NOVEL NETWORK EXPLORATION THROUGH MRNA CHANGES IN THE SKELETAL MUSCLE AFTER RESISTANCE EXERCISE

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) has been regarded as a syndrome of modified lifestyle disorder. The thrifty genotype has been used to elucidate the amplified incidence and prevalence of T2DM in high risk ethnic populations such as Pima Indians, Latino and African Americans, Japanese and Pacific people. Exercise moves the crucial imperfections linked with reduced metabolic capacity and fatty oxidation are interrelated to the etiology of obesity and insulin resistance.

Objectives: To investigate the statistically significant changes in skeletal muscle mRNA expression with resistance exercise after 16 weeks, using the microarray data and identify the statistically significant genes related to energy metabolism and skeletal muscle remodelling in response to exercise in the SPIRIT study cohort.

Materials and Methods: This randomized clinical trial was carried out at Massey University, New Zealand from 1st July 2015 to 31st December 2015. Eighteen participants with type 2 diabetes were recruited in aerobic exercise training for 16 weeks. mRNA was extracted from the skeletal muscle biopsy sample. Examination of the gene set, molecular and physiological function analysis and network construction was performed in Ingenuity Pathways Analysis. A total of 20,000 genes associated with the human genome were probed.

Results: Total 653 genes (3.3% of the human genome) were found to change in expression, with statistical significance, after 16 weeks of intervention. a total of 143 genes had a fold change ≥1.2. Out of the 143 genes, 120 showed a fold change ≥1.3 and 20 genes had a fold change ≥1.4.

Conclusion: The resistance exercise could be an important rehabilitation tool to stimulate skeletal muscle flexibility in people with T2DM and obesity.

Key words: Type 2 diabetes mellitus, Persistent resistance training, Resistance exercise

INTRODUCTION

Type 2 diabetes (T2D) is disease of modern age associated with 'thrifty genotype[1] and physical inactivity. These genotypes with food profusion and inactivity store plenty of fat. The fat in the body leads to such as obesity and skeletal muscle atrophy. These changes lead to T2D in people with "thrifty" genes.[2] These changes has been linked with increased incidence and prevalence T2D in certain ethnic populations such as Pima Indians, Latino and African Americans, Japanese and Pacific people.[3-5]

The rate of incidence of T2D is rapidly rising in New Zealand[6] and its incidence is more in Pacific Islanders.[7] Sukala et al[8] reported in the SPIRIT study that 16 weeks of exercise training either in the form of aerobic (AER) or resistance could be an intervention for T2D in Pacific Islands population. The clinical exercise trial that was performed (SPIRIT study)[9] showed improvement in quality of life in both AER and resistance groups.[10] The muscle of T2D subjects is associated with lipid deposits[11], decreased activities of key enzymes[12,13] atypical mitochondria[14,15] and lowered insulin senstivity.[16]

Skeletal muscle responds to exercise in people with T2D as it increases glucose utilizationl[17], oxidation of fat[18] and mitochondrial function.[19] This improved muscle metabolism with exercise has also been associated with improved insulin resistance. The weakened metabolic flexibility and increased lipid deposition and hence weakened muscle plasticity is associated with aetiology of T2D.

MATERIALS AND METHODS

This randomized clinical trial was carried out at Massey University, New Zealand from 1st July 2015 to 31st December 2015. Eighteen participants with type 2 diabetes were recruited to participate in aerobic exercise training for 16 weeks. The mRNA was isolated from

muscle tissue samples (~10 mg) using mirVana™ miRNA Isolation Kit (Applied Biosystems/Ambion, Austin, TX). Concentration of RNA was determined by NanoDrop® spectrophotometer ND-1000 (Nano Drop Technologies, Wilmington, DE) and RNA was used for mRNA gene expression profiling and all signalling data and all annotation files were exported for statistical analysis and integration (Partek 6.6, St Louis, MO). Statistically significant genes in the microarray data obtained are defined by Robust p value (ROBP< 0.005). Quality was determined with an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA). The RNA isolated The Ingenuity Software was used to interrogate the microarray gene selection to construct molecular system models from the functions related with skeletal muscle plasticity and glucose and lipid handling in the skeletal muscle tissue of SPIRIT participants. Networks/Modules identifying disease related functions were overlapped. Examination of the gene set and physiological function analysis was performed. The networks were constructed by using Ingenuity Pathways Analysis (IPA) software (Winter Release 2015, Ingenuity® Systems). The IPA software recognises the flow of upstream transcriptional controllers in the gene database incorporated.

RESULTS

In the RE, fibrosis, angiogenesis and immune and inflammatory cell assembly were the key functions recognized (Figs. 1-2). The increased leukocyte migration, new vessel formation, muscle development, and reduced fibrosis were mainly revealing remodelling in the skeletal muscle. The well-designed connection between diseased module fibrosis and vasculogenesis is shown in Figure 1 and diseased module fibrosis and leukocyte migration in Figure 2. The key hub genes that are elaborated in both fibrosis and vasculogenesis modules and overlap with the disease module insulin resistance were PPARG, IGFBP7, CAV1, PIK3R1, COL1A2, BDNF, IGF2 (Fig. 1).

In response to 16 weeks of RE, cellular movement, haematological system development and function, immune cell trafficking, inflammatory response, tissue and cellular growth and development were the top ranked functional modules. The identified increased leukocyte migration, vasculogenesis, muscle development, and decreased fibrosis were highly indicative of connective tissue remodelling. The upstream regulators associated with the topmost networks in the skeletal muscle after 16 weeks of RE (Figs. 1-2, Table 1).

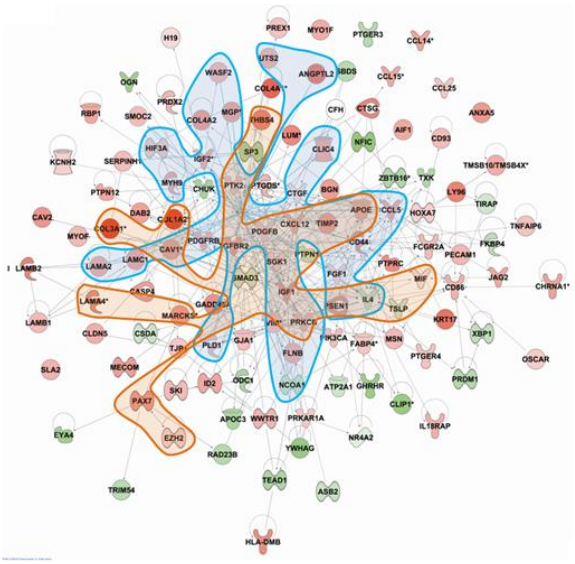

Figure 1: The disease module is fibrosis (regulation directional Z-Score -2.3, function p-value 7.79E-08, blue) and functional remodelling module is vasculogenesis (Z-score 2.3, p= 2.36.23E-06, brown). The two modules are overlapped to show the functional connectivity between the disease and functional adaptation modules leading to molecular plasticity associated with improved tissue metabolic function. The pink colour of the symbol represents upregulation of the gene and green colour represents down regulation.

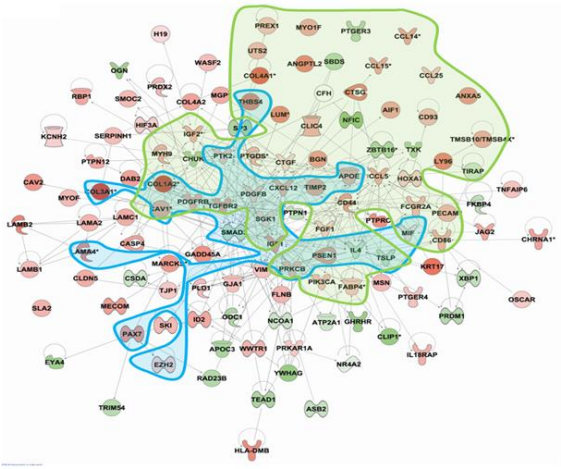

Figure.2: The disease module is fibrosis (-2.3, function p-value 7.79E-08, blue) and functional remodelling module is leukocyte migration (Z score unspecified 2.5, p= 1.23E-06, green). The two networks are overlapped together to show the functional connectivity between the diseased and functional module associated with improved fibrosis and extra-cellular remodelling associated with leukocytes. The pink colour of the symbol represents upregulation of the gene and green colour represents down regulation

Table 1: Predicted activation status of upstream regulatory factors associated with the top-ranked functional networks determining molecular regulation of skeletal muscle plasticity to resistance training in grade 3 obese T2DM adults

Upstream regulator	Molecular function	Predicted activation state	Regulation z-score	p-value of overlap
NFKBIA	Other	Activated	2.4	9.10E-06
HIF1A	Transcription regulator	Activated	2	5.90E-05
ERBB2	Kinase	Activated	2.2	3.70E-10
SMAD3	Transcription regulator	Activated	2.2	3.00E-06
FBN1	Other	Inhibited	-2.2	2.90E-07
EDN1	Cytokine	Activated	2.1	2.20E-06
HOXA9	Transcription regulator	Activated	2	1.70E-04
IL1B	Cytokine	Activated	3.7	1.50E-13
SMAD2	Transcription regulator	Activated	2.2	1.20E-04

ACOX1, peroxisomal acyl-coenzyme A oxidase 1; IL4, interleukin 4; IL6, interleukin MAPK1, mitogen-activated protein kinase 1, PTEN, phosphatase and tensin homolog; RELA, V-rel reticuloendotheliosis viral oncogene homolog A, PPARA, peroxisome proliferator-activated receptor alpha WISP2, WNT 1-inducible-sginalling pathway protein 2, CEBPB, CCAAT/enhancer binding protein beta; CTGF, connective tissue growth factor

DISCUSSION

A balanced exploration of the mRNA microarray data results was performed. The statistically significant genes (ROBP <0.005) were categorised rendering to their fold change. The functional and disease module intra-tissue cellular network modelling method was applied using IPA software to create modules/networks related with skeletal muscle structure and function (Figures 1 and 2). The hub genes (genes that are connected in both the disease and normal physiology networks) involved in skeletal muscle placticity related to muscle structure and metabolic functions were identified in relation to RE (Table 1). The genome networks were appraised to identify key networks. The modules offered allusive changes in fibrosis, vasculogenic modifications in reaction of the exercise. That induced introduced. mitochondrial transcriptional factors (mtTFA, mtTFB1 and mtTFB2) genes[20]-related with glucose metabolism.[21,22]

Both anti-fibrotic and pro-angiogenic modules were topmost disease and functional routes in response to resistance training. Increased tissue fibrosis is a well-known component contributing to disease state in obesity and T2D.[23] In skeletal muscle, excessive connective tissue accumulation is regarded to be triggered by disease associated pathways including spurs like TGFB1. TGFB1 is a protein structurally related similar to a cytokine.[24] It performs multiple functions related with production, proliferation and differentiation of immune cells. It is associated with immunity and chronic low-grade inflammation.[23] The increased endomyosin thickening that increases the physical transit distance between the capillary lumen and the myocellular insulin receptor, hypovascularity and impaired insulin signalling in capillary endothelial cells appear to be key components of skeletal muscle tissue insulin resistance.[13] Meanwhile, ECM and cytoskeleton remodelling are consistent with the literature[22-26], changes that may lead to reduced insulin resistance through the connective tissue mechanism. In this study, an important upstream regulator associated with AER training that was identified, was CEBPB (Fig. 2). Therefore, understanding the complex molecular mechanism(s), driving reduced fibrosis and vasculogenic plasticity could be beneficial in discovery of the cellular processes regulating improved tissue functional capacity in skeletal muscle with metabolic dysfunction.

The involvement of leukocytes in skeletal muscle regeneration from injury, fibrosis and myogenesis is well established, but identification of an inflammatory-related immune-cell associated regulatory molecular programme directing extracellular matrix remodelling from a within-subject longitudinal exercise-training intervention in grade 3 obese adults with T2DM is a new finding. Sedentary obese and diabetic adults have been reported to exhibit increased collagen deposition and profound thickening of the endomysium (the extracellular matrix layer surrounding individual muscle fibres).[27] Increased collagen and other extracellular matrix gene expression were seen in skeletal muscle from healthy adults but has not been demonstrated in T2DM subjects until this study.

The SPIRIT cohort had elevated blood lipid content shown by elevated levels of low density lipoproteins and triglycerides. Improved insulin

sensitivity results have been shown by lowering of blood lipids.[9] Strengths of the current network approach via the IPA software include construction of reliable disease and functional modules/networks based on the comprehensive human tissue specific transcriptome. A consequent weakness is the dataset complexity.

The key hypothesis underlying the network modular approach was that the disease modules/networks may represent dysfunctional-tissue phenotype associated with disease. Moreover, these phenotypic changes/dysfunctions associated with disease could be a consequence of changes in the key genes intersecting or overlapping upon several cellular and molecular processes of physiological significance. As a result of this interdependency, key functional and disease regulators, and associated gene regulatory connectivity was readily identified from centrally connected hubs. Additionally, the functions utility in IPA analysis provided further valuable insight into the control networks matching with muscle plasticity. It also informs on the complexity of the multiple molecular systems governing disease-attenuating plasticity to chronic exercise training in type-2 diabetic skeletal muscle. However these results should be considered with caution due to small number of participants (17 in total). Furthermore the significant mRNA changes observed in this study from the microarray data were not further validated by real-time PCR, due to unavailability of the muscle tissue.

CONCLUSION

These results suggest that exercise could be an important rehabilitation tool in people with T2D and obesity.

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