The Effect of 10 Weeks of Peri-Training Whey Protein Supplementation on Systemic, Metabolic, and Skeletal Muscle Molecular Responses in Type-2 Diabetes

A Thesis

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ABSTRACT

Introduction: Type-2 diabetes (T2D) is a modern global epidemic associated with multiple health complications and economic burden. Exercise improves glycaemic control in populations with T2D with greater insulin sensitivity, muscle hypertrophy, and reduced emotional distress as possible mediators. Milk protein supplementation has been shown to produce similar benefits, raising the potential of an adjunct therapy. Therefore, the primary purpose of the thesis was to determine if whey-protein supplementation can promote skeletal muscle plasticity associated with improved glycaemic control in exercising men with T2D. Secondary aims were to determine if improvements in functional capacity and glycaemic control led to better mood and quality of life.

Methods: In a randomized, double blind clinical trial, 24 non-insulin dependent middle-aged men with T2D were allocated to a pre- and post-training whey-carbohydrate (20 grams-10 grams) supplement or isocaloric carbohydrate-only control. Participants completed 45 high-intensity endurance and resistance exercise sessions over 10 weeks. Insulin sensitivity was determined from glucose disposal rates (GDR) during a euglycaemic insulin clamp, with fasting blood glucose concentration (FBG) and the homeostatic model of assessment of insulin resistance (HOMA-IR) providing secondary measures of glycaemic control. Insulin-mediated haemodynamics; microvascular blood flow (mBF) and microvascular blood volume (mBV) were assessed at the vastus lateralis (VL) muscle via near-infrared spectroscopy. VL muscle biopsies were used to determine capillarity, intramyofibrillar mitochondrial and lipid density, citrate synthase (CS) and cytochrome c oxidase (COX) activity, and mRNA content of angiogenic and mitochondrial markers: eNOS, VEGFA, VEGFR2, PGC1-α, CS, NRF1. Aerobic capacity (VO_2peak), strength (1-repetition maximum), VL muscle and subcutaneous adipose thickness, and survey-rated mood and quality of life (DASS42; SF-36) were also assessed.

Results: There were substantial increases in GDR (27.5%; 90%CI 1.2%, 60.7% and 24.8%; -5.4%, 64.8%), capillarisation (24.5%; -0.1%, 55.0 and 26.3%; 1.9%, 56.6%), and mitochondrial density (24.3%; 13.8%, 35.8% and 26.7%; 16.8%, 37.5%) in the control and whey groups respectively, with no group differences. Lipid density, COX enzyme activity, VL muscle thickness, VO_2peak, 1RM strength, mood, and quality of life were also substantially increased with no group differences. Exercise training had no effect on microvascular haemodynamics; however, whey supplementation produced likely and possible improvements in mBV (16.8%; -4.3%, 42.6%) and mBF (5.9%; -3.7%, 16.3%) respectively at rest and likely improvements in both mBV (17.5%; -3.7%, 43.5%) and mBF (10.2%; 0.3%, 21.1%) under insulin-stimulated conditions. Regression analysis of the pooled 10-week change outcomes showed a positive relationship between the change in lipid density and the change in GDR (r = 0.29); negative associations between basal mBV and FBG (r=.27) and HOMA-IR (r=.30); a negative association between basal mBF and HOMA-IR (r=.48); and a positive association (r = 0.39) between the total DASS score and the change in FBG.

Conclusion: Peri-training whey protein supplementation elevated microvascular blood kinetics in middle-aged men with T2D; but did not accentuate the substantial improvements produced by the intense mixed-mode exercise training on tissue and cellular remodelling, insulin sensitivity, glycaemia, exercise capacity, mood or quality of life. The findings support the use of adjunct whey protein supplementation for elevating microvascular blood kinetics in populations with T2D, an outcome that could potentially improve the treatment of vascular diseases where microcirculation contributes to disease pathology and therefore warrants further exploration. The observation that myocellular lipid density was increased by

intense exercise training and not detrimental to insulin sensitivity supports recent evidence that lipid accrual may be a favourable adaptation to exercise in populations with T2D.

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Having finally completed this study, I am extremely thankful to quite a few people who participated. I am very much obliged for the commitment of a number of research institutions who through physical, intellectual and financial investment made this project possible: Massey University, CCDHB Endocrine and Research Centre, Otago Medical School, Environmental Science Research (ESR), and the Wellington Hospital Clinical Trials Unit. I am grateful to the study volunteers who let the research team prod and poke them quite a few times and the staff at the medical centres who sent them: Island Bay; Port Nicholson; Newtown; Kelburn; Thorndon; Miramar; The Terrace; Brooklyn; City; Courtney; Kilbirnie; Capital; and Te Aro Health.

I am extremely thankful to the French interns, Romain, Marine, Alexandria, Rachel, Julia, Cassandre, Julie, and Laurie, who analysed a mountain of data that I would probably still be looking at if I tried to do it on my own. I acknowledge the great work of Brooke and Shang who made every day so much easier for me and kept the participants showing up each morning. Thanks to the Massey staff who helped out or gave input when I needed it; Stu, Brandon and Jill; Marilyn, Sally, Sarah, Jim Clarke and Nick Kim; and Barry Clarke for getting involved at the hospital; Chris Harris who provided technical assistance when it came to inventing things, usually in his own time; Margaret McMillan, who kept with us till the end.

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STATEMENT OF CONTRIBUTION

Study conception and design was by Kim Gaffney, Dr David Rowlands and Dr Lee Stoner. Ethics proposal was written by Kim Gaffney and Dr David Rowlands. Participants were recruited and the study co-ordinated by Kim Gaffney. Supplements were designed by Kim Gaffney and Dr David Rowlands. Exercise training sessions were supervised by Kim Gaffney and PhD candidate Adam Lucero. Research assistants helped with exercise sessions, supplement production, data collection and analysis of microscopy imaging throughout the project.

CHAPTER 4: NIL WHEY PROTEIN EFFECT ON GLYCAEMIC CONTROL AFTER INTENSE MIXED-MODE TRAINING IN TYPE-2 DIABETICS.

Maximal cycling workload and breath-gas collection and ECG testing was by Kim Gaffney, Adam Lucero, and Dr James Faulkner. Insulin-clamps were supervised by Kim Gaffney, Adam Lucero, and one of three general practitioners Patricia Whitfield, Brian Corley and Nick Oscroft, with assistance from Dr David Rowlands and Dr Barry Clarke. NIRS measurement was completed by Adam Lucero or Kim Gaffney. General blood analysis was conducted at Wellington Hospital and insulin concentration the Nutrition Laboratory, Massey University, Palmerston North. Statistical analyses were performed by Dr David Rowlands and Kim Gaffney. The manuscript was written and prepared by Kim Gaffney with guidance from Dr David Rowlands and feedback from Dr Lee Stoner, Dr James Faulkner, Dr Jeremy Krebs, and Dr Patricia Whitfield.

CHAPTER 5: WHEY SUPPLEMENTATION IMPROVES MICROCIRCULATION AFTER 10 WEEKS IN EXERCISING MEN WITH T2D.

NIRS data collection methods were developed by Adam Lucero, Kim Gaffney, Dr Lee Stoner, and Dr David Rowlands. Data collection was performed by Adam Lucero and Kim Gaffney. Capillary density methods were developed by Kim Gaffney with assistance from Jane Anderson and St John Wakefield at the Otago Medical School EM Laboratory; images were analysed by Kim Gaffney with help from research assistants; PCR analysis of mRNA was performed by Jane Clapham and Donia McCartney at Environmental Science Research. Data analysis was conducted by Kim Gaffney, Adam Lucero, and Dr David Rowlands.

CHAPTER 6: THE EFFECT OF 10 WEEKS OF PERI-EXERCISE WHEY PROTEIN SUPPLEMENTATION ON MITOCHONDRIAL CONTENT IN MEN WITH TYPE-2 DIABETES.

Muscle biopsies were conducted by a research general practitioner with assistance from Kim Gaffney. Tissue was processed for analysis by Kim Gaffney. Electron imaging methods were designed by Kim Gaffney, Dr David Rowlands, and Dr St John Wakefield. Electron microscopy preparation and imaging was performed by the Electron Microscopy Lab at Otago Medical School, Wellington NZ. Imaging analysis protocols were designed by Kim Gaffney and Adam Lucero and analysis was conducted by research assistants. Mitochondrial enzyme analysis was conducted by Adam Lucero. Statistical analysis was conducted by Dr David Rowlands, Kim Gaffney and Adam Lucero.

CHAPTER 7: NIL WHEY PROTEIN EFFECT ON MOOD AFTER 10 WEEKS OF EXERCISE IN TYPE-2 DIABETES.

Psychometic analysis methods were developed by Kim Gaffney. Data was collected by Kim Gaffney and Adam Lucero. Data was prepared by research assistants and analysed by Kim Gaffney and Dr David Rowlands.

RESEARCH ETHICS

Ethics approval was obtained from the Northern B Health and Disability Ethics Committee, Wellington, NZ for the study conducted. The potential risks, and management of the risks involved are detailed below:

The main risks in this study were 1) tissue sampling, which were minimized through strict adherence to Hospital and University safety protocols, and; 2) exercise risks, which were minimized through baseline health screening and ECG testing during maximal exercise, and by having exercise physiologists supervise each exercise session.

Social and psychological risks were minimised by ensuring privacy and confidentiality of participants throughout data collection and data storage periods. Initially we obtained informed consent and communicated to participants their right to discontinue or withdraw.

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LIST OF ABBREVIATIONS

1RM	1-repetition maximum
Akt	protein kinase B
AS160	Akt substrate of 160 kDa
COX	citochrome c oxidase
CS	citrate synthase
DASS	Depression, Anxiety and Stress Scale
eNOS	endothelial nitric oxide synthase
FBG	fasting blood glucose
FMD	flow-mediated dilation
GDR	glucose disposal rate
GLUT4	glucose transporter 4
HOMA-IR	homeostatic model assessment of insulin resistance
IRS	insulin receptor substrate
mBF	microvascular blood flow
mBV	microvascular blood volume
MPS	muscle protein synthesis
NRF1	nuclear respiratory factor 1
NIRS	near-infrared spectroscopy
NO	nitric oxide
PGC1-α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	phosphoinositide 3-kinase
ROS	reactive oxygen species
SF-36	Short-form (36) health survey
T2D	type-2 diabetes
VEGFA	vascular endothelial growth factor A
VEGFR2	vascular endothelial growth receptor 2
VO_2peak	peak oxygen consumption