Title Page

Nutrition and vascular function

A thesis submitted to the University of Adelaide by

Jennifer Beatrice Keogh MSc

Department of Medicine

University of Adelaide

South Australia

For the degree of

Doctor of Philosophy

October 2007

Table of contents

For Peter who believed I could do it

Declaration of originality

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 or material published or written by another person except where due acknowledgement has been made. I give consent to a copy of my thesis being made available in the University Library.

The author acknowledges that copyright of published works contained within this thesis listed below resides with the copyright holder/s of those works.

Signed……

Jennifer Beatrice Keogh

Date……..

October 2007

Publications arising from this thesis

Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is impaired by a high-saturated fat diet but not by a high-carbohydrate diet. Arteriosclerosis Thrombosis and Vascular Biology 2005; 25(6):1274-1279. Impact Factor: 7.053

Clifton PM, Keogh JB**,** Foster PR, Noakes M. Effect of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. Int J Obes (Lond). 2005;29(12):1445-51. Impact factor 3.5

Keogh JB, Brinkworth GD and Clifton PM. Effects of weight loss on a low carbohydrate diet on flow mediated dilatation, endothelial markers and adiponectin. Br J Nutr. 2007 Oct;98(4):852-9. Epub 2007 May 10 Impact Factor: 2.967

Keogh JB, Luscombe-Marsh ND, Noakes M, Wittert GA, Clifton PM. Long-term weight maintenance and cardiovascular risk factors are not different following weight loss on carbohydrate-restricted diets high in either monounsaturated fat or protein in obese hyperinsulinaemic men and women. Br J Nutr. 2007;97(2):405-10. Impact Factor:2.967

In press

Keogh JB, Ho JT, O'Loughlin P, Bornstein SR, Lewis JG, Torpy DJ and Clifton PM. Moderate weight loss reduces renin and aldosterone but does not influence basal or stimulated pituitary-adrenal axis function. Horm Metab Res. Accepted January 2007 Impact Factor: 2.049

Keogh JB, Brinkworth GD, Noakes M, Belobrajdic DP, Buckley JD, CliftonPM Effects on endothelial function and markers of cardiovascular disease risk in subjects with abdominal obesity of weight loss on a very low carbohydrate diet. AJCN. Accepted January 2007 Impact Factor: 6.562

Published abstracts arising from this thesis

Keogh JB and PM Clifton. Adiponectin blood pressure and weight loss. Asia Pac J Clin Nutr 2006;15(Suppl):50

Keogh J, Luscombe N, Foster P, Noakes M, Wittert G, Clifton P
Long term weight maintenance with two carbohydrate restricted diets.
Obesity Reviews 2005;6(Suppl):6

Keogh JB, Torpy DJ, Brinkworth GD, PM Clifton. Salt and blood pressure: relationship with obesity, weight loss and direct effects on vascular function. Asia Pac J Clin Nutr 2005;14(Suppl):59

Clifton P, Keogh J, Brinkworth G, Torpy D, Ho J. Salt and blood pressure. Relationship with obesity, weight loss and direct effects on vascular function. Circulation 2005; 112(17):U741

Acknowledgements

I would like to thank Professor Peter Clifton and Associate Professors David Torpy and Manny Noakes for their advice and encouragement and for creating the environment that made these studies possible.

I would also like to thank the Australian Society for the Study of Obesity for the award of the Janet Bryson PhD scholarship which I received during 2006.

All the studies in the thesis were conducted in the clinical research unit at the Commonwealth Scientific and Industrial Research Organisation Human Nutrition, Adelaide, South Australia and the contribution of the clinical research unit staff is gratefully acknowledged as follows:

Clinical trial managers: Kathryn Bastiaans, Vanessa Courage Anne McGuffin and Julia Weaver for co-ordination of the studies.

Dietitians: Xenia Cleanthous, Paul Foster, Julianne McKeough and Gemma Williams for assistance with the dietary interventions.

Nurses: Lindy Lawson and Rosemary McArthur for taking blood samples and blood pressure measurements.

Laboratory staff: Cherie Keach, Mark Mano, Laura Nehez, Cathryn Seccafien, Candita Sullivan and Julie Turner for assisting with the biochemical assays.

Dr Damien Belobrajdic supervised the laboratory analysis of adhesion molecules, tPA, PAI-1 and adiponectin in chapter 6.

Dr Jui Ho measured aldosterone and renin in chapter 7.

Flow mediated dilatation and pulse wave velocity:

Ms Jessica Grieger measured FMD and PWV in Chapter 3 and submitted this data as part of her honours thesis (Department of Physiology, University of Adelaide). The data was reanalysed in keeping with the aims and structure of the thesis and the manuscript

from this study was writted by Jennifer Keogh. Further details of Jennifer's role to this study are outlined below in the contribution.

In chapters 3, 4, 5 and 6 FMD and PWV were measured by Ms Jodie Avery, Dr Grant Brinkworth and Mr Tom Wycherley respectively. Dr Brinkworth also measured the AI in chapter 5.

I would like to thank Leanne Griffiths CHN librarian for her help providing journal articles.

Financial support:

I gratefully acknowledge the support of Unilever Australasia (a Division of Unilever Australia Limited) and Slim-Fast Nutrition Institute, USA (Chapter 3).

I thank the Diabetes Australia Research Trust for support of the study in Chapter 5, the National Heart Foundation of Australia and the National Health and Medical Research Council (NHMRC) of Australia for project grants to conduct the study in chapter 6 and the NHMRC for support of the study in Chapter 8.

I thank the volunteers who gave their time so generously to make the studies possible.

Contribution

Jennifer Keogh's contribution to the studies in the thesis

Chapter 3
The effect of a high saturated fat diet compared with a high MUFA, high PUFA or a high carbohydrate diet on FMD
Protocol design and development
Ethics submission
Development and implementation of the dietary protocol
Counselling of volunteers
Performed the data entry from the food records
Performed statistical analysis and interpretation of the study data
Write-up of the study data for publication
Presented data at national conferences

Chapter 4
The effect of weight loss on inflammatory and endothelial markers and FMD and PWV using two low fat diets
Performed data entry from the food records
Performed statistical analysis and interpretation of the study data
Write-up of the study data for publication
Presented data at national and international conferences

Chapter 5
Effects of weight loss on a low carbohydrate/low saturated fat diet on FMD, adhesion molecules, adiponectin, AI, BP and PWV following short-term weight loss and long-term follow-up
Protocol design and development
Ethics submission
Dietary protocol design
Counselling of volunteers
Performed waist, BIA and seated blood pressure measurements
Performed the data entry from the food records
Performed statistical analysis and interpretation of the study data
Write-up of the study data for publication
Presented data at national conferences
Presented at the European Congress on Obesity 2007

Chapter 6
Effect of a very low carbohydrate/ high saturated fat diet during weight loss on FMD, AI, BP, adiponectin and adhesion molecules during weight loss
Participation in protocol design and development
Development of the dietary protocol
Performed all augmentation index measurements before and after weight loss
Performed laboratory analyses for lipids, CRP, adiponectin, PAI-1, tPA, CAMs
Performed statistical analysis and interpretation of the study data
Performed write-up of the study data for publication
Presented at the European Congress on Obesity 2007

Chapter 7
Effects of weight loss on AI and the BP response to salt in adults
Protocol design and development
Ethics submission
Development and implementation of the dietary protocol
Counselling of volunteers
Performed blood (seated) pressure measurements
Performed the data entry from the food records
Performed statistical analysis and interpretation of the study data
Performed write-up of the study data for publication
Presented data at national conferences

Chapter 8
Effect of long term weight maintenance on CVD risk factors
Counselling of volunteers
Performed data entry from the food records
Performed statistical analysis and interpretation of the study data
Write-up of the study data for publication
Presented at the European Congress on Obesity 2006
Presented data at national conferences

Index of tables and figures

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

Abbreviations

Acetylcholine (ACh)

Angiotensin converting enzyme (ACE)

Augmentation index (AI)

Australian Clinical Trials Registry (ACTR)

Blood Pressure (BP)

Body mass index (BMI)

Cardiovascular disease (CVD)

Cellular adhesion molecules (CAMS)

Cholesteryl ester transfer protein (CETP)

Coefficient of variation (CV)

Coronary artery disease (CAD)

C-reactive protein (CRP)

Diabetes Mellitus (DM)

Diastolic Blood Pressure (DBP)

Dietary Approaches to Stop Hypertension (DASH)

Disability-adjusted life years (DALYs)

Docosahexaenoic acid (DHA)

Eicosapentaenoic acid (EPA)

Enzyme-Linked ImmunoSorbent Assay (ELISA)

Endothelial NO synthase (eNOS)

Flow independent dilatation (FID)

Flow mediated dilatation (FMD)

Gylceryl trinitrate (GTN)

High density lipoprotein cholesterol (HDL-C)

3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA)

Impaired fasting glucose (IFG)

Impaired glucose tolerance (IGT)

Intercellular adhesion molecule-1 (ICAM-1),

Low density lipoprotein cholesterol (LDL-C),

Methacholine (MCh)

Monounsaturated fat (MUFA)

Myocardial Infarction (MI)

Nitric oxide (NO)

Plasma renin activity (PRA)

Plasminogen activator inhibitor 1 (PAI-1),

Polycystic ovarian syndrome (PCOS)

Polyunsaturated fat (PUFA)

Pulse wave velocity (PWV)

Relative risk (RR)

Systolic Blood Pressure (SBP)

Tissue plasminogen activator (tPA)

Total cholesterol (TC)

Trans fatty acids (TFA)

Transient ischaemic attacks (TIA)

Triglyceride (TG)

Tumour necrosis factor-alpha (TNFα)

UK Prospective Diabetes Study (UKPDS))

Vascular cell adhesion molecule-1 (VCAM-1)

Very low density lipoprotein (VLDL)

Thesis summary

Common risk factors for CVD such as hyperlipidaemia, hypertriglyceridemia, low HDL-C, obesity, insulin resistance, impaired glucose tolerance, inflammation and hypertension may increase the risk of atherosclerosis through altering vascular function. Modification of dietary intake and weight loss can ameliorate these risk factors and may impede the development of atherosclerosis. CVD risk can be assessed by measurement of both traditional e.g. lipid levels, glucose and blood pressure and novel risk markers of CVD e.g. FMD, levels of adhesion molecules, inflammatory markers and adipokines. Changes in these measurements are used to determine effects, if any, of dietary interventions. The studies in this thesis focus on the relationship between nutrition and vascular function and the effects of modifying dietary composition either with, or without weight loss. The primary hypotheses addressed were that a high saturated fat diet would have adverse effects on markers of CVD risk., that short and long term weight loss would have beneficial effects on these markers, that a conventional low fat, high glycaemic load diet would also have adverse effects on these markers and that weight loss would attenuate the BP response to salt. Six studies were conducted to address these hypotheses.

The effects of saturated fat were investigated in chapters 3 and 6. In chapter 3, a high saturated fat diet impaired FMD and increased the level of the adhesion molecule P-selectin compared with a high MUFA, a high PUFA, or a low fat, high glycaemic load diet in weight stability. The high fat, high glycaemic load caused increases of 23-39% in TG and decreases of 10-15% in HDL-C but despite these adverse effects there was no change in FMD. In chapter 6, subjects on a very low carbohydrate/high saturated fat diet lost approximately 1 kg more weight over 8 weeks than those on a conventional low fat diet. While other CVD risk factors, glucose, insulin, E and P-selectin, ICAM-1 and

PAI-1 levels all improved FMD did not change in either diet. Reductions in LDL-C and CRP were greater on the conventional diet.

The effects of weight loss on CVD risk factors were also investigated in the studies in chapters 4, 5, 7 & 8. In chapter 4, moderate weight loss using 2 different low fat diets resulted in improvements in PAI-1 and sICAM-1 but there was no change in FMD. Similarly in chapter 5 weight loss on a low carbohydrate/low saturated fat diet did not change FMD but there were other benefits including reductions in glucose and insulin, LDL-C, adhesion molecules, VCAM1 and ICAM1. Adiponectin did not change after short term weight loss in either of the studies in chapters 5 or 6. In chapter 7 salt loading increased ambulatory day time BP and this response was not altered by short term moderate weight loss. The long term effects of weight loss were investigated in chapters 5, 7 and 8. In chapter 5, after 52 weeks, there was sustained weight loss of 5% but no change in FMD while adiponectin levels increased and LDL-C and insulin were substantially reduced. In chapter 7 the BP response to salt loading remained unchanged despite weight loss maintenance. Finally in chapter 8 weight loss was predicted by protein intake and there were reductions in CVD risk demonstrated by decreases in insulin, TG and CRP and increases in HDL-C.

The studies in this thesis demonstrate that moderate weight loss has beneficial effects on traditional and novel cardiovascular disease risk markers but does not have a beneficial effect on FMD regardless of dietary composition. A high saturated fat diet has detrimental effects on novel CVD risk markers in weight stability but weight loss attenuates this effect. A high saturated fat diet may have detrimental effects on adhesion molecules in weight stability and may attenuate the beneficial effects of weight loss on LDL-C and CRP. Moderate long term weight loss maintenance has beneficial effects on most but not all CVD risk markers.