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EFFECT OF AEROBIC AND RESISTANCE TRAINING;

THE SKELETAL MUSCLE MITOCHONDRIAL FUNCTION IN THE SPIRIT STUDY COHORT

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ABSTRACT... Objective: The aim of this investigation was to examine the effect of aerobic and resistance training on the skeletal muscle mitochondrial function. Setting: Wellington Hospital New Zealand, Massey University, Wellington Campus New Zealand. Period: Sep 2008- Sep 2011 Results: There was a very large effect (6.7 ± 1.2) in the AER group for BHAD activity whereas in the PRT group a large effect (2.7 ± 1.2) for BHAD activity was observed. There was an increase in CS activity in both groups (PRT; p = 0.007, AER; p=0.03) however, the activity increase was more in the PRT group (effect size = 1.8 ± 1.3). COX activity was raised in both groups as well though the effect size in the PRT group was 2.3 ± 1.2 meaning a very large change with PRT exercise compared to a moderate effect (1.0 ± 1.2) with AER exercise. Conclusion: Overall these findings suggest that both PRT and AER exercise can be effective therapeutic modalities for the induction of changes at the cellular level in muscle of people with T2DM.

Key words: T2DM, type 2 diabetes Mellitus, PRT, progressive resistance training, AER, aerobic training)

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INTRODUCTION

It is well known that the production of the energy molecule, adenosine triphosphate (ATP) and oxidation of fatty acids are chief functions of the mitochondria, the power house of a cell[1]. There is evidence that mitochondria in the muscle cells are capable of getting rid of non-esterified fatty acids by oxidation and can protect against fatty acid induced insulin resistant disorders such as type 2 diabetes mellitus (T2DM), obesity and metabolic syndrome. Mitochondria perform the oxidation of fatty acids by a process called β-oxidation. The by-products of the β-oxidation metabolic pathway are recycled by the electron transport chain (ETC) in mitochondria. Studies have shown that fatty acid oxidation in mitochondria of individuals with fatty acid/lipid associated disorders is hampered gradually and progressively to an extent that lipid moieties start accumulating inside the muscle cytosol in the form of intramuscular triglycerides (IMTG)[2] and inside the mitochondria[3]. Accumulation of lipids within the muscle has gained considerable attention over the past 10 years because of their association with insulin resistance and related disorders. Low mitochondrial enzyme activity and the accumulation of IMTG within the muscle cell are hallmark features of insulin resistant disorders such as T2DM, obesity and metabolic syndrome.

The rate at which the muscle cell can convert ADP to ATP is directly proportional to the metabolic activity of the cell. The metabolic activity of a muscle cell can be defined as, "A process by which the cell utilises glucose or fatty acids to produce energy in the form of ATP"[4]. Thus the activities that can enhance the metabolic activity of a cell such as exercise can be helpful in diseases associated with cellular metabolic derangements like T2DM, obesity and metabolic syndrome. Exercise has been reported to be associated with improvements in insulin resistance by stimulating mitochondrial biogenesis, increasing mitochondrial enzyme activity[5] and decreasing production of FFA derivatives in the muscle cell[6]. These effects clearly strengthen the argument for the clinical application of exercise for the management of T2DM; however, the availability

of data demonstrating mitochondrial adaptations to exercise is still limited. The majority of clinical exercise trials have been conducted on populations having a BMI <30 and in most of the trials the ethnicity of the population has not been specified. Globally there are ethnic variations in the prevalence of obesity and T2DM so the response to exercise in terms of metabolic adaptations in particular at-risk ethnic populations such as Pacific Islanders still needs to be investigated.

The SPIRIT(south pacific Island resist diabetes with intense training) study is the first clinical exercise trial that investigated the impact of exercise on glycaemic control in a NZ Pacific Islands population that had T2DM and grade 3 obesity (BMI>35) as discussed by Sukala et al[7]. The SPIRIT study cohort had no improvement in insulin sensitivity as measured by HOMA-IR. HOMA-IR is not the gold standard method for determining insulin sensitivity however, it was clear that 16 weeks of exercise training whether it was aerobic or resistance training, did not seem to have much impact on this cohort. Mitochondrial function[4] is disturbed in people with insulin resistance states such as T2DM, obesity and metabolic syndrome and exercise has shown to improve insulin signalling[8], lipid oxidation[9] and mitochondrial oxidative capacity[3] yet these cellular and metabolic pathways have not been examined specifically in a Polynesian population with T2DM and obesity. Therefore the molecular mechanisms underpinning the exercise physiology in the skeletal muscle, the major tissue for glucose disposal[10] and thus vulnerable to insulin resistance[9], needed to be examined in the SPIRIT study cohort. Does mitochondrial enzyme activity increase after 16 weeks of exercise? Does aerobic exercise provide more changes metabolically and cellularly compared to resistance training?

Mitochondrial activity can be examined by measuring the activity of three well-known targeted key mitochondrial enzymes COX[11], CS[12] and BHAD[13]. The impact of exercise on fat metabolism in the muscle cell can be determined by examining the activity of BHAD[6], one of the key enzymes in the beta-oxidation pathway and by determining IMTG content[14]. Therefore the purpose of this study is to investigate the three key mitochondrial enzymes related with energy metabolism; COX, enzyme in electron transport chain, CS, an enzyme of citric acid cycle and BHAD a key enzyme of β-oxidation before and after 16 weeks of AER and PRT exercise.

METHODS

The SPIRIT study was initially intended to be a randomised controlled trial comparing a resistance training group to a non-exercise control group. A sample size of 12 per group was determined a priori based on a previous study of similar design by Castaneda et al[14] and is illustrated below.

T-tests: Means: Difference between two independent means (two groups)
Analysis: A priori: Compute required sample size

Input	Tail(s)	=	Two
	Effect size d	=	1.2162423
	α err prob	=	0.05
	Power (1- β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output	Noncentrality parameter δ	=	2.979173
	Critical t	=	2.073873
	Df	=	22
	Sample size group 1	=	12
	Sample size group 2	=	12
	Total sample size = 24		

However, upon initiation of subject recruitment, many subjects stated they would drop out if randomised to a non-exercise control group because they knew exercise could potentially improve their diabetes and facilitate weight loss. After further consultation with cultural liaisons, the substitution of an aerobic exercise group in place of the control group was deemed a feasible and acceptable option to participants. The method for the collection and storage of the muscle biopsy tissue that was used for measuring mitochondrial enzyme activity and determining IMTG and mitochondrial content has been discussed by Sukala et al[7]. The standard procedure for the collection of skeletal muscle tissue is obtaining a muscle biopsy from the vastus lateralis muscle[15]. Biopsies from the right vastus lateralis were collected under local anaesthesia (1% Xylocaine, Astra Zeneca Ltd, Auckland, New Zealand) at 0 and 16 weeks using a 5 mm Bergstrom needle with applied suction. The 16 week muscle biopsy was collected ~72 hours following the final exercise session to avoid the confounding effect of the acute transcriptome and signalling response to acute exercise. The snap frozen tissue was broken into pieces by orienting longitudinally in Tissue-Tek optimal cutting temperature (OCT) embedding medium (Sakura Finetek Ltd, Tokyo, Japan) and snap frozen again in liquid nitrogen-cooled isopentane and then stored in Eppendorf cryotubes at −80 °C until analysed. The standard enzyme assays were used previously described[1,2,3]. All enzyme assays were performed in flat bottomed 96 well plates (Greiner bio-one) by microplate spectrophotometry (Bio-Rad benchmarkplus). All reagents for the enzyme assays were ordered from Sigma-Aldrich (Auckland, New Zealand). Each SPIRIT study SM biopsy sample was assayed in triplicate for each enzyme measured. Statistical analysis was done using Microsoft Excel 2007 and SPSS (Statistical software package for social sciences version 20.0). Within group effect was analysed by paired t-test and between group effect was analysed by two-way analysis of variance (ANOVA). Enzyme activity and obesity marker results are presented as mean ± SD and $P < 0.05$ was considered statistically significant. Data for the enzyme analysis was expressed as the effect of exercise training expressed as standardised difference (effect size), where the within group baseline standard deviation was used as the denominator. Effect size thresholds were calculated for small standardised difference, according to Cohen, 1986 and are as follows; 0.2= small, 0.6= moderate 1.2= large, 2.0= very large.

RESULTS

Using the final optimised assay for COX, CS and BHAD, the respective enzyme activities were determined in the SPIRIT study participant skeletal muscle at 0 and 16 weeks after exercise.

All experiments were repeated in triplicates. Figure 1 is a bar graph showing the average baseline (0 week) and 16 week mitochondrial enzyme activity results for the SPIRIT study cohort. The effect of PRT and AER training on mitochondrial enzyme activity in the SPIRIT study cohort is presented in Table I.

Within the AER group there was a statistically significant increase in enzyme activity for all three mitochondrial enzymes examined (Table I). For the PRT group there was a statistically significant increase in COX and CS activity within the group (Table I). Between groups there were statistically significant differences for all three mitochondrial enzymes (Table I). The effect size shows whether the increase in activity is a small or large effect. For the AER group, the increase in BHAD activity is a very large effect, whilst the increase in COX activity is a moderate effect and CS activity a small effect (Table II). For the PRT group the increase in activity for all three mitochondrial enzymes examined was large to very large effect.

Enzymes	AER		PRT		P value
Enzyme	Post-Pre difference	Effect size (ES)[a]	Post-Pre difference	Effect size (ES)[a]	Between group
	P-value within group		P-value within group		
COX	0.011	1.0 ± 5.2	0.005	2.3 ± 1.3	0.007
CS	0.03	0.4 ± 1.5	0.007	1.8 ± 1.3	0.004
BHAD	0.03	6.7 ± 1.2	0.078	2.7 ± 1.2	0.008

Table-I. Effect of Aerobic and Resistance Training on Mitochondrial Enzyme activity

Data are the effect of exercise training expressed as standardised difference (effect size), where the within group baseline standard deviation was used as the denominator. ES Thresholds 0.2= small, 0.6= moderate 1.2= large, 2.0= V large. Threshold is for small standardised difference, according to Cohen, 1986. Data expressed as ES ± SD for n= 8 in AER group and n=8 in PRT group. COX represents cytochrome c oxidase; CS represents citrate synthase and BHAD represents beta-hydroxyacyl-CoA dehydrogenase. P< 0.05 is considered statistically significant.

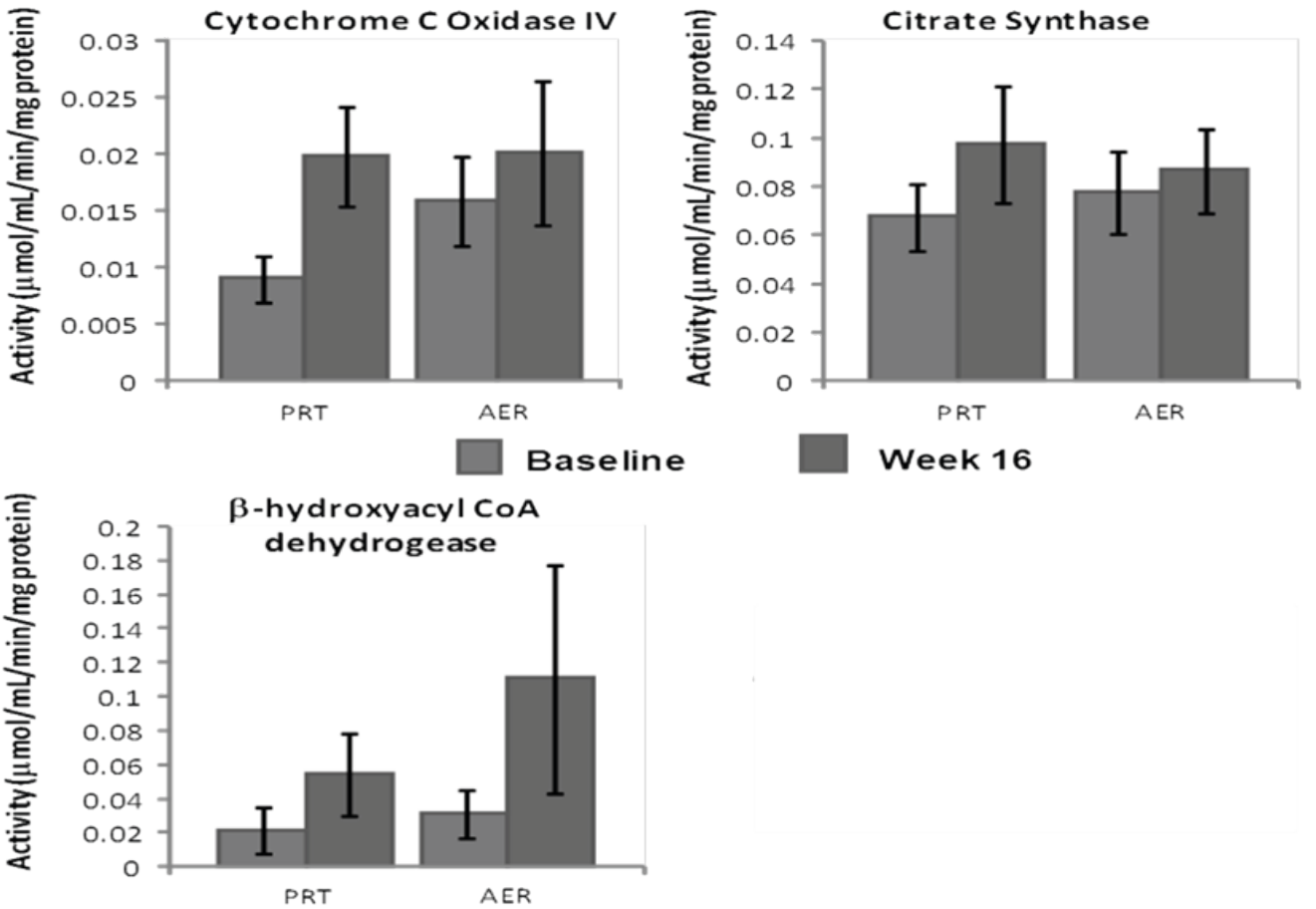

Figure 1: Activity of key mitochondrial enzymes in Aerobic (AER) and resistance (PRT) groups at baseline and at 16 weeks after intervention. The blue bars show the baseline (0 weeks) results and red bars show the results after16 weeks. Data is expressed as mean ± SD. n=8 for Aerobic group and n=8 for PRT group.

SUMMARY

With optimisation of any assay the aim is to obtain an intra-assay CV of less than 10% and inter-assay CV less than 15%. All three mitochondrial enzyme assays optimised in this study provided intra-assay CV and inter-assay CV of less than 5%, indicating excellent reproducibility. The enzyme results indicate increased mitochondrial

Effect	Fold change	Effect size	Confidence interval for effect size	P-value
AER change 16-0 weeks	1.89	0.9	0.69	0.007
PRT change 16-0 weeks	1. 38	0.67	0.67	0.04
PRT-AER change 16-0 weeks	0.87	0.23	0.9	0.604
PRT-AER change at 16 weeks	1.02	0.02	0.64	0.939

Table-II. Effect of exercise training expressed as standardised difference and effect size

Data are the effect of exercise training expressed as standardised difference (effect size), where the within group baseline standard deviation was used as the denominator. ES Thresholds 0.2= small, 0.6= moderate 1.2= large, 2.0= V large. Threshold is for small standardised difference, according to Cohen, 1986. n= 8 in AER group and n=8 in PRT group

function after 16 weeks of AER or PRT exercise as shown by the detection of 1.1- 3 fold increase in the enzyme activity of the three key mitochondrial enzymes in both exercise groups.

DISCUSSION

The aim of this investigation was to examine skeletal muscle mitochondrial function in the SPIRIT study participants. In order to demonstrate the effect of 16 weeks of AER and PRT training on mitochondrial function the enzyme activity of three key mitochondrial enzymes, COX, CS and BHAD was examined. The results presented in Table I demonstrate that the activity of these key enzymes was significantly increased in the AER training group after 16 weeks of exercise intervention and in the PRT group only the CS and COX enzymes increased in activity significantly. The effect size was calculated to illustrate the magnitude of the change after AER and PRT training. There was a very large effect (6.7 ± 1.2) in the AER group for BHAD activity whereas in the PRT group a large effect (2.7 ± 1.2) for BHAD activity was observed. There was an increase in CS activity in both groups (PRT; p = 0.007, AER; p=0.03) however, the activity increase was more in the PRT group (effect size = 1.8 ± 1.3). COX activity was raised in both groups as well though the effect size in the PRT group was 2.3 ± 1.2 meaning a very large change with PRT exercise compared to a moderate effect (1.0 ± 1.2) with AER exercise.

The novelty of this study is that although there was no improvement in the glycaemic control and insulin resistance of the SPIRT study participants after 16 weeks of aerobic or resistance training[7], the ability to investigate cellular functionality in this cohort could be performed. The results presented in this chapter support the notion that exercise is capable of inducing physiological changes at cellular and molecular level. These cellular and molecular changes in the skeletal muscle, the main tissue for glucose disposal[10], are important as they are showing that previously metabolically inflexible skeletal muscle tissue has become metabolically flexible after 16 weeks of exercise training in this cohort. These cellular and metabolic changes could lead to macrophysiological changes in SPIRIT study cohort such as glycaemic control and may underscore the value of exercise in the insulin resistance related disorders such as obesity, T2DM and metabolic syndrome.

There is accumulative evidence that demonstrates that there is lower activity of mitochondrial enzymes with insulin resistance in people with T2DM[1,16,18,19]. It has been postulated that in insulin resistance states due to over-saturation of lipids in the muscle cells, mitochondria in the muscle cell gradually lose the capacity to oxidise fatty acids[20], decrease overall mitochondrial function and can lead to disturbed mitochondrial morphology. Exercise has been a very valuable tool to improve mitochondrial function and structure in skeletal muscle of individuals with T2DM[21-24]. The SPIRIT study is the first investigation that has demonstrated that 16 weeks of PRT and AER training in grade 3 obese Pacific Island people with T2DM leads to statistically significant increases in mitochondrial enzyme activities. This finding supports the hypothesis that exercise changes in this cohort are delayed regarding the potential benefits of exercise at the cellular level and is also in agreement with previously reported studies demonstrating the effects of aerobic

and resistance exercise on muscle tissue[21,25-27]. This is an important skeletal muscle functional adaptation because insulin sensitivity is positively correlated with muscle oxidative capacity[4] and the derangement in the oxidative capacity has been linked with insulin resistance in physically inactive tissue[1,5,28,29]. Oxidative enzymes are abundant in type 1 muscle fibres exhibiting greater capillary density. Exercise has been shown to increase capillary density with an associated improvement in oxidative capacity of muscle. In the SPIRIT study cohort, an increase in capillary density was found after 16 weeks of aerobic exercise[30] which is also associated with the increase in oxidative enzyme function in this study. These results suggest that both aerobic and resistance exercise can generate transformations at the cellular level in muscle overloaded with lipids in grade 3 obese type 2 diabetes participants.

There was a statistically significant increase in COX and CS activities (Table I) in both AER and PRT groups and BHAD activity in AER group (Table I). These findings are in line with the findings of previous exercise studies of comparable duration (≥ 16weeks)[29] but differs from some of the exercise studies (≥ 16 weeks) which clearly demonstrated improved insulin sensitivity along with improvement in the mitochondrial function and decrease in IMTG content[3,27]. In SPIRIT study participants there was no significant change in the insulin sensitivity determined by HOMA-IR in either AER or PRT groups[7,30]. In a clinical exercise trial with T2DM participants (n=20), Toledo et al.[29] reported that there was a 7.1 ± 0.8% weight loss observed with an associated 67 ± 17% decrease in the IMTG content and increased activity of mitochondrial enzymes. This study employed moderate to intensive daily exercise for 16-20 weeks however, the ethnicity of the participants was not identified. The difference of this study compared to the SPIRIT study was the BMI of the participants, which was 30 ± 1.5 (classified as grade 1 obesity), compared to BMI of 43.8 ± 9.5 (grade 3 obesity) in the SPIRIT participants[7]. Schrauwen and colleagues[31] compared the mitochondrial activity and IMTG in obese non-diabetic and BMI matched diabetic participants. They showed that there was similar IMTG content in the BMI matched T2DM relative to the obese individuals but the mitochondrial activity was markedly diminished in the T2DM individuals. These results may suggest that mitochondrial function as well as IMTG content may be important in people with T2DM.

Amati et al[33] reported that there was a twofold increase in IMTG content quantified by EM images in the sedentary obese subjects when compared to the lean counterparts and trained athletes. He found that the IMTG content was increased in both obese and trained athletes but the level of ceramide and DAG was higher in obese individuals. In this study the IMTG levels dropped in both AER and PRT groups after 16 weeks exercise howeve,r the ceramide and DAG levels were not determined. Bajpeyi[32] compared effect of short term exercise for 10 consecutive days on lean, obese (BMI 38.8± 1.7) and T2DM subjects (BMI=35.5±2.5) and reported that the reduction of IMTG by 35% quantified by oil-o-red staining technique was only observed in T2DM individuals. No change in IMTG was observed in grade 3 obese individuals (BMI= 38.8 ± 1.7) without diabetes[19]. These results are suggestive that the response to exercise is not universal; it seems quite variable and could be dependent on the metabolic status of the population under study. When these results are compared to the results of the SPIRIT study participants who were grade 3 obese as well as having diabetes, 16 weeks of AER or PRT exercise has been able to produce morphological and functional changes in the skeletal muscle and reduction of IMTG (28% in PRT, 48% in AER) is equivalent to that observed in Bajpeyi et. al study[32]. These results may suggest that metabolic adaptation in the SPIRIT cohort is occurring although not immense enough to produce the macro physiological changes such as change in HbA1c, and it is possible that these could occur if exercise was continued for longer duration. Furthermore, the increase in mitochondrial enzyme activity after 16 weeks of exercise could indicate that skeletal muscle is regaining metabolic flexibility[28,33].

The decreased density in lipid droplets (1.89 fold drop in AER group; 1.38fold drop in PRT group) in the muscle cell and increased activity of the BHAD enzyme (P=0.03 for AER group; P=0.078 for PRT group), a key mitochondrial enzyme involved in oxidation of fatty acids, could suggest that there was increased lipid handling and trafficking[1,12,34-36] in the skeletal muscle of the SPIRIT study participant. Exercise-induced changes in mitochondria can be beneficial for well-being of people with T2DM and obesity[20,23,28,37]. The results from the SPIRIT study (Table I) show that after 16 weeks of training the mitochondrial enzymes related with fatty acid oxidation (BHAD), electron transport chain (COX) and citric acid cycle (CS) are significantly increased in the PRT and AER exercise groups. These results are comparable with Baldi and Snowling[25] study where improvement in the mitochondrial enzymes activity was observed after resistance training of 10 weeks of moderate intensity in T2DM subjects.

Overall the mitochondrial enzyme data suggests that exercise is inducing changes at the cellular level in spite of lack of improvement in glycaemic control and insulin sensitivity noted for the SPIRIT study cohort[7,30]. Furthermore these results also support the notion that exercise can provoke some favourable changes in the metabolically unbalanced muscle fibre and is capable in altering the fatty acid oxidation in the muscle cell by improving the activity of the mitochondrial enzyme BHAD. However, to our knowledge there is no study found to date that has reported the change in function of mitochondria after exercise in grade 3 obese individuals with T2DM. The morphological and functional transformation of mitochondria by exercise could open new horizons in investigation regarding the role of mitochondria in exercise rehabilitation. The change in mitochondrial function and fatty acid metabolism in the muscle cell after exercise supports that it is not only IMTG that are responsible for the metabolic disturbances in muscle tissue but the role of mitochondria is also critical as proposed by Martins and colleagues[15].

Lastly although the data regarding the role of mitochondria and intramyocellular lipids in Pacific peoples with T2DM and obesity is consistent with previous research in this area[2,32,38] what was not investigated was the role of lipid products like ceramide and DAG in SPIRIT study participants. Ceramide and DAG are metabolically active fat particles that interfere with insulin signalling[15,33] and the study of these lipid products along with their transporter proteins such as Perlipin 2 and 5 would be important to further confirm the lipid trafficking within in the sketelal muscle building a relationship between mitochondria and lipid metabolism affecting insulin signalling pathway.

CONCLUSIONS

Overall these findings suggest that both PRT and AER exercise can be effective therapeutic modalities for the induction of changes at the cellular level in muscle of people with T2DM. These results supplement the current data available regarding the crucial role of mitochondria in metabolic dysfunction observed in T2DM and obesity which are collectively named as “diabesity” in the relevant literature[12,39,40]. The key strength of this study was that this is the first study of its kind in which investigation of mitochondrial and muscle lipid biomarkers were performed on a T2DM cohort with grade 3 obesity and in a Polynesian population.

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