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

Thesis submitted for the Degree of Doctor in Philosophy

At The University of Adelaide

Faculty of Health Sciences

Department of Paediatrics

September 2007

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.

Professor Jennifer Couper, has been my guide and mentor since the beginning of my research career. Her kindness, amazing energy and availability at all times even under the most stressful circumstances have inspired me deeply. I am privileged to have had the opportunity to work with her and my indebtedness to her extends well beyond this thesis.

Dr Esko Wiltshire, (Paediatric Endocrinologist), has been like an older brother to me guiding me from the most simple things in research to the most complex ones. His clarity of thought and ability to "laterally" extend an argument has been inspirational. His continuous guidance via email and in overseas meetings has been invaluable.

Roger Gent (Chief Paediatric ultrasonographer, Women's and Children's Hospital) and Lino Piotto (Paediatric ultrasonographer, Women's and Children's Hospital) were responsible for excellent ultrasound scans at a very early morning almost any day of the week. I am very thankful to them and especially to Roger, without whose enthusiasm this project would have not been possible.

Mr Craig Hirte (Statistician) for his invaluable long hours with amazing statistical explanations that actually made statistics looks easy!

The University of Adelaide scholarship and the International Postgraduate Research Scholarship that funded me to do this research.

To all the children and their families, who kindly participate in the trials coming at very early hours in the morning, without whom it would have not been possible.

To my parents, Gustavo and Hilda Sophie, for the years of love and support that made my research career possible going out of Colombia. For their ongoing support from Colombia. Muchísimas gracias no es ni será suficiente!

To my "Australian mother", Margaret Brown, not only for encouraging me to do research and to always ask the right research question as a good Research Fellow, but also for the everyday invaluable support as an adopted mum.

To my other parents, Karl and Delele, for the continuing support and looking after me as my parents and my other parents. Thank you very much.

To Karl and Natalia…. who not only support me through this hard work and studying but also helped me being part of one of the trials as healthy volunteers.

Finally I want to acknowledge the unbelievable, unending love and support of my husband, Mellick, who has never stopped me from pursuing new ideas. He has encouraged me to go through this research pathway from the very beginning and I am sure he will continue to do so. He has been my personal supervisor as his PhD preceded mine by 10 years.

TABLE OF CONTENTS

PUBLICATIONS RELEVANT TO THIS THESIS

- **Peña AS,** Wiltshire EJ, McKenzie K, Gent R, Piotto L, Hirte C, Couper JJ**.** Vascular endothelial and smooth muscle function relate to body mass index and glucose in obese and nonobese children. ***J Clin Endocrinolol Metab*** 2006;91:4467-4471

- **Peña AS,** Wiltshire EJ, Gent R, Hirte C, Couper JJ**.** Folic acid improves endothelial function in children and adolescents with type 1 diabetes. ***J Pediatr*** 2004; 144:500-4.

- **Peña AS,** Wiltshire EJ, Gent R, Hirte C, Couper JJ**.** Folic acid does not improve endothelial function in obese children and adolescents. ***Diabetes Care*** 2007; 30: 2122-2127.

- Wiltshire EJ, Gent R, Hirte C, **Pena A,** Thomas DW, Couper JJ. Endothelial dysfunction relates to folate status in children and adolescents with type 1 diabetes. ***Diabetes*** 2002; 51: 2282-6.

ABSTRACTS RELEVANT TO THIS THESIS

- **Peña AS,** Wiltshire EJ, Gent R, Hirte C, Couper JJ**.** Effect of folic acid on endothelial function in children and adolescents with type 1 diabetes (Oral A3) Australian Paediatric Endocrine Group (APEG). Darwin, August 22nd -24th, 2002.
- **Peña AS,** Wiltshire EJ, Gent R, Hirte C, Couper JJ**.** Effect of folic acid on endothelial function in children and adolescents with type 1 diabetes. Meetings and Proceedings & Abstract Book (Oral 121-Page 60). Australian Diabetes Society & Australian Diabetes Educators Association (ADS &ADEA) Annual Scientific Meeting. Adelaide, 25th - 27th September 2002.
- **Peña A,** Wiltshire EJ, Gent R, Hirte C, Couper JJ**.** Folic Acid Improves Endothelial Function in Children and Adolescents with Type 1 Diabetes. ***Diabetes*** 2003, 52 Supplement 1: A15.
- **Peña AS**, MacKenzie K, Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Obese children and children with type 1 diabetes have similar severity of endothelial dysfunction (Oral SO4.2). APEG. Auckland, New Zealand, 1 - 3rd December, 2004.
- **Peña AS**, MacKenzie K, Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Obese children and children with type 1 diabetes have similar degrees of endothelial and smooth muscle dysfunction. ***Hormone Research***, September 2005: 268.
- **Peña AS**, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper J. Vascular endothelial and smooth muscle function relate to body mass index in non-obese and obese children (Oral 15). 14th Annual Scientific Meeting Australian Society for the Study of Obesity (ASSO). Adelaide, October 28th – 30th, 2005.

- **Peña AS,** Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Folic acid does not improve endothelial function in obese non-diabetic children and adolescents. ***Obesity reviews*** 2006, 7 Supplement 2: 192.
- **Peña AS**, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper J. Vascular endothelial and smooth muscle function relate to BMI and glucose in normal children and those with obesity or type 1 diabetes. ***Pediatric Diabetes*** 2006; 64 Supplement 5: abs020.
- **Peña AS,** Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Folic acid does not improve endothelial function in obese non diabetic children and adolescents. (Oral SO5.1). APEG, Hobart, 20-22nd September 2006.
- **Peña AS**, Wiltshire E, MacKenzie K, Gent R, Piotto L, Hirte C, Couper J. Progression of vascular disease in adolescents with type 1 diabetes and obese non diabetic adolescents (Oral S12.02). APEG, Hobart, 20-22nd September 2006
- **Peña AS,** Wiltshire E, Gent R, Piotto L, Hirte C, Couper J. Folic acid does not improve endothelial function in obese non diabetic children and adolescents (Page 72 - Oral SO5.1). The Royal Australian College of Physicians RACP Congress. Melbourne, 6-10th May 2007.

LIST OF SPECIAL ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
APEG	Australian Paediatric Endocrine Group
ATL	Advanced Technology Laboratories
BMI	Body Mass Index
CI	Confidence Interval
CRP	C-reactive protein
CV	Coefficient of variation
DEXA	Dual Energy X-ray Absorptiometry
eNOS	endothelial Nitric Oxide Synthase
ECG	Electrocardiogram
ET-1	Endothelin-1
FMD	Flow Mediated Dilatation
FABF	Forearm Arterial Blood Flow
GTN	Glyceryl Trinitrate Mediated Dilatation
HbA1c	Haemoglobin A1c, glycosylated haemoglobin
HDL	High Density Lipoprotein
HsCRP	High Sensitive C reactive protein
LDL	Low Density Lipoprotein
MTHFR	Methylenetetrahydrofolate reductase
NO	Nitric Oxide
PAI-1	Plasminogen Activator Inhibitor-1
RCF	Red Cell Folate

SD	Standard Deviation
SE	Standard Error of Mean
tHcy	Total Plasma homocyst(e)ine
TNF-α	Tumour Necrosis Factor α
tPA	Tissue Plasminogen Activator
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
VD	Vessel Diameter
vWF	von Willebrand Factor

LIST OF FIGURES

LIST OF TABLES