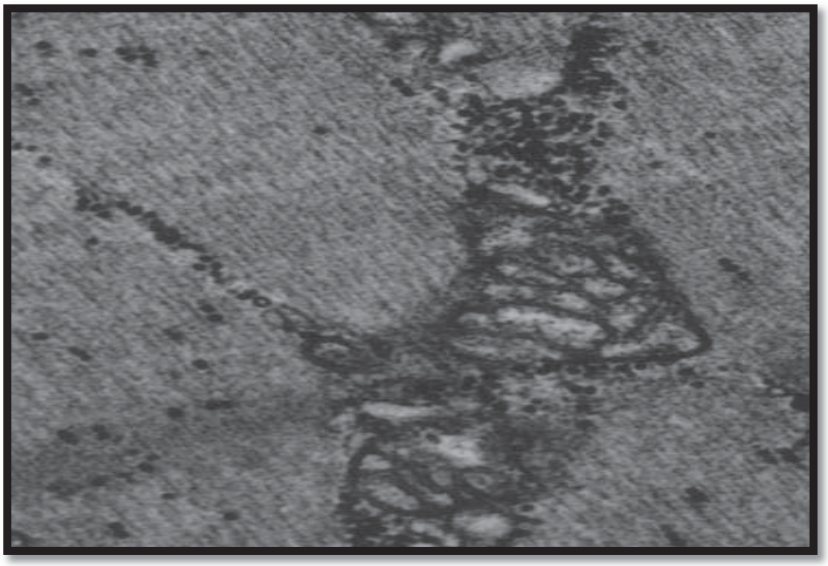
THE SOUTH PACIFIC ISLANDS RESIST DIABETES WITH INTENSE TRAINING (SPIRIT) STUDY

Investigation of obesity markers and morphological, functional and genetic changes in the skeletal muscle



A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Health Sciences

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ABSTRACT

The skeletal muscle (SM), the major tissue for disposal of excess blood glucose, plays a big role in development of insulin resistance leading to type 2 diabetes mellitus (T2DM). Lipid accumulation and decline in mitochondrial activity in SM has been observed in people with T2DM. Several studies have demonstrated that exercise has the ability to increase SM lipid oxidation and mitochondrial activity and hence is effective as a treatment strategy for people with T2DM for improving blood glucose control and insulin sensitivity.

The SPIRIT study was the first clinical randomised exercise trial involving a cohort of Polynesian New Zealanders with T2DM. The uniqueness of this study is that it is the first clinical trial in Polynesian population with grade 3 obesity (n=18; BMI 43.8 ± 9.5 kg/m^2) and T2DM. The SPIRIT cohort underwent 16 weeks of progressive resistance training (PRT) or aerobic exercise (AER) training. The cohort showed no changes in HbA1c levels after 16 weeks of exercise and hence no improvement in their blood glucose control. This was an unexpected result and led to the following hypothesis which underlines this PhD study – "In skeletal muscle of SPIRIT cohort, metabolic adaptation to exercise is delayed due to metabolic inflexibility".

To investigate this hypothesis, mitochondrial function and morphology, lipid droplet content and changes in gene expression pre and post exercise intervention were examined in the SM. Since the SPIRIT cohort showed no changes in weight, waist circumference and BMI, examination of the concentration of specific obesity markers pre and post exercise training also occurred.

Mitochondrial function was examined pre and post 16 weeks exercise intervention by measuring the SM activity of three key mitochondrial enzymes; citrate synthase (CS) involved in Krebs cycle, beta-hydrxoyacyl-CoA dehydrogenase (BHAD) involved in fat oxidation and cytochrome c oxidase (COX) involved in electron transport chain. The PRT cohort showed statistically significant increases in activity for COX (P=0.005) and CS (P=0.007) with very large effect size (2.3 ± 1.3 and 1.8 ± 1.3 respectively). AER exercise led to significant increases in the activity for all three enzymes COX (P=0.01), CS (P=0.03), BHAD (P=0.03) with moderate effect size for both COX and CS activity but very large effect for BHAD (6.7 ± 1.2). For all three enzymes there were statistically significant differences (P<0.05) between the AER and PRT groups. These results demonstrate increased mitochondrial activity and functioning after 16 weeks of PRT or AER exercise.

To further investigate the morphology of pre and post SM tissue the electron microscope images were examined for quantification of intramyocellular triglyceride (IMTG) content. There was a 48% statistically significant decrease (P=0.007) in IMTG (lipid droplets) in the AER group and there was a 28% statistically significant decrease (P=0.04) in IMTG content in the PRT group. The reduction in lipid droplet accumulation in the SM and associated increase in skeletal muscle BHAD activity (enzyme involved in oxidation of fatty acids in the mitochondria) demonstrates the benefit of exercise for the SPIRIT cohort.

The Ingenuity Pathways Analysis software was used to investigate the microarray gene data obtained for the SM of the SPIRIT cohort. The results indicate changes in gene expression associated with early phase connective tissue remodelling, for both forms of exercise by upregulation of genes like IGF-1, TGFBR2, PDGFRB in the resistance training group and COL4A1, COL3A1, MYH11, BGN, ACTA2, CD300LG, A2M, GPR116 in the aerobic training group. The AER training group also showed significant changes in SM mRNAs associated with glucose and lipid handling. Two key mRNAs that had increased expression after 16 weeks of AER exercise were *PPARGC1A* (gene encodes for PGC1-α, a regulator of energy metabolism) and *PPARG* (gene encodes for protein peroxisome proliferator-activated receptor gamma) regulates fatty acid storage and glucose metabolism.

Of the specific markers related to obesity that were examined only sex hormone binding globulin (a marker of insulin sensitivity) showed a statistically significant increase (P=0.01) in the PRT group. Seven of the nine PRT participants had an increase in SHBG levels, indicating a possible improvement in insulin sensitivity for these individuals. Statistically significant positive correlation of SHBG and statistically significant negative correlation of cortisol were established with number of exercise sessions attended in both groups meaning that greater exercise sessions may have positive impact on altering the metabolic profile of the individual.

The results of this PhD study have shown that exercise has induced changes in the skeletal muscle of the SPIRIT cohort. The increased mitochondrial enzyme activity and function, decreased IMTG content, increased fat oxidation and improved functional plasticity of the skeletal muscle are changes occurring at the functional, structural and genetic levels which denounce the hypothesis that metabolic adaptation in the SPIRIT cohort was delayed due to the SM being metabolically inflexible. These findings have demonstrated that exercise enhances metabolic flexibility in tissue that could be metabolically inactive e.g. tissue such as SM tissue in grade 3 obese individuals with T2DM.

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LIST OF ACRONYMS

1RM	1 Repetition Maximum
ACSM	American College of Sports Medicine
AER	Aerobic exercise
ANOVA	Analysis of Variance
BHAD	Beta hydroxyacyl-CoA dehydrogenase
BMI	Body Mass Index
COX	Cytochrome Oxidase
CS	Citrare Synthase
CV	Coefficient of Variability
FFA	Free Fatty Acids
GLUT4	Glucose Transporter 4th isoform
HbA1c	Haemoglobin A1c (glycated haemoglobin)
HOMA2-IR	Homeostasis Model Assessment Insulin Resistance
IPA	Ingenuity Pathway Analysis
PRT	Progressive Resistance Training
QOL	Quality of life
T2DM	Type 2 diabetes Mellitus
IMTG	Intramuscular Triglyceride
IMCL	Intramyocellular lipid
LD	Lipid Droplet
GWR	Greater Wellington Region
SPIRIT	South Pacific Islands Resist Diabetes with Intense Training