Vascular endothelial and smooth muscle function in children at risk of cardiovascular disease and the effect of folic acid supplementation

ALEXIA SOPHIE PEÑA VARGAS (MD)

Department of Endocrinology and Diabetes
Women’s and Children’s Hospital

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Faculty of Health Sciences
Department of Paediatrics

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ABSTRACT

Cardiovascular disease secondary to atherosclerosis is the most common cause of human morbidity and mortality. An early and fundamental event in the development of atherosclerosis is abnormal vascular endothelial and smooth muscle function. This can be measured by flow mediated dilatation and glyceryl trinitrate mediated dilatation in children at risk of atherosclerosis. Folic acid improves endothelial function (flow mediated dilatation) in adults with coronary artery disease. No studies have previously investigated the effects of folic acid on vascular function in at risk children with diabetes or obesity.

In a cross sectional study an evaluation of vascular endothelial and smooth muscle function and their determinants was performed in 159 children with type 1 diabetes, 58 children with obesity, and 53 healthy children. Children with type 1 diabetes and children with mild to moderate obesity had comparable and severe vascular dysfunction but different determinants. Vascular function in healthy and obese children related to both body mass index and weight (adjusted for age and sex), and blood glucose. Children with obesity had lower folate levels and higher homocysteine levels than children with type 1 diabetes, an abnormal lipid profile and raised inflammatory markers.

A randomised double blind placebo controlled cross over trial of 8 weeks of folic acid supplementation was performed in 38 children with type 1 diabetes. In these children, folic acid improved endothelial function with a sustained increase in folate levels but independent of homocysteine levels. Folic acid did not improve smooth muscle function.

A randomised double blind placebo controlled parallel trial of 8 weeks folic acid supplementation was performed including 53 obese children. Folic acid did not improve
vascular function in obese children in spite of sustained increase in folate levels, and a
decrease in homocysteine levels.

It was concluded that children with type 1 diabetes and obesity have comparable
and severe endothelial and smooth muscle function. Determinants of vascular function in
children, including weight and glucose, represent a continuum effect. Folic acid
supplementation improved endothelial function in children with type 1 diabetes but not in
children with obesity, whose metabolic changes causing endothelial dysfunction differ
from children with diabetes.
DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to a copy of this thesis, when deposited in the University Library being available for loan and photocopying.

………………………….                                                September 28th 2007
Alexia Sophie Peña Vargas          Date
DEDICATION

To Mellick
ACKNOWLEDGMENTS

It is impossible to adequately acknowledge the help of the people who have been instrumental in this thesis and to whom I am deeply indebted.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>APEG</td>
<td>Australian Paediatric Endocrine Group</td>
</tr>
<tr>
<td>ATL</td>
<td>Advanced Technology Laboratories</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow Mediated Dilatation</td>
</tr>
<tr>
<td>FABF</td>
<td>Forearm Arterial Blood Flow</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl Trinitrate Mediated Dilatation</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Haemoglobin A1c, glycosylated haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>HsCRP</td>
<td>High Sensitive C reactive protein</td>
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<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
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<tr>
<td>MTHFR</td>
<td>Methylene tetrahydrofolate reductase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen Activator Inhibitor-1</td>
</tr>
<tr>
<td>RCF</td>
<td>Red Cell Folate</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error of Mean</td>
</tr>
<tr>
<td>tHcy</td>
<td>Total Plasma homocyst(e)ine</td>
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<tr>
<td>TNF-α</td>
<td>Tumour Necrosis Factor α</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue Plasminogen Activator</td>
</tr>
<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>VD</td>
<td>Vessel Diameter</td>
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Chapter 1: Introduction
1.1 Problem statement

Cardiovascular disease is the most common cause of morbidity and mortality in adulthood. The term cardiovascular disease includes diseases of the heart and/or blood vessels (arteries and veins) such as coronary heart disease, atherosclerosis, stroke, peripheral vascular disease, hypertension, arrhythmia and valvular heart disease. Coronary heart disease, including myocardial infarction and angina, is the most common cause of mortality in adulthood; it causes one of every five deaths in United States. Atherosclerosis is a disease under the category of diseases of arteries, arterioles and capillaries including arteriolosclerosis, arteriosclerosis, arteriosclerotic vascular disease, atheroma, degeneration, arteritis and endarteritis; and excluding cerebral, coronary, pulmonary and mesenteric atherosclerosis. For this thesis I will use the term atherosclerosis as the disease of all arteries in the body specifically heart, brain and lower extremities, that causes coronary heart disease, stroke and peripheral vascular disease, respectively.

Risk factors for cardiovascular disease and atherosclerosis include family history of cardiovascular disease, diabetes, obesity, abnormal lipid profile, cigarette smoking, inactivity and chronic renal failure. These risk factors can be present in childhood and track into adulthood; childhood may be a time when the first changes of atherosclerosis are reversible. I will be focusing my thesis on two risk factors for atherosclerosis that have increased in children over recent decades: type 1 diabetes and obesity.

Type 1 diabetes is the most common type of diabetes in childhood and is a chronic illness characterized by insulin dependency due to destruction of the insulin producing cells (pancreatic β cells). The incidence of type 1 diabetes varies around the
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world with areas of high, medium or low incidence \(^6\), but is increasing by an average of 3\% per year in all areas, including Australia \(^7\)-\(^{12}\). Adults with type 1 diabetes have high morbidity and mortality mainly related to cardiovascular disease \(^{13}\)-\(^{16}\). This cardiovascular morbidity and mortality has been reduced but not eliminated in spite of intensive management of type 1 diabetes as justified in the Diabetes Control and Complications Trial (DCCT) \(^{17}\) and its follow up study, the Epidemiology of Diabetes Interventions and Complications Trial (EDIC) \(^{18}\)-\(^{20}\).

Obesity in childhood is defined as body mass index (BMI) \([\text{weight (kg)} / \text{height}^2]\) above 95\(^{\text{th}}\) percentile for age and sex, which correlates with obesity in adulthood defined as BMI above 30 Kg/m\(^2\) \(^{21}\). The worldwide prevalence of obesity in childhood and adulthood has doubled over the last decade \(^{22}\)-\(^{27}\). Childhood obesity is an independent risk factor for adult obesity \(^{28}\),\(^{29}\) and causes serious long-term morbidity and mortality \(^{30}\),\(^{31}\). Obesity in childhood relates to atherosclerosis and coronary heart disease risk factors in adulthood \(^{32}\),\(^{33}\).

Atherosclerosis begins and progresses during childhood and adolescence \(^{34}\)-\(^{37}\). A critical, early and potentially reversible event in the development of atherosclerosis is an abnormality of endothelial and/or smooth muscle function, termed vascular dysfunction \(^{38}\)-\(^{40}\). Other events involved in the development of atherosclerosis are inflammation, lipid and calcium deposition, and vessel wall proliferation. The progression of these events causes increased intima media thickness and arterial intraluminal obstruction with subsequent decrease in blood and oxygen supply to the tissues.

Vascular function (endothelial and smooth muscle function) has been measured non invasively using ultrasound of the brachial artery as a surrogate marker of atherosclerosis, as first described in 1990 \(^{41}\)-\(^{45}\). Endothelial function can be assessed with flow mediated
dilatation (FMD) which measures brachial artery diameter changes in response to changes in blood flow; these diameter changes are related to the amount of nitric oxide released by the endothelial cells. Abnormal FMD or endothelial dysfunction correlates with abnormal coronary angiography in adults 46-50. Smooth muscle function can be assessed with glyceryl trinitrate mediated dilatation (GTN), which measures vasodilatation produced by smooth muscle cells in response to glyceryl trinitrate, which is an exogenous nitric oxide donor. Abnormal GTN or smooth muscle dysfunction occurs in adults at risk of atherosclerosis and in adults with coronary atherosclerosis 51,52.

There is little data on vascular function particularly smooth muscle function in children with type 1 diabetes or obesity. A better understanding of vascular function determinants in these children in comparison to healthy children is required in order to test interventions early in life. Childhood interventions may improve vascular function and potentially prevent atherosclerosis at a reversible stage. Optimal metabolic control in type 1 diabetes, 53,54 and optimal diet and exercise in obesity 55,56, can be difficult to achieve in children, especially in adolescents.

Folic acid supplementation has been used to improve endothelial dysfunction in adults with coronary heart disease and high homocysteine levels 57,58. Folic acid supplementation in childhood has only been used to improve endothelial dysfunction in children with chronic renal failure and high homocysteine levels 59. Even though there is no evidence of the effects of folic acid supplementation on endothelial function in children with type 1 diabetes, our previous work showed that folate levels relate to endothelial function in these children 60.
1.2 Hypothesis

In light of the above problem statement and the background discussed in Chapter 2, I generated the following hypotheses.

1) Children with type 1 diabetes have comparable degree of vascular endothelial and smooth muscle dysfunction to children with obesity.

2) Determinants of vascular endothelial and smooth muscle function in non diabetic children include body mass index, waist circumference, lipids, inflammatory markers and folate status.

3) Folic acid supplementation improves endothelial and smooth muscle function in children with type 1 diabetes.

Following the initial results of the folic acid intervention trial in children with type 1 diabetes, the following hypothesis was also generated:

4) Folic acid supplementation improves endothelial function in children with obesity as in children with type 1 diabetes.

1.3 Overall objectives and aims

The overall objectives of this thesis were: to measure vascular endothelial and smooth muscle function in children and adolescents at risk of cardiovascular disease, namely children with type 1 diabetes and obesity; and to test the effects of folic acid supplementation as a strategy to improve vascular endothelial and smooth muscle function in these children.
The specific aims of this thesis were:

1) To compare vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity and healthy children.

2) To evaluate the determinants of vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity, and healthy children.

3) To evaluate the effects of folic acid supplementation on endothelial and smooth muscle function in children with type 1 diabetes.

4) To evaluate the effects of folic acid supplementation on endothelial and smooth muscle function in children with obesity.

1.4 Research strategy

As vascular endothelial and smooth muscle function measured by FMD and GTN are early, and potentially reversible events in atherosclerosis, a literature review of vascular function in general and vascular function in type 1 diabetes and obesity was carried out together with a review of folic acid and its effects on vascular function. This explanatory background is included in Chapter 2.

In Chapter 3, I investigate and compare vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity, and healthy children in a cross sectional study. In addition in this Chapter I include determinants of vascular function identified in the literature review such as body size measurements, lipids, glucose, insulin and inflammatory markers, and their relationship to vascular function. Chapter 3 relates to hypothesis one and two, and specific aims one and two.
Chapter 4 investigates the effects of folic acid supplementation on vascular endothelial and smooth muscle function, and folate status in children with type 1 diabetes using a double blind randomised placebo controlled cross over trial. *Chapter 4 relates to hypothesis three and specific aim three.*

In Chapter 5, I evaluate the effects of folic acid supplementation on vascular endothelial and smooth muscle function, folate status and inflammatory markers in children with obesity using a double blind randomised placebo controlled parallel trial. *Chapter 5 relates to hypothesis four and specific aim four.*

In Chapter 6, I discuss the findings of all studies in view of research validity and recently published data. I will also review future research questions and the implications of the findings for children with type 1 diabetes and obesity.

In Chapter 7 the main findings of this thesis are summarized with a final conclusion.
2 Chapter 2. Literature Review
In this Chapter I will start with a general overview of vascular function and its evaluation. I will review the endothelial and smooth muscle function evaluation using FDM and GTN. Then, I will review vascular dysfunction in diabetes mellitus, its etiopathogenesis and treatment. Next, I will revise vascular dysfunction in obesity. Finally I will review folic acid in general and folic acid as a treatment option for endothelial dysfunction investigating folic acid effects on vascular function and trials using folic acid to improve endothelial function.

2.1 Vascular endothelial and smooth muscle function

The wall of the artery consists of three layers. The inner layer or intima is the vascular endothelial cell layer; this is thinnest layer and is in direct contact with the blood in the lumen of the artery. The media layer is the vascular smooth muscle cell layer. The outer layer or adventitia contains fibroblast cells and connective tissue. I will describe the function of the endothelial and smooth muscle cell layer.

2.1.1 Endothelial function

Vascular endothelial cells are specialized epithelial cells. Besides being part of the internal wall of the arteries these cells have 3 main functions. The first is the regulation of arterial blood flow with production of nitric oxide (NO) and other substances, the second is the regulation of haemostasis, and the third is the promotion of growth and angiogenesis. All these functions are interrelated; for example NO both regulates blood flow and contributes to haemostasis by inhibiting platelet adhesion. Throughout this thesis I will
be referring to endothelial function as that related to the production of NO and vasodilatation, unless otherwise specified.

NO was first identified as the endothelial dependent relaxation factor by Furchgott in 1978. NO is a short-lived free radical molecule produced by endothelial cells. It causes vasodilatation maintaining basal vessel tone and facilitating blood flow regulation inside the arteries. The vasodilatory effect of NO is mediated by the activation of guanylate cyclase and subsequent production of cyclic guanosine monophosphate. NO is produced from L-Arginine and oxygen in an oxidative reaction catalysed by endothelial Nitric Oxide Synthase (eNOS) and the cofactors calcium, tetrahydrobiopterine, flavin adenine dinucleotide and heme proteins. NO is rapidly degraded to nitrate in a reaction using heme proteins (haemoglobin, myoglobin, or cytoglobin) or is degraded to peroxynitrate in a reaction using superoxide.

NO can be measured directly or indirectly. Direct measurements of NO include chemiluminescence, electroanalysis and spectrophotometric reactions, which are technically difficult due to instability of NO and scant availability of NO from tissues. Indirect evaluation of NO include blood or tissue measurements of the NO metabolites (nitrate and nitrite) using fluorometric or colorimetric methods, which also have technical limitations due to the continuous interconversion between nitrate and nitrite. Other indirect measurements assess NO vasodilatory effect using methods that evaluate in vivo vasodilatation of the coronary or peripheral arteries. These methods are coronary angiography, intracoronary ultrasonography, venous occlusion plethysmography measuring also forearm blood flow and brachial artery FMD. FMD is the only non invasive method and the most widely used. FMD will be further reviewed at the end of the section.
Endothelial cells regulate haemostasis by producing coagulation and thrombolytic proteins or protein binding sites. The pro-coagulation proteins produced by the endothelial cells are von Willebrand factor (vWF)\(^77\), a glycoprotein part of the coagulation cascade that interacts with platelets, and plasminogen activator inhibitor-1 (PAI-1), an inhibitor of fibrinolysis\(^78\). The thrombolytic proteins produced by the endothelium are tissue plasminogen activator (tPA) and urokinase, which promote fibrinolysis by converting plasminogen to plasmin, which in turn degrades fibrin in the clot\(^79\). All of these proteins can be measured directly in blood and are part of the evaluation of the haemostatic component of endothelial function assessment\(^80\).

Other substances produced by the endothelial cells are involved in the regulation of blood flow and haemostasis. These substances are prostacyclin, thromboxane A2, platelet activating factor (PAF) and endothelin-1 (ET-1), and can be increased in endothelial injury becoming circulating markers of endothelial dysfunction\(^81\).

Endothelial cells promote formation of new blood vessels and remodelling of existing vessels by interaction with vascular endothelial growth factor super family (VEGF), platelet derived growth factor (PDGF), basic fibroblast growth factor (βFGF), and transforming growth factor β (TGF-β)\(^82\). VEGF, PDGF, βFGF and TGF-β can be measured in blood to evaluate the mitogenic component of endothelial function.

In addition, there are other markers of endothelial activation such as circulating vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (E-selectin)\(^83,84\). Levels of these soluble adhesion molecules can be measured in blood.
2.1.2 Smooth muscle function

Vascular smooth muscle cells are part of the structural wall of the arteries and their function is contraction and relaxation in response to a stimulus or signals, as with any other type of muscle cell in the body. The contraction and relaxation of these cells cause arterial contraction and dilatation regulating blood flow inside the arteries \(^{85}\). The main vasodilatory stimulus by which smooth muscle cells react is NO, which can be produced endogenously by the endothelium or given exogenously as a NO donor \(^{86}\). The main vasoconstrictory stimulus is ET-1 \(^{87}\).

Smooth muscle function and endothelial function can be evaluated in vivo using the same methods as they both assess vasodilatation in response to NO. Evaluation of smooth muscle function assesses vasodilatation in response to exogenous NO (nitroglycerin, sodium nitroprusside and glyceryl trinitrate), while evaluation of endothelial function assesses vasodilatation in response to endogenous NO. As smooth muscle function assesses a vasodilatory response to exogenous but not endogenous NO, it is an endothelial independent response. Methods that assess smooth muscle function are coronary angiography using nitroglycerin \(^{88}\), intracoronary ultrasonography using nitroglycerin \(^{75}\), venous occlusion plethysmography using sodium nitroprusside \(^{76}\), and GTN using glyceryl trinitrate \(^{43}\). GTN is the only non invasive method and uses sublingual glyceryl trinitrate in comparison to other methods that use intra-arterial nitroglycerin or sodium nitroprusside.

Even though the evaluation of smooth muscle function can occur during the same procedure as the evaluation of the endothelial function it has not been investigated as frequently in clinical studies. Research has used outcomes of endothelial function more than smooth muscle function.
2.1.3 FMD and GTN in the evaluation of endothelial and smooth muscle function

FMD and GTN are safe, non-invasive, accurate and reproducible methods for evaluation of the endothelial and smooth muscle function. FMD and GTN are safe and non-invasive as they use ultrasound to obtain images of the vasodilatatory changes in brachial artery in relation to NO. The accuracy of FMD and GTN has been established with the use of a wide range of in vivo arterial diameters in comparison to in vitro phantom cylinders resembling “arteries”. FMD and GTN are reproducible and consistent when performed by experienced sonographers using a standardised technique for the procedure itself and for the vessel diameter measurements. Recently, a standardised technique has been reported in consensus guidelines for the ultrasound assessment of FMD using the brachial artery.

FMD measures the endothelial function related to the production of NO. NO is released by the endothelial cells in response to changes in blood flow that generate shear stress. FMD is a feasible and reproducible technique for assessing endothelial function in large populations and is a surrogate marker of atherosclerosis that is present before any clinical evidence of vascular disease. Lower brachial FMD levels (endothelial dysfunction) correlate to coronary endothelial dysfunction evaluated by coronary angiography in adults. Recently, abnormal FMD has been identified as a good predictor of future cardiovascular events in adults with ischemic heart disease, cardiomyopathy, arrhythmias, valvular disease or congenital heart disease.

GTN measures vasodilatation produced by smooth muscle cells in response to an exogenous nitric oxide donor, which is glycercyl trinitrate. This is an endothelial independent response. Lower GTN levels (or smooth muscle dysfunction) are an
independent risk factor in adults at risk of atherosclerosis and in adults with coronary atherosclerosis \(^{51,52}\).

Brachial FMD and GTN have been widely used in the evaluation of endothelial and smooth muscle function in adults with or at risk of cardiovascular disease \(^{41,95-101}\). Normal ranges for FMD and GTN were reported by Adams in a cohort of 207 adults with no identifiable major risk factors for atherosclerosis \(^{43}\).

FMD and GTN have been measured in children with specific diseases and in most cases using the femoral rather than the brachial artery. There is a strong correlation between FMD and GTN in most control and disease groups. FMD measured in the brachial and femoral artery is reduced in hypercholesterolemic children \(^{41,102-104}\). Femoral GTN is lower in children with familial hypercholesterolemia. \(^{102}\) Femoral FMD but not GTN is reduced in children with homozygous homocystinuria \(^{105}\). Brachial FMD is reduced in children who had low birth weight \(^{106}\) and children with lower levels of physical activity \(^{107}\). Brachial FMD and GTN are lower in adolescents with type 1 diabetes \(^{60,108}\), and in severely obese children \(^{109}\). Recently, brachial FMD and GTN ranges for 105 healthy children and adolescents were published \(^{44}\).

### 2.2 Vascular dysfunction and diabetes

I will begin this section with a review of the etiopathogenesis of vascular dysfunction in diabetes, without distinction of the type of diabetes or the population involved, unless it is specified. Then I will explore vascular dysfunction in type 1 diabetes and type 2 diabetes. Finally I will summarize the available interventions for vascular dysfunction in diabetes.
2.2.1 General etiopathogenesis

The factors that contribute to vascular dysfunction in diabetes are hyperglycaemia, inflammation, procoagulatory state and total plasma homocyst(e)ine (tHcy) [tHcy refers to the combined plasma pool of homocysteine, homocystine or disulphide of homocysteine and mixed disulphides involving homocysteine and homocysteine thiolactone] \(^{110}\). There are other etiological factors associated with vascular dysfunction and cardiovascular disease such as obesity, abnormal lipid profile, insulin levels, genetics, ethnicity, in utero growth restriction, ageing, hypertension, inactivity and cigarette smoking \(^{111}\). Obesity, abnormal lipid profile and insulin levels will be reviewed in the vascular dysfunction and obesity section. Genetics, ethnicity, in utero growth restriction, ageing, hypertension, inactivity and cigarette smoking will not be further reviewed in this thesis as they occur independent of diabetes.

2.2.1.1 Hyperglycaemia

Sustained glucose levels above the normal range and/or highly variable glucose levels, conditions common to all types of diabetes, are the main factors involved in the pathogenesis of endothelial dysfunction through different mechanisms (Figure 1).
Chronic hyperglycaemia causes endothelial damage through several different mechanisms. These mechanisms are autooxidation of glucose\textsuperscript{112,113}, activation of polyalcohol pathway with the formation of sorbitol and fructose from glucose\textsuperscript{114-116}, production of diacylglycerol with subsequent activation of protein kinase C (PKC)\textsuperscript{117-119}, production of advance glycation end products\textsuperscript{120,121} and shunting the glucose into the hexosamine pathway\textsuperscript{122}. All of the mentioned mechanisms generate reactive oxygen species (superoxide, peroxynitrite, hydrogen peroxide) and therefore increase oxidative stress (Figure 1)\textsuperscript{123-126}. Oxidative stress can also be increased by reduction of antioxidant defence mechanisms such as superoxide dismutase, catalase and glutathione peroxidase\textsuperscript{127-129}.

Oxidative stress but in particular peroxynitrite, a NO metabolite, causes changes in prostacyclin synthetase with subsequent decrease in prostacyclin, which is a vasodilator.
Furthermore chronic hyperglycaemia causes reduction of the availability of NO. Decrease in NO is caused by reduction in NO production by interference with eNOS and/or excessive NO inactivation \textsuperscript{131-135}. eNOS can also be affected by activation of PKC and increased reactive oxygen species (Figure 1) \textsuperscript{136,137}.

Acute hyperglycaemia also causes additional endothelial abnormalities including apoptosis in cultured human endothelial cells \textsuperscript{138,139}, and endothelial activation increasing vascular and leukocyte adhesion molecules \textsuperscript{140}.

Hyperglycaemia causes abnormalities of smooth muscle cell function through similar mechanisms to those producing endothelial damage (Figure 1). Oxidative stress causes smooth muscle dysfunction \textsuperscript{126} and abnormal apoptosis regulation in cultured smooth muscle cells \textsuperscript{141,142}. PKC activation causes abnormal smooth muscle cell proliferation \textsuperscript{143,144}. In addition hyperglycaemia causes increments in Angiotensin II, which is not only a vasoconstrictor but also an inducer of smooth muscle cell proliferation \textsuperscript{145}.

2.2.1.2 Inflammation

Hyperglycaemia increases markers of inflammation such as interleukin-6 and tumour necrosis alpha (TNF-\(\alpha\)) in adults with normal glucose tolerance and impaired glucose tolerance \textsuperscript{146}. Interleukin-6 and C-reactive protein (CRP), another marker of inflammation, are elevated in adults who later develop type 2 diabetes \textsuperscript{147,148}.

CRP is increased in adults in the normal population and in children with type 1 diabetes and relates to cardiovascular disease \textsuperscript{149-151}. CRP levels are related to diabetes control and BMI in adults with type 1 diabetes. \textsuperscript{152} Higher CRP levels are associated with microalbuminuria and atherosclerosis measured by intima media thickness in adults with diabetes \textsuperscript{153-155}.  

\footnotesize{Alexia Sophie Peña Vargas, September2007}
2.2.1.3 Procoagulatory state

Coagulation abnormalities such as activation of coagulation factors, a decrease in fibrinolysis, and changes in platelet function occur in diabetes, and predispose to vascular function abnormalities and atherosclerosis. Thrombomodulin, platelet aggregation and vWF are increased in adults with type 1 and type 2 diabetes. Thrombomodulin, vWF, platelet count and aggregation, and plasma viscosity are increased in adults with type 1 diabetes and precede diabetic complications. Fibrinogen, factor VII and protein C relate to albumin excretion ratio in adults with type 1 and type 2 diabetes, and vWF and protein S relate to albumin excretion ratio in type 1 diabetes.

vWF, tPA and prothrombin factors are increased in children with type 1 diabetes. Higher levels of vWF in these children precede the development of microalbuminuria by several years.

2.2.1.4 Total plasma homocyst(e)ine -tHcy

Homocysteine is an amino acid that causes endothelial dysfunction. tHcy is considered an independent risk factor for cardiovascular disease. Abnormalities in glucose homeostasis i.e. impaired glucose tolerance and diabetes do not predispose to abnormal tHcy levels, and in fact tHcy is reduced in children and adults with type 1 diabetes compared to controls due to glomerular hyperfiltration. In spite of overall low tHcy levels, there is a controversy about the association between tHcy levels and diabetic complications. Some studies report no association between tHcy levels and micro and macrovascular complications of type 1 diabetes, whilst others report a positive association between tHcy levels and complications in type 1 and type 2 diabetes.
2.2.2  Vascular dysfunction in adults and children with diabetes

Young adults with type 1 diabetes have endothelial and smooth muscle dysfunction \(^{172}\). There is controversy about the relation between glycaemic control and vascular dysfunction. Some report no relation \(^{173}\), whilst others report a relation between HbA1c and vascular dysfunction \(^{174}\). Adults with type 1 diabetes have vascular dysfunction preceding the development of other diabetic vascular complications \(^{173-175}\). Vascular dysfunction is worse in adults with type 1 diabetes and microalbuminuria than those without microalbuminuria \(^{174,176}\). Endothelial and smooth muscle dysfunction in young adults with type 1 diabetes relates to LDL cholesterol and diabetes duration \(^{172}\). Endothelial dysfunction relates to LDL particle size and LDL vitamin E content \(^{177}\).

Adults with type 2 diabetes have endothelial dysfunction measured by plethysmography and FMD \(^{178-181}\); data on smooth muscle function in this population is inconsistent. Some report normal function measured by plethysmography or GTN \(^{178,180,181}\); and some report abnormal function measured by plethysmography \(^{179}\).

Children with type 1 diabetes for 3.3-14.9 years have vascular dysfunction as measured by FMD and GTN in comparison to controls. These abnormalities in large vessels precede the abnormalities in small vessels such as retinopathy and microalbuminuria \(^{108}\). We have shown that children with type 1 diabetes of short duration (average of 5.7 years) and without any diabetic complications have lower FMD and GTN than controls. Endothelial dysfunction in these children was independently related to folate status, but not related to diabetes duration or control \(^{60}\). There is no data about vascular function in children or adolescents with type 2 diabetes.
2.2.3 Interventions for vascular dysfunction in diabetes

In this section, interventions for vascular dysfunction will be reviewed with the exception of intensive diabetes therapy and folic acid. Intensive diabetes therapy must be the first line in the management for vascular dysfunction as it has been shown to decrease but not eliminate the progression of atherosclerosis measured by arterial intima media thickness \(^{182}\); and decrease the progression of microvascular complications. Folic acid will be reviewed in the last section of this chapter.

The following interventions have been used for vascular dysfunction in adults with diabetes with variable benefits: angiotensin converting enzyme inhibitors (ACE inhibitors), angiotensin type 1 receptor antagonists, lipid lowering agents, antioxidants such as vitamin C and E, and L-arginine.

ACE inhibitors are recommended treatment for microalbuminuria but not for endothelial dysfunction per se \(^{183-185}\). ACE inhibitors decrease angiotensin II, a potent vasoconstrictor, and increase bradykinin which is a potent vasodilator that increases NO \(^{186}\). ACE inhibitors (Enalapril and Quinapril) do not improve FMD or GTN in normotensive adults with type 1 diabetes \(^{187,188}\). Quinalapril improves E-selectin but not other markers of endothelial dysfunction such as ET-1, PAI-1, t-PA or vWF \(^{188}\). Perindopril, another ACE inhibitor, does not improve endothelial function in adults with type 1 diabetes \(^{189}\).

Angiotensin type 1 receptor antagonists (Losartan) inhibit the effect of angiotensin II by blocking its receptor. Losartan improves endothelial function in adults with type 2 diabetes \(^{190,191}\). There is no data about the effects of Losartan in type 1 diabetes.

Lipid lowering agents such as statins (atorvastatin or simvastatin) or fibrates (fenofibrate or gemfibrozil) improve endothelial function independent of lipid lowering
effect in adults with hyperlipidemia \(^{192-195}\). Atorvastatin, an inhibitor of the 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA), improves FMD but not GTN in adults with type 1 diabetes \(^{196}\). Simvastatin, another HMG-CoA inhibitor, does not improve endothelial or smooth muscle function in adults with type 2 diabetes \(^{180}\). Gemfibrozil, an activator of peroxisome proliferators-activated receptor alpha (PPAR-\(\alpha\)), improves FMD in adults with type 2 diabetes \(^{197}\).

Vitamin C or ascorbic acid improves endothelial function by increasing NO availability through different mechanisms \(^{198}\). Vitamin C improves NO synthesis with increments in tetrahydrobiopterine \(^{199}\) and decreases NO degradation decreasing oxygen derived free radicals \(^{200}\). Vitamin C and E improve endothelial dysfunction related to postprandial hyperglycaemia in healthy adults \(^{201}\). Vitamin C improves FMD in adults with impaired glucose tolerance \(^{202}\) and improves endothelial function in adults with type 1 or type 2 diabetes \(^{203,204}\).

Vitamin E or alpha-tocopherol has some antioxidant properties which are beneficial for the endothelium \(^{205}\). Vitamin E increases antioxidants such as glutathione and decreases lipid peroxidation end products (malondialdehyde) in children with type 1 diabetes \(^{206}\). Vitamin E improves endothelial function in adults with type 1 diabetes \(^{207,208}\), but does not improve endothelial markers such as VCAM-1 and P-selectin, or LDL oxidation \(^{207,209}\). Vitamin E does not change smooth muscle function in adults with type 1 diabetes \(^{208}\). Vitamin E improves FMD in adults with type 2 diabetes \(^{210}\).

L-arginine, the precursor of NO, has been trialled for vascular dysfunction in adults with coronary artery disease and hyperlipidemia \(^{211,212}\). L-arginine has beneficial effects in vascular function of rats with shorter duration of diabetes but has no effect in rats with
longer duration of diabetes. Short term oral L-arginine does not change FMD or GTN in adults with type 1 diabetes.

At the start of my project and to my knowledge there were no trials evaluating any intervention for vascular dysfunction in children or adolescents with diabetes. The only trial evaluating any intervention for vascular dysfunction in children was a trial using vitamin E and C in children with hyperlipidemia, which showed an improvement in endothelial dysfunction in these children.

2.3 Vascular dysfunction and obesity

In this section, I will first review the etiopathogenesis of vascular dysfunction in obesity. Then, I will review the studies of vascular function in obese individuals. Finally I will revise interventions for vascular dysfunction in the obese population.

2.3.1 General etiopathogenesis

Etiological factors specifically involved in vascular dysfunction in obesity include adiposity, abnormal lipid profile, inflammation, insulin levels, hyperglycaemia, haemostasis abnormalities and tHcy. These occur in addition to other traditional risk factors for atherosclerosis such as family history of cardiovascular disease, ethnicity, ageing, hypertension, inactivity and cigarette smoking that also occur with obesity and will not be reviewed in this theses. As hyperglycaemia was discussed in the previous section it will not be further reviewed in this section.
2.3.1.1 Adiposity

Adiposity is the main etiological factor involved in vascular dysfunction in obesity, and it interrelates with other etiological factors (Figure 2). Adiposity means increase in adipose tissue, which can be subcutaneous and/or visceral. Adiposity, especially visceral adiposity, causes abnormal production of fat tissue derived products or adipocytokines. Adiposity increases angiotensinogen, leptin, macrophage chemo attractant protein-1 (MCP-1), CRP, interleukin-6, TNF-α, resistin, PAI-1 and free fatty acids; and decreases adiponectin. Angiotensinogen is a precursor of angiotensin I and II which are vasoconstrictors and inductors of smooth muscle cell proliferation. Leptin increases oxidative stress causing endothelial and smooth muscle dysfunction and also relates to insulin resistance. MCP-1 is increased by leptin and it causes recruitment and activation of monocytes promoting inflammation. CRP, interleukin-6 and TNF-α are inflammatory markers that can cause direct damage to the endothelium, decrease NO production and increase oxidative stress. Resistin is associated with insulin resistance. PAI-1 is also produced by endothelial cells, and in excess causes impaired fibrinolysis. Free fatty acids cause endothelial dysfunction, decreasing NO production and cause insulin resistance. Low adiponectin levels are related to decreased NO production, increased insulin levels and inflammation. Furthermore adiposity causes abnormal lipid profile, inflammation, insulin resistance, hyperglycaemia, other coagulation abnormalities and increased tHcy levels that also contribute to vascular dysfunction in obesity (Figure 2).
Figure 2. Increased adipose tissue and vascular dysfunction

Adiposity, along with abnormal lipid profile, impaired glucose tolerance and high blood pressure define metabolic syndrome. Metabolic syndrome is strongly associated with vascular dysfunction, and coronary heart disease.

Adiposity can be measured using BMI, waist and hip circumference, dual energy X-ray absorptiometry (DEXA) and magnetic resonance spectroscopy. BMI defines obesity and the degree of adiposity i.e. adults with BMI higher than 30 kg/m² are classified as obese and adults with BMI between 25 and 29 kg/m² are classified as overweight. The definition of obesity and overweight status in children is extrapolated from adult BMI cut offs of 30 and 25 kg/m², respectively and adjusted for age and sex. In other words children with a BMI higher than 95th percentile for age and sex corresponds to adults with a BMI higher than 30 kg/m² and will be classified as obese; and children with a BMI between 85th
and 94th percentile corresponds to adults with a BMI between 25 and 29 kg/m² and will be classified as overweight. The degree of obesity in children according to age and sex can be expressed as BMI z-score or BMI standard deviation from the mean of the population of the same age and sex. BMI z-scores can be calculated using EpiInfo data base version 3.2.2 and Centers for Disease Control 2000 standardized reference charts.

BMI in the non obese range (BMI <30 kg/m²) is one of the determinants of endothelial function in healthy adults, and BMI in the overweight-obese range (BMI >25 kg/m²) is independently associated with coronary endothelial dysfunction and atherosclerosis. Higher BMI in adolescence is associated with higher morbidity and mortality from coronary heart disease in adulthood independent of adult weight. BMI, along with abnormal lipid profile, is associated with the extent of atherosclerotic lesions in the coronary arteries and aorta in children and young adults who died from causes other than cardiovascular disease.

Visceral adiposity (central adiposity) can be evaluated by measurement of waist and hip circumference. Higher waist to hip ratio relates to endothelial dysfunction and intima media thick ness in overweight adults. Higher waist circumference independent of BMI in a large cohort of adults is associated with increased risk of abnormal lipid profile, diabetes, hypertension and metabolic syndrome. In children there is emerging data that waist circumference is a more informative measure of visceral obesity than BMI. Waist circumference data adjusted for age and sex in children was recently published.

Adiposity measured by DEXA as total body fat and total lean mass is related to endothelial dysfunction in adults. Higher total body fat is related to high blood pressure and abnormal lipid profile in children and adolescents.
Regional fat distribution can be accurately measured by magnetic resonance spectroscopy\textsuperscript{254,255}. Abnormal central regional fat distribution is related to endothelial dysfunction and other markers of atherosclerosis in obese and non-obese adults\textsuperscript{256}.

2.3.1.2 Abnormal lipid profile

Abnormalities in the lipid profile such as an increase in total cholesterol, LDL cholesterol and/or triglycerides; and/or decrease in HDL cholesterol per se cause endothelial dysfunction in adults and children\textsuperscript{41,102,104,257,258}. Higher cholesterol even within the normal range is associated with endothelial dysfunction in healthy adults\textsuperscript{259}. In a large cohort of adults with mean cholesterol of 5.2 mmol/L, higher cholesterol is associated also with smooth muscle dysfunction\textsuperscript{51}.

Abnormal lipid profile in obesity also relates to vascular dysfunction. The abnormal lipid profile in obesity relates to increased oxidative stress that causes endothelial dysfunction\textsuperscript{260}. Low HDL and apolipoprotein A-1 relate to endothelial dysfunction and arterial stiffness in severely obese children\textsuperscript{109}. High triglyceride levels caused by experimental obesity are associated with endothelial dysfunction in animal studies\textsuperscript{231}.

The abnormal lipid profile that occurs in obesity also relates to other cardiovascular risk factors. Hypertriglyceridermia in obese adults relates to increased PAI-1\textsuperscript{261}. Hypertriglycerideremia, high LDL cholesterol and low HDL cholesterol in obese adults are related to visceral adiposity and insulin resistance\textsuperscript{262-265}. High total cholesterol levels in obese children are related to high blood pressure\textsuperscript{266,267}. Low HDL and apolipoprotein A-1 in severely obese children are related to visceral adiposity and insulin resistance\textsuperscript{109}.

2.3.1.3 Inflammation

Inflammation, measured by inflammatory markers such as CRP, TNF-α and interleukin-6, relates to vascular dysfunction and other cardiovascular risk factors in non
obese children and adults. High CRP levels relate to endothelial dysfunction in healthy children. CRP, TNF-α and interleukin-6 are related to fibrinogen levels, insulin resistance and cardiovascular risk factors.

Inflammation also occurs in obesity and relates to endothelial dysfunction. CRP relate to the degree of obesity measured by BMI in adults. CRP, TNF-α and interleukin-6 increase with adiposity. CRP relates to adiposity, markers of endothelial activation and vWF in adults; and relates to coagulation markers such as D-dimer and PAI-1 in obese adolescents.

2.3.1.4 Insulin levels

Insulin levels in obese or non-obese population relate to endothelial function, cardiovascular disease and other cardiovascular risk factors such as high homocysteine levels, coagulation abnormalities, abnormal lipid profile and blood pressure.

Insulin levels independent of obesity or blood glucose levels are associated with endothelial dysfunction. Normal insulin levels regulate endothelial function in vitro and in vivo; however high insulin levels and insulin resistance are associated with endothelial dysfunction in young normal weight normoglycaemic adults and in obese non-diabetic adults. In addition high insulin levels and insulin resistance relates to endothelial dysfunction and arterial stiffness in severely obese children.

High insulin levels in adults are independently associated with cardiovascular disease such as myocardial infarction, death from coronary heart disease and abnormalities on electrocardiogram on meta-analysis.

Hyperinsulinemia in adults is related to high tHcy levels and coagulation abnormalities. Hyperinsulinemia and insulin resistance both occur in obese children.
and are related to other cardiovascular risk factors such as high homocysteine levels, blood pressure and lipid profile.

2.3.1.5 Abnormalities in haemostasis

Haemostasis is regulated by pro-coagulation proteins such as vWF, PAI-1 and coagulation factors; and by thrombolytic proteins such as tPA, urokinase, protein C and S. Haemostasis regulation is affected by obesity and it will affect NO mediated vasodilatation, the main endothelial function examined through this theses.

The haemostasis abnormalities that occur in obesity include abnormal production of pro-coagulation or thrombolytic proteins; and relate to cardiovascular risk factors. Excessive production of PAI-1 occurs in obesity as PAI-1 is not only produced by the endothelial cells but also by adipose tissue (Figure 2). PAI-1 is increased in obese children and relates to low fitness levels. Increase in coagulation factors, tPA, antithrombin III and/or protein C occurs in obesity. Factor VII, factor VIII, fibrinogen, plasminogen and antithrombin III are increased in obese adults and relate to body weight and insulin resistance. PAI-1, Fibrinogen, D-dimmer and tPA are increased in obese children, and are related to insulin resistance.

Abnormalities in haemostasis also relate to other cardiovascular risk factors such as abnormal lipid profile in obese and non obese subjects. High fibrinogen levels independently relate to adiposity and low HDL cholesterol levels in large cohort of adults. High levels of Factor VII, Factor VIII, plasminogen and antithrombin III are related to hypertriglyceridemia and hypercholesterolemia in adults. D-dimer and P-selectin, but not vWF are associated with hypercholesterolemia in obese children.
2.3.1.6 tHcy

Increments in tHcy levels per se cause endothelial dysfunction. tHcy causes endothelial damage via changes in NO availability, increments in oxidative stress, coagulation abnormalities and insulin resistance. Furthermore an increase in tHcy promotes growth of vascular smooth muscle cells.

Higher tHcy levels occur in obesity and are related to cardiovascular risk factors. tHcy are increased in the obese and are inversely related to folate status and diet as in the normal population. Higher tHcy levels correlate with other cardiovascular risk factors such as high insulin levels in obese adults and children.

2.3.2 Vascular dysfunction in adults and children with obesity

Endothelial dysfunction occurs in overweight and obese adults. Overweight but otherwise healthy adults have lower FMD that is independently related to waist to hip circumference ratio. Obese adults have brachial and coronary endothelial dysfunction that is related to adiposity, visceral fat and insulin resistance. Obese normotensive adults have a similar degree of endothelial dysfunction to lean hypertensive adults.

Smooth muscle function measured by GTN and plethysmography using sodium nitroprusside is comparable in obese and non-obese adults.

There is scant data about vascular function in obese children. Children with severe obesity (BMI z-score +3.0 to +8.1) have both endothelial and smooth muscle dysfunction as they have significantly reduced FMD and GTN in comparison to controls (BMI z-score -1.98 to +0.98). In these children FMD is positively related to Apolipoprotein A-1 and...
negatively related to insulin resistance \(^{109}\). Children with obesity (BMI 29 +/- 5.5 kg/m\(^2\) not adjusted for age or sex) have reduced FMD but not GTN, which are related to BMI \(^{308}\).

2.3.3 **Interventions for vascular dysfunction in obesity**

Interventions for vascular dysfunction in obesity should start with interventions targeting the obesity itself, which are diet and exercise \(^{55,56}\).

Different types of diets in obesity have beneficial effects on vascular function and cardiovascular risk factors. Very low calorie diet (800 calories a day) for 2 weeks improves endothelial function independent of changes in weight, lipid profile or insulin resistance in obese adults with essential hypertension \(^{309}\). Low calorie, low-fat diet (1600 kilocalories a day, 25% of energy from fat) alone or in combination with a serotonergic agonist (dexfenfluramine) for 3 months improves endothelial function, adiposity, lipids and insulin resistance in obese normotensive adults; this improvement relates to weight loss \(^{310}\). Low calorie diet does not improve smooth muscle function in spite of improvement in weight, lipids and insulin resistance \(^{309}\). Low calorie diet with 55% carbohydrate, 25% fat and 20% protein for 7 months improves insulin sensitivity, cardiac output and systolic blood pressure in proportion to weight reduction \(^{311}\).

Diet and exercise in combination have beneficial effects on vascular function that are greater than their individual effect. Low fat, high-fibre diet combined with daily exercise for 45-60 minutes for 3 weeks improves lipid profile, blood pressure, insulin sensitivity, NO availability and oxidative stress independent of changes in BMI in obese adults \(^{312}\). Diet and physical training decrease percentages of body fat and coagulation markers such as PAI-1, tPA and D-dimer in obese children \(^{273,313}\).
Even though diet and exercise should be routine in the management of vascular dysfunction in obesity, they are very difficult to implement, reinforce and maintain particularly in children and adolescents. Moreover it has only been the intensive regimens described in the fore mentioned trials that have improved vascular function and cardiovascular risk factors for relatively short duration. Therefore in obesity there is a clearly need to develop and evaluate other simpler interventions such as vitamin supplementation as additional options in the management of vascular function.

2.4 Folic acid and endothelial dysfunction

In this section I will start with a general overview of folic acid followed by a revision of folic acid effects on the endothelium and the trials using folic acid as an intervention for endothelial dysfunction. This section includes articles published after the work of this thesis started as they introduce important information about the effects of folic acid on the endothelium.

2.4.1 Folic acid overview

Folic acid was discovered by Wills in 1931 as a yeast extract that corrected macrocytic anaemia of pregnancy. Folic acid has had different names such as \textit{Lactobacillus Cassei} factor, liver factor \textit{Lactobacillus Cassei} factor, vitamin Bc, vitamin B9 and vitamin M, but its current name came from one of its sources the spinach leaf or \textit{folium} in Latin.

Folic acid or pteroylmonoglutamic acid is one of vitamin B complex vitamins. It can be found in the form of folic acid, folate or as tetrahydrofolic acid. Folic acid is an inactive
compound produced synthetically from a pteridine ring, p-aminobenzoic acid and glutamic acid (Figure 3). Folate is the natural form of folic acid and it is present in a wide source of foods such as leafy green vegetables, legumes, dry fruits, citrus juices, liver, kidney, yeast, cereal, wholegrain breads, sunflower seeds and especially folate fortified food. Its bioavailability is approximately 50%. Tetrahydrofolic acid is produced by reduction of folic acid and is the active form of folic acid\textsuperscript{316}.

\textbf{Figure 3. Synthetic folic acid molecule. Modified from Elliot, 1997} \textsuperscript{316}.

Folic acid is involved in the synthesis of nucleic acids, erythropoiesis and amino acid interconversions; i.e. adequate concentrations of 5-methyltetrahydrofolate along with vitamin B6 and B12 are necessary for the conversion of homocysteine to methionine. 5-methyltetrahydrofolate is reduced from 5, 10'-methylene tetrahydrofolate by the enzyme methylenetetrahydrofolate reductase [MTHFR] (Figure 4).
Vascular function in children at risk of cardiovascular disease and the effects of folic acid

NOTE: This figure is included on page 33 of the print copy of the thesis held in the University of Adelaide Library.

Figure 4. Metabolism of homocysteine and the role of folic acid. Modified from Guba, 1996

Recommended dietary allowances of folic acid for children older than 14 years and adults are 0.4 mg a day of dietary folate equivalent (0.1 mcg of food folate = 0.5 mcg of folic acid taken in fasting state = 0.6 mcg of folic acid taken with food) with higher recommended dietary allowances during pregnancy (0.6mg/day) and breastfeeding (0.5mg/day)\textsuperscript{317,318}. Folic acid is a water soluble substance absorbed in the proximal part of the small intestine and reduced and methylated in the liver to 5-methyltetrahydrofolinic acid. It is well distributed in the body but 50% of the stores are in the liver. Folic acid is excreted mainly in the urine\textsuperscript{319}.

Folic acid can be measured in serum, red blood cells and cerebrospinal fluid. Normal serum and red cell folate levels are 5-45 nmol/L and 180-900 nmol/L, respectively;
and vary according to the method used for measurement. Red cell folate levels are good indicators of folic acid stores.

Folic acid supplementation is required in folic acid deficiency, prevention of neural tube defects, hyperhomocysteinemia and homocystinuria. Folic acid deficiency requires different folic acid doses according to the severity of the deficiency that can be caused by nutritional deficit, gastrointestinal diseases, liver disease, chronic renal failure and use of medications including anticonvulsants (phenytoin, barbiturates, and valproate), methotrexate, nitrofurantoin or sulfasalazine. Prevention of neural tube defects requires a minimum of 0.4 mg/day of folic acid 1 month before conception and 3 months after conception; higher doses will be required if there is a prior history of neural tube defect. Hyperhomocysteinemia improves with daily supplementation of 0.5 -5mg of folic acid and 0.5 mg of Vitamin B12 a day.

Other uses of folic acid supplementation include prevention of cancer and cardiovascular disease. Population studies have shown that high folate intake reduces the risk of colorectal and breast cancer; and low folate intake increases the risk of these cancers. Folic acid in cardiovascular disease will be described in the next sections.

Folic acid is a very safe vitamin as it is water soluble; even with higher doses toxicity has not been reported. Rare side effects include allergic reactions and gastrointestinal symptoms such as nausea, abdominal distension and flatulence.

2.4.2 Effects of folic acid on vascular function

The known effects of folic acid on the endothelium are mediated via three different mechanisms: the reduction of tHcy, improvement of NO availability and antioxidant effects. There is scant literature on the effects of folic acid on smooth muscle cells; but one
trial has shown that folic acid decreases proliferation of smooth muscle cells through its tHcy lowering effect \(^{325}\).

The effect of folic acid lowering effects on tHcy levels and its effects on endothelium are well studied. Folate status, measured by serum folate and red cell folate, relates to tHcy levels in large cohorts of adults and children \(^{326-328}\). Fortified folate diet improves folate status and tHcy levels in adults \(^{329,330}\). Folic acid supplementation at a dose of 0.5-5 mg a day improves folate status and reduces tHcy levels up to 25% according to a meta-analysis. This reduction in tHcy is related to basal tHcy levels and folate status i.e. the maximum reduction is achieved with higher baseline tHcy and lower baseline folate status \(^{322}\). Folic acid supplementation improves folate status and endothelial function through a reduction in tHcy levels in adults with hyperhomocysteinemia \(^{58,331}\) and coronary artery disease \(^{332}\). Folic acid does not change smooth muscle function in this population. Furthermore, folic acid has some effects on coagulation abnormalities caused by high tHcy levels, it decreases fibrinogen and vWF, and increases plasminogen and antithrombin III \(^{333}\).

Folic acid improves endothelial function by improving NO availability which occurs with improvement of NO synthesis and/or a reduction of NO degradation. Folic acid improves NO synthesis by improving eNOS action and by increasing an eNOS cofactor (tetrahydrobiopterine) in \textit{in vivo} studies \(^{334,335}\). Folic acid improves endothelial function by increasing NO synthesis and decreasing NO degradation in adults with familial hypercholesterolemia; these effects are independent of lowering tHcy \(^{336,337}\).

Folic acid has antioxidant properties as it reduces reactive oxygen species such as superoxide and improves antioxidant defence mechanisms like superoxide dismutase, glutathione peroxidase and malonyldialdehyde \(^{333,334,338}\). Folic acid supplementation
improves folate status and endothelial function via reduction of superoxides in adults with coronary artery disease. This endothelial effect occurs independently of any change in tHcy levels\textsuperscript{339,340}.

Folic acid supplementation improves endothelial function independent of the tHcy lowering effects and with no changes in serum nitrite/nitrate in adults with coronary artery disease\textsuperscript{57}. This may suggest other mechanisms by which folic acid has a beneficial effect on the endothelium.

2.4.3 \textbf{Interventional trials using folic acid to improve endothelial function}

Folic acid alone has been used as intervention for endothelial dysfunction in adults, but not children, with different diseases using different regimens (Table 1). The majority of these folic acid intervention trials show an improvement on endothelial function (FMD) in diseases such as hyperhomocysteinemia, coronary artery disease and chronic renal failure\textsuperscript{57,58,331,332,339-341}. However, folic acid does not improve endothelial function in adults with an unfavourable methylenetetrahydrofolate reductase (MTHFR) genotype that predisposes to higher tHcy levels\textsuperscript{342}, nor in adults with chronic renal failure\textsuperscript{343,344}. In addition, folic acid supplementation prevents endothelial dysfunction caused by exposure to a lipid load in healthy adults\textsuperscript{345}. 
**Table 1. Trials evaluating the effects of folic acid on endothelial function (FMD)**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Subjects</th>
<th>Age</th>
<th>Sex</th>
<th>Condition</th>
<th>Study design</th>
<th>Folic acid</th>
<th>Dose</th>
<th>Duration</th>
<th>Main Review</th>
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<tr>
<td>58</td>
<td>17 (aged 54[10] years) 15 males Hyperhomocysteinemia No other cardiovascular risk factors</td>
<td>RPCT</td>
<td>DB</td>
<td>CO</td>
<td>WO</td>
<td>10 mg/d</td>
<td>8 weeks</td>
<td>FMD</td>
<td>FMD improved by 2.2%</td>
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<td>GTN no change</td>
<td>Serum folate increased</td>
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<td></td>
<td>Folate decreased by 15%</td>
<td>Lipids no change</td>
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<td></td>
<td></td>
<td></td>
<td>tHcy decreased by 15%</td>
<td>Vit B12 no change</td>
</tr>
<tr>
<td>233</td>
<td>20 (age not reported) Hyperhomocysteinemia (tHcy &gt; 13umol/L) No other cardiovascular risk factors</td>
<td>RPCT</td>
<td>DB</td>
<td>CO</td>
<td>WO</td>
<td>5 mg/d</td>
<td>6 weeks</td>
<td>FMD</td>
<td>FMD improved by 2.5%</td>
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<td></td>
<td>GTN no change</td>
<td>Folate increased</td>
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<td></td>
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<td></td>
<td>tHcy decreased by 28%</td>
<td></td>
</tr>
<tr>
<td>332</td>
<td>75 (59[10] years) 59 males Coronary artery disease (tHcy &gt;9umol/L)</td>
<td>RPCT</td>
<td>DB</td>
<td>Parallel</td>
<td>5 mg/d or Folic acid, Vit C (2g/d) &amp; Vit E (800IU/d)</td>
<td>4 months</td>
<td>FMD</td>
<td>FMD improved</td>
<td></td>
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<td></td>
<td>GTN no change</td>
<td>Folate decreased by 475%</td>
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<td>tHcy decreased by 11%</td>
<td>Vitamin B12 no change</td>
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<td></td>
<td></td>
<td>B12 reduced related to tHcy change</td>
<td>B12, lipids &amp; MDA No change</td>
</tr>
<tr>
<td>338</td>
<td>52 (57[8] years) 44 males Coronary artery disease</td>
<td>RPCT</td>
<td>DB</td>
<td>CO</td>
<td>WO</td>
<td>5 mg/day</td>
<td>6 weeks</td>
<td>Also acute 5-MTHF 50ug/min</td>
<td>FMD improved</td>
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<td>GTN no change</td>
<td>Serum folate increased</td>
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<td>Folate decreased by 19%</td>
<td>Vitamin B12 no change</td>
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<td>tHcy decreased by 11%</td>
<td>MDA and TAC no change</td>
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<td></td>
<td>B12, lipids &amp; MDA No change</td>
<td>Acute 5-MTHF reduced superoxide</td>
</tr>
<tr>
<td>57</td>
<td>90 (63 [46-79] years) 79 males Coronary artery disease (stenosis &gt;50%)</td>
<td>RPCT</td>
<td>DB</td>
<td>Parallel</td>
<td>5 mg/d</td>
<td>12 weeks</td>
<td>FMD</td>
<td>FMD improved but not significantly</td>
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<td>GTN no change</td>
<td>Serum folate increased</td>
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<td>Folate decreased by 24% not related to FMD change</td>
<td>Nitrate/nitrite no change</td>
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<td></td>
<td>Vit B12 no change</td>
<td>vWF no change</td>
</tr>
<tr>
<td>240</td>
<td>33 (55.5 [7] years) 30 males Coronary artery disease</td>
<td>RPCT</td>
<td>DB</td>
<td>Parallel</td>
<td>5mg/d</td>
<td>6 weeks</td>
<td>FMD</td>
<td>FMD improved not related to tHcy</td>
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<td>GTN no change</td>
<td>Serum folate increased</td>
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<td>Folate decreased by 11%</td>
<td>5-MTHF increased</td>
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<td>tHcy decreased but not acutely</td>
<td>Vit B12 no change</td>
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<td></td>
<td></td>
<td>FMD change before tHcy change</td>
<td>Acute 5-MTHF reduced superoxide</td>
</tr>
<tr>
<td>241</td>
<td>29 (aged 52[8] years) 23 males tHcy &gt;50th percentile No other cardiovascular risk factors</td>
<td>Open-label</td>
<td>10 mg/d</td>
<td>1 year</td>
<td>FMD</td>
<td>FMD improved by 1.5%</td>
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<td>GTN no change</td>
<td>Serum folate increased</td>
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<td></td>
<td>Folate decreased by 69%</td>
<td>tHcy decreased by 12%</td>
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<td></td>
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<td></td>
<td>tHcy decreased by 11%</td>
<td>Triglycerides increased</td>
</tr>
<tr>
<td>59</td>
<td>25 (12[7-17] years) 13 males Children with CRF (no dialysis) tHcy 9.85 (3.57)</td>
<td>RPCT</td>
<td>DB</td>
<td>CO</td>
<td>WO</td>
<td>5mg/m2</td>
<td>8 weeks</td>
<td>FMD</td>
<td>FMD increased by 1.26%</td>
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<td>GTN no change</td>
<td>Folate increased</td>
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<td></td>
<td></td>
<td>tHcy decreased</td>
<td>TAC no change</td>
</tr>
</tbody>
</table>

*Table adapted from Alexia Sophie Peña Vargas, September 2007*
<table>
<thead>
<tr>
<th>Ref</th>
<th>Subjects</th>
<th>Age</th>
<th>Sex</th>
<th>Condition</th>
<th>Study design</th>
<th>Folic acid Dose Duration</th>
<th>Main Review</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>342</td>
<td>126 (39[12] years)</td>
<td>53 males</td>
<td>Healthy</td>
<td>MTHFR genotypes (CC, CT and TT)</td>
<td>RPCT DB and single blinded CO</td>
<td>400 mcg/d or diet with -400mcg/d -4 months</td>
<td>FMD Folate thCyl vWF Ag</td>
<td>FMD no change Folate increased by 79% with tablets and by 46% with diet thCyl decreased by 16% with tablets and by 14% with diet vWF Ag no change Changes were related to phenotype</td>
</tr>
<tr>
<td>343</td>
<td>35 (45.3 [2.6] years)</td>
<td>19 males</td>
<td>CRF on dialysis for 6 (1) years</td>
<td>Hyperhomocysteinemic</td>
<td>RPCT Open</td>
<td>1 and 5 mg/day ± Betain 2g 2/week (46 weeks) then 15 mg/day (4 weeks)</td>
<td>FMD GTN Folate thCyl vWF Thromb TPA PAI-1</td>
<td>FMD no change GTN no change Folate increased thCyl decreased but not normalize Other variables no change except for E selectin that increased Betaine no additional effect</td>
</tr>
<tr>
<td>344</td>
<td>100 (62[22-84] years)</td>
<td>67 males</td>
<td>CRF tHcy &gt; 12umol/L</td>
<td></td>
<td>RPCT DB Parallel</td>
<td>5 mg/day 12 weeks</td>
<td>FMD GTN Folate RCF thCyl Nitrite-nitrate Vit B12, vWF</td>
<td>FMD no change GTN no change Folate increased RCF increased thCyl decreased by 29.4% Nitrate-nitrite no change VWF no change</td>
</tr>
<tr>
<td>345</td>
<td>20 (23 [3.4] years)</td>
<td>10 males</td>
<td>Healthy</td>
<td></td>
<td>RPCT DB CO Washout 8 weeks</td>
<td>10mg/day 2 weeks</td>
<td>Acute 50g fat load FMD GTN Folate thCyl Lipids MDA</td>
<td>FMD remains the same with folic acid after lipid load compared to placebo that decreased GTN No change Folate increased Lipids no change MDA remains the same with folic acid after lipid load compared to placebo that decreased</td>
</tr>
<tr>
<td>346</td>
<td>130 (45.3 [7.6] years)</td>
<td>66 males</td>
<td>Siblings of patients with premature atherothrombotic disease</td>
<td></td>
<td>RPCT DB Parallel</td>
<td>Folic acid 5mg/d plus vit B6 (250mg/d) -2 years</td>
<td>FMD GTN CCA stiffness Folate thCyl Vit B6 Vit B12 BP</td>
<td>FMD no change GTN no change CCA stiffness no change Folate increased thCyl decreased by 40.1% Vitamin B6 increased Vitamin B12 no change BP decreased</td>
</tr>
</tbody>
</table>

RPCT=Randomised placebo controlled trial, DB=Double blind, CO=Cross over, WO=washout, Vit= vitamin, N/N= Nitrate/Nitrite, MDA= Plasma malondialdehyde, TAC=Total antioxidant capacity, 5-MTHF=5-methyltetrahydrofolate, CRF Chronic renal failure, Thromb=Trombomodulin, CCA= common coronary artery, BP=blood pressure.

Folic acid regimens in the mentioned trials used different doses and with minimal side effects. All the trials with the exception of two had a short intervention period of less
than 12 weeks \(^{341,346}\). Folic acid doses varied from 0.4 –10 mg a day; 0.4 mg a day was insufficient to improve endothelial function \(^{342}\); 5 mg a day was the most common dose used with beneficial endothelial effects in most cases; and 10 mg a day was sufficient in all trials to improve endothelial function \(^{58,333,341}\). Folic acid was safe with almost non-existent side effects even with the highest dose (10 mg a day) taken for the longest period (1 year); there were only few subjects in only one trial that used 5 mg of folic acid combined with Vitamin C and E and they reported abdominal pain, diarrhoea and rash \(^{332}\).

Folic acid supplementation has also been used in combination with other vitamins such as vitamin B6, B12 and antioxidant vitamins, as an intervention for endothelial dysfunction and cardiovascular disease, with beneficial endothelial effects \(^{347-352}\). Folic acid in combination with vitamin B6 does not improve endothelial function in siblings of patients with premature atherosclerosis \(^{346}\).

Prior to the beginning of this work and to my knowledge there were no trials evaluating the effects of folic acid on vascular function in children or adults with either diabetes or obesity.

2.4.4 Folic acid, homocysteine and other markers of cardiovascular disease

Folic acid stores, homocysteine and its determinants such as MTHFR polymorphisms are associated with markers of cardiovascular disease including carotid stiffness, carotid intima media thickness; and strokes, ischemic heart disease and thrombosis. Lower folic acid stores measured by red cell folate and independent of homocysteine levels are associated with increased carotid intima media thickness in a large cohort of healthy adults \(^{353}\). Adults homozygous for the MTHFR TT allele have higher homocysteine levels and higher risk for strokes, ischemic heart disease and deep vein thrombosis in comparison to adults with other MTHFR polymorphisms according to meta-
analysis \textsuperscript{354,355}. This association between MTHFR polymorphism and ischemic heart disease decrease with higher folate intake. \textsuperscript{356}

Meta-analysis of cohort studies show a decrease in serum homocysteine by 3 \(\mu\)moll/L lowers the risk of ischemic heart disease by 16\%, deep vein thrombosis by 25\% and stroke by 24\%. This is taking into account adjustment for other confounding factors for cardiovascular disease such as age, sex, smoking, blood pressure and cholesterol \textsuperscript{355}. Folic acid supplementation trials have shown limited and variable folate effects on cardiovascular disease in adults. Long term supplementation with folic acid (5mg/day) and vitamin B6 (250 mg/day) improve blood pressure and abnormalities in exercise electrocardiogram but have no effect on carotid artery stiffness or carotid and femoral flow velocity in siblings of patients with premature heart disease \textsuperscript{346,350}. Long term supplementation with folic acid (5 mg/day) does not improve carotid artery stiffness in adults with chronic renal failure \textsuperscript{357}. A large multivitamin supplementation trial with folic acid (2.5 mg/day), vitamin B6 (50 mg/day) and vitamin B12 (1 mg/day) did not reduce the risk of death from cardiovascular disease or myocardial infarction in adults older than 55 years and with pre-existent vascular disease or diabetes \textsuperscript{358}.

Meta-analysis of folic acid supplementation trials studying stroke show that folic acid at a dose of 0.5-15mg/day for 24 to 72 months in adults with history of coronary artery disease, end-stage renal disease or oesophageal dysplasia significantly reduces the risk of stroke. Folic acid effects are greater if every day diet is not fortified with folic acid, homocysteine decreases more than 20\% and the duration of supplementation is longer than 36 months \textsuperscript{359}. In addition folic acid supplementation may be cost effective in the primary and secondary prevention of coronary heart disease of adults between 35 and 84 years of age according to a cost effect analysis. \textsuperscript{360}.

\textit{Alexia Sophie Peña Vargas, September 2007}
3 Chapter 3: Vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity and healthy children.


NOTE: This publication is included on pages 41-60 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1210/jc.2006-0863
NOTE: Statement of authorship appears in the print copy of the thesis held in the University of Adelaide Library.
This chapter evaluates and compares vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity and healthy children and includes the paper entitled “Vascular endothelial and smooth muscle function relate to body mass index and glucose in obese and non obese children”, which reported the main results of the cross sectional evaluation of vascular function in the 3 group of children (Type 1 diabetic, obese and healthy children)

The impact factor of the journal where this paper has been published is 5.799

Even though this paper was only published in November 2006 it has already been cited once.

Data from this paper has been presented in National and International Meetings (See abstracts relevant to this thesis page XV):

- APEG Annual Scientific Meeting. Auckland, New Zealand. 1-3rd December, 2004 (Oral presentation SO4).
- ESPE/LWPES 7th JOINT MEETING PAEDIATRIC ENDOCRINOLOGY. Lyon, France. 21st – 24th September, 2005 (Poster presentation P22-922).
- APEG Annual Scientific Meeting. Hobart, Australia. 20-22nd September, 2006 (Oral Presentation S12.02).
4 Chapter 4. Folic acid improves endothelial function in children and adolescents with type 1 diabetes


NOTE: This publication is included on pages 61-76 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1016/j.jpeds.2003.12.049
NOTE: Statement of authorship appears in the print copy of the thesis held in the University of Adelaide Library.
The paper presented in this chapter is the first description of folic acid effects on endothelial function in children and adolescents with type 1 diabetes.

The impact factor of the journal where this paper has been published is 3.991.

This paper has been cited 12 times in the peer-reviewed literature.

Data from this paper has been presented in National and International meetings (see abstracts relevant to this thesis page XV):

- APEG Annual Scientific Meeting. Darwin, Australia. 22\textsuperscript{nd} – 24\textsuperscript{th} August, 2002 (Oral presentation A3).
- Australian Diabetes Society & Australian Diabetes Educators Association (ADS &ADEA) Annual Scientific Meeting. Adelaide, Australia. 25\textsuperscript{th} - 27\textsuperscript{th} September 2002 (Oral presentation 121).
- American Diabetes Association 63\textsuperscript{rd} Scientific Sessions. New Orleans, USA. 13-17\textsuperscript{th} June, 2003 (Oral presentation 64).
5 Chapter 5. Folic acid does not improve endothelial function in obese children and adolescents


NOTE: This publication is included on pages 77-96 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.2337/dc06-2505
NOTE: Statement of authorship appears in the print copy of the thesis held in the University of Adelaide Library.
The paper presented in this chapter is the first description of folic acid effects on endothelial function in obese children and adolescents.

The impact factor of the journal where this paper has been published is 7.912.

This paper has not been cited as it was just published in August 2007.

Data from this paper has been presented in National and International Meetings (See abstracts relevant to this thesis Page XV)

This paper was awarded with the “Professor John Chalmers Prize” in recognition of the best paper presented at the South Australian Annual Trainee Research Presentations (2006).

This paper was also awarded with the “Royal Australian College of Physicians Trainee Research Award (Paediatrics and Child Health)” in recognition of the best paper presented at National RACP Advance Trainee Research Award Session (2007).

The trial presented in this paper was registered in the Australian Clinical Trial Registration: ACTRN01260600457549 (URL: http://www.actr.org.au/).
6 Chapter 6. Discussion
In this chapter, I will first discuss the findings of this research in respect to research validity and limitations. Those findings will be interpreted in the context of current evidence. Then, the clinical implications of this research for subjects with type 1 diabetes and/or subjects with obesity will be discussed. Finally, I will explore future research options in the area studied.

6.1 Study results

6.1.1 Vascular endothelial and smooth muscle function in children with type 1 diabetes, children with obesity and healthy children

A cross-sectional case-control design was used to evaluate vascular function in children with type 1 diabetes, children with obesity and healthy children. This type of design has inherent advantages such as smaller sample size being adequate and the possibility of obtaining quick answers studying many risk factors. This design has also disadvantages such as the possibility of bias error, the limited use to certain disease outcomes related to definition of the cases or controls, and the limitation in demonstration of causality due to lack of a time sequence. Bias error that can occur in the selection of cases or controls (selection bias) was reduced in this design as cases were selected from one source and clearly defined in relation to inclusion and exclusion criteria. Controls were not a random sample of the general population but were age-matched controls. They were selected from three different sources: relatives of cases, friends of cases, and relatives or friends from the hospital staff. In spite of this fact controls from different sources were comparable. Another limitation in this study was the difference in the number of participants in each group; however vascular function differences between groups were
large enough to be detected. An increase in the total number of participants would have improved even further precision in the study reducing random error. Other biases besides selection bias that can affect the validity (lack of systematic error) in this type of study include self-selection bias, information bias and confounding factors. Self-selection bias in this study could have particularly occurred in the obese group and it was unavoidable; this is because only the obese children and their families who acknowledge their condition and the need of treatment would have come to the hospital and would have been asked to be part of the study. Information bias is more likely to occur in case control studies evaluating exposures not diseases itself as in my study; information bias or disease misclassification in the case of my study was unlikely as the information on disease came from casenotes and use of insulin in the case of children with type 1 diabetes and from current accurate measurements in the case of children with obesity. Confounding factors are defined by these criteria: they must be a risk factor for the disease, they must be associated with the exposure under study in the source population and they must not be affected by the disease. Lipid profile could have been a confounding factor in this study as it is a risk factor for vascular dysfunction, but it does not satisfy the full criteria for a confounding factor as lipid profile can be affected by obesity and type 1 diabetes. Lipid profile was included in the analysis of the data taking into account these considerations. Confounding factors such as smoking, hypertension, use of multivitamins, or other medications affecting vascular function were removed during the selection of the study population as they were part of the exclusion criteria. Other confounding factors such as age and puberty were controlled with the age-matched selection process.
The first finding of this study was that children with type 1 diabetes and no other vascular diabetic complications have similar degree of vascular dysfunction as children with mild to moderate obesity. This confirmed the original (null) hypothesis that children with type 1 diabetes have a comparable degree of endothelial and smooth muscle dysfunction to children with obesity, and rejected the alternative hypothesis. This result has not been shown before, but children with type 1 diabetes have a similar degree of endothelial dysfunction as children with other vascular conditions such as Kawasaki disease. There is no data about smooth muscle function in this trial. Moreover, children with type 1 diabetes have comparable arterial structural abnormalities, increased carotid intima media thickness, to children with obesity.

The second finding of this study was that endothelial and smooth muscle function related to BMI and weight adjusted for age and sex (BMI z-score and weight z-score, respectively) in obese and healthy children. This result confirmed the original (null) hypothesis that determinants of vascular function include BMI, and rejected the alternative hypothesis. The relationship between vascular function and BMI has been shown in adults from the community including smokers and hypertensive subjects; and in healthy adults with no cardiovascular risk factors. In children, endothelial function has been related to BMI above 95th percentile, but not across all percentiles of BMI as shown in my research cohort. I have shown an independent relationship between smooth muscle function and BMI z-score in obese and non-obese or healthy children; this result has also been shown in healthy children, children with type 1 diabetes and/or hypercholesterolemia.

Smooth muscle function was also related to BMI z-score as a continuum in children with type 1 diabetes; and smooth muscle function was significantly lower in obese diabetic
Vascular function in children at risk of cardiovascular disease and the effects of folic acid

compared to non obese diabetic children. This is consistent with the finding that macrovascular complications including cardiovascular disease in type 1 diabetes are predicted by BMI\textsuperscript{398}. In addition microvascular diabetic complications such as retinopathy and neuropathy are more prevalent in overweight subjects with type 1 diabetes\textsuperscript{399}.

The third finding of this study was that vascular smooth muscle function was related to blood glucose within the normal range in healthy children and in the non diabetic group which includes healthy and obese children. This result is original to my knowledge. It links glycaemia to early signs of atherosclerosis in children without diabetes and does suggest that smooth muscle function is a sensitive maker of early vessel disease. HbA1c has been related to atherosclerotic plaques found post-mortem in non-diabetic youth without other cardiovascular risk factors\textsuperscript{373}. In addition HbA1c relates to cardiovascular events and deaths in a large cohort of non diabetic adults from a population study\textsuperscript{374}.

Endothelial function was not related to blood glucose in non diabetic children (healthy and obese) or in children with type 1 diabetes, but it was related in the whole cohort of children (non diabetic and diabetic). The lack of relationship between blood glucose and endothelial function in non-diabetic children can be explained by two reasons: the small number of participants studied (n=111) and the lack of wide range of glucose levels in the participants studied that were either children with normal or abnormal glucose levels (mean = 4.8 mmol/L or mean = 13.6 mmol/L, respectively). The relationship between glucose and endothelial function appeared in the whole group with more subjects (n=270) and a wider range of glucose levels (range from 4.1-23.1). This finding was also consistent with previous studies in larger cohort of adults including adults with impaired fasting glucose\textsuperscript{375}.
6.1.2 Folic acid supplementation and its effects on endothelial function in children with type 1 diabetes

A randomised double-blind placebo controlled cross over trial was used to evaluate the effects of folic acid on endothelial function in children with type 1 diabetes. Randomization and allocation was done by an independent body (Pharmacy department, Women’s and Children’s Hospital, Adelaide) and was adequate as there were no differences in the baseline characteristics of the two intervention groups defined by first intervention received; this reduced the chance of selection bias. The design was double-blind i.e. the participants and investigators (Alexia Peña and Esko Wiltshire) who assessed outcomes of folic acid intervention were blinded; this reduced the chance of observation bias. The control group received a placebo tablet with identical characteristics to the intervention group, made by the same pharmaceutical company.

Cross over designed trials have strengths and weakness. The strengths are the possibility of being able to compare interventions to placebo in the same participant and in a smaller study population, which makes trials more feasible. Weaknesses include limitations to its use in chronic and stable diseases that may benefit from a rapid and short duration intervention; and order effects such as carry over effect and treatment period interaction; requiring complex analysis. Carry-over effect is when the effects of an intervention are still present during the evaluation of another intervention. In this trial there was a carry-over effect for serum folate and RCF but there was no carry-over effect for FMD, the main outcome in this trial. This carry over effect was similar to the one reported in a trial of children with chronic renal failure and could have been avoided with a longer washout period. The treatment period interaction effect is the difference between interventions that can be explained by the order in which the interventions were given to
the participants; there was no treatment period interaction effect for serum folate, RCF, tHcy or FMD in this trial.

The flow chart participant and recruitment as per CONSORT statement is shown in Figure 10. Fifty-two children were eligible for the trial of whom 13 did not participate. The main reasons for refusal were lack of interest (9), needle phobia (2) and multivitamin consumption (2). The analysis was done by intention to treat which means that all participants were included in the analysis regardless of whether they completed the trial or not.

**Figure 10. Flow chart participant and recruitment of diabetic trial**

The main finding of this study was that short-term folic acid supplementation improved endothelial function in children with type 1 diabetes. This confirmed the original (null) hypothesis that folic acid improves endothelial function in children with type 1 diabetes.
diabetes, and rejected the alternative hypothesis. This result has not been shown before but it was consistent with very recent publications in children with type 1 diabetes and adults with type 2 diabetes shown in Table 9. This table includes my trial highlighted in gray and a trial from our group highlighted in blue.\textsuperscript{367,388}
Table 9. Trials with evaluating the effects of folic acid on endothelial function (FMD or FABF) in diabetes

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Study Design</th>
<th>Folic acid</th>
<th>Main Reviews</th>
<th>Main Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>Design</td>
<td>Dose/Route</td>
<td>Duration</td>
<td>Side effects</td>
</tr>
<tr>
<td>T1DM children</td>
<td>RPCT</td>
<td>5 mg/day</td>
<td>Oral</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Mean 8 years and range 1-24 years</td>
<td>DB</td>
<td>Orally alone or combined with vit B6 (100mg/day)</td>
<td>8 weeks</td>
<td>No side effects</td>
</tr>
<tr>
<td>T2DM adults</td>
<td>Open label</td>
<td>5-MTHF (1ug/100ml)</td>
<td>Acute IV Infusion</td>
<td>Side effects not reported</td>
</tr>
<tr>
<td>T2DM adults</td>
<td>RPCT</td>
<td>5 mg/day</td>
<td>Orally</td>
<td>4 weeks</td>
</tr>
<tr>
<td>T2DM adults</td>
<td>RPCT</td>
<td>10mg/day</td>
<td>Orally</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>

Data are mean (SD). RPCT Randomised placebo controlled trial, DB Double blind, CO Cross over, Vit 6 Vitamin B6, 5-MTHF 5-methyltetrahydrofolate, FABF Forearm arterial blood flow, SP Sodium nitroprusside, PWV Pulse wave velocity
Folic acid does not decrease tHcy levels in children with type 1 diabetes as it does in adults with type 2 diabetes. This can be related to the lower tHcy levels in children in general in comparison to adults and in children with type 1 diabetes compared to age-matched controls. In spite of the differences of folic acid effect on tHcy levels in children and adults with diabetes, folic acid improves endothelial function in all published trials including my trial. This along with the lack of relationship between endothelial function improvement and tHcy changes suggests that folic acid effect on endothelial function is independent of tHcy changes (Table 9). This is also consistent with trials in adults with other conditions than diabetes, which have shown that folic acid improves endothelial function directly by increasing antioxidant status and/or nitric oxide levels. My research did not investigate antioxidant status or measure directly nitric oxide levels.

6.1.3 Folic acid supplementation and its effects on endothelial function in children with obesity

A randomised double-blind placebo controlled parallel trial was used to evaluate the effects of folic acid on vascular function in children with obesity. Randomization was adequate as there were no differences in baseline characteristics between the two intervention groups; this reduced selection bias. The design was double blind i.e. the participants and investigator (Alexia Pena), who assessed outcomes of folic acid intervention, were blinded reducing observation bias. A parallel design compared participants who were exposed to only one of the study interventions i.e. folic acid or placebo; this is the simplest and easiest interventional trial design. Participants in this trial were selected from the hospital, not the community, which limits generalization of the
results. Participation rate was low however, comparable to other trials in obesity\textsuperscript{392,393} and probably influencing a good retention during the study period.

The flow chart participant and recruitment as per CONSORT statement\textsuperscript{401} is shown in Figure 11. One hundred and ninety four obese children were eligible for the trial of whom 138 did not participate. The main reasons for refusal were lack of interest (88), living in the countryside (18), time constrains (16), needle phobia (10), difficulties taking tablets (4). Of the 56 children screened, two had the first assessment and did not wish to continue and one has vitamin B12 deficiency and was excluded. The analysis was done by intention to treat which means that all participants were included in the analysis regardless of whether they completed the trial or not.

![Flow chart participant and recruitment of obese trial](image)

\textbf{Figure 11. Flow chart participant and recruitment of obese trial}

The main finding of this study was that short-term folic acid supplementation did not improve endothelial function in children with obesity. This result rejected the original (null) hypothesis that folic acid improves endothelial function in children with obesity as it
did in children with type 1 diabetes, and confirmed the alternative hypothesis. The power of this result on a retrospective calculation was 76% at a 5% significance level to detect a difference in endothelial function (FMD) of 3 +/- 3.3%. A smaller change in FMD, even though not clinically significant, could have occurred in obese children with the intervention but the number of subjects included in this study was unable to detect this difference if it did exist. Folic acid effects on endothelial function have not been shown before in adults or children. The evidence of use of folic acid in obesity is limited to a trial in overweight adults that showed beneficial effects of folic acid (2.5mg/day) in addition to a hypo-caloric diet on inflammatory markers and insulin sensitivity. Endothelial function was not assessed in this trial 386.

The finding that short term supplementation of daily folic acid (5mg/day) did not improve endothelial function in obese children as it did in children with type 1 diabetes is very interesting. Possible explanations for this finding are: the differences in baseline characteristics, compliance between two groups, and responses to folic acid. Moreover it could be that a higher dose of folic acid is required in obese children compared to diabetic children.

Even though obese children have a similar degree of vascular dysfunction compared to diabetic children, other baseline characteristics besides the ones that define obesity and diabetes were different in these 2 groups. Obese children have dyslipidemia, a higher level of inflammation, higher tHcy and lower folate status in comparison to diabetic children. Inflammation and lipid abnormalities may be more important components of the underlying mechanism for endothelial dysfunction in obesity than in type 1 diabetes.

Compliance was slightly lower in obese children compared to diabetic children. This might be one of the causes of differences in endothelial response to the same folic
acids supplementation. However, lower compliance in the obese children was sufficient to produce effects of folic acid i.e. serum folate and red cell folate increased and tHcy decreased.

The differences in the response to folic acid supplementation between obese and diabetic children were unexpected (Table 10). Folic acid did not improve endothelial function in obese children compared to diabetic children in spite of increasing serum folate and RCF to a higher level than in diabetic children. Folic acid decreased tHcy levels in obese but not in the diabetic children, in whom tHcy levels are not raised. This might demonstrate differences in folic acid mechanism of action in obese children compared to diabetic children i.e. folic acid reduced tHcy levels without endothelial effects. This was also shown in children with chronic renal failure and hyperhomocysteinemia. Moreover other endothelial effects of folic acid such as antioxidant activity and increment in nitric oxide levels may have been unachievable in the obese population with the dose used in this trial; for example folic acid can be insufficient to increase antioxidant activity when baseline antioxidant status is markedly reduced and tHcy levels are high. Perhaps a higher dose of folic acid will have some endothelial effects in obesity as dose dependent effects of folic acid occur for its homocysteine lowering effect. However, this seems unlikely as 5 mg a day of folic acid is already ten times higher the recommended dietary allowance of folic acid.
Table 10. Folic acid effects in obese children and children with type 1 diabetes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Folic acid effects in obese children</th>
<th>Folic acid effects in T1DM children</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD</td>
<td>No change</td>
<td>Increased by 2.58%</td>
</tr>
<tr>
<td>GTN</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Serum folate</td>
<td>Increased by 100%</td>
<td>Increased by 50%</td>
</tr>
<tr>
<td>RCF</td>
<td>Increased by 67%</td>
<td>Increased by 56%</td>
</tr>
<tr>
<td>tHcy</td>
<td>Decreased by 12%</td>
<td>No change</td>
</tr>
<tr>
<td>Compliance</td>
<td>80.12%</td>
<td>91.1%</td>
</tr>
</tbody>
</table>

In addition the complexity of vascular dysfunction in obesity might require both diet and exercise for other strategies to work. Intensive diet and exercise have shown to improve endothelial function in overweight and obese children.

6.2 Implications of this research

The finding that children with type 1 diabetes have similar degrees of vascular dysfunction compared to children with mild to moderate obesity is relevant for clinical practice. Children with type 1 diabetes, their families and treating doctors are fully aware of the severity of diabetes and its vascular complications. On the contrary, most obese children and their families do not recognize obesity as a disease and have difficulties appreciating the high risk of vascular complications. In view of these different perceptions of disease this new finding has several and important implications for treating doctors and obese children. It gives evidence of the presence of asymptomatic obesity complications at
an early age in children with mild to moderate degree of obesity and highlights the severity of the disease. It can promote treating doctors to become more proactive managing obesity. And, it will potentially help obese children and their families become more receptive to interventions.

This research has also shown that children with type 1 diabetes who are obese have worse vascular function compared to non-obese children with type 1 diabetes independent of their diabetes control. This evidence has implications in the clinical practice as it will reinforce the need to be aggressive in weight management in diabetes and the need to optimize insulin treatment avoiding weight gain using either newer insulin analogues or pump therapy.

The finding that lower weight and BMI, even in the normal range, are related to better vascular function has major implications in the promotion of health, which should not only focus on obesity but also on achieving lower weight in children within the normal weight range. Any strategies that contain or reduce weight gain early in life may be beneficial for vascular function and therefore prevent cardiovascular disease.

The conclusion that lower blood glucose levels within the normal range are related to better vascular function in non-diabetic children is also an important finding with implications in the promotion of health in childhood. Prior to this research, the idea of maintaining lower blood glucose levels has been promoted only in adults with impaired fasting glucose and carbohydrate intolerance but not in children. Maintaining lower blood glucose levels even in the normal range from childhood may also have an effect on vascular function and later development of cardiovascular disease.

The finding that folic acid supplementation improves endothelial function in children with type 1 diabetes without side effects is a very promising finding, which needs
more investigation including other endpoints such as vascular structural changes (intima media thickness). Folic acid is a cheap, well tolerated and feasible strategy for prevention of early atherosclerosis in children with type 1 diabetes potentially reducing long term morbidity and mortality from cardiovascular disease in diabetes.

The finding that folic acid supplementation did not improve endothelial dysfunction in children with obesity in spite of improvement of tHcy levels, highlight the complexity of vascular dysfunction in obesity and the difficulties for its management, without adequate exercise and weight loss.

6.3 Future research

The finding of BMI and blood glucose as determinants of vascular function even in healthy children needs to be further investigated. Longitudinal observation of BMI and blood glucose changes along with other determinants of vascular function in healthy children, children with abnormal BMI (obese children) or T1DM is required as BMI and insulin resistance change dramatically during puberty.

Even though a randomized double blind placebo controlled trial provides evidence about short term beneficial effects of folic acid supplementation on endothelial function in children with type 1 diabetes, longer term folic acid supplementation trials with vascular structural endpoints are required before adopting a change in clinical practice regarding management of vascular dysfunction or prevention of atherosclerosis in children with type 1 diabetes. These trials can also provide further information about mechanisms of action of folic acid on endothelial function which are interestingly independent of the tHcy lowering effects and probably relate to antioxidant or nitric oxide effects.
Investigation of other simpler strategies in the management of endothelial
dysfunction in children with obesity is required in addition to diet and exercise, which are
difficult to implement in this population. More research is required into mechanisms of
vascular dysfunction in children with obesity in order to develop targeted therapies,
especially when diet and exercise are not successful. This research should include
oxidative stress and inflammatory markers which may play a more important role in
vascular dysfunction in obesity in comparison to type 1 diabetes.
Chapter 7. Summary and final conclusions
The aims of this thesis were to evaluate early signs of atherosclerosis, endothelial and smooth muscle dysfunction, in children with type 1 diabetes and children with obesity; and to test the effects of early folic acid supplementation on vascular function in these children.

From the literature review it was evident that vascular dysfunction was not well studied in diabetic and obese children. Furthermore simple strategies like folic acid as an intervention for vascular dysfunction, which has been evaluated in adults, has not been evaluated in children at risk of cardiovascular disease.

The first part of the thesis addressed vascular function and its determinants in children with type 1 diabetes, children with mild to moderate obesity, and healthy children. I have shown that children with type 1 diabetes have comparable and severe vascular dysfunction to children with obesity, but different determinants. Children with type 1 diabetes have a more favourable cardiovascular risk factor profile with a lower degree of inflammation, lower LDL to HDL ratios, lower triglycerides, lower tHcy and higher folate status in comparison to children with obesity. This highlights potential different underlying mechanisms for vascular dysfunction in these children. In addition children with type 1 diabetes who are obese are at higher risk of vascular dysfunction than non obese diabetic children. BMI, weight and blood glucose levels are important determinants of vascular function in non diabetic non obese children.

The second part of this theses showed that folic acid improves endothelial function in children with type 1 diabetes independent of their diabetes control. The endothelial effects of folic acid in these children are independent of homocysteine and likely to relate to antioxidant or nitric oxide effects, which were not directly measured in this work.
The last part of this theses showed that folic acid does not improve endothelial function in children with obesity in spite of a significant increase in folate status and decrease in homocysteine levels. The lack of folic acid effect on endothelial function in obese compared to diabetic children is consistent with different underlying mechanisms for vascular dysfunction in these two groups of children. It also suggests different mechanisms of action of folic acid in obesity in comparison to type 1 diabetes, i.e. reduction of homocysteine levels is more important in obesity when tHcy is higher than in type 1 diabetes. Children with obesity are less likely to respond to additional interventions that will not include weight management.

In conclusion children with type 1 diabetes and children with obesity have similar degree of vascular dysfunction but only children with type 1 diabetes respond to folic acid. This highlights the likely different underlying mechanisms of vascular dysfunction and folic acid effects on the endothelium in these children, that need to be further evaluated. BMI, weight and blood glucose are important determinants of vascular function in non diabetic, non obese children. Follow up of these risk factors in a cohort of healthy children and children at risk of cardiovascular disease over time extend these findings and help to implement strategies to promote weight and blood glucose control as an important prevention of cardiovascular disease from childhood and even before pathologies develop.

Finally if we can also show that folate slows the progression of vascular structural changes in children with type 1 diabetes, then we can justify routinely supplementing folate in these children.
8 Chapter 8. Appendices
8.1 Appendix A: Ethics Committee approvals, Drug and Therapeutics

Committee approval and information sheet of cross sectional study

24th November 2004

A/Prof J Cooper
Endocrinology & Diabetes
WCH

Dear Jenny


At its meeting on 24th November 2004, the WCH Research Ethics Committee approved your request to extend ethical approval for a further three years. Please note the amended approval number above reflecting the extension, and use it in any future communications.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
WCH RESEARCH ETHICS COMMITTEE

Alexia Sophie Petra Vargas, September 2007
26th May 2000

Dr J Cooper
University Dept of Paediatrics
WCH

Dear Jenny


Thank you for your letter dated 9th May in which you clarified matters raised by the scientific assessment obtained by the WCH Research Ethics Committee. All matters have been addressed and final approval is given for the study to proceed. I enclose herewith the CTN form for the study, duly signed.

I remind you approval is given subject to:

• immediate notification of any adverse events to subjects;
• immediate notification of any unforeseen events that might affect continued ethical acceptability of the project;
• submission of any proposed changes to the original protocol. Such changes must be approved by the Committee before they are implemented;
• submission of a brief annual report on the state of progress of the study, and a final report when it is completed.

Approval is given for a period of three (3) years only, and if the study is more prolonged than this, a new submission will be required. Please note the approval number above indicates the month and year in which approval expires and it should be used in any future communication.

Yours sincerely

ÅFNE BAMPTON
CHAIR
WCH RESEARCH ETHICS COMMITTEE

cc: Ms A Sutcliffe, DTC

Alexia Sophia Felix Vargas, September 2007
Vascular function in children at risk of cardiovascular disease and the effects of folic acid

Women's and Children's Hospital
ADELAIDE

Pharmacy Department Ph: (08) 8204 7330
Fax: (08) 8204 6049
Email: phmcyadren2@email.adelaide.edu.au

13 April 2000
Ms Anne Bampton
Chairman
Research Ethics Committee
WOMEN'S & CHILDREN'S HOSPITAL

Dear Anne,

Re: The Association of Flow Mediated Dilatation and Homocysteine Metabolism in Children and Adolescents with Diabetes, Dr Wilshire

At its meeting on 13 April 2000 the DTC decided to recommends approval of this CTN study to the REC

Yours sincerely

Anne Sculiffe
on behalf of
SECRETARY
DRUG & THERAPEUTICS COMMITTEE

C/- Pharmacy Department - ext 7339
cc. Dr E Wilshire

When replying to Ms Bampton please send copy to DTC

Investigator please respond to concerns raised quoting the corresponding DTC number above.

Alexia Sophie Peha Vargas, September 2007
Research Study: Information Sheet

The Association of Flow Mediated Dilatation with Homocysteine in Children and Adolescents with Diabetes

1) What is the study for and why is it being done?
As you know we are undertaking a study looking at an amino acid called homocysteine and blood vessel function in children and adolescents with diabetes, to help work out ways of preventing the blood vessel complications that occur in diabetes. We would like to extend this study by using a new technique called flow-mediated dilatation (FMD) to look directly at the way the blood vessels are working. As with the blood tests, we need a group of children and teenagers who don’t have diabetes and for the comparison group.

2) What would I be asked to do if I took part in the study?
Flow mediated dilatation uses ultrasound to examine an artery in an arm and works as follows:

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

The whole procedure takes about 30 minutes altogether, although the ultrasound measurements take about 1 minute each.

3) Are there any risks or side-effects associated with Flow Mediated Dilatation?
The procedure is very safe and has been used in a large number of children and adults, including some adults and adolescents with diabetes. The first part of the test, where the blood pressure cuff is left up can be uncomfortable, although most children have not been too bothered by it. The medication, GTN, is very safe and remains active for only a very short period of time. It can cause brief headaches in some people, although in practice this has not been a common problem when used as part of this ultrasound test in children. GTN can also cause lower blood pressure, which we will be monitoring, light-headedness, facial flushing and a fast heart rate, although these have also all been very uncommon when used in children.

4) What will be done with this information?
We will then compare the results of this test with some of things we have been measuring in your blood. We will try to work out if homocysteine is involved in diabetes complications. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. All of the information will remain confidential.
5) Do I have to take part in the study?

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

6) Can I change my mind later if I decide to participate?

Yes, you can choose to leave the study at any time.

7) Will the study benefit me in any way?

We can’t be certain that you will get any benefit from taking part. However, by examining the blood vessel directly we hope that we will get better information that will help us have new ways, to help prevent the complications of diabetes.

8) Do you have permission to do the study?

We have got permission from the Research Ethics Committee at the Women’s and Children’s Hospital to do this study. Other doctors have also looked carefully at the study and thought it was a good study to do. We have been given some money from Juvenile Diabetes Foundation Australia to do this research, as they thought this was an important area to study.

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

Please contact Dr Alexia Peña at any time. She can be paged through the Women’s and Children’s Hospital on (08)-81617000, pager 4199, or extension 18134, or after-hours is available on 0405373297. You can also call Dr Jenny Couper on 81617000, page 4127, or 81616402.
8.2 Appendix B: Consent forms of cross sectional study

WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

CONSENT FORM

I ____________________________________________________________________________

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

“The Association of Flow Mediated Dilatation and Homocysteine Metabolism in Children and Adolescents with Diabetes”

as a normal control subject.

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

2. I understand that my child will 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 and my child.

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

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

6. I understand that there will be no payment to my child for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
9.  
a) I consent to specimens of blood and urine being taken from my child and being used in the above project.

b) I do / do not consent to the blood and 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.

10.  I understand that my child is free to stop donating samples, or undertaking Flow Mediated Dilatation studies, at any stage, without giving any reason, and that his/her action of donating/not donating a sample will not affect (i) his/her prospects in any position or (ii) any other conceivable situation.

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

Relationship to subject: ..................................................

Full name of subject: ......................................................

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: .............................

Version dated 29/2/00
WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

CONSENT FORM

I ___________________________________________________________________

hereby consent to my involvement in the research project entitled:

“The Association of Flow Mediated Dilatation and Homocysteine Metabolism in Children and Adolescents with Diabetes”

as a normal control subject.

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 while information gained in the study may be published, I will not be identified and information will be confidential.

5. 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 hospital.

6. I understand that there will be no payment to me for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
9. a) I consent to specimens of blood and urine being taken from me and being used in the above project.

b) I do / do not consent to the blood and 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.

10. I understand that I am free to stop donating blood and urine samples, or undertaking Flow mediated Dilatation studies, at any stage, without giving any reason, and that my action of donating/not donating a sample will not affect (i) my prospects in any position or (ii) any other conceivable situation.

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

Full name of subject: ..............................................................

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

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

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

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

Version dated 29/2/00
CONSENT FORM

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

“The Association of Flow Mediated Dilatation and Homocysteine Metabolism in Children and Adolescents with Diabetes”

1. The nature and purpose of the research project described on the attached Information Sheet have been explained to me and my child. 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 and my child.

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

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

6. I understand that there will be no payment to my child for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
9  
a) I consent to specimens of blood and urine being taken from my child and being used in the above project.

b) I do / do not consent to the blood and 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.

Signed: ..........................................................  
Relationship to Patient: ......................................................  
Full name of patient: ..............................................................  
Dated:...............................  
I certify that I have explained the study to the parent and child and consider that he/she understands what is involved.  

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

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

Dated 29/2/00
WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

CONSENT FORM

I ___________________________________________________________________

hereby consent to my involvement in the research project entitled:

1. “The Association of Flow Mediated Dilatation and Homocysteine Metabolism in Children and Adolescents with 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 take 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 while information gained in the study may be published, I will not be identified and information will be confidential.

5. 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 hospital.

6. I understand that there will be no payment to me for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
a) I consent to specimens of blood and urine being taken from me and being used in the above project.

b) I do / do not consent to the blood and 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.

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

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

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

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

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

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

Dated 8/2/99
8.3 Appendix C: Suitable site for imaging the brachial artery with ultrasonic markers
8.4 Appendix D: Pulsed doppler signal showing arterial flow before, during and after arterial occlusion

Ultrasound of the brachial artery at 60 degrees

Normal arterial flow before arterial occlusion

Decrease arterial flow during arterial occlusion

Increase in arterial flow after release of arterial occlusion (reactive hyperaemia)
8.5 Appendix E: Vessel diameter before and after the increase in blood flow – 
Flow mediated dilatation

Vessel diameter measurement pre FMD (0.29 cm) 
Vessel diameter measurement post FMD (0.36 cm) 
in the same position as measurement pre FMD
8.6 Appendix F: Vessel diameter before and after glyceryl trinitrate spray – Glyceryl trinitrate mediated dilatation

Vessel diameter measurement pre GTN (0.32 cm)  Vessel diameter measurement post GTN (0.40 cm) in the same position as measurement pre GTN
8.7 Appendix G: Ethics Committee approvals, Drug and Therapeutics

Committee approval and information sheet of folic acid intervention trial in
children with type 1 diabetes

2nd July 2001
Dr J Cooper
Dept of Diabetes & Endocrinology
WCH

Re: A randomised, controlled, crossover clinical trial of folic acid (folate) to improve
do endothelial function in children and adolescents with type 1 diabetes. REC1215/5/2004

I refer to your letter dated 17th May 2001 in which you responded to matters raised by the Drug &
Therapeutics Committee and to the revised Information Sheet you subsequently provided. I also refer to a
letter from the DTC dated 22nd June advising its concerns had been addressed. Final approval is given as
you may proceed with the study once you have obtained TGA approval of the enclosed CTN application.

I remind you approval is given subject to:

• immediate notification of any serious or unexpected adverse events to subjects;
• immediate notification of any unforeseen events that might affect continued ethical acceptability of the
  project;
• submission of any proposed changes to the original protocol. Such changes must be approved by the
  Committee before they are implemented;
• immediate advice, giving reasons, if the protocol is discontinued before its completion;
• submission of a brief annual report on the state of progress of the study, and a final report when it is
  completed.

Approval is given for a period of three (3) years only, and if the study is more
prolonged than this, a new submission will be required. Please note the approval number above
indicates the month and year in which approval expires and it should be used in any future
communication.

Yours sincerely

PAUL NEUENING
Chair
WCHR RESEARCH ETHICS COMMITTEE

Cc: DTC

Alexia Sophie Pfeiffer Vargas, September 2007
22 June 2001

Chairman,
Research Ethics Committee
WOMEN'S & CHILDREN'S HOSPITAL

Dear Sir/Madam,

A randomised, controlled, crossover clinical trial of folic acid (folate) to improve endothelial function in children and adolescents with type 1 diabetes. REC1215

The DTC has considered the investigator's responses in her letter of 17 May 2001 and is satisfied that all its concerns have been addressed.

Yours sincerely

Anne Stoliffe
on behalf of
SECRETARY
DRUG & THERAPEUTICS COMMITTEE

C/- Pharmacy Department - ext 7350
cc  A/Prof J Cooper

Alastia Sophie Feke Vargas, September 2007
9 October 2001

Our Ref: 01/08/998
Please include the reference number and study title in all correspondence

Eiko Wiltshire
Department of Paediatrics
Wellington School of Medicine

Dear Eiko

01/08/998 - A randomised, controlled, crossover clinical trial of folic acid (folate) to improve endothelial function in children and adolescents with type 1 diabetes

Thank you for your letter received in this office on 9 October, and enclosing an amended consent form for this study.

As you have satisfactorily addressed the points raised in my letter of 28 September and the amendment to the consent form satisfies the point about blood storage and any future research, final approval for this study is granted by the Chairperson under delegated authority from the Wellington Ethics Committee. It is a condition of Ethics Committee approval that you provide a brief progress report no later than 1 October 2002 and at the completion of the study, a copy of any report/publication for the Committee's records. Please notify the Committee if the study is abandoned or changed in any way.

The Committee certifies that it is satisfied that this project is not conducted principally for the benefit of the manufacturer or distributor of the medicines or item in respect of which the trial is carried out. Participants may be eligible for ACC coverage should injury or harm result from their participation in this study.

I hope our research goes well.

Yours sincerely

Sharon Cole
CHAIRPERSON

Wellington Ethics Committee
Room 425, Fourth Floor
Community & Support Services
Wellington Hospital
Private Bag 7022
Wellington South
Phone 044 555 586 ext. 5858
Fax 044 555 5840
Email: claire@wecc.org.nz

Accredited by Health Research Council

Alexia Sophie Pehe Vargas, September 2007
Research Study: Information Sheet

A randomised, controlled, crossover clinical trial of folic acid (folate) to improve endothelial function in children and adolescents with type 1 diabetes

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

Our diabetes unit has been studying how blood vessels work in children for several years. We have found that if children with diabetes have good folate stores their blood vessels work optimally and we therefore want to find out whether giving the vitamin, folate (folic acid) to children improves the way their blood vessels function. The importance of this is that very early changes in blood vessel function (as determined by an ultrasound test that we use) are related in the long term to how healthy the blood vessels are in adult life.

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

You/your child would take one folate tablet every day for 2 months and for another 2 months take a blank tablet or placebo tablet. You and the researchers and your doctor would not know whether you were taking the folate tablet or the blank tablet, as this is to ensure that it is the folate which is helping blood vessel function and not some other effect. The way that we determine how the blood vessel is working is to use a technique called flow-mediated dilatation via an ultrasound test to examine an artery in your arm and this works as follows:

1. An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it. An electrocardiogram recording is also taken.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow is measured again with ultrasound, to check this difference. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after a period of 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

The whole procedure takes about 30 minutes, although the ultrasound measurements take about 1 minute each.

This ultrasound test would be performed at the beginning of the study and after the 2-month period of taking either folate tablets or blank tablets. It would mean you would perform 4 studies in total. At the beginning of the study and after the 2 month period of taking either folate tablets or blank tablets we would measure in a blood test the levels of folate in your body and also the levels of homocysteine which is an amino acid which is important in blood vessel function in children and adolescents. This involves four blood tests, with local anaesthetic cream (EMLA) if you wish to use this to numb the skin. Maybe your child has probably used this cream in the past. The local anaesthetic cream rarely causes some irritation of the skin.

Therefore in summary the whole study takes 6 months. The first 2 months you take either a folate tablet or a blank tablet every day for 2 months. Then there is a 2 month period where you take neither tablet to ensure that any effects of the folate tablet have gone before the second part of the study, when you take either folate tablets or blank tablets depending on which one you took in the first part of the study.

Alexia Sophie Peña Vargas, September 2007
This is called a crossover study and ensures that every body gets the therapy and the blank tablet. It also allows us to see for each person in the study, the effect of folate on their blood vessel function, compared with the effect of the blank tablet.

3) Are there any risks of side effects associated with Flow Mediated Dilatation?
The procedure is very safe and has been used in a large number of children and adults, including adults and adolescents with 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, GTN, is very safe and remains active for only a very short period of time. It can cause brief headaches in some people, although in practice this has not been a problem when used as part of this ultrasound test in children. GTN can also cause lower blood pressure, which we will be monitoring, light-headiness, facial flushing and a fast heart rate, although these have also all been very uncommon when used in children.

4) Are there any risks or side effects associated with folate tablets 5mg daily?
Folate is a vitamin which occurs naturally in many foods including leafy green vegetables and it is also in many cereals that have been supplemented with folate. It is safe and no side effects of folate have been described at this dose. The dose has been chosen as an amount of folate that we know in adults, improves blood vessel function. At higher dose of folate: nausea, diarrhoea and wheezing have been rarely reported.

5) What will be done with this information?
We will then compare the results of this test with the substances we measure in your blood. We will try to work out if folate and homocysteine are involved in improving blood vessel function. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. All of the information will remain confidential.

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

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

8) Will the study benefit me in any way?
We can't be certain that you will get any benefit from taking part. However, we may improve blood vessel function in children with diabetes and this may be very useful information for you as an individual to have.

9) Do you have permission to do the study?
We got permission from the Research Ethics Committee at the Women’s and Children’s Hospital to do this study. Other doctors have also looked carefully at the study and thought it was a good study to do. We have been given money from Juvenile Diabetes Foundation Australia and Channel 7 Children’s Research Foundation of SA to do this research, as they thought this was an important area to study.

10) What if I have other questions about the study?
Please contact Dr Alexia Pena, Research Fellow, at any time. She can be paged through the Women's and Children's Hospital on (08) 8204 7000, pager 4199, or extension 6035, or after-hours is available on (08) 8356 7753. You can also call Associate Professor Jenny Couper on (08) 8204 7000, page 4127 or (08) 8204 6242.

Alexia Sophie Peña Vargas, September 2007
Research Study: Information Sheet

You are invited to take part in a research study to see whether the vitamin folic acid improves the way blood vessels work in young people with diabetes.

1) What is the study for and why is it being done?
Dr Wiltshire and the diabetes unit where he worked in Adelaide have been studying how blood vessels work in children with diabetes. We have found that if children with diabetes have good folate stores their blood vessels work optimally and we therefore want to find out whether giving the vitamin, folate (folic acid) to young people with diabetes improves the way their blood vessels function. The importance of this is that very early changes in blood vessel function (as determined by an ultrasound test that we use) are related in the long term to how healthy the blood vessels are in adult life and anything that improves the way blood vessels work may reduce the chances of blood vessel complications in the future.

2) What would I be asked to do if I took part in this study?
The whole study takes 6 months. The first 2 months you take either a folate tablet or a blank tablet every day for 2 months. Then there is a 2 month period where you take neither tablet to ensure that any effects of the folate tablet have gone before the second part of the study. Then you take the other tablet for 2 months. This is called a crossover study and ensures that everybody gets the therapy and the blank tablet. It also allows us to see for each person in the study, the effect of folate on their blood vessel function, compared with the effect of the blank tablet. You would be randomly selected to have either the folate for the first two months and the blank tablet for the second two months, or to have the blank tablet first and the folate second. You, the researchers and your doctor would not know whether you were taking the folate tablet or the blank tablet.

We use an ultrasound test to see how the blood vessel is working. It works as follows:

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

The whole procedure, which happens in the ultrasound department of Wellington Hospital, takes about 30 minutes, although the ultrasound measurements take about 1 minute each. We record the ultrasound pictures onto videotape and do the measurements later. We keep the videotapes stored securely so the measurements can be checked. You would not be identified on the videotape.
This ultrasound test would be performed at the beginning of the study and after the 2-month period of taking either folate tablets or blank tablets. It would mean you would have 4 studies in total. At the beginning of the study and after the 2 month period of taking either folate tablets or blank tablets we would measure in a blood test the levels of folate in your body and also the levels of homocysteine which is an amino acid and which is important in blood vessel function in children and adolescents. This involves three blood tests. We would give you local anaesthetic cream if you wish to use this to numb the skin for the blood tests.

The ultrasound and blood tests are best done in the fasting state. We would arrange them to be first thing in the morning, and we would organise breakfast to be as soon as the test was finished. You would just need to bring your insulin.

3) **Are there any risks of side effects associated with Flow Mediated Dilatation?**

The procedure is very safe and has been used in a large number of children and adults, including adults and young people with diabetes. The first part of the test, where the blood pressure cuff is left up can be uncomfortable, although most children have not been too bothered by it. The medication, GTN, is very safe and remains active for only a very short period of time (10-15 minutes). It can cause brief headaches in some people, although in practice this has not been a problem when used as part of this ultrasound test in children. GTN can also cause lower blood pressure, which we monitor, light-headedness, facial flushing and a fast heart rate, although these have also all been very uncommon when used in children.

4) **Are there any risks or side effects associated with folate tablets 5mg daily?**

Folate is a vitamin which occurs naturally in many foods including leafy green vegetables and it is also in many cereals that have been supplemented with folate. It is completely safe and no side effects of folate have been described at this dose in young people. A theoretical side-effect of using folic acid is that it might mask the symptoms of vitamin B12 deficiency, but this is an extremely rare condition in young people and we will check your vitamin B12 levels at the beginning. The dose has been chosen as an amount of folate that we know improves blood vessel function in adults.

5) **Are there any costs associated with the study?**

There are no costs to you associated with being involved in the study. We will reimburse you for any transport costs you need to attend the appointments and provide your breakfast on the ultrasound days.

6) **What happens if anything goes wrong?**

In the unlikely event of a physical injury as a result of your participation in this study, you will be covered by the accident compensation legislation with its limitations. If you have any questions about ACC please feel free to ask the researcher for more information before you agree to take part in this trial.

7) **What will be done with this information?**

We will then compare the results of this test with some of the things we have been measuring in your blood. We will try to work out if folate and homocysteine are involved in improving blood vessel function. The ultrasound tests and blood test results will be stored securely and will not be used for any other study without your permission. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. All of the information will remain confidential and no information which could identify you will be used in any reports from the study.
8) **Do I have to take part in the study?**
No, not at all. You should only take part in this part of the study if you want to be invited. If you choose not to take part it will not affect your usual care or treatment in any way.

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

10) **Will the study benefit me in any way?**
We can’t be certain that you will get any benefit from taking part. However, we feel likely that folate will improve blood vessel function in children with diabetes and this will be very useful information for you as an individual to have.

We will let you know the results of your ultrasound studies once all the studies have been done and the overall study results. There will be a delay in getting this information to you.

11) **Do you have permission to do the study?**
We have permission from the Wellington Ethics Committee to do this study. Other organisations have also looked carefully at the study and thought it was a good study to do. We have been given some money from the Wellington Medical Research Foundation to do this research and they thought this was an important area to study.

12) **What if I have other questions about the study?**
Please contact Dr Esco Wiltshire, Paediatrician, at any time. He can be paged at Wellington Hospital on (04) 3855999, pager 6912, or extension 6192. If you have any questions or concerns about your rights as a participant in this study you may wish to contact the Health and Disability Advocate Telephone: Mid and lower North Island 42 36 38 (0800 4 ADNET) or the Wellington Ethics Committee, Wellington Hospital, 3855999, Ext 5185.
8.8 Appendix H: Consent forms of folic acid intervention trial in children with type 1 diabetes

**WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE**

**CONSENT FORM**

I ..............................................................................................................................................................

hereby consent to my involvement in the research project entitled:

“Randomized, controlled, cross-over clinical trial of Folic Acid to improve endothelial function in children and adolescent 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 take 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 while information gained in the study may be published, I will not be identified and information will be confidential.

5. 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 hospital.

6. I understand that there will be no payment to me for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
9   a) I consent to a specimen of blood being taken from me and being used in the above project.

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

10. I understand that I am free to stop donating blood or undertaking Flow mediated Dilatation studies, at any stage, without giving any reason, and that my action of donating/not donating a sample will not affect (i) my prospects in any position or (ii) any other conceivable situation.

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

Full name of subject: ..............................................................

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

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

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

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

“Randomized controlled, cross over clinical trial of Folic Acid to improve endothelial function in children and adolescent with Type 1 Diabetes”

1. The nature and purpose of the research project described on the attached Information Sheet have been explained to me. I understand it, and agree to my child taking part.
2. I understand that my child will 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 and my child.
4. I understand that while information gained in the study may be published, my child will not be identified and information will be confidential.
5. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my child's relationship with this hospital.
6. a) I consent to specimens of blood being taken from my child and being used in the above project.
   b) I do / do not consent to the blood samples being used in any other research project, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.
7. I understand that my child is free to stop donating samples, or undertaking Flow Mediated Dilatation studies, at any stage, without giving any reason, and that his/her action of donating/not donating a sample will not affect (i) his/her prospects in any position or (ii) any other conceivable situation.

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

Relationship to subject: ......................................................

Full name of subject: ..............................................................

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

“A randomised, controlled, crossover clinical trial of folic acid (folate) to improve endothelial function in children and adolescents with type 1 diabetes”

REQUEST FOR INTERPRETER
(to be included on all consent forms)

<table>
<thead>
<tr>
<th>Language</th>
<th>I wish to have an interpreter.</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>I wish to have an interpreter.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>E hiaha ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.</td>
<td>Ae</td>
<td>Kao</td>
</tr>
<tr>
<td>Samoan</td>
<td>Oute mana’o ia iai se fa’amatala upu.</td>
<td>Ioe</td>
<td>Leai</td>
</tr>
<tr>
<td>Tongan</td>
<td>Oku ou fiema’u ha fakatonulea.</td>
<td>Io</td>
<td>Ikai</td>
</tr>
<tr>
<td>Cook Island</td>
<td>Ka inangaro au i tetai tangata uri reo.</td>
<td>Ae</td>
<td>Kare</td>
</tr>
<tr>
<td>Niuean</td>
<td>Fia manako au ke fakaaoaga e taha tagata fakahokohoko kupu.</td>
<td>E</td>
<td>Nakai</td>
</tr>
<tr>
<td>Other languages</td>
<td>Other languages to be added following consultation with relevant communities.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. I have read and I understand the information sheet dated June 28th, 2001, for volunteers taking part in the study designed to test whether folic acid improves blood vessel function in young people with diabetes. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

2. I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health care or continuing health care.

3. I have had this project explained to me by ______________________________________________________________________.

4. I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

5. I understand that the treatment, or investigation, will be stopped if it should appear harmful to me.

6. I understand the compensation provisions for this study.

7. I have had time to consider whether to take part.

8. I know whom to contact if I have any side effects to the study.

9. I know whom to contact if I have any questions about the medication or the study.
10. I consent to storage of ultrasound images for further use in this research project…….YES/NO

11. I consent to storage of ultrasound images for use in future research projects that have received ethical approval from the Wellington Ethics Committee………………………YES/NO

12. I consent to the researchers storing a specimen of my blood for its later use as a part of this study or other research………………………………………………………………………………YES/NO

OR

13. I consent to blood samples being destroyed at the end of the study………………YES/NO

14. I would like the researcher to discuss the outcomes of the study with me ………YES/NO

15. I understand that my GP will be informed of my participation in this study/the results of my participation in this study, if I wish…………………………………………………………………………………………YES/NO

16. I ________________________________ hereby consent to take part in this study.

Date

Signature (or proxy consent)  Signature of witness

Full names of Researchers  Name of witness

Contact Phone Number for researchers: 3855999, page 6912

Project explained by:
Project role
Signature
Date

(Note: A copy of the consent form to be retained by participant and (in the case of patients) a copy to be placed in the medical file.)

Version dated 28/6/2001
8.9 Appendix I: Ethics Committee approval, Drug and Therapeutics Committee approval and information sheet of folic acid intervention trial in obese children

21st November 2003

Dr A Pena
Endocrinology & Diabetes
WCH

Re: Effects of folic acid on endothelial function in obese children and adolescents
REC13/131/1/2004

Thank you for your letter dated 12th November 2003 in which you responded to matters raised by the WCH Research Ethics Committee at its October 2003 meeting. All matters have been addressed and the study may proceed once you have forwarded the attached CN form and payment to TGA. Until the form and payment have been forwarded, anyone receiving the proposed therapeutic agent will be considered, in a medical emergency, to have been given an unregistered drug. You must also submit a copy of the TGA acknowledgment to the Research Committee as soon as you receive it.

I remind you approval is given subject to:

- Immediate notification of any adverse or unexpected adverse events to subjects;
- Immediate notification of any unforeseen events that might affect continued ethical acceptability of the project;
- Submission of any proposed changes to the original protocol. Such changes must be approved by the Committee before they are implemented;
- Immediate advice, giving reasons, if the protocol is discontinued before its completion;
- Submission of a final annual report on the state of progress of the study, and a final report when it is completed.

Approval is given for a period of three (3) years only, and if the study is more prolonged than this, a new submission will be required. Please use the approval number above indicating the month and year in which approval expired so it should be used in any future communication.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
WCH RESEARCH ETHICS COMMITTEE

Cc: DTC

Alexia Sophie Peha Vargas, September 2007
Vascular function in children at risk of cardiovascular disease and the effects of folic acid

14 November 2003

Dr T Zullevics
Chair
RESEARCH ETHICS COMMITTEE

Dear Tamara,

Effects of folic acid on endothelial function in obese children and adolescents
(Assisted Prof J Cooper) REC1513

The DTC Clinical Trials Review Committee at its meeting of 13 November 2003 considered the letter from the Investigators dated 27 October 2003. All issues have now been answered, and the DTC recommends approval of this trial.

Yours sincerely

Assoc Prof R McKinnon
Chairman
Clinical Trials Group
DRUG & THERAPEUTICS COMMITTEE

C/- Pharmacy Department - ext 17350
c.c. Ms Brenda Penny, REC
Dr J Cooper

When replying to Chair of REC, please forward a copy to DTC

Alexia Sophie Peña Vargas, September 2007
Information Sheet

FOLATE COVER STUDY

Folic acid for children who are overweight

Effects of folic acid on blood vessel function in overweight children and adolescents

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

Our endocrine unit has been studying how blood vessels work in children for several years. We have found that conditions such as diabetes affect the way the blood vessels work. We have also shown that a vitamin like folic acid improves blood vessel function in children with type 1 diabetes. Overweight children also have abnormal blood vessel function. We therefore want to find out whether giving folic acid to overweight children improves the way their blood vessels function. The importance of this is that very early changes in blood vessel function (as determined by an ultrasound test that we use) are related in the long term to how healthy the blood vessels are in adult life.

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

You/your child would take either a folic acid or a placebo tablet every day for 2 months. You, the researchers and your doctor would not know whether you were taking the folic acid tablet or placebo tablet (a tablet without any effect or “dummy” tablet). This is to ensure that it is the folic acid which is helping blood vessel function and not some other effect. The way that we determine how the blood vessel works is a technique called flow-mediated dilatation. This technique uses an ultrasound to examine an artery in your arm and include four parts:

1) An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it. An electrocardiogram recording is also taken. This is a measurement of the electrical activity of the heart using 3 electrodes placed on the trunk.

2) A blood pressure cuff is blown up around the arm for 4-5 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow are measured again with ultrasound, to check this difference. If the blood vessel is not working properly, it will not dilate as much.

3) Another ultrasound picture is taken after a period of 15 minutes rest, to allow the vessel to return to its initial size.

4) And lastly, an ultrasound picture is taken after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

The whole procedure takes about 30 minutes, although the ultrasound measurements take about 1 minute each.

This ultrasound test would be performed twice before starting the tablets, after 2-months of taking either folic acid or placebo tablets and after 2 months of stopping the tablets. It would mean you would have 4 visits in total. Each visit includes the ultrasound procedure, weight and height measurement, and a blood test.

The blood test will measure levels of folic acid in your body and levels of homocysteine which is an amino acid which is important in blood vessel function in children and adolescents. It will also include a lipid assessment (cholesterol and triglycerides). The amount of blood required for these tests on each visit is 20 ml. There will be a total of 4 blood samples collected (80ml). Taking blood causes brief discomfort or pain, much like a pinprick. This can be minimised by using a simple local anaesthetic cream (EMLA or AnGEL) and by comforting the child. Temporary bruising can occur and infection is possible but extremely rare. Your child has probably used this cream in the past. The local anaesthetic cream rarely causes some irritation of the skin.

Therefore in summary the whole study takes 5 months and 4 visits. The first 2 visits are baseline assessments within 4 weeks, then the third visit will be after 2 months of having either folic acid or placebo tablet treatment, and the fourth visit will be 2 months after stopping the tablets.
assessments within 4 weeks, then the third visit will be after 2 months of having either folic acid or placebo tablet treatment, and the fourth visit will be 2 months after stopping the tablets.

**Are there any risks of side effects associated with Flow Mediated Dilatation?**

The procedure is very safe and has been used in a large number of children and adults, including overweight adults and adolescents. The first part of the test, where the blood pressure cuff is left up can be uncomfortable, although most children have not been bothered by it. The medication, GTN, is very safe and remains active for only a very short period of time. It can cause brief headaches in some people, although in practice this has not been a problem when used as part of this ultrasound test in children. GTN can also cause a temporary drop in blood pressure, which we will be monitoring, light-headiness, facial flushing and a fast heart rate, although these have also all been very uncommon when used in children and adolescents.

**Are there any risks or side effects associated with folic acid tablets 5mg daily?**

Folic acid or folate is one of the B group vitamins. It is found in many foods including leafy green vegetables, wholegrain breads, cereals, dried beans and peas. It is also found in many cereals that have been supplemented with folic acid. Folic acid has been used in pregnant women and anemic patients. It is safe and no side effects of folic acid have been described at 5 mg dose. The dose has been chosen as an amount of folic acid that we know in adults and children improves blood vessel function. At higher dose of folic acid diarrhoea and wheezing have been rarely reported.

**What will be done with this information?**

We will then compare the results of the ultrasound and blood tests while you were on folic acid or placebo tablets treatment and after you finish the treatment. We will try to work out if folic acid and homocysteine are involved in improving blood vessel function. We will also write articles about the study and publish these, or talk about the study at conferences. All of the above information will remain confidential.

**Do I have to take part in the study?**

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

**Can I change my mind later if I decide to participate?**

Yes, you can choose to leave the study at any time.

**Will the study benefit me in any way?**

We can't be certain that you will get any benefit from taking part. However, we may improve blood vessel function in overweight children and this may be very useful information for you as an individual to know.

**Do you have permission to do the study?**

We have obtained permission from the Research Ethics Committee at the Women's and Children's Hospital to do this study. You can contact the Secretary of this Committee, Ms Brenda Penny on (08) 81616521.

**What if I have other questions about the study?**

Please contact Dr Alexia Pena, Research Fellow, at any time. She can be paged through the Women's and Children's Hospital on (08) 81617000, pager 4199 or (08) 81618134, or after-hours she is available on 0405373297. You can also call Associate Professor Jenny Couper on (08) 81617000, pager 4127 or (08) 8161 6242.
8.10 Appendix J: Consent forms of folic acid intervention trial in children with obesity

WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

PARENT CONSENT FORM

I ______________________________________________________________

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

“Effects of folic acid on blood vessel function in obese children and adolescents”

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 and my child.

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

5. I understand that my child 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 hospital.

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

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

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

03_11_03 Version 2
9. a) I consent to a specimen of blood being taken from my child and being used in the above project.
   
b) I do / do not consent to my child’s blood samples being used in any other research project, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

10. I understand that my child is free to stop donating blood or undertaking Flow mediated Dilatation studies (ultrasound studies) at any stage; without giving any reason, and the action of donating/not donating a sample will not affect (i) my prospects in any position or (ii) any other conceivable situation.

Signed: ..................................................Name..............................................

Relationship to the participant child..........................................................

Full name of participant child: ........................................................................

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

I certify that I have explained the study to the parent and child and consider that they understand what is involved.

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

Title: ................................................................

Dated: ..................................................
WOMEN'S & CHILDREN'S HOSPITAL RESEARCH ETHICS COMMITTEE

CHILD CONSENT FORM

I ________________________________ hereby consent to my involvement in the research project entitled:

“Effects of folic acid on blood vessel function in obese children and adolescents”

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 take 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 while information gained in the study may be published, I will not be identified and the information will be confidential.

5. 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 hospital.

6. I understand that there will be no payment to me for taking part in this study unless specified in the Information Sheet.

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

8. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
9  a) I consent to a specimen of blood being taken from me and being used in the above project.

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

10. I understand that I am free to stop donating blood or undertaking Flow mediated Dilatation studies (ultrasound studies), at any stage, without giving any reason, and that my action of donating/not donating a sample will not affect (i) my prospects in any position or (ii) any other conceivable situation.

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

Full name of subject: ..............................................................

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

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

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

Title: .......................................................

Dated: .........................
9 Chapter 9. Bibliography


Vascular function in children at risk of cardiovascular disease and the effects of folic acid


105. Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, Deanfield JE. Impaired endothelial function occurs in the systemic arteries of


131. Srinivasan S, Hatley ME, Bolick DT, Palmer LA, Edelstein D, Brownlee M, Hedrick CC. Hyperglycaemia-induced superoxide production decreases eNOS


151. Romano M, Pomilio M, Vigneri S, Falco A, Chiesa PL, Chiarelli F, Davi G. Endothelial perturbation in children and adolescents with type 1 diabetes:


271. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction:


349. Woodside JV, Young IS, Yarnell JW, Roxborough HE, McMaster D, McCrum EE, Gey KF, Evans A. Antioxidants, but not B-group vitamins increase the resistance of low-density lipoprotein to oxidation: a randomized, factorial design, placebo-controlled trial. *Atherosclerosis*. 1999;144:419-427.


