT FORM

LAY TITLE: Does Metformin improve blood vessel health in youth with type 1 diabetes?

SCIENTIFIC TITLE: Does Metformin improve vascular function and structure in youth with type 1 diabetes?

I _____

hereby consent to my involvement in the research project entitled:

Does Metformin improve blood vessel health in youth 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 taking part.

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

5. I understand that there will be no payment to me for taking part in this study.

6. 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.

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

8. a) I consent to a specimen of the following blood and urine being taken from me and being used in the above project.

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

9. I agree to the accessing of my medical records.

11. I understand that my information will be kept confidential as explained in the information sheet except where there is a requirement by law for it to be divulged.

Signed: .....

Full name of patient: .....

Dated:.....

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

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

Dated: .....

Version 2. Updated on 17/11/2011

10. Chapter 10: Bibliography

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