Dynamic proteome profiling of human muscle responses to high-intensity interval training

By

Kanchana Srisawat

A thesis submitted in partial fulfilment of the requirements of
Liverpool John Moores University
for the degree of Doctor of Philosophy

January 2019

Declaration

The work presented in this thesis was carried out at the Research Institute of Sport and Exercise Sciences, Liverpool John Moores University. Unless otherwise stated, it is the original work of the author. While registered as a candidate for the degree of Doctor of Philosophy, for which submission is now made, the author has not been registered as a candidate for any other award. This thesis has not been submitted in whole, or in part, for any other degree.

Some of works were published and presented as follow;

1. **Publication**
    a. Srisawat K, Shepherd SO, Lisboa PJ, Burniston JG. A Systematic Review and Meta-Analysis of Proteomics Literature on the Response of Human Skeletal Muscle to Obesity/Type 2 Diabetes Mellitus (T2DM) Versus Exercise Training. Proteomes. 2017;5(4):30. doi:10.3390/proteomes5040030
    b. Hesketh S, Srisawat K, Sutherland H, Jarvis J, Burniston J. On the Rate of Synthesis of Individual Proteins within and between Different Striated Muscles of the Rat. Proteomes. 2016;4(1):12. doi:10.3390/proteomes4010012
2. **Conferences proceeding**
    a. A systematic review and Meta-analysis of proteomics literature on the response of human skeletal muscle to obesity/type 2 diabetes mellitus (T2DM) vs exercise/stimulated exercise/healthcare intervention, A Celebration of Women in Research at LJMU 2018, March 2018
    b. A systematic review and Meta-analysis of proteomics literature on the response of human skeletal muscle to obesity/type 2 diabetes mellitus (T2DM) versus exercise training, Public Health PhD Symposium, Liverpool John Moores University, Redmonds Building, Brownlow Hill, Liverpool, L3 5UG, Poster presentation, July 2017
    c. A systematic review of proteomics literature on human skeletal muscle alteration to metabolic syndrome: **obesity and type 2 diabetes mellitus (T2DM)**. LJMU RISES PGR Conference, Liverpool – May 2016

Kanchana Srisawat
Research Institute of Sport and Exercise Science,
Liverpool John Moores University,
Byrom Street,
Liverpool, Merseyside,
L3 3AF, UK

Abstract

Skeletal muscle is the most abundant tissue in the body and it plays a central role in whole-body metabolism. Physical inactivity and obesity are associated with poor metabolic health due to insulin resistance of peripheral tissues, in particular skeletal muscle. Conversely, a physically active lifestyle or formal exercise training are associated with adaptations in skeletal muscle that improve its substrate metabolism and insulin sensitivity. Such changes in muscle function are underpinned by changes to the muscle proteome, i.e. relative increases or decreases in the abundance of select proteins. Proteomic analysis of human muscle biopsy samples involves non-targeted analysis of a large number of proteins and can generate new hypotheses. To date, the majority of proteomic studies report protein abundances only and the dynamic aspects (i.e. synthesis and degradation) of proteins in human muscle has seldom been investigated, but it is likely that the quality of muscle proteins i.e. turnover rate may also contribute to muscle function.

We have used deuterium oxide to label newly synthesised proteins in vivo and then analysed muscle biopsy samples using peptide mass spectrometry techniques that are capable of identifying individual proteins and measuring their relative abundance and fractional rate of synthesis. We have used this method to investigate 2 research questions:

(i) what changes occur in the skeletal muscle proteome associated with sedentary lifestyle and obesity, and

(ii) what is the dynamic proteome response of human muscle to high-intensity interval training?

Our cross-sectional study of obese vs trained individuals (N = 4, in each group) revealed novel proteins that had differences in abundance and synthesis rate. Proteins including carbonic anhydrase 3, heat shock protein β-1, glyceraldehyde 3 phosphate dehydrogenase, sarcoplasmic/endoplasmic reticulum calcium ATPase 1, creatine kinase muscle type, and type IIx myosin heavy chain) are more abundant and have a lower rate of turnover in the muscle of individuals with obesity. Therefore, these may be more likely to accrue deleterious post-translational modifications that may affect their activity or interaction with other proteins. We then performed a longitudinal study to investigate responses to 10-week high-intensity interval training (HIT) programme in the muscle of participants with obesity. HIT increased the turnover rate of individual muscle proteins and in particular we discovered greater abundance of 2 novel proteins, desmin and α-actinin-3, which have not been previously reported in regard to endurance exercise. In conclusion, we have applied a new dynamic proteome profiling method to investigate complex physiological responses associated with obesity and exercise training in human muscle *in vivo*. Our data support the hypothesis that obesity is associated with changes to the turnover rate as well as abundance of muscle proteins. Our findings bring new insight and a new avenue of research investigating protein quality control as a mechanism underpinning the deleterious effects of obesity and physical inactivity. HIT training was able to counter some of the effects associated with obesity and sedentary behaviour and this thesis highlights a number of key muscle proteins that are suitable for future targeted research.

Acknowledgement

I would like to pay special thankfulness to the Royal Thai Government (Strategic plan and policy Scholarship) who support full-scholarship cover 4 years. Office of the Civil Service Commission (the Prime Minister as the Chairman), Department of Disease control Ministry of Public Health, Thailand, and Office of Educational Affair, Royal Thai Embassy, London, UK who provide kindly and warmly support when I am in either Kingdom of Thailand or the United Kingdom.

I would like to pay my deeply gratitude, greatest appreciation and warmth to the person below who made my research successful and assisted me at every point to achieve my goal:

- My supervisory team
    - Professor Jatin Burniston for the majority of thesis contents are depending on his correction. For his performance on protein turnover rate calculation, research design, training in Proteomic laboratory, strong knowledge, and massive academic intention. This thesis cannot be accomplished without him. Special thankfulness for our reconciliation.
    - Dr Sam Shepherd for his solace, kindness, and guided to handle with 8 participants, sample collection, support in clinical issues, ELISA laboratory, muscle biopsy in related to his expertise, HIT session, collaborated on behalf of supervisory team.
    - Professor Paulo Lisboa for his guide in statistical analysis, meta-analysis, a chair of supervision meeting, and guided plan that make me feel comfortable with.
- Special thanks to my examiners: Professor Craig Sale and Dr. Juliet Strauss who have given the perfectly feasible feedbacks and recommendations to fulfil the gaps of this work.
- RISES staff, Dr Juliet Strauss, Dr Matt Cocks for collecting muscle biopsies, and Phlebotomy training by Dr, Neil Chester and Dr Dominic Doran.
- Participants (Dean, Matt, Martin, Phil, John, John T, David, and Peter) include Miss Katie Hesketh who is nicely help me to advertise, recruit, and organize meeting sessions for health check, collecting sample and, training, Liverpool John Moores University Student Governance, Liverpool student union, student counseling,
- My Mom and Dad, my sisters, my boyfriend, close-friends, friends, my colleagues in Thailand, without whom I was nothing; they also support morally and emotionally.

Finally, yet importantly, I would like to express my appreciation to particle factors which are much advantage to fulfil my years of PhD, i.e., Mendeley references manager, Overleaf, LaTeX creators, trips to different places. YouTube, game online, Thai spicy food, and international events.

Table of Contents

List of Table

List of Figures

Glossary

%labelled	percentage of labelling
%synthesis	percentage synthesis
1D SDS-PAGE	One Dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
1D/2D	One dimension or two dimensions
2D SDS-PAGE	Two-Dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
2D-DIGE	2-D Difference Gel Electrophoresis
AMSTAR	Assessing Methodological Quality of Systematic Reviews
ANOVA	Analysis of Variance
AUC	area under the curve
BLAST	Basic Local Alignment Search Tools
BMI	Body mass index
BP	Blood pressure
BSA	Bovine Serum Albumin
C	carbon
CA	Cancer
CHD	coronary heart disease
CI	Confidence Interval
CRF	chronic renal failure
CVD	cardiovascular disease
D	heavy hydrogen
DALYs	Disability Adjusted life years
DAVID	The Database for Annotation, Visualization and Integrated Discovery
DEXA	Dual–energy X–ray absorptiometry
DIGE	Difference gel electrophoresis
DM	Diabetes mellitus
DNA	Deoxy ribonucleic acid
DOAJ	Directory of Open Access Journals
DTT	Dithiothreitol
DVAID	The database for annotation, visualization and integrated discovery
ECG	electrocardiogram
ECM	Extracellular matrix
ELISA	enzyme-linked immunosorbent assay
ENV71	enterovirus 71 infection
ES	Effect size
FDR	False discovery rate
FMw	Fragment Molecular weight
FSR	Fractional synthesis rate FSR.
GC-MS	gas chromatography mass spectrometry
GLUT4	Glucose transporter type 4
GP	General Practice
H	light hydrogen
HbAb1C	glycated haemoglobin
HDMSE	High Definition Mass Spectrometry
HFHSD	High fat high sugar diet
HIT	High intensity interval training
HPLC-ESI-MS/MS	high-performance liquid chromatography/electrospray ionization tandem mass spectrometry
HR	Hazard ratio

	Glossary (continue)
HSCC	hypopharyngeal squamous cell carcinoma
hSGLT3	insulin-independent glucose transporter
HSPB6	heat shock protein β-6
HT	Hypertension HT
IAA	iodoacetamide
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
IFCO	Intermediate filament cytoskeleton organisation
IGT	glucose intolerant
IR	insulin resistance
IRMS	Isotopic ratio mass spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	liquid-chromatography-mass spectrometry
LE	Lean-healthy
LEOW	lean with slightly overweight
MALDI-TOF	matrix-assisted laser desorption/ionization
MIDA	Mass isotopomer distribution analysis
MMP-7	matrix metalloproteinase-7
MOB	morbidly obese BMI >40 kg.m2
MOWSE	Molecular Weight Search
MPE	mole per enrichment
mRNA	messenger Ribonucleic acid
MS	Mass spectrometry
MVPA	Moderate to Vigorous Physical Activity
MW	molecular weight
MYH1	Myosin-1
MYH2	Myosin-2
MyHC	Myosin heavy chain
N	nitrogen
NAFLD	non-alcoholic fatty liver disease
NCD	Non communicable disease
NEAA	Non-essential amino acid
NGT	non-glucose tolerance
NHS	National Health Service
NHST	null hypothesis significant test
NICE	the National Institute for Health and Care Excellence, UK
nLC-MS/MS	Nano liquid tandem mass spectrometry
NMES	Neuromuscular electrical stimulation
NMES	Neuromuscular Electrical stimulation
NW	normal weight BMI 18-24.99 kg.m2
O	oxygen
OB	Obese-sedentary or Obese/Obesity
OBT2DM	Obese with type II diabetes mellitus
OGTT	2-hour Oral glucose tolerance test
OMIM	Online Mendelian Inheritance in Man
OR	Odd ratio
OW	overweight BMI 25-29.99 kg.m2
OXPHOS	Oxidative phosphorylation
PAGE	Polyacrylamide gel electrophoresis
PCr	phosphocreatine

	Glossary (continue)
PDC	dehydrogenase enzyme complex PDC
PEST	Proline, Glutamic acid, Serine, and Threonine.
pI	Isoelectric point
PICO	population, intervention, comparator, outcome
PI3K-Akt	Phosphoinositide 3 kinase
PLR	*Plantaris*
PRISMA	the Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTMs	post-translational modifications
RA	rectus abdominus
RCTs	Randomised Clinical Trials
RE	bed rest with resistive exercise
RF	rectus femoris
RP-HPLC	Reverse phase high performance liquid chromatography
RQ1	research question 1
RQ2	research question 2
RR	Relative risk
RVE	bed rest a countermeasure or vibration
S	sulphur
SD	Standard Deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	Standard Error
SEM	standard error of mean
SLC2A	solute carrier protein
SPSS	Statistical Package for the Social Sciences
T1DM	Diabetes Mellitus type I
T2DM	Diabetes Mellitus type II
T3DM	Diabetes Mellitus type III
TCA	Tricarboxylic acid cycle
TENA	Tenascin C
TFA	trifluoroacetic acid
TiO2	Titanium dioxide
TLR4	toll-like receptor 4
TOF	Time of flight
UPS	Ubiquitin Proteasome system
VAT	visceral adipose tissue
VL	Vastus lateralis
$VO_{2\ max}$	maximum oxygen uptake
WHO	World Health Organization

List of Gene ontology (coverage UniProtKB keywords)

GO:0006936	**muscle contraction**
GO:0006939	smooth muscle contraction
GO:0006937	regulation of muscle contraction
GO:0030049	muscle filament sliding
GO:0006941	striated muscle contraction
GO:0045933	positive regulation of muscle contraction
GO:0045932	negative regulation of muscle contraction
GO:0061061	muscle structure development
GO:0007517	muscle organ development
GO:0007525	somatic muscle development
GO:0042692	muscle cell differentiation
GO:0045445	myoblast differentiation
GO:0045104	intermediate filament cytoskeleton organization
GO:0099185	postsynaptic intermediate filament cytoskeleton organization
GO:0045109	intermediate filament organization
GO:0060052	neurofilament cytoskeleton organization
GO:0045105	intermediate filament polymerization or depolymerization
GO:0006006	**Glucose Metabolic Process**
GO:0019574	sucrose catabolic process via 3'-ketosucrose
GO:0046430	non-phosphorylated glucose metabolic process
GO:0019664	mixed acid fermentation
GO:0006094	gluconeogenesis
GO:0010906	regulation of glucose metabolic process
GO:1902924	poly(hydroxyalkanoate) biosynthetic process from glucose
GO:0019655	glycolytic fermentation to ethanol
GO:0010907	positive regulation of glucose metabolic process
GO:0006007	glucose catabolic process
GO:0019650	glycolytic fermentation to butanediol
GO:0045239	**TCA enzyme complex**
GO:0042709	succinate-CoA ligase complex
GO:0030062	mitochondrial tricarboxylic acid cycle enzyme complex
GO:0045240	dihydrolipoyl dehydrogenase complex
GO:0045246	cytosolic tricarboxylic acid cycle enzyme complex
GO:0045242	isocitrate dehydrogenase complex (NAD+)
GO:0006629	**Lipid metabolic process**
GO:0008202	steroid metabolic process
GO:0016042	lipid catabolic process
GO:0044255	cellular lipid metabolic process
GO:0045834	positive regulation of lipid metabolic process
GO:1903509	liposaccharide metabolic process
GO:1902898	fatty acid methyl ester metabolic process
GO:0019216	regulation of lipid metabolic process
GO:0008610	lipid biosynthetic process
GO:1903173	fatty alcohol metabolic process
GO:0045833	negative regulation of lipid metabolic process
GO:1900555	emericellamide metabolic process
GO:0006119	**OXPHOS**
GO:0042776	mitochondrial ATP synthesis coupled proton transport
GO:1903862	positive regulation of oxidative phosphorylation
GO:0042773	ATP synthesis coupled electron transport
GO:0002082	regulation of oxidative phosphorylation
GO:0090324	negative regulation of oxidative phosphorylation

List of Gene Ontology (continue)

GO:0006810	Transport
GO:0006856	eye pigment precursor transport
GO:0006811	ion transport
GO:0044766	multi-organism transport
GO:0050892	intestinal absorption
GO:0099504	synaptic vesicle cycle
GO:0006836	neurotransmitter transport
GO:0006858	extracellular transport
GO:0006839	mitochondrial transport
GO:1901998	toxin transport
GO:0051051	negative regulation of transport
GO:0046907	intracellular transport
GO:0046903	secretion
GO:0009914	hormone transport
GO:0051050	positive regulation of transport
GO:1901678	iron coordination entity transport
GO:0010496	intercellular transport
GO:0099111	microtubule-based transport
GO:0042044	fluid transport
GO:0070633	transepithelial transport
GO:0071702	organic substance transport
GO:0016192	vesicle-mediated transport
GO:0098657	import into cell
GO:0055085	transmembrane transport
GO:0051049	regulation of transport
GO:0036465	synaptic vesicle recycling
GO:0071705	nitrogen compound transport
GO:0051180	vitamin transport
GO:0051181	cofactor transport
GO:0042908	xenobiotic transport
GO:0015893	drug transport
GO:0015915	fatty-acyl group transport
GO:0035459	cargo loading into vesicle
GO:0007300	ovarian nurse cell to oocyte transport
GO:0010232	vascular transport
GO:0100020	regulation of transport by transcription from RNA polymerase II promoter
GO:0007034	vacuolar transport
GO:0015669	gas transport
GO:0072348	sulfur compound transport
GO:0008219	**Cell death**
GO:0070265	necrotic cell death
GO:0012501	programmed cell death
GO:0070997	neuron death
GO:0060548	negative regulation of cell death
GO:0010941	regulation of cell death
GO:0036473	cell death in response to oxidative stress
GO:0010942	positive regulation of cell death
GO:0016209	**Anti-oxidant activity**
GO:0004362	glutathione-disulfide reductase activity
GO:0045174	glutathione dehydrogenase (ascorbate) activity
GO:0004601	peroxidase activity
GO:0050605	superoxide reductase activity

	List of Gene Ontology (continue)
GO:0004784	superoxide dismutase activity
GO:0032542	sulfiredoxin activity
GO:0004791	thioredoxin-disulfide reductase activity
GO:0051920	peroxiredoxin activity
GO:0005694	**Chromosomal protein**
GO:0005700	polytene chromosome
GO:0000803	sex chromosome
GO:0000228	nuclear chromosome
GO:0044427	chromosomal part
GO:0000229	cytoplasmic chromosome
GO:0030849	autosome
GO:0000793	condensed chromosome
GO:0004089	**carbonate dehydratase activity**
GO:0004089	carbonate dehydratase activity
GO:0015976	carbon utilization
GO:0050796	Regulation of Insulin secretion
GO:0046676	negative regulation of insulin secretion
GO:0032024	positive regulation of insulin secretion
GO:0061178	regulation of insulin secretion involved in cellular response to glucose stimulus
GO:0005576	**Extracellular region**
GO:0006860	extracellular amino acid transport
GO:0043083	synaptic cleft
GO:0044421	extracellular region part
GO:1900115	extracellular regulation of signal transduction
GO:1900116	extracellular negative regulation of signal transduction
GO:0006859	extracellular carbohydrate transport
GO:0006858	extracellular transport
GO:0098595	perivitelline space
GO:0010367	extracellular isoamylase complex
GO:0097579	extracellular sequestering of copper ion
GO:0043230	extracellular organelle
GO:0099544	perisynaptic space
GO:0048046	apoplast
GO:1903561	extracellular vesicle
Other	
GO:0019538	Protein metabolic process
GO:0002376	immune system process
GO:0042592	homeostatic process
GO:0051716	cellular response to stimulus
GO:0023052	signaling
GO:0016021	integral component of membrane
GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process
GO:0006351	transcription, DNA-templated

Chapter I

General Introduction

Contents

Obesity and sedentary behaviour as risk factors for T2DM

Diabetes is ranked 7th amongst the top 10 leading causes of death and killed approximately 1.6 million people globally in 2016 (World Health Organization, 2018). The majority of diabetes cases are type 2 diabetes (T2DM; Type 2 Diabetes mellitus) which is largely preventable (Melmed *et al.*, 2016) through the correct selection of healthy lifestyle choices. If left untreated, T2DM most severe symptoms include shock, unconsciousness, and ultimately death (Gilbert, 1992). Appropriate therapeutic management of T2DM patients could improve their quality of life (World Health Organization, 2016) and healthy life expectancy (Seuring, Archangelidi and Suhrcke, 2015) by preventing comorbidities (e.g. retinopathy, neuropathy, or vascular diseases). Individuals living with T2DM have peripheral insulin resistance accompanied by impaired glucose tolerance (IGT), obesity, and a significantly heightened risk of cardiovascular disease (CVD) (Ferrannini *et al.*, 1991; Grundy *et al.*, 2004). The World Health Organisation (WHO) Multi-national Study of Vascular Disease in Diabetes (MSVDD) reported data from 10 Centers (London, Switzerland, Warsaw, Berlin, Zagreb, Hong Kong, Tokyo, Havana, Arizona and Oklahoma) and concluded that 52 % of all CVD deaths occurred in patients diagnosed with T2DM (Morrish *et al.*, 2001).

According to Diabetes UK (2013), the most common risk factors of T2DM, include genetic predisposition, obesity (OB), hypertension (HT), poor mental health, and physical inactivity. Typically, the accompanying presence of obesity is regarded as a risk factor that exacerbates the effect of NCD (Non-communicable disease). The prevalence of obesity is high, 39 % of adult (18 years or over) were overweight, and 11 % of males and 15 % of females were obese in 2014. In total, 9 risk factors are stated as the modifiable risk factors of CVD; T2DM, HT, tobacco use, alcohol consumption, physical inactivity, mental disorders, unhealthy diet, cholesterol/lipids, and overweight/obesity. The modifiable risk factors imply that to prevent CVD and all NCD, it is necessary to develop national intervention plans/strategies. Modification in behavioural factors is the most effective and least costly intervention, particularly compared to pharmaceutical interventions, and the management of body weight has been a key objective of government health guidelines. The molecular basis of NCD is unknown but is polygenic and highly dependent on the environment factors including lifestyle choices such as diet and exercise habits

As highlighted by WHO, overweight and obesity are comorbidities of T2DM that accounted for 3.4 million deaths per year and 93.6 million disability-adjusted life years (DALY) in 2010 (World Health Organization 2014). Moreover, Public Health England reported that 90 % of adults with T2DM are either obese or overweight (Public Health England 2014). More locally, NHS Liverpool recorded 297 deaths due to T2DM according to death certificate registrations (Office for National Statistics, 2011). In England and Wales, obesity was the cause of 0.03% male and 0.04% female deaths in 2011 (Office for National Statistics, 2011). Although the percentage of deaths seems small, obesity has a wider notable

negative impact on quality of life including poor mental health such as loss of self-esteem and reduction in working effectiveness, and limitations to physical capacity due to effects on the respiratory system such as sleep apnea, and reduced daily activity levels and exercise intolerance.

Obesity and T2DM are also outcomes of sedentary lifestyle that involves physical inactivity, and may be accompanied by other risk factors such as tobacco use and alcohol consumption. T2DM may be largely preventable through positive lifestyle choices, and arguably the most critical component of a healthy lifestyle is physical activity. For example, there is irrefutable evidence that low levels of physical activity or low cardiorespiratory fitness increase the risk of T2DM and CVD (Warburton, Nicol and Bredin, 2006). In contrast, regular exercise confers a lower risk of death even in individuals that smoke cigarettes or display other established CVD risk factors such as HT or dyslipidaemia (Blair *et al.*, 1996).

Overweight and obesity have well-recognised metabolic risk factors and often coincide with insufficient physical activity habits. Research illustrates that insulin homeostasis plays an important role in metabolic diseases such as diabetes. Moreover, clinical diagnosis of elevated blood glucose (glucose intolerance) is underpinned by impaired-insulin secretion or peripheral insulin insensitivity/ insulin resistance that is largely associated with skeletal muscle metabolism (DeFronzo and Tripathy, 2009). A number of papers (e.g. (Hittel *et al.*, 2005; Hwang *et al.*, 2010; Lefort *et al.*, 2010; Al-Khalili, *et al.*, 2014)) report that insulin resistance is implicated with molecular dysfunction within skeletal muscle (Hittel *et al.*, 2005; Hwang *et al.*, 2010; Al-Khalili, *et al.*, 2014), such as mitochondrial malfunction (Lefort *et al.*, 2010), alteration of oxidative stress response (Al-Khalili, *et al.*, 2014), and to disruption proteasome function (Al-Khalili, *et al.*, 2014). Skeletal muscle is the largest organ for insulin-mediated glucose disposal which is directed by the canonical insulin receptor signalling cascade. This signalling pathway becomes more or less effective depending on skeletal muscle quality, and one of the major effectors of muscle quality is physical activity. A sedentary lifestyle can lead to obesity and skeletal muscle dysfunction, which impairs fat oxidation, alters lipid intermediates and inhibits the normal signalling downstream of the insulin receptor. This lessens glucose disposal in muscle and in-turn leads to impaired whole-body metabolism and greater disposal of fats in the liver and adipose tissue. Conversely, increasing physical activity by exercise training, such as endurance training or resistive exercise, is affirmed to counteract these adverse outcomes by improving muscle mass and insulin sensitivity. Concomitantly, regular muscle activity also helps control whole body metabolism because it involves regular cycles of release and use of fatty acids and helps maintain normal blood glucose level. Therefore, mechanisms associated with exercise not only improve muscle metabolism but also boost insulin responsiveness.

In many countries, the prevalence of inactivity exceeds that of obesity, alcohol misuse and smoking combined. For example, in the UK approximately two-thirds of adults fail to meet the minimum

recommendation of 30 minutes moderate exercise, 5 times a week (British Heart Foundation, 2015). Habitual activity is often categorised by the number of steps walked each day, i.e. less than 5,000 steps/day (inactive), 5,000-7,499 steps/day (low active), 7,500-9,999 steps/day (somewhat active), 10,000-12,499 steps/day (active), and 12,500 steps/day (highly active) (Tudor-Locke and Bassett, 2004). A recent study (Yuenyongchaiwat, 2016) reported sedentary-obese subjects who undertook 10,000 steps per day, had improved physical and mental health as well as weigh reduction. Moreover, a further literature has recommended moderate-to-vigorous physical activity (MVPA) of 10,000 steps/day or walking 100 steps/minute as being sufficient physical activity for healthy adult population. Others advocate a minimum range for 'free-living physical activity' and recommend 7,000-8,000 steps/day based on the philosophy 'some physical activity is better than none' for health promotion (Tudor-Locke *et al.*, 2011). However, lower than recommended physical activity would likely increase detrimental outcomes including obesity (Flegal *et al.*, 2002) or other non-communicable diseases (U.S. Department of Health and Human Services; Center for Disease Control and Prevention, 1996). A Meta-analysis on television viewing, as an example of one aspect of modern lifestyle, found every 2 hours of TV viewing per day associated with a relative risk (RR) of T2DM morbidity of 1.20 (95%CI, 1.14-1.27)(Grøntved and Hu, 2011). A 6y-follow-up study revealed 3,757 (7.5%) of 50,277 women became obese (BMI ≥ 30 $kg.m^{-2}$)(Hu *et al.*, 2003) and long-term TV viewing was associated with elevated blood lipid levels (Jakes *et al.*, 2003). Therefore, reducing sedentary behaviour by increasing physical activity such as walking and cycling, is supported by 72 countries (The 6th ISPAH International congress on physical activity and health, 2016) and is a key aspect of WHO guidelines for sustainable development (World Health Organisation (WHO), 2018) .

The contribution of skeletal muscle to whole body metabolism

In recent years, interest in the role of skeletal muscle in whole body metabolism and the aetiology of T2DM has increased. Skeletal muscle accounts for 40 % of body mass in adults (Owen *et al.*, 1978; MacIntrosh, Gardiner and McComas, 2006; Li and Larsson, 2010). Skeletal muscle is important in human movement, and the protein composition of muscle dictates muscle contractile properties, in particular the myosin heavy chain (MyHC) isoform composition of muscle has a major influence on the velocity of shortening of muscle fibre, i.e. slow-twitch fatigue resistant and fast-twitch fatigable (MacIntrosh, Gardiner and McComas, 2006; Li and Larsson, 2010).

Muscles are arranged in repeating structures called sarcomeres that consist of thick and thin filament proteins (actin and myosin) that account for ~50 % of the protein content of muscle (Houston, 1999; Wilborn and Willoughby, 2004). Muscles are innervated by somatic efferent α-motor neurons that innervate numerous myofibers to constitute individual motor units. Signals from the cerebral cortex,

transmitted by α-motor neurons, terminate at the motor end-plate and result in the release of acetylcholine at neuromuscular junction, which is the connection between motor neuron branches and the muscle fiber. This instigates an action potential that travels along the length of muscle fiber and T-tubule system, which consists of invaginations of the surface membrane into the interior of the fiber (Jones, Round and Haan, 2004). This wave of depolarisation through the T-tubule system instigates the release of calcium ion (Ca^{2+}) from the sarcoplasmic reticulum via the ryanodine channels (Jones, Round and Haan, 2004).

This process is called excitation-contraction coupling (EC-coupling) and is the beginning of the interaction between the thin-filament (actin) and the thick filament (myosin) which is responsible for muscle contraction (Jones, Round and Haan, 2004). Muscle thin filaments consist of actin, troponin, and tropomyosin with proportion 7:3:2, respectively (Hill *et al.*, 1992). Tropomyosin at the resting state prevents cross bridge formation between myosin and actin. However, to allow muscle contraction, tropomyosin will move from the active site of actin to instigate the cross-bridge cycle. This process is activated by the troponin-tropomyosin complex in response to calcium ion (Ca^{2+}) binding to Troponin C, which changes the conformation of the troponin-tropomyosin complex and allows the myosin head to interact with actin. Muscle thick filament contains bundles consisting of 2 myosin heavy chain that each has 1 regulatory light chain and 1 essential light chain associated with neck region of the myosin heavy chain molecule. The globular head of the myosin heavy chain is made by N-terminal of protein and has actin binding sites and ATPase activity. These 2 sites are essential for cross bridge cycle which transforms chemical energy to mechanical energy (metabolism) (Hill and White, 1968).

The contractile characteristics of fast-twitch muscle are determined by myosin heavy chain (MyHC) type IIa (gene MYH2), or IIx (gene MYH1) isoforms whereas slow-twitch expresses MyHC type I (gene MYH7). In rat, skeletal muscle there are different proportions or heterogeneity between both muscle fibre types. For example, soleus (SOL) represents a distinctive slow-fibre type because it is a postural muscle. On the hand, extensor digitorum longus (EDL) exhibits a more dominant fast-fiber phenotype. These characteristics relate to patterns of protein expression (particularly metabolic enzymes) in addition to the dominant myosin heavy chain isoform and other contractile proteins mentioned above. Fast-twitch muscle fibres rely heavily on the glycolytic pathway whereas slow-twitch fibres have a greater potential to use aerobic metabolic pathways, e.g. they may rely more on the oxidation of fatty acids. In human, muscles exhibit a mixed fibre type distribution. For example the vastus lateralis which is commonly used for molecular studies in humans contain about 10 % type IIx, 30 % type IIa and 60 % type I but these proportions can be markedly different depending on the age and physical activity status of the individual (Cobley *et al.*, 2016).

A unique property of muscle is its plasticity and ability to change phenotype, particularly in response to physical activity or sedentary behaviour, which can change the contractile properties of muscle and cause associated changes in muscle metabolism (St-Jean-Pelletier *et al.*, 2017). In relation to chronic disease prevention, muscle activity contributes the greatest magnitude of effect (e.g. compared to dietary habits) and so provides a potential free form of medication. This is particularly true for T2DM patient that suffer from metabolic dysfunction in muscle that impact whole body/ peripheral insulin sensitivity and glucose control. The main function of metabolism is to produce energy and synthesise biochemical substances via multiple metabolic pathways. In muscle, the central metabolic pathways include carbohydrate, lipid, and nitrogen metabolism as well as high-energy phosphates (e.g. phosphocreatine) (Bronk, 1999). These subdivisions of metabolic pathway include cell catabolic and biosynthetic activities (Bronk, 1999). Carbohydrate pathways include glycolysis, pentose phosphate, glycogen synthesis and degradation (Bronk, 1999). Nitrogen metabolism covers protein synthesis, nucleotide synthesis, nucleotide catabolism, amino acid metabolism, and urea synthesis (Bronk, 1999). Lipid metabolism comprises fatty acid oxidation, fatty acid synthesis, cholesterol synthesis, ketone body metabolism, and synthesis of phospholipids and triacylglycerols (Bronk, 1999). In muscle, catabolic activities are important to break down storage polymers in muscle tissue (e.g. muscle glycogen) into smaller molecules (e.g. glucose) in preparation for energy-producing pathways. The main catabolic pathways in muscle that produce substrates for energy metabolism (i.e. glycogenolysis/glycolysis and β-oxidation of fatty acids) provide intermediates for the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) which serve to drive oxidative phosphorylation (OXPHOS). OXPHOS is the major ATP producing process in muscle and resynthesise ATP from ADP at complex V of the mitochondrial respiratory chain on the inner mitochondrial membrane. The Phosphocreatine and glycolytic pathways are able to resynthesise ATP independently of a supply of oxygen but the capacity for ATP resynthesis without oxygen is limited, hence, phosphocreatine becomes depleted and by-products are produced. Note also that most of muscle ATP resynthesis is aerobic, i.e. at rest and during recovery, as well as contributing to ATP requirements during exercise (Salway, 2004). Therefore, increases in physical activity through daily activity or exercise training are associated with adaptations to the muscle metabolic pathways that provide the energy for muscle activity (Salway, 2004). Evidence suggests that skeletal muscle dysfunction associated with obesity and T2DM involves impairments in aerobic/ oxidative metabolism and inflexibility in transitioning between different substrate usages. In particular, an accumulation of muscle lipid intermediates, including ceramides (Central metabolic points in sphingolipid biosynthesis and breakdown) is significantly greater in OB-T2DM people in comparison with obese-non-diabetic subjects' (Broskey *et al.*, 2018) and this has been linked to dysfunction of the muscle insulin signaling pathway (Salway, 2004).

Glucose and fatty acids are stored in form of glycogen and triglyceride, respectively. However, there is no storage molecule of amino acids because proteins are functional macromolecules. When the body needs amino acids, muscle is the primary source of proteins that are broken-down into peptides and then amino acids (Lippi *et al.*, 2006). Muscle is major repository of amino acids and is an insulin sensitive tissue. In healthy people, muscle is responsible for the majority of insulin-mediated glucose uptake after a protein-containing meal. Fats are also deposited in muscle as intra and inter cellular lipid stores and amino acids can be released from muscle to support metabolism or provide amino acid substrates for other tissues. On the other hand, people with chronic disease such as diabetes have reduced skeletal muscle function and glucose uptake in response to insulin is impaired because of dysfunctional insulin receptor signalling that disrupts the transport of amino acids and other macronutrients through cell membrane (Salway, 2004).

Peripheral insulin resistance is a major hallmark of T2DM. Clinically this is evident as poor blood glucose homeostasis because peripheral tissues, in particular skeletal muscle, become insensitive to the actions of insulin. Therefore, (after a meal or oral glucose tolerance test; OGTT) relatively less glucose is deposited in muscle and this places a greater burden on the liver to convert excess glucose for storage in adipose tissue as fatty acids. An insulin resistant state is associated with glucose intolerance, which means it takes a relatively greater time to reduce blood glucose level after each meal, and this raises the risk for glycation modifications to proteins (e.g HbA1C) that are associated with protein dysfunction. For example, a key diagnostic criterion of T2DM and is described in ICD-11 code 5A11 (International Statistical Classification of Diseases and Related Health Problems 11th Revision for ICD-11 for Mortality and Morbidity Statistics 2018). The NHS, UK (National Health Service) diagnosis criteria is HbAb1C level ≥ 48 mmol.mol^{-1} or OGTT: resting blood glucose > 7 mmol.mol^{-1} and increasing to >11 mmol.mol^{-1} after glucose administration. HbAb1C is recommended to diagnose blood glucose intolerance because it is an accurate reference that can be applied internationally.

Insulin controls blood glucose levels by stimulating the transport of glucose molecules through the cell membrane of target tissues, in particular skeletal muscle. Insulin binding to its receptor on muscle results in a cascade of downstream events. The insulin signalling cascade consists of 4 main actions; i. Glucose storage and uptake, ii. Protein synthesis, iii. Lipid synthesis, and iv. Mitogenic response. In muscle, a key function of insulin is to trigger membrane protein families to uptake glucose, amino acids, and fatty acids, the most well-known of these is GLUT4 for glucose. Overall scheme of insulin toward glucose, amino acids, and free fatty acids (FFA) is shown in **Figure 1**.

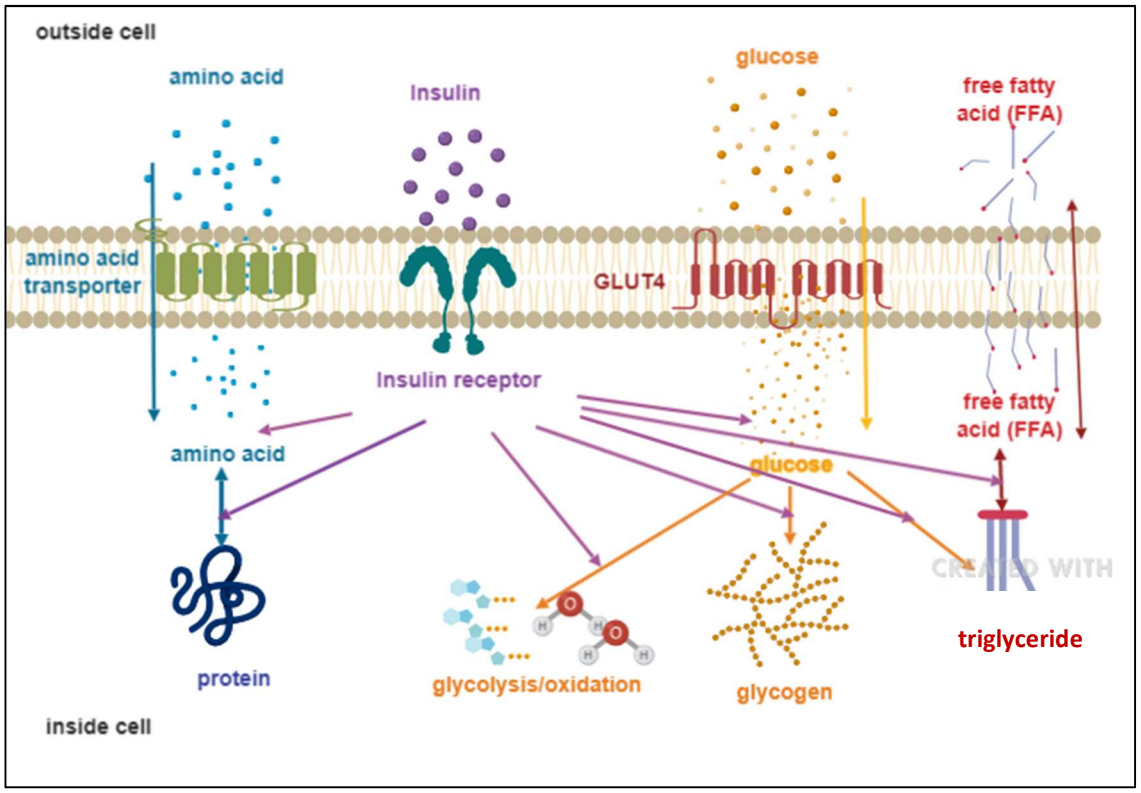


Figure 1 Insulin regulated metabolism i.e. Glucose, amino acid, and lipid

Insulins main function is to store carbohydrate, protein, and lipid. Meanwhile it inhibits degradation of these macromolecules. The Signal transduction of insulin action that regulates carbohydrate, lipid, and protein synthesis are included. Graphic was drawn on website BioRender (https://biorender.com/).

One of the effects of this signalling cascade is that stores of GLUT-4 protein are brought to the cell membrane. When GLUT-4 is in the cell membrane, it transports glucose from the blood to within the cell. This is the main mechanism by which insulin stimulation of muscle increases the capacity of muscle to take up glucose. As a result, blood glucose levels normally reduce when insulin signalling functions properly. In obese/ glucose intolerant individuals, blood glucose levels remain elevated for a relatively longer period (demonstrated in 2-h-oral glucose tolerance test, OGTT) because of resistance to the actions of insulin that leads to a less than adequate response of peripheral tissues to take up glucose. When glucose remains high in blood and cannot be transferred into muscle cells effectively the excess glucose will be metabolised to lipid in the liver and contribute to dyslipidaemia. Consequently, lipids are distributed throughout the body and form other diagnostic criteria such as cholesterol level (LDL, total cholesterol). Dyslipidaemia may also disrupt glucose uptake and lead to further muscle insulin resistance (Ragheb *et al.*, 2009). Obese and T2DM subjects have high LDL and total cholesterol level, but low levels of HDL in comparison with Lean-healthy people (Alshehri, 2010). In addition, excess free fatty acids can deleteriously effect liver and can induce inflammation and fibrosis. The adverse effect of free fatty acid is known as Non-alcoholic fatty liver disease (NAFLD). A population study (N = 2,020) with 31

years follow-up reported that childhood obesity/overweight can predict the risk of NAFLD in adulthood (Cuthbertson *et al.*, 2018).

In healthy individuals skeletal muscle is responsible for the majority of insulin mediated glucose disposal (Grundy *et al.*, 2004), whereas, resistance to the effects of insulin is a characteristic feature of type 2 diabetes (Petersen *et al.*, 2007). Dysfunction of the insulin signalling cascade is one of the major hypotheses of peripheral insulin resistance where signalling downstream of the insulin receptor becomes inhibited by lipid intermediates and this means fewer GLUT-4 transporters move to the cell membrane and less glucose is deposited in muscle. However, it is likely that there are other processes that also contribute to muscle dysfunction. Based on the strong protective effect of exercise training against T2DM risk we hypothesis that a deeper understanding of the mechanisms of muscle adaptation to exercise will contribute to the discovery other additional mechanisms that might also modulate muscle insulin sensitivity.

Endurance exercise as a preventative countermeasure against T2DM risk

The link between exercise capacity and all-cause mortality is irrefutable and has been reviewed extensively in recent years (Warburton, Nicol and Bredin, 2006; Warburton *et al.*, 2010). A person's health status can be measured by their maximum aerobic capacity (VO_2 max) using a graded exercise test (Myers *et al.*, 2002). Using this technique, low aerobic capacity has consistently been shown to be a stronger predictor of early mortality than other established risk factors. As such exercise capacity is a primary contributor factor to the prevalence of NCD, including CVD, cancer (CA), T2DM, and chronic respiratory disease (Warburton, Nicol and Bredin, 2006; Booth, Roberts and Laye, 2012). According to the American Heart Association, statistical data suggest physical inactivity and low aerobic capacity causes atherosclerotic cardiovascular disease including hypertension and insulin resistance, and significantly reduces life expectancy. Whereas, regular exercise improves disease incidence rates and is linked to an enhanced quality of life (Thompson *et al.*, 2003). Exercise is established as one of the key medical intervention methods of chronic disease prevention (Booth, Roberts and Laye, 2012) as well as primary and secondary prevention of premature death for T2DM (Warburton, Nicol and Bredin, 2006). Moreover, it has been recommended to reduce the risk of non-communicable diseases by integrating moderate-intensity exercise at least 60 min and 150-300 min per week in children and adult, respectively (World Health Organisation, 2015).

In a non-diabetic population (N = 2,324) with high resting glucose levels (i.e. glucose intolerance), lifestyle change including exercise was more effective than Metformin treatment in controlling glucose intolerance (Knowler *et al.*, 2002). Although WHO recommends moderate intensity exercise (50-60% maximum heart rate), High intensity interval training (HIT) is an attractive alternative choice. A

systematic review and meta-analysis of the comparison between the effects of moderate-intensity continuous training (MICT) vs HIT revealed that both modes of exercise had similar effectiveness and improved the body composition of overweight and obese participants (Wewege *et al.*, 2017). However, sedentary lifestyles common in modernized societies have limited physical activity which is due to multiple factors such as free time, access to facilities or distractions such as television. Moreover, continuous exercise may not be well tolerated, and adherence may be low in obese participants. Therefore, HIT with its time-efficiency and high-impact becomes a better choice and it has been introduced for overcoming the limitations imposed by modern lifestyles. HIT is well documented to increase aerobic capacity, improve insulin sensitivity, and is a time-efficient training strategy (Cocks *et al.*, 2013; Shepherd *et al.*, 2013). In a systematic review and meta-analysis of 26 RCT (n=318) there was a positive impact of HIT and it was demonstrated as an effective alternative to traditional training that reduce volume of activity (Gist *et al.*, 2014). Work from our group (Shepherd *et al.*, 2015) has not only reported advantages on physical health (cardio-metabolic fitness) but also revealed evidence of improvements in psychological wellbeing.

Aerobic exercise causes adaptation of the cardiovascular system (Torok *et al.*, 1995; Meyer *et al.*, 2003; Vincent, Bourguignon and Vincent, 2006; Burniston, 2009; Randers *et al.*, 2014), muscle mass (Salanova *et al.*, 2014, 2015), metabolism (Sheffield-Moore *et al.*, 2004; Cuthbertson *et al.*, 2005; Lefort *et al.*, 2010; Egan *et al.*, 2011; Hipkiss, 2011; Hody *et al.*, 2011; Hussey *et al.*, 2013), and weight control (Oscai, 1973; STEFANICK, 1993; Volek, VanHeest and Forsythe, 2005). The mitochondrial capacity of muscle is increased by endurance exercise training and this can help to counteract functional declines associated with sedentary behaviour, disease or ageing. At the molecular level, the major role of mitochondrial is to replenish (resynthesise) cellular ATP via oxidative phosphorylation; mitochondria also have roles in apoptosis, calcium handling and redox regulation. During exercise, the energy consumption of skeletal muscle is increased and the heightened requirement for energy (in the form of ATP) is met by the metabolism of metabolic substrates, including intracellular stores (e.g. phosphocreatine, muscle glycogen and intramyocellular triacylglycerides) and circulating substrates (e.g. blood glucose and non-esterified free fatty acids). The activity of glycogen phosphorylase rises under the effect of adrenaline release (from central nervous system) and muscle contraction by exercise. Adrenaline is accompanied by noradrenaline, which stimulate the hormone-sensitive lipase of adipose tissue to ensure that muscles have increased supply of fatty acid from adipose tissue. This phenomenon also prevents muscle from depleting the blood glucose level too much. Adrenaline secretion also increases oxygen supply, improving blood flow to muscle, increasing heart rate, and ensuring arterial blood supply. Central nervous system can also send signals to medulla to anticipate exercise to increase muscle activity. Even before muscle activity occurs; Adrenaline and Noradrenaline can flood the muscle with glucose phosphate and the blood with fatty acids. This ensures that the sudden demand of exercise will not cause

too great a reduction the level of blood glucose. Release of fatty acids is necessary during exercise since the represent the body's main energy store.

Consistent with the mechanism discussed regarding impaired glucose tolerance, studies also demonstrate a key role of the glucose transporter protein (GLUT) in the muscle response to exercise. The GLUT protein family consists of 3 protein classes based on sequence similarity and are encoded by the solute carrier protein (SLC2A) gene family (Joost and Thorens; Uldry and Thorens, 2004). With regards to insulin sensitive tissue, GLUT4 or insulin-dependent glucose transporter is the dominant isoform in skeletal muscle (Roberts, Hevener and Barnard, 2013). According to Wang et al (2009), the review reported 5(Hughes *et al.*, 1993; Dela *et al.*, 1994; Christ-Roberts *et al.*, 2004; Holten *et al.*, 2004; Kim, Lee and Kim, 2004) out of 7(Fritz *et al.*; Hughes *et al.*, 1993; Dela *et al.*, 1994; Christ-Roberts *et al.*, 2004; Holten *et al.*, 2004; Kim, Lee and Kim, 2004; Castaneda F, Layne JE, 2006) studies found GLUT4 mRNA/protein expression are increased by either resistance or aerobic exercise(Wang *et al.*, 2009). However, it is unlikely that Glut4 translocation is the only mechanism responsible for the beneficial effects of exercise in obese sedentary individuals. New 'omic' technologies such as proteomics are a proven method for unbiased generation of new hypotheses and the non-targeted approach of omic techniques is more likely to discover novel information than hypothesis-led research that may be biased by misinterpretation. That is, because we do not fully understand the mechanisms responsible for the metabolic dysfunction of muscle or the preventive effects of regular exercise, we are not in a position to predict which proteins or processes are of greatest importance and therefore should be elevated above others when selecting future targets for hypothesis-led research. **The first aim of this thesis is to synthesise evidence from previous studies on what changes occur in the skeletal muscle proteome associated with sedentary behaviours and obesity versus muscle adaptations to exercise training.**

Proteomic evidence that has been published recently provides analysis of the skeletal muscle proteome from models of exercise (Burniston and Hoffman 2011) and insulin resistance (Deshmukh, 2016). Burniston (Burniston, 2008) used treadmill running in rats and was the first proteomic work to investigate the muscle response to exercise. In addition to detecting cardinal features of the muscle response to exercise, the analysis found novel changes in enzymes such as aconitase and phosphoglucomutase which were not previously linked with the muscle exercise response. Our laboratory (Burniston, Kenyani, *et al.*, 2014) has also used a model of artificial selection on high running capacity (HCR) and low running capacity (LCR) to discover muscle proteins that segregate with differences in innate muscle aerobic capacity. In recent years, proteomic methods and technology have been developed including sample preparation techniques and mass spectrometry for advanced protein identification to provide in-depth protein profiles of diabetic skeletal muscle. Approximately, 8,039 out of 10,218 individual proteins were identified in skeletal muscle and included metabolic processes,

mitochondria, and enzymes of core metabolic pathways (Deshmukh *et al.*, 2015; Deshmukh, 2016). In addition, 10 proteins are found to be approximately 50% more abundant in diabetic muscle, including, myosin heavy chain (isoform 1 and 4), titin, nebulin, calcium ATPase 1, α-actin, creatine kinase, α-actinin-3, and glycogen phosphorylase (Deshmukh *et al.*, 2015). However, these works were conducted in rodent skeletal muscle which has a different myofiber profile from human muscle; for example slow myosin of rat demonstrates a higher velocity of contraction than that of human (Canepari *et al.*, 2000).

The development of proteomics research has the potential to make a prominent impact on health-related studies. For example, the gathering of proteomic evidence has been used to raise multiple new hypotheses regarding pathology (metabolic disorder, OB, T2DM) and muscle adaptation to exercise training (Wang *et al.*, 2009; Burniston and Hoffman, 2011; Malik *et al.*, 2013; Deshmukh, 2016). However, these past reviews of the literature have been hypothesis-led or argument-led and have not been collected systematically. Multiple biases may have been incorporated by the reviewers such as selective bias (narrative review) or methodological bias (hypothesis-led review). Therefore, this thesis uses an evidence-synthesis methodologies (systematic review and meta-analysis) in **Chapter II** in order to interpret the findings from the current proteomics literature in an unbiased manner and provide a clearer foundation for the current experimental work.

The previous proteomics literature has highlighted numerous proteins that are either more or less abundant in the muscle of OB/T2DM condition or after exercise training. The abundance of each protein is determined by the balance between its rates of synthesis versus degradation. Therefore, the synthesis and degradation of muscle proteins (i.e. turnover) is a key regulatory method involved in muscle responses to disease or exercise training. **A key aim of this thesis is to investigate whether obesity and exercise training effect the synthesis and degradation of proteins in human muscle**. More recently stable isotope tracer methodologies have begun to be incorporated in to proteomic studies (e.g.(Lam *et al.*, 2014)) and this can bring important new understanding of differences in protein turnover that may underpin differences in protein abundance. The analytical techniques and computational methods for measuring individual protein turnover rate are still in the early stages of development and so **Chapter III** focuses on establishing proteomic techniques for the analysis of stable isotope (deuterium) incorporation in to proteins in rodents, before attempting this work in human participants (reported in Chapters IV and V).

Muscle protein turnover itself may also be effected by obesity and T2DM. Non-targeted proteomic investigations from 3 leading laboratories in the field of muscle and type 2 diabetes (Hwang *et al.*, 2010; Ghosh *et al.*, 2011; Al-Khalili, *et al.*, 2014) have each highlighted that differences in the ubiquitin proteasome system co-occur with differences in muscle insulin sensitivity. Therefore, the loss of muscle insulin responsiveness in T2DM patients may cause muscle atrophy through effects on protein

degradation. In addition there is evidence that short periods of high-fat feeding blunt muscle protein synthesis in humans (Stephens *et al.*, 2015), and pharmacological inhibition of the proteasome in cultured human myotubes causes insulin resistance (Al-Khalili, de Castro Barbosa, *et al.*, 2014). However, the effect of insulin resistance on muscle protein synthesis in vivo is not as clearly defined. Protein synthesis may be maintained in well-controlled diabetes at the muscle (Halvatsiotis *et al.*, 2002) and whole body level (Gougeon *et al.*, 2008) but, nonetheless, patients with diabetes have exacerbated age-related declines in muscle mass (Workeneh and Bajaj, 2013). Therefore, an underlying role of defects in muscle protein synthesis cannot be ruled out. The difficulty in interpretation of the current literature in part arises from the use of amino acid tracers to investigate protein turnover (Jaleel *et al.*, 2008). First exogenous amino acid tracers are subject to metabolism and transport in vivo, which may be defective in T2DM. Second, amino acid tracers cannot be used to measure individual proteins because the level of incorporation of label in to each protein is relatively low and cannot be detected using current measurement techniques. Therefore, currently data only exist on the synthesis rate measured across mixtures of muscle proteins which is unlikely to give a clear indication of the effects of disease or exercise training.

To begin to understand the effects of obesity and a sedentary lifestyle on the turnover of individual muscle proteins, **Chapter IV** asks the question "**what differences exist in the abundance and synthesis rate of proteins between sedentary obese individuals and lean endurance-trained athletes.**" We use methods of deuterium oxide administration in vivo and peptide mass spectrometry that were established in Chapter III to investigate the muscle proteome of 2 independent groups of male volunteers (i.e. cross-sectional study). Our results are the first to show that there are differences in both the abundance of muscle proteins and the synthesis rate of individual proteins.

Changes in muscle protein synthesis are involved in the adaptation to exercise and have most commonly been investigated in connection with resistance exercise training (Phillips *et al.*, 1997), However, endurance training may also be associated with changes in the average synthesis rate of mixed muscle proteins (Short *et al.*, 2004). Most recent studies have confirmed that HIT improves quality of life, and has the benefit of being time-efficient. Our group (Holloway et al 2009) performed the first proteomic analysis of human muscle responses to exercise and found significant changes in the abundance of muscle proteins, including metabolic enzymes. Therefore, **Chapter V** asks the question **"What changes occur in the skeletal muscle proteome of obese humans that partake in sustainable time–efficient high intensity interval exercise intervention?"** This chapter presents a longitudinal study of high-intensity interval training (HIT) using the group of obese sedentary individuals that were studied in Chapter IV. Consistent with Chapter IV, deuterium labelling and peptide mass spectrometry are used to show that the response of human muscle to HIT involves both changes to the abundance of muscle proteins and also changes to the rate of synthesis of individual muscle proteins.

Chapter II Evidence Synthesis of Proteomic study under the effect of obesity/type2diabetes/insulin resistance and Endurance exercise intervention

Contents

Summary

We performed a systematic review and meta-analysis of proteomics literature that reports human skeletal muscle responses in the context of either pathological decline associated with obesity/T2DM and physiological adaptations to exercise training. Literature was collected from PubMed and DOAJ databases following PRISMA guidelines using the search terms 'proteom*', and 'skeletal muscle' combined with either 'obesity, insulin resistance, diabetes, impaired glucose tolerance' or 'exercise, training'. Eleven studies were included in the systematic review, and meta-analysis was performed on a subset (4 studies) of the reviewed literature that reported the necessary primary data. The majority of proteins (n = 73) more abundant in the muscle of obese/T2DM individuals were unique to this group and not reported to be responsive to exercise training. The main response of skeletal muscle to exercise training was a greater abundance of proteins of the mitochondrial electron transport chain (ETC), tricarboxylic acid cycle (TCA) and mitochondrial respiratory chain complex I assembly. In total, 5 proteins (**NDUA8**; NADH dehydrogenase [ubiquinone] 1 α-subcomplex subunit 8, **NDUB8**; NADH dehydrogenase [ubiquinone] 1 β-subcomplex subunit 8, mitochondrial, **NDUS2**; NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial, **AATM**; Aspartate aminotransferase, mitochondrial, and **ATPB**; ATP synthase subunit β, mitochondrial) were less abundant in muscle of obese/T2DM individuals and were also reported to be more abundant in the muscle of endurance-trained individuals, suggesting one of the major mechanisms of exercise-induced protection against the deleterious effects of obesity/T2DM occurs at complex I of the electron transport chain.

Introduction

The incidence of OB and T2DM has reached epidemic proportions and is a major burden on both individual patients and their healthcare providers. NCD including metabolic syndrome and T2DM may be largely preventable through correct selection of lifestyle choices, and arguably the most critical component of a healthy lifestyle is physical activity. For example, there is irrefutable evidence that low levels of physical activity or low cardiorespiratory fitness increase the risk of T2DM and CVD (Warburton, Nicol and Bredin, 2006). In contrast, regular exercise confers a lower risk of death even in individuals that smoke cigarettes or display other established CVD risk factors such as hypertension or dyslipidaemia (Blair *et al.*, 1996)

Despite the robust link between exercise capacity and all-cause mortality, the mechanisms responsible for the benefits of exercise are poorly understood. Nevertheless, changes that occur in skeletal muscle are thought to be particularly important because this tissue represents approximately 40% of body mass and is responsible for both physical work and the majority (~80%) of insulin-stimulated glucose uptake (DeFronzo et al., 1981). Moreover, skeletal muscle exhibits profound plasticity in response to changes in the environment including physical activity/inactivity and diet. Muscle is also an easily accessible tissue in humans and currently there is a wealth of literature reporting human muscle adaptation to exercise training or diseases such as T2DM. Most of this literature is generated through hypothesis-led reductionist experiments. Without disregarding the importance of these works, we have favoured the use of post-genomic hypothesis generating techniques, in particular proteomics. Our chosen approach is based on the premise that the proteome is a highly relevant level of study because it is the product of genetic, epigenetic and post-transcriptional processes, and the function of a cell/tissue is determined by its protein complement. Moreover, we regard the proteome as being the interface between the genome and the environment and, therefore, ideally located for the study of complex polygenic processes that are highly responsive to environment factors. Last but not least, proteomic studies use comprehensive non-targeted analysis to generate data that can be interrogated by unsupervised statistical techniques and, therefore, have the potential to discover entirely new information.

We (Burniston and Hoffman, 2011) and others (Wang *et al.*, 2009; Deshmukh, 2016) have previously conducted narrative reviews of muscle proteomics literature from human studies investigating the benefits of exercise or the patho-biochemical changes that occur in OB individuals or T2DM patients. However, a major shortcoming of the previous reviews (including our own) is that the selection of articles by the reviewing authors could have introduced bias. This is not a trivial matter because it is entirely at odds with the unbiased hypothesis generating nature of proteomics and other -omic sciences. Moreover, because we do not fully understand the mechanisms responsible for the metabolic dysfunction of muscle or the preventive effects of regular exercise, we are not in a position to predict

which proteins or processes are of greatest importance and therefore should be elevated above others when selecting future targets for hypothesis-led research.

In an attempt to address this issue, we have performed a systematic review of proteomics literature, thereby matching the non-targeted data collection of proteomics with the systematic/non-biased selection of research literature. Initially, our aim was to systematically review proteomic data relating to the effects of exercise in the muscle of humans that are OB, glucose intolerant (IGT) or have T2DM. Our initial literature search revealed that to date just one article (Hussey *et al.*, 2013) reported such data, whereas most publications focused on either the pathophysiological adaptation associated with metabolic disorders/disease or the physiological adaptation to exercise training. Based on the rationale that some exercise-induced adaptations may directly oppose patho-biochemical changes associated with obesity and impaired glucose tolerance (van Tienen *et al.*, 2012)we chose to systematically review each sub-area independently in an attempt to find contrasts that may indicate proteins common to each scenario. Therefore, the aim of this study was to review proteomic studies that have investigated human skeletal muscle in the context of either (RQ1) pathological decline associated with OB, insulin resistance (IR), or T2DM and (RQ2) physiological adaptations in response to exercise training. Specifically, we were driven by the questions, "**what changes occur in the skeletal muscle proteome of humans that are OB, exhibit signs of the metabolic syndrome or diagnosed with T2DM**" and "**what changes occur in the skeletal muscle proteome of humans that partake in regular exercise.**"

A meta-analysis was also conducted to increase the precision of the evidence on protein abundance data, with respect to RQ1 and RQ2, obtained from the systematic review. This chapter therefore consists of two sections: Section 1 is the systematic review and Section 2 is the meta-analysis. The systematic review used only original articles selected following the PRISMA guidelines, formulated the findings based on PICO, and assessed the quality of evidence obtained by the Delphi list and AMSTAR. The meta-analysis was conducted on publications derived from the systematic review that provided sufficient statistical information to be eligible for inclusion in the meta-analysis. The results were presented and compared with those generated from the systematic review but only considered individual proteins that have been reported in repeated studies.

Systematic Review

Section 1 first describes the methodology used to select eligible studies to be included in the systematic review using the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and the PICO principle (P; patient, problem or population, I; intervention, C; comparison, control or comparator, O; Outcome measure), and a quality assessment of the review. The results and main findings are then presented followed by a discussion surrounding individual protein function and expression that may have either a direct or an indirect impact on human health through metabolic disease or exercise training. Herein exercise was limited to endurance training which included High intensity interval training (HIT).

Methodology

Eligibility criteria of potentially inclusive studies

The present study first set strict criteria that were to be used to evaluate studies that could be potentially included in the systematic review. The initial eligibility criteria were English, original, peer-reviewed articles that used proteomics methodologies to study human skeletal muscle. For the purposes of this review, we define proteomics as non-targeted investigations at the protein level that use comprehensive/high-throughput analysis techniques to generate new information and hypotheses. The general aim of included articles must focus on identifying individual proteins as well as an outcome (e.g. change in protein abundance) in response to either disease or exercise training.

Study Selection

The systematic review process was conducted according to Fortmann et al (Fortmann *et al.*, 2013). Abstracts were independently screened by two investigators against pre-specified inclusion criteria. To eliminate confounding factors which may interfere with the systematic analysis, we only included articles that reported individual protein abundance changes as an outcome measure. Articles were excluded if the methodology was unclear, for example if the method of protein abundance measurement could not be identified. A consensus meeting was held to resolve potential conflicts regarding the inclusion of studies. A third investigator was available to resolve conflicts but in all cases a consensus was reached between the primary investigators during the consensus meeting.

Subject-group definitions

RQ1, pathological decline due to disease

BMI (Body Mass Index) was used to stratify participants. To be included in the systematic review participants must be overweight (OW; BMI >25 to 29.99 $kg.m^{-2}$), obese (OB; BMI 30-39.99 $kg.m^{-2}$) or morbidly obese (MOB; BMI >40 $kg.m^{-2}$. Type 2 Diabetes mellitus (T2DM) risk was assessed by biological evidence, participants were stratified as having impaired glucose tolerance (IGT; an individual with intermediate hyperglycemia i.e., fasting plasma glucose 6.1–6.9 $mmol.L^{-1}$ or 110–125 $mg.dL^{-1}$)(World Health Organization and International Diabetes Federation, 2006), or T2DM (fasting plasma glucose ≥7.0 $mmol.L^{-1}$ or 126 $mg.dL^{-1}$, or 2 h oral glucose tolerance plasma glucose concerntration ≥11.1 $mmol.L^{-1}$ or 200 $mg.dL^{-1}$) (World Health Organization and International Diabetes Federation, 2006).

RQ2, exercise training intervention for aerobic muscle adaptation

For the purposes of this review, traditional forms of exercise (i.e. continuous/ endurance) have been included as well as exercise training which includes endurance exercise in the form of High intensity interval training (HIT).

Definition of Exercise training

Endurance training includes activities that increase breathing and heart rate such as walking, jogging, swimming, and biking. Endurance activity keeps heart, lungs and circulatory system healthy and improves overall fitness.

HIT is a type of endurance training that involves repeated bouts of high intensity interspersed with periods of recovery. This type of endurance exercise must include time interval, training session duration, frequency of exercise, and target intensity of the high-intensity component (e.g. approximately 80% maximum oxygen uptake)(Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013; Schild *et al.*, 2015).

The PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)

The systematic review was conducted according to PRISMA (Moher *et al.*, 2009). Briefly, four stages of literature appraisal including identification, screening, eligibility, and inclusion were used. PubMed and the DOAJ databases were searched using terms consisting of proteom* AND skeletal muscle AND obesity OR insulin resistance OR diabetes. Searches were limited to the period between 1996 and 2015, and the last literature search was performed on 10th December 2015.

For research question 1 (RQ1): proteom* AND skeletal muscle AND obesity OR insulin resistance OR diabetes. On the other hand, research question 2 (RQ2) used key words of: proteom* AND skeletal muscle AND exercise.

PICO for article assessment (P; patient, problem or population, I; intervention, C; comparison, control or comparator, O; Outcome measure)

The guidelines of the National Institute for Health and Care Excellence (NICE, UK) were used to structure our systematic review and data was extracted from articles according to the PICO model ('Developing NICE guidelines: the manual | 4-developing-review-questions-and-planning-the-evidence-review | Guidance and guidelines | NICE', no date). This method was developed in accordance with the Cochrane collaboration systematic review database. The tool can be used to appraise and approve high-quality evidence especially in health research, thereby helping to make decisions on the effectiveness of clinical treatments (*Cochrane, Trusted evidence, Informed decisions, Better health.*, no date). In the present study, **P**articipants were subset according to RQ1 and RQ2, and **I**ntervention or **I**ndicators such as BMI, glucose tolerance and training status were extracted where relevant. The **C**omparator was health status (RQ1) or training status (RQ2) and the **O**utcome data collected were the identity and relative abundance of muscle proteins.

AMSTAR (Assessing the Methodological Quality of Systematic Reviews)

No single tool can be used to fully assess multi-dimensional evidence, particularly clinical-based evidence. Nonetheless, we applied the AMSTAR checklist (Shea *et al.*, 2007, 2009) to give insight to the strength and robustness of our study method for the systematic review. Previous reviews in this field likely include bias because they cited hypothesis-led literature (Wang *et al.*, 2009) or generated selective bias based on choices regarding the publications cited (Deshmukh, 2016). Therefore, we integrated AMSTAR to evaluate whether our systematic review contains non-selective bias. This will help inform future use/inclusion of our work in this field of literature. Our review could answer 'YES" to 9 out of 11 (82%) AMSTAR questions and therefore does not contain non-selective bias.

A Criteria list for Quality Assessment of Randomised Clinical Trials (RCT) for Conducting Systematic Review (The Delphi List)

The Delphi List (Verhagen *et al.*, 1998) has been developed for appraising the of outcome research studies. The majority of data included in our review was generated from RCT that may require specific quality assessment using the Delphi list. For example, the scale or hierarchy of outcome measures may be ambiguous. This tool assesses core study i.e., subject, method, and outcome. All 3 dimensions are expected to perform

randomly. The strength of each component of evidence is scored in 4 levels i.e., Low Quality (0.0% - 25.0%), Medium Quality (25.1% - 50.0%), Medium High Quality (50.1% - 75.0%), and High Quality (75.1% - 100.0%). The method of evaluation was done by 3 blinded-assessors. The results of the 20 articles included in RQ1 and RQ2 are available in the supplementary material.

Meta-analysis in individual protein study

There is a large body of literature from experiments that have been conducted for a diverse range of purposes, including the assessment of health technologies, evaluating the cost-effectiveness of drug interventions, or the validation of alternative health interventions. Particularly with regard to exercise interventions, it is common to find inconsistent treatment or intervention strategies because of differences in the intensity, duration and mode of the exercise. Outcome measures may also vary amongst publications or parallel research may be performed in different populations and using different designs. In this study, a meta-analytic method was used in addition to systematic review of the literature, to further identify individual protein changes associated with the independent variables: RQ1 disease-related muscle dysfunction or RQ2 exercise-induced muscle adaptation. Our systematic review highlighted numerous proteins that exhibit different levels of abundance but it is not clear whether these differences are important at the population level. The aim of a meta-analysis is to combine data from all common proteins that have been reported to be significantly different in abundance in the primary literature. An advantage of meta-analysis is that it can reduce or control the risk of bias or bias', i.e., selection bias, performance bias, attrition bias, detection bias, and reporting bias. Although our systematic review assessed the quality of evidence, a statistical method such as meta-analysis is required to increase the precision of the review and to find effects that are important at a population level.

Meta-analysis is unlike null-hypothesis significance testing (NHST). The purpose of meta-analyses is to measure the magnitude of treatment/intervention effect (i.e. effect size (ES)) or the strength of a relationship between 2 variables in a population. Meta-analysis originally emerged from medical research in the mid-1980s and has since been applied in numerous other areas such as psychology and politics. We are using meta-analysis to improve the precision of protein data reported to be significant in the primary research literature. Meta-analysis may also answer give insight to the effectiveness of treatments or health care interventions such as exercise training. By considering the magnitude of the ES or estimating the ES range (Confidence Interval, CI) meta-analysis provides more reliable results that can be used to inform policy decisions. ES can be derived from primary outcome data using the Mean difference (Cohen's *d*), Pearson product moment correlation (*r*), Odd ratio (OR), Relative Risk (RR), or Hazard Ratio (HR). When summarising primary research data, different models may be used, e.g. fixed-

or random-effect models, depending on the design of the original studies. The primary output data from meta-analyses include tables and graphics such as Forest plots and Funnel plots.

Table 1 demonstrate summary point of new statistic vs conventional statistics

Keys	Dichotomous method	Estimation Thinking	Meta-analytic thinking
Statistical inference	NHST reject H_0 vs accept H_0	CIs point estimate NHST as true effect	ES (LL, UL) combined estimation (small, medium, large)
Question type	Yes/no	Why/what	Yes, then why/what
Guidance for Decision	Clear but misleading incomplete picture	Give more information reveal underlying	Give more information reveal underlying
General use	Independent research	Population study e.g. medical/policy research	Population study evidence synthesis
Difference	p-value (without direction) descriptive statistic e.g. M, SD, SE	OR, HR,RR (with direction)	Effect size index (OR, HR, RR, d, r) (with direction) (true effect = 0 or 1)

Methodology

The purpose of meta-analytical studies is to i) increase power or increase the chance of detecting a true statistically significant result if one exists, ii) improve precision especially when analysing evidence based on multiple sources of information, iii) answer questions that may be broader than the specific primary experiment, and iv) settle controversies arising from apparently conflicting studies. Data were extracted from studies that performed NHST to provide information on significant differences in protein abundance. Such studies do not provide information on whether these effects are important at a population-level. To perform Meta-analysis on proteomics data, there are some points to be considered, i.e., types of study design, reported statistical values in the primary studies, the ES that is appropriate to primary data, and data interpretation. Therefore, we took the following 4 steps, spanning data preparation through to interpretation.

Definition of criteria, variables, and effect of study

All papers included in the meta-analysis were obtained from our systematic review which reported significantly different proteins. Therefore, protein data should include sufficient statistical information for further analysis,

including exact p-value, mean, standard error of mean (SEM), or standard deviation (SD). However, it was necessary to exclude some studies that were eligible for inclusion in our systematic review but did not report the required statistic information and, instead, only reported fold-change values.

Independent variables were disease (RQ1) and endurance exercise (RQ2). For exercise studies, either a history of exercise training (long-term endurance trained) or a specific exercise intervention were included. Whereas dependent variables were protein abundance changes either under the effect of disease or in response to habitual exercise or an exercise training intervention.

Data preparation and extraction

Data were acquired for proteins that were reported to be significant in the primary research articles. Data were categorised into 2 sets according to experimental design; Independent groups and repeated measures studies. Secondly, the ES of all continuous data was calculated alongside, Cohen's *d* and Pearson Product Moment correlation or *r*. Some studies did not provide exact p-value data (e.g. only P<0.05 was reported) and raw p−values could not be converted directly to Pearson Product moment correlation (*r*). In such cases it was necessary to transformed data to Z-scores, and then back-calculate to find the true *t-test* value.

Cross-sectional study contained control and intervention/treatment group for comparison in a single event (time). Ten articles (Hojlund et al., 2003; Hittel et al., 2005; Lefort et al., 2010; Thingholm et al., 2011; Giebelstein et al., 2012; Al-Khalili, Barbosa, et al., 2014; Al-Khalili, de Castro Barbosa, et al., 2014; Caruso et al., 2014; Schild 2015) out of the 18 publications were of a Cross-sectional experimental design. However, these articles reported different statistical data i.e., mean ± SD, mean ± SE, and *p*−value. Therefore, data were converted into Pearson Product moment correlation (*r*). This data transformation was most often necessary for studies included in RQ1.

Quasi-experimental design was most common in publications from RQ2. Three (Holloway *et al.*, 2009; Egan *et al.*, 2011; Schild *et al.*, 2015) out of 20 studies used this design. The main statistical outcomes were reported based on Student's *t−test* distribution. Notably, 2 studies (Hussey et al., 2013; Schild et al., 2015) used both cross-sectional and longitudinal experimental designs. A flowchart documenting data extraction is presented in **Figure 2.**

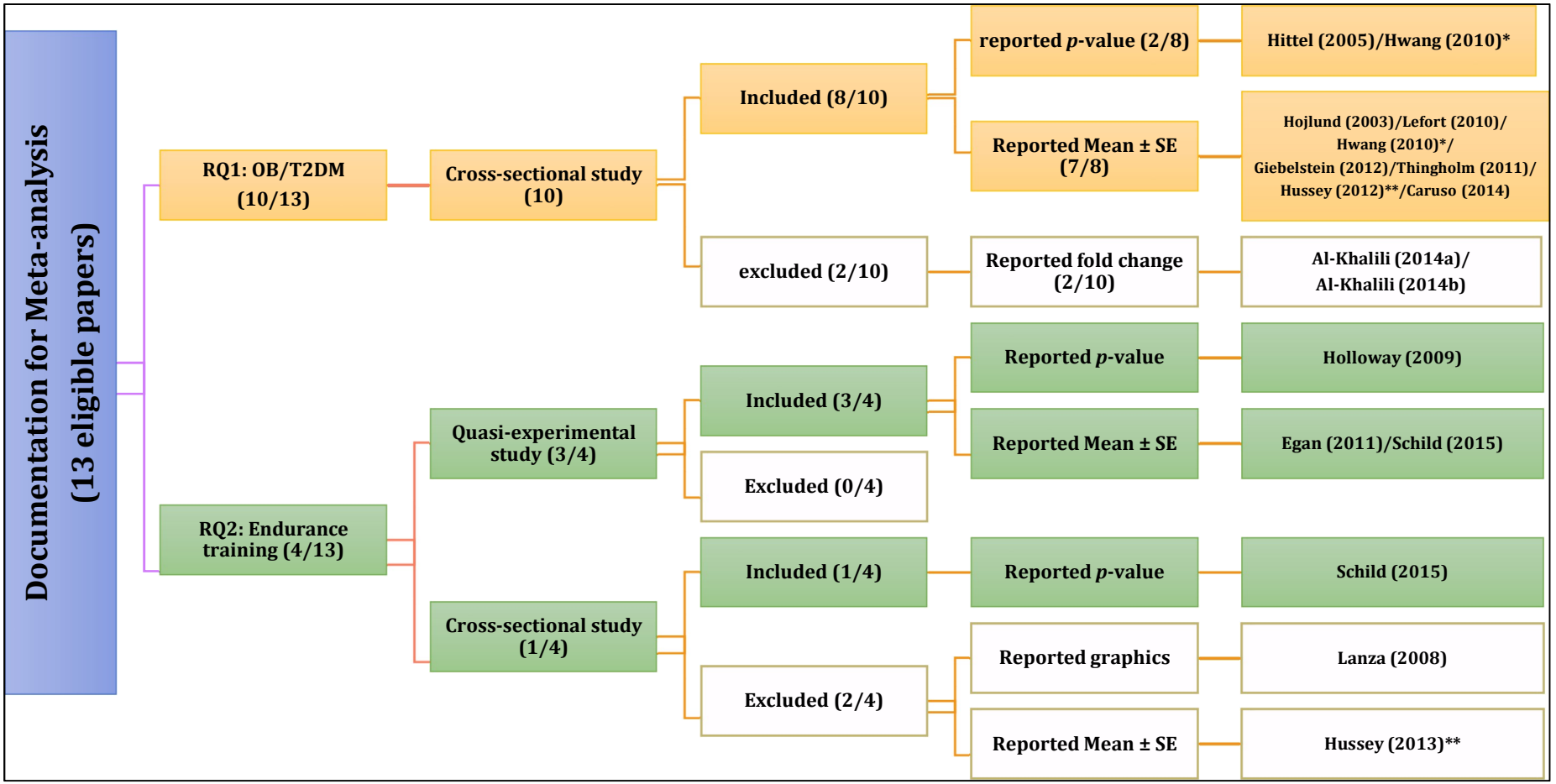


Figure 2 Documentation for Meta-analysis.

Initially, 13 studies met meta-analysis criteria. Despite Hussey et al (Hussey *et al.*, 2013) performed pre vs post endurance training within T2DM subjects, the statistical report has not sufficient for meta-analysis in RQ2. Schild et al (Schild *et al.*, 2015) have conducted their studies in 2 different design; cross-sectional and quasi-experimental study. * is for paper that reported both *p*-value and Mean ± SE and ** is for paper that reported in both RQ1 and RQ2.

Meta-analysis calculations

Primary data collected from original research articles was compiled in Excel. Data transformations were also conducted in Excel. Data were then analysed by Comprehensive Meta-Analysis software (Biostat, Englewood, NJ).

Summary Effect gives a summary of the magnitude of the effect across repeated studies on a single statistical analysis/ dependent outcome (i.e. particular protein). Essentially, it presents r based on Z-score or z_r. The Forest plot is a graphic ouptput of Meta-analysis that displays the summary effect. Ordinarily, the ES of each individual study is presented as a box while the summary effect is illustrated by a diamond shape in the forest plot. Each individual study must be combined and weighted before a conclusion can be drawn. Two methods can be used to combine replicated studies, i.e., Fixed- and Random- models. Each method considers variance within the original studies but there are some differences in concepts. ES and *p* – value when represented as r conform to a Chi-square (c^2) distribution that can be interpreted into small ($0.00 \leq r \leq 0.10$), medium ($0.10 < r \leq 0.30$), and large ($0.30 < r \leq 0.50$) effects, similar to Cohen's *d*. Fixed model assume all the combined studies have the same variance. On the other hand, the Random method accommodates differences in variance between studies.

However, r has to convert to Fisher's z scale (z_r) in Meta-analysis because the variance of *r* (V_r) strongly depends on the level of correlation. Therefore, summary effect is based on Fisher's z, and then z_r will be converted back to *r* for presentation under c^2 distribution. Both models of summary effect are back transformed to apply NHST. Correlation indicates the direction of variables but does not apply to represent the magnitude of ES. For this reason, to combine the *p*- value, we apply to NHST to test the hypothesis that the effect in all studies is zero.

Two-step meta-analysis

The current study utilised a complicated data input process. Each RQ was bifurcated in to a summary effect for the single study and a summary effect from aggregated studies. A tool for determining which summary effect is most appropriate is heterogeneity. Heterogeneity (I^2) has been used to consider to what extent the result of studies are consistent. The Cochrane's *Q* statistic is applied to determine I^2 by summing the squared deviations of each study's estimate from the overall meta-analytic estimate, and then weighting each study's contribution in the same manner as in the final meta-analysis. Where Q is the observed true variation to the within study error and df is its degree of freedom or expected variation within study error. A grading guide of I^2 interpretation is available in **Table 2**.

Table 2 Guide to interpretation the heterogeneity (I^2)

I^2	Interpretation
0% to 40%	Might not be important
30% to 60%	May represent moderate heterogeneity
50% to 90%	May represent substantial heterogeneity
75% to 100%	Considerable heterogeneity

The decision to use either Fixed or Random effect model was based on our research questions. This was not dependent on *p*-value for heterogeneity. However, *p*-value could imply the distribution across the studies and determine whether there is real variance among the effects. Where I^2 is higher and *p*- value is significant or *p*- value < 0.05, the Fixed-model would not fit the data. In this study, there were 2 levels of data that had to combine. The first dataset relied on Fixed-effect assumption because some studies reported more than 1 protein abundance value. This was required to combine primary ES in order to consolidate aggregated studies. On the other hand, Random-effect method was integrated in the second level of analysis. This assumed that all included studies were performed in different populations that had different variations. In addition, heterogeneity has been applied to investigate the dispersion of data.

Data interpretation

Concept of Assumption

To interpret data, we have to determine 3 components of hypothesis test; heterogeneity, fixed-effect model, and random effect model.

Heterogeneity: NHST or H_0 is that the studies share a common effect size.
Fixed effect model; NHST or H_0 is that the common effect is zero.
Random effects model: NHST or H_0 is that the mean of the true effects is zero.

For example, did all individuals that were included in the repeated studies were assumed homogeneous or they shared a common effect size? Thus, Fixed-effect model was applied under the assumption that the common effect is zero or variance between studies is zero. If p-value falls under 0.05 (*p*-value <0.05), the conclusion indicates that disease or exercise has a significant effect on individual protein abundance. On the other hand, if variance between studies is true (i.e. NHST of heterogeneity test is rejected), the Random-effect will be applied. Therefore, the conclusion will be considered following p-value (*p*-value < 0.05). In this case, Fixed-effect could not be used because all the included experiments do not share a common variance.

Potential protein regulation

Each protein was presented by the assumption of summary effect. However, summary effect indicates how either disease or intervention effect on protein abundance change or strength of the relationship between 2 variables (Cohen, 1988). Therefore, the effect size index in this study would not likely specify actual direction of protein abundance. Reasonably, these assumptions based on increase and/or decrease abundance/fold change/mean difference, variance, and relative weight regarding sample size. Therefore, to determine the direction of individual protein's regulation under these effects, itis also useful to refer back to the original input data.

Results

RQ1: Effect of OB/T2DM on individual protein abundance change

RQ1 Article Selection

In total, 235 publications were collected during the initial searches and 138 were included after elimination of duplicates. Each abstract was screened against the eligibility criteria and a further 101 publications were excluded. The majority (n = 69) of the excluded publications reported data from animal models, whilst n = 28 were review articles, and n = 4 reported the medicinal effects of plant extracts. Therefore, 37 full-text articles were assessed in detail, and 10 publications were retained for further assessment (Figure 3). The earliest reported study meeting the eligibility criteria was Højlund et al. (Hojlund et al., 2003) published in 2003, and therefore the current review considers literature over a 13 year period from 2003 to 2016.

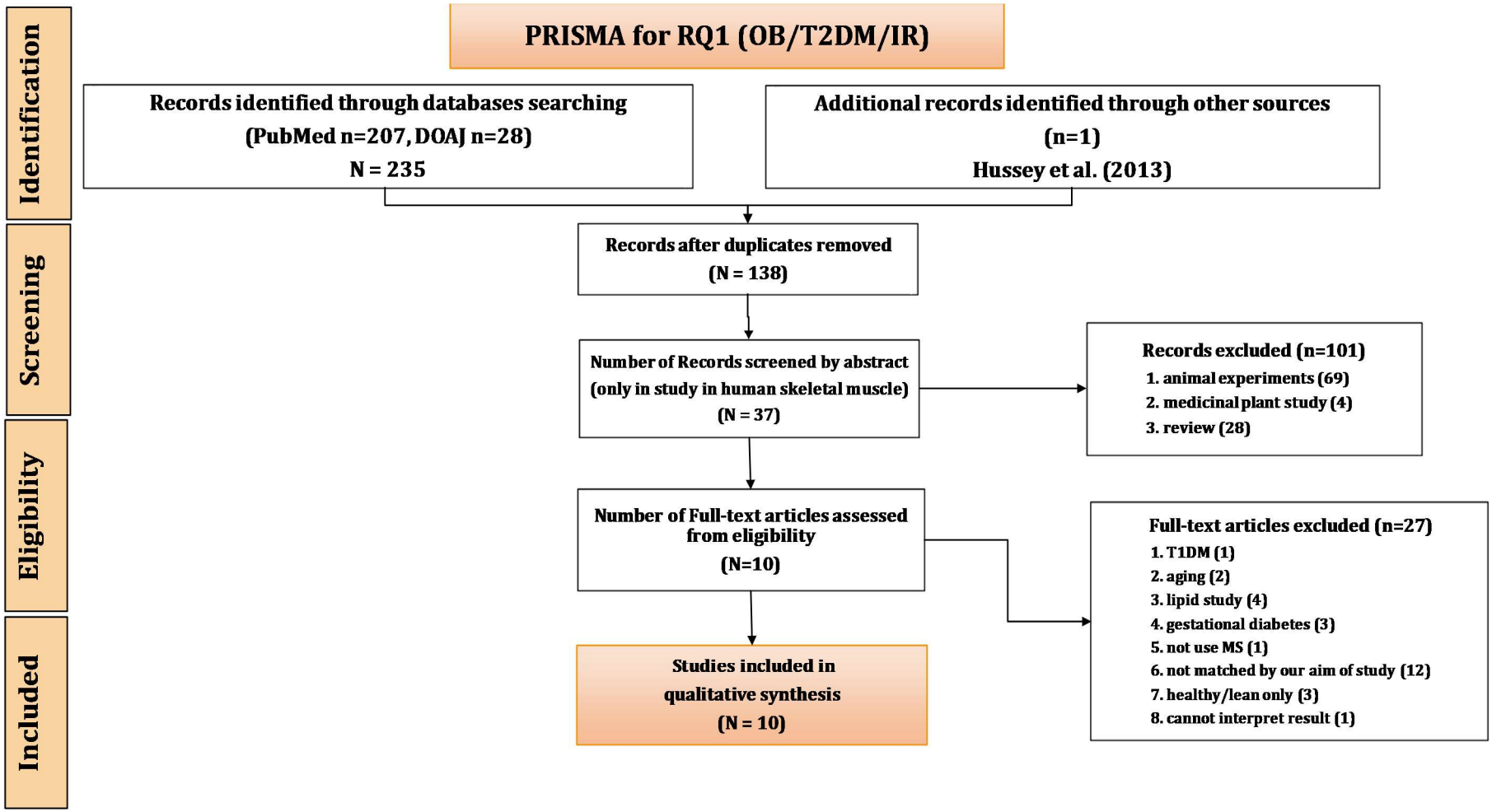


Figure 3 Systematic review of muscle proteomics data relating to human obesity and T2DM.

A systematic review was conducted according to the PRISMA method. In total, 235 publications were collected in the initial searches and 138 were included after elimination of duplicates. Each abstract was screened against the eligibility criteria and a further 101 publications were excluded. The majority (n = 69) of the excluded publications reported data from animal models, while n = 28 were review articles and n = 4 reported the medicinal effects of plant extracts. Therefore, 37 full-text articles were assessed in detail and in all 10 publications were retained for outcome quality assessment using The Delphi List.

RQ1 Data Extraction

The content of articles relevant to RQ1 using the PICO system are summarised in **Appendix 1**, which includes Population, Indicator, Comparison, and Outcome measures **(Appendix 1).** The population of participants in the literature reviewed was entirely of white Caucasian ethnicity. The number of participants ranged from 8 (Hwang *et al.*, 2010) to 10 (Hojlund *et al.*, 2003) per study, and the total number of participants included in the current systematic review was n = 94 control and n = 182 case. Two publications reported co-morbidities such as pre-hypertension in obese participants (Hwang *et al.*, 2010) or hypertension amongst participants with T2DM (Al-Khalili, Barbosa, *et al.*, 2014). BMI was the primary indicator used to define obesity and on average the BMI of participants in the case groups ranged from 30.2 to 57.3 $kg \cdot m^{-2}$ (Hittel *et al.*, 2005). In all studies, age was matched between case and control groups. The age of control participants ranged from 33 (Hwang *et al.*, 2010) to 51 years (Thingholm *et al.*, 2011; Giebelstein *et al.*, 2012), while obese participants were between 34 (Hwang *et al.*, 2010) and 50 years (Thingholm *et al.*, 2011; Giebelstein *et al.*, 2012), and T2DM patients were between 43 (Hojlund *et al.*, 2003) and 58 years (Caruso *et al.*, 2014). Sex-specific differences have not been investigated in the proteomics literature to date. Hittel et al. (Hittel *et al.*, 2005) used females only and analysed samples of rectus abdominus, which is unique to this study. The majority of studies (Hojlund *et al.*, 2003; Hwang *et al.*, 2010; Giebelstein *et al.*, 2012; Caruso *et al.*, 2014) recruited roughly equal numbers of males and females but in 2 two studies (Thingholm *et al.*, 2011; Al-Khalili, Barbosa, *et al.*, 2014) the sex of the participants was not reported. The majority of studies (Hwang *et al.*, 2010; Thingholm *et al.*, 2011; Hussey *et al.*, 2013; Al-Khalili, *et al.*, 2014; Al-Khalili, *et al.*, 2014; Caruso *et al.*, 2014) investigated biopsy samples obtained from the vastus lateralis. Højlund et al. (Hojlund *et al.*, 2003) and Hussey et al. (Hussey *et al.*, 2013) report direct analysis of biopsy material and regenerated human myocytes, whereas three studies (Al-Khalili *et al.*, 2011; Al-Khalili,*et al.*, 2014; Caruso *et al.*, 2014) were conducted entirely in human myotube cell cultures.

All outcome data were semi-quantitative, i.e., relative protein abundance. In total, the abundance of 156 proteins was reported to be statistically different between case (obese/T2DM) and control muscle **(Appendix 2).** There were 89 proteins that were more abundant in muscle of obese/T2DM patients and 3 (myosin heavy chain 2 **(MYH2)**, glutamine tRNA ligase **(SYQ)** and glycogen phosphorylase, **(PYGM)**) of these were repeated in more than one study. The most common biological processes amongst these proteins is the tricarboxylic acid (TCA) cycle, gluconeogenesis and muscle contraction. A total of 61 non-redundant proteins were less abundant in obese/T2DM patients, and only one protein (myosin regulatory light chain 2 ventricular cardiac muscle isoform, **MLRV_HUMAN**) was repeated in more than one study. Gene ontological analysis found biological process enriched in this group included muscle filament proteins, mitochondrial electron transport chain—NADH to ubiquinone, and mitochondrial ATP synthesis coupled proton transport.

RQ1 Meta-Analysis

Six (Hojlund *et al.*, 2003; Hittel *et al.*, 2005; Hwang *et al.*, 2010; Thingholm *et al.*, 2011; Giebelstein *et al.*, 2012; Hussey *et al.*, 2013)out of 11 studies included in the systematic review reported data in a format amenable to meta-analysis. The other five studies were excluded from the meta-analysis because they reported relative differences in protein abundance between groups but did not provide data regarding group averages or the distribution (i.e., SD or SEM or *p*-value) of values within each group. Moreover, numerous proteins were found significantly different in one study only and were therefore not applicable to meta-analysis. Thirteen proteins (**ECH1**; Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial, **HBA**; Hemoglobin subunit α, **MLRS**; Myosin regulatory light chain 2, skeletal muscle isoform, **MLRV**; Myosin regulatory light chain 2, ventricular/cardiac muscle isoform , **MYH1**; Myosin-1, **MYH2**; Myosin-2, **PYGM**; Glycogen phosphorylase, muscle form, G3P; Glyceraldehyde-3-phosphate dehydrogenase, **NDUS2**; NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial, **ATP5F1**; ATP synthase F(0) complex subunit B1, mitochondrial, **ATPB**; ATP synthase subunit β, mitochondrial, **COX2**; Cytochrome c oxidase subunit 2, and **APOA1**; Apolipoprotein A-I) were reported in more than one study. Two proteins (**MYH1** and **MYH2**, which mainly function in muscle) exhibited statistical difference that revealed a large effect of disease on protein abundance. Forest plots of these proteins are presented in **Figure 4.**

Protein	Reference	Proteomic method	*r*	LL	UL	*p* -value	control	T2DM/OB/IR lation (95% CI)	Relative weight	*p* -value	I^2
MYH1	Lefort 2010	1D&LC-MS/MS	0.80	0.60	0.91	0.00	8	8	60.74	0.32	0.00
P12882	Hussey 2013	1D&LC-MS/MS	0.65	0.27	0.86	0.00	6	6	39.26		
	Summary		**0.75**	**0.58**	**0.86**	**0.00****	**14**	**14**			
MYH2	Lefort 2010	1D&LC-MS/MS	0.74	0.48	0.88	0.00	8	8	43.88	0.86	0.00
Q9UKX2	Thingholm 2011	LC-MS/MS	0.76	0.56	0.88	0.00	10	10	56.12		
	Summary		**0.75**	**0.60**	**0.85**	**0.00****	**18**	**18**			

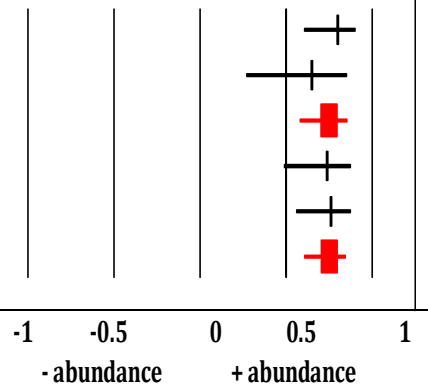


Figure 4 Forest plots of meta-analysis outcomes of muscle proteomics data relating to human obesity and T2DM.

Two out of 13 proteins were significantly different (**MYH1** and **MYH2**). Effect size (ES) of each protein was combined by two studies (k = 2) and was presented based on weighted Pearson product moment correlation (r) with 95% Confidence interval (95% CI). Both proteins were illustrated large effect size and were analysed by fixed-model (r_{MYH1} = 0.750 (0.577, 0.858), p_{MYH1} = 0.000, I^2_{MYH1} = 0.00%, p = 0.324; and r_{MYH2} = 0.751(0.603, 0.849), p_{MYH2} = 0.000, I^2_{MYH2} = 0.00%, p = 0.861).

RQ2: Effect of exercise/stimulated exercise training on individual protein abundance change

RQ2 Article Selection

The systematic review of muscle proteomics data relating to human muscle responses to exercise (i.e., RQ2) was separate to the review of literature relevant to RQ1. In total, 127 publications were collected in the initial searches, and two (Lanza *et al.*, 2008; Hussey *et al.*, 2013) were manually integrated for screening. Forty-one papers were included after elimination of duplicates, and when each abstract was screened against the eligibility criteria, a further 48 publications were excluded. The majority (n = 31) of the excluded studies were conducted without identifying the protein response to exercise or were performed in non-human animal models, and 17 were review articles **(Figure 5).** Therefore, four full-text articles passed the quality threshold and were retained for further assessment. The earliest studies meeting these criteria were Lanza et al. (Lanza *et al.*, 2008) reporting a cross-sectional comparison of habitually trained versus untrained individuals, and Holloway et al. (Holloway *et al.*, 2009) reporting longitudinal analysis in response to a six-week regimen of high-intensity interval training.

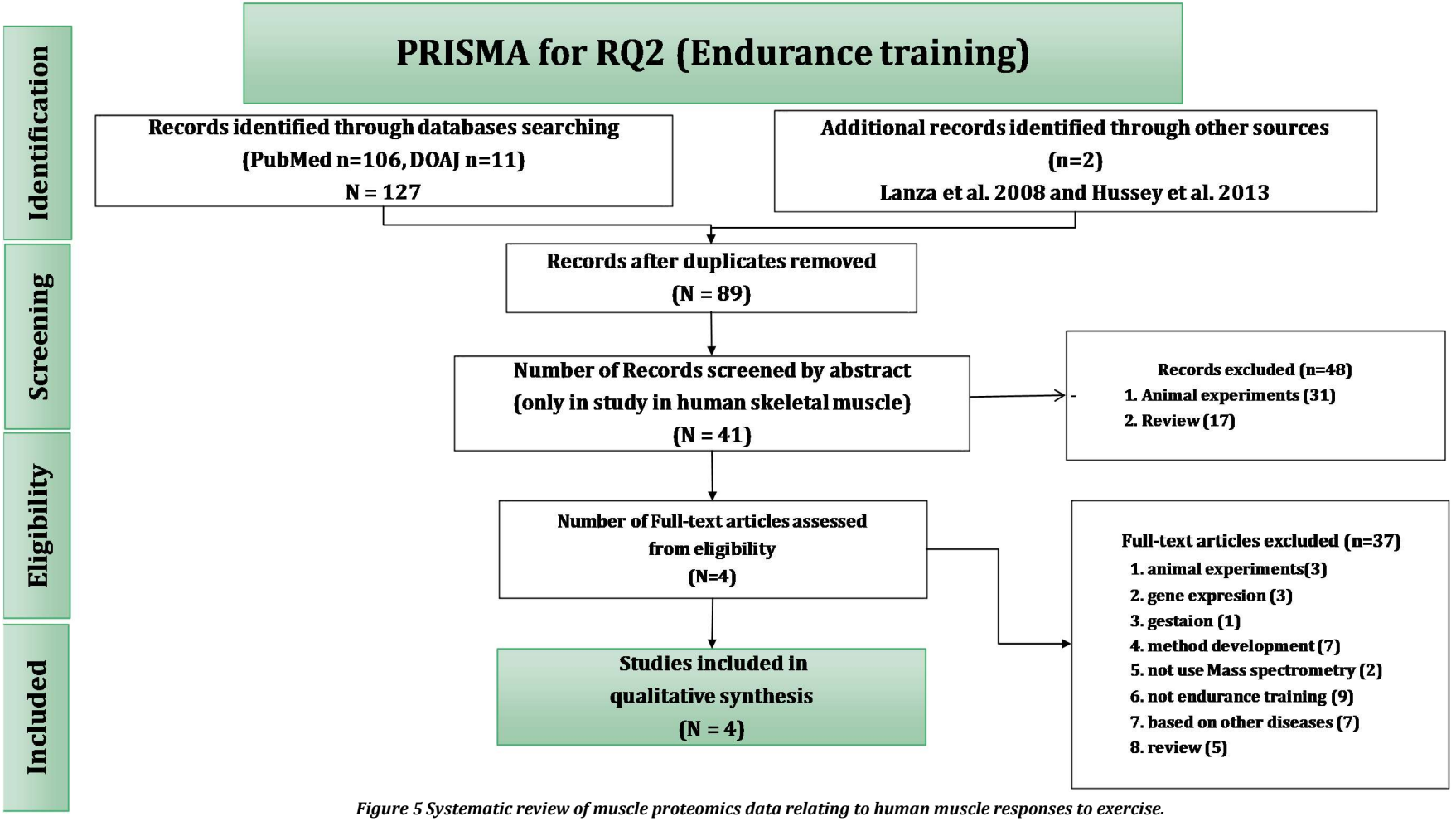


Figure 5 Systematic review of muscle proteomics data relating to human muscle responses to exercise.

A systematic review was conducted according to the PRISMA method. In total, 127 publications were collected in the initial searches and one was manually integrated for screening. Then, 89 were included after elimination of duplicates. Each abstract was screened against the eligibility criteria, and a further 48 publications were excluded. The majority (n = 31) of the excluded publications conducted work in animal or were review articles (n = 17). Therefore, four full-text articles underwent quality assessment, and in all, four publications were retained for outcome quality assessment using the Delphi List.

RQ2 Data Extraction

The content of articles relevant to RQ2 using the PICO system are summarized in **Appendix 2.** The number of participants in uncontrolled longitudinal trials (Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013) ranged between 5 (Holloway *et al.*, 2009) and 8 (Egan *et al.*, 2011), whereas randomised controlled studies (Lanza *et al.*, 2008; Schild *et al.*, 2015) used between 5 and 10 in each group. The total number of participants included in the current systematic review was 77 (n = 32 control, n = 32 case and n = 13 in pre- vs post-exercise interventions). These studies included both males and females (Lanza *et al.*, 2008; Hussey *et al.*, 2013), males only (Holloway *et al.*, 2009; Egan *et al.*, 2011), or did not report the sex of their participants (Schild *et al.*, 2015). Three publications (Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013) report the effects of an endurance exercise intervention, whereas two (Lanza *et al.*, 2008; Schild *et al.*, 2015) reported cross-sectional analysis of trained versus untrained populations. The endurance exercise interventions comprised of training programmes that used cycling (80%VO_{2max} without session duration reported, 7 day/week for 14 days) (Egan *et al.*, 2011), or a four-week intervention involving a combination of continuous moderate-intensity cycling (55% VO_{2max}, 60 min, 3 day/week) and high intensity intermittent cycling (70%VO_{2max}, 6 × 5 min, 2 day/week) (Hussey *et al.*, 2013), or treadmill running for six weeks (6 × 1 min intervals at 90–100% VO_{2max}, 3 day/week) (Holloway *et al.*, 2009).

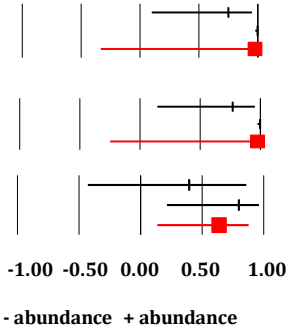
Comparisons were made between trained and untrained individuals using either a cross-sectional design (Lanza *et al.*, 2008; Schild *et al.*, 2015) or within-subject longitudinal designs that were either uncontrolled (Holloway *et al.*, 2009; Egan *et al.*, 2011) or randomised controlled trials (Hussey *et al.*, 2013). Consistent with RQ1, all outcome data were semi-quantitative, i.e., relative protein abundance. In total, the abundance of 75 proteins was reported to be statistically different between exercise trained and untrained muscle **(Appendix 2).**

Sixty-eight non-redundant proteins were more abundant in muscle of exercise-trained individuals, and two proteins (ATP synthase subunits α and β, ATPA and ATPB) were repeated in more than one study. The most common biological processes included the tricarboxylic acid cycle, mitochondrial electron transport chain—NADH to ubiquinone, mitochondrial respiratory chain complex I assembly, gluconeogenesis. Seven proteins were less abundant in exercise-trained muscle, and one protein (**CYRAB**; α-B-crystallin,) was reported by more than one publication.

RQ2 Meta-Analysis

Meta-analysis was conducted on data collected from longitudinal studies (Holloway *et al.*, 2009; Egan *et al.*, 2011; Schild *et al.*, 2015) that investigated the effects of exercise training. Meta-analysis was not performed on data from cross-sectional studies because Lanza et al. (Lanza *et al.*, 2008) did not report group averages or the distribution (i.e., SD or SEM) of values within each group, which left only Schild et al. (Schild *et al.*, 2015). Five proteins (CRYAB, ATPA, NDUA8, NDUFA13, and ATPB) were reported in more than one study. Among these, three proteins were found to be statistically different and are presented in **Figure 6**.

Uniprot	Protein	Reference	Biopsy	Intervention	Proteomic method	*r*	LL	UL	*p* -value	sample size	Correlation and 95%CI	Relative weight	*p* -value	I^2
P51970	**NDUA8**	Egan 2011	VL	2w-endurance(cycling)	2D-DIGE & LC-MS/MS	0.751	0.098	0.952	0.029	8		52.15	0.002*	89.97%
		Schild 2015	VL	>5y-endurance	LC-MS/MS	0.999	0.977	1.000	0.000	5		47.85		
		Summary				**0.978**	**0.000**	**1.000**	**0.050***	**13**				
Q9P0J0	**NDUFA13**	Egan 2011	VL	2w-endurance(cycling)	2D-DIGE & LC-MS/MS	0.770	0.143	0.956	0.023	8		52.32	0.002*	89.15%
		Schild 2015	VL	>5y-endurance	LC-MS/MS	0.998	0.975	1.000	0.000	5		47.68		
		Summary				**0.977**	**0.000**	**1.000**	**0.050***	**13**				
P06576	ATPB	Holloway 2009	VL	6w-endurance	1D & LC-MS/MS	0.394	-0.430	0.860	0.352	8		50.00	0.285	1.70%
P06576.	ATPB	Egan 2011	VL	2w-endurance(cycling)	2D-DIGE & LC-MS/MS	0.798	0.213	0.962	0.015	8		50.00		
		Summary				**0.638**	**0.134**	**0.880**	**0.017***	**16**				



Oxidative phosphorylation

Figure 6 Forest plots of meta-analysis outcomes of muscle proteomics data relating to human muscle responses to endurance exercise.

The summary effect of 3 individual proteins and abundance change were analysed by Mixed-model method and depicted in pink rectangular box. All proteins function mainly in the oxidative phosphorylation pathway. The effect size (ES) of each protein was combined for two studies (k = 2) (Holloway 2009, Egan 2011, or Schild 2015) and was presented based on weighted Pearson product moment correlation (r) with 95% Confidence interval (95% CI). All proteins large effect size and presented from trivial to very large range when refer to population (95% CI) i.e., r_{NDUA8} = 0.978 (0.000, 1.000), p_{NDUA8} = 0.050, I^2_{NDUA8} = 89.97%, r_{NDUA13} = 0.977 (0.000, 1.000), p_{NDUA13} = 0.050, I^2_{NDUA13} = 89.15%, and r_{ATPB} = 0.638(0.134, 0.880), p_{ATPB} = 0.017, I^2_{ATPB} = 1.70%.

Internal Quality Assessment

Publications were scored using the criteria of the Delphi list (Verhagen *et al.*, 1998). The quality of evidence presented in the 11 articles collected under RQ1 was ranked as medium to high (62.5–75.0%). Evidence collected under RQ2 was sub-divided in to cross-sectional (case versus control) or longitudinal (within subject) designs. The longitudinal studies were ranked medium high, whereas the case-control studies were ranked high.

Using the AMSTAR evaluation method (Shea *et al.*, 2007, 2009), the quality of our current systematic review was ranked 'high' (81.2%). The score for our current work was less than 100% because it was not possible to conduct meta-analysis on data from all studies and, secondly, because we did not set specific criteria for handling grey literature.

Discussion

We have systematically reviewed proteomics literature on human muscle in the context of either (i) the pathophysiological decline associated with obesity, insulin resistance or type 2 diabetes mellitus or (ii) physiological adaptations in response to exercise training. Our systematic review and meta-analyses highlight a shift toward a fast-twitch myofibre profile and a decrease in the abundance of electron transport chain components, particularly NADH-ubiquinone subunits of complex I, in the muscle of obese and T2DM individuals. In contrast, the main response of the muscle proteome to exercise training was a greater abundance of proteins of the mitochondrial electron transport chain, tricarboxylic acid cycle and mitochondrial respiratory chain complex I assembly. Although comparatively few proteins were shared across the two different systematic review questions, RQ1 and RQ2, our systematic review is consistent with the paradigm that some exercise-induced adaptations may directly oppose patho-biochemical changes associated with obesity/impaired glucose tolerance/T2DM.

Figure 7 presents a Venn diagram showing the number of significantly different proteins that were reported within each research question. The greatest number (n = 73) of proteins was reported to be specifically more abundant in the muscle of obese/T2DM patients **(Figure 6 and Appendix 1).** Gene ontology analysis discovered significant (Fisher's with BH corrected $p < 0.05$) enrichment of the biological process phrases "muscle filament sliding" (**MYH2**; Myosin-2, **MYH3**; Myosin-3, **MYH8**; Myosin-8, **MYL1**; Myosin light chain 1/3, skeletal muscle isoform, and **TNNT3**; Troponin T, fast skeletal muscle) and "protein stabilisation" (**CDC37**; Hsp90 co-chaperone Cdc37, **TCPD**; T-complex protein 1 subunit delta, **TCPQ**; T-complex protein 1 subunit theta, **G3P**; Glyceraldehyde-3-phosphate dehydrogenase, **HS90B**; Heat shock protein HSP 90-β, and **P85A**; Phosphatidylinositol 3-kinase

regulatory subunit α) amongst this group. In addition, there was significant enrichment of the KEGG pathways "biosynthesis of amino acids" and "PI3K-Akt signalling (phosphatidylinositol 3-kinase and Protein Kinase B)" that are conspicuous features of the wider literature relating to the patho-biochemical adaptation of muscle in T2DM patients. Individual proteins within the highlighted PI3K-Akt signalling pathway, such as tenascin-C (**TENA**), may constitute interesting targets for follow-up studies.

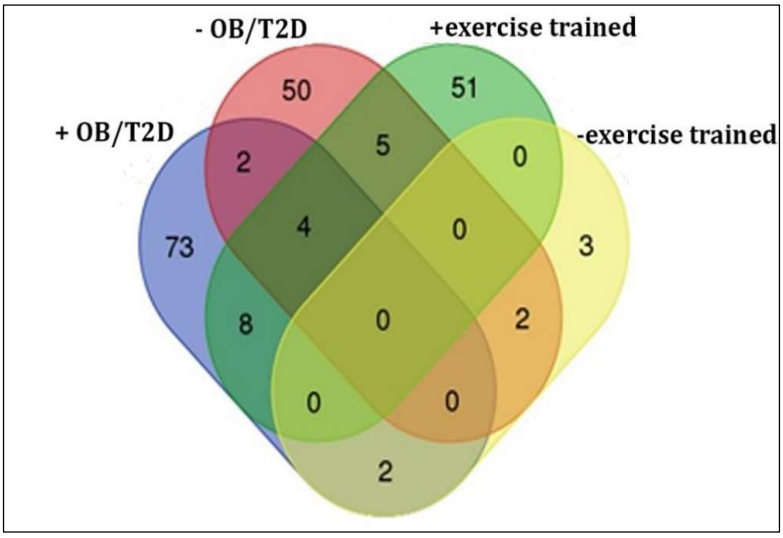


Figure 7 Venn diagram of non-redundant proteins extracted by systematic review of literature relating to RQ1 and RQ2.

Proteins reported under RQ1 to be greater in abundance in the muscle of obese/T2DM muscle (+OB/TSDM, n = 73) or less abundant in the muscle of obese/T2DM muscle (−OB/T2DM, n = 50) are overlaid with proteins reported under RQ2 that were either more abundant in exercise-trained muscle (+EXERC, n = 51) or less abundant in exercise trained muscle (−EXERC, n = 3).

Tenascin C (**TENA**) is a glycoprotein of the extracellular matrix that is associated with the molecular signature of tissue injury (Midwood *et al.*, 2011). Typically, the expression of tenascin-C is low or undetectable in healthy tissues but transiently increases in response to injury, including damaging eccentric exercise of skeletal muscle (Chen *et al.*, 2003). Tenascin-C was initially identified as a toll-like receptor 4 (**TLR4**) agonist that mediates the sustained local inflammatory response associated with arthritic joint disease (Midwood *et al.*, 2009). Subsequently tenascin-C has also been implicated in the chronic low-grade inflammation associated with obesity. Catalán et al. (Catalán *et al.*, 2012)reports tenascin-C gene expression is significantly elevated in visceral adipose tissue (but not subcutaneous adipose tissue) of obese humans, and elevated levels of tenascin-C expression also occur in the adipose tissue of animal models of either genetic or diet-induced obesity. Similarly, increases in hepatic tenascin-C expression occur in obese individuals with non-alcoholic fatty liver disease (NAFLD) (JH *et al.*, 2016), and in each of these scenarios tenascin-C interacts with TLR4 to augment local tissue inflammation. It should be noted that our review found that tenascin C was upregulated in cultured myotubes and not 'adult' skeletal muscle, and therefore future studies should investigate tenascin C in skeletal muscle

directly. This is important because chronic inflammation in obese individuals is associated with muscle insulin resistance, and it could be hypothesised that the tenascin-C/TLR4 signalling axis is also involved in this response.

We compared the outcomes of each review question by investigating proteins that are common or distinct between the case and control groups of RQ1 and RQ2 **(Figure 7)**. In exercise-trained individuals, 51 proteins were specifically more abundant and biological process phrases enriched in this group included mitochondrial electron transport, NADH to ubiquinone, tricarboxylic acid cycle, and mitochondrial respiratory chain complex I assembly. This profile shared close similarity to the biological process phases that were enriched amongst the 50 proteins that were less abundant in muscle of obese/T2DM individuals, which included metabolic proteins and specifically enzymes of the electron transport chain. However, when compared at the level of individual proteins just five proteins were shared between these groups. Proteins that were less abundant in obese/T2DM muscle but more abundant in exercised muscle include: NADH ubiquinone oxidoreductase subunits A8 (**NDUA8**), B8 (**NDUB8**) and S2 (**NDUS2**); glutamic-oxaloacetic transaminase 2 (**AATM**) and ATP synthase β (**ATPB**). Therefore, a difference in the abundance of subunits of complex I (NADH ubiquinone oxidoreductase) is one of the primary hypotheses generated from our systematic review of proteomics literature. In contrast, Fast-twitch myofibrillar proteins, **MLRS**, and **SERCA1** (Sarcoplasmic/endoplasmic reticulum calcium ATPase 1) were more abundant in muscle of obese individuals and less abundant in exercise trained muscle.

Currently, Hussey et al. (Hussey *et al.*, 2013) is the only proteomic work to directly investigate exercise responses in T2DM muscle and reports a dominant increase in the abundance of enzymes of the malate-aspartate shuttle which serves to transport NADH from the cytosol to within the mitochondria. This adaptation is a long established effect of endurance exercise in the muscle of healthy human participants (Holloszy and Coyle, 1984). However, Hussey et al. (Hussey *et al.*, 2013) did not detect any change in the abundance of complex I subunits in T2DM patients. The question arises whether this is because the ability to change the abundance of complex I components is lost/diminished in obesity/T2DM, or if the study was not capable of detecting this effect. Hussey et al. (Hussey *et al.*, 2013) reports GeLC-MS/MS analysis, which achieves a good depth of proteome coverage, but differential analysis of protein abundances was performed by spectral counting, which can suffer from missing data that makes comparative analyses difficult. In addition, the exercise intervention was relatively short (a four-week duration), and whilst there was a trend for improved glucose tolerance, no statistically significant improvement in fasting glucose or insulin values, OGTT, or Matsuda index was reported (Hussey *et al.*, 2013). Therefore, targeted analysis is required in the future to resolve whether defects in the abundance and assembly of complex I subunits are a key contributing factor to the patho-biochemical adaptation of muscle in T2DM patients.

Within the systematic reviews, eight proteins were reported to be more abundant in both obese/T2DM and exercise-trained muscle. These proteins included 2 enzymes of the TCA cycle (**CISY**; citrate synthase, and **ACON**; aconitase) and enzymes involved in glycolysis (**PGAM2**; phosphoglycerate mutase 2,), fatty acid oxidation (**ACADVL**; acyl-CoA dehydrogenase, very long chain), and the electron transport chain (**SDHA**; succinate dehydrogenase complex flavoprotein subunit A) in addition to **EFTU** (Tu translation elongation factor, mitochondrial), and **MCCB** (methylcrotonoyl-CoA carboxylase 2), which contributes to the catabolism of branched chain amino acids leucine and valine.

We also performed a meta-analysis to increase the precision of the results obtained from the systematic review. Meta-analysis of data related to RQ1 ("pathological decline associated with obesity, insulin resistance or type 2 diabetes mellitus") revealed a significant increase in the abundance of fast-twitch myosin heavy chain isoforms (MYH1 and MYH2) in obesity and T2DM. Three myosin isoforms are typically expressed in human muscle: slow-twitch type I (MYH7) and fast-twitch isoforms IIa (MYH2) and IIx (MYH1). The proportion of each isoform differs as a consequence of genetic background and habitual activity level and might typically be expected to be 50–60% type I, 20–30% IIa and 10–20% IIx (Haggmark and Thorstensson, 1979). A greater abundance of MYH1 and MYH2 is consistent with the higher proportion of fast twitch fibres and the lower oxidative capacity of skeletal muscle often observed in obese and T2DM individuals (Lefort *et al.*, 2010; Hussey *et al.*, 2013).

In contrast, meta-analysis of data collected under RQ2 ("physiological adaptations in response to exercise training") identified just three proteins (**NDUA8**; NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 8, **NDUFA13**; NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 13, and **ATPB**; ATP synthase subunit β, mitochondrial) that were significantly more abundant after endurance exercise training. **ATPB** is a subunit of complex V and **NDUA8** and **NDUFA13** are responsible for subunit I of the mitochondrial respiratory chain. The reported upregulation of these OXPHOS proteins in response to endurance-type exercise training signifies an improved capacity for oxidative ATP production.

We have conducted systematic reviews and meta-analyses to consolidate knowledge from the existing proteomics literature relating to the effects of obesity and type 2 diabetes or exercise training on human skeletal muscle. Because our work used hypothesis generating proteomics data our results highlight questions for future research rather than conclusive outcomes at the population level. We have been able to select targets and generate hypotheses from more robust data than is available from each of the individual studies conducted so far. Nonetheless, our work was necessarily limited by the small number and breadth of the current proteomics literature, and in particular few papers reported data amenable to meta-analysis. In addition, the rapid pace of technical development in the field of proteomics over the

past decade has resulted in relatively poor equivalency of data across the current body of literature. For example, contrary to our hypothesis, we do not report widespread and diametrically opposite responses between the muscle of obese and type 2 diabetics compared exercise-trained individuals. This could be an indication that the underlying mechanisms are so complex that changes in the proteins in response to training are different to the proteins that change in response to disease yet lead to diametric yet opposing whole-body responses (e.g., increase or decrease in insulin sensitivity), or it could be an artefact caused by the wide variety of proteomics techniques that have been applied in this area. In particular, it can be confusing to compare 2D gel data that resolves proteins to their constituent species or proteoforms with LC-MS/MS profiling data that reports the overall abundance of all species of each protein.

Until recently, differential analysis of muscle using LC-MS was challenging and in our previous narrative review (Burniston and Hoffman, 2011) we advocated the continued use of 2D gel-based proteomics because this suffers less from issues caused by missing data that occurs in LC-MS/MS work flows, including differential labelling and spectra counting techniques. More recently we (Malik *et al.*, 2013) reported label-free LC-MS profiling of skeletal muscle using data-independent acquisition and Progenesis Quantitative Informatics for Proteomics, which enabled high-throughput and comprehensive analysis of the skeletal muscle proteome. In the future, more wide spread application of robust platforms such as this will generate more parallel data with greater equivalency of proteome coverage across studies from different laboratories. In addition to this technological advance, our systematic review also highlights a number of areas for improvement in experiment design. In the proteomics literature to date, few studies report the medical history or full medical characteristics of the participants. For example, the majority of literature did not include information regarding hypertension or other cardiovascular risk factors such as chronic kidney disease that are common co-morbidities in obese/type 2 diabetic patients. The literature reviewed herein generally used equal numbers of male and female participants but as yet sexual dimorphism in the muscle response to obesity or exercise training has not been specifically investigated. In addition, ethnic backgrounds other than white Caucasian need to be investigated, especially in light of epidemiological evidence that Asian and Polynesian populations amongst others are at a greater risk of T2DM than white Caucasians (Lee, Brancati and Yeh, 2011)

Limitations

We took a systematic approach to reviewing the literature relevant to this thesis and where possible we performed meta-analysis of the published data relating to the effects of exercise or obesity/ T2DM on the human skeletal muscle proteome. Despite our use of a systematic approach, there are limitations due to the relatively limited availability of literature in this field and a tendency for articles to not include in-depth physiological characterisation of the participants. Therefore, the majority of publications on obesity and T2DM did not include information regarding hypertension or other cardiovascular risk factors such as chronic kidney disease that are common co-morbidities in OB/T2DM patients. We noted that there had been a relatively large number of narrative reviews in proteomics relating to muscle, T2DM or exercise but, as far as we are aware, ours was the first systematic review of the proteomics literature. Narrative reviews, and to some extent systematic reviews, can be biased or have a risk of bias in the interpretation of the literature so we attempted to also include statistical methods (i.e. meta-analysis) to assess the combined results available from the published literature, with the aim of increasing precision and to answer to what extent and how the data relate to the underlying effects of obesity/T2DM or exercise on the human muscle proteome. We found that sample size across the proteomic studies was modest, and this is likely due to the invasive and costly nature of analysing human muscle samples using proteomic techniques. This small sample size increases the risk of misinterpretation in our meta-analysis because the variance and distribution of the data may still not be a close reflection of the population as a whole. Nonetheless, ours was the first attempt to apply systematic and meta-analytical approach to reviewing this literature and we were able to summaries important outcomes from this work that had not been highlighted in the body of narrative reviews from this area. A particular difficulty in reviewing the proteomic literature arose from the broad range of different experimental designs of original studies and different analytical techniques that had been employed to study the muscle proteome. This challenge must be considered carefully because it could lead to inappropriate selection of statistical parameter or designs. Some proteins have been excluded from the analysis because it derived from different design that showed unclear method of analysis or reporting of data. Finally, data extraction is a crucial step in meta-analysis. Some information was lost because of a lack of accurate reporting, e.g. p-value < 0.00. Sexual dimorphism in the population has not been particularly investigated regarding obese muscle or in response to exercise. Moreover, all of the included literature used a similar ethnic background, White Caucasian. Asian and Polynesian populations are at the greater risk of T2DM than white Caucasian. In the light of this epidemiological evidence, we should consider ethnic diversity in future studies. The publications reviewed reported numerous proteins; however, few of them were reported more than 1 publication (an exception was ATPA). Although there were numerous proteins which exhibited notable differences in abundance, we could not provide the magnitude effect in the population using Meta-analysis.

Conclusions

In conclusion, skeletal muscle of obese and T2DM individuals is marked by a shift toward a fast-twitch myofibre profile and a decrease in the abundance of glycolytic enzymes and electron transport chain components, particularly NADH-ubiquinone subunits of complex I. A handful of proteins associated with fatty acid or amino acid metabolism and the tricarboxylic acid cycle were more abundant in both obese/T2DM patients and exercise-trained individuals, which may be consistent with the paradigm known as the athlete's paradox. The majority of proteins (n = 73) that are significantly more abundant in the muscle of obese/T2DM individuals were unique to this group and were not reported to be responsive to exercise training. Functional annotation of these proteins found enrichment of the biological process "protein stabilisation" and the PI3K-Akt pathway. Amongst the proteins listed in the PI3K-Akt pathway was tenascin C, which may represent an important target for more focused follow-up studies in muscle. The main response of skeletal muscle to exercise training was a greater abundance of proteins of the mitochondrial electron transport chain, tricarboxylic acid cycle and mitochondrial respiratory chain complex I assembly. In total, three proteins were less abundant in muscle of obese/T2DM individuals and were also reported to be more abundant in the muscle of endurance-trained individuals suggesting one of the major mechanisms of exercise-induced protection against the deleterious effects of obesity/glucose intolerance and T2DM occurs at complex I of the electron transport chain.

Chapter III

Preliminarily studies of human muscle protein profiles (abundance) and computational method for protein turnover rate in rat skeletal muscle

Contents

Summary

Chapter II synthesised data from proteomic studies that used mass spectrometry to produce human protein profiles for specific patho-physiologies and exercise interventions. This data was restricted to protein abundance data, and no data currently exist on the balance between the synthesis and breakdown of individual proteins in OB/T2DM or the effects of endurance exercise training. If a protein becomes more or less abundant, it is important to investigate the mechanism underlying this change. Proteins exist in a continuous cycle of synthesis and breakdown, and a change in abundance can occur by changes to (i) the rate of synthesis, (ii) the rate of breakdown or (iii) changes in both the rate of synthesis and rate of breakdown. This chapter aims to develop methods that may be able to give information on both changes in protein abundance and the rate of turnover (synthesis and breakdown) of muscle proteins. The chapter consists of 2 sections describing pilot studies of (1) human protein abundance profiling of archived human skeletal muscle and (2) the measurement of individual protein synthesis rates in fast-twitch skeletal muscle of rats that were administered deuterium oxide *in vivo*.

Section (1) found differences in protein abundance across muscle samples from T2DM, OB and Lean individuals that were consistent with the outcomes from our systematic review of the published literature (Chapter I). Four out of the 82 proteins profiled exhibited statistically significant differences. The abundance of heart-type fatty acid binding protein (**FABPH**) was more abundant in OB and T2DM muscle, which may be consistent with the 'athlete's paradox' and may indicate higher lipid storage in muscle resulted insulin resistance. In addition, sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) enzymes (SERCA1, SERCA2, and SERCA3) revealed pronounced differences in abundance and have not been previously reported.

Section 2 aimed to describe the method for calculation individual protein turnover rate. The study was conducted in laboratory rats that received deuterium oxide (^2H_2O or D_2O). Rat (*Rattus norvegicus*) is mammalian species that has a skeletal muscle structure similar to human (Homo sapiens) but the smaller body mass of rats (e.g. ~500 g) means that it is a simpler and more cost-effective model for pilot experiments. The study utilised *plantaris* muscle which has been characterised as predominated fast-twitch muscle similar to human VL which will be used in the next Chapters. The calculation method included non-linear model analysis to determine protein synthesis rate (*k*). This calculation was based on multiple parameters i.e., percent enrichment of the precursor pool (*p*) and the probability of light hydrogen (H) substitution to heavy hydrogen (D) based on each peptide amino acid sequence. To obtained k, protein mixtures were retrieved from *plantaris* and analysed by LC-MS/MS (Liquid chromatography–tandem mass spectrometry). The raw data show differences in deuterium incorporation into protein over each time point because heavy (D) and light hydrogen (H) effect the molar fraction of the peptide mono isotopic peak (FM_0). This method is appropriate to calculate protein synthesis at an individual protein level and will also be used in further studies in Chapter IV and V. Our preliminarily results demonstrate that the synthesis rate of individual proteins is different within *plantaris* muscle.

Introduction

Current proteomic approach

Proteomics relates to the specific study of proteins and is under a branch of molecular biology and biochemistry. Proteomics covers fundamental and advanced study across 4 major areas (Liebler, 2002), i.e., non-targeted discovery, post-genomics, system biology, and bioinformatics. Non-targeted discovery relies on Mass spectrometry (MS) and separation tools such as gel electrophoresis and liquid chromatography. MS enables the identification of proteins from complex mixtures such as muscle homogenates. In addition, peptide mass spectra can also be used to report stable isotope incorporation for the calculation of protein synthesis. Proteomics is a Post-genomic science because the interpretation of mass spectrometry data requires genomic information created by the sequencing of the genome. Systems biology, is a research approach/philosophy based on the interpretation of comprehensive non-targeted data, such as DNA, mRNA and protein profiling. Bioinformatics is the combination of math, physics, statistics, and biological understanding for analysing data such as protein structure, content, quantity, charge, dynamic change, or post-translational modification, to raise greater understanding and generate new hypotheses. This is facilitated by powerful software collections, which can be used for interpreting data and evaluating proteins with different properties.

Studies performed in humans have used a broad range of different proteomic techniques, including: one-dimensional denaturing gel electrophoresis (1D-SDS-PAGE) followed by LC-MS/MS(Holloway *et al.*, 2009; Hwang *et al.*, 2010; Lefort *et al.*, 2010; Hussey *et al.*, 2013; Caruso *et al.*, 2014), two-dimensional-difference in-gel electrophoresis (2D-DIGE) followed by LC-MS/MS (Holloway et al., 2009; Egan et al., 2011; Giebelstein et al., 2012; Al-Khalili, Barbosa, et al., 2014; Al-Khalili, et al., 2014) or MALDI-TOF (Hojlund *et al.*, 2003; Hittel *et al.*, 2005; Moriggi *et al.*, 2010; Gondin *et al.*, 2011; Salanova *et al.*, 2014, 2015; Brocca *et al.*, 2015) or LC-MS/MS only (Lanza *et al.*, 2008; Thingholm *et al.*, 2011; Schild *et al.*, 2015).The studies discovered novel proteins from either predominated fast muscle fibre type (type IIa and type IIx) or slow fibre type (type I) from human subjects.

Various quantitative methods have been used, for example 2D gel-based studies rely on protein quantification by densitometry of gel images (e.g. Holloway et al) which can give highly detailed information on each proteoform of a protein, which may include different splice variants or post-translational states. More recently, quantitation by mass spectrometry has become popular using either isobaric labels (e.g. isotopic tracer for relative and absolute quantitation; iTRAQ) or label-free quantitation based on MS ion counts. Our laboratory has experience across all of the common proteomic technologies and we have optimised techniques such as an automated 'off-line' LC-MALDI analysis of

iTRAQ labelled samples (Holloway *et al.*, 2009) to minimise the loss of data that can occur during online analysis of iTRAQ-labelled samples. More recently, we have worked to improve label-free analysis of skeletal muscle proteins (Malik et al., 2013; Burniston, et al., 2014) which can be more time efficient (2 hour per sample) and more reproducible than quantitative in vitro labels such as iTRAQ. Further development has been achieved using high-definition mass spectrometry ($HDMS^E$; Burniston et al 2014) which combines ion mobility separation in the mass spectrometer (i.e. HDMS) with the standard separation of peptides by reverse-phase liquid chromatography (RPLC). This method improves the resolution of peptide mixture by separating peptide ions based on either low or high mobility in the gas phase of the instrument.

A study by Hussey et al (Hussey *et al.*, 2013)is amongst the most relevant work to the area of this thesis. Hussey et al (2013) used label-free LC-MS/MS profiling and reported significantly different protein abundance change regarding both T2DM and endurance training (cycling). The main findings highlighted insulin disposal in T2DM subjects was improved after 8 weeks training. Moreover, they found novel proteins from metabolic pathways such as oxidative phosphorylation (OXPHOS), the tricarboxylic acid (TCA) cycle and glycolysis were significantly up regulated. Since this work, we have developed label-free profiling techniques that are more reproducible than those used in Hussey et al and other studies. Our LC-MS profiling combined with Progenesis (Waters Corporation, MA) data analysis is able to profile the most abundant metabolic enzymes and contractile proteins in muscle to a high level of reproducibility (Burniston, 2008; Malik *et al.*, 2013; Sollanek *et al.*, 2017) and will be used in the current thesis to investigate protein abundances.

Protein turnover rate study and deuterium oxide (D_2O, heavy water)

Nowadays, proteomic studies routinely provide effective technologies for identifying proteins from complex mixtures and reporting individual protein abundances. In this chapter, we aim to extend proteomic analysis to include the measurement of protein turnover based on the rate of stable isotope incorporation in vivo. In order to develop this technique for human studies, animal experiments are an appropriate choice for simulating the human system. Therefore, our pilot study selected rat for the initial investigation because their body size is smaller, which minimizes the amount of deuterium oxide required. However, rat models are inadequate to investigate polygenetic diseases such as T2DM so our latter studies will be conducted in humans.

Protein consists of long chain amino acid residue that undergo synthesis and breakdown in a continuous cycle. Disuse, aging, diseases, and major life style change such as physical activity and nutrition have a crucial impact on the break down and synthesis rate of muscle. To detect the rate of synthesis and

breakdown (i.e. turnover rate) of proteins, amino acids must be traced in vivo. Radioisotopes and stable isotopes have been used to label amino acid molecules by incorporating amide/amine group such as substitution with isotopes of C, N, or H , including: L-[l-^{13}C]leucine (Matthews *et al.*, 1982), [^{15}N]glycine(Waterlow, Garlick and Millward, 1978; Waterlow, Golden and Garlick, 1978), or L-[$^{2}H_5$]phenylalanine(Matthews *et al.*, 1982). To study protein turnover rate in human skeletal muscle, these amino acid tracer must be infused intra-venously to enrich precursor pool (Wagenmakers and Wagenmakers, 1999).

Studies using stable isotope-labelled amino acids in vivo rely on gas chromatography mass spectrometry (GC-MS) analysis of amino acids hydrolysates to measure isotope abundances to calculate Fractional synthesis rate (FSR). This approach is adequate for monitoring average FSR in whole tissues or subcellular fractions (e.g. myofibrillarr or mitochondrial) (Yarasheski *et al.*, 1992). Moreover, Jaleel et al (Jaleel *et al.*, 2008) reports the combination of stable isotope labelling in vivo with the proteomic separation technique of 2DIGE. Skeletal muscle samples were isolated from rats 25 min after a bolus administration of 15 mg of ring-[$^{13}C_6$] phenylalanine/kg.body mass^{-1} and muscle mitochondrial proteins were separated using 2DIGE. Proteins were identified from in-gel tryptic digests using LC-MS/MS. FSR was calculated from the isotopic enrichment measured using GC-MS/MS by selective ion monitoring of phenylalanine derivatives. The marriage of these standard workflows enabled FSR to be calculated for 68 mitochondrial proteins and 23 non-mitochondrial proteins. Synthesis rates spanned a 5-fold range in mitochondria and 10-fold range when considering the sarcoplasmic and myofibrillarr proteins that were also detected in the mitochondrial fraction. Myosin heavy chain exhibited the lowest FSR (0.16 ± 0.04 %/h) and the E2 component of the mitochondrial branched-chain α-keto acid dehydrogenase complex had the greatest FSR (1.5 ± 0.42 %/h). Pathway analysis found FSR did not cluster within functional groups, including Tricarboxylic acid (TCA) (Electron transport chain, ETC), ETC and β-oxidation etc. or cellular locations (i.e. membrane proteins vs mitochondrial matrix). This work represents a significant step forward and could be used to measure short-term differences in FSR in laboratory rodents. That said, the dual use of both LC-MS/MS and GC-MS to identify proteins and quantify FSR is a laborious process that cannot be easily automated.

More recently, advances in GC-MS technology (Wilkinson et al., 2014), i.e. using high-temperature conversion and GC-pyrolysis Isotope Ratio Mass Spectrometry (IRMS), have enabled more sensitive detection of deuterium labelling. This enabled the analysis of protein synthesis at low (i.e. 0.2 %) mole per enrichment (MPE) of the precursor pool. Participants (8 young males) were given a bolus of 150 ml 70 % 2H2O and then performed unilateral resistance exercise (knee extension) 4 sets of 8 reps at 80 % 1RM) performed on days 0, 2, 4, 6 and 8. Whey protein (20 g) was provided after each exercise session and biopsy samples were taken from both the exercised leg and the counter-lateral leg prior to the exercise bout on days 0, 2, 4 and 8. Body water enrichment was monitor in saliva samples and peaked

at 0.2 % MPE 24 h after bolus administration and followed an exponential decay with an estimated half-life of 11 ± 0.9 days. Biopsy samples were fractionated to myofibrillar and sarcoplasmic, FSR for myofibrillarr was 1.45 ± 0.1/day in the control leg and 1.97 ± 0.13 %/d in the exercise leg over the first 2 days of the protocol, i.e. significantly greater $p<0.05$. In the future, GC-pyrolysis IRMS could be combined with proteomic separation techniques such as 2D-DIGE (e.g. similar Jaleel et al (Jaleel et al., 2008)) but the throughput of this approach would be relatively limited and overly complicated. That is, while 2D-DIGE is a powerful tool for comparative analysis of proteoforms it is laborious and has limited ability to resolve proteins all proteins (discussed in (Malik et al., 2013)). Therefore, in the first instance, it would be preferable to use more robust and high-throughput combination of LC-MS/MS that are already used for broad-scale profiling of muscle protein abundances.

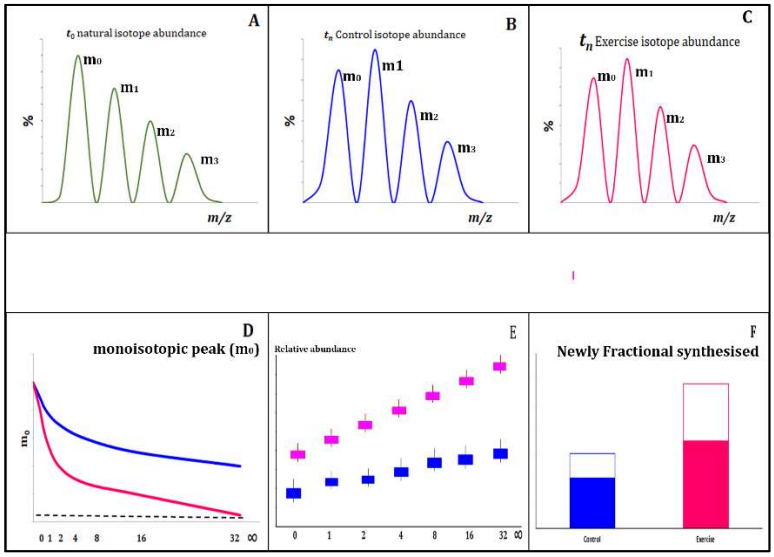


Figure 8 Measurement of protein synthesis by peptide mass spectrometry.

Mass spectrometry resolves peptides as 'envelopes' of peptide mass isotopomers, which represent the natural abundance of C, H, N, O and S isotopes (A). The mass isotopomer with the lowest mass in the envelope is known as the monoisotopic peak (m0) because it consists entirely of primary isotopes (i.e. ^{12}C, ^{1}H, ^{14}N, ^{16}O etc). The second isotopomer of the envelope is composed of peptides that contain at least one stable secondary isotope (i.e. ^{13}C, ^{2}H, ^{15}N etc). The rate constant for the synthesis of individual proteins can be calculated from the change in the peptide mass isotopomer distribution (B and C). The incorporation of deuterium in to a protein is evident from the decrease in the fractional abundance of the monoisotopic peak (m_0), which contains natural isotopes only (D). Relative abundance can also be measured based on normalised ion intensities (E). Therefore, data can be expressed to show both the change in protein abundance and the fraction of protein that has been newly synthesised (F).

Labelling of newly synthesised proteins by deuterium affects the mass isotopomer distribution of the peptide ions detected by mass spectrometry (**Figure 8A-C**). Essentially, the incorporation of deuterium (^{2}H) in to newly synthesised proteins alters the natural distribution of the peptide mass isotopomer envelope. This change in the fractional abundance of each isotopomer contains information regarding both the enrichment of deuterium in the precursor pool and the fractional synthesis rate of the parent protein.

Equilibration of deuterium with body water and the exchange of ^2H in to C–H bonds of amino acids each occur rapidly. Therefore, the rate of protein synthesis is the single limiting factor to the incorporation of deuterium in to protein. Hence, the decrease in the fractional abundance of the monoisotopic peak (**Figure 8D**) follows a first-order exponential decay that matches the reciprocal rise in deuterium incorporation. The rate constant (k) of this process describes the fractional synthesis rate of the parent protein (Equation 1).

$$\boldsymbol{f_t = f_{max}\,(1 - e^{-kt})} \text{ ----------Equation 1}$$

The fraction (*f*) of newly synthesised protein at time (*t*) points during labelling. The asymptotic or maximal value (f_{max}) is calculated from the measured enrichment of deuterium in the precursor pool taking in to account the elemental composition of the peptide and the maximum integration number (*n*) for deuterium labelled amino acids (e.g. glycine n = 2, alanine n = 4 and glutamine/ glutamic acid n = 5). Please note the above is a brief presentation of the key measurements and calculations, more thorough explanations and empirical evidence of the underlying principles are available in (Hellerstein and Neese, 1999; Busch *et al.*, 2006) amongst others.

Peptide mass spectrometry of deuterium labelled rat tissues has been reported recently. Kim and colleagues (Kim *et al.*, 2012) found a modest correlation (Spearmen ρ = 0.50) between synthesis rates of proteins in heart and liver and many mitochondrial proteins ranked differently between cardiac and liver mitochondria, which suggests tissue-specific regulation. Moreover, the physiochemical properties of proteins did not have a strong influence on their rate of turnover, for example, there was a weak inverse relationship between protein abundance and turnover rate. Moreover, there was no relationship between turnover rate and molecular weight (MW), isoelectric point (pI) , hydrophobicity or motifs such as PEST (Proline, Glutamic acid, Serine, and Threonine). In the heart, protein located on the outer mitochondrial membrane had a generally greater turnover rate than proteins of the inner mitochondrial membrane, which may suggest a particular contribution of extra-mitochondrial degradation processes such as the Ubiquitin Proteasome system (UPS).

A follow-up study (Lam *et al.*, 2014) measured changes in protein synthesis are involved in cardiac hypertrophy induced by isoprenaline. Pathological growth of the heart is associated with a shift toward greater utilisation of glucose and deuterium labelling in vivo reveals isoprenaline increases the synthesis rates of glycolytic enzymes without affecting their abundance (Lam *et al.*, 2014). This is consistent with the mechanism of action of enzymes such as triosephosphate isomerase, which become irreversibly damaged during catalysis (Hipkiss, 2011). Thus, elevations in glycolytic flux require concomitantly greater rates of protein synthesis to prevent depletion of the viable enzyme pool. This is an important example of information

not captured by studies reporting protein abundances only. Similar mechanisms are likely to also underpin exercise-induced adaptations. For example, high levels of aerobic capacity, whether achieved by selective breeding (Burniston *et al.*, 2011) or endurance training (Burniston, 2009), are associated with a greater abundance of enzymes involved in fatty acid oxidation in the heart. Interestingly, Hunter et al (Hunter *et al.*, 2008) reports lesser ubiquitination of the β-oxidation enzyme, trifunctional protein-α (ECHA) in response to aerobic exercise, and so provides a further indication that regulation of cardiac metabolic enzymes can be achieved by either changes in abundance or turnover. As yet, equivalent work has not been conducted in skeletal muscle and we believe these exciting findings open new horizons to understand physiology and the health benefits of exercise.

A shortcoming of the present literature is that comprehensive analysis of protein synthesis rates is not routinely matched with equivalent analysis of protein abundances. Therefore, protein turnover (i.e. synthesis, abundance and degradation) has not yet been fully described. For instance, Lam et al (Lam *et al.*, 2014), reports synthesis rates for almost 3,000 cardiac proteins but performed abundance measurements on just a handful of targets (i.e. glycolytic enzymes). We will use our experience in muscle proteomics using label-free quantitation (Burniston, Connolly, *et al.*, 2014) to analyse both the rate of synthesis and the abundance of each protein. Label-free profiling defines the isotopic envelope of each peptide and records the total ion abundance of all mass isotopomers over the duration of the chromatographic peak for that peptide. Label-free quantitation of protein abundances will be performed using Progenesis QI for Proteomics (Nonlinear Dynamics Ltd, Newcastle upon Tyne, UK). Log transformed MS data will be normalised by inter-sample abundance ratio and used to investigate differences in protein abundance across independent groups (i.e. LE, OB with IGT) by one-way analysis of variance. To control False discovery rate (FDR), p-value distributions will be used to calculate q-values and a criterion FDR of <10% will be set. This statistical approach considers the biological variation across each protein and is, therefore, more sophisticated than arbitrarily implementing a threshold based on fold-change.

Aim of the study

The aim of this chapter is to establish methods for studying the abundance and turnover rate of individual proteins. These techniques will then be applied in human studies on muscle protein turnover rate in response to exercise training which will be describe in Chapter IV and V. Therefore, this Chapter described preliminarily experiments which have been conducted in both human and rat skeletal muscle. This chapter uses archived human Vastus lateralis from lean (LE), obese (OB) and T2DM participants to produce initial data for measuring protein abundance in human muscle. Separate analysis of fast-twitch *plantaris* muscle from rats administered deuterium oxide in vivo is then used to establish the techniques and calculation for measuring the synthesis rate of individual proteins.

Section 1; Proteome profiling of human skeletal muscle from Lean (LE), Obese (OB), and T2DM individuals

Methods

Subjects

Proteomic analysis was performed on muscle (Vastus lateralis) biopsy samples from 9 participants (4 males and 5 females) from a previous study that had a cross-sectional design comprising 3 independent groups; LE (BMI ≤ 30 $kg.m^{-2}$, OB (BMI>30 $kg.m^{-2}$), T2DM (BMI>30 $kg.m^{-2}$). T2DM patients were diagnosed by their GP (General Practitioner) following ICD-10 (International Statistical Classification of Diseases and Related Health Problems 10^{th} Revision) code E11 and/or blood test according to NHS (National Health Service) diagnosis criteria: HbAb1C level ≥ 48 $mmol.mol^{-1}$ or OGTT: resting blood glucose > 7 $mmol.mol^{-1}$ and increasing to >11 $mmol.mol^{-1}$ after glucose administration. The collection of samples used in this study was ethically approved (08/H1202/86) and each participant gave their informed consent. Originally, the samples were collected during elective orthopaedic (knee replacement) surgery. Due to the nature of the biopsies being obtained during surgery, the sample yield was enough for some of the preliminary work completed in this section.

Preparation of muscle samples for proteomic analysis

Proteins were extracted from archived (-80 ^{0}C) human vastus lateralis muscle using methods developed in-house and optimised for tissues such as cardiac and skeletal muscle that contain relatively large amounts of connective tissue. As a prerequisite to mechanical homogenisation, muscle samples were pulverised in a mortar and pestle cooled in liquid nitrogen. An accurately weighed portion (100 mg) of muscle powder was used to ensure equivalent extraction of protein from each sample. Mechanical homogenisation was performed in a buffered solution containing detergent (Triton X-100) and protease inhibitors. Muscle powder was mixed with 10 volumes of homogenisation buffer (1% Triton X-100, 5 mM EDTA, 50 mM Tris pH 7.4, including Roche Complete protease inhibitor, and Roche PhosphoSTOP phosphatase inhibitor). Samples were homogenised on ice using Polytron homogeniser at full speed for 20 s. Homogenates were incubated on ice for 15 min, in the weak detergent and low salt buffer to solubilise membranes and separate myofibrils from nuclei and mitochondria. Centrifugation (12,000 x g, at 4 ºC for 45 min) was used to pellet the myofibrillar aggregates and the supernatant, containing soluble proteins, was retained but was not analysed in the current work.

Protein assay

Bradford Assay Bradford (Bradford, 1976) was used for detecting protein quantity at wavelength 595 nm (Magellan software, TECAN). Samples were diluted 1:10 with ddH_2O. Five microliter of each sample was dispensed into microtitre plate wells in triplicates. A Standard curve was constructed using a BSA as a reference standard and serial dilution to achieve a concentration range of 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, and 1.75µg/µl. Standards were pipetted in triplicate and 245 µl of Bradford reagent was added to each well to give a final solution of 250 µl equivalent to 1:50 of protein: dye ratio. Samples were incubated for 5 min at room temperature before spectrophotometric detection at a wavelength of 595 nm, which is the protein-dye absorbance maximum in the blue spectrum.

Interpolation of sample protein concentrations was performed by standard curve analysis calculated between a minimum of 2 base points (x, y). The adjacent base points will be joined by mean of a straight line **(Figure 9)**.

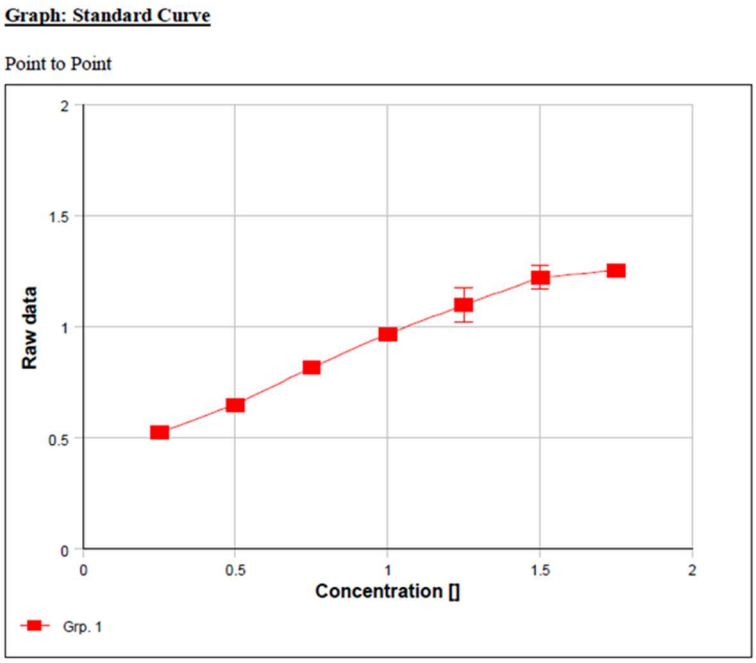


Figure 9 Standard curve of ALBU protein at 5 different concentrations (0.00, 0.25, 0.50, and 0.75 µg/µl)

Graph was plotted by point-to-point method. Each point represents the absorbance change at protein concentration of 0.250, 0.500, 0.750, 0.100, 0.125, 0.150, and 0.175 µg/µl

Tryptic in-solution digestion

An aliquot of each muscle sample containing 50 µg protein was precipitated in 5 volumes of ice-cold acetone at -20 ^{0}C for 1 h. Samples were centrifuged at 5,000 x g at room temperature for 5 min. Supernatant containing unwanted substances including nucleic acids and salts that may interfere with protein analysis was discarded and the pellet was dried for approximately 10 min at room temperature. The protein pellet was solubilised in 400 µl of 0.1% Rapigest SF (Waters, USA) in 50 mM Ammonium bicarbonate (Ambic). The solution was centrifuging through 5 kDa MW cut-off spin columns at 15,000 x g at room temperature for 30 min to remove Triton x 100 detergent. This step was repeated and then samples were incubated at 80 ^{0}C for 15 min to heat denature the proteins. Disulphide bonds were disrupted by the addition of Dithiothreitol (DTT) at 6.5 µM in 50 mM Ambic and the samples were incubated at 60 ºC for 15 min. Free cysteines were modified (carbamidomethyl) by 13.5 µM iodoacetamide (IAA) in 50 mM Ambic while incubated at 4 ºC for 30 min and protected from light. Sequencing grade trypsin (Promega) was added at a protein to enzyme ratio of 50:1 and the solution and incubated overnight at 37 ^{0}C. This incubation allows trypsin to cleave proteins into peptide fragments at the carboxylic (C-terminus) side of lysine and arginine residues except when followed by proline. Trypsin activity is greatest when incubated at high pH condition (i.e. Ambic, ~pH 11). The reaction is stopped by the addition of 2 µl concentrated Trifloroacetic acid (TFA), and incubated at 37^{o}C for 30 min. The addition of acid also degrades the acid-labile surfactant (Rapigest SF) and after centrifuged at 15,000 x g for 10 min at room temperature, the supernatant, containing tryptic peptides, is stored in clean Lo-bind (-20 ^{0}C) Eppendorf tubes.

Label-free profiling using LC-MS data

All samples were analysed by label-free LC-MS profiling as reported previously (Malik *et al.*, 2013). Label-free quantitation was performed using Progenesis Quantitative Informatics for Proteomics (QI-P, version 4.1; Non-linear dynamics, http://www.nonlinear.com). The processing of data consisted of 4 stages (i) mass and retention time alignment, (ii) normalisation, (iii) quantitation and (iv)protein identification. Protein abundances were normalisation before validation and identification. The purpose of normalisation was to exclude noise in the data that interferes with the result. Noise is a random background signal which may interfere with an analytical method. By eliminating or minimising noise, the chance to detect the true signal or ion in the sample is increased. The scalar factor (α_k) was applied for normalising peptide ions and filter out random noise. The concept for calculating the scalar factor is based on the median and mean absolute deviation of all detected abundances. This method aims to reduce bias and is widely used across proteomics and genomics studies (A Waters Company: nonlinear dynamics, no date). Firstly, it is essential to determine global scaling factor by calculate Normalised Factor (NF) or within fraction normalisation as follow:

$$y_i' = \alpha_k y_i \quad \text{---------- Equation 2}$$

Where y_i' is normalised abundance of peptide ion **i** on sample k

α_k is the scalar factor for sample k

y_i is the measure peptide ion abundance of peptide ion **i** on sample k

After that, normalise process will determine to all proteins. A peptide ion will be calculated its distribution by using log (ratio) value. Ideally, the scatter plot would present close to 0.0. This indicates that up-regulation and down-regulation has the same weight and distributes to both directions equally. Model for Log (ratio) is as follow:

$$R_{i,x} = \frac{Ab_{i,x}}{Ab_{i,NR}} \text{---------- Equation 3}$$

Such that $R_{i,x}$ is the ratio of the abundance of the peptide ion **i** in run x and peptide ion **i** in the normalisation reference (NR)

$Ab_{i,x}$ is the abundance of the peptide ion **i** in run x

$Ab_{i,NR}$ is peptide ion **i** in the normalisation reference (NR)

NR would be automatically selected by the software based on NF form equation **(1)**. Then the log transformation was applied for generating normal distribution and weighing up and down regulation equally. The number of Log $\mathbf{R_{i,x}}$ will plot in the scatter plot. Note that model **(1)** and **(2)** were retrieved from nonlinear dynamics; Proteins were identified by searching mass spectra against the Swiss-Prot database using a locally implemented MASCOT server (www.matixscience.com). Differential quantitative analysis was performed on normalised ion abundances of peptides unique to each parent protein and data were analysed by one-way analysis of variance (ANOVA) within Progenesis QI-P. Post-hoc analysis has performed by SPSS (Statistical Package for the Social Sciences) software version 23 (IBM inc.). Variables were compared by mean under Student t-test. The measurement was taken on one variable of a matched paired subjects; LE vs OB, OB vs T2DM, and LE vs T2DM.

Bioinformatics analyses

All 6 significantly different proteins were obtained for Pathway annotation analysis by DAVID (The Database for Annotation, Visualization and Integrated Discovery) (https://david.ncifcrf.gov/home.jsp) (Matthews *et al.*, 1982). The method of algorithm has been described(Huang, Sherman and Lempicki, 2009). Briefly, a list of gene/protein has produced based on Uniprot accession number or protein code (http://www.uniprot.org). Then, functional annotation tool is used to provide information on the shared features (e.g. biological function or metabolic pathway) across the proteins. This step is primarily

based on co-occurrence or group similar gene that provide similar function (protein families) following biological pathway, disease, protein-protein interaction, homologies, gene tissue expression, etc. The grouping algorithm has performed based on Kappa statistics and fuzzy heuristic clustering. A chance of gene sharing information is a chance to enhance statistical power of clustering. A kappa statistic's concept is agreement, which range from 0-1 (Cohen, 1960). The higher number is the higher degree of relationship between interested genes/proteins. The fuzzy heuristic clustering allows considering gene more than 1 cluster. First step is to form multiple seeds (protein/gene input), then only initial group with closely related number will be maintained (kappa score ≥ 0.4, seed > 3). The steps perform loop running until the secondary step is stable and represent the fuzziness capability of algorithm.

Results

Clinical Characteristics of subjects

T2DM participants were not only diagnosed as diabetic state but also met obese criteria. Although OB subjects revealed obesity, they have not been diagnosed ICD-10 (International Statistical Classification of Diseases and Related Health Problems 10th Revision) code E11 for Type 2 diabetes mellitus. However, one OB participant had hypertension but was not taking any prescribed medication. One LE had complications, i.e., Asthma and heart condition while the other was considered hypertensive., T2DM patients were receiving standard insulin treatment, and one participant in this group mildly hypertensive (BP = 131/68 mmHg). All clinical characteristics were presented in **Table 3.**

Table 3 Clinical characteristics of participants in each independent group

	Gender	Age (Y)	BMI kg.m^2	Medical condition	Blood pressure
Lean	Male	87	25.8	Asthma/cardiac conditions	
(LE)	Male	54	28.4		
	Female	62	26.3		147/102 mmHg
Obese	Male	59	39.7		125/68 mmHg
(OB)	Male	53	40.6		
	Female	62	40.3		
T2DM	Female	60	48.4		
	Female	78	38.6	Insulin-dependent	
	Female	52	35.3		131/68 mmHg

Proteomic analysis

In total 89 proteins were confidently identified based on MOWSE probability statistic generated by the Mascot search engine. The Mowse score represents -10 log(p), therefore a p-value of 10^{-20} gives a MOWSE score of 200. A significance threshold of 0.05 is used to determine the MOWSE score at which the protein identification becomes confident. The exact cut-off value changes depending on the size of the database being searched and the number of queries (i.e. peptides) submitted to each search. For LC-MS/MS data submitted to the UniProt 'Human' database, a MOWSE score of 30 is usually sufficient to confidently identify a peptide. The accuracy and sensitivity of modern-day instruments means that MOWSE scores from >100 to 1000 are common and represent unambiguous protein identifications. Throughout this thesis, all proteins were categorised based on UniProt Knowledgebase (UniProtKB) keywords or that we emphasize 2 aspects i.e., Molecular function and Biological process. The keywords are derived from Gene Ontology (GO) databases but are more specific to the protein of interest than general GO systems, which describe attributes based on genes and gene products in any organism. Regarding the controlled vocabulary in UniProtKB keywords, all 82 proteins were grouped and shown in **Figure 10a and Figure 10b.** muscle proteins (14), transferase (11), oxidoreductase (10), myosin (8), and motor protein (8) were contained majority proteins based on Molecular function (**Figure 10a**). For biological process (**Figure 10b**) contained major group of proteins involve in glycolysis (13) and transport process (11).

Of the 89 proteins identified, 82 were eligible for quantitative analysis, i.e. contained at least 1 unique/non-conflicting peptide. For statistical analysis, ANOVA initially found 2 significant differences in abundance of individual proteins among 3 groups and showed in **Figure 11.** A quality control has been taken and the relevant factors obtained in **Table 4. Table 4** summarises the alignment and normalisation data from Progenesis QI-P. Alignment similarity scores ranged from 28.6% (LE) to 87.4% (T2DM). After normalisation, the normalisation factor (NF) ranged between 0.77 and 3.56. The Log (ratio) was less than zero (-0.11) in LE participants but greater than 0.55 in T2DM.

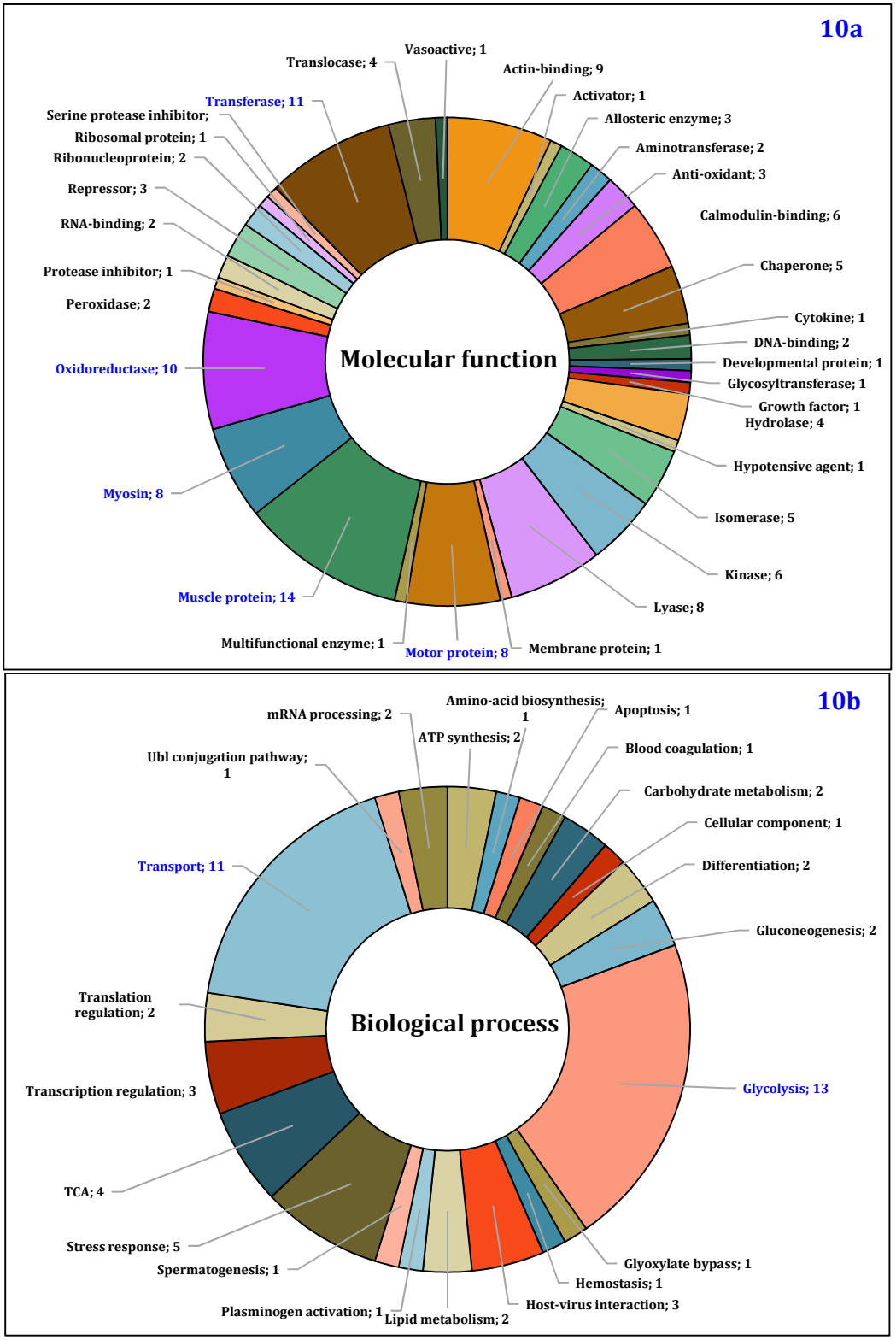


Figure 10 A number of 82 proteins from protein profile by LC-MS/MS

All 82 proteins were categorised into either Molecular function (10a) or Biological process (10b) based on to **UniProtKB keyword(s)** (https://www.uniprot.org) to reduce bias. Further information of particular proteins across molecular function and biological process available in **Appendix 3.**

Table 4 Quality control data from Progenesis QI-P.

Group of study		*Vectors**	*Similarity score (%)***	*Normalisation Factor (NF)*	*Log (ratio)*
LE	**1**	1162	65.1	0.77	-0.11
	2	1443	55.6	1.00 (Ref.)	0.00 (Ref.)
	3	1047	28.6	1.14	0.057
OB	**1**	1150	70.6	1.08	0.032
	2	897	49.6	1.13	0.051
	3	831	21.4	1.12	0.049
T2DM	**1**	651	73.3	3.56	0.55
	2	Ref.	Ref.	1.07	0.33
	3	1307	87.4	1.10	0.041

Information regarding the identity of the 82 proteins profiled in the current work is available in **Appendix 4.** One-way ANOVA discovered 2 proteins (**AT2A2**; Sarcoplasmic/endoplasmic reticulum calcium ATPase 2, and **FABPH**; Fatty acid binding protein, heart type) differed significantly ($p > 0.05$) abundance among LE, OB, T2DM muscle (FDR; False discovery rate, $q = 0.05$)

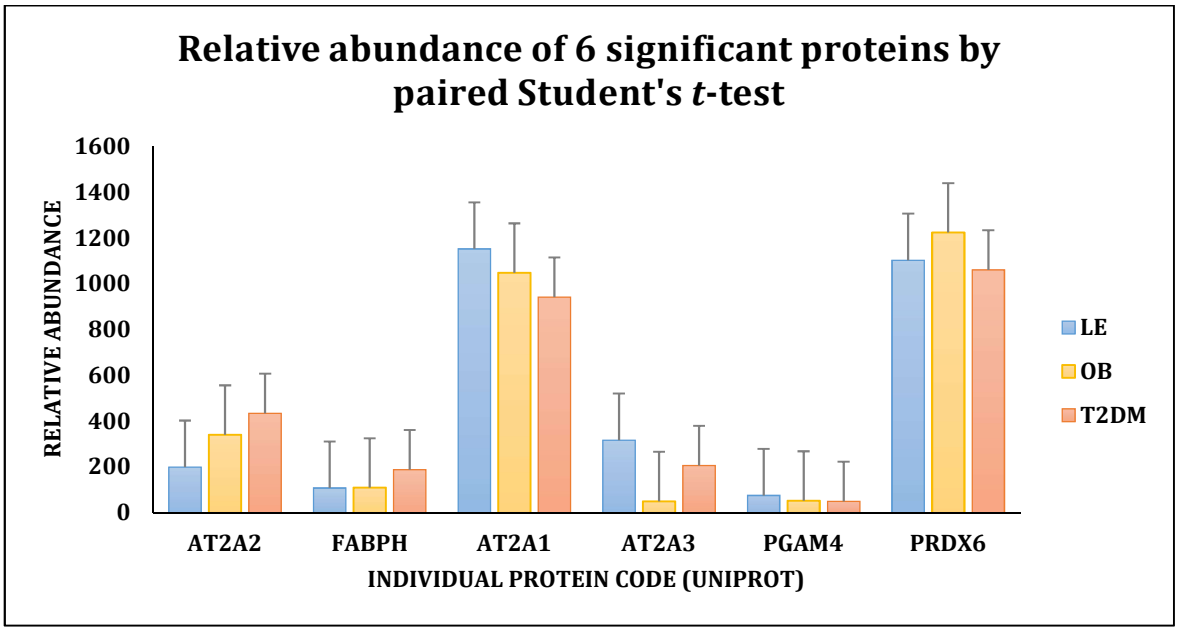


Figure 11 Difference in relative abundance of 6 proteins among LE, OB, and T2DM

Label-free profiling detected statistically (p-value < 0.05) significant differences in the relative abundances of AT2A2, FABP, AT2A1, AT2A3, PGAM4, and PRDX6. Data are presented as Mean ± SE, n=3 in each group. In this regard, 2 proteins (AT2A2 and FABPH) was significantly up-regulation in T2DM/OB, 2 proteins (AT2A1 and PRDX6) showed less abundance in OB/T2DM, and the remaining 2 proteins (AT2A3 and PGAM4) found down-regulation in OB/LE. Shading bar represents subject group that reported statistically significant to abundance whereas non-shading bar was for subjects' group that did not show any difference in abundance against other groups (p-value < 0.05 by paired *Student's t-test* analytical method).

Table 5 reported significantly different proteins abundance at different probability level.

Protein	*p*-value (ANOVA)	*p*-value (Student's t-test)		
		LE vs OB	OB vs T2DM	LE vs T2DM
AT2A2	**0.01***	0.06	0.04*	0.35
FABPH	**0.04***	**0.94**	**0.02***	**0.06**
AT2A1	**0.07**	**0.43**	**0.02***	**0.15**
AT2A3	**0.07**	**0.02***	**0.19**	**0.44**
FHL1	0.07	0.08	0.42	0.20
ZN644	0.07	0.74	0.14	0.05
PGAM4	**0.11**	**0.01***	**0.83**	**0.18**
TNNI1	0.31	0.85	0.07	0.37
PRDX6	**0.33**	**0.32**	**0.02***	**0.74**

At 5% level of significant by ANOVA found 2 proteins (**AT2A2** and **FABPH**) significantly different in abundance change and added **AT2A1** at 10% level of significant. Extended analysis by paired-matched test, 6 proteins found coverage 3 out of 5 significant proteins at 10% level. In addition, 2 proteins (**FHL1** and **ZN644**) have not revealed significantly different by paired-match test at both 5% and 10% level. * Significant at $p < 0.05$

Discussion

The current preliminary experiment profiled 82 proteins in the muscle of LE, OB and T2DM patients. We found 2 proteins (**AT2A2** and **FABPH**) that exhibited statistically significant ($p < 0.05$ and $p < 0.1$, respectively) differences in abundance between the independent groups **(ANOVA).** There are 6 and 9 individual proteins that were found by post-hoc analysis using *Student's t-test* ($p < 0.05$ and $p < 0.1$, respectively). The lack of further statistical differences is likely due to low n values. Despite the trend of the abundance profiles for the majority (29) of other proteins profiled was in accordance with our findings from the systematic review of published literature (**Table 6)**. To set a critical value for the statistical analysis test, the data was determined under non-parametric test (F distribution) and t-distribution (extended analysis) and it is more likely recommended to considerate 5% more than 10% level as the maximum acceptable probability for determining statistical significance (**Table 5)**. This indicates 5% is appropriate for the current test because 10% would become less biologically meaningful. That is, significant test among 3 different groups found 2 significant proteins at $p < 0.05$. However, paired-match subjects test found these 2 proteins were statistically different at $p < 0.1$. Therefore, $p < 0.05$ was used to reveal essential information in both among (by ANOVA) and paired-match test (by *t*-test).

Technical differences are the most likely explanation for the difference in findings between our current LC-MS analysis, which reports changes in overall protein abundance only, compared to data in our systematic

review, which included data from gel-based studies that resolve proteins as multiple proteoforms. In addition, we used participants of an older age (52-87 y) compared to studies from systematic review (33-58y). Our experiment using LC-MS/MS only is similar to Thingholm et al (2011) which reported 30 proteins related to systematic review (RQ1). In the current work, only 4 proteins (**FABPH**, **HBA**; Haemoglobin subunit α, **HSPB6**, and **MYH3**) were common with the reported proteins from the previous LC-MS/MS analysis (Thingholm *et al.*, 2011). The current data discovered only FABPH exhibits a significantly different abundance among 3 different subject groups.

Table 6 Comparison of proteins from this study with previous reports.

Accession	Description	Molecular function	Biological process	Reference(s)	regulation	
					current study	previous study
FABPH*	Fatty acid-binding protein, heart	cytoskeletal protein binding icosatetraenoic acid binding long-chain fatty acid binding long-chain fatty acid transporter activity oleic acid binding	Transport protein	(Thingholm *et al.*, 2011)	OB*<LE<T2DM*	OBT2DM>LE
AT2A2 **	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	Translocase	Transport Calcium transport Ion transport	NA	T2DM*<OB<LE*	NA
AT2A1*	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1			(Lefort *et al.*, 2010)	OB*<T2DM*<LE	OB>LE
AT2A3*	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3			NA	T2DM<OB*<LE*	NA
PGAM4*	Probable phosphoglycerate mutase 4	Hydrolase Isomerase	Glycolysis	NA	T2DM>OB*>LE*	NA
PRDX6*	Peroxiredoxin-6	Antioxidant Hydrolase Multifunctional enzyme Oxidoreductase Peroxidase	Lipid metabolism Lipid degradation	NA	T2DM*>OB*>LE	NA

FABPH and **AT2A2** found significantly different in abundance change regarding ANOVA whereas the remaining proteins (**AT2A1**, **AT2A3**, **PGAM4**, and **PRDX6**) showed significantly different respecting pair-matched analysis (*t*-test). *significant at $p < 0.05$ and **significant at $p < 0.01$.

The finding that **FABPH** abundance was significantly greater in T2DM muscle **(Figure 11)** is consistent with previous study (Thingholm *et al.*, 2011), which was included in our systematic review. FABPH serves as a 'sink' for long-chain free fatty acids entering the muscle cell (Binas and Erol, 2007) which are latter directed to either β-oxidation or triacylglycerol storage. Previously we (Malik *et al.*, 2013)

reported a greater abundance of **FABPH** was associated with high aerobic capacity in selectively breed rats and physically active humans. This finding may be consistent with the phenomena termed the 'athlete's paradox' where both endurance trained athletes and obese individuals exhibit an enhanced capacity to uptake and store fatty acids in muscle, discussed (Goodpaster *et al.*, 2001). The study by Goodpaster et al (Goodpaster *et al.*, 2001) revealed that skeletal muscle insulin resistance may be mediated by lipid oxidative capacity of muscle. They also demonstrate excess muscle lipid accumulation and greater insulin sensitivity in athlete subjects, suggesting that lipid storage in muscle is not a key distinguishing feature. However, our data show a further elevation in **FABPH** in T2DM muscle and it is not clear whether this might relate to a worsening of the muscle insulin resistance in these patients.

We also discovered that the sarcoplasmic/endoplasmic reticulum ATPase 2 (**SERCA or AT2A2**) was more abundant in muscle of OB and T2DM individuals. This is novel because this protein was not included in the literature of our systematic review. **SERCA** proteins are responsible for the re-uptake of Ca^{2+} in to the sarcoplasmic reticulum and therefore muscle relaxation following contraction and consist of 3 isoforms which derive from different variants (Gélébart *et al.*, 2003). **SERCA2** is most abundant in slow-twitch and cardiac muscle whereas **SERCA1** is more abundant in fast-twitch skeletal muscle. **SERCA3** may also be involves in muscle excitation/contraction but is most abundant in tissues include lymph node and cancer cell lines. Our findings contrast with the literature included in the systematic review.

Only **AT2A1** or **SERCA1** has been reported previously and is up-regulated in the muscle of OB participants (Lefort *et al.*, 2010). Surprisingly, this preliminarily study (by pair-matched test) discovered the opposed response and sarcoplasmic/endoplasmic reticulum ATPase families (**SERCA1** or **AT2A1**) was more abundant in muscle of LE individuals. Therefore, our findings may indicate a shift toward a slower contracting phenotype in OB/T2DM muscle, which is not consistent with the findings from systematic review that had been reported in Chapter II. This discrepancy could be due to differences in the age or physical activity of study participants. Ageing is associated with a selective loss of type II fast-twitch fibres and this may have influenced our results. That is, because samples from LE group are from later life their muscle may contain a higher proportion of slow-twitch fibres that are likely to contain **SERCA2**. We also found evidence of over expression in OB/T2DM compared to LE group in protein **PRDX6** (Peroxiredoxin-6) which functions as an anti-oxidant enzyme in lipid metabolic process. An apparent benefit of **PRDX6** may be cell protection against oxidative stress by detoxifying peroxides and in phospholipid homeostasis (Kang, Baines and Rhee, 1998; Chen *et al.*, 2000).

Interestingly, we have identified **AT2A3** or **SERCA3** which has been reported its specific function toward Mg^{2+}, catalyses the hydrolysis of ATP coupled with the transport of Ca^{2+}, and involved with muscle excitation/contraction coupling process (Martin *et al.*, 2002; Bobe *et al.*, 2004). **SERCA3** showed

down-regulation in OB/LE that was similar to the trend in **PGAM4** (Probable phosphoglycerate mutase 4). **PGAM4** functions in the glycolytic pathway and catalyses 3-phosphoglycerate to 2-phosphoglycerate. Impaired **PGAM4** is associated with an intolerance to physical activity. The trend of these proteins (SERCA3 and PGAM4) to be of greater abundance in LE compared to OB may also be related to the higher proportion of slow-twitch fibre as described previously.

Limitation

It was necessary to conduct our preliminarily studies in 2 different species. Pre-existing human muscle samples were used to develop the techniques for label-free proteome profiling and muscle from laboratory rats that had consumed deuterium oxide was used to develop the techniques for calculating the fractional synthesis rate of new proteins. Our use of archived human muscle samples, which had been stored since 2009 presented unforeseen challenges to the analysis and we suspect that decomposition of the samples meant that fewer than expected proteins were resolved and analysed using our proteomic techniques. Nonetheless, this work was a valuable training experience and we were able to detect some significant differences in protein abundance between LE, OB, and T2DM samples, but given the known technical issues in extracting protein from these samples the results must be interpreted with caution. Other factors that must be taken in to consideration when interpretation these data are that the numbers of samples in each group were quite modest and the samples were collected from later life subjects so age-related differences in the muscle proteome may be a confounding factor. For example, one individual reported a cardiac condition and was taking medication i.e. Metformin and Atenol which could confound the interpretation of the data regarding the differences between LE, OB and T2DM muscle. In older adult populations, it is also likely that some participants may have undiagnosed or underlying disease state or that participants may suffer from more than 1 disease. Despite these limitations, our proteome profiling resolved more than 80 proteins and highlighted 2 proteins (FABPH and SERCA2) that were markedly revealed greater abundance in obese/T2DM individual. The design of experiment was limited. The cross-sectional design was appropriate for exploring both human protein profile and rat protein turnover calculation. However, to strengthen the findings future work should extend the analysis to a larger number of samples. Although we applied both ANOVA and *Student's t-test*, our result relied on ANOVA. The reason we chose ANOVA was to reduce the reporting bias regarding statistical parameter. In molecular analysis, Wilcoxon's rank test is also recommended as an appropriate method to reduce bias especially in the analysis of very modest sample size.

Conclusion

We have successfully used archived human Vastus lateralis from Lean (LE), Obese (OB) and T2DM participants to produce initial data for measuring protein abundance in human muscle. Our analysis revealed multiple protein differences that agreed with systematic review (Chapter II). There were 82 proteins to produce protein profile in human, however, only 4 proteins **(FABPH, HBA, HSPB6, MYH3)** found similar to previous study (Thingholm *et al.*, 2011). **SERCA** protein families play an important role in muscle contraction and relaxation. Our study reports **SERCA 2** protein (by ANOVA) which is different from **SERCA 1** protein in Chapter II (section 1 systematic review). Moreover, **FABPH** is acquired greater abundance in T2DM as well as a sink for long-chain free fatty acid that store in skeletal muscle. This supports 'athlete's paradox' but may also indicate higher lipid storage in muscle resulted insulin resistance.

Section 2; Preliminary study of Protein turnover rate computation in rat skeletal muscle (Plantaris) model

The purpose of this section is to describe the computational method for calculating individual protein turnover rate. The study was conducted on rat samples, which provides a cheaper experiment and slightly simpler calculation method because precursor enrichment is at a constant steady-state. The approach relies on stable isotope labelling in vivo using deuterium oxide or heavy water (^2H_2O or D_2O). Our later studies in Chapter IV and Chapter V are performed in human skeletal muscle which requires substantially greater volumes of stable isotope (D_2O). Therefore, this pilot study was selected to explore and initially apply the proteomic approach for determining the turnover rate of individual proteins. The distribution of D_2O labelling and incorporation in to protein is similar between humans and rodents but it is simpler to introduce this technique using the animal model. Note that this pilot study was performed in *plantaris* muscle which has been characterised as predominated fast-twitch locomotive muscle and in that sense is similar to human VL which will be used in the next Chapters.

Methods

Sample preparation

Experimental procedures were conducted under the British Home Office Animals (Scientific Procedures) Act 1986 and were approved by the local ethical review committee. The male Wistar rats (500±32 g body weight) were bred in-house in a conventional colony, housed in controlled conditions of 20°C, 45% relative humidity, and a 12 h light (0600-1800 hours) and 12 h dark cycle, with water and food available ad libitum. The animals consumed 5% v/v heavy water (D_2O or ^2H_2O) in drinking water for 0, 10, 20, and 30 days (n=3, in each). Deuterium administration was initiated by an intraperitoneal injection of 10 µL.g 99% D_2O saline, and was maintained by administration of 5% (v/v) D_2O in drinking water, which was refreshing daily. Animals were asphyxiated with rising concentration of CO_2 and killed by cervical dislocation. Each muscle was cleaned of fat and connective tissue and then weighed before being frozen in liquid nitrogen and stored at -80°C. Five tissues were obtained and kept in Cryotube, *plantaris* (PL). Samples were processed consistent with the method descried in the previous subsection on Human muscle proteomic analysis. Briefly, muscles were pulverised in motar and pestle with liquid nitrogen and weighed 100 mg per sample was homogenised using Polytron homogenizer full speed for 20s in 10 volumes of lysis buffer (5 mM EDTA, 50 mM Tris pH 7.4, 1% Triton X-100, Roche complete protease inhibitor, and Roche complete PhosphoSTOP tablet). Solution was incubated on ice for 15 min

before centrifugation at 12,000 g at 4°C for 45 min. Supernatant (sarcoplasmic proteins) and pellet (myofibrillar fraction) were separated into individual tubes and were kept at -80°C.

Muscle processing and mass spectrometry analysis

Sarcoplasmic protein solution was aliquot in quantity of 100 μg for each sample. An aliquot containing 100 μg protein was precipitated in 5 volumes of acetone at -20°C for 1 hour. Proteins were extracted from *Plantaris* (PLR) and analysed by liquid chromatograph-mass spectrometry as described in the previous section (Chapter III, Section 1) and Hesketh et al 2016. In addition to label-free quantitation of protein abundances (i.e. as reported in Section 1 analysis of LE, OB, and T2DM muscle) the mass isotopomer distribution of each peptide was also collected and analysed using mMass software (http://www.mmass.org). The explanation of this software was described as part of bioinformatics (data processing).

Protein synthesis calculation

Mass Isotopomer Distribution Analysis (MIDA)

The synthesis of individual muscle proteins was investigated using methods modified from Price et al (2012)(Price *et al.*, 2012). Each peptide mass spectra have a characteristic distribution of M_0, M_1, M_2, and so on, molecular species (mass isotopomers). The proportion of mass isotopomers can be represented as a frequency histogram of the mass isotopomer distribution and gives information on the relative abundances of 'light'/primary isotopes and secondary 'heavy' stable isotopes in the elemental composition of each peptide.

A model for specific synthesis rate of each peptide was generated from observed peptide mass spectra (from LC-MS/MS analysis) across a time series of measurements, i.e., day 10, day 20, and day 30, whereas control (no label) is used as reference. The main value of interest is the molar fraction of the mono isotopic peak (M0) because this represents the proportion of unlabelled protein and was expected to decline as the amount of labelled protein increases as a function of new protein synthesis.

The key output variables of this study were the rate of protein synthesis (k). The key input variables are the number of exchangeable hydrogen -(H->D)(Commerford, Carsten and Cronkite, 1983) of each peptide, Normalised peak intensities of Molar fraction of the mono isotopic peak at each time point (FM_0 at day 0, 10, 20 ,30), and probability of labelling by precursor (p) which is equivalent to the level of body water enrichment of ^2H. The decline in the mono isotopic peak is expected to follow an exponential decay for the form:

$$f_t = f_{max}\, x\, (e^{-kt})$$

Where f_t is fraction of the monoisotopic peak at t

f_{max} is maximum fraction of the monoisotopic peak (i.e. at day 0, with no D_2O label incorporated)

k is protein turnover rate assume constant in every time point

t is time point during labelling

Gas-chromatography mass spectrometry (GC-MS)

Body water enrichment of ^2H was measured in plasma samples against external standards constructed by adding ^2H_2O to phosphate buffered saline over the range from 0.0 to 3.0 % in 0.5 % increments. The ^2H enrichment of aqueous solutions was determined after exchange with acetone similar to described in Mccabe et al (McCabe *et al.*, 2006). Samples were centrifuged at 12,000 g, 4 ºC for 10 min and 20 µl of plasma supernatant or standard was reacted overnight at room temperature with 2 µl of 10 N NaOH and 4 µl of 5 % (v/v) acetone in acetonitrile. Acetone was then extracted in to 500 µl chloroform and water was captured in 0.5 g Na2SO4 prior to transferring a 200 µl aliquot of chloroform/acetone to an auto-sampler vial.

Samples and standards were analysed in triplicate using an Agilent 5973N mass selective detector coupled to an Agilent 6890 gas chromatography system. A CD624-GC column (30 m x 0.25 mm x 1.40 µ m) was used in all analyses. Samples (1 µl) were injected using an Agilent 7683 autosampler. The temperature program began at 50 °C and increased by 30 °C/min to 150 °C, and was held for 1 min. The split ratio was 50:1 with a helium flow of 1.5 ml/min. Acetone eluted at approximately 2.0 min. The mass spectrometer was operated in the electron impact mode (70 eV) and selective ion monitoring of m/z 58 and 59 was performed using a dwell time of 10 ms/ion.

This method is to measure percent enrichment of the precursor pool (p), i.e. body water. A blood sample was used to measure precursor pool enrichment of D_2O or p by GC-MS. Enrichment was calculated using the equilibrium concept to measure δD or δ^2H (Scrimgeour *et al.*, 1993). Where δD is the corrected D after correction for natural abundance (i.e. D0 sample).

Bioinformatics

Protein identification

Protein are fragmented into peptides before MS analysis and bioinformatics analysis is used to identify proteins based on the mass and amino acid sequences of the observed peptide ions. Lists of mass spectra peaks are searched against protein databases but a peptide could identify more than 1 protein. Therefore, it is essential to identify protein by using BLAST (Basic Local Alignment Search Tools) algorithm with database through search engines such as MASCOT. The MASCOT server was developed by Matrixscience Company and provides a free service for small dataset (http://www.matrixscience.com). A list such as peptide mass fingerprint or more complex peptide fragmentation data can be input to MASCOT search engine. This step would blast candidate protein to FASTA database of known proteins such as NCBI, Swiss Prot, and etc. After that, candidate peptides can be matched to officially reported peptide sequence in the databases. The output contains multiple scoring for determining protein homology/identification such as MOWSE score, protein properties (pI, protein mass). In this study, MOWSE score is the determining factor that was used to include peptides for MIDA analysis. MOWSE score was developed by Papin et al (Pappin, Hojrup and Bleasby, 1993) supports concept where peptide alone provide sufficient information to identify protein. The score is given based on the frequency of a Fragment Molecular weight (FMw) being found in a protein of a given molecular weight range (Pappin, Hojrup and Bleasby, 1993). In MASCOT search, it will present probability based MOWSE score against a number of hits; a higher score represents a more significant/ certain identification.

Data processing

Here, mMass has been applied for collecting peak intensity data for mass isotopomer distribution analysis (MIDA) calculations. The mMass software has been disseminated as a free software (http://www.mmass.org/download/) and it contains multiple functions for proteomics data processing, including mass spectra visualisation, sequence editing, protein database identification, mass formula and peak picking, In this study, mMass has been used for visualising mass spectra and collecting mass isotopomer information. For each peptide, 3 peaks (M_0, M_1, and M_2 mass isotopomers (**Figure 12**) with the high intensity and clear pattern were collected for analysis.

Regarding MIDA concept, the algorithm definition was described as a systematic approach to polymerization biosynthesis (Hellerstein and Neese, 1992, 1999). In this study, each molecular species (mass isotopomers) were collected as a characteristic distribution of the first, second, and third peak were given as M_0, M_1, and M_2, respectively. Then, the proportion of mass isotopomer were applied to normalise peak intensities, the main output for onward analysis was the molar fraction of the

monoisotopic peak (FM0), which is the fraction of the monoisotopic (M_0) peak relative to the sum of all (M_0, M_1, or M_2) peak intensities.

The first step to determine the molar fraction from mono isotopic peak is to consider high quality peak. A peptide pattern with high resolution indicates high-quality peaks that can be selected for analysis (**Figure 12A).** Next, each mass isotopomer peak was measured and labelled as M_0, M_1, and M_2 (**Figure 12B**). Finally, measurement values were contained in the table on the right-hand side as showed in **Figure 2C**.

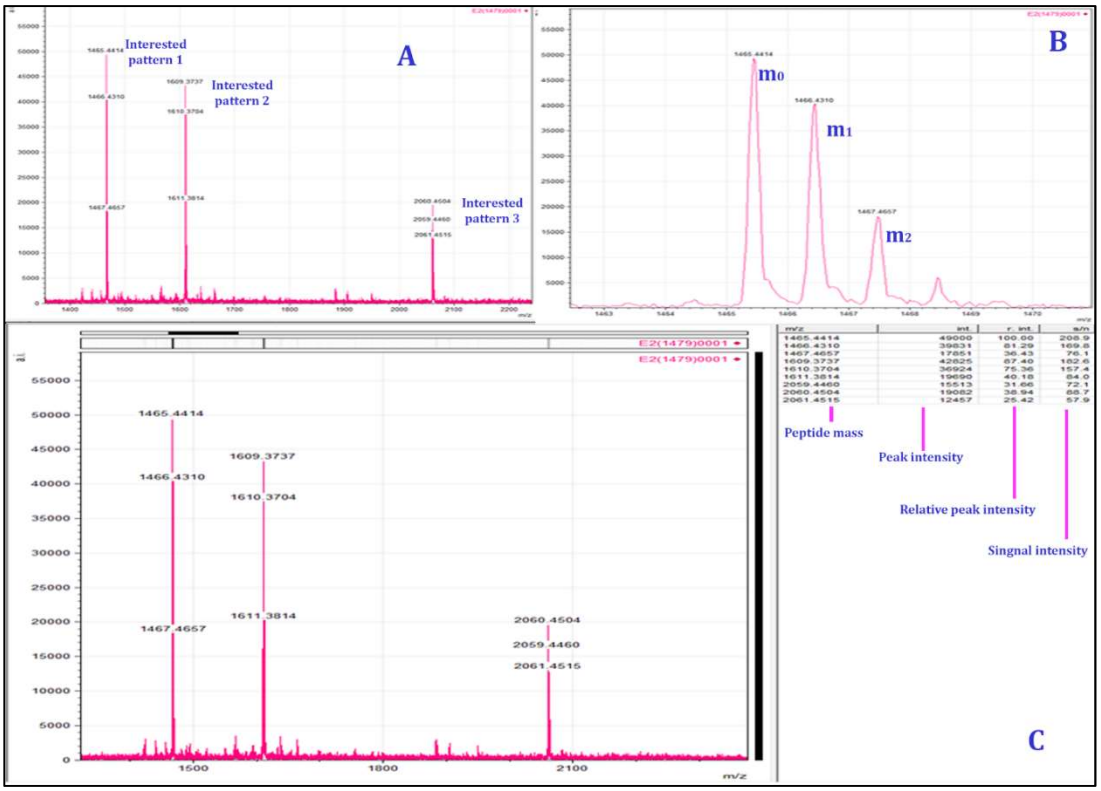


Figure 12 Schematic pattern of isotopomer that were collected for studying.

Overall figure from the screen of mMass software reveals 3 peptides of interest. Figure 12b reveals pattern of 3 peaks of isotopomer that we measured. Figure 1c demonstrates important numbers that have been determined; peptide mass, peak intensity, relative peak intensity and signal intensity. Note that peptide mass has been used for calculation protein turnover rate.

Protein turnover rate calculation

The aim of this module is to individually calculate the rate of protein synthesis by determining FSR which requires a non-linear curve fitting similar to reported in (Camera *et al.*, 2017). The assumption is that the relative mass isotopomer pattern changes in relation to the incorporation of heavy isotope (M_1, M_2,…M_n) where the first mass isotopomer (M0) is unlabelled. The rate of decrease in M_0 abundance follows first-order exponential decay equal to the rate of protein turnover (k) (Lam *et al.*, 2014). The steps of FSR or newly synthesis protein/peptide are

1. Measure peak intensities of monoisotopic mass (M_0, M_1, M_2,…M_n) at each time point (Day 0, Day 10, Day 20, and Day 30)
2. Normalised peak intensity $\frac{M0}{\sum M}$ to give the molar fraction of the monoisotopic peak at each time point
3. Use semi-log plot to calculate the slope (i.e. gradient m = turnover rate, **k**) for the change in the molar fraction of the monoisotopic peak

$$y = c + mx \text{----------} \textbf{\textit{Equation 4}}$$

Where

y	is lnFM0 or log of the molar fraction of the monoisotopic peak at any time point (presented on y-axis)
c	$\ln F_{max}$ or log of the molar fraction of the monoisotopic peak at day 0 (i.e. without D_2O labelling)
m	constant protein turnover rate (**k**) by exponential decay model regarding MIDA (slope of semi-log model)
x	is time point of study at day 0, 10, 20, and 30 (presented on x-axis)

Such that $$-k_t = ln\frac{FM0_{max}}{FM0_t} \text{----------} \textbf{\textit{Equation 5}}$$

4. Extract peptide sequence information from MASCOT report to determine C, H, N, O number
5. Calculate the probability of labelling by precursor (**D**) by determining exchangeable sites which is depending on relative specific activity of each amino acid as described in Commerford et al (Commerford, Carsten and Cronkite, 1983).
6. Divide k by D to reveal **observed *k***, which is specific to each peptide regarding its amino acid residue which contain different CHNO number.
7. Other parameter that have to be determined is precursor enrichment (***p***). This is measured by GC-MS
8. Finally, FSR is the result of **observed *k*** divided by ***p***. the result present in %synthesis per day.

Results

Example results were obtained for peptides that were highly abundant and well resolved in fast-twitch muscle (*plantaris*). There were 4 proteins (MYG; Myoglobin, ALDOA; Fructose bisphosphate aldolase A, KCRM; Creatine kinase, and ENOB; B-enolase) that had a high MOWSE score and provided enough information to input in to the model. These selected proteins were shown in **Table 7.**

Table 7 Four selected proteins from PLR and information

Protein (Swiss Prot name/Uniprot accession No.)	Mass	MOWSE score	Matched	peptides
Myoglobin (MYG_RAT/Q9QZ76)	17203	870	17	2
Fructose-bisphosphate aldolase A (ALDOA_RAT/P05065)	39783	307	16	3
Creatine kinase M-type (KCRM_RAT/P00564)	43246	213	21	4
B-Enolase (ENOB_RAT/P15429)	47326	126	10	2

Four proteins **MYG_RAT** (Q9QZ76; Myoglobin), **ALDOA_RAT** (P05065; Fructose-bisphosphate aldolase A), **KCRM_RAT** (P00564; Creatine kinase, M-type), and **ENOB_RAT** (P15429; β-enolase) were presented for practising calculation. The proteins were considered by high MOWSE score and manifested a multiple matched by other proteins in the database. The unique peptides are the peptides that specific to each protein. Protein mass were also shown in table. Protein Mass were range from 47,326 kDa (ENOB_RT) to 17,203 kDa (MYG_RAT) whereas MOWSE score range from 870 (MYG_RAT) to 126 (ENOB).

These 4 proteins (MYG, ALDOA, KCRM, and ENOB) provided completed information across 4 time points of deuterium administration, i.e, day 0, day 10, day 20, and day 30. Among these, average MYG_RAT synthesis rate (% per day) illustrated the highest rate (2.52% per day) followed by KCRM_RAT (1.04% per day), ENOB_RAT (0.73% per day), and ALDOA_RAT (0.56% per day) were presented in **Table 8.**

Table 8 Summary of MIDA calculation.

Protein	Peptide	m/z	different number of labelling site (*n*)	%precursor pool enrichment of D_2O (*p*)	FSR (%/day)	Average protein synthesis (%/day)
MYG	YSGDFGADAQGAMSK	752	23.20	3.43	2.30	2.52
	YSGDFGADAQGAMSK	760	23.20	3.43	2.74	
ALDOA	GILAADESTGSIAK	666	24.98	3.43	0.84	0.56
	QLLLTADDR	522	24.98	3.43	0.39	
	LQSIGTENTEENR	745	26.61	3.43	0.44	
KCRM	VLTPDLYNK	456	9.29	3.43	1.22	1.04
	LMVEMEK	456	10.72	3.43	1.54	
	FEEILTR	454	13.45	3.43	0.66	
	GQSIDDMIPAQK	659	25.71	3.43	0.75	
ENOB	GNPTVEVDLHTAK	691	15.26	3.43	0.41	0.73
	TAIQAAGYPDK	568	20.02	3.43	1.06	

Essential parameters for MIDA calculation were presented in regard to Peptide, protein mass/charge, different number of labelling site (*n*), percentage of precursor pool enrichment of deuterium oxide (*p*), Newly Fractional synthesised (FSR), and the average FSR.

Discussion

Muscle proteins analysed in this study had synthesis rates that seem to correlate inversely with fast-twitch muscle function. That is, fast-twitch muscle has a low capacity of oxidative metabolism but a high contraction speed for rapid movement. **MYG** is involved in intracellular oxygen transport and had a higher rate of synthesis (2.52%/day) than the glycolytic enzymes (**ALDOA** and **ENOB**) or the high-energy phosphate enzyme **KCRM**. Our data on **MYG** is similar to the synthesis rate (2.03%/day at day 10 of D_2O corporation) measured from rat's triceps muscle (Shankaran *et al.*, 2015) but inconsistent against FSR measurement at 28 days of D_2O corporation (0.39%/day) (Shankaran *et al.*, 2015). **KCRM** which is responsible for re-synthesising ATP by oxidising phosphoryl group from phosphocreatine (PCr) to ADP had a synthesis rate of 1.04%/day in *plantaris* muscle, which is greater than the previously reported value in triceps muscle (0.36%/day) (Shankaran *et al.*, 2015), quadriceps muscle (0.30%/day) (Jaleel *et al.*, 2008), EDL (~0.36%/day) (Hesketh et al., 2016), and SOL (0.71%/day) (Hesketh et al., 2016). In glycolysis metabolism, **ENOB** (0.73%/day) and **ALDOA** (0.56%/day) found similar FSR to quadriceps (0.77%/day) (Jaleel *et al.*, 2008) and triceps (0.48%/day) (Shankaran *et al.*, 2015), respectively.

The present study outlines higher FSR magnitude than have been reported previously (Jaleel *et al.*, 2008; Hesketh *et al.*, 2016; Shankaran *et al.*, 2016) and showed in **Table 9**. Although **KCRM** and **ENOB** turnover rate are similar after both 14 and 28 days of deuterium incorporation (Shankaran *et al.*, 2016), our finding (30 days of D_2O consumption) discovers FSR of **KCRM** (1.04%/day) is higher than that manifests in **ENOB** (0.73%/day). This finding contrasts the previous study in 14 days experiment (Hesketh *et al.*, 2016). Early studies in rat's FSR were conducted in different muscle such as EDL(Hesketh *et al.*, 2016), quadriceps(Jaleel *et al.*, 2008), and triceps(Shankaran *et al.*, 2016). Instead, our study performed in single muscle (PLR) which revealed fast muscle fibre phenotype whereas quadriceps muscle in Jaleel et al (2008) study (Jaleel *et al.*, 2008) was mixed muscle type and showed regions of predominately slow and fast fibres. Therefore, **KCRM** in our study presented higher FSR than those reported in quadriceps but ENOB was similar to FSR in quadriceps muscle. PLR is predominated fast-twitch muscle fibre type and has unique ability to switch to slow muscle fibre type after functional loading (Caiozzo *et al.*, 2000; Parsons *et al.*, 2004) whereas EDL is dominated fast-twitch fibre only which was manifested lower FSR (**KCRM** and **ENOB**) than that was accounted in SOL (slow-twitch fibre)(Hesketh *et al.*, 2016). Both EDL and SOL are widely used research models because of their distinctive fibre types, that is, fast- and slow-twitch, respectively. Our finding highlights that differences in muscle fibre type can give rise to differences in protein turnover rate. Therefore, it is possible that changes in muscle phenotype due to lifestyle factors such as exercise training could also result in changes in protein synthesis rates.

Table 9 Four proteins from the current trial compare to previous investigations.

Protein	**Present study**	**Hesketh et al 2016** (Hesketh *et al.*, 2016)	**Shankaran et al 2015** (Shankaran *et al.*, 2015)	**Jaleel et al., 2008** (Jaleel *et al.*, 2008)
	D_2O LC-MS/MS PLR % synthesis per day	**D_2O 2D-DIGE&MALDI-TOF EDL/SOL % synthesis Per day**	**D_2O LC-MS triceps %synthesis Per day**	**[ring-$^{13}C_6$]phenylalanine LC&GC MS Hind limb quadriceps FSR+-SEM (%/h)**
MYG	2.52	NA	2.03/0.39	NA
ALDOA	0.56	NA	1.93/0.48	NA
KCRM	1.04	0.36*/0.71*	1.30/0.36	0.30 ± 0.07
ENOB	0.73	0.57*/1.29*	1.37/0.38	0.77 ± 0.14

*digits were estimated from figure in the study which provide %synthesis after 14 days (the maximum duration in the experiment).

The study by Hesketh et al (Hesketh *et al.*, 2016) reported FSR after 14 days of deuterium labelling and used MALDI-TOF mass spectrometry, which may be different to LC-MS/MS analysis by quadrupole

instrument (Kasumov *et al.*, 2013). Although the samples were labelled by D_2O, the calculation method was different from the current calculation. Hesketh et al 2016 relied on binomial model following Xiao et al (Xiao *et al.*, 2008) while our study performed by exponential model following Lam et al (Lam *et al.*, 2014)and Camera et al (Camera *et al.*, 2017). The binomial model analysises isotope changes after a fixed time period of incorporation (synthesis) whereas exponential model uses a time series across the period of investigation. Although D_2O incorporation in to peptide mass isotopomers occurs according to a multinomial distribution pattern (Hellerstein and Neese, 1999), the exponential model is a better fit for a time series determination (at least 4 time points). In addition, our previous experiment was limited to 14 days of experiment which did not reach a plateau for any of the protein synthesis and resulted incomplete protein renewal data. Our current experiment is of longer duration (30 days of D_2O investigation) and reveals evidence that was more robust and was able to calculate average FSR which covered the rise-to-plateau period of the standard curve. In our experiment, MPE body water was measured from plasma which was less prone to error than the method used in Hesketh et al (Hesketh *et al.*, 2016) which determined MPE from muscle peptides only. Measurement of precursor enrichment from muscle peptides requires higher levels of label incorporation than are necessary for the determination of protein synthesis rate (***k***). Therefore, the current method MPE in plasma by GC-MS which gives more accurate data. For that reason, our experiment contains high accuracy and validity procedure which is possibly contribute to further work in more complexity species such as human.

Limitation

The determination of fractional synthesis rates was performed in rat *Plantaris* tissue. We selected 4 proteins which revealed high MOWSE score, i.e. confident identification. The highest MOWSE score was associated with the lowest protein mass whereas the lowest MOWSE score was the largest protein mass. This is because a greater proportion (i.e. percentage of the entire sequence) of the low-mass protein was analysed. The skeletal muscle structure of rodents is similar to human but our work used locomotor leg muscles. Human (*Homo sapiens sapiens*) are Bipedal whereas Rodent (*Rattus sp.*) are Quadrupedal. Therefore, the skeletal structures and posture are different, and this results in different muscle fibre proportions. We have reported deuterium incorporation in rat, which is a simplified version of the method that will be used in humans. The protein kinetic method to measure in synthesis in human is more complicated because it must also incorporate the non-linear rise in precursor enrichment.

Conclusion

This chapter has successfully used fast-twitch *plantaris* muscle from rats administered deuterium oxide in vivo to establish the techniques and calculations for measuring the synthesis rate of individual proteins. *Plantaris* is predominated fast-twitch muscle and the synthesis of ALDOA was similar to triceps muscle. However, *Plantaris* would likely also have slow-twitch twitch fibres and some proteins (ENOB) had similar FSR to mix muscle types such as quadriceps. The synthesis rate of ENOB was between previously published values for fast- (EDL) and slow-twitch fibre (SOL). Interestingly, central cellular energy protein (Creatine kinase, KCRM) of *Plantaris* had a higher FSR compared to all other muscle types that have been reported, i.e. mixed-muscle (quadriceps), fast-twitch fibre (EDL and triceps), and slow-twitch fibre (SOL). When combined, Section 1 and Section 2 of this chapter provide the complete analysis technique for studying both the abundance and synthesis rate of individual proteins in human muscle.

Chapter IV

Cross-sectional analysis Of Obese vs Trained

Contents

Summary

There are clear differences in the functional properties of muscle of lean healthy individuals compared to obese sedentary persons that are at a heightened risk of T2DM. However, the current proteomics literature (reviewed in Chapter II) only considers differences in protein abundance as a mechanism for the differences in muscle function. There is evidence to suggest that the quality of muscle proteins, i.e. their turnover rate, may also contribute to muscle function and this Chapter aims to establish whether differences exist in the synthesis rate as well as abundance of individual proteins in the muscle of lean active individuals versus obese sedentary persons.

The study recruited 8 individuals that were either obese or sedentary (OB; n=4) or of normal body weight and actively involved in endurance exercise training (TR; n=4). The physiological characteristics of the participants was recorded and muscle was collected at 4 time points (0, 4, 9, and 14 day) during a period of heavy water consumption. Proteomic and bioinformatic analyses of the muscle samples were consistent with the methods developed in Chapter III.

In total 231 proteins were identified and the abundance of 43 proteins was significantly ($p < 0.05$) different between OB and TR groups. Synthesis data was collected for 155 proteins and the fractional synthesis rate (FSR) of 52 proteins was notably different of OB vs TR. Of these, we report 8 proteins that meet statistical criteria for both differences in abundance and FSR. These are categorised into 3 groups i.e. increased both abundance and FSR in OB compared to TR muscle (**ALDOA**; Fructose-bisphosphate aldolase A), increased abundance but decreased FSR (**CAH3**; Carbonic anhydrase 3, **HSPB1**; Heat shock protein β-1, **G3P**; Glyceraldehyde 3 phosphate dehydrogenase, **AT2A1**; Sarcoplasmic/endoplasmic reticulum calcium ATPase 1, **KCRM**; Creatine kinase, muscle type, and **MYH1**; Myosin-1), an decreased abundance but increased FSR (**ACTN2**; α actinin-2).

This study is has generated highly novel data that is unique in the current literature. We report protein specific differences in the synthesis rate and abundance of proteins do exist between the muscle of TR and OB humans. Our finding that some proteins that are more abundant in OB muscle also have a lower rate of synthesis suggest that differences in protein degradation or protein quality control are associated with differences in muscle function and metabolism.

Introduction

Skeletal muscle plays an important role in whole body protein metabolism and is an insulin sensitive tissue (Jensen *et al.*, 2011). Many chronic and metabolic diseases have a large effect on muscle and, for example, reduce muscle mass, diminish muscle strength and interfere with the metabolic function of muscle (Wolfe, 2006). Many factors are involved with these adverse effects such as unhealthy dietary habits and a sedentarily lifestyle which are each associated with the increased prevalence of obesity in the general population. Moreover, excess dietary intake together with physical inactivity are causes of energy imbalance. Loss of muscle mass also becomes a problem and may be associated with effects on muscle protein turnover, for example, lower rates of protein turnover indicates a lesser rate of replacement of old protein by new protein which has a detrimental effect on muscle function and metabolism. In the resting state, the synthesis of protein and breakdown of protein are responsible for a major component of muscle the energy expenditure. Muscle protein turnover has most commonly been investigated using stable isotope-labelled amino acids that are delivered by an intravenous infusion. Muscle samples are then collected and analysed to measure the amount of stable isotope-labelled amino acid, and this gives a measure of the relative amount of new protein that has been synthesised during that period, i.e. fractional synthesis rate (FSR) (Kim *et al.*, 2016). This technique is able to measure the average rate of synthesis across mixed muscle proteins and it has commonly been used to investigate muscle protein synthetic responses to hormones, feeding or resistance exercise. In healthy adults in the post-absorptive resting state, the average turnover of muscle proteins is estimated to be approximately 1.8 %/day (Tipton *et al.*, 2003). The energy requirements for this level of protein turnover are largely provided by oxidation of fat to provide ATP (Rasmussen and Wolfe, 1999). Currently there is no clear indication that muscle protein turnover is substantially different in obese people but this is in part due to a lack of literature investigating this issue.

Obesity is a state which is characterized by a disproportionately increased fat mass but may also be associated with greater muscle mass particularly in postural or locomotor muscles (Hibbert, Broemeling and Isenberg, 1994). However, this does not indicate increase energy expenditure, which may indicate protein turnover is lower in obese individuals. Increased body fat is associated with elevated free fatty acid levels in plasma which alter muscle substrate utilization and also has an effect on muscle insulin sensitivity (Wolfe, 2006). This is accompanied by the observed elevations in intramuscular triglyceride (IMTG) content in muscle of obese people (He, Watkins and Kelley, 2001). The accumulation of IMTG in muscle promotes dysregulation of lipid metabolism in muscle and resulted in insulin resistance via the action of lipid intermediates (Wolfe, 2006). The effects of obesity on skeletal muscle protein turnover have not been widely investigated and the majority of literature has focused on whole body amino acid and protein metabolism (Katsanos and Mandarino, 2011). In addition to its effects on glucose metabolism, the stimulation of muscle by insulin also influences muscle protein turnover, in particular

insulin inhibits protein degradation at fasting or post-absorptive levels. Currently it is not clear whether this mechanism is affected by obesity, high levels of plasma free fatty acids or muscle insulin resistance. Muscle protein turnover is highly responsive to protein feeding or amino acid delivery, particularly of essential amino acids. Some studies report that obesity is not associated with differences in the basal level of protein turnover between healthy-weight or obese individuals, but the response of muscle protein synthesis to essential amino acids is significantly blunted in obese persons (Murton *et al.*, 2015). However, other studies that have investigated protein turnover in specific subcellular fractions of human muscle report an overall reduced turnover rate of protein in muscle from obese individuals in the basal state, and when stimulated with amino acid, the mitochondrial fraction of obese muscle exhibits a lesser response compared to health-weight control (Guillet *et al.*, 2009).

A shortcoming of the literature on muscle protein turnover is that it does not consider protein-specific differences. The techniques that have been used, i.e. amino acid tracers and GC-MS analysis of muscle hydrolysates, are not capable of investigating the abundance or turnover rate of individual proteins in human muscle. The introduction of proteomic techniques including liquid chromatography-tandem mass spectrometry (LC-MS/MS) described in Chapter III, has meant that it is now possible to investigate large numbers of proteins in human muscle. However, so far, this literature only reports differences in protein abundance, for example between healthy and obese/ T2DM individuals. In our synthesis of evidence from the current literature (Chapter II) we used systematic review and meta-analysis to show that the muscle of obese or T2DM individuals has a greater proportion of fast-twitch fibres that express myosin heavy chain type IIx (MYH1) and IIa (MYH2). We also found that chronic inflammation in muscle is likely to be associated with insulin resistance and TENA/TLR4 (ECM protein remodeling) is involved in this response. These findings from the literature on human muscle are consistent with more mechanistic studies that have been performed in laboratory animal models.

A High fat high sugar diet (HFHD) negatively effects skeletal muscle and is associated with chronic inflammation in muscle. The main focus of HFHD studies has been on extracellular matrix proteins (ECM). ECM proteins, especially tenascin, hyarulonic acid, and fibronectin, are responsive to muscle injury and change in abundance after cellular inflammation (Calve, Odelberg and Simon, 2010). Particularly, Tenascin-C (TENA) is responsive to contractile activity and mechanical stress (Flück *et al.*, 2000) in skeletal muscle and is a substrate of matrix metalloproteinase-7 (MMP-7) associated with infarction of the left-ventricle (Chiao *et al.*, 2010). In addition, TENA is activated by Toll-like receptor 4 (TLR-4) as well as endogenous protein associated with a proinflamatory state (Midwood *et al.*, 2009; Catalán *et al.*, 2012). Our systematic review (Chapter II) found TENA exhibited the greatest increase in abundance in T2DM myotube cell (Thingholm *et al.*, 2011) and is associated with the muscle ECM and in particular is localised to the neuromuscular junction. Skeletal muscle TENA abundance is associated with obesity and may be involved in a 'dynamic tissue crosstalk' mechanism in diet-induced obesity

(Samdani *et al.*, 2015). According to HFHSD model, TENA is a ligand of the integrin receptor and interaction of these proteins leads to GLUT4 impairment and contributes to muscle insulin resistance (Howard *et al.*, 2012). Furthermore, TENA found more abundance in regard to patient with diabetic and venous ulcer (Loots *et al.*, 1998). Interestingly, this protein is not found in healthy people (Midwood *et al.*, 2009, 2011). Exercise study found TENA increases in abundance after eccentric exercise (Chen *et al.*, 2003; Raastad *et al.*, 2010). This reported up to 11.2 fold in comparison with control group (Chen *et al.*, 2003).

Based on the evidence generated by systematic review and meta-analysis, this Chapter aims to investigate proteome-wide differences in muscle protein turnover of TR and OB individuals by cross-sectional experimental design. There are multiple factors that can influence and have an effect on protein abundance. However, few studies have investigated protein turnover rate which would likely be the underlying mechanism of changes in protein abundance. The systematic review highlighted hypotheses regarding muscle inflammation and extracellular matrix proteins, e.g. TENA, which probably indicate broader changes across multiple proteins that will be further investigated here using a proteomic approach. This Chapter focuses on risk factors which relate to human lifestyles such diet and physical inactivity which result in insulin resistance, glucose intolerance, and may affect muscle protein changes in (i) abundance and (ii) FSR of each protein. Prior to embarking on these highly advanced research methods, I have undertaken training in fundamental proteomics skills including muscle processing, label-free quantitative profiling using LC-MS data (Chapter III), and protein turnover rate calculation using MALDI-TOF (Hesketh *et al.*, 2016) and LC-MS/MS data (rat with deuterium oxide labelling). This Chapter extends from this groundwork and uses the model illustrated in rat muscle (Chapter III) to investigate differences in the synthesis rate and abundance of individual proteins in the muscle of healthy-weight active individuals versus obese sedentary individuals.

Methods

Ethical approval

Four lean, trained (TR) and 4 obese, sedentary (OB) male participants were recruited into the study (for characteristics, see Table 15), which was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and conformed with the Declaration of Helsinki. Written, informed consent was obtained from volunteers following a verbal and written explanation of the nature and risks involved in the experimental procedure. The OB group were engaged in less than two 30 min sessions of physical activity per week in the preceding year whereas the TR group undertook at least 3 sessions of structured endurance-based exercise for > 60 minutes per week for at least 1 year (as assessed using the Paffenbarger physical activity questionnaire).

Participant recruitments

After the protocol was approved by NHS Research Committee, the advertisement was disseminated throughout multiple avenues, mainly via poster and email. The potential participants were initially screened by questionnaire and preliminarily health check that included blood pressure, weight, height, and BMI. All participants were recruited initially under age-matched criterion. Therefore, our study obtained closely age-matched participants which gave a low standard deviation. Our inclusion criteria consisted of control group (TR) and an obese/overweight sedentary group (OB). The TR group met criteria of healthy-male which engaged in aerobic exercise training more than 3 sessions of 60 minutes per week for at least 2 years and BMI is less than 25 $kg.m^{-2}$. An OB group consists of otherwise healthy males that engaged in less than 2 sessions of 30 min per week for at least 1 year and BMI was over 28 $kg.m^{-2}$. All participants were age between 30 – 45 y. Participants were given information sheet and signed consent form prior began the study.

Pre-experimental procedures

Prior to entering the study, subjects attended the laboratory and performed a progressive exercise test to exhaustion on an electronically braked cycle ergometer (Lode BV, Groningen, The Netherlands) in order to determine maximum aerobic power (W_{max}) and peak oxygen uptake (VO_{2peak}) using an on-line gas collection system (Moxus metabolic cart, AEI Technologies, Pittsburgh, Pennsylvania, USA). The test consisted of initially cycling at 95W, followed by sequential increments of 35W every 3 min until cadence was reduced to <50 rpm, at which point the test was terminated. The highest value obtained in the last 30 s of the test was taken as VO_{2peak}. Eligibility for the OB group was confirmed if subjects had a VO_{2peak} <35 $ml.min^{-1}.kg^{-1}$, and for the TR group if VO_{2peak} >45 $ml.min^{-1}.kg^{-1}$. Body composition was also analysed

at this visit using dual-energy X-ray absorptiometry (DEXA). QDR software (Hologic Inc., MA, USA) was used to determine fat mass and fat-free mass on a region-specific basis. Relative fat mass was calculated as absolute fat mass as a proportion of total region mass.

On a separate occasion, subjects attended the laboratory after an overnight fast (>10 h), having refrained from vigorous exercise in the preceding 48 h period, and underwent an oral glucose tolerance test (OGTT) to determine insulin sensitivity. A 20G cannula was inserted into the antecubital vein of one arm, and a 3-way stopcock attached to permit multiple blood sampling and cannula flushing. A resting blood sample (10 ml) was taken immediately before subjects consumed a 25% glucose beverage (75g glucose in 250 ml water). Following this, further blood samples were collected at 15, 30, 45, 60, 90, and 120 minutes. Isotonic saline (B Braun Ltd., UK) was used to keep the cannula patent throughout the test. Blood samples were collected into serum separator and EDTA-containing vacutainers. Serum and plasma samples were obtained through centrifugation at 1,000 g for 10 min at 4°C and stored at -80^{0}C for subsequent analysis. Plasma glucose concentrations were determined spectrophotometrically using a semi-automatic analyser in combination with a glucose oxidase kit (Randox Laboratories, Antrim, UK). Insulin concentrations were determined using a commercially available direct insulin enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fisher Scientific, UK). Insulin sensitivity(Matsuda and DeFronzo, 1999; DeFronzo and Matsuda, 2010) was calculated based on plasma glucose and insulin concentrations using the following equation:

$$Matsuda\ (ISI) = \frac{10000}{\sqrt{(FPG\ x\ FPI)\ (mean\ OGTT\ insulin)(mean\ OGTT\ glucose)}} Equation\ 6$$

Where FPG is Fasting Plasma glucose and FPI is Fasting plasma insulin

Experimental protocol

For the main experimental protocol subjects were required to consume deuterium for 14 days. Throughout this period saliva (every day) and blood samples (every 2nd day) were collected, and muscle biopsies were obtained at baseline (day 0), and after 4, 9, and 14 days of deuterium consumption. Moreover, daily energy and macronutrient intake and physical activity was monitored throughout the experimental protocol. All procedures are detailed below.

Muscle biopsy procedures

On arrival at the laboratory after an overnight fast (>10 h), the thigh of one leg was prepared for muscle biopsy collection. Briefly, an incision was made in the vastus lateralis after local anaesthetic (0.5%

marcaine) was administered under the skin and over the fascia and a muscle biopsy (~100 mg) was taken using the conchotome technique. Skeletal muscle samples were first blotted to remove excess blood, and visible fat and collagen were removed through dissection. Muscle tissue (~40 mg) was snap frozen in liquid nitrogen and stored at -80^{0}C for subsequent analysis. In total, subjects received two muscle biopsies from each leg in a randomised order over the 14-day experimental period.

Deuterium consumption

Deuterium oxide ($^{2}H_2O$ or D_2O) labelling of newly synthesised proteins was achieved by oral consumption of D_2O (Sigma-Aldrich, UK) for 14 days. Deuterium (^{2}H) is the stable isotope of protium (^{1}H) and has a natural abundance of 0.0156 %. D_2O is a non-hazardous clear liquid that does not emit harmful ionising radiation and is not classified as dangerous according to Directive 67/548/EEC. No long-term adverse effects of deuterium have been reported in humans in the substantial body of clinical research literature. However, acute side effects including nausea and vertigo may occur when deuterium is given to humans as large (i.e. >150 ml) boluses(Jones and Leatherdale, 1991). Therefore, in the current study subjects consumed D_2O in 50 ml boluses four times each day (totalling 200 ml per day) approximately 3-4 hours apart. This administration protocol has been used previously without causing side effects(Peng, Ho and Taylor, 1972; Klein and Klein, 1986; Jones and Leatherdale, 1991; Busch *et al.*, 2006) and will raise the enrichment of deuterium in body water to ~2.5 %. This enrichment is an order of magnitude below the concentration (i.e. >25 % enrichment) required to cause isotope effects in biological systems that could change the conformation or stability of biopolymers and, for example, impair enzymatic processes. The target level of enrichment is also far below the lethal dose range of 30-40 % observed in mice, rats and dogs(Jones and Leatherdale, 1991).

Blood and saliva collection and analysis

Saliva samples were collected into a cryotube (Fisher Scientific, UK) using passive drool for a period of 60 s prior to the first drink each day. Samples were stored at -80^{o}C for later analysis. Venous blood samples were collected every 2nd day during the experimental period from an antecubital vein. Blood samples were collected into serum separator vacutainers, centrifuged at 1,000 g for 10 min at 4°C and stored at -80^{0}C for subsequent analysis i.e. precursor enrichment measurement.

Diet and physical activity

Habitual physical activity was monitored throughout the 14-day experimental protocol using a triaxial accelerometer (GT3X+, ActiGraph, LLC) worn around the hip. Minimum wear time was defined as ≥9 hours of registered time. Raw data were exported via ActiLife software (version 6.13.3) and the

proportion of the day spent in sedentary, light, and moderate-to-vigorous physical activity was calculated. Dietary intake was recorded through MyFitnessPal for at least 7 days during the experimental protocol. Average daily energy and macronutrient intake was calculated using Nutritics software (Nutritics Ltd., Dublin, Ireland).

Precursor pool enrichment measurement

Blood and saliva samples were measured by GC-MS by interpolation from D_2O standards (McCabe *et al.*, 2006) and previously reported in Camera (Camera *et al.*, 2017).

Human muscle protein turnover rate analysis

Proteomic analysis and FSR procedure were conducted following method, which has been described in Chapter III and previously reported in Camera 2017. The concept is to combine both human protein profiling and applied FSR which was trial in rat model. Briefly, sample preparation, protein assay, and tryptic in-solution digestion were performed following human protein profile method as described in Chapter III-part A followed by LC-MS/MS. Finally, protein synthesis calculation was done by MIDA concept as described in Chapter III-part B.

Statistical analysis

For health and characteristic data*, Student independent t-test* has been applied for NHST between TR and OB group by using SPSS version 23 (IBM). Insulin and glucose data were calculated by number of areas under the curve, which was plotted by each time point (0, 30, 60, 90, and 120 min). For protein abundance change, ANOVA has been applied to analysis regarding non-parametric assumption (*F* distribution) and sample size is low which lead the data contain bias. Therefore, we applied Protein abundance change presented in the log-scale graph and cut of at $p < 0.05$. The FDR or *q*-value were also present in the **Table 11 and 12.** FDR has been applied to test false positive among rejected hypothesis result. Regarding to the low value of protein abundance, log transformation has been applied to present direction of protein abundance change **(Figure 13 and Figure 14).**

Results

Physical and health data measurement

Data pertaining to body composition, insulin sensitivity, exercise capacity, physical activity and habitual dietary intake in TR and OB individuals is presented in **Table 10.** By design, OB individuals had a higher BMI and lower aerobic fitness compared to the TR group ($p_{BMI} < 0.05$, $p_{VO2max} < 0.01$). Although glucose and insulin area under the curve (AUC) was not different between groups, TR individuals displayed greater insulin sensitivity (Matsuda insulin sensitivity index) compared to OB ($p < 0.01$). Abdominal fat and lean mass in comparison of TR and OB were measured a. visceral adipose tissue (VAT), b. percentage of fat, c. fat mass, and d. Lean mass from TR (n=4) were lower than those that retrieved from OB (n=4). The pattern of VAT, percentage of fat, and fat mass presented was substantially higher in abdominal fat of OB than that found in TR. As a further matter, lean mass revealed slightly higher mass in OB than LE. All data from OB and LE showed significant differences ($p_{VAT} < 0.01$, $p_{\%Fat} < 0.05$, $p_{Fat\ mass} < 0.05$, and $p_{Lean\ mass} < 0.01$). Regarding physical activity, the number of participants were 3 for each group. OB (n=3) revealed slightly higher percent in sedentarily and light activity lifestyle than TR (n=3). Contrastingly, OB showed less walking step than TR. However, no statistically different was found between 2 groups. The table also disclosed daily dietary consumption throughout 2-week-experiment. OB (n=3) had higher calorie intake than TR (n=2) but they ingested slightly less (*NS*) fat than TR.

Table 10 physical and health data of TR and OB (p<0.05, ** p<0.01).*

	TR (n = 4)		OB (n = 4)		*p*
	Mean ± SD	**(min-max)**	**Mean ± SE**	**(min-max)**	
Physical characteristics					
Age (y) (min, max)	38 ± 7	(32-45)	37± 5	(33-45)	
Weight (kg)	75.6 ± 4.4	(70.65-82.45)	110.0 ± 18.2	(87.2-136.2)	0.04*
Height (cm)	177 ±0.50	(171.5-185)	179.7 ± 3.6	(176-183.5)	0.5
BMI (kg/m²)	24.2 ± 2.1*	(21.3-27.2)	34.0 ± 5.0	(28.15-40.45)	0.04*
V_{O2peak} (mL/min/kg)	45.5 ± 6.9**	(40.2-57.0)	26.2 ± 3.8	(20.5-30.8)	0.01**
Insulin sensitivity					
Glucose (AUC)	703.25 ± 81.5	(589.1-780.0)	979.25 ± 211.4	(359.0-1,215.0)	0.09
Insulin (AUC)	5422 ± 2627	(3,144.4-9,151.5)	13,426 ± 7162	(9,170.0-21,694.8)	0.05
Matsuda index	5.7 ± 1.4*	(4.3-7.3)	1.7 ± 0.6	(1.2-2.4)	0.02*
Body composition (excluded head)					
VAT (g)	319.3 ± 39.6**	(287.0-377.0)	766.25 ± 166.0	(574.0-973.0)	0.01**
%fat	18.5 ± 4.7*	(14.3, 24.3)	30.2 ± 7.5	(22.4-39.6)	0.04*
Fat mass (g)	13,353.5 ± 3891.5*	(9,584, 17,551.1)	32,932.1 ± 14,110.2	(18,721.3, 51,451.3)	0.05*
Lean mass (g)	55,881.3 ± 2988.3**	(52,470.3, 59,619.6)	70,319.4 ± 5,716.4	(62,692.8, 76,400.1)	0.01**
Physical activity					
Sedentary (%/day)	63.4 ± 3.0	(60.6-66.6)	64.9 ± 3.7	(61.2, 68.6)	0.63
Light activity (%/day)	28.2 ± 5.5	(21.9-32.1)	27.9 ± 4.4	(23.5, 32.3)	0.93
MVPA (%/day)	8.7 ± 3.4	(6.2-12.5)	7.3 ± 6.1	(1.2, 13.4)	0.75
Step counts (steps/day)	9,681.9 ± 2,789.3	(7,221.0-12,712.0)	6,471.9 ± 2,125.8	(4,401-8,649)	0.19
Dietary consumption					
Total calories intake (kcal/day)	1,756 ± 28.3	(1,736.1-1,776.2)	2,003 ± 814.8	(1,063.8-2,519.9)	0.06
Fat (%)	37.0±10.0	(29.8-43.9)	28.7±6.3	(22.7-35.4)	0.35
Carbohydrate (%)	42.1±5.1	(38.6-45.7)	44.0±8.3	(38.9-53.6)	0.19
Proteins (%)	14.9±1.0	(14.2-15.7)	18.6±1.3	(17.6-20.0)	0.1

The subjects have not performed any intervention/training. However, they consumed only D_2O 200 ml (50 ml x 4 aliquots x 14 days) and muscle samples were collected at baseline (day 0), day 4, day 9, and day 14. Therefore, this experiment covered 2 weeks of study.

Proteomic analysis

Human protein profile

Protein abundance profiling

Protein abundance profiles were analysed following the methodology that has been described in Chapter III section 1. Both myofibrillar and sarcoplasmic proteins were analysed and we identified 100 and 131 proteins, respectively **(Figure 13)** in these fractions and found 20 proteins were common to both fractions. The majority of proteins were myofibrillar/ muscle-contraction proteins or cytoskeleton proteins. Interestingly, the myofibrillar fraction also exhibited a cluster of proteins from the insulin secreted signalling pathway (**ITPR1;ITPR2;ITPR3;RL19** or Inositol 1,4,5-trisphosphate receptor type 1; Inositol 1,4,5-trisphosphate receptor type 2; Inositol 1,4,5-trisphosphate receptor type 3; 60S ribosomal protein L19) which were not found in the sarcoplasmic fraction. However, these proteins were not significantly different between OB and TR muscle. All proteins are listed by their main functional and biological process categories in **Appendix 5.**

We discovered 25 individual proteins from the soluble fraction, and 18 proteins from myofibrillar fraction which displayed significant differences in abundance between OB/TR ($p < 0.05$). Among these, three proteins were found in both fractions; **ENOB and ENOG** (Beta-enolase & Gamma-enolase), **FHL1** (Four and a half LIM domains protein 1), and **FLNC** (Filamin-C). All proteins analysed (n=52) are reported in **Table 11** and **Table 12**. In addition, all statistical test of all proteins had been presented **in Appendix 6 (sarcoplasm and myofibrillar fraction).**

Direction of abundance change in individual proteins is plotted in **Figure 13** (sarcoplasmic fraction) and **Figure 14** (myofibrillar fraction). The majority of proteins that were different between OB and TR groups were muscle specific proteins (muscle contraction and muscle development), cytoskeletal proteins or were involved in glucose metabolism.

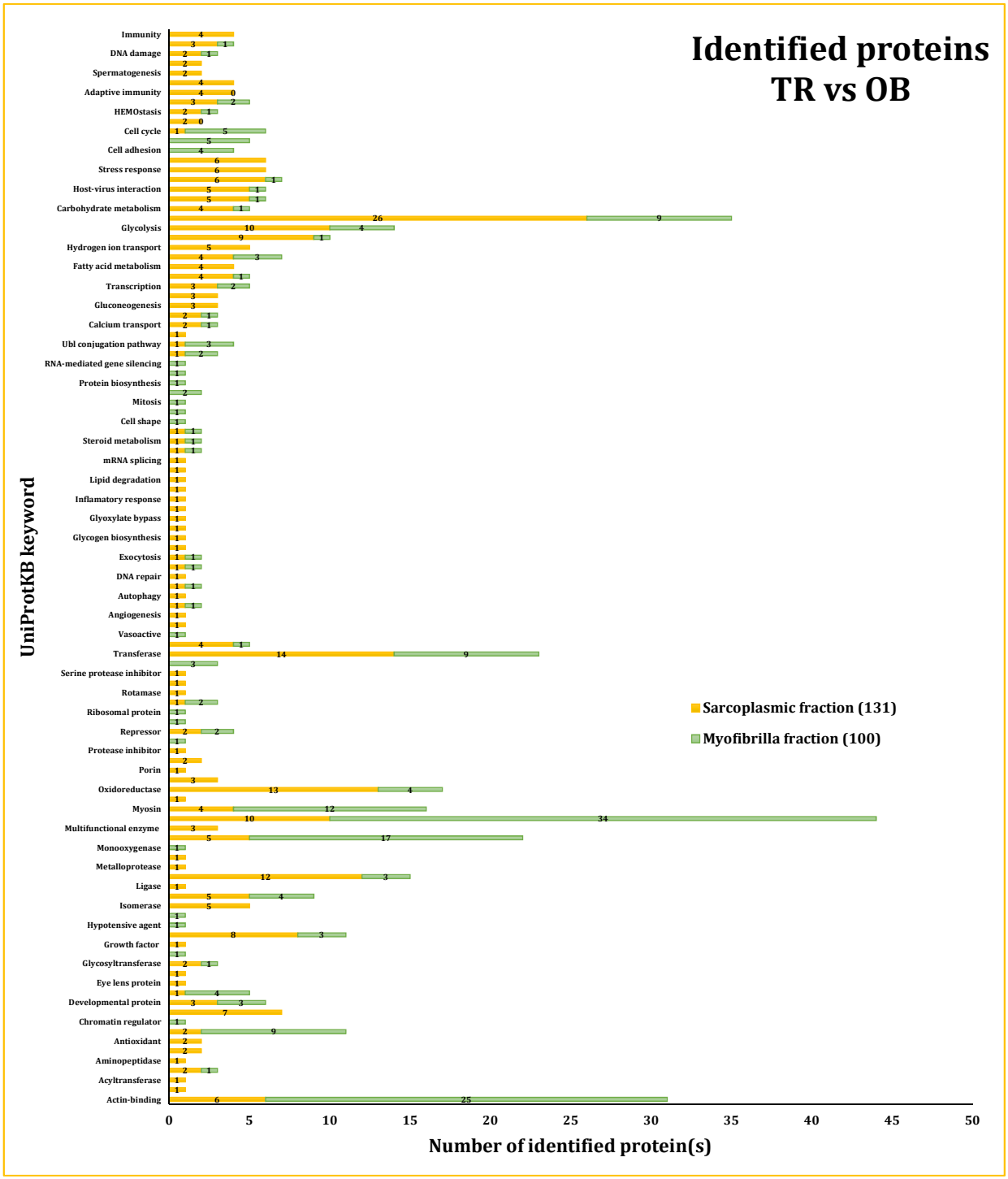


Figure 13 human muscle protein profile of TR and OB individual from different fraction; sarcoplasma and myofibrils.

Sarcoplasmic fraction was revealed Transport proteins (26/131), Transferase functional proteins (14/131), and Oxidoreductase functional proteins (13/131) were as the first 3 major groups among other proteins, respectively. However, Myofibrillar fractions was detected muscle proteins (34/100) was the most major group followed by actin-binding function (25/100) and Motor protein (17/100). Sarcoplasmic fraction (131 proteins) revealed a greater number of proteins than that were measured in myofibrillar fraction (100 proteins). Among this, 20 proteins were found in both fractions. All protein list by Molecular function and Biological process (UniProtKB) were given in **Appendix 5**

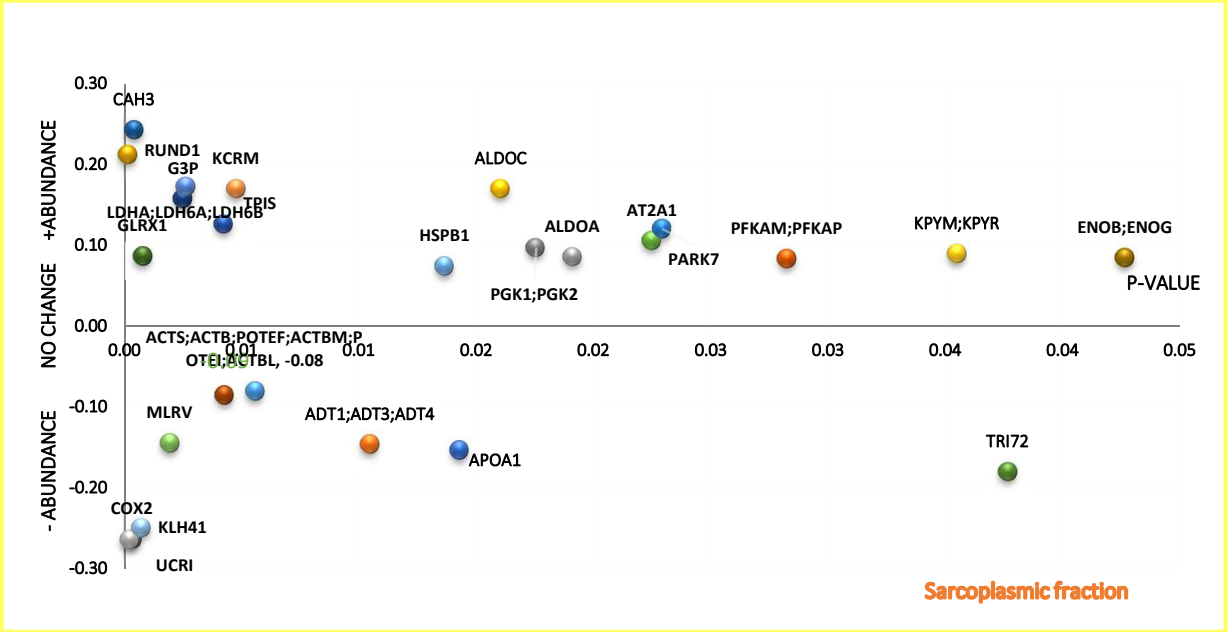


Figure 14 Log fold change of 25 significantly different protein abundance (p<0.05) from sarcoplasmic fraction.

Among these, 16 proteins showed up-regulation in OB meanwhile 9 proteins displayed down-regulation. Most abundance change proteins were function in glucose metabolism followed by myofibrillar proteins. All 7 glucose proteins were increased activity in OB (ALDOA, ALDOC, ENOB&ENOG, G3P, PFKAM&PFKAP, PGK1&PGK2, and TPIS). A cluster of actin protein (ACTS;ACTB;POTEF;ACTBM;POTEI;ACTBL) and cardiac-specific protein (MLRV) showed down-regulation in OB whereas other muscle proteins elevated its level (AT2A1, KCRM, and KPYM&KPYR).

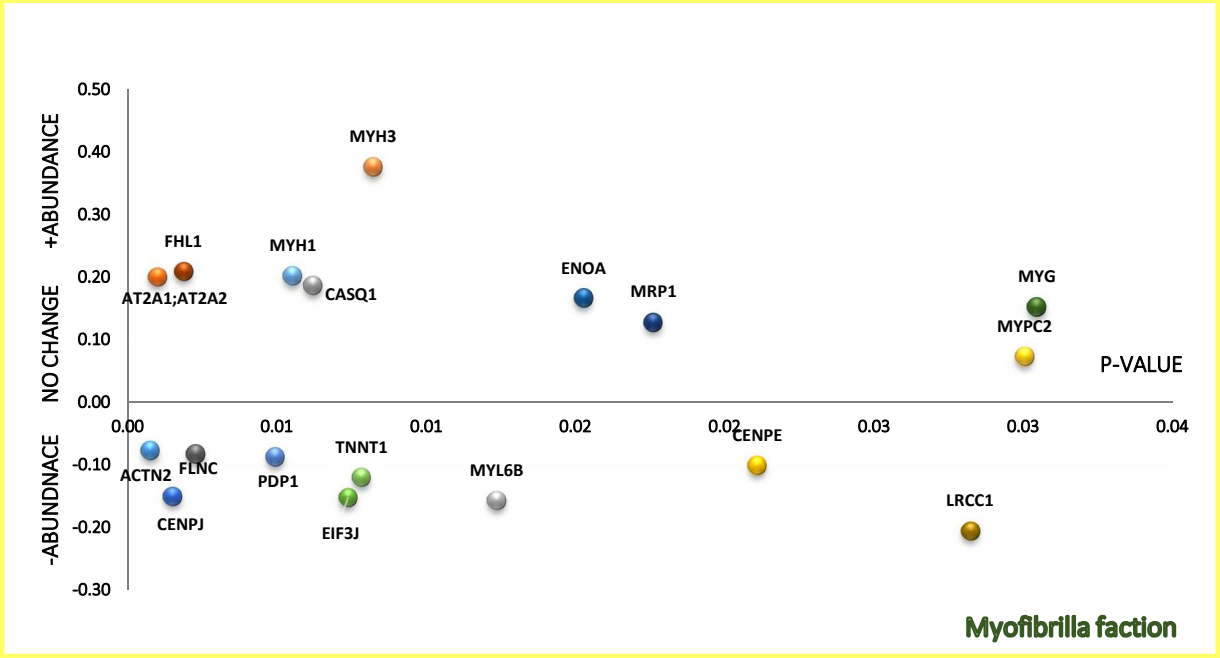


Figure 15 Log fold change of 18 significantly different protein abundance (p<0.05) from myofibrillar fraction.

Five myofibrillar protein were up-regulation (MYH1, MYH3, MYG, MYPC2 and AT2A1&AT2A2) but 2 (MYL6B and TNNT1) muscle specific proteins found decreased its level in OB. Structural proteins found both increased (CASQ1 and FHL1) and decreased abundance (EIF3J and FLNC).

Protein synthesis rate profiling

The fractional synthesis rate (FSR) of individual proteins was analysed using the methods described in Chapter III section 2. FSR data was calculated for a total of n=75 proteins in the sarcoplasmic fraction, of which n=22 were increased, n=34 decreased, and n=4 proteins exhibited no difference between groups. In addition, n=14 proteins could only be measured in either OB or TR group and therefore were not suitable for difference testing. In the myofibrillar fraction, a total of 40 proteins were analysed and the FSR of n= 14 was greater in OB group, whereas the FSR of N=17 proteins were less in the OB compared to TR group. Two proteins (***HBA****; α-haemoglobin and* ***A1AT***; *Alpha-1-antitrypsin*) were not different between groups and 7 proteins could be measured in only one of the groups and so were not submitted for difference testing. The protein-specific FSR data were then matched with the equivalent protein-specific abundance data and are reported in **Table 11** (8 proteins which showed statistically change in both abundance and FSR, **Table 12** reported 11 proteins were found statistically significance in abundance and provided FSR data. Forty-four proteins which reveal statistically alteration in FSR only were shown in **Appendix 7**.

Table 11 Eight (8) out of 231 proteins from either Sarcoplasmic or Myofibrillar fractions that show statistically significant in both protein abundance change and fractional synthesis rate (FSR)

	Protein accession	UniProtKB	abundance change		Newly fractional synthesis rate (FSR)			Fraction
			Fold change of OB/TR	*p*	OB FSR±SD (%/day)	TR FSR±SD (%/day)	*p*	
Increased abundance and FSR (+,+)								
1	**ALDOA*,****	Lyase/ RNA binding/Glycolysis/ protein homotetramerization	1.22	0.02	2.3±0.0	1.19 ±0.00	0.00	Sarcoplasm
Increased abundance but decreased FSR (+,-)								
2	**CAH3**,***	Lyase	1.75	0.00	0.73±0.0	1.62±0.00	0.01	Sarcoplasm
3	**HSPB1*,****	Chaperone/ identical protein binding/ protein folding chaperone/protein homodimerization activity/ protein kinase binding/ RNA binding/ Stress response/regulation of protein phosphorylation/ response to virus/ retina homeostasis	1.18	0.01	1.3±0.00	2.79±0.00	0.00	Sarcoplasm
4	**G3P**,****	Oxidoreductase/ Transferase/aspartic-type/ endopeptidase inhibitor activity/ microtubule binding/ Apoptosis/ Transferase/ Glycolysis/ Translation regulation/ antimicrobial humoral immune response mediated by antimicrobial peptide/ cellular response to interferon-gamma/ defense response to fungus/ killing by host of symbiont cells/ killing of cells of other organism/ microtubule cytoskeleton organization/ negative regulation of endopeptidase activity/ negative regulation of translation/ neuron apoptotic process/ peptidyl-cysteine S-trans-nitrosylation/ positive regulation by organism of apoptotic process in other organism involved in symbiotic interaction/ positive regulation of cytokine secretion/ protein stabilization/ regulation of macroautophagy	1.44	0.00	1.04±0.00	16.44±0.02	0.00	Sarcoplasm
5	**AT2A1*,****	Translocase/ ATPase activity/ ATP binding/calcium ion binding/ calcium-transporting ATPase activity/ Calcium transport/ Ion transport/ Transport/ calcium ion transport/ negative regulation of striated muscle contraction/ positive regulation of fast-twitch skeletal muscle fiber contraction/ regulation of striated muscle contraction	1.27	0.02	1.43±0.00	12.96±0.04	0.00	Sarcoplasm
6	**KCRM**,****	Kinase/ Transferase/ ATP binding	1.48	0.00	0.86±0.00	0.93±0.00	0.00	Sarcoplasm
7	**MYH1*,****	Actin binding/ Calmodulin binding/ Motor protein/ Muscle protein/ Myosin	1.59	0.01	0.83±0.00	50.75±0.04	0.00	Myofibrils
Decreased abundance but increased FSR (-,+)								
8	**ACTN2**,****	Actin binding/cytoskeletal protein binding/ FATZ binding/ integrin binding/ion channel binding/phosphatidylinositol-4,5-bisphosphate binding/ protein dimerization activity/ protein domain specific binding/protein homodimerization activity/ titin Z domain binding/cell adhesion/focal adhesion assembly/microspike assembly/phospholipase C-activating angiotensin-activated signaling pathway/positive regulation of cation channel activity/positive regulation of endocytic recycling/protein homotetramerization/protein localization to plasma membrane/regulation of apoptotic process/sarcomere organization	0.84	0.0	0.94±0.00	0.88±0.00	0.00	Myofibrils

Among these 8 notably proteins, only **ALDOA** (Fructose-bisphosphate aldolase A) found increased in both abundance and FSR whereas only **ACTN2** (A-actinin-2) found decreased in both abundance and FSR. Majority of proteins were found increased in abundance but reduced FSR in OB. These proteins are **CAH3** (Carbonic anhydrase 3), and **HSPB1** (Heat shock protein β-1), **G3P** (Glyceraldehyde 3 phosphate dehydrogenase), **AT2A1** (Sarcoplasmic/endoplasmic reticulum calcium ATPase 1), **KCRM** (Creatine kinase, muscle type), and **MYH1** (Myosin-1). * represent $p < 0.05$, and ** is for $p < 0.01$. All proteins were categorised follow UniProtKB for Molecular function/Biological process categories.

Table 12 Eleven (11) out of 231 proteins report statistically significant in abundance only and provide FSR data.

Protein accession		UniProtKB	abundance change		Newly fractional synthesis rate (FSR)			Fraction
			Fold change of OB/TR	*p*	OB FSR±SD (%/day)	TR FSR±SD (%/day)	*p*	
Reported statistically significant in abundance only								
1	COX2	cytochrome-c oxidase activity/Electron transport/ Respiratory chain/ Transport/ mitochondrial electron transport, cytochrome c to oxygen	0.55	0.00	2.95±0.00	6.56±0.00	NA	Sarcoplasm
2	TPIS	Isomerase/ Lyase/ methylglyoxal synthase activity/ protein homodimerization activity/ triose-phosphate isomerase activity/ Gluconeogenesis/ Glycolysis/ glyceraldehyde-3-phosphate biosynthetic process/ methylglyoxal biosynthetic process	1.34	0.00	0.91±0.00	0.98±0.00	NA	Sarcoplasm
3	CASQ1	Muscle protein/ positive regulation of release of sequestered calcium ion into cytosol/ positive regulation of store-operated calcium channel activity/ protein polymerization/regulation of skeletal muscle contraction by regulation of release of sequestered calcium ion/ regulation of store-operated calcium entry/ sarcomere organization	1.54	0.01	0.71±0.00	0.7±0.00	NA	Myofibrils
4	APOA1	Cholesterol metabolism/ Lipid metabolism/ Lipid transport/ Steroid metabolism/ Sterol metabolism/ Transport/ chemorepellent activity/ integrin-mediated signaling pathway/ negative chemotaxis/ peptidyl-methionine modification/ positive regulation of cholesterol efflux/ positive regulation of phagocytosis/ positive regulation of phospholipid efflux/ positive regulation of Rho protein signal transduction/positive regulation of stress fiber assembly/ positive regulation of substrate adhesion-dependent cell spreading/ protein oxidation/ protein stabilization	0.70	0.01	4.21±0.00	3.41±0.00	NA	Sarcoplasm
5	TRI72	phosphatidylserine binding/ Exocytosis/ Transport/ exocytosis/ muscle organ development/ muscle system process/ plasma membrane repair/ protein homooligomerization	0.66	0.04	2.67±0.00	2.28±0.00	NA	Sarcoplasm
6	KLH41	myofibril assembly/ protein ubiquitination/ regulation of myoblast differentiation/ regulation of myoblast proliferation/ skeletal muscle cell differentiation	0.54	0.00		7.7±0.00	NA	Sarcoplasm
7	MLRV	Motor protein/ Muscle protein/ Myosin/ heart development/ positive regulation of the force of heart contraction/ regulation of the force of heart contraction	0.72	0.00		0.67±0.00	NA	Sarcoplasm
8	PARK7	Chaperone/ Hydrolase/ Protease/ RNA-binding/ copper ion binding/ kinase binding/mercury ion binding/mRNA binding/peptidase activity/protein homodimerization activit/signaling receptor binding/ Autophagy/ DNA damage/ DNA repair/ Fertilization/ Inflammatory response/ Stress response/ autophagy/ cellular response to hydrogen peroxide/detoxification of copper ion/detoxification of mercury ion/DNA repair/glucose homeostasis/guanine deglycation/guanine deglycation, glyoxal removal/guanine deglycation, methylglyoxal removal/inflammatory response/ insulin secretion/mitochondrion rganization/negative regulation of cell death/negative regulation of extrinsic apoptotic signaling pathway/negative regulation of protein binding/negative regulation of reactive oxygen species biosynthetic process/positive regulation of acute inflammatory response to antigenic stimulus/positive regulation of NAD(P)H oxidase activity/protein deglycosylation/regulation of androgen receptor signaling pathway/regulation of inflammatory response/regulation of neuron apoptotic process/single fertilization	1.32	0.02		1.37±0.00	NA	Sarcoplasm
9	MYL6B	Motor protein/ Muscle protein/ Myosin	0.70	0.01	6.72±0.00			Myofibrils
10	CISY	Transferase/citrate (Si)-synthase activity/ RNA binding/ Tricarboxylic acid cycle/ carbohydrate metabolic process	0.82	0.00	14.11±0.00			Sarcoplasm
11	MYPC2	Actin-binding/ Muscle protein/ Cell adhesion	1.18	0.03	2.27±0.00			Myofibrils

Five (5) proteins (**COX2**; Cytochrome C oxidase subunit 2, **TPIS**; Triosephosphate isomerase, **CASQ1**; Calsequestrin-1, **APOA1**; Apolipoprotein A-I , and **TRI72**; Tripartite motif-containing protein 72) reveal remarkably abundance change but their FSR is unable to measure because of variance is about zero. Six (6) proteins found FSR value in only 1 group i.e. OB and TR. The remaining 17 proteins are unable to measure their FSR. * represent $p < 0.05$, and ** is for $p < 0.01$. All proteins were categorised follow UniProtKB for Molecular function/Biological process categories.

Discussion

Effect of OB on skeletal muscle: abundance and fractional synthesis rate (FSR) change

The current study reported protein abundances and protein turnover in TR vs OB individuals using dynamic proteomic profiling. The analysis included more than 200 individual proteins from the soluble and myofibrillar fractions of muscle. The majority of proteins were assigned to functional groups that are specific to muscle fibres or muscle cell structure. Interestingly, we have found 6 out of the 8 proteins that had a greater abundance in OB muscle also had a lesser the rate of fractional synthesis (FSR). These proteins include **CAH3**; Carbonic anhydrase 3, **HSPB1**; Heat shock protein beta-1, **G3P**; Glyceraldehyde-3-phosphate dehydrogenase, **AT2A1**; Sarcoplasmic/endoplasmic reticulum calcium ATPase 1, **KCRM**; Creatine kinase M-type, and **MYH1**; Myosin-1. This outcome may be evidence that the abundance of these proteins is primarily regulated by degradative processes; that is, the greater protein abundance is mediated by selective inhibition of degradation of these proteins in OB muscle. This may indicate an adverse effect of obesity on skeletal muscle with the consequence that relative quality of these proteins may be less. In addition, we found the intermediate filament cell organisation protein (**ACTN2**; Alpha actinin-2) was less abundant and had a lesser synthesis rate in OB muscle, which may indicate defects in both quality and expression of this intermediate filament protein and could influence cell structure and organisation. Conversely, **ALDOA** (Fructose-bisphosphate aldolase A) was found to be more abundant and had a greater synthesis rate in OB muscle, and this may be part of the cell response to high glucose/ poor glucose homeostasis in OB participants.

The effects of obesity and T2DM on muscle metabolism is a major research area. Skeletal muscle is accessible in humans using the percutaneous biopsy technique and this has enabled a substantial body of literature to be accumulated on the molecular responses of human muscle to obesity/ T2DM but relatively few of these studies have investigated differences in muscle protein turnover. Likewise, comparatively few studies have used proteomic techniques (reviewed in Chapter II) to conduct non-targeted analysis and generate new hypotheses. Some of the most recent proteomic work that has been conducted in human muscle of obese and T2DM patients has raised the hypothesis that protein turnover may be affected. This may be consistent with the growing body of evidence highlighting that maintaining the correct turnover of proteins is important to maintain protein quality, and disruptions to this process may be associated with cellular deteriorations due to ageing or disease. Currently, there is uncertainty regarding the effects of obesity on muscle protein turnover.

Many studies on mixed muscle protein turnover have been published in regards to multiple aspects such as feeding and resistive training exercise (Beals *et al.*, 2018), protein-rich food ingestion (Beals *et al.*, 2017), fasted-state elevated plasma amino acid (Tran, Kras, *et al.*, 2018) and increased nutrient delivery

(Murton *et al.*, 2015). In obese adults (Beals *et al.*, 2018) reports pork feeding coupled with resistive training elicits normal sarcoplasmic protein synthesis responses but the protein synthetic response of the myofibrillar fraction is diminished in comparison to normal weight individuals. Another study from the same laboratory (Beals *et al.*, 2017) investigating protein-rich food ingestion on mitochondrial protein synthesis and found the effect in sedentary adults (normal weight and overweight, n = 9 in each group) is similar to obese individuals (n = 9) but indicated that adiposity and chronic low-grade inflammation may increase muscle TLR4 (Toll-like receptor 4) signaling protein (Beals *et al.*, 2017). Therefore, it was concluded that mitochondrial protein synthesis may contribute to metabolic derangements in obesity (Beals *et al.*, 2017). However, these studies do not investigate individual mitochondrial protein synthesis rates. The current literature on protein synthesis response across mixed protein may easily be confounded if different groups of proteins exhibit opposite changes in synthesis rate, which would result in no overall change to the average rate of synthesis.

Muscle protein synthesis rates remain responsive to increases in plasma amino acids in obese participants (Tran, *et al.*, 2018). This study measured protein synthesis at 2 time points i.e. fasted-state and infused amino acid state. They reported that adults with obesity (n = 10) had a lower rate of muscle protein synthesis at baseline but were able to increase the fractional rate of synthesis in response to amino acid infusion in comparison with lean subjects (n = 10). The finding indicates responsiveness of mixed muscle synthesis to plasma amino acid concentration is maintained. This group also investigated the fractional synthesis rate of mitochondrial ATP synthase β-subunit **(ATPB)** and ATP synthase activity (Tran, *et al.*, 2018). A reduction in F1-ATPase synthesis rate was reported in obese/insulin resistant participants, which coincided with a lesser abundance and catalytic activity of ATPB protein. These events indicate an impaired/defective capacity for ATP generation via ATP synthase in muscle from obesity/insulin resistant individuals.

A previous review (Wolfe, 2006) raised concerns about the potential adverse effects of diseases such as obesity on skeletal muscle protein metabolism, as well as on central/ whole body metabolism. However, there is currently limited evidence to suggest that muscle of obese individuals has a substantial difference in protein turnover, despite reports that mechanisms involved with protein turnover rate or synthesis are impaired (Katsanos and Mandarino, 2011). A study on muscle protein synthesis in older obese participants reported impaired protein turnover, but the exact role or contribution of physical inactivity levels on the decline in metabolic health remains ambiguous (Murton *et al.*, 2015). The main findings of early reports reveal protein abundance data only and shows a greater abundance in fast-twitch myofibrillar proteins but lesser abundance in mitochondrial protein. However, these prior studies on protein synthesis reported only mixed protein data and so report average FSR. This type of data could mask changes at the individual protein level. That is, when mixed-protein samples are

analysed an increase in the FSR of some proteins will be counteracted by decreases in the FSR of others and could lead investigators to report no overall difference in muscle protein synthesis rate.

A literature search found 75 publications related to fractional synthesis rate and human skeletal muscle analysis. The majority of these studies used different amino acid-specific isotope tracers and a range of analytical methods to investigate the precision and accuracy of the techniques for determining the fractional synthesis of new protein in human skeletal muscle. Although many experiments analysed fractional synthesis rate, they used isotope-labelled amino acids such as ^{13}C (Koopman *et al.*, 2007), ^{14}C (Lund *et al.*, 2017), ^{15}N (Phillips *et al.*, 1997), and ^{2}H (Dickinson *et al.*, 2010; Shankaran *et al.*, 2016). These 3 stable isotopes are widely used as isotopic tracers for determining rate of protein synthesis and breakdown. ^{13}C and ^{15}N are generally labelled by infusion, which is considered an invasive approach in humans and has limitations regarding study duration and the type of exercise that can be performed. Recently, deuterium oxide/ heavy water has become more popular than the previous isotopes because it can be taken in the drinking water – meaning that studies can be conducted over a longer duration and can include a broad range of exercise and lifestyle interventions. The application of ^{2}H as a tracer is unique because it labels C—H bonds of amino acids intracellularly, e.g. alanine has numerous H that can exchange with deuterium to give approximately 3.7 times higher enrichment compared to body water (Gasier, Fluckey and Previs, 2010). So, in addition to being a less invasive method appropriate for applying in human subjects, the use of deuterium oxide also improves the sensitivity of the technique and enables individual proteins to be analysed. Two previous studies (Robinson *et al.*, 2011; Scalzo *et al.*, 2014) have employed deuterium oxide in human and exercise context and were aimed at studying aging and aerobic training. However, these works were conducted to measure DNA synthesis rate as well as mixed protein turnover in muscle (Robinson *et al.*, 2011; Scalzo *et al.*, 2014). Although Scalzo et al (Scalzo *et al.*, 2014) reported data on muscle protein synthesis of sprint interval training (6-week-SIT) the data were based on separate GC-MS and LC-MS analyses with the result did not provide the exact protein turnover rate at a protein-specific level. Therefore, Shankaran et al (Shankaran *et al.*, 2016) is the first report on human muscle that used deuterium oxide and proteomic techniques, but this work reports protein synthesis rates only and did not consider the abundance of individual proteins.

The current study is unique in that we report protein abundance profiles and protein-specific turnover in TR vs OB individuals. Initially, we identified 231 proteins and the abundance of 43 proteins was significantly ($p < 0.05$) different between OB and TR skeletal muscle. The fractional synthesis rate (FSR) was measured across 155 proteins and 52 proteins reported statistically significant difference between TR and OB groups (**Appendix 7**). These proteins were matched to our abundance data and found 19 proteins have both abundance and FSR data in either OB or TR or both groups (**Table 11 and 12)**. The novelty of the present work is that we can investigate novel information on both protein abundance and fractional synthesis rate of human muscle proteins. This is important because a change in synthesis rate

may either be associated with accretion of that protein or may represent a greater turnover of the protein without any change in protein abundance (i.e. if the degradation rate of the protein is also increased).

Our data reveals that **CAH3** (Carbonic anhydrase 3) exhibits greater abundance (1.75-fold change, $p = 0.00$) but lesser FSR in OB (FSR_{OB} = 0.73 %/day, FSR_{TR} = 1.62 %/day, $p = 0.01$). This suggests deterioration in protein quality which has not previously been reported. CAH3 is a ubiquitous metalloenzyme, which catalases the reversible hydration of carbon dioxide to the bicarbonate ion. This isoform is expressed highly in skeletal muscle but appears to be more abundant in type-I fibre than type-II muscle fibres in human and rat (Harju *et al.*, 2013). CAH3 is also involved with oxidative stress response and may protect cells from apoptosis (RÄISÄNEN *et al.*, 1999) via a cellular anti-oxidant role in response to high reactive oxygen species (ROS)(Cabiscol and Levine, 1995). Our data indicates obesity is associated with lesser **CAH3** quality, which may have an impact on redox balance and could promote a pro-apoptotic state. In support of this interpretation, **HSPB1** (heat shock protein family B member 1) exhibited a similar pattern of alteration to **CAH3** where HSPB1 abundance was greater in OB (abundance = 1.18-fold greater, $p = 0.01$) but FSR was less in OB compared to TR (FSR_{OB} = 1.30 %/day, FSR_{TR} = 2.79 %/day, p=0.00). **HSPB1** is also known as heat shock protein family 27, which may protect cells under adverse conditions such as infection, inflammation, exposure to toxins, elevated temperature, injury, and disease. In particular, heat shock proteins block or protect against processes that lead to programmed cell death (apoptosis). In addition, they appear to be involved in activities such as cell movement (motility), stabilization of cell structure (the cytoskeleton), protein-folding and the stabilization of newly synthesised proteins, or repairing damaged proteins. Heat shock proteins also appear to play a role in muscle contraction or remodelling of myofibrils in response to exercise. HSBP1 is reported to be up-regulated in response to proteasome inhibition which causes molecular derangement and has been hypothesised to contribute to muscle dysfunction in obese/ T2DM muscle (Al-Khalili, *et al.*, 2014)

Our analysis found Creatine kinase, muscle type (**KCRM**) was of greater abundance in OB compared to TR (1.18-fold change, $p = 0.00$) but lesser protein quality (FSR_{OB} = 0.86 %/day, FSR_{TR} = 0.93 %/day, $p = 0.00$). **KCRM** plays an important role in striated muscle energy metabolism, i.e. via the resynthesis ATP from phosphocreatine (PCr). This protein is also crucial for the spatial distribution of energy throughout the cell, and is a major component of the muscle M-band. The greater abundance of KCRM in OB muscle may be a compensatory mechanism to enable a greater contribution PCr as substrate for ATP resynthesis in response to poor regulation of glucose and fatty acid metabolism. We also report a greater abundance of glycolytic enzymes (e.g. Triosephosphate isomerase (**TPIS**) with a trend for a reduced FSR compared to TR muscle. The glycolytic enzyme, TPIS, is of particular interest because the abundance of this protein decreases in response to endurance training (reported in the next Chapter, i.e. in response to 10-week-HIT intervention). The greater abundance of TPIS in OB compared to TR (1.34-fold change, $p = 0.00$)

but lesser quality (FSR_{OB} = 0.91 %/day, FSR_{TR} = 0.98 %/day, *NS*) may have consequences on Glyceraldehyde 3-phosphate abundance, which is a substrate of **G3P** (glyceraldehyde 3 phosphate dehydrogenase). **TPIS** is involved in glycerol and glucose metabolism. Major role is to synthesise D-glyceraldehyde 3-phosphate from glycerone phosphate. Glyceraldehyde 3-phosphate is a substrate of **G3P** and has been reported to be increased in abundance in OB muscle. We also found **G3P** had a significantly greater abundance in OB muscle, which is consistent with previous reports on abundance (Hittel *et al.*, 2005; Giebelstein *et al.*, 2012). In addition, our novel dynamic proteome profiling method enabled us to also report G3P synthesis rate is significantly altered in OB compared to TR muscle.

Association of body composition and training status on the muscle proteome

Our primary aim was to investigate differences in the skeletal muscle proteome (in particular protein turnover) between groups of humans that have markedly different disease risk profiles. Non-communicable diseases, including T2DM, can arise for a complex interaction between genetic heritage and environmental factors, such as diet and exercise factors. Lifestyle factors such as poor diet and physical inactivity often cooccur in the general population, and the majority of health interventions are targeted at individuals that are overweight and physically inactive. Therefore, we chose to use both body composition and training status to optimise differences between our healthy (TR) and unhealthy (OB) groups. By design, OB and TR participants differed ($p < 0.05$) in BMI, VO_{2max}, insulin sensitivity index (Matsuda index), and body composition (VAT, %fat, fat mass, and lean mass) and, therefore, represent discrete populations with markedly disease risk profiles. Our current cross-sectional analysis confirms the health status of OB subjects differs from TR participants, and that this is associated with differences in muscle protein abundance and synthesis rate. A limitation of this design, however, is that we cannot investigate whether the differences in the muscle proteome between OB and TR groups are specifically associated with either obesity or physical inactivity in isolation.

We found OB is associated with down-regulation of insulin regulated secretion protein cluster (**ADT1; ADT3; ADT4**; ADP/ATP translocase-1,3, and 4 = 0.71-fold change, $p = 0.01$). Moreover, this protein cluster is also up-regulated in response to our 10-week-HIT protocol (Chapter V). Dysregulation of insulin regulated secreted proteins would likely effect glucose homeostasis mechanisms that interfere with glycaemic control and have consequences for β-cell function/ demand. Other proteins that may be associated with differences in insulin sensitivity, include the glycolytic enzymes **G3P** (Glyceraldehyde-3-phosphate dehydrogenase), **ALDOA** (Fructose-bisphosphate aldolase A) and **TPIS** (Triosephosphate isomerase). **G3P** is a multifunctional enzyme, its main function is in the glycolytic pathway where it converts glyceraldehyde-3-phosphate into 1,3-Bisphosphoglycerate and in the process converts NAD+ and Pi in to $NADH+H^+$. G3P also has roles in cell apoptosis or cell death where it is translocated to the nucleus under oxidative stress (Nakajima *et al.*, 2009). Over expression of **G3P** can effect fatty acid gene promoters and this is associated with greater adiposity (Rolland *et al.*, 1995). G3P has also been reported in association with oxidative stress in

neurodegenerative disease such as Alzheimer (Mazzola and Sirover, 2001), and these findings illustrate that G3P should not be thought of solely as a glycolytic enzyme.

Muscle **ALDOA** functions as a scaffolding protein that builds a multi-enzyme complex to localize glycolysis at the Z-line of muscle (Gizak, Rakus and Dzugaj, 2003; Rakus *et al.*, 2003; Mamczur *et al.*, 2005). **ALDOA** catalyzes an early step of glycolysis that coverts fructose 6 phosphate into glyceraldehyde 3 phosphate which is a substrate of **G3P** (glyceraldehyde 3 phosphate dehydrogenase). Moreover, **TPIS** which is also an enzyme that function to produce substrate for **G3P** in gluconeogenesis was less abundant. Therefore, in the glucose metabolism data we show distinctive changes that highlight **G3P** as the marker of change. These findings are consistent with observations that of greater abundance of glycolytic proteins in muscle of long-term unhealthy/ sedentary individuals that have poor glucose and lipid homeostasis. Accordingly, we measured low expression in lipid metabolic proteins (**APOA1**; Apolipoprotein A-I) in OB subjects, and this profile changed after endurance exercise **(APOA1** abundance; OB/TR = 0.70-fold change, p = 0.01 in Chapter V). **APOA1** is involved with transport cholesterol from tissue to liver for excretion and is the main component of good cholesterol (high density cholesterol, HDL).

Our study is the first report on both **G3P** abundance and dynamic change. We found **G3P** is more abundant (1.44-fold change, p = 0.00) in the muscle of obese individuals, which is consistent with other reports (Hittel *et al.*, 2005; Giebelstein *et al.*, 2012). However, we also add new information to show **G3P** synthesis rate is less in obese compared to trained individuals (FSR; OB = 1.04 %/day, TR = 16.04 %/day, p = 0.00), which could indicate an accumulation of damaged protein in muscle of OB participants. Shankaran (Shankaran *et al.*, 2016) recruited sedentary subjects to undertake 3-w-SIT, the sedentary baseline revealed similar rate of FSR ($FSR_{sed.}$=1.0%/day) to ours (FSR_{OB}=1.04%/day). This would suggest that the greater abundance of **G3P** is due to lesser protein degradation and accumulation of damaged protein. This adds new data, in addition to studies from systematic review report G3P abundance in both OB (Giebelstein et al., 2012) and MOB with early-onset of T2DM (Hittel *et al.*, 2005). A report (Giebelstein *et al.*, 2012) on OB (Vastus lateralis) found increased expression of G3P in OB compared to LE and reported high number of multiple gel spots relating to glycolytic enzymes in OB. This change indicated shift-toward of glycolytic enzyme in fast-twitch muscle fibres consistent with our finding regarding **SERCA1** and **MLRV**. However, this change in glycolytic pattern may not alter muscle fibre type distribution, i.e. traditional myosin heavy chain profile, as reported in a previous study (Giebelstein *et al.*, 2012). A report (Hittel *et al.*, 2005) conducted in Rectus abdominus of OB and MOB specimen also found a greater abundance of **G3P** and this was associated with changes in Adenylate kinase (**AK**) and Aldolase A abundance. The authors also hypothesised a long-term unhealthy diet and lack of exercise contribute to loss of glucose and lipid homeostasis, which may lead to compensatory mechanisms in chronic decreased muscle.

We found VO_{2max} in OB was less than that was measured in TR which indicated impaired oxygen capacity uptake in OB condition. We revealed down-regulation (0.55-fold change, $p = 0.00$) and lessen FSR (FSR_{OB} 2.95 %/day, FSR_{TR} 6.56 %/day) of OB/TR in **COX2** (cytochrome C oxidase subunit2, Complex IV). **COX2** or cytochrome C oxidase is highly expressed in heart and predominantly slow-twitch skeletal muscle fibres. COX2 functions in Complex IV of the electron transport chain. Complex IV plays an important role in reducing oxygen into water. **COX 2** was reported greater abundance in muscle of long-term (over 5 years) endurance trained individuals (Schild *et al.*, 2015) and **COX2** is a commonly used as a marker of muscle OXPHOS response to exercise training. For example, a report on High capacity running (HCR) which compared to Low capacity running (LCR) found that the measurement of **COX2** from HCR revealed higher abundance than **COX2** from LCR (1.14 fold change) (Malik *et al.*, 2013). A lesser abundance of COX2 in OB muscle may indicate defects in mitochondrial metabolic function. However increased FSR of COX2 in OB would suggest that protein quality is maintained. Our current study is the first to report both abundance and FSR changes of **COX2** protein from OB skeletal muscle. Although earlier literature revealed **COX2** protein abundance changes, the synthesis rate of this protein in human muscle has not been investigated. The current finding showed **COX2** protein abundance is less in OB (0.55-fold change, $p = 0.00$) and this in-line with previous articles (Hittel *et al.*, 2005; Giebelstein *et al.*, 2012; Hussey *et al.*, 2013). However, the FSR (FSR_{OB} 0.94 %/day, FSR_{TR} 0.88 %/day) was less than reported in a previous study ($FSR_{pre\text{-}HIT}$ = 1.27 %/day, $FSR_{post\text{-}HIT}$ = 1.33 %/day) (Shankaran *et al.*, 2016) that used sedentary lean participants. This difference may be associated with the different experimental design of Shankaran (Shankaran *et al.*, 2016), which compared a sedentary group against an exercise training group. Despite our report FSR is less than the FSR reported by previous work (Shankaran *et al.*, 2016), the pattern is similar in that FSR_{COX2} from trained is higher than OB.

Our current work provides data on 19 proteins **(Table 11** and **Table 12)** that were statistically different in abundance and FSR, and a further 11 proteins reported significant differences in abundance and a trend for difference between FSR in OB versus TR. We discovered 6 novel proteins (**TNNI1**; Troponin I, slow skeletal muscle, **CASQ1**; Calsequestrin 1, **CAH3**, **TPIS**; Triosephosphate isomerase, **FLNC**; Filamin C, and **KLH41**; Kelch-like protein 41) that were statistically significant in abundance and have not previously been reported, i.e., were not included in the meta-analysis dataset **(Table 13, no. 14-19)**. Of these, only **CASQ1, CAH3**, and **TPIS** provide both abundance and FSR data, which can be used to indicate protein quality. Some of our data are consistent with the proteasome inhibition experiment by Al-Khalili et al (Al-Khalili, *et al.*, 2014) i.e. **HSPB1**, and profiling of human myotube by Al-Khalili et al. (Al-Khalili, *et al.*, 2014) i.e. **PARK7, ENOA**, and **CISY**. We included 10 proteins in the meta-analysis dataset and revealed 5 proteins **(ALDOA, APOA1, KCRM, MYH1,** and **TRI72)** were not notably different in abundance between OB and TR populations. In contrast, we discovered 5 proteins (**ACTN2;** α-actinin-2, **AT2A1;** Sarcoplasmic/endoplasmic reticulum calcium ATPase 1, **COX2;** Cytochrome c oxidase subunit 2, **G3P;** Glyceraldehyde-3-phosphate dehydrogenase, and **MLRV;** Myosin regulatory light chain 2, ventricular/cardiac muscle isoform) which, consistent to previous literature, exhibited a significantly different abundance between OB

and TR. Among these, **G3P, AT2A1**, and **COX 2** found in sarcoplasmic fraction whereas **ACTN2** was detected in the myofibrillar fraction. **G3P** and **AT2A1** were greater in abundance but had lesser turnover rate in OB, whereas **COX2** was less in both abundance and FSR, but **ACTN2** reported lessen abundance but elevated FSR.

Our main findings highlight 5 individual proteins (**ACTN2, G3P, SERCA1, COX2, and MLRV**) that showed robust information regarding differences in abundance and, fractional synthesised, and were highlighted from our previous meta-analysis, including: **ACTN2, SERCA1, COX2, G3P**, and **MLRV**. We reported protein quality which indicate either accumulate damage (**SERCA1**; abundance 1.27, FSR_{OB} = 1.4 %/day, FSR_{TR} =13.0 %/day, p = 0.00, **G3P**; abundance 1.44-fold change, FSR_{OB} = 1.0 %/day, FSR_{TR} = 16.4%/day, p = 0.00), post-translational modification (**COX2**; abundance 0.55, FSR_{OB} = 2.9 %/day, FSR_{TR} = 6.6 %/day, p = NA), or effective quality control (**ACNT2**; abundance 0.84, FSR_{OB} = 0.94 %/day, FSR_{TR} = 0.88 %/day, p = 0.0.

Table 13 the current data compares with other studies in systematic review in Chapter II.

	Accession	Our study			Shankaran 2015	systematic review (RQ1 only)	Reference(s)	meta-analysis	Effect size significant
		Abundance	OB_FSR	TR_FSR	Sedentary FSR				
		% per day							
1	**ACTN2**	**0.84****	**0.9****	**0.8**	**1.0**	**0.58**	(Hwang *et al.*, 2010)	**include**	**Yes**
2	**AT2A1**	**1.27***	**1.4**	**13.0**	**1.8**	**0.75**	(Lefort *et al.*, 2010)	**include**	**Yes**
3	**COX2**	**0.55***	**3.0**	**6.5**	**1.3**	**0.74**	(Hussey *et al.*, 2013)	**include**	**Yes**
4	**G3P**	**1.44****	**1.0**	**16.4**	**1.0**	**1.81-2.00** **1.52-1.75**	(Hittel *et al.*, 2005) (Giebelstein *et al.*, 2012)	**include**	**Yes**
5	**MLRV**	**0.72**	**NA**	**0.7**	**1.2**	**0.37-1.85**	(Giebelstein *et al.*, 2012)	**include**	**Yes**
6	ALDOA	1.22*	2.3	1.2	1.5	1.04 1.76-2.60	(Lefort *et al.*, 2010) (Hittel *et al.*, 2005)	include	No
7	APOA1	0.70	4.2	3.4	10.4	1.04 1.32	(Lefort *et al.*, 2010) (Thingholm *et al.*, 2011)	include	No
8	KCRM	1.48**	0.9	2.0	0.9	1.03-1.18	(Giebelstein *et al.*, 2012)	include	No
9	MYH1	1.59*	0.8	50.8	1.6	1.74 2.70	(Lefort *et al.*, 2010; Hussey *et al.*, 2013)	include	No
10	TRI72	0.66	2.7	2.3	1.5	0.85	(Lefort *et al.*, 2010)	include	No
11	HSPB1	1.18*	4.0	2.8	2.2	1.4	(Al-Khalili, *et al.*, 2014)	exclude	NA
12	PARK7	1.32	NA	1.4	NA	1.25	(Al-Khalili, *et al.*, 2014)	exclude	NA
13	CISY	0.82	14.1	NA	1.3	1.25	(Al-Khalili *et al.*, 2014)	exclude	NA
14	TNNT1	0.76	6.9	5.3	2.0			exclude	NA
15	CASQ1	1.54	0.71	0.70	1.4			exclude	NA
16	CAH3	1.75**	0.7	1.6	0.6		NA	exclude	NA
17	TPIS	1.34	0.9	1.0	0.8			exclude	NA
18	FLNC	0.83	4.6	NA	2.3			exclude	NA
19	KLH41	0.54	NA	7.7	3.7			exclude	NA

Four (4) proteins (**ACTN2**; α-actinin-2, **AT2A1**; Sarcoplasmic/endoplasmic reticulum calcium ATPase 1, **COX2;** Cytochrome c oxidase subunit 2, and **G3P;** Glyceraldehyde-3-phosphate dehydrogenase) reported complete information for comparison (both abundance and FSR). Other 6 proteins were included in meta-analysis but only **MLRV** (Myosin regulatory light chain 2, ventricular/cardiac muscle isoform) found significantly different in abundance change under the effect of pathophysiology. The result of newly fractional synthesis rate (FSR) also compares to other study by Shankaran et al (2015). Our reported FSR showedsimilarly to the publication by Shankaran et al (2015).

Our present data is consistent with previous work (Shankaran *et al.*, 2016), we report **ACTN2, AT2A1, COX2**, and **G3P** synthesis rates and in addition we are the first to report protein abundance data alongside dynamic FSR data to give insight to protein quality. Our current study identified 231 proteins and measured FSR of 155 proteins across OB and TR. In total, 43 of 231 and 52 of 155 proteins were statistically different in abundance and/or FSR ($p < 0.05$), respectively. The range of FSR was from 0.65 %/day (**MYL1**; Myosin light chain 1/3, skeletal muscle isoform and **PDRX2**; Peroxiredoxin-2) to 40.91 %/day (**ENOB**; Beta-enolase) in OB and from 0.58 %/day (**MYL1**) to 50.75 %/day (**MYH1**; Myosin-1) in TR. The previous work (Shankaran *et al.*, 2016) measured 513 proteins and reported 273 proteins with FSR that range from 2.2% to 70.3% per week or 0.31% to 10.04% per day. The minimum FSR in Sedentary baseline was protein **SPYL2** (Synaptophysin-like protein 2) and the maximum was protein **CO3** (Complement C 3). Overall, we report a different range of FSR when compared with Shankaran et al, however, individual proteins between ours and the previous report revealed similar FSR such as **G3P** (Glyceraldehyde 3 phosphate dehydrogenase, FSR_{OB} = 1.0 %/day, FSR_{SED} = 1.0 %/day). This may suggest sedentary behaviour (reported in Shankaran et al) is not dissimilar from obesity and physical inactivity (reported herein). On the other hand, some protein such as **COX2** (Cytochrome C oxidase subunit-2) were higher FSR_{OB} (3.0 %/day) than FSR_{SED} (1.3 %/day) participants studied by Shankaran et al. This is possibly because our OB subject remained inactive during the experiment and we observed no statistical difference in physical activity **(Table 13)** between OB and TR. Therefore, some proteins, e.g. associated with oxidative capacity or electron transport chain may have a greater FSR when compared against sedentary individual. Shankaran et al (Shankaran *et al.*, 2016) investigated sedentary individuals that performed 3-week of Sprint Interval cycling (longitudinal study of pre- and post-sprint interval training). This Chapter extracted data from the sedentary FSR component of the Shankaran et al experiment because our design was cross-sectional rather than longitudinal. We reported similar value of FSR to the previous study (Shankaran *et al.*, 2016) but we determined the statistically significant change regarding abundance data before we consider FSR differences. It is equally important to know whether there are differences in either the abundance or turnover rate of proteins between OB and TR muscle. Some proteins may not change in abundance because there are compensatory changes in both synthesis and degradation, i.e. turnover rate will be different but the abundance will be unchanged.

The control group reported in Shankaran et al, was sedentary whereas our experiment required Athlete-trained (TR) condition which is assumed to be a healthy control. Comparing against a control group of sedentary individuals may be inappropriate because a sedentary lifestyle is one cause of obesity and muscle characteristics may not be different especially in cross-sectional study. Therefore, healthy/ active control indicates greater different against the OB case group. The most important point to emphasise is that Shankaran et al (Shankaran *et al.*, 2016) investigated the response to exercise

training in normal participants – they did not investigate obese people. We have investigated the difference between OB and TR to learn whether obesity and sedentary behaviour is associated with a different turnover of muscle proteins compared to healthy active people. This is an entirely different question to that of Shankaran et al (Shankaran *et al.*, 2016). It was important for us to ask our question because it was not clear from the available literature whether obesity is associated with a change in the turnover of muscle proteins.

Five proteomics publications (Lefort *et al.*, 2009; Hwang *et al.*, 2010; Thingholm *et al.*, 2011; Hussey *et al.*, 2013; Formentini *et al.*, 2017) have investigated obese subjects but this literature reported only protein abundance differences. Hwang et al (Hwang, *et al.*, 2010) raised the hypothesis 'Changes in the extracellular matrix might lead to altered mitochondrial function by means of altered mechano-signal transduction produced by changes in intermediate filament protein abundances' The hypothesis was based on data showing lower abundance of cytoskeletal proteins (e.g. **ACTN2, MYOZ1**; Myomezine-1) which function in actin capping and Z-disc components and a reduction in the major intermediate filament protein (**DESM**; Desmin). There was also a decrease in mitochondrial proteins (**COX6C** and **QCR7**), which may indicate a link between sarcomere and mitochondria. The proposed mechanism may involve intermediate filament proteins that integrate signals of force transduction between the sarcomere and extracellular matrix protein (e.g. integrin and the dystroglycan complex). This would regulate the function in respect to cell organisation in skeletal muscle and therefore also require mitochondrial adaptation. The proposed mechanism is based on data of an interaction between extracellular matrix and mitochondria described by mutation of Collagen 6 protein in Bethlem muscular dystrophy. This specific change to intermediate filament protein abundance was associated with mitochondrial adaptation. Although we have identified increased abundance of 1 protein from extracellular matrix (**FIBG**; Fibrinogen gamma chain), this protein was not statistically significant. Interestingly, our findings include **FLNC** which functions in cell junction and assembly and also is a key protein involved in cell-extracellular matrix interaction. Therefore, our report is in line with the previous hypothesis and we provide further supporting evidence, which covers new proteins i.e., lesser abundance and fractional synthesis rate of intermediate filament protein **(ACTN2)**, cell junction and cell-extracellular matrix interaction protein (**FLNC**), and mitochondrial protein **(COX2).**

Our findings seem to be distinct from the hypothesis of (Lefort *et al.*, 2010) that 'Higher ROS in OB would be associated with higher thermodynamic driving forces as a result of low metabolic turnover rate'. Our data reported no OXPHOS protein which found consistent with previous report (Lefort *et al.*, 2010). They also highlight greater abundance in muscle contraction proteins (e.g. **MYH1, MYH2**, and **SERCA1**) as well as a result of insulin resistant in muscle tissue. Moreover, these proteins are specific to fast-twitch muscle fibre and probably denote a shift in myofibrillar profile. Our study reported mitochondrial protein showed a trend of lesser expression in OB, including **COX2**, **UCRI** (Cytochrome b-c1

complex subunit Rieske, mitochondrial), and tricarboxylic acid protein e.g. **CISY** (Citrate synthase, mitochondrial).

The hypothesis of molecular arrest as an underlying effect of molecular derangement of skeletal muscle of T2DM and protein turnover has been tested by inhibiting the ubiquitin proteasome system (UPS) (Al-Khalili, *et al.*, 2014). The UPS influences oxidative stress responses, protein dynamics, and gene expression. The study by Al-Khalili et al was performed on human myotube cell cultures that were derived from Lean and T2DM subjects. The main findings suggest that T2DM is caused by impaired insulin induced protein turnover associated with dysfunction of the proteasome-degradation system. The disruption to proteostasis maintenance systems may be linked to glucose intolerance or insulin resistance through the intracellular insulin signalling pathway, metabolism and oxidative stress responses. Proteins that were responsive to the inhibition of the UPS, and exhibited a greater expression, were associated with program cell death such as **HSPB1** (Heat shock protein beta-1). On the other hand, proteins which function mainly in cytoskeletal cell structure i.e. **WDR1** (WD repeat-containing protein 1) became less abundant after UPS inhibition. This indicates some of the deterioration in muscle in obese/ T2DM may be due to dysregulation of the UPS.

Studies performed on cultured myotubes from human muscle aim to exclude environment factors such as exercise and diet in order to investigate intrinsic differences. However, myotubes are not fully differentiated mature myofibres so a limitation is that myosin isoform expression, muscle fibre cytoskeleton, and development of insulin resistance may not be faithfully replicated. The current thesis reports abundance and dynamic changes to some muscle contraction proteins of both fast-twitch (**SERCA1, MYH1, MYPC2**) and slow-twitch (**MLRV**, and **TNNI1**) myofibres, and intermediate filament proteins (e.g. **ACTN2**) which may not be replicated in primary cell cultures. **ACTN2** is a key muscle protein and co-localises with **PHB2** (Prohibitin-2) and other cytoskeletal proteins. We reported statistically significant differences in abundance (**ACTN2**; abundance = 1.20-fold greater in TR, $p = 0.00$) and turnover kinetics of **ACTN2** (FSR_{OB} = 0.94%/day, FSR_{TR} = 0.88%/day, $p = 0.00$). Our findings add synthesis information to previously reported (Hussey *et al.*, 2013) lesser abundance of **ACTN2** in OB. The lesser abundance of **ACTN2** may be explained by molecular remodelling of proteins from multiple processes such as structural (cell cytoskeleton), extracellular matrix, and mitochondrial proteins. Hussey et al report down-regulation of mitochondria in T2DM muscle evidenced by NADH availability and mitochondrial proteins (**COX2, AATM, MDHM, UCR9, ATP5F1**, and **ATPA)**, and changes to cytoskeletal proteins prohibitin-2 (**PHB2**) and α-actinin 2 (**ACTN2**). Our report is consistent with Hussey et al and also highlighted mitochondrial protein abundance changes (e.g. **COX2** down-regulation in OB). Although we did detect **PHB2**, we were not able to detect a difference in abundance or synthesis rate. **ACTN2** was reduced (0.84-fold change) in abundance in muscle of OB individuals, however, ACTN2 FSR was relatively greater in OB individuals (FSR_{OB} = 0.94%/day, FSR_{TR} = 0.88%/day, $p = 0.00$). Our

work also discovered 2 other muscle cytoskeletal proteins, **KLH41** (Kelch-like protein 41) and **FHL1** (Four and a half LIM domains protein 1) that were of less abundance in OB muscle. Hwang et al (2010) (Hwang *et al.*, 2010) raised the hypothesis that insulin resistance in muscle may alter mitochondrial function via mechano-transduction events that result from a reduction in intermediate filament proteins such as **ACTN2** and **MYOZ1** but overexpress in microtubule proteins (Hwang *et al.*, 2010). This difference in protein expression may interfere with extracellular matrix protein function and may be associated with proinflammatory cytokine production which in turn effect mitochondrial protein regulation. Moreover, our previous publication hypothesised from non-bias review of literature that TENA /TLR4 signalling pathway may be involved in muscle insulin resistance as a consequence of muscle inflammation (Srisawat *et al.*, 2017). This current thesis has identified a cluster of abundance differences ($p < 0.05$) of cytoskeleton proteins (**KLH41**; Kelch-like protein 41, **FHL1**; Four and a half LIM domains protein 1, and **FLNC**; Filamin-C) consistent with the hypothesis that muscle cytoskeleton remodelling is a major component in muscle dysfunction associated with obesity and physical inactivity.

We found that **SERCA1** protein was increased in abundance (1.27-fold greater in OB, $p = 0.02$) but the synthesis rate of **SERCA1** was less in OB than TR muscle (FSR_{OB}=1.4%/day, FSR_{TR}=13.0%/day, $p = 0.00$). Shankaran et al (Shankaran *et al.*, 2016) reports the FSR of **SERCA1** (FSR_{pre} = 1.8%/day, FSR_{post} = 2.2%/day) and found **SERCA1** FSR increased in response to a programme of sprint interval training. The greater abundance but lower FSR of SERCA1 in OB muscle may indicate a negative effect on protein quality compared to TR. Lefort et al (Lefort *et al.*, 2010) also reported a difference in **SERCA1** abundance in OB muscle (0.58-fold less than control). **SERCA1** is responsible for controlling Ca^{2+} ion levels inside cells and has an important role in muscle contraction and relaxation by storing calcium. The **SERCA** proteins are Ca^{2+}ATPases that use ATP to pump Ca^{2+} back in to the sarcoplasmic reticulum (SR) and therefore particularly influence muscle relaxation rate. **SERCA1** is most prominent in type II fast-twitch muscle fibres (Periasamy and Kalyanasundaram, 2007). In insulin resistant and diabetic patients, changes in SERCA1 function may relate to endoplasmic reticulum (ER) stress or may be an indicator of changes to myofibre phenotype. We found up-regulation in other fast-twitch specific muscle proteins (**MYH1**; Myosin-1 and **MYPC2**; Myosin-binding protein C, fast type) which also indicate a difference in muscle fibre phenotype between OB and TR. However, we cannot rule out ER stress, which has been reported to occur in response to lipotoxicity or fatty acid excess caused by consuming a high-fat diet (Ghemrawi, Battaglia-Hsu and Arnold, 2018). An excess of palmitate, in particular, may interfere with lipid distribution in skeletal muscle and worsen ER stress (Peng *et al.*, 2011). Our data also found lower abundance in lipid metabolism protein (**APOA1**; Apolipoprotein A-1) and a greater abundance of stress responsive proteins (**HSPB1**; Heat shock protein beta-1 and **PARK7**; Protein/nucleic acid deglycase DJ-1).

Our study also found differences in a second calcium-handling protein, (**CASQ1**; Calsequestrin-1), which has a moderately affinity for Ca^{2+} but highly capacity for calcium binding protein. However, **CASQ1** was

of greater abundance (1.54-fold change, p = 0.01) in OB muscle. **CASQ1** is a sarcoplasmic reticulum (SR) Ca^{2+} binding protein in the lumen of the SR whereas SERCA1 is a Ca^{2+} pump on the SR membrane that pumps Ca^{2+} in to the SR lumen. Note also that **CASQ1** is an important regulator of mitochondrial Ca^{2+} so the different responses between **SERCA1** and **CASQ1** might be associated with the roles of these proteins in 2 different organelles: **SERCA1** in the sarcoplasmic reticulum and **CASQ1** in the mitochondria. We report no difference in FSR of **CASQ1** between OB and TR (FSR_{OB} = 0.71%/day, FSR_{TR} = 0.70%/day, p = NA). Based on the greater abundance the absolute synthesis **CASQ1** may also be greater in OB compared to TR highly active muscle. Consistent with our findings, Ferguson et al (Ferguson *et al.*, 2014) reported **CASQ1** was significantly decreased in abundance in rat soleus muscle by physical activity.

Our study identified the slow-twitch isoform of myosin regulatory light chain i.e. **MLRV** (Myosin regulatory light chain 2, ventricular/cardiac muscle isoform), was significantly less in OB (abundance = 0.72, p = 0.00) muscle, which is in line with other reports (Hojlund *et al.*, 2003; Giebelstein *et al.*, 2012). **MLRV** is involved with muscle contraction and is specific to cardiac muscle and slow-twitch skeletal muscle. The lesser abundance of cardiac/slow-skeletal muscle specific protein would be consistent with a change in muscle phenotype property that showed a shift-toward fast-twitch glycolytic pattern in OB (our results and (Giebelstein *et al.*, 2012)). We reported fast-twitch proteins (**SERCA1, MYH1** and **MYH3)** are greater in abundance but have a lower rate of synthesis in OB muscle. Muscle contraction is triggered by calcium release from the SR and we found greater abundance of **SERCA1,** in addition to **MYH1**, and **MYH3** which are dominant in fast-twitch fibres. We also identified the abundance of slow-twitch myosin heavy chain **MYH7** is less in OB muscle, however, this change was not statistically significant (abundance 0.82-fold change, p = 0.07). Interestingly, FSR of **MYH7** (FSR_{OB} = 4.59 %/day, FSR_{TR} = 1.18 %/day, p = 0.00) was greater in OB than TR which indicate adverse effect of obesity on slow-twitch fibre proteins but the overall outcome is a lesser abundance due to protein degradation.

Our data also report down-regulation of another slow-twitch specific protein, **TNNI1** (Troponin I, slow skeletal muscle). Taken together, our data are consistent with previous literature (Hwang *et al.*, 2010; Lefort *et al.*, 2010; Thingholm *et al.*, 2011; Hussey *et al.*, 2013) and the outcome of our systematic review and meta-analysis that the muscle of OB/T2DM patients likely has a greater proportion of fast-twitch fatigable fibres. Obesity can be an outcome of a long-term sedentary lifestyle which includes physical inactivity that may affect muscle fibre proportions. Therefore, endurance exercise training may be a targeted and effective intervention to counteract this adverse outcome.

Limitations

The major limitation of the work reported in this chapter is that we cannot tell whether the differences in muscle proteins are due to obesity or physical activity in isolation. Participants in both OB and TR groups reported similar activity levels i.e. sedentary, light activity, moderate to vigorous physical activity (MVPA) and step counts but had markedly different peak exercise capacities and body mass index. Our primary statistical analysis filtered out some proteins because they did not meet the minimum quality threshold for data analysis. This is typical of all proteomics work in that the requirements needed to identify a protein are easily meet but it is much more difficult to quantify a protein, and more difficult again to both quantify a protein and measure its rate of synthesis. Generally, Student's t-test has been recommended to test between 2 groups and ANOVA is used for 3 group comparisons. This is appropriate when data is normally distributed. Proteomic data may be distributed following F-curve which is highly skewed in small sample sizes. Student's t-test could result in reporting bias, therefore, we applied ANOVA for the primary statistical analysis.

Conclusion

The current study is the first to report both the abundance and turnover of individual proteins in human skeletal muscle of obese sedentary compared to lean trained individuals. Over 200 proteins were identified and we reported FSR in 52 proteins, the majority of proteins included in our analysis were myofibrillar and cytoskeleton proteins. We report differences in protein abundance and turnover between OB and TR muscle that extend the previous literature on protein abundance only that was included in our systematic review and meta-analysis (Chapter II). We have found 6 proteins that had greater abundance but lesser rate of fractional synthesis, including: **CAH3**, **HSPB1, G3P, AT2A1, KCRM,** and **MYH1**. **ALDOA** showed both greater abundance and FSR whereas **ACTN2** decreased in both abundance and FSR. This provides new insight in to the adverse effect of obesity on skeletal muscle and points to a lesser quality of some proteins in the muscle of obese individuals.

Chapter V

Longitudinal Analysis of Muscle Proteome Responses to High-Intensity Interval Training (HIT) in Obese Individuals

Contents

Summary

Obesity and a sedentary lifestyle are associated with poor metabolic function in skeletal muscle that contributes to poor whole-body metabolism and glucose homeostasis. In the previous chapter we reported dynamic proteome profiling of muscle in obese sedentary (OB) vs lean trained (TR) individuals using a cross-sectional experiment design. We were able to confirm our working hypothesis that obesity and low physical activity are associated with adverse effects on both the abundance and turnover of skeletal muscle proteins.

The current chapter reports follow-on work conducted in the OB individuals reported in Chapter IV. The OB participants (N = 4) performed a 10-week-HIT program and during the final 2 weeks of the exercise intervention, deuterium labelling was performed and biological samples were collected at 4 time points (day 0, 4, 9, 14) consistent with the methods reported in Chapter IV. The clinical and physiological characteristics were also measured to enable longitudinal analysis of physiological performance and muscle proteome dynamics from baseline (i.e. Chapter IV data) to after HIT (i.e. new data reported in this Chapter).

HIT improved the insulin sensitivity (17%, Matsuda index: OB_{pre} = 1.70±0.6, OB_{post} = 2.00±1.0, p = 0.00) and maximum oxygen uptake (VO_{2max}, 9%: OB_{pre} = 26.2±3.6 $kg.m^{-2}$, OB_{post} = 28.6±6.0 $kg.m^{-2}$, p = 0.25). We identified 231 proteins and 41 of them showed statistically significant (p < 0.05) changes in abundance between pre vs post study. Fractional synthesis rate (FSR) were obtained 97 proteins and 31 proteins had a significantly (p < 0.05) different FSR after HIT.

Five proteins: **COX2** (cytochrome C oxidase subunit 2), **TPIS** (Triosephosphate isomerase), **ALDOA** (Fructose-bisphosphate aldolase A), **ACTN2** (α-actinin 2), and **MYH1** (myosin-1). Moreover, **CAH3** (Carbonic anhydrase 3), **G3P** (Glyceraldehyde-3-phosphate dehydrogenase), **AT2A1** (Sarcoplasmic/endoplasmic reticulum calcium ATPase 1), and **KCRM** (Creatine kinase M-type) that were significantly different between OB and TR muscle (reported in Chapter IV) demonstrated changes in abundance or turnover rate that indicate a positive response to the 10-week HIT protocol.

Introduction

The link between exercise capacity and all-cause mortality is irrefutable and has been reviewed extensively in recent years(Warburton, Nicol and Bredin, 2006). A person's health status can be defined by their $V_{O2\,max}$ measured using a graded exercise test (Myers *et al.*, 2002). Using this technique, low aerobic capacity has consistently been shown to be a stronger predictor of early mortality than other established risk factors such as a primary contributor factor to the prevalence of non-communicable diseases, including T2DM, cardiovascular disease and chronic respiratory disease (Warburton, Nicol and Bredin, 2006; Booth, Roberts and Laye, 2012). In many countries the prevalence of inactivity exceeds that of obesity, alcohol misuse and smoking combined, for example, in the UK approximately two-thirds of adults fail to meet the minimum recommendation of 30 minutes moderate exercise, 5 times a week (British Heart Foundation, 2015).

Exercise is recommended by national and international bodies, including the World Health Organisation, American Heart Association and UK Government Guidelines. It is recommended that physical activity should be integrated in to daily lifestyle in order to maintain independence and prevent non-communicable disease, including metabolic syndrome and the development of obesity, T2DM and cardiovascular disease. According to the American Heart Association, statistical data suggest physical inactivity and low aerobic capacity causes atherosclerotic cardiovascular disease associated with hypertension and insulin resistance, and significantly reduces life expectancy. However, regular exercise improves disease incidence rates and or prevents worsening or progression of disease and therefore enhances the quality of life of patients and lessens the burden on healthcare systems (Thompson *et al.*, 2003).

Exercise causes adaptation of the cardiovascular system (Torok *et al.*, 1995; Meyer *et al.*, 2003; Vincent, Bourguignon and Vincent, 2006; Burniston, 2009; Randers *et al.*, 2014), muscle mass (Salanova *et al.*, 2014, 2015), muscle metabolism (Sheffield-Moore *et al.*, 2004; Cuthbertson *et al.*, 2005; Lefort *et al.*, 2010; Egan *et al.*, 2011; Hipkiss, 2011; Hody *et al.*, 2011; Hussey *et al.*, 2013), and weight control (Oscai, 1973; STEFANICK, 1993; Volek, VanHeest and Forsythe, 2005). In healthy individuals skeletal muscle is responsible for the majority of insulin mediated glucose disposal (Grundy *et al.*, 2004), whereas, resistance to the effects of insulin is a characteristic feature of type 2 diabetes (Petersen *et al.*, 2007) and a complex environmental adaptation to lifestyle factors such as physical inactivity and poor dietary habits. Exercise and a generally active lifestyle are a key intervention that can largely prevent chronic diseases (Booth, Roberts and Laye, 2012) as well as counteract or slow the progression of diseases such as T2DM (Warburton, Nicol and Bredin, 2006). It is recommended that in order to reduce the risk of non-communicable diseases, adults should incorporate moderate-intensity exercise of at least 150-300 min per week in to their habitual lifestyle (World Health Organisation, 2015).

In Chapter IV we reported dynamic proteome profiling of muscle in obese sedentary (OB) vs lean trained (TR) individuals using a cross-sectional experiment design. We were able to confirm our working hypothesis that obesity and low physical activity are associated with adverse effects on both the abundance and turnover of skeletal muscle proteins. In particular, we discovered several proteins (including: **CAH3**, **HSPB1, G3P, AT2A1, KCRM,** and **MYH1**. **ALDOA)** had a greater abundance but lesser rate of fractional synthesis in the muscle of obese sedentary individuals. This provides new insight in to the adverse effect of obesity on skeletal muscle and points to a lesser protein quality in the muscle of obese individuals but it is not clear whether these outcomes were due to obesity per se or the sedentary lifestyle of the obese participants.

This Chapter aims to determine the muscle proteome response in a metabolic disease-prone phenotype (OB) by longitudinal analysis of a 10-week programme of high-intensity interval training (HIT). HIT has been implemented as an optimal form of endurance exercise. HIT increases aerobic capacity, improves insulin sensitivity and is a more time-efficient training strategy than continuous low-intensity endurance exercises, such as jogging. HIT demonstrates benefits in terms of increases in aerobic capacity, improved insulin sensitivity, and is a time-efficient training strategy (Cocks *et al.*, 2013; Shepherd *et al.*, 2013). Moreover, in a systematic review and meta-analysis of 26 randomised controlled trials of HIT that enrolled (n = 318) participants (Wang *et al.*, 2009), a clear positive impact of HIIT on clinical outcomes was demonstrated. HIT is associated with positive muscle adaptations including improvements in glycogen synthase abundance (Christ-Roberts *et al.*, 2004), glucose transporter isoform 4 (GLUT4) (Hughes *et al.*, 1993; Dela *et al.*, 1994; Christ-Roberts *et al.*, 2004; Kim, Lee and Kim, 2004), insulin signaling proteins, and lipid metabolism (Bruce *et al.*, 2004; Kim, Lee and Kim, 2004). Therefore, HIT is recommended as an effective alternative strategy for endurance training that has a reduced volume of activity/ time commitment.

Methods

Study design

To investigate the effects of HIT on muscle proteome dynamics, data collected in Chapter IV will serve as the baseline data, including the physiological characteristics of the obese (OB participants and the dynamic muscle proteome data). The current chapter reports the HIT intervention and data collected 'post-exercise' in order to perform longitudinal analysis of the effects of HIT on the muscle proteome dynamics of obese individuals' that are now physically active, i.e. undertaking a prescribed exercise training programme.

In this study, the four sedentary, obese (OB) subjects from Chapter IV took part in a 10-week high intensity interval training (HIT) intervention. During the final two weeks (week 9 and 10) of the training intervention, subjects repeated the experimental protocol i.e., daily deuterium consumption, saliva collection, physical activity monitoring, recording of dietary consumption; bi-daily visits to the laboratory for blood sample collection, and 4 time visits (day 0, 4, 9, and 14) to the laboratory for muscle biopsies (identical in all respects to that described in Chapter IV). Post-training assessments of exercise capacity (VO_{2max}), insulin sensitivity (oral glucose tolerance test) and body composition (DEXA) were performed 72 h following the final training session, and were identical in all respects to those described in Chapter IV.

HIT intervention program

The HIT training protocol was similar to the protocol used by Gillen et al.(Gillen *et al.*, 2013) Subjects performed repeated bouts of cycling at 100% maximum aerobic power (W_{max}) for 60 s, interspersed with 60 s low-intensity recovery cycling at 50 W maintaining a cadence <50 rpm. Subjects trained three times per week for 10 weeks, and were excluded from the study if they were absent from more than two sessions. Initially, subjects performed 4 intervals per training session, and this increased by 2 intervals every 2 weeks, such that subjects performed 8 intervals per training session in weeks 9 and 10.

Statistical analysis

For health and characteristic data, *Student independent t-test* has been applied for NHST before and after study by using SPSS version 23 (IBM). Insulin and glucose data were calculated by area under the curve, which was plotted by each time point (0, 30, 60, 90, and 120 min). For protein abundance change, ANOVA was applied in order to accommodate non-parametric (*F* distribution) and control potential bias due to low sample size. Protein abundance changes were presented in log-space and a cut off at $p < 0.05$ was used to determine statistical significance. The FDR or *q*-value were also present in the **Appendix 7.**

FDR has been applied to test false positives among the rejected hypothesis results. Log transformation of protein abundance changes was applied to present increases or decreases in protein abundance equidistant about zero **(Figure 16 and Figure 17).**

Results

Physical and Health data

Data pertaining to body composition, insulin sensitivity, exercise capacity and habitual dietary intake in TR and OB individuals is presented in **Table 11**. No significant changes were detected for any of the reported variables ($p<0.05$), but training increased VO_{2peak} ~9% and improved insulin sensitivity by 17%**.** Habitual energy and macronutrient intake were not different from pre- to post-training.

Table 14 Physical and health data prior to and after the HIT intervention.

	Pre-HIT (n = 4)		**Post-HIT (n = 4)**		***p*-value**
	Mean ± SD	**(min-max)**	**Mean ± SE**	**(min-max)**	
Physical characteristics in OB group					
Age (y)			38 ± 5 (33,45)		
Weight (kg)	110.0 ± 18.2	(87.2-136.2)	109.6 ± 19.5	(83.9-137.6)	0.72
Height (cm)	179.7 ± 3.6	(176-183.5)	179.7 ± 3.6	(176-183.5)	0.39
BMI ($kg.m^{-2}$)	34.0 ± 5.0	(28.15-40.45)	33.8 ± 5.3	(27.09-40.86)	0.59
V_{O2peak} ($L.min^{-1}$)	26.2 ± 3.8	(20.5-30.8)	28.6 ± 6.0	(19.1-34.9)	0.25
Insulin sensitivity					
Glucose (AUC)	979.25 ± 211.4	(359.0-1,215.0)	862.75 ± 119.0	(413.0-1,000.0)	0.99
Insulin (AUC)	13,426 ± 7162	(9,170.0-21,694.8)	12970.33 ± 6727.4	(8,795.0-20,731.0)	0.8
Matsuda index	1.7 ± 0.6	(1.2-2.4)	**2.00 ± 1.0**	**(1.1-3.1)**	**0.00****
Abdominal fat (excluded head)					
VAT (g)	766.25 ± 166.0	(574.0-973.0)	765.50 ± 181.5	(537.0-979.0)	0.98
%fat	30.2 ± 7.5	(22.4-39.6)	29.7 ± 6.1	(22.4-38.8)	0.3
Fat mass (g)	32,932.1 ± 14,110.2	(18,721.3-51,451.3)	32,349.3 ± 13,967.8	(18,167.2-33,600.8)	0.75
Lean mass (g)	70,319.4 ± 5,716.4	(62,692.8-76,400.1)	70,627.5 ± 7,484.9	(60,479.0-78,372.3)	0.23
Physical activity					
Step counts (n=2)	5,383.00 ± 1388.8	(4,401-8,649)	5,564.50 ± 1645.4	(3,919.1, 6,728.0)	0.5
Dietary consumption					
Total calories intake (kcal/day)	2,003 ± 814.8	(1,063.8-2,519.9)	1,716.33 ± 177.1	(1598.9-1920.0)	0.57
Fat (%)	28.7±6.3	(22.7-35.4)	29.1±3.2	(26.5-32.8)	0.65
Carbohydrate (%)	44.0±8.3	(38.9-53.6)	47.9±6.7	(40.2-52.0)	0.77
Proteins (%)	18.6±1.3	(17.6-20.0)	15.9±2.4	(13.3-18.0)	0.26

Proteomic analysis

Human protein profile

This study is a longitudinal experiment and aims to produce dynamic proteome profiling data on the response of human muscle to HIT. Proteomic analysis of the sarcoplasmic fraction identified 131 proteins and the myofibrillar fraction included 100 proteins (**Figure 16**). There were 16 proteins that changed significantly in abundance **(Figure 17)** in the sarcoplasmic fraction and 30 proteins **(Figure 18)** from myofibrils were alter in abundance significantly ($p < 0.05$). Further information of the identified proteins including statistical significance of exercise responsive proteins is provided in **Appendix 7**.

The direction (i.e. either increase or decrease in response to HIT) of abundance changes in individual proteins is plotted in **Figure 17** (sarcoplasmic fraction) and **Figure 18** (myofibrillar fraction). The majority of proteins were well-defined muscle proteins specific to the myofibrillar and cytoskeletal structures, and 3 clusters of myofibril proteins (**TPM2;TPM4, KCRS;KCRU,** and **ACTS;ACTB;POTEF;ACTBM;POTEI;ACTBL**) were found to be more abundant in the sarcoplasmic fraction after HIT. Cytoskeletal proteins (n=9) were a major group of proteins in the myofibrillar fraction. One cytoskeletal protein (**DYH7**; Dynein heavy chain 7, axonemal) showed up-regulation but 8 others (**TAOK2;**, Serine/threonine-protein kinase TAO2, **MPP9;** M-phase phosphoprotein 9, **MICA3;** [F-actin]-monooxygenase MICAL3, **KLHL5;** Kelch-like protein 5, **IF122;** Intraflagellar transport protein 122 homolog, **ERC2;** ERC protein 2, and **DESM;** Desmin) demonstrated down-regulation in response to exercise.

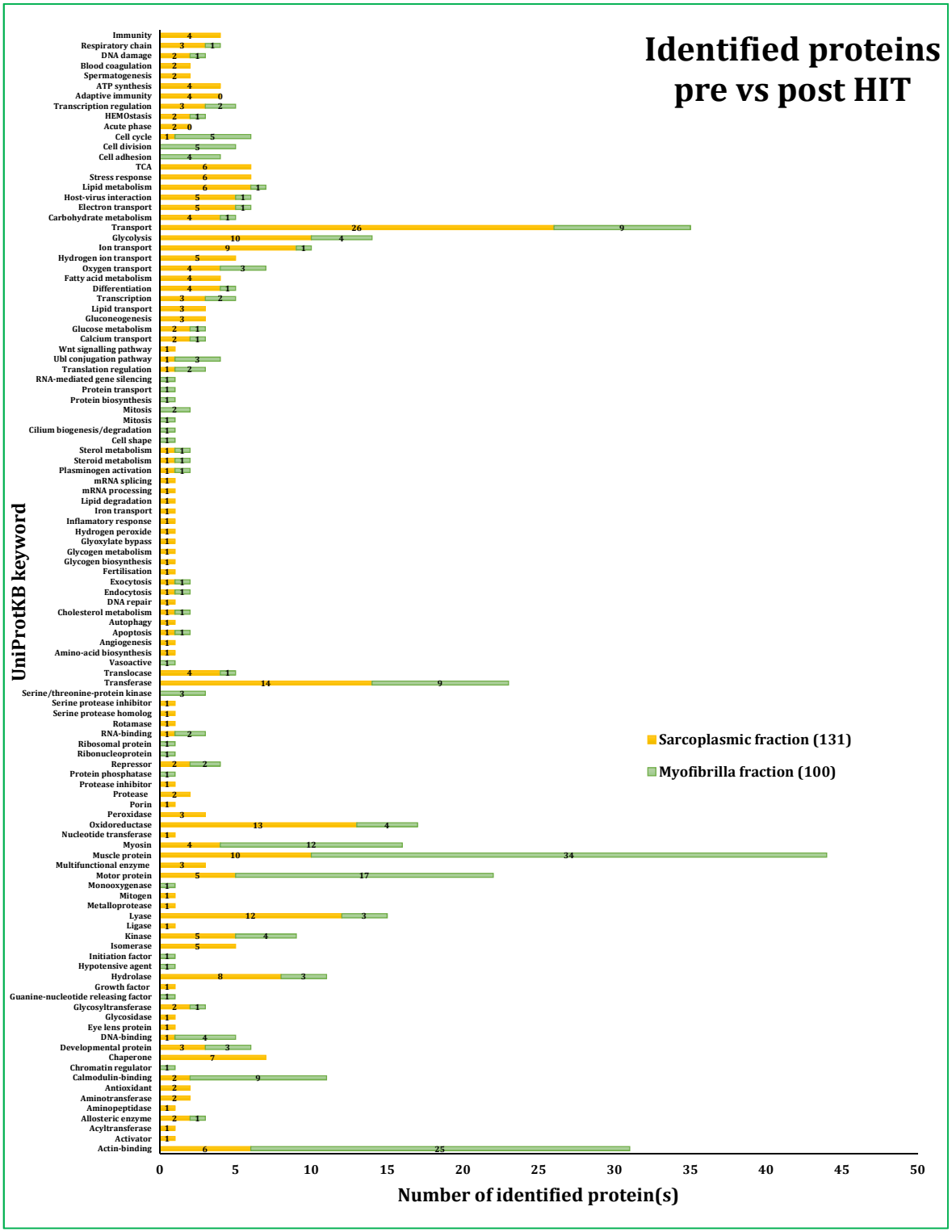


Figure 16 Human muscle protein profile of OB individual from different fraction; sarcoplasma and myofibril.

Sarcoplasmic fraction was revealed Transport proteins (26/131), Transferase functional proteins (14/131), and Oxidoreductase functional proteins (13/131) were as the first 3 major groups among other proteins, respectively. However, Myofibrillar fractions was detected muscle proteins (34/100) was the most major group followed by actin-binding function (25/100) and Motor protein (17/100). Sarcoplasmic fraction (131 proteins) revealed a greater number of proteins than that were measured in myofibrillar fraction (100 proteins). Among this, 20 proteins were found in both fractions. All protein list by Molecular function and Biological process (UniProtKB) were given in **Appendix 8**.

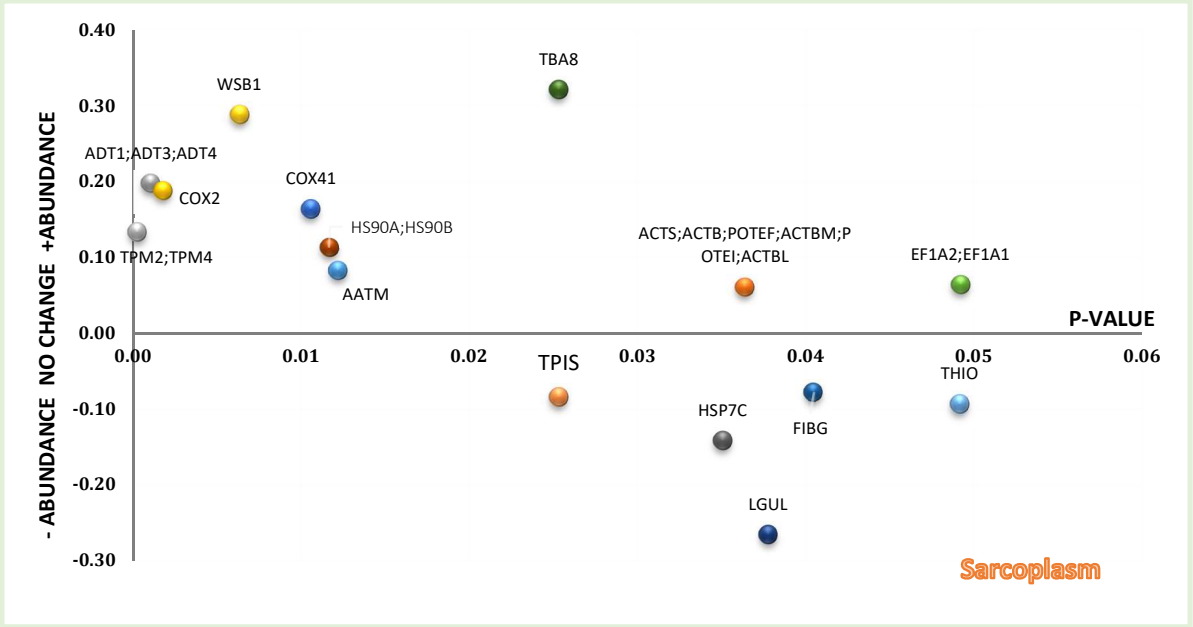


Figure 17 Changes in the abundance of sarcoplasmic proteins in response to HIT.

Data are presented as the Log fold-change in normalised abundance after within-subject analysis of baseline (Pre-) versus post-exercise (Post) samples (n= 4, in each group). These proteins revealed up regulation in 10 proteins and down regulation in 6 proteins from the profile. Most abundance change in proteins were function in muscle (ACTS&ACTB&POTEF&ACTBM&POTEI&ACTBL, KCRS&KCRU, and TPM2&TPM. Further information were given in **Appendix 9.**

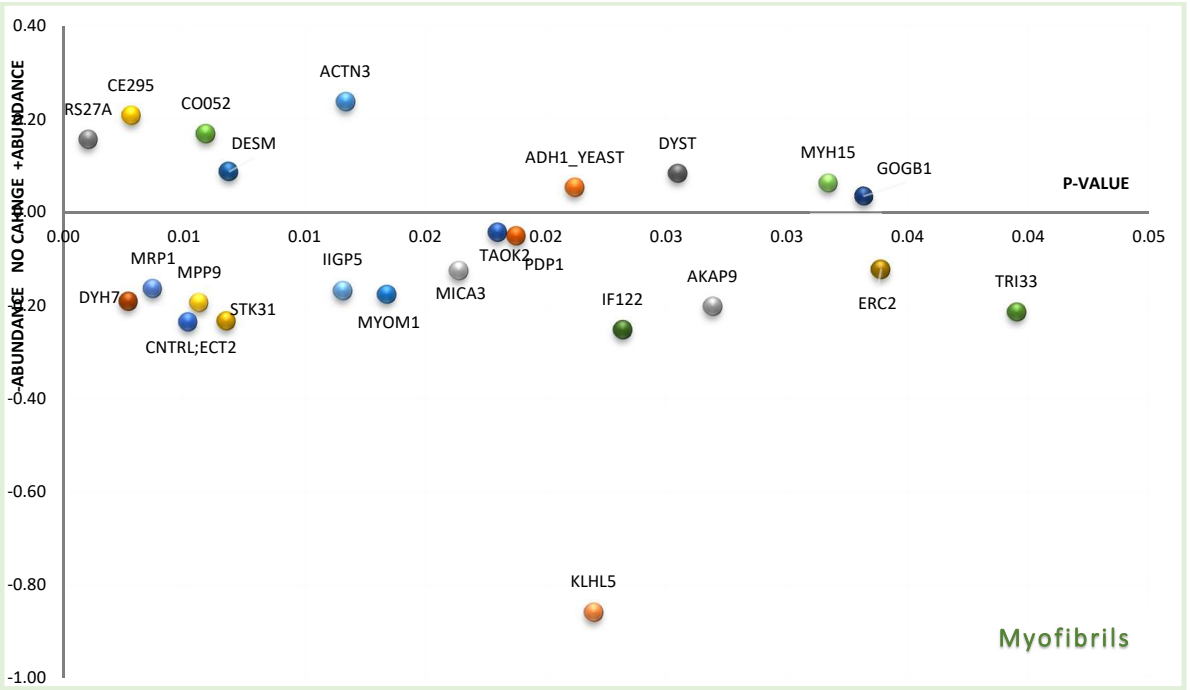


Figure 18 Changes in the abundance of myofibrillar proteins in response to HIT

Data are presented as the Log fold-change in normalised abundance after within-subject analysis of baseline (Pre-) versus post-exercise (Post) samples (n= 4, in each group). Among these, 10 proteins increased abundance whereas 15 proteins decrease its regulation. Eight (8) proteins which function in cytoskeletal structure found down-regulation, but only DESM increased abundance regarding HIT.

Human Protein turnover rate study

A number of proteins from sarcoplasmic fraction (n=66) were increased (n=21), decreased (n=16), but some proteins (n=29) could only be measured in either pre or post samples so could not be submitted for difference testing. Analysis of the myofibrillar fraction included peptides from 45 proteins which increased (n=23), decreased (n=15), and 7 proteins could only be detected at one time-point. The data on statistically significant differences in protein abundances and synthesis rates were combined and **Table 15** reports 1 protein from sarcoplasmic fraction and 2 proteins from myofibrillar fraction that exhibited significant differences in both abundance and synthesis rate in response to the HIT intervention. Proteins that had statistically significant differences in abundance only or FSR only are reported in **Table 16. Table 17** presents a comparison of the dynamic proteome response to HIT against the previously reported (Chapter IV) cross sectional analysis of OB versus TR muscle.

Table 15 Three (3) highlight proteins that show statistical significance in both abundance and FSR.

	Protein accession	Function	abundance change		Newly fractional synthesis rate (FSR)			Fraction
			Fold change of OB/TR	*p*	Pre-HIT FSR±SD (%/day)	Post-HIT FSR±SD (%/day)	*p*	
Reported statistically significant in abundance only								
1	**ACTN3**	**Actin binding/ focal adhesion assembly/muscle filament sliding/negative regulation of calcineurin-NFAT signaling cascade/negative regulation of glycolytic process/ negative regulation of oxidative phosphorylation/ positive regulation of glucose catabolic process to lactate via pyruvate/positive regulation of skeletal muscle tissue growth/regulation of aerobic respiration/ regulation of apoptotic process /regulation of the force of skeletal muscle contraction/ response to denervation involved in regulation of muscle adaptation/ skeletal muscle atrophy/ transition between fast and slow fiber**	**1.73**	**0.01***	**0.66±0.00**	**0.96±0.00**	**0.60**	**Myofibrils**
2	**DESM**	**Muscle protein/ intermediate filament organization**	**1.22**	**0.01***	**11.90±0.03**	**3.43±0.00**	**0.00****	**Myofibrils**
3	COX2	cytochrome-c oxidase activity/ Electron transport/ Respiratory chain/ Transport/ mitochondrial electron transport, cytochrome c to oxygen	1.54	0.00	2.95±0.00	3.91±0.00	NA	**Sarcoplasm**

COX2 (Cytochrome C oxidase subunit 2) and **ACTN3** (α-actinin-3) showed greater expression and revealed increased FSR in response to 10-wee-HIT. However, **DESM** (Desmin) were revealed increased abundance but reduced FSR after exercise. The remaining protein FSR were presented in **Appendix9.** (*$p < 0.05$ and **$p < 0.01$)

Table 16 Proteins that show statistical significance in either abundance only or FSR only.

Protein accession		Function	abundance change Fold change of Pre-HIT/ Post-HIT	*p*	Newly fractional synthesis rate (FSR) Pre-HIT FSR±SD (%/day)			Post-HIT FSR±SD (%/day)			*p*	Fraction
Reported statistically significant in abundance only												
1	**AATM**	**Aminotransferase, Transferase/L-aspartate:2-oxoglutarate aminotransferase activity/RNA binding/ 2-oxoglutarate metabolic process/aspartate catabolic process/aspartate metabolic process/ Lipid transport, Transport fatty acid transport/ glutamate metabolic process/response to ethanol**	**1.21**	**0.01***	**2.93**	**±**	**0.00**					**Sarcoplasm**
2	**TPIS**	**Isomerase/ Lyase/ methylglyoxal synthase activity/ protein homodimerization activity/ triose-phosphate isomerase activity/ Gluconeogenesis/ Glycolysis/ glyceraldehyde-3-phosphate biosynthetic process/ methylglyoxal biosynthetic process**	**0.82**	**0.03***	**0.91**	**±**	**0.00**					**Sarcoplasm**
Report statistically significant in FSR only												
3	**ALDOA**	**Lyase/ RNA binding/Glycolysis/ protein homotetramerization**	**0.93**	**0.39**	**2.02**	**±**	**0.00**	**1.52**	**±**	**0.00**	**0.04***	**Myofibrils**
4	**MYH1**	**Actin binding/ Calmodulin binding/ Motor protein/ Muscle protein/ Myosin**	**0.84**	**0.20**	**0.78**	**±**	**0.00**	**1.06**	**±**	**0.00**	**0.00****	**Myofibrils**
5	**ACTN2**	**Actin binding/cytoskeletal protein binding/FATZ binding/ integrin binding/ion channel binding/phosphatidylinositol-4,5-bisphosphate binding/ protein dimerization activity/ protein domain specific binding/protein homodimerization activity/ titin Z domain binding/cell adhesion/focal adhesion assembly/microspike assembly/phospholipase C-activating angiotensin-activated signaling pathway/positive regulation of cation channel activity/positive regulation of endocytic recycling/ protein homotetramerization/protein localization to plasma membrane/regulation of apoptotic process/sarcomere organization**	**0.96**	**0.36**	**1.01**	**±**	**0.00**	**1.46**	**±**	**0.00**	**0.00****	**Myofibrils**
6	**MYPC2**	**Actin-binding/ Muscle protein/ Cell adhesion**	**0.95**	**0.35**	**1.95**	**±**	**0.00**	**1.81**	**±**	**0.00**	**0.00****	**Myofibrils**
Report non-statistically significance in both abundance and FSR but highlight in OB vs TR study												
7	CAH3	Lyase	0.93	0.50	0.73	±	0.00	1.39	±	0.00	NA	Sarcoplasm
8	HSPB1	Chaperone/ identical protein binding/ protein folding chaperone/protein homodimerization activity/ protein kinase binding/ RNA binding/ Stress response/regulation of protein phosphorylation/ response to virus/ retina homeostasis	1.08	0.36	1.30	±	0.00					Sarcoplasm

Protein accession		Function	abundance change Fold change of Pre-HIT/ Post-HIT	*p*	Newly fractional synthesis rate (FSR) Pre-HIT FSR±SD (%/day)			Post-HIT FSR±SD (%/day)			*p*	Fraction
9	G3P	Oxidoreductase/ Transferase/aspartic-type/ endopeptidase inhibitor activity/ microtubule binding/ Apoptosis/ Transferase/ Glycolysis/ Translation regulation/ antimicrobial humoral immune response mediated by antimicrobial peptide/ cellular response to interferon-gamma/ defense response to fungus/ killing by host of symbiont cells/ killing of cells of other organism/ microtubule cytoskeleton organization/ negative regulation of endopeptidase activity/ negative regulation of translation/ neuron apoptotic process/ peptidyl-cysteine S-trans-nitrosylation/ positive regulation by organism of apoptotic process in other organism involved in symbiotic interaction/ positive regulation of cytokine secretion/ protein stabilization/ regulation of macroautophagy	0.96	0.28	1.68	±	0.00	1.63	±	0.00	0.49	Myofibrils
10	AT2A1	Translocase/ ATPase activity/ ATP binding/calcium ion binding/ calcium-transporting ATPase activity/ Calcium transport/ Ion transport/ Transport/ calcium ion transport/ negative regulation of striated muscle contraction/ positive regulation of fast-twitch skeletal muscle fiber contraction/ regulation of striated muscle contraction	0.83	0.05	1.43	±	0.00	0.65	±	0.00	NA	Sarcoplasm
11	AT2A1				1.93	±	0.00					Myofibrils
12	KCRM	Kinase/ Transferase/ ATP binding	0.93	0.44	0.93	±	0.00	0.76	±	0.00	NA	Sarcoplasm
Incomplete information in this study but found statistical significance in abundance in OB vs TR study												
13	PARK7	Chaperone/ Hydrolase/ Protease/ RNA-binding/copper ion binding/ kinase binding/mercury ion binding/mRNA binding/peptidase activity/protein homodimerization activit/signaling receptor binding/ Autophagy/ DNA damage/ DNA repair/ Fertilization/ Inflammatory response/ Stress response/ autophagy/ cellular response to hydrogen peroxide/detoxification of copper ion/detoxification of mercury ion/DNA repair/glucose homeostasis/guanine deglycation/guanine deglycation, glyoxal removal/guanine deglycation, methylglyoxal removal/inflammatory response/ insulin secretion/mitochondrion rganization/negative regulation of cell death/negative regulation of extrinsic apoptotic signaling pathway/negative regulation of protein binding/negative regulation of reactive oxygen species biosynthetic process/positive regulation of acute inflammatory response to antigenic stimulus/positive regulation of NAD(P)H oxidase activity/protein deglycosylation/regulation of androgen receptor signaling pathway/regulation of inflammatory response/regulation of neuron apoptotic process/single fertilization	0.83	0.06	0.77	±	0.00					Sarcoplasm
14	CASQ1	Muscle protein/ positive regulation of release of sequestered calcium ion into cytosol/ positive regulation of store-operated calcium channel	0.83	0.16	0.71	±	0.00	1.60	±	0.00	NA	Myofibrils

	Protein accession	Function	abundance change Fold change of Pre-HIT/ Post-HIT	*p*	Newly fractional synthesis rate (FSR) Pre-HIT FSR±SD (%/day)			Post-HIT FSR±SD (%/day)			*p*	Fraction
		activity/ protein polymerization/ regulation of skeletal muscle contraction by regulation of release of sequestered calcium ion/ regulation of store-operated calcium entry/ sarcomere organization										
15	KLH41	myofibril assembly/ protein ubiquitination/ regulation of myoblast differentiation/ regulation of myoblast proliferation/ skeletal muscle cell differentiation	0.78	0.17	3.11	±	0.00	1.59	±	0.00	NA	Myofibrils
16	APOA1	Cholesterol metabolism/ Lipid metabolism/ Lipid transport/ Steroid metabolism/ Sterol metabolism/ Transport/ chemorepellent activity/ integrin-mediated signaling pathway/ negative chemotaxis/ peptidyl-methionine modification/ positive regulation of cholesterol efflux/ positive regulation of phagocytosis/ positive regulation of phospholipid efflux/ positive regulation of Rho protein signal transduction/positive regulation of stress fiber assembly/ positive regulation of substrate adhesion-dependent cell spreading/ protein oxidation/ protein stabilization	1.11	0.19	4.21	±	0.00					Sarcoplasm
17	MLRV	Motor protein/ Muscle protein/ Myosin/ heart development/ positive regulation of the force of heart contraction/ regulation of the force of heart contraction	0.94	0.46	4.52	±	0.00	6.13	±	0.02	0.39	Myofibrils
18	MYL6B	Motor protein/ Muscle protein/ Myosin			13.10	±	0.01	9.19	±	0.00	NA	Myofibrils
19	CISY	Transferase/ citrate (Si)-synthase activity/ RNA binding/ Tricarboxylic acid cycle/ carbohydrate metabolic process	0.97	0.54	13.74	±	0.00					Sarcoplasm
20	TRI72	phosphatidylserine binding/ Exocytosis/ Transport/ exocytosis/ muscle organ development/ muscle system process/ plasma membrane repair/ protein homooligomerization	1.13	0.28	2.67	±	0.00					Sarcoplasm

AATM (Aspartate aminotransferase, mitochondrial) and **TPIS** (Triosephosphate isomerase) are proteins from sarcoplasmic fraction that showed statistically significant in abundance but revealed FSR in OB pre-HIT only. **ALDOA** (Fructose-bisphosphate aldolase A), **MYH1** (Myosin-1), **ACTN2** (α-actinin 2), and **MYPC2** (Myosin-binding protein C, fast-type**)** reported FSR remarkably change FSR in response to HIT and the remaining 14 proteins were reported their abundance/FSR in previous Chapter.

Table 17 The comparison and trend of abundance change and FSR among 3 different case groups across 23 proteins.

			abundance change			Newly fractional synthesis rate (FSR)				Trend	
			Fold change (FC)			Chapter IV		Chapter V			
		Function	Chapter IV		Chapter V	FSR±SD (%/day)					
Protein accession			TR	OB	Post-HIT	TR	OB or Pre-HIT		Post-HIT	abundance	FSR
1	**ACTN3**	**Muscle contraction**			**1.73** **0.01***			**0.66** **0.60**	**0.96**	**OB<post-HIT**	**OB<post-HIT**
2	**DESM**	**Cytoskeleton/ structure**			**1.22** **0.01***			**11.90** **0.00****	**3.43**	**OB<post-HIT**	**post-HIT<OB**
3	**COX2**	**OXPHOS**	**1.82** **0.00****	**0.55**	**1.54** **0.00****	**6.56** **NA**	**2.95**	**2.95** **NA**	**3.91**	**OB<post-HIT<TR**	**OB<post-HIT<TR**
4	**TPIS**	**Glucose metabolism**	**0.75** **0.01***	**1.34**	**0.82** **0.03***	**0.98** **NA**	**0.91**	**0.91**		**TR<post-HIT<OB**	**OB<TR**
5	**AATM**	**Lipid metabolism**	**1.06** **0.42**	**0.94**	**1.21** **0.00****	**36.48** **0.00****	**2.93**	**2.93**		**post-HIT>TR>OB**	**OB<TR**
6	**ALDOA**	**Glucose metabolism**	**0.82** **0.02***	**1.22**	**0.93** **0.39**	**1.19** **0.00****	**2.30**	**2.02** **0.04***	**1.52**	**TR<post-HIT<OB**	**TR<post-HIT<OB**
7	**ACTN2**	**Muscle contraction**	**1.2** **0.00****	**0.84**	**0.96** **0.36**	**0.94** **0.00****	**0.88**	**1.01** **0.00****	**1.46**	**OB<post-HIT<TR**	**OB<TR<post-HIT**
8	**MYH1**	**Muscle contraction**	**0.63** **0.01***	**1.59**	**0.84** **0.2**	**50.75** **0.00****	**0.83**	**0.78** **0.00****	**1.06**	**TR<post-HIT<OB**	**OB<post-HIT<TR**
9	CAH3	Carbonate dehydratase activity	0.57 **0.00****	1.75	0.93 0.5	1.62 0.01*	0.73	0.73 NA	1.39	TR<post-HIT<OB	OB<post-HIT<TR
10	HSPB1	Cell death	0.84 0.01*	1.18	1.08 0.36	2.79 0.00**	1.30	1.3		TR<post-HIT<OB	OB<TR
11	G3P	Glucose metabolism	0.70 **0.00****	1.44	0.96 0.28	16.44 **0.00****	1.04	1.68 0.49	1.63	TR<post-HIT<OB	post-HIT<OB<TR
12	AT2A1	Muscle contraction	0.79 0.02	1.27	0.83 0.05	12.96 **0.00****	1.43	1.43 NA	0.65	TR<post-HIT<OB	post-HIT<OB<TR
13	KCRM	Muscle contraction	0.68 **0.00****	1.48	0.93 0.44	0.93 **0.00****	0.86	0.93 NA	0.76	TR<post-HIT<OB	post-HIT<OB<TR
14	MLRV	Muscle contraction	1.4 **0.00****	0.72	0.94 0.46	0.67		4.52 0.39	6.13	OB<post-HIT<TR	OB<post-HIT
15	MYPC2	Muscle contraction	0.85 0.03*	1.18	0.95 0.35		2.27	1.95 **0.00****	1.81	TR<post-HIT<OB	post-HIT<OB
16	PARK7	Cell death	0.76 0.02*	1.32	0.83 0.06	1.37		0.77		TR<post-HIT<OB	NA
17	CASQ1	Cytoskeleton/ structure	0.65 0.01*	1.54	0.83 0.16	0.70 NA	0.71	0.71 NA	1.6	TR<post-HIT<OB	TR<OB<post-HIT
18	KLH41	Cytoskeleton/ structure	1.84 **0.00****	0.54	0.78 0.17	7.70		3.11 NA	1.59	OB<post-HIT<TR	post-HIT<TR
19	APOA1	Lipid metabolism	1.43 0.01*	0.70	1.11 0.19	3.41 NA	4.21	4.21		OB<post-HIT<TR	TR<OB
21	MYL6B	Muscle contraction	1.44 0.01*	0.70			6.72	13.1 NA	9.19	OB<TR	post-HIT<OB
22	CISY	TCA	1.22 **0.00****	0.82	0.97 0.54		14.11	13.74		OB<post-HIT<TR	NA
23	TRI72	Transport	1.52 0.04*	0.66	1.13 0.28	2.28 NA	2.67	2.67		OB<post-HIT<TR	TR<OB

DESM (Desmin) and **ACTN3** (α-actinin 3) are discovered their remarkably change in overabundance and increased FSR in response to 10-week-HIT (shading in **'green'**). The remaining of them were found across Chapter IV. **ALDOA** (Fructose-bisphosphate aldolase A), **ACTN2** (α-actinin 2), and **MYH1** (Myosin-1) were highlighted in Chapter IV and also found their FSR showed significant but not revealed notably change in abundance regarding *p*-value. **COX2** (cytochrome C oxidase subunit 2), **TPIS** (Triosephosphate isomerase), and **AATM** (Aspartate aminotransferase, mitochondrial) reported significantly in abundance in response to HIT but do not reveal complete data of FSR. Shading **'yellow'** indicate proteins which were reported significantly in both abundance and FSR in chapter IV.

Discussion

Effect of High intensity interval training (HIT) on OB skeletal muscle

The current study reported protein abundances and protein turnover in OB individuals in response to 10-week-HIT using dynamic proteomic profiling. The analysis included more than 200 individual proteins from the soluble and myofibrillar muscle fractions. We discovered interesting patterns in protein response, for example DESM increased in abundance significantly but its synthesis rate was less after exercise training, which might suggest regulation primarily by protein degradation. Our current work also highlights changes in both abundance and FSR of 5 proteins that were reported to be different between the muscles of OB vs TR individuals in Chapter IV. Moreover, the direction of changes induced by HIT are similar to the TR condition reported in Chapter IV. These proteins are **COX2** (cytochrome C oxidase subunit 2), **TPIS** (Triosephosphate isomerase), **ALDOA** (Fructose-bisphosphate aldolase A), **ACTN2** (α-actinin 2), and **MYH1** (myosin-1). Moreover, **CAH3** (Carbonic anhydrase 3), **G3P** (Glyceraldehyde-3-phosphate dehydrogenase), **AT2A1** (Sarcoplasmic/endoplasmic reticulum calcium ATPase 1), and **KCRM** (Creatine kinase M-type), which have key roles in muscle metabolism and contraction.

Previous literature on endurance exercise

Similar to Chapter IV, we searched for literature relevant to our current experiment in order to extend the search terms beyond our systematic review (Srisawat *et al.*, 2017) by not limiting the search to hypothesis-generating criteria. We found 4 studies used deuterium oxide as a stable isotope tracer for determining fractional synthesis rates of proteins in response to 3-week-SIT (Shankaran *et al.*, 2016), acute-resistance exercise (Gasier *et al.*, 2012), 9-day-resistive exercise (Camera *et al.*, 2017), and long-term bedrest with 8-day-resistive exercise (Wilkinson *et al.*, 2014). However, 3 (Gasier *et al.*, 2012; Wilkinson *et al.*, 2014; Shankaran *et al.*, 2016) focused on fractional synthesis rate (FSR) without conducting protein abundance measurements. Only 1 article (Camera *et al.*, 2017) reported both protein abundance and FSR responses but this work studied muscle adaptation to the effect of resistance exercise. We found 1 study (Short *et al.*, 2004) performed aerobic training and analysed FSR but focused on outcomes related to aging and used a short-term invasive method for labelling proteins, i.e. inserting vein catheter for infusion C-13 and N-15 isotope-labelled amino acids, and therefore did not report synthesis data on a protein-by-protein basis.

We found 108 publications which reported outcome measures in human muscle biopsy samples in response to endurance exercise. Therefore, despite the research area for human exercise being very large (~145,000 publications), less than one-tenth of one percent of studies reports analysis of invasive

techniques (i.e. muscle biopsy) to collect detailed molecular information. Regarding hypothesis-generating studies, we obtained 4 articles (Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013; Schild *et al.*, 2015) that reported protein abundance in response to endurance training. These are relevant to our current study for discussion but their reports are limited to protein abundance changes in response to endurance training and do not include data on protein synthesis rates. Therefore, the role of protein quality and the synthesis of proteins as an underlying mechanism to the changes in protein abundance still remains a largely unexplored topic. The first proteomic study on abundance changes was released in 2009 (Holloway *et al.*, 2009). Holloway et al (Holloway *et al.*, 2009) investigated the effect of exercise training on human skeletal muscle which was similar to the current thesis. They conducted 6-week-endurance (treadmill) exercise and analysed vastus lateralis muscle proteome by 1D and 2DGE coupled with LC-MS/MS. The report revealed that exercise increased maximal oxygen uptake (6.5%, p = 0.0001) and this was associated with a greater abundance of OXPHOS proteins and mitochondrial trifunctional protein, which is involved in beta-oxidation of fatty acids. Moreover, the 2DGE analysis detected changes in multi-spot proteins which indicate changes in protein phosphorylation or other post-translational modifications in response to exercise training. However, these data are from healthy-weight individuals and the number of experiments that consider responses to exercise in people with T2DM or obesity is small.

Our current study reported protein quality, and is the first work to combine both protein abundance and protein synthesis rate in one experiment. Although our early proteomic work (Holloway *et al.*, 2009) was the first to report longitudinal of effect of endurance exercise on human muscle, it did not include analysis of protein quality (i.e. turnover rates). Two years later, Egan (Egan *et al.*, 2011) reported more advanced analysis of protein abundance data by applying 2D-DIGE and LC-MS/MS and demonstrated that 2-weeks of high intensity exercise induced changes in electron transport chain system, increased oxygen transport, TCA, phosphor-transfer enzymes, and remodelling of skeletal muscle mitochondrial proteome. This work was more advanced than Holloway et al (Holloway *et al.*, 2009) because it used fluorescent DIGE labelling which affords better resolution of the proteome (i.e. more proteins analysed) and more accurate quantification (i.e. because technical variation is improved by using fluorescent dyes). However, the data reported in the current chapter extend beyond these previous studies because we conducted a longer period (10 week) of HIT in individuals at risk of T2DM.

Hussey et al (Hussey *et al.*, 2013) reports proteomics analysis of an endurance training program which consisted of high intensity training 3 d/wk interspersed with moderate intensity training 2d/wk. The duration in this trial was 4 weeks. Up-regulation was found in malate-aspartate shuttle proteins (e.g. **AATM;** Aspartate aminotransferase, mitochondria**, AATC;** Aspartate amino transferase, cytoplasm), which contribute to NADH availability after exercise training. Moreover, they also raised the hypothesis that Exercise training may co-ordinate an increase in the synthesis of mitochondrial proteins, by

enhancing mitochondria-cytoskeleton interaction based on the observation of a 25% increment of **ANXA2** (Annexin A2) proteins in response to 6-week-endurance, but they did not attempt to measure protein synthesis. Nevertheless, they had previously reported cytoskeletal proteins (**PHB2**; Prohibitin 2, **ANXA2**, **PDLIM3**; PDZ and LIM domain protein 3) are required for short movement of mitochondria and alteration of these cytoskeletal proteins may be associated with mitochondrial dysfunction in T2DM. Essentially this process is termed cortical actin cytoskeletal organisation and is associated with proteins such as **ACTN2** and **ANXA2**. Therefore, an enhanced mitochondria-cytoskeletal interaction may occur in response to exercise and may be associated with the synthesis of new mitochondrial proteins. Our current study found similar findings regarding greater abundance of malate-aspartate shuttle proteins (**AATM**), OXPHOS proteins (**COX2**), and decreased glycolytic proteins (**TPIS**). However, we extend these data on protein abundance changes and for the first time also report the effects of HIT on the turnover rate of human muscle proteins. Our result on FSR found the increment in both abundance and FSR of mitochondrial protein **COX2** (abundance = 1.54, p = 0.00; FSR_{pre} = 3.0%/day, FSR_{post} = 3.9%/day, p = 0.07) and cytoskeletal protein ACTN3 (abundance = 1.73, p = 0.01; FSR_{pre} = 0.6%/day, FSR_{post} = 1.0%/day, p = 0.00) in response to 10-week-HIT. In addition, DESM protein was more abundant (abundance = 1.22, p = 0.01; FSR_{pre} = 12.0%/day, FSR_{post} = 3.4%/day, p = 0.00), but the FSR of DESM was less after the HIT intervention. In regard to these changes, it is suggested that HIT not only counteracts the adverse health effect but also improve obese skeletal muscle in a gradual manner. Our 10-week-HIT intervention in an OB population was a longer period of intervention than the previous literature which have been conducted under 2-wk (Egan *et al.*, 2011), 4 wk (Hussey *et al.*, 2013), 6wk-endurance exercise (Holloway *et al.*, 2009), and 3-wk-SIT (Shankaran *et al.*, 2016), and this may explain why we are able to detect some novel changes such as the response of desmin.

A retrospective study (Schild *et al.*, 2015) reported data from athletes (ET) with over 5 years training history (> 5 year endurance trained with VO2max > 57 $ml.min^{-1}.kg^{-1}$) defined as control group and untrained (UT) group (sedentary with VO_{2max} <47 $ml.min^{-1}.kg^{-1}$). The UT group performed endurance exercise with moderate intensity (60% of VO_{2max}) including 10 min of warm-up. The duration of cycling was 60 min in total. Vastus lateralis samples were collected before and after training (3 h post-exercise). The design of this study included 2 different comparisons, i.e. cross-sectional comparison vs ET vs UT and longitudinal comparison of UT prior to and after exercise. Therefore, this publication is similar to the design of our current work but i) the VO_{2max} of our participants was less than in the previous study and there were statistically significant differences between TR vs OB, ii) we applied 10-week-HIT (80-100% intensity) with 1 min bout and 1 min rest, from 4-8 intervals, iii) the VO_{2max} for UT of previous study (Schild *et al.*, 2015) was higher than might be expected for a population defined as 'sedentary' whereas our study set VO_{2max} of sedentary-obese lower than 25 $ml.min^{-1}.kg^{-1}$, and iv) our case group was clearly defined as obesity (OB) with BMI between 29 – 39 $kg.h^{-2}$ whereas Schild et al (Schild *et al.*, 2015) recruited UT with BMI between 20.7 -30.7 $kg.h^{-2}$ which the lower bound was in the range of healthy-

weight individuals. The proteomic method was similar to our laboratory which contained non-bias off-gel LC-MS/MS but measured abundance data only. Schild et al (Schild *et al.*, 2015) reported 92 significant proteins which responded to long-term endurance training whereas ours found 41 notable proteins in response to 10-week-HIT. This indicates our protocol of training was effective and altered almost half of proteins (41/92 = 44.6%) that were found to be different in long-term (>5 y) endurance trained humans. Schild et al (Schild *et al.*, 2015) reported alterations in proteins which function in the OXPHOS system, TCA cycle, fatty acid utilization, malate-aspartate shuttle, and amino acid metabolism. However, only 1 prominent protein was consistent with our study i.e. increased abundance of **COX2** (Cytochrome C oxidase subunit 2), this difference in outcome may be due to the different training modes, i.e. HIT in our current work versus continuous endurance training reported in Schild et al.

Overall, the previous publications measured only protein abundance (Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013; Schild *et al.*, 2015), or only FSR (Shankaran *et al.*, 2016), or contributed both abundance and FSR but in response to resistance exercise training (Camera *et al.*, 2017), or used invasive amino acid labelling for mixed protein FSR measurement (Short *et al.*, 2004). We are the first group to measure both abundance and FSR on a protein-by-protein basis in human muscle and the current work reports the first data on the muscle response to 10-week-HIT. We identified 231 individual proteins, 46 statistically significant changes in abundance, and measured FSR in 106 proteins, whereas in a previous study (Camera *et al.*, 2017) our group identified 90 proteins and found n=28 statistically significant differences in response to resistance exercise. Although Shankaran (Shankaran *et al.*, 2016) identified 513 proteins and measured FSR across 273 proteins, they did not report protein abundance changes across these 273 proteins, which is key to understanding whether changes in synthesis rate lead to changes in protein abundance. Nevertheless, to discuss our protein FSR data the closest relevant data is the former study by Shankaran et al (Shankaran *et al.*, 2016) and Camera et al. (Camera *et al.*, 2017) which performed under 3w-SIT and 9-d-REX, respectively. We reported 5 proteins (**COX2, TCA, ACTN3, DESM**, and **TPIS**) matched with the previous literature and exhibiting statistically significant responses in FSR. These common proteins which have changed in dynamic features may indicated a key role for regulating their turnover in the muscle response to exercise.

Recently, an article (Shankaran *et al.*, 2016) on FSR in response to 3-w-SIT reported higher FSR in glycolytic and skeletal protein when compared against a sedentary group. This work also stated that primary functional impact on the FSR related to muscle anaerobic power and contractile strength. The current thesis consisted of similar stable isotope (D_2O) labelling but also measured protein abundance to investigate protein quality. Our current study used a 10-w-intervention which is a longer period than Shankaran et al and we are the first to highlight the abundance and synthesis changes of novel proteins i.e. **ACTN3, COX2**, and **AATM**. Our training protocol likely imposed a more sustained metabolic demand on the muscle which can be noted by the change in VO_{2max} and depends on the frequency, intensity, and

duration of sprint efforts as well as the recovery between bouts. Shankaran et al (Shankaran *et al.*, 2016) used 3-week SIT involving 4-8 bouts of 30 second maximal effort with 4 minutes recovery periods between each bout. Participants performed 9 sessions where the last session consisted of 4 bouts. However, they did not report any data on VO_{2max} change. On the other hand, our protocol of 10-week-HIT consisted of a progressive protocol over a total of 30 sessions involving 1-minute work periods at 100 % VO_2 max interspersed with 1-minute recovery periods. Furthermore, we reported 9% change in VO_2 max, indicating our protocol induced muscle adaptation by observing increased metabolic demand as increase maximum capacity of oxygen uptake.

Recently, our laboratory (Camera *et al.*, 2017) reported the first proteomic analysis of deuterium oxide (D_2O)-labelled human muscle that included both abundance and synthesis measurements of individual proteins. Participants consumed D_2O coupled with High-fat-low-carbohydrate-diet (HFLC) throughout the exercise training regimen (9-day-resistive exercise, 9d-REX) whereas in the current study we used 2 separate labelling periods (i.e. at baseline and at the 9^{th} – 10^{th} week of HIT training). Camera et al established the new measurement method for studying protein dynamics and reported both protein abundance and FSR/breakdown in human tissue. This study demonstrated different patterns of response on a protein-by-protein basis, these results were in response to resistive exercise of 9 days duration. Our present outcome measures were in line with previous report (Camera *et al.*, 2017) but we reported the effects of an endurance intervention, i.e. high-intensity interval training (HIT).

Effect of High intensity interval training (10-week-HIT) on fitness and insulin sensitivity

Working through the previous literature, we found gaps and limitations among the experiments such as lack of protein quality / synthesis data (Holloway *et al.*, 2009; Egan *et al.*, 2011; Hussey *et al.*, 2013; Schild *et al.*, 2015), recall bias because of retrospective designs (Schild *et al.*, 2015), lack of protein abundance data (Shankaran *et al.*, 2016), articles not related to endurance exercise (Gasier *et al.*, 2012; Wilkinson *et al.*, 2014; Camera *et al.*, 2017), and use of amino acid tracer methods to measure mixed muscle protein synthesis (Short *et al.*, 2004). Thus, the work presented in the current thesis reports a novel study and contributes to the advancement of knowledge on protein abundance and protein quality responses to a sustainable training regimen (10-week-HIT). Our longitudinal study reported numerous proteins in response to 10 weeks HIT and demonstrate protein abundance profiles and protein turnover data. Our data found that after 10 week of HIT in OB individuals increased VO_{2max} 9 % and increased insulin sensitivity 17 % which is consistent with previous reports (Holloway *et al.*, 2009; Cocks *et al.*, 2013; Shepherd *et al.*, 2013). Increased insulin sensitivity may be related to the effect of exercise on insulin signalling networks and possible changes to the post-translational modifications of proteins involved in the insulin signalling cascade (Deshmukh, 2016). A phosphoproteomic study by Hoffman et al (Hoffman *et al.*, 2015) found 1,004 phosphosites on 562 proteins that were responsive to a single bout

of high-intensity exercise. Interestingly, we found a greater abundance of insulin-regulated proteins (**ADT1;ADT3;ADT4**; ADP/ATP translocase-1,3,and 4 = 1.57 fold change, $p = 0.00$). This change suggests the responsiveness to insulin is elevated in response to HIT (Malin *et al.*, 2016). Exercise has been considered as insulin mimetic, particularly high-intensity or long duration exercise interventions have been associated with an increase in skeletal muscle glucose uptake through enhancing insulin sensitivity (Rose, Kiens and Richter, 2006). We also identified muscle glycogen phosphorylase tended to be of greater abundance (**PYGM;PYGL**; Glycogen phosphorylase, muscle form; Glycogen phosphorylase, liver form = 1.16 fold change, $p = 0.46$), but this did not reach statistical significance.

We also have observed an augmented VO_{2max} after 10-week-HIT. The 9% increment of VO_{2max} suggests HIT is associated with an increase in oxidative capacity of muscle. Taken together with our protein data, OXPHOS proteins in complex IV (**COX2**; cytochrome C oxidase subunit2, and **COX41**; Cytochrome C oxidase subunit 4 isoform 1, mitochondrial), were elevated in abundance (**COX2** = 1.54-fold change, $p = 0.00$, and **COX41** = 1.46-fold change, $p = 0.01$), and similar increases in the malate-aspartate shuttle protein (AATM) also supports the interpretation that the increment in maximum oxygen uptake in response to HIT exercise is associated with muscle adaptation. OXPHOS proteins greater in abundance were electron transport chain proteins, especially Complex IV which plays an important role for reducing oxygen into water. This is inconsistent with 2-weeks-endurance study (Egan *et al.*, 2011) which reported increased abundance in OXPHOS proteins from Complex I (**NDUA8**; NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 8, and **NDUFA13**; NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 13) and Complex V (**ATPA**; ATP synthase subunit α, mitochondrial, and **ATPB;** ATP synthase subunit β, mitochondrial).

The majority of significant changes in protein abundance were obtained from the myofibrillar fraction, which was more responsive than reported in Chapter IV (TR vs OB). Our findings indicate that HIT could reduce cell inflammation which we observed as an increase in structural muscle proteins (e.g. **DESM**; Desmin = 1.22-fold change, $p = 0.01$, and **ACTN3**; α actinin-3 = 1.73-fold change, $p = 0.01$), lesser abundance of extracellular matrix protein (**FIBG**; Fibrinogen gamma chain = 0.84-fold change) and elevated mitochondrial protein (e.g. **AATM**; Aspartate aminotransferase, mitochondrial = 1.21-fold change, $p = 0.01$). However, microtubule proteins such as **TBA8** (Tubulin α-8 chain = 2.09, $p = 0.01$) remained at a higher abundance, indicating that OB skeletal muscle may require an intervention longer than 10-wk in order to be fully restored. Nevertheless, the findings indicate that long-term HIT is able to counteract some of the adverse effect of obesity, perhaps by reducing muscle cell inflammation.

The pattern of protein responses between OB and TR (Chapter IV, cross-sectional study) and the response of OB to HIT (this Chapter) are presented in **Table 17**. The majority of proteins revealed that post-HIT, proteins tend to improve their quality more towards the TR condition. In particular, we report

specific improvements in not only muscle contraction and intermediate filament proteins but also metabolic enzymes. Across glucose metabolic process, we found 3 proteins i.e. **TPIS**, **ALDOA**, and **G3P** changed to become more similar to TR condition after 10-week-HIT. These proteins exhibited statistically significant changes in either abundance (**TPIS**; 0.82-fold change, p = 0.03), FSR (**ALDOA**; pre-HIT = 2.02 %/day, post-HIT = 1.52 %/day, p = 0.04), or showed a trend for alteration (**G3P**; abundance; 0.96-fold change, p = 0.28, $FSR_{pre\text{-}HIT}$ = 1.68 %/day, $FSR_{post\text{-}HIT}$ = 1.63 %/day, p = 0.49). In Chapter IV we found **ALDOA** was less abundant and had a lower FSR in TR (abundance; TR = 0.82-fold change or OB = 1.22-fold change, p = 0.02, FSR_{TR} = 1.19 %/day, FSR_{OB} = 2.30 %/day, p = 0.00), whereas the abundance of **G3P** was less in TR muscle but its FSR was greater compared to OB (abundance; TR = 0.70-fold change or OB = 1.44-fold change, p = 0.00, FSR_{TR} = 16.44 %/day, FSR_{OB} = 1.04 %/day, p = 0.00). The abundance of **TPIS** was also less in TR (abundance; TR = 0.75-fold change or OB = 1.34-fold change, p = 0.01) and there was no difference in its synthesis rate (FSR_{TR} = 0.98 %/day, FSR_{OB} = 0.91 %/day, p = NA). In the current Chapter, we report that after OB completed their 10-week-HIT program, **ALDOA** abundance decreases (0.93-fold change, p = 0.39) and this is associated with significant decreased in its FSR ($FSR_{pre\text{-}HIT}$ = 2.02 %/day, $FSR_{post\text{-}HIT}$ = 1.52 %/day, p = 0.04). We also report that both **TPIS** and **G3P** exhibit changes in a similar trend towards the TR condition, i.e. down-regulation but there was no notable change in FSR. The outcomes indicate glucose metabolism may be improved in response to the HIT intervention.

ALDOA in muscle has been reported as a scaffolding protein that builds a multi-enzyme complex glycolysis at the Z-line of muscle (Gizak, Rakus and Dzugaj, 2003; Rakus *et al.*, 2003; Mamczur *et al.*, 2005). **ALDOA** is involved in early step of glycolysis that covert fructose 6 phosphate into glyceraldehyde 3 phosphate which is a substrate of **G3P** (glyceraldehyde 3 phosphate dehydrogenase). Moreover, **TPIS** which is also an enzyme that functions to produce substrate for **G3P** in glycolysis was less abundant. Therefore, in the glucose metabolism data we show distinctive changes that highlight **G3P** as the marker of change. Although **G3P** found decreased FSR which indicate impaired protein quality, the change was not statistically significant (p = 0.49). The hypothesis is supported that over expression of glycolytic proteins in long-term unhealthy/ sedentary individuals is associated with a loss of glucose and lipid homeostasis. Thus, this chapter demonstrate that HIT can counteract the loss of glucose and lipid homeostasis and reduce the compensation by increasing muscle mitochondrial proteins to improve muscle function similar to trained-athletes (TR). We also found greater expression in lipid metabolic proteins (**APOA1**; Apolipoprotein A-I) which was similar change to TR but did not remarkably change regarding statistical value **(**abundance; TR = 1.43-fold change, p = 0.01 and $OB_{post\text{-}HIT}$ = 1.11-fold change, p = 0.19). **APOA1** is involved with transport cholesterol from tissue to liver for excretion and is the main component of good cholesterol (high density cholesterol, HDL).

Previous chapter reported **MYH1** and **SERCA1** showed statistically significant differences in abundance and FSR (**SERCA**; TR = 0.79-fold change, OB = 1.27-fold change, p = 0.02, $FSR_{pre\text{-}HIT}$ = 1.8%/day, $FSR_{post\text{-}HIT}$ = 2.2%/day, p = 0.00 and **MYH1**; TR = 0.63-fold change, OB = 1.59-fold change, p = 0.01, $FSR_{pre\text{-}HIT}$ = 50.75 %/day, $FSR_{post\text{-}HIT}$ = 0.83 %/day, p = 0.00). However, this post-HIT study did not find notable changes in abundance of either **MYH1** (0.84-fold change, p = 0.20) or **SERCA1** (0.83-fold change, p = 0.50) but we did observe a trend for positive changes. We were able to measure FSR of **MYH1** (pre-HIT = 0.78 %/day, post-HIT = 1.06 %/day, p = 0.00) and report an increase after HIT, which is similar to the trend towards the TR condition. These trends may indicate the improvement of muscle fibre type proportion that reduce type IIx of fast-twitch muscle. Moreover, the change may suggest a reduction in ER stress consistent with **CASQ1** down-regulation in response to physical activity (Ferguson *et al.*, 2014). We also found **CASQ** showed a trend toward lesser abundance but increased FSR (abundance 0.83-fold change, p = 0.16, $FSR_{pre\text{-}HIT}$ 0.71 %/day, $FSR_{post\text{-}HIT}$ 1.6 %/day, p = NA) which is similar to the pattern in TR muscle (abundance 0.65-fold change, p = 0.01, FSR_{TR} = 0.70 %/day, FSR_{OB} = 0.71 %/day, p = NA).

CAH3 (Carbonic anhydrase 3) was found to have a lesser protein quality in OB (abundance; TR = 0.57-fold change or OB 1.75-fold change, p = 0.00, FSR; FSR_{OB} = 0.73 %/day, FSR_{TR} = 1.62 %/day, p = 0.01). In contrast, our 10-week HIT trended of improve CAH3 in both abundance and FSR (abundance; post-HIT = 0.93-Fold change, p = 0.50, $FSR_{pre\text{-}HIT}$ = 0.73 %/day, $FSR_{post\text{-}HIT}$ = 1.39 %/day, p = NA) which is similar to TR outcome. This indicates handling of reactive oxygen species (ROS) (Cabiscol and Levine, 1995) may be improved by exercise and would likely lessen cell death signalling (S. *et al.*, 1998). Moreover, this effect may be associated with a transition in muscle fibre type proportion from type II fibre toward more type I fibre (Harju *et al.*, 2013).

Our current data can also be used to extend the findings or interpretation of our meta-analysis. We found **COX2** showed significant differences in abundance in both Chapters (IV and V) and was presented in **Table 21**. In addition, we discovered 2 proteins **(DESM** and **ACTN3)** had elevated abundance in response to high intensity exercise. These proteins were not highlighted in the previous analysis (Srisawat *et al.*, 2017) and therefore represent new information. Moreover, the abundance changes in OB are opposite to the alterations that occurred in response to exercise training. This indicates our intervention could counteract against adverse outcome of metabolic risk factors such as OB and physical inactivity. We also found changes in some important myofibrillar and cytoskeletal proteins (e.g. titin, nebulin, and myosin light chain) that had been highlighted in a previous narrative review (Deshmukh, 2016). Therefore, alternative patterns of response may be involved with muscle contraction proteins, cytoskeletal protein, metabolic proteins (OXPHOS, glucose, lipid), and ECM that were not captured in our current work.

Table 18 Comparison with other previous publications

	Accession	Function	Current study FSR (%/day) 10-week-HIT			Shankaran 2015 (%/day) 3-w-SIT			Camera 2017 (%/day) 9-d-REX		systematic review (RQ2 only)	Meta-analysis	Effect size significant
			abundance	Pre-HIT	Post-HIT	abundance	Pre-SIT	Post-SIT	abundance	Post-REX			
1	**ACTN3**	**Muscle contraction**	**1.73***	**0.6**	**1.0**	**NA**	**0.96**	**0.66**	**NA**		**NA**	**NA**	**NA**
2	**DESM**	**Cytoskeleton/ structure**	**1.22***	**12.0**	**3.4**	**NA**	**NA**	**NA**	**down**		**4.25**	**NA**	**NA**
3	**COX2**	**OXPHOS**	**1.54****	**3.0**	**3.9**	**NA**	**1.27**	**1.34**	**NA**		**Down (Schild *et al.*, 2015)**	**Included**	**Yes**
4	TPIS	Glucose metabolism	0.82*	0.9	NA	NA	0.77	1.18	down	0.72	Up (Egan *et al.*, 2011)	Included	No
5	AATM	Lipid metabolism	1.21*	0.1	NA	NA	1.06	1.46	NA	NA	Up (Egan *et al.*, 2011)	Included	No

The current protein abundance and FSR that showed significantly different in abundance change regarding extended meta-analysis. **DESM** (Desmin), **ACTN3** (α-actinins 3), **COX2** (Cytochrome c oxidase subunit 2), **AATM** (Aspartate aminotransferase, mitochondrial), **TPIS** (Triosephosphate isomerase)

We have extended the meta-analysis from our previously published (Srisawat *et al.*, 2017) dataset. Our extended meta-analysis found that **COX2** abundance showed notable down-regulation in OB (0.55-fold change, p = 0.00) but increased its abundance in response to HIT (1.54-fold change, p = 0.00). This protein was not specifically highlighted in our previous meta-analysis (Srisawat *et al.*, 2017) because its abundance changed in only 1 study (down-regulation in T2DM individuals; (Hussey *et al.*, 2013)) or up-regulation in over 5 y of endurance trained (Schild *et al.*, 2015). Hussey (Hussey *et al.*, 2013) performed the analysis across T2DM individual which trained under the 4-week-endurance program whereas our experiment intervened 10-week-HIT which is consistent with long-term endurance trained (Schild *et al.*, 2015).

The present finding indicated our intervention (10-week-HIT) could improve/counteract adverse outcomes of metabolic risk factors such as OB. Some of the proteins highlighted in our proteomic analysis have previously been investigated and reported in the hypothesis-led literature and are discussed individually below.

Our current work demonstrates that **DESM** is up-regulated (1.22-fold change, p = 0.01) but decreased in synthesis rate (pre-HIT 12.0%/day, post-HIT 3.4%/day, p = 0.00) after training. Notably, DESM was not included in the meta-analysis so this represents new information that **DESM** is increased in abundance in response to (10 weeks) high intensity interval training (HIT) whereas there was no match with previous shorter period of HIT. Desmin (Z-band related protein) (Deasy *et al.*, 2007) is responsive to other simulated exercise interventions (Moriggi *et al.*, 2010; Gondin *et al.*, 2011; Salanova *et al.*, 2014) including, eccentric exercise (Hody *et al.*, 2011), but decreased in abundance after resistance exercise training (9-d-REX) (Camera *et al.*, 2017) . However, desmin has been reported to be more abundant in

multiple muscle types such as soleus in response to resistive exercise (Moriggi *et al.*, 2010), rectus femoris in response to eccentric exercise (Hody *et al.*, 2011), and vastus lateralis in response to resistive exercise (Salanova *et al.*, 2014). This indicates mechanical force may be key to the effect on Z-band remodelling (Malm and Yu, 2012) and greater muscle regeneration (Gallanti *et al.*, 1992). These studies investigated muscle in pathophysiological states compared with normal muscle and found that desmin regeneration in response to mechanical force across all muscle types. Our new data is consistent with studies on **DESM** involving 8-w-SIT (Woolstenhulme *et al.*, 2005). This experiment demonstrated that **DESM** was increased in abundance by 60 % in response to training. Our current study reported similar findings and indicated that HIT is associated with muscle improvements in protein regulation and cell structure. HIT increases mechanical force/ intermediate filament proteins which co-localise with myofibrillar proteins and may cause remodeling of the myofibrillar structure. Short periods of aerobic exercise may not be able to change myofibrillar structure, but periods of 10-week-HIT (current study) or 8-w-SIT (Woolstenhulme *et al.*, 2005) are able to bring about a change in cytoskeleton cell organisation.

Our study is the first to report on **ACTN3** (α-actinin 3) regarding its abundance and FSR in response to 10-week-HIT. Former literature (Yang, Garton and North, 2009) reported this Z-line protein is restricted to type II muscle fibres that are associated with forceful high-velocity contractions. Moreover **ACTN3** is a genetic marker of athletic performance regarding high muscle strength (Norman *et al.*, 2009). Its role has been highlighted regarding the regulation of muscle fibre differentiation and/or contraction (Mills *et al.*, 2001). Although Mills et al (2001) analysed rat muscle, they also investigated human specimens. Their investigation based on Western blot analysis revealed that **ACTN3** is expressed before fast-myosin isoforms during muscle development (Mills *et al.*, 2001). This experiment also reported that **ACTN3** expression is low, but our experiment was able to detect ACTN3 and contribute both abundance and synthesis data to the literature. **ACTN3** has a role in thin filament organisation and interactions between the sarcomere and the muscle membrane (Mills *et al.*, 2001). There is some evidence that Sarcomeric α-actinin binds other thin filament and Z-line proteins including nebulin, myotililn, CapZ and myozenin (Papa *et al.*, 1999; Salmikangas *et al.*, 1999), the intermediate filament proteins, synemin and vinculin (McGregor *et al.*, 1994; Bellin *et al.*, 1999). Our data likely indicate improvements in muscle strength and performance in response to high-intensity training. However, a report (Venckunas *et al.*, 2012) on ACTN3 (R577X-premature stop codon) polymorphism suggested that **ACTN3** protein also has a small protective role against muscle damage after eccentric exercise and improves stress-sensor signalling. This study investigated muscle biopsies (vastus lateralis) of 19 young healthy men and compared 2 different ACTN3 genotypes i.e. with ACTN3 (genotype RR) and ACTN3 deficiency (genotype XX). ACTN3 mRNA expression was measured but more knowledge on ACTN3 at the protein level is required. Our study reported greater abundance of **ACTN3** protein associated with greater FSR (pre-HIT 0.6%/day, post-HIT 1.0%/day). This may indicate the effect of long-term HIT is associated with improved high-velocity contraction, muscle development, and greater muscle strength.

Cytochrome C oxidase (complex IV) has been reported to increase 2-fold in association with a 60 % increase in mitochondria in response to endurance exercise (Holloszy, 1967). Complex IV of the mitochondrial electron transport chain plays an important role in reducing oxygen into water. Our finding reported COX2 showed up-regulation (**COX2** = 1.54-fold change, $p = 0.00$) and greater FSR after training (FSR_{pre} = 3.0 %/day, FSR_{post} = 3.9 %/day, $p = 0.07$). This effect was not evident after a 2-wk endurance intervention (Egan *et al.*, 2011). **COX2** protein (coded by MT-CO2 gene) functions to support the endurance training increase oxygen capacity (Egan *et al.*, 2011; Schild *et al.*, 2015). The finding also indicates the link to increase malate-aspartate shuttle protein, i.e. over expression in **AATM**, and is consistent with the 9 % gain in VO_{2max} in our experiment.

AATM (Aspartate aminotransferase, mitochondrial) has been mentioned as a component of the Malate-Aspartate shuttle which is an important NAD+/ NADH redox cycle in muscle (Hussey *et al.*, 2013). AATM is also involved in the cellular uptake of long-chain fatty acids and facilitates sarcolemma fatty acid transport. The Malate-Aspartate shuttle proteins have been reported to increase in abundance in response to endurance training and this enhances the capacity for electron transfer from the cytosol into mitochondria (Holloszy and Coyle, 1984). Our study reported AATM significantly increased in abundance 1.21-fold change ($p = 0.01$) but we were unable to obtain FSR data from post-training samples. In addition, we found a trend for the abundance of **AATM** to be greater in TR muscle (Chapter IV; 1.06-fold difference, $p = 0.42$). Fundamentally, AATM function couples with **MDHC** (Malate dehydrogenase, cytoplasmic) protein, which is responsible for converting malate to oxaloacetate and releasing NADH and H^+. Moreover, the product of this reaction is important for synthesis of amino acid and fatty acid. Up-regulation of MDHC has been highlighted previously (Giebelstein *et al.*, 2012) in regard to OB and T2DM muscle. However, there was no equivalent change of **MDHC** in respect to endurance exercise vs OB/T2DM protein abundance. MDHC is also involved in NADH redox balance through aspartic acid synthesis (requiring **AATM** protein) via malate-aspartate shuttle. Our current study reported **AATM** abundance was greater after 10-week-HIT, however, we are unable to clarify the quality of **AATM**. Shankaran et al used 3-wk SIT and reported only AATM FSR data and found that **AATM** synthesis rate is increased in response to endurance training. Taken together, our report and previous data by others (Shankaran *et al.*, 2016) indicated that **AATM** may be improved in protein quality and abundance, which may link to positive outcomes of cellular uptake and transport of long-chain fatty acids and the shuttling/ transfer of electrons between cytosol and mitochondria.

TPIS (Triosesphosphate isomerase) is involved in glycolysis as part of glucose metabolism. Its major role is to synthesis glyceraldehyde 3-phosphate from glycerone phosphate. Glyceraldehyde 3-phosphate is a substrate of **G3P** protein that has been reported to increase in abundance in OB, presented in our previous study (Chapter IV) and by others (Hittel *et al.*, 2005; Giebelstein *et al.*, 2012). On the other hand,

we found reduced abundance in response to 10-week-HIT (0.82-fold change). We could not identify its quality but (Shankaran *et al.*, 2016) report an increment in FSR (pre-test 0.77%/d, post-test 1.18%/d) after 3-w-SIT. Typically endurance training would be expected to increase glycogen sparing consistent with previous review(Holloszy and Coyle, 1984) and studies in rat skeletal muscle (Burniston, 2008). Consistent with this, we did find a glycogen phosphorylase had a greater abundance (**PYGM; PYGL**; Glycogen phosphorylase, muscle form; Glycogen phosphorylase, liver form = 1.16-fold change, $p = 0.46$) but it was not statistically significant. We can measure FSR of PYGM which is the isoform specific to skeletal muscle and report that FSR is significantly decreased in response to HIT (**PYGM**; FSR_{pre} = 1.9 %/day, FSR_{post} = 1.8 %/day, $p = 0.01$). Therefore, we require further evidence to clarify the protein quality of TPIS, PYGM and other glycolytic proteins.

Limitations

We conducted a longitudinal training study which spanned a 3-month study period with a high participant burden, nonetheless our participants engaged and attended all visits. Our participants consumed deuterium oxide in their drinking water and some reported mild side-effects such as stomach bloating or nausea. These symptoms are reported as a normal side effect if participants drink over 150 ml as a single bolus, and in our study, they were reported early in the labelling protocol. In the latter days, participants drank deuterium oxide after meals and these symptoms did not occur. It was challenging to collect venous blood samples from obese participants especially on days when they had not performed exercise. However, saliva samples were readily collected over the entire duration of experiment (14 days of pre-study and 14 days of last training). The measurement of MPE body water from blood and saliva revealed similar values. Therefore, we can use MPE data from either blood or saliva samples for FSR calculations. Muscle biopsies from obese participants were difficult to collect too; there was a large volume of fat and generally bleeding lasted longer so extra care was taken to close the incision site. All participants reported they were satisfied and felt well cared for after completion of the study. Studies included in our systematic review reported a number of trained participants were n = 21 (Lanza *et al.*, 2008), n = 8 (Egan *et al.*, 2011), n =5 (Hussey *et al.*, 2013; Schild *et al.*, 2015), n = 6 (Holloway *et al.*, 2009). But the development of more intricate and sophisticated proteomic analysis is often reported in more modest numbers of participants. For example, Hoffman et al (Hoffman *et al.*, 2015) reports the first wide-spread phosphoproteomic analysis of the human muscle response to exercise and also used a sample size of N =4. Our current report is a pioneering study which will open new avenues for further research and was limited to a small number of participants for practical and financial reasons. In particular, deuterium labelling was exceptionally costly and limited the number of participants that

could be studied, but deuterium oxide is safe and easy to administer stable isotope for human studies and is essential to the proteomic profiling techniques we have used.

Conclusion

This Chapter aimed to determine if muscle proteins from a metabolic prone disease phenotype (OB) are improved by longitudinal high-intensity interval training (HIT). Our analysis encompasses more than 200 proteins and reported abundance and FSR data. Our results support the hypothesis that exercise can counteract against changes in muscle metabolic processes and chronic inflammation by improving intermediate filament proteins that may be associated with changes in extracellular matrix that effect mechanical transduction and improve mitochondrial protein function.

Chapter VI

General Discussion and Future direction of research

Contents

General Discussion

This work aims to contribute to the basic scientific understanding of the disease burden in humans associated with metabolic dysfunction and glucose intolerance that may result in insulin resistance and T2DM. The major risk factors that predispose individuals to this disease burden are related to lifestyle factors, including physical inactivity and poor dietary habits that lead to obesity and a low capacity for exercise. Skeletal muscle is a key insulin-responsive tissue that makes a major contribution to whole-body metabolism. Skeletal muscle is a highly malleable tissue that adapts to physical activity/ inactivity, by altering its protein composition, including myofibrillar protein profile and metabolic enzymes. As yet we do not fully understand the underlying mechanisms that lead to protein-specific changes in abundance, either associated with dysfunction or improvements in the metabolic health of muscle. In part this is because the majority of data represent static information about the abundance of proteins in either diseased or healthy muscle. Such literature provides important information about molecular outcomes that can be associated with physiological/ functional indices of health, but they cannot answer questions about how/why the change in protein abundance occurred. This thesis contributes to a new era of dynamic proteome studies that can give insight to the relative contributions of synthesis and degradation to changes in protein abundance in human muscle.

We began by first synthesising evidence from the existing literature on human muscle proteome changes associated with sedentary behaviour and obesity versus muscle adaptation to exercise training. We used formal systematic review and meta-analysis methods to increase the robustness of the literature review and we reported prominent proteins that were associated with either maladaptation of muscle to obesity/ T2DM or positive adaptations associated with exercise training. In particular we report that the muscle of obese or T2DM patients has a greater proportion of fast-twitch myosin heavy chains (**MYH1** and **MYH2**) and our review also raised a novel hypothesis regarding muscle inflammation and the role of tenascin-C in muscle pathophysiology associated with T2DM. Our review of positive muscle adaptation focused on data from endurance training studies which highlight that exercise causes beneficial adaptations to the cardiovascular system, muscle mass, and muscle metabolism. Our evidence was obtained from non-bias proteomic studies rather than hypothesis-led literature so the outcomes of our review are hypotheses rather than conclusions. We reported proteins from OXPHOS and other mitochondrial proteins shift-toward a greater abundance in endurance trained muscle, which is consistent with the wider literature. However, all previous experiments reported only protein abundance data. The protein quality and protein synthesis rate or turnover are still unknown/ unreported. We still do not know whether obesity should be associated with increased or decreased protein synthesis but some literature from primary human muscle cultures suggests that the proteasome may be dysfunctional in T2DM muscle. Therefore, we investigated whether obesity and exercise training effect the synthesis and degradation of proteins in human muscle. We employed the

stable isotope, deuterium oxide, for labelling proteins in vivo, which is new and relatively less invasive than previous methods that used intravenous infusion of amino acid tracers.

Our preliminarily method development experiment (Chapter III) on human protein profile reported 2 proteins, i.e. **FABPH** and **SERCA2**, that were more abundant in the muscle of OB/T2DM. Interestingly, SERCA2 is related to an elevated proportion of slow-twitch muscle fibre and was greater in abundance in OB and T2DM muscle which is opposite to the outcome of our systematic review and meta-analysis. The most likely explanation for this difference in finding is that the samples used in our method development work were collected from older adults that may have a different myofibre profile (i.e. greater abundance of slow-twitch fibres) compared to the wider literature on T2DM in middle-aged patients.

We also described a method for investigating protein turnover rate of individual proteins that can be applied in human muscle as well as muscle from non-human animal models. Our method uses deuterium oxide labelling in vivo coupled with peptide mass spectrometry to identify proteins, measure their relative abundance and calculate their fractional synthesis rate. The resulting method is more comprehensive than previous techniques because it can report synthesis data on a protein-by-protein basis rather than average synthesis rate across protein mixtures in human skeletal muscle. That is, previous studies in humans have been conducted using amino acid tracers, which require intravenous infusion and muscle samples must be hydrolysed to amino acids before analysis of isotope incorporation. This is a proven method for measuring fractional synthesis rates but the hydrolysis to amino acids breaks the link to the identity of individual proteins. Therefore, the existing literature reports only synthesis data of whole muscle or muscle fractions such as the myofibrillar fraction (mixture of ~50 proteins) and does not report the abundance of individual proteins.

The main experiments of this thesis are reported in Chapters IV and V and used different designs i.e. cross-sectional design of obesity vs trained (Chapter IV) and longitudinal study of obesity with 10-week-high-intensity-interval-training (Chapter V). The cross-sectional study began with the question of whether differences exist in the abundance and synthesis rate of proteins between sedentary obese individuals and lean endurance-trained athletes. The main experiments were conducted using deuterium oxide administration in vivo and peptide mass spectrometry for protein and peptide analysis. The protein data indicate the difference in relative abundance between OB vs TR muscle and highlighted proteins from the cell cytoskeleton, OXPHOS, muscle contraction, and glycolytic pathways. In particular, proteins less abundant in slow-twitch muscle (**ACTN2, COX2**, and **MLRV**) but more abundant in fast-twitch fibre (**SERCA1**) and glycolytic metabolism (**G3P**) were highlighted. We also measured the fractional synthesis rate of the majority of these proteins. We raised 3 hypotheses:

i) Muscle inflammation evidenced by muscle-cytoskeleton-proteins (low abundance but high FSR in **ACTN2**) indicate loss of protein homeostasis, ii) OB skeletal muscle dysfunction is associated with defects in the protein quality of proteins responsible for Ca++ handling. (SERCA1 showed greater abundance but decreased FSR), and iii) Higher Reactive Oxygen Species (ROS) in OB (**COX2**) could be associated with higher thermodynamic driving forces, which result from low metabolic turnover rate (low abundance and FSR in **COX2** protein). This may indicate a compensatory mechanism to increase insulin sensitivity (high abundance in **G3P**). Our data extend the existing literature by also showing that these proteins had a lesser turnover rate in OB muscle, which may indicate that protein quality is adversely affected.

As a countermeasure against the adverse effect of obesity, we started to investigate a longitudinal experiment which involved a 10-week regimen of high-intensity interval training (Chapter V). This work investigates the question of "what changes occur in the skeletal muscle proteome of obese humans that partake in sustainable time–efficient high intensity interval exercise intervention?" We discovered 2 prominent proteins which have never been reported in the literature in this context. These proteins showed overexpression in abundance **DESM** and **ACTN3** and had either lower FSR (**DESM**) or higher FSR (**ACTN3**) after training. Both proteins play a major role in cell cytoskeletal organisation. These findings highlight new avenues for future research. Other proteins that we report to be different in abundance and FSR in OB include **COX2**, up-regulation of **AATM** and down-regulation in **TPIS.** We have raised 3 hypotheses: i) HIT improves aerobic capacity and also has a positive effect on human muscle adaptation by remodelling muscle proteins associated with intermediate filament and mitochondrial proteome. An aspect of this response may include **ACTN3** which previously has only been reported during muscle development or resistance training interventions, ii) Increased synthesis of mitochondrial proteins and mitochondrial protein remodelling is enhanced by endurance training. This suggests there may be an interaction or mechanism shared between mitochondrial and cytoskeletal proteins **(AATM, COX2, ACTN3, DESM)**, and iii) HIT promotes glucose uptake or glycogen storage in muscles by increasing insulin sensitivity that primarily involves (**TPIS**) and substrate for the G3P reaction.

General limitations

Our proteomics analysis has generated a large amount of entirely new information regarding the abundance and fractional synthesis of individual proteins in the muscle of OB and TR participants. Our work used a 'bottom-up' proteomic approach which encompasses mass spectrometry analysis of peptide digests to derive data about muscle proteins. Therefore, this analysis is not optimal for discovering post-translational modifications, which usually is achieved by either a 'top-down' proteomics approach (e.g. 2D gel electrophoresis) or through the enrichment of specific post-translational modifications (e.g. phosphorylation enrichment using TiO_2). We have retrieved 4,616 peptide fragments (2,473 from myofibrillar fraction and 2,143 from sarcoplasmic fraction), these were part of 123 proteins. This was because each protein is digested in to numerous tryptic peptides. LC-MS/MS is a very powerful approach to analysis large numbers of proteins; however, a limitation of LC-MS/MS is that further optimisation and additional processing steps, such as fractionation, are required to gain deeper insight (i.e. analysis of a greater number of proteins) in to the muscle proteome. Muscle is a particularly challenging substrate for proteomic analyses because it contains a small number of highly abundant proteins (i.e. myofibrillar proteins and metabolic enzymes). In an attempt to minimise this challenge we routinely fractionate muscle in to myofibrillar and soluble protein fractions (Burniston, Connolly, *et al.*, 2014). Nonetheless the complexity of the soluble muscle fraction is still too great for complete separation by 1-dimension liquid chromatography and state-of the-art work in cataloguing the muscle proteome (Deshmukh *et al.*, 2015) uses additional fractionation procedures but this creates addition challenges to data analysis for abundance and synthesis measurements. Therefore, it will require a sustained period of time to further optimise our techniques to increase the number of proteins that can be reported.

The combined challenges of invasive sample collection and costly sample analysis mean that generally the number of participants reported in proteomic studies is less than is common in classical physiological studies. Studies included in our systematic review had n values ranging from 4 (Hussey *et al.*, 2013) to 20 individuals (Lefort *et al.*, 2010)). It is common for the development of more intricate and sophisticated proteomic analysis to be reported in more modest numbers of participants. For example, Hoffman et al reports the first wide-spread phosphoproteomic analysis of the human muscle response to exercise and used a sample size of n =4. Hoffman et al (Hoffman *et al.*, 2015) measured protein phosphorylation after a single bout of HIT. The human muscle peptides were labelled by iTRAQ and phosphopeptides were enriched using titanium dioxide. A limitation of this study is the design, which measure proteins from pre vs post-acute HIT which is a short period and proteins would not likely result in significant changes in protein abundance. Our cross-sectional study consisted of a similar number of participants (N=8; n_{TR}=4, n_{OB}=4) but arguably the burden on our participants was much greater. Our analysis of protein synthesis rates relies upon a time-series of 4 sampling points. Moreover, the daily

consumption of deuterium oxide (4 shots at equidistant times throughout the day), daily saliva collection and regular venepuncture to collect venous blood were additional burdens on the participants. We obtained 4 percutaneous needle biopsies from each participant over a 14-d period. Therefore, we were also acutely aware of the heightened risk of potential complications such as infection/ poor wound healing, discomfort and anxiety regarding the sample collection procedures. Overall, our participants were satisfied with our experiment and they remained committed until the last visit. However, the initial recruitment for our study was challenging and we had contact with potential participants who were eligible for the study but were concerned about muscle biopsy procedure, so they were not enrolled in the study.

Chapter V has been conducted to study the effect of 10 weeks (3 visit/week) of high intensity interval training (HIT) on muscle proteome dynamics in obese humans. Baseline data had already been collected from the obese participants as part of the work conducted in Chapter IV. A limitation of the study is that we performed a comparison of pre vs post HIT but we did not include non-exercised control group due to cost implications of deuterium oxide labelling and proteomic analysis. A full case-control study would require a matched-control group without HIT intervention to compare with case (OB with exercise intervention). However, it is important to acknowledge that our aim was to discover new questions (i.e. generate hypotheses) rather than to prove answers to know questions. It is expected that the current proteomics work will be followed up using a more controlled/ targeted investigation of the hypothesis raised by our work.

Future direction of research

This thesis has used hypothesis generating research designs to build new ideas about the response of human skeletal muscle to either pathological conditions of obesity and T2DM or the beneficial effects of exercise training. Our synthesis of evidence from the existing literature (Chapter II) highlighted tenascin-C which is an extracellular matrix protein involved with chronic inflammation in obese muscle and insulin resistance. Our review found up-regulation of TENA in primary myotubes cultured from obese muscle but this could be associated with an 'injury' response caused by the isolation process or culture conditions. Therefore, it is important to conduct further studies that specifically test whether the abundance of tenascin-C is greater in muscle biopsies of obese versus trained individuals. Unfortunately, TENA was not detected by the proteomic methods employed in the current thesis. Therefore, more in-depth proteomic analysis is required, or more targeted measurement of TENA using techniques such as western blotting could be used to test this hypothesis. For example, a cross-sectional analysis of 3 groups i.e. Trained, OB, and T2DM, could be used to investigate if the differences in TENA found in muscle cell culture are also evident in biopsy samples taken in vivo.

In Chapter IV we investigated differences in the abundance and synthesis rate of proteins between the muscle of obese and trained individuals. This cross-sectional analysis enabled us to detect significant differences in both abundance and synthesis rate of individual proteins. This is the first data of its type in these populations and currently the only other study to report equivalent dynamic proteome profiling in human muscle is Camera et al, (Camera *et al.*, 2017) which studied responses to a resistance exercise training protocol. Chapter IV highlights several proteins that were more abundant in OB muscle and had a lesser synthesis rate in OB muscle, which indicates the quality of these proteins may be adversely affected in obese individuals. The proteins exhibiting these responses, include **SERCA1** (Sarcoplasmic/endoplasmic reticulum calcium ATPase 1, Muscle-Calcium protein) and **G3P** (Glyceraldehyde-3-phosphate dehydrogenase, Glucose metabolism protein). The cross-sectional design used in Chapter IV cannot distinguish whether the differences in the muscle proteome between trained and obese participants is due to their obesity or their sedentary behaviour/ lack of exercise. However, our data does provide evidence that the muscle of people at risk of developing T2DM is characterised by accumulation of specific proteins with relatively low rates of turnover. This is an important step forward for the field because previously it was not clear whether obesity or T2DM had affected protein synthesis in skeletal muscle. Indeed, there were conflicting theories and data regarding protein turnover in an insulin resistant state and our new data help to clarify that protein homeostasis is disrupted in the muscle of people at risk of non-communicable disease. In the future, similar studies could investigate the effects of obesity or physical inactivity in isolation, which may give more detailed insight, e.g. to obesity-specific effects. That said, non-communicable disease often arises from poor lifestyle choices and it is common for physical inactivity and poor dietary habits to cooccur in the general population.

Future studies could also make greater use of lifestyle questionnaires to give insight in to behavioural factors that associate with muscle molecular data. This may give new evidence to support preventive medicine, health and medical research, as well as policy decisions. Regarding our –omic approach, we relied on mass spectrometry to conduct a non-biased investigation and collect information on a proteome-wide scale. The advantage of our analytical method is that it provides new information and can generate new hypothesis or questions, which might not otherwise be raised by traditional research approaches. However, future studies are required to confirm our proteomic discoveries and to investigate in more detail the underlying mechanisms such as post-translational modifications that may be associated with disruptions to protein homeostasis. Notably, a major direction arising from our data is that of protein homeostasis and dysfunction of protein degradation in the muscle of individuals at risk of non-communicable disease. We highlight several proteins that could be used as biomarkers of these effects in future studies, and provide a specific read-out on the effects of pharmacological or lifestyle interventions aimed at preventing disease.

In Chapter V we investigated the effects of high-intensity exercise training in obese participants. The muscle proteome response included proteins that changed significantly in abundance or significantly in synthesis rate and only 3 proteins exhibited a 'dual' response, i.e. significant differences in both synthesis and abundance. This could be due to the repeated measures design, i.e. proteins that changed in abundance during weeks 1-8 of the HIT protocol when deuterium oxide was not consumed by the participants cannot be investigated for effects on synthesis rate. **COX2** (Cytochrome C oxidase subunit 2, Oxidative phosphorylation), **ACTN2** (α-actinin-2, Muscle-Cytoskeleton), and **MLRV** (Myosin regulatory light chain 2, ventricular/cardiac muscle isoform, cardiac/smooth muscle contraction protein) were less abundant in OB (**COX2**; 0.55 fold change, p = 0.00, **ACTN2**; 0.84 fold change, p = 0.00, **MLRV**; 0.72 fold change, p = 0.00) but FSR increased in response to HIT trained muscle (**COX2**; abundance 1.54, p = 0.00, FSR_{pre} = 2.95 %/day, FSR_{post} = 3.91 %/day, p = NA, **MLRV**; $FSR_{pre\text{-}HIT}$ 4.5%/day, $FSR_{post\text{-}HIT}$ 6.1%/day, p = 0.39). This represents novel evidence of the muscle dynamic proteome response to endurance exercise training. In addition, we highlight novel responses of proteins such as **DESM** and **ACTN3**, which are not associated with low-intensity endurance exercise but are often found to be responsive to resistance exercise. This suggests that HIT is able to instigate some of the adaptations of skeletal muscle to resistance exercise at the same time as producing adaptations that are traditionally associated with endurance training. Our study used a 10-week HIT intervention that was longer than used in other closely related studies. However, we did not include a non-exercise control group so we cannot be certain that some of our findings may have occurred in the absence of exercise training. Nonetheless, our exercise intervention improved the health status of the participants, i.e. increasing insulin sensitivity and aerobic fitness (VO_2 peak), and previous work, including meta-analysis of randomised controlled trials, that used HIT, have reported a statistically significant effect of this type of training.

Our separate analyses of cross-sectional differences between OB and TR, and longitudinal analysis of OB prior to and after HIT discovered that endurance exercise counteracts some of the protein-specific

effects of obesity and physical inactivity in skeletal muscle. This general outcome is slightly at odds with the conclusion from our systematic review and meta-analysis of the previous literature, which struggled to find a clear opposite effect of exercise against OB/T2DM at the individual protein level. This difference is likely explained by the fact that the analytical techniques used in Chapter IV and V were consistent, whereas data reviewed in Chapter II were from numerous investigations that used a selection of different proteomic techniques.

Outcomes from the current studies may give insight to future drug development and exercise physiology research. Targeted studies could be conducted to validate the discoveries of individual proteins reported herein that change in either protein abundance or synthesis rate. However, an advantage of broad proteomic data, such as ours, is that higher-level questions can be generated and then tested in future work. For example, our analysis suggests an association between the response of mitochondrial proteins including OXPHOS (e.g. **COX2**) and Malate-Aspartate shuttle (e.g. **AATM**) systems, and the response of the muscle cytoskeleton (e.g. **ACTN3** and **DESM**). This raises hypotheses regarding the interrelationship between mitochondria and the muscle cytoskeleton. Our data regarding these highlighted proteins report increases in both abundance and fractional synthesis rate in response to longitudinal HIT. Therefore, future research in this question may be particularly focused to synthetic process, i.e. encompassing gene expression, post-transcriptional processes and translation, rather than protein degradative mechanisms.

Conclusion

We have found the existing proteomic literature on human muscle in the context of the pathophysiological effects of obesity, glucose intolerance and T2DM or the beneficial effects of endurance exercise only considers 'static' measurements of protein abundance or post-translational modification. Generally, the outcomes of this literature are consistent with data from hypothesis-led research and show that the muscle of obese/ T2DM patients has relatively poor mitochondrial function and exhibits a shift toward a more fast-twitch phenotype. There has been uncertainty regarding the effects of obesity or T2DM on muscle protein turnover, which may largely be due to the necessary reliance on amino acid tracers to study the average protein synthesis across mixtures of proteins. We have established proteomics techniques that can now report the synthesis rate of individual proteins in addition to measuring each protein's abundance. Using this new technology, we report the muscle from obese people has selective changes in protein turnover and some proteins in obese muscle were of a lower quality. Proteins that accumulate and have a slower turnover rate are more likely to become damaged and aggregate which can be a factor in the development of disease. We report exercise training improves the turnover rate of many muscle proteins and this novel finding is likely to be a key factor underlying the protective effect of exercise against metabolic diseases.

References

A Waters Company: nonlinear dynamics (no date) *How does normalisation work in TransOmics™ Informatics?* Available at: http://www.nonlinear.com/transomics/metabolomics/v1.0/faq/how-normalisation-works.aspx (Accessed: 11 April 2019).

Al-Khalili, L. *et al.* (2011) 'An iTRAQ proteomic study reveals an association between diet-induced enhanced fatty acid metabolism and the development of glucose intolerance in prediabetic mice.', *Molecular & cellular proteomics : MCP*. Protein Structural Biology and Proteomics and Metabolomics Core Facility, Pennington Biomedical Research Center, Louisiana State University System, Louisiana, USA., United States: Elsevier Science, 10(3), pp. 254–266. doi: 10.1016/j.bbalip.2015.01.013.

Al-Khalili, L., Barbosa, T. de C., *et al.* (2014) 'Profiling of human myotubes reveals an intrinsic proteomic signature associated with type 2 diabetes', *Translational Proteomics*. Elsevier, 2(C), pp. 25–38. doi: 10.1016/j.trprot.2013.12.002.

Al-Khalili, L., de Castro Barbosa, T., *et al.* (2014) 'Proteasome inhibition in skeletal muscle cells unmasks metabolic derangements in type 2 diabetes.', *American journal of physiology. Cell physiology*, 307(9), pp. C774-87. doi: 10.1152/ajpcell.00110.2014.

Alshehri, A. M. (2010) 'Metabolic syndrome and cardiovascular risk.', *Journal of family & community medicine*. Medknow Publications, 17(2), pp. 73–8. doi: 10.4103/1319-1683.71987.

Beals, J. W. *et al.* (2017) 'Protein-Rich Food Ingestion Stimulates Mitochondrial Protein Synthesis in Sedentary Young Adults of Different BMIs', *The Journal of Clinical Endocrinology & Metabolism*. Oxford University Press, 102(9), pp. 3415–3424. doi: 10.1210/jc.2017-00360.

Beals, J. W. *et al.* (2018) 'Altered anabolic signalling and reduced stimulation of myofibrillar protein synthesis after feeding and resistance exercise in people with obesity', *The Journal of Physiology*. John Wiley & Sons, Ltd (10.1111), 596(21), pp. 5119–5133. doi: 10.1113/JP276210.

Bellin, R. M. *et al.* (1999) 'Molecular characteristics and interactions of the intermediate filament protein synemin. Interactions with alpha-actinin may anchor synemin-containing heterofilaments.', *The Journal of biological chemistry*, 274(41), pp. 29493–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10506213 (Accessed: 2 December 2018).

Binas, B. and Erol, E. (2007) 'FABPs as determinants of myocellular and hepatic fuel metabolism.', *Molecular and cellular biochemistry*, 299(1–2), pp. 75–84. doi: 10.1007/s11010-005-9043-0.

Blair, S. N. *et al.* (1996) 'Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women.', *JAMA*, 276(3), pp. 205–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8667564 (Accessed: 11 February 2016).

Bobe, R. *et al.* (2004) 'Identification, Expression, Function, and Localization of a Novel (Sixth) Isoform of the Human Sarco/Endoplasmic Reticulum Ca $^{2+}$ ATPase 3 Gene', *Journal of Biological Chemistry*, 279(23), pp. 24297–24306. doi: 10.1074/jbc.M314286200.

Booth, F. W., Roberts, C. K. and Laye, M. J. (2012) 'Lack of exercise is a major cause of chronic diseases.', *Comprehensive Physiology*, 2(2), pp. 1143–211. doi: 10.1002/cphy.c110025.

Bradford, M. M. (1976) 'A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding', *Analytical Biochemistry*. Academic Press, 72(1–2), pp. 248–254. doi: 10.1016/0003-2697(76)90527-3.

British Heart Foundation (2015) *Physical Activity Statistics 2015*. Available at: https://www.bhf.org.uk/research/heart-statistics/heart-statistics-publications/physical-activity-statistics-2015 (Accessed: 29 February 2016).

Brocca, L. *et al.* (2015) 'Human skeletal muscle fibre contractile properties and proteomic profile: adaptations to 3 weeks of unilateral lower limb suspension and active recovery', *The Journal of Physiology*, 593(24), pp. 5361–5385. doi: 10.1113/JP271188.

Bronk, J. R. (J. R. (1999) *Human metabolism : functional diversity and integration*. Addison Wesley Longman. Available at: https://books.google.co.th/books/about/Human_Metabolism.html?id=6lpFAQAAIAAJ&redir_esc=y (Accessed: 21 April 2019).

Broskey, N. T. *et al.* (2018) 'Skeletal muscle ceramides and daily fat oxidation in obesity and diabetes', *Metabolism*, 82, pp. 118–123. doi: https://doi.org/10.1016/j.metabol.2017.12.012.

Bruce, C. R. *et al.* (2004) 'Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type 2 diabetes', *Diabetologia*, 47(1), pp. 23–30. doi: 10.1007/s00125-003-1265-7.

Burniston, J. G. (2008) 'Changes in the rat skeletal muscle proteome induced by moderate-intensity endurance exercise', *Biochimica et biophysica acta*. Research Institute for Sport and Exercise Sciences (RISES) and Institute for Health Research (IHR), Liverpool John Moores University, 15-21 Webster Street, Liverpool, L3 2ET, UK. jatin.burniston@ntlworld.com: Elsevier Pub. Co, 1784(7–8), pp. 1077–1086. doi: 10.1016/j.bbapap.2008.04.007.

Burniston, J. G. (2009) 'Adaptation of the rat cardiac proteome in response to intensity-controlled endurance exercise.', *Proteomics*. Germany, 9(1), pp. 106–115. doi: 10.1002/pmic.200800268.

Burniston, J. G. *et al.* (2011) 'Proteomic analysis reveals perturbed energy metabolism and elevated oxidative stress in hearts of rats with inborn low aerobic capacity.', *Proteomics*. Germany, 11(16), pp. 3369–3379. doi: 10.1002/pmic.201000593.

Burniston, J. G., Kenyani, J., *et al.* (2014) 'Conditional independence mapping of DIGE data reveals PDIA3 protein species as key nodes associated with muscle aerobic capacity', *Journal of Proteomics*. Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool L3 3AF, UK. Electronic address: j.burniston@ljmu.ac.uk.: Elsevier, 106, pp. 230–245. doi: 10.1016/j.jprot.2014.04.015.

Burniston, J. G., Connolly, J., *et al.* (2014) 'Label-free profiling of skeletal muscle using high-definition mass spectrometry.', *Proteomics*, 14(20), pp. 2339–44. doi: 10.1002/pmic.201400118.

Burniston, J. G. and Hoffman, E. P. (2011) 'Proteomic responses of skeletal and cardiac muscle to exercise', *Expert review of proteomics*. England, 8(3), pp. 361–377. doi: 10.1586/epr.11.17.

Busch, R. *et al.* (2006) 'Measurement of protein turnover rates by heavy water labeling of nonessential amino acids.', *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1760(5), pp. 730–44. doi: 10.1016/j.bbagen.2005.12.023.

Cabiscol, E. and Levine, R. L. (1995) 'Carbonic anhydrase III. Oxidative modification in vivo and loss of phosphatase activity during aging.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 270(24), pp. 14742–7. doi: 10.1074/JBC.270.24.14742.

Caiozzo, V. J. *et al.* (2000) 'MHC polymorphism in rodent plantaris muscle: effects of mechanical overload and hypothyroidism', *Am J Physiol Cell Physiol*, 278, pp. C709–C717. Available at: https://www.physiology.org/doi/pdf/10.1152/ajpcell.2000.278.4.C709 (Accessed: 26 April 2018).

Calve, S., Odelberg, S. J. and Simon, H.-G. (2010) 'A transitional extracellular matrix instructs cell behavior during muscle regeneration', *Developmental Biology*, 344(1), pp. 259–271. doi: 10.1016/j.ydbio.2010.05.007.

Camera, D. M. *et al.* (2017) 'Dynamic proteome profiling of individual proteins in human skeletal muscle after a high-fat diet and resistance exercise.', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 31(12), pp. 5478–5494. doi: 10.1096/fj.201700531R.

Canepari, M. *et al.* (2000) 'Functional diversity between orthologous myosins with minimal sequence diversity', *Journal of Muscle Research & Cell Motility*. Kluwer Academic Publishers, 21(4), pp. 375–382. doi: 10.1023/A:1005640004495.

Caruso, M. *et al.* (2014) 'Increased interaction with insulin receptor substrate 1, a novel abnormality in insulin resistance and type 2 diabetes.', *Diabetes*. United States, 63(6), pp. 1933–1947. doi: 10.2337/db13-1872.

Castaneda F, Layne JE, C. C. (2006) *Skeletal muscle sodium glucose co-transporters in older adults with type 2 diabetes undergoing resistance training*, *J Med Sci 3(3)*. doi: 10.7150/ijms.3.84.

Catalán, V. *et al.* (2012) 'Increased tenascin C and toll-like receptor 4 levels in visceral adipose tissue as a link between inflammation and extracellular matrix remodeling in obesity', *Journal of Clinical Endocrinology and Metabolism*. The Endocrine Society, 97(10), pp. E1880-9. doi: 10.1210/jc.2012-1670.

Chen, J.-W. *et al.* (2000) '1-Cys Peroxiredoxin, a Bifunctional Enzyme with Glutathione Peroxidase and Phospholipase A $_2$ Activities', *Journal of Biological Chemistry*, 275(37), pp. 28421–28427. doi: 10.1074/jbc.M005073200.

Chen, Y.-W. *et al.* (2003) 'Molecular responses of human muscle to eccentric exercise', *Journal of Applied Physiology*, 95, pp. 2485–2494.

Chiao, Y. A. *et al.* (2010) 'In vivo matrix metalloproteinase-7 substrates identified in the left ventricle post-myocardial infarction using proteomics.', *Journal of proteome research*. United States: NIH Public Access, 9(5), pp. 2649–2657. doi: 10.1021/pr100147r.

Christ-Roberts, C. Y. *et al.* (2004) 'Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects', *Metabolism*, 53(9), pp. 1233–1242. doi: 10.1016/j.metabol.2004.03.022.

Cobley, J. *et al.* (2016) 'Age- and Activity-Related Differences in the Abundance of Myosin Essential and Regulatory Light Chains in Human Muscle', *Proteomes*, 4(2), p. 15. doi: 10.3390/proteomes4020015.

Cochrane, Trusted evidence, Informed decisions, Better health. (no date). Available at: http://www.cochrane.org/.

Cocks, M. *et al.* (2013) 'Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males', *Journal of Physiology-London*, 591(3), pp. 641–656. doi: 10.1113/jphysiol.2012.239566.

Cohen, J. (1960) 'A Coefficient of Agreement for Nominal Scales', *Educational and Psychological Measurement*. Sage PublicationsSage CA: Thousand Oaks, CA, 20(1), pp. 37–46. doi: 10.1177/001316446002000104.

Cohen, J. (1988) *Statistical power analysis for the behavioral sciences*. L. Erlbaum Associates.

Commerford, S. L., Carsten, A. L. and Cronkite, E. P. (1983) 'The Distribution of Tritium among the Amino Acids of Proteins Obtained from Mice Exposed to Tritiated Water', *Radiation research*, 94(1), pp. 151–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6856764 (Accessed: 19 April 2016).

Cuthbertson, D. *et al.* (2005) 'Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle.', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 19(3), pp. 422–4. doi: 10.1096/fj.04-2640fje.

Cuthbertson, D. J. *et al.* (2018) 'Longitudinal analysis of risk of Non-Alcoholic Fatty Liver Disease (NAFLD) in adulthood', *Liver International*. Wiley/Blackwell (10.1111). doi: 10.1111/liv.13993.

Deasy, B. M. *et al.* (2007) 'A role for cell sex in stem cell-mediated skeletal muscle regeneration: female cells have

higher muscle regeneration efficiency.', *The Journal of cell biology*, 177(1), pp. 73–86. doi: 10.1083/jcb.200612094.

DeFronzo, R. *et al.* (1981) 'The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization.', *Diabetes*, 30(12), pp. 1000–7.

DeFronzo, R. A. and Matsuda, M. (2010) 'Reduced Time Points to Calculate the Composite Index', *Diabetes Care*, 33(7). Available at: http://care.diabetesjournals.org/content/33/7/e93.long (Accessed: 2 June 2017).

DeFronzo, R. A. and Tripathy, D. (2009) 'Skeletal muscle insulin resistance is the primary defect in type 2 diabetes.', *Diabetes care*. American Diabetes Association, 32 Suppl 2(Suppl 2), pp. S157-63. doi: 10.2337/dc09-S302.

Dela, F. *et al.* (1994) 'Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM.', *Diabetes*, 43(7), pp. 862–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8013748 (Accessed: 14 March 2016).

Deshmukh, A. S. *et al.* (2015) 'Deep proteomics of mouse skeletal muscle enables quantitation of protein isoforms, metabolic pathways and transcription factors.', *Molecular & Cellular Proteomics: MCP*. Max-Planck Institute of Biochemistry, Germany; American Society for Biochemistry and Molecular Biology. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=25616865&site=ehost-live.

Deshmukh, A. S. (2016) 'Proteomics of Skeletal Muscle: Focus on Insulin Resistance and Exercise Biology', *Proteomes*. MDPI AG, 4(1), p. 6. doi: 10.3390/proteomes4010006.

'Developing NICE guidelines: the manual | 4-developing-review-questions-and-planning-the-evidence-review | Guidance and guidelines | NICE' (no date). NICE.

Dickinson, J. M. *et al.* (2010) 'A Method To Study In Vivo Protein Synthesis In Slow- And Fast-twitch Human Muscle Fibers', *Medicine and Science in Sports and Exercise*, 42, p. 64.

Egan, B. *et al.* (2011) '2-D DIGE analysis of the mitochondrial proteome from human skeletal muscle reveals time course-dependent remodelling in response to 14 consecutive days of endurance exercise training.', *Proteomics*. Germany, 11(8), pp. 1413–1428. doi: 10.1002/pmic.201000597.

Ferguson, D. P. *et al.* (2014) 'Differential skeletal muscle proteome of high- and low-active mice.', *Journal Of Applied Physiology (Bethesda, Md.: 1985)*. Children's Nutritional Research Center, Baylor College of Medicine, Houston, Texas; American Physiological Society, 116(8), pp. 1057–67. doi: 10.1152/japplphysiol.00911.2013.

Ferrannini, E. *et al.* (1991) 'Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome.', *Diabetologia*, 34(6), pp. 416–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1884900 (Accessed: 26 February 2016).

Flegal, K. M. *et al.* (2002) 'Prevalence and trends in obesity among US adults, 1999-2000.', *JAMA*, 288(14), pp. 1723–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12365955 (Accessed: 10 December 2018).

Flück, M. *et al.* (2000) 'Rapid and reciprocal regulation of tenascin-C and tenascin-Y expression by loading of skeletal muscle.', *Journal of cell science*. The Company of Biologists Ltd, (20), pp. 3583–91. doi: 10.1038/ng0997-104.

Formentini, L. *et al.* (2017) 'Mitochondrial H(+)-ATP synthase in human skeletal muscle: contribution to dyslipidaemia and insulin resistance.', *Diabetologia*. Germany, 60(10), pp. 2052–2065. doi: 10.1007/s00125-017-4379-z.

Fortmann, S. P. *et al.* (2013) 'Vitamin and Mineral Supplements in the Primary Prevention of Cardiovascular Disease and Cancer: An Updated Systematic Evidence Review for the U.S. Preventive Services Task Force', *Annals of Internal Medicine*. American College of Physicians, 159(12), pp. 824–834. doi: 10.7326/0003-4819-

159-12-201312170-00729.

Fritz, T. *et al.* 'Low-intensity exercise increases skeletal muscle protein expression of PPARdelta and UCP3 in type 2 diabetic patients.', *Diabetes/metabolism research and reviews*, 22(6), pp. 492–8. doi: 10.1002/dmrr.656.

Gallanti, A. *et al.* (1992) 'Desmin and vimentin as markers of regeneration in muscle diseases.', *Acta neuropathologica*, 85(1), pp. 88–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1285499 (Accessed: 2 December 2018).

Gasier, H. G. *et al.* (2012) 'Acute resistance exercise augments integrative myofibrillar protein synthesis', *Metabolism*. W.B. Saunders, 61(2), pp. 153–156. doi: 10.1016/J.METABOL.2011.07.001.

Gasier, H. G., Fluckey, J. D. and Previs, S. F. (2010) 'The application of 2H2O to measure skeletal muscle protein synthesis.', *Nutrition & metabolism*, 7(1), p. 31. doi: 10.1186/1743-7075-7-31.

Gélébart, P. *et al.* (2003) 'Identification of a new SERCA2 splice variant regulated during monocytic differentiation.', *Biochemical and biophysical research communications*, 303(2), pp. 676–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12659872 (Accessed: 20 August 2016).

Ghemrawi, R., Battaglia-Hsu, S.-F. and Arnold, C. (2018) 'Endoplasmic Reticulum Stress in Metabolic Disorders.', *Cells*. Multidisciplinary Digital Publishing Institute (MDPI), 7(6). doi: 10.3390/cells7060063.

Ghosh, S. *et al.* (2011) 'Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance.', *Diabetes*, 60(8), pp. 2051–60. doi: 10.2337/db11-0121.

Giebelstein, J. *et al.* (2012) 'The proteomic signature of insulin-resistant human skeletal muscle reveals increased glycolytic and decreased mitochondrial enzymes.', *Diabetologia*. Germany, 55(4), pp. 1114–1127. doi: 10.1007/s00125-012-2456-x.

Gilbert, P. (1992) *Depression: The Evolution of Powerlessness*, *East Sussex: Lawrence Erlbaum Associates Ltd.* Psychology Press. Available at: http://www.amazon.com/Depression-Evolution-Powerlessness-Dr-Gilbert/dp/0863772218 (Accessed: 19 August 2014).

Gillen, J. B. *et al.* (2013) 'Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women', *Obesity*, 21(11), pp. 2249–2255. doi: 10.1002/oby.20379.

Gist, N. H. *et al.* (2014) 'Sprint Interval Training Effects on Aerobic Capacity: A Systematic Review and Meta-Analysis', *Sports Medicine*, 44, pp. 269–279. doi: 10.1007/s40279-013-0115-0.

Gizak, A., Rakus, D. and Dzugaj, A. (2003) 'Immunohistochemical localization of human fructose-1,6-bisphosphatase in subcellular structures of myocytes.', *Histology and histopathology*, 18(1), pp. 135–42. doi: 10.14670/HH-18.135.

Gondin, J. *et al.* (2011) 'Neuromuscular electrical stimulation training induces atypical adaptations of the human skeletal muscle phenotype: a functional and proteomic analysis.', *Journal of applied physiology (Bethesda, Md. : 1985)*. United States, 110(2), pp. 433–450. doi: 10.1152/japplphysiol.00914.2010.

Goodpaster, B. H. *et al.* (2001) 'Skeletal Muscle Lipid Content and Insulin Resistance: Evidence for a Paradox in Endurance-Trained Athletes', *The Journal of Clinical Endocrinology & Metabolism*. Oxford University Press, 86(12), pp. 5755–5761. doi: 10.1210/jcem.86.12.8075.

Gougeon, R. *et al.* (2008) 'Determinants of whole-body protein metabolism in subjects with and without type 2 diabetes.', *Diabetes care*, 31(1), pp. 128–33. doi: 10.2337/dc07-1268.

Grøntved, A. and Hu, F. B. (2011) 'Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: a meta-analysis.', *JAMA*. NIH Public Access, 305(23), pp. 2448–55. doi: 10.1001/jama.2011.812.

Grundy, S. M. *et al.* (2004) 'Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood

Institute/American Heart Association conference on scientific issues related to definition.', *Circulation*, 24(3), pp. 433–8. doi: 10.1161/01.CIR.0000111245.75752.C6.

Guillet, C. *et al.* (2009) 'Changes in Basal and Insulin and Amino Acid Response of Whole Body and Skeletal Muscle Proteins in Obese Men', *The Journal of Clinical Endocrinology & Metabolism*. Oxford University Press, 94(8), pp. 3044–3050. doi: 10.1210/jc.2008-2216.

Haggmark, T. and Thorstensson, A. (1979) 'Fibre types in human abdominal muscles', *Acta Physiologica Scandinavica*, 107(4), pp. 319–325. doi: 10.1111/j.1748-1716.1979.tb06482.x.

Halvatsiotis, P. *et al.* (2002) 'Synthesis rate of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes.', *Diabetes*, 51(8), pp. 2395–404. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12145150 (Accessed: 26 February 2016).

Harju, A.-K. *et al.* (2013) 'Carbonic anhydrase III: A neglected isozyme is stepping into the limelight', *Journal of Enzyme Inhibition and Medicinal Chemistry*. Taylor & Francis, 28(2), pp. 231–239. doi: 10.3109/14756366.2012.700640.

He, J., Watkins, S. and Kelley, D. E. (2001) 'Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity.', *Diabetes*. American Diabetes Association, 50(4), pp. 817–23. doi: 10.2337/DIABETES.50.4.817.

Hellerstein, M. K. and Neese, R. A. (1992) 'Mass isotopomer distribution analysis: a technique for measuring biosynthesis and turnover of polymers.', *The American journal of physiology*, 263(5 Pt 1), pp. E988-1001. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1443132 (Accessed: 27 December 2014).

Hellerstein, M. K. and Neese, R. A. (1999) 'Mass isotopomer distribution analysis at eight years: theoretical, analytic, and experimental considerations', *American Journal of Physiology - Endocrinology and Metabolism*, 276(6 Pt 1), pp. E1146-70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10362629 (Accessed: 26 February 2016).

Hesketh, S. *et al.* (2016) 'On the Rate of Synthesis of Individual Proteins within and between Different Striated Muscles of the Rat', *Proteomes*. Multidisciplinary Digital Publishing Institute, 4(1), p. 12. doi: 10.3390/proteomes4010012.

Hibbert, J., Broemeling, L. and Isenberg, J. (1994) *Determinants of Free-Living Energy Expenditure in Normal Weight and Obese Women Measured by Doubly Labeled Water*. doi: 10.1002/j.1550-8528.1994.tb00043.x.

Hill, L. E. *et al.* (1992) *THE JOURNAL OF BIOLOGICAL CHEMISTRY Analysis of Troponin-Tropomyosin Binding to Actin TROPONIN DOES NOT PROMOTE INTERACTIONS BETWEEN TROPOMYOSIN MOLECULES**. Available at: http://www.jbc.org/content/267/23/16106.full.pdf (Accessed: 21 April 2019).

Hill, T. L. and White, G. M. (1968) 'On the sliding-filament model of muscular contraction. 3. Kinetics of cross-bridge fluctuations in configuration.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 61(2), pp. 514–21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5245985 (Accessed: 21 April 2019).

Hipkiss, A. R. (2011) 'Energy metabolism and ageing regulation: metabolically driven deamidation of triosephosphate isomerase may contribute to proteostatic dysfunction.', *Ageing research reviews*, 10(4), pp. 498–502. doi: 10.1016/j.arr.2011.05.003.

Hittel, D. S. *et al.* (2005) 'Proteome analysis of skeletal muscle from obese and morbidly obese women.', *Diabetes*. United States, 54(5), pp. 1283–1288.

Hody, S. S. *et al.* (2011) 'Human Muscle Proteome Modifications after Acute or Repeated Eccentric Exercises', *Medicine and science in sports and exercise*. United States, 43(12), pp. 2281–2296. doi:

10.1249/MSS.0b013e318222edf3.

Hoffman, N. J. *et al.* (2015) 'Global Phosphoproteomic Analysis of Human Skeletal Muscle Reveals a Network of Exercise-Regulated Kinases and AMPK Substrates.', *Cell metabolism*. NIH Public Access, 22(5), pp. 922–35. doi: 10.1016/j.cmet.2015.09.001.

Hojlund, K. *et al.* (2003) 'Proteome analysis reveals phosphorylation of ATP synthase beta -subunit in human skeletal muscle and proteins with potential roles in type 2 diabetes.', *The Journal of biological chemistry*. United States, 278(12), pp. 10436–10442. doi: 10.1074/jbc.M212881200.

Holloszy, J. and Coyle, E. (1984) 'Adaptations of skeletal muscle to endurance exercise and their metabolic consequences.', *Journal of Applied Physiology*, 56(4), pp. 831–838. doi: 10.1152/jappl.1984.56.4.831.

Holloszy, J. O. (1967) 'Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle.', *The Journal of biological chemistry*, 242(9), pp. 2278–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4290225 (Accessed: 18 December 2018).

Holloway, K. V. *et al.* (2009) 'Proteomic investigation of changes in human vastus lateralis muscle in response to interval-exercise training', *Proteomics*. Germany, 9(22), pp. 5155–5174. doi: 10.1002/pmic.200900068.

Holten, M. K. *et al.* (2004) 'Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes.', *Diabetes*, 53(2), pp. 294–305. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14747278 (Accessed: 14 March 2016).

Houston, M. E. (1999) 'Gaining weight: the scientific basis of increasing skeletal muscle mass.', *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee*, 24(4), pp. 305–16. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10470448 (Accessed: 21 April 2019).

Howard, M. L. *et al.* (2012) 'Mutation of Gtf2ird1 from the Williams-Beuren syndrome critical region results in facial dysplasia, motor dysfunction, and altered vocalisations', *Neurobiology of Disease*, 45, pp. 913–922. doi: 10.1016/j.nbd.2011.12.010.

Hu, F. B. *et al.* (2003) 'Television Watching and Other Sedentary Behaviors in Relation to Risk of Obesity and Type 2 Diabetes Mellitus in Women', *JAMA*. American Medical Association, 289(14), p. 1785. doi: 10.1001/jama.289.14.1785.

Huang, D. W., Sherman, B. T. and Lempicki, R. A. (2009) 'Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources', *Nature Protocols*. Nature Publishing Group, 4(1), pp. 44–57. doi: 10.1038/nprot.2008.211.

Hughes, V. A. *et al.* (1993) 'Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance.', *The American journal of physiology*, 264(6 Pt 1), pp. E855-62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8333511 (Accessed: 14 March 2016).

Hunter, C. J. *et al.* (2008) 'Initial signaling response to acute exercise bout is similar in hearts of rats bred for divergent exercise capacities.', *Frontiers in bioscience : a journal and virtual library*, 13, pp. 347–55. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17981552 (Accessed: 26 February 2016).

Hussey, S. E. *et al.* (2013) 'Effect of Exercise on the Skeletal Muscle Proteome in Patients with Type 2 Diabetes', *Medicine And Science In Sports And Exercise*. Department of Physiology, The University of Melbourne, Australia.: Lippincott Williams & Wilkins, 45(6), pp. 1069–1076. doi: 10.1249/MSS.0b013e3182814917.

Hwang, H. *et al.* (2010) 'Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes.', *Diabetes*. United States, 59(1), pp. 33–42. doi: 10.2337/db09-0214.

Jakes, R. W. *et al.* (2003) 'Television viewing and low participation in vigorous recreation are independently associated with obesity and markers of cardiovascular disease risk: EPIC-Norfolk population-based study',

European Journal of Clinical Nutrition, 57(9), pp. 1089–1096. doi: 10.1038/sj.ejcn.1601648.

Jaleel, A. *et al.* (2008) 'In vivo measurement of synthesis rate of individual skeletal muscle mitochondrial proteins.', *American journal of physiology. Endocrinology and metabolism*, 295(5), pp. E1255-68. doi: 10.1152/ajpendo.90586.2008.

Jensen, J. *et al.* (2011) 'The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise.', *Frontiers in physiology*. Frontiers Media SA, 2, p. 112. doi: 10.3389/fphys.2011.00112.

JH, B. *et al.* (2016) 'Diet-Induced Obesity Enhances Progression of Hepatocellular Carcinoma through Tenascin-C/Toll-Like Receptor 4 Signaling.', *Am J Pathol*, 186, pp. 145–58.

Jones, D. A. (David A. ., Round, J. M. and Haan, A. de. (2004) *Skeletal muscle from molecules to movement : a textbook of muscle physiotherapy for sport, exercise and physiotherapy*. Churchill Livingstone.

Jones, P. J. H. and Leatherdale, S. T. (1991) 'Stable isotopes in clinical research: safety reaffirmed.', *Clinical science (London, England : 1979)*. Portland Press Limited, 80(4), pp. 277–80. doi: 10.1042/CS0800277.

Joost, H. G. and Thorens, B. 'The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review).', *Molecular membrane biology*, 18(4), pp. 247–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11780753 (Accessed: 7 March 2016).

Kang, S. W., Baines, I. C. and Rhee, S. G. (1998) 'Characterization of a mammalian peroxiredoxin that contains one conserved cysteine.', *The Journal of biological chemistry*, 273(11), pp. 6303–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9497358 (Accessed: 21 April 2019).

Kasumov, T. *et al.* (2013) 'Assessment of cardiac proteome dynamics with heavy water: slower protein synthesis rates in interfibrillar than subsarcolemmal mitochondria.', *American journal of physiology. Heart and circulatory physiology*. United States, 304(9), pp. H1201-14. doi: 10.1152/ajpheart.00933.2012.

Katsanos, C. S. and Mandarino, L. J. (2011) 'Protein Metabolism in Human Obesity: A Shift in Focus From Whole-Body to Skeletal Muscle', *Obesity*. John Wiley & Sons, Ltd, 19(3), pp. 469–475. doi: 10.1038/oby.2010.290.

Kim, H. J., Lee, J. S. and Kim, C. K. (2004) 'Effect of exercise training on muscle glucose transporter 4 protein and intramuscular lipid content in elderly men with impaired glucose tolerance.', *European journal of applied physiology*, 93(3), pp. 353–8. doi: 10.1007/s00421-004-1214-2.

Kim, I.-Y. *et al.* (2016) 'Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research.', *Experimental & molecular medicine*. Korean Society for Biochemistry and Molecular Biology, 48(1), p. e203. doi: 10.1038/emm.2015.97.

Kim, T.-Y. *et al.* (2012) 'Metabolic labeling reveals proteome dynamics of mouse mitochondria.', *Molecular & cellular proteomics : MCP*. American Society for Biochemistry and Molecular Biology, 11(12), pp. 1586–94. doi: 10.1074/mcp.M112.021162.

Klein, P. D. and Klein, E. R. (1986) 'Stable Isotopes: Origins and Safety', *The Journal of Clinical Pharmacology*. Blackwell Publishing Ltd, 26(6), pp. 378–382. doi: 10.1002/j.1552-4604.1986.tb03544.x.

Knowler, W. C. *et al.* (2002) 'Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin.', *The New England journal of medicine*, 346(6), pp. 393–403. doi: 10.1056/NEJMoa012512.

Koopman, R. R. *et al.* (2007) 'Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis', *American Journal of Physiology-Endocrinology and Metabolism*. United States: American Physiological Society, 293(3), pp. E833–E842. doi: 10.1152/ajpendo.00135.2007.

Lam, M. P. Y. *et al.* (2014) 'Protein kinetic signatures of the remodeling heart following isoproterenol stimulation.', *The Journal of clinical investigation*. American Society for Clinical Investigation, 124(4), pp. 1734–44. doi: 10.1172/JCI73787.

Lanza, I. R. *et al.* (2008) 'Endurance exercise as a countermeasure for aging.', *Diabetes*. American Diabetes Association, 57(11), pp. 2933–42. doi: 10.2337/db08-0349.

Lee, J. W. R., Brancati, F. L. and Yeh, H.-C. (2011) 'Trends in the Prevalence of Type 2 Diabetes in Asians Versus Whites', *Diabetes Care*, 34(2).

Lefort, N. *et al.* (2009) 'Proteome profile of functional mitochondria from human skeletal muscle using one-dimensional gel electrophoresis and HPLC-ESI-MS/MS.', *Journal of proteomics*. Netherlands, 72(6), pp. 1046–1060. doi: 10.1016/j.jprot.2009.06.011.

Lefort, N. *et al.* (2010) 'Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle.', *Diabetes*. United States, 59(10), pp. 2444–2452. doi: 10.2337/db10-0174.

Li, M. and Larsson, L. (2010) 'Force-generating capacity of human myosin isoforms extracted from single muscle fibre segments.', *The Journal of physiology*. Wiley-Blackwell, 588(Pt 24), pp. 5105–14. doi: 10.1113/jphysiol.2010.199067.

Liebler, D. C. (2002) *Introduction to Proteomics: Tools for the New Biology*, *Humana Press*. Totowa, New Jersey: Humana Press Inc. Available at: http://www.humanapress.com (Accessed: 25 December 2014).

Lippi, G. *et al.* (2006) 'Biochemistry, physiology, and complications of blood doping: Facts and speculation', *Critical Reviews in Clinical Laboratory Sciences*, 43, pp. 349–391. doi: 10.1080/10408360600755313.

Loots, M. A. M. *et al.* (1998) 'Differences in Cellular Infiltrate and Extracellular Matrix of Chronic Diabetic and Venous Ulcers Versus Acute Wounds', *Journal of Investigative Dermatology*, 111(5), pp. 850–857. doi: 10.1046/j.1523-1747.1998.00381.x.

Lund, J. *et al.* (2017) 'Exercise in vivo marks human myotubes in vitro: Training-induced increase in lipid metabolism.', *PloS one*. United States, 12(4), p. e0175441. doi: 10.1371/journal.pone.0175441.

MacIntrosh, B. R., Gardiner, P. F. and McComas, A. J. (2006) *Skeletal Muscle : Form and Function*. second edi. United States of America: Human Kinetics, Inc.

Malik, Z. A. *et al.* (2013) 'Label-Free LC-MS Profiling of Skeletal Muscle Reveals Heart-Type Fatty Acid Binding Protein as a Candidate Biomarker of Aerobic Capacity', *Proteomes*. MDPI AG, 1(3), pp. 290–308. doi: 10.3390/proteomes1030290.

Malin, S. K. *et al.* (2016) 'Exercise Intensity Modulates Glucose-Stimulated Insulin Secretion when Adjusted for Adipose, Liver and Skeletal Muscle Insulin Resistance.', *PloS one*. Edited by A. Philp. Public Library of Science, 11(4), p. e0154063. doi: 10.1371/journal.pone.0154063.

Malm, C. and Yu, J.-G. (2012) 'Exercise-induced muscle damage and inflammation: re-evaluation by proteomics.', *Histochemistry and cell biology*. Germany, 138(1), pp. 89–99. doi: 10.1007/s00418-012-0946-z.

Mamczur, P. *et al.* (2005) 'The effect of calcium ions on subcellular localization of aldolase-FBPase complex in skeletal muscle', *FEBS Letters*, 579(7), pp. 1607–1612. doi: 10.1016/j.febslet.2005.01.071.

Martin, V. *et al.* (2002) 'Three Novel Sarco/endoplasmic Reticulum Ca $^{2+}$ -ATPase (SERCA) 3 Isoforms', *Journal of Biological Chemistry*, 277(27), pp. 24442–24452. doi: 10.1074/jbc.M202011200.

Matsuda, M. and DeFronzo, R. A. (1999) 'Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp.', *Diabetes Care*, 22(9). Available at: http://care.diabetesjournals.org/content/22/9/1462 (Accessed: 12 June 2017).

Matthews, D. E. *et al.* (1982) 'Relationship of plasma leucine and alpha-ketoisocaproate during a L-[1-13C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment.', *Metabolism: clinical and experimental*, 31(11), pp. 1105–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7132737

(Accessed: 15 April 2018).

Mazzola, J. L. and Sirover, M. A. (2001) 'Reduction of glyceraldehyde-3-phosphate dehydrogenase activity in Alzheimer's disease and in Huntington's disease fibroblasts.', *Journal of neurochemistry*, 76(2), pp. 442–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11208907 (Accessed: 2 July 2016).

McCabe, B. J. *et al.* (2006) 'Reproducibility of gas chromatography–mass spectrometry measurements of 2H labeling of water: Application for measuring body composition in mice', *Analytical Biochemistry*. Academic Press, 350(2), pp. 171–176. doi: 10.1016/j.ab.2006.01.020.

McGregor, A. *et al.* (1994) 'Identification of the vinculin-binding site in the cytoskeletal protein alpha-actinin.', *The Biochemical journal*. Portland Press Limited, 301 (Pt 1(1), pp. 225–33. doi: 10.1042/BJ3010225.

Melmed, S. *et al.* (2016) *Williams textbook of endocrinology*. Available at: https://www.sciencedirect.com/book/9780323297387/williams-textbook-of-endocrinology (Accessed: 23 August 2018).

Meyer, K. *et al.* (2003) 'Eccentric exercise in coronary patients: Central hemodynamic and metabolic responses', *Medicine and Science in Sports and Exercise*, 35, pp. 1076–1082. doi: 10.1249/01.mss.0000074580.79648.9d.

Midwood, K. *et al.* (2009) 'Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease', *Nature Medicine*, 15(7), pp. 774–781. doi: 10.1038/nm.1987.

Midwood, K. S. *et al.* (2011) 'Advances in tenascin-C biology', *Cell Mol Life Sci.*, 68(19), pp. 3175–99. doi: 10.1007/s00018-011-0783-6.

Mills, M. *et al.* (2001) 'Differential expression of the actin-binding proteins, α-actinin-2 and -3, in different species: implications for the evolution of functional redundancy', *Human Molecular Genetics*, 10(13), pp. 1335–1346. doi: https://doi.org/10.1093/hmg/10.13.1335.

Moher, D. *et al.* (2009) 'Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.', *PLoS medicine*. Public Library of Science, 6(7), pp. 264–269. doi: 10.1371/journal.pmed.1000097.

Moriggi, M. *et al.* (2010) 'Long term bed rest with and without vibration exercise countermeasures: effects on human muscle protein dysregulation.', *Proteomics*. Germany, 10(21), pp. 3756–3774. doi: 10.1002/pmic.200900817.

Morrish, N. J. *et al.* (2001) 'Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes.', *Diabetologia*, 44 Suppl 2, pp. S14-21. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11587045 (Accessed: 30 November 2015).

Murton, A. J. *et al.* (2015) *Obesity appears to be associated with altered muscle protein synthetic and breakdown responses to increased nutrient delivery in older men, but not reduced muscle mass or contractile function*. Available at: http://diabetes.diabetesjournals.org/content/diabetes/early/2015/05/22/db15-0021.full.pdf (Accessed: 27 December 2018).

Myers, J. *et al.* (2002) 'Exercise Capacity and Mortality among Men Referred for Exercise Testing', *New England Journal of Medicine*. Massachusetts Medical Society, 346(11), pp. 793–801. doi: 10.1056/NEJMoa011858.

Nakajima, H. *et al.* (2009) 'Glyceraldehyde-3-phosphate dehydrogenase aggregate formation participates in oxidative stress-induced cell death.', *The Journal of biological chemistry*, 284(49), pp. 34331–41. doi: 10.1074/jbc.M109.027698.

Norman, B. *et al.* (2009) 'Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes', *Journal of Applied Physiology*, 106, pp. 959–965. doi: 10.1152/japplphysiol.91435.2008.

Office for National Statistics (2011) 'http://www.ons.gov.uk/ons/search/index.html?newquery=diabetes'. Available at: http://www.ons.gov.uk/ons/search/index.html?pageSize=50&sortBy=none&sortDirection=none&newquery=obesity (Accessed: 26 April 2015).

Oscai, L. B. (1973) *Exercise and Sport Sciences Reviews*, *Vol.1 issue 1*. Available at: http://journals.lww.com/acsm-essr/Citation/1973/00010/The_Role_of_Exercise_in_Weight_Control.8.aspx (Accessed: 29 February 2016).

Owen, O. E. *et al.* (1978) 'Interrelationships among key tissues in the utilization of metabolic substrate.', *Adv. Mod. Nutr.*, 2, pp. 517–550.

Papa, I. *et al.* (1999) 'Alpha actinin-CapZ, an anchoring complex for thin filaments in Z-line.', *Journal of muscle research and cell motility*, 20(2), pp. 187–97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10412090 (Accessed: 2 December 2018).

Pappin, D. J. C., Hojrup, P. and Bleasby, A. J. (1993) 'Rapid identification of proteins by peptide-mass fingerprinting', *Current Biology*. Cell Press, 3(6), pp. 327–332. doi: 10.1016/0960-9822(93)90195-T.

Parsons, S. A. *et al.* (2004) 'Genetic Loss of Calcineurin Blocks Mechanical Overload-induced Skeletal Muscle Fiber Type Switching but Not Hypertrophy', *Journal of Biological Chemistry*, 279(25), pp. 26192–26200. doi: 10.1074/jbc.M313800200.

Peng, G. *et al.* (2011) 'Oleate Blocks Palmitate-Induced Abnormal Lipid Distribution, Endoplasmic Reticulum Expansion and Stress, and Insulin Resistance in Skeletal Muscle', *Endocrinology*, 152(6), pp. 2206–2218. doi: 10.1210/en.2010-1369.

Peng, S. K., Ho, K. J. and Taylor, C. B. (1972) 'Biologic effects of prolonged exposure to deuterium oxide. A behavioral, metabolic, and morphologic study.', *Archives of pathology*, 94(1), pp. 81–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5064213 (Accessed: 19 January 2018).

Periasamy, M. and Kalyanasundaram, A. (2007) 'SERCA pump isoforms: their role in calcium transport and disease.', *Muscle & nerve*, 35(4), pp. 430–42. doi: 10.1002/mus.20745.

Petersen, K. F. *et al.* (2007) 'The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome.', *Proceedings of the National Academy of Sciences of the United States of America*, 104(31), pp. 12587–94. doi: 10.1073/pnas.0705408104.

Phillips, S. M. *et al.* (1997) 'Mixed muscle protein synthesis and breakdown after resistance exercise in humans.', *The American journal of physiology*. United States, 273(1 Pt 1), pp. E99-107. doi: 10.1152/ajpendo.1997.273.1.E99.

Price, J. C. *et al.* (2012) 'Measurement of human plasma proteome dynamics with 2H2O and liquid chromatography tandem mass spectrometry', *Analytical Biochemistry*. Academic Press, 420(1), pp. 73–83. doi: 10.1016/J.AB.2011.09.007.

Raastad, T. *et al.* (2010) 'Changes in calpain activity, muscle structure, and function after eccentric exercise.', *Medicine and science in sports and exercise*, 42(1), pp. 86–95. doi: 10.1249/MSS.0b013e3181ac7afa.

Ragheb, R. *et al.* (2009) 'Free fatty acid-induced muscle insulin resistance and glucose uptake dysfunction: evidence for PKC activation and oxidative stress-activated signaling pathways.', *Biochemical and biophysical research communications*. United States, 389(2), pp. 211–216. doi: 10.1016/j.bbrc.2009.08.106.

RÄISÄNEN, S. R. *et al.* (1999) 'Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis', *The FASEB Journal*, 13(3), pp. 513–522. doi: 10.1096/fasebj.13.3.513.

Rakus, D. *et al.* (2003) 'Colocalization of muscle FBPase and muscle aldolase on both sides of the Z-line.', *Biochemical and biophysical research communications*, 311(2), pp. 294–9. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/14592412 (Accessed: 15 January 2019).

Randers, M. B. *et al.* (2014) 'Exercise performance and cardiovascular health variables in 70-year-old male soccer players compared to endurance-trained, strength-trained and untrained age-matched men', *Journal of Sports Sciences*, 32, pp. 1300–1308. doi: 10.1080/02640414.2014.898857.

Rasmussen, B. B. and Wolfe, R. R. (1999) 'REGULATION OF FATTY ACID OXIDATION IN SKELETAL MUSCLE', *Annual Review of Nutrition*, 19(1), pp. 463–484. doi: 10.1146/annurev.nutr.19.1.463.

Roberts, C. K., Hevener, A. L. and Barnard, R. J. (2013) *Comprehensive Physiology*, *Comprehensive Physiology*. Edited by R. Terjung. Hoboken, NJ, USA: John Wiley & Sons, Inc. doi: 10.1002/cphy.c110062.

Robinson, M. M. *et al.* (2011) 'Long-term synthesis rates of skeletal muscle DNA and protein are higher during aerobic training in older humans than in sedentary young subjects but are not altered by protein supplementation.', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 25(9), pp. 3240–9. doi: 10.1096/fj.11-186437.

Rolland, V. *et al.* (1995) 'Evidence of increased glyceraldehyde-3-phosphate dehydrogenase and fatty acid synthetase promoter activities in transiently transfected adipocytes from genetically obese rats.', *The Journal of biological chemistry*, 270(3), pp. 1102–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7836367 (Accessed: 2 July 2016).

Rose, A. J., Kiens, B. and Richter, E. A. (2006) 'Ca2+-calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise', *Journal of Physiology-London*, 574, pp. 889–903. doi: 10.1113/jphysiol.2006.111757.

S. *et al.* (1998) *Carbonic anhydrase III protects cells from hydrogen peroxide induced apoptosis*, *Pathophysiology*. Elsevier. Available at: https://www.infona.pl/resource/bwmeta1.element.elsevier-95c80135-6f24-3c01-b885-ab511659fcb0 (Accessed: 3 January 2019).

Salanova, M. *et al.* (2014) 'Disuse deterioration of human skeletal muscle challenged by resistive exercise superimposed with vibration: evidence from structural and proteomic analysis.', *The FASEB Journal*. United States, 28(11), pp. 4748–4763. doi: 10.1096/fj.14-252825.

Salanova, M. *et al.* (2015) 'Vibration mechanosignals superimposed to resistive exercise result in baseline skeletal muscle transcriptome profiles following chronic disuse in bed rest', *Scientific reports*. England, 5, p. 17027. doi: 10.1038/srep17027.

Salmikangas, P. *et al.* (1999) 'Myotilin, a novel sarcomeric protein with two Ig-like domains, is encoded by a candidate gene for limb-girdle muscular dystrophy', *Human Molecular Genetics*. Oxford University Press, 8(7), pp. 1329–1336. doi: 10.1093/hmg/8.7.1329.

Salway, J. G. (2004) *Metabolism at a glance*. Blackwell Pub. Available at: https://www.wiley.com/en-ag/Metabolism+at+a+Glance%2C+3rd+Edition-p-9781118682074 (Accessed: 21 April 2019).

Samdani, P. *et al.* (2015) 'A Comprehensive Inter-Tissue Crosstalk Analysis Underlying Progression and Control of Obesity and Diabetes', *Scientific Reports*. Nature Publishing Group, 5, p. 12340. doi: 10.1038/srep12340.

Scalzo, R. L. *et al.* (2014) 'Greater muscle protein synthesis and mitochondrial biogenesis in males compared with females during sprint interval training', *The FASEB Journal*, 28(6), pp. 2705–2714. doi: 10.1096/fj.13-246595.

Schild, M. *et al.* (2015) 'Basal and exercise induced label-free quantitative protein profiling of m. vastus lateralis in trained and untrained individuals.', *Journal of proteomics*. Netherlands, 122, pp. 119–132. doi: 10.1016/j.jprot.2015.03.028.

Scrimgeour, C. M. *et al.* (1993) 'A simplified method for deuterium/hydrogen isotope ratio measurements on water samples of biological origin', *Biological Mass Spectrometry*. John Wiley & Sons, Ltd., 22(7), pp. 383–387.

doi: 10.1002/bms.1200220704.

Seuring, T., Archangelidi, O. and Suhrcke, M. (2015) 'The Economic Costs of Type 2 Diabetes: A Global Systematic Review', *PharmacoEconomics*. Springer International Publishing, 33(8), pp. 811–831. doi: 10.1007/s40273-015-0268-9.

Shankaran, M. *et al.* (2015) 'Proteome-wide muscle protein fractional synthesis rates predict muscle mass gain in response to a selective androgen receptor modulator in rats', *Am J Physiol Endocri-nol Metab*, 310, pp. E405–E417. Available at: https://www.physiology.org/doi/pdf/10.1152/ajpendo.00257.2015 (Accessed: 27 April 2018).

Shankaran, M. *et al.* (2016) 'Circulating protein synthesis rates reveal skeletal muscle proteome dynamics', *Journal of Clinical Investigation*. United States, 126(1), pp. 288–302. doi: 10.1172/JCI79639.

Shea, B. J. *et al.* (2007) 'Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews.', *BMC medical research methodology*, 7, p. 10. doi: 10.1186/1471-2288-7-10.

Shea, B. J. *et al.* (2009) 'AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews.', *Journal of clinical epidemiology*, 62(10), pp. 1013–20. doi: 10.1016/j.jclinepi.2008.10.009.

Sheffield-Moore, M. *et al.* (2004) 'Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise.', *American journal of physiology. Endocrinology and metabolism*, 287(3), pp. E513-22. doi: 10.1152/ajpendo.00334.2003.

Shepherd, S. O. *et al.* (2013) 'Sprint interval and traditional endurance training increase net intramuscular triglyceride breakdown and expression of perilipin 2 and 5', *The Journal of physiology*. Blackwell Publishing Ltd, 591(Pt 3), pp. 657–75. doi: 10.1113/jphysiol.2012.240952.

Shepherd, S. O. *et al.* (2015) 'Low-Volume High-Intensity Interval Training in a Gym Setting Improves Cardio-Metabolic and Psychological Health', *PLOS ONE*. Edited by S. E. Alway. Fitness Information Technology, Inc, 10(9), p. e0139056. doi: 10.1371/journal.pone.0139056.

Short, K. R. *et al.* (2004) 'Age and aerobic exercise training effects on whole body and muscle protein metabolism.', *American journal of physiology. Endocrinology and metabolism*, 286(1), pp. E92-101. doi: 10.1152/ajpendo.00366.2003.

Sollanek, K. J. *et al.* (2017) 'Global Proteome Changes in the Rat Diaphragm Induced by Endurance Exercise Training.', *PloS one*. Public Library of Science, 12(1), p. e0171007. doi: 10.1371/journal.pone.0171007.

Srisawat, K. *et al.* (2017) 'A Systematic Review and Meta-Analysis of Proteomics Literature on the Response of Human Skeletal Muscle to Obesity/Type 2 Diabetes Mellitus (T2DM) Versus Exercise Training.', *Proteomes*. Multidisciplinary Digital Publishing Institute (MDPI), 5(4), p. 30. doi: 10.3390/proteomes5040030.

St-Jean-Pelletier, F. *et al.* (2017) 'The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men', *Journal of Cachexia, Sarcopenia and Muscle*, 8(2), pp. 213–228. doi: 10.1002/jcsm.12139.

STEFANICK, M. L. (1993) *Exercise and Sport Sciences Reviews*, *Vol. 21 issue 1*. Available at: http://journals.lww.com/acsm-essr/Citation/1993/01000/Exercise_and_Weight_Control.12.aspx (Accessed: 29 February 2016).

Stephens, F. B. *et al.* (2015) 'Lipid-induced insulin resistance is associated with an impaired skeletal muscle protein synthetic response to amino acid ingestion in healthy young men.', *Diabetes*, 64(5), pp. 1615–20. doi: 10.2337/db14-0961.

The 6th ISPAH International congress on physical activity and health (2016) *THE BANGKOK DECLARATION ON*

PHYSICAL ACTIVITY FOR GLOBAL HEALTH AND SUSTAINABLE DEVELOPMENT. Bangkok. Available at: www.ispah.org (Accessed: 10 December 2018).

Thingholm, T. E. *et al.* (2011) 'Characterization of human myotubes from type 2 diabetic and nondiabetic subjects using complementary quantitative mass spectrometric methods.', *Molecular & cellular proteomics : MCP*. United States, 10(9), p. M110.006650. doi: 10.1074/mcp.M110.006650.

Thompson, P. D. *et al.* (2003) 'Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical', *Circulation*, 107(24), pp. 3109–16. doi: 10.1161/01.CIR.0000075572.40158.77.

van Tienen, F. H. J. *et al.* (2012) 'Physical Activity Is the Key Determinant of Skeletal Muscle Mitochondrial Function in Type 2 Diabetes', *The Journal of Clinical Endocrinology & Metabolism*, 97(9), pp. 3261–3269. doi: 10.1210/jc.2011-3454.

Tipton, K. D. *et al.* (2003) 'Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion', *American Journal of Physiology-Endocrinology and Metabolism*. American Physiological SocietyBethesda, MD , 284(1), pp. E76–E89. doi: 10.1152/ajpendo.00234.2002.

Torok, D. J. *et al.* (1995) 'CARDIOVASCULAR-RESPONSES TO EXERCISE IN SPRINTERS AND DISTANCE RUNNERS', *Medicine and Science in Sports and Exercise*, 27, pp. 1050–1056. doi: 10.1249/00005768-199507000-00014.

Tran, L., Kras, K. A., *et al.* (2018) 'Lower Fasted-State but Greater Increase in Muscle Protein Synthesis in Response to Elevated Plasma Amino Acids in Obesity.', *Obesity (Silver Spring, Md.)*. NIH Public Access, 26(7), pp. 1179–1187. doi: 10.1002/oby.22213.

Tran, L., Langlais, P. R., *et al.* (2018) 'Mitochondrial ATP synthase β-subunit production rate and ATP synthase specific activity are reduced in skeletal muscle of humans with obesity', *Experimental Physiology*. John Wiley & Sons, Ltd (10.1111). doi: 10.1113/EP087278.

Tudor-Locke, C. *et al.* (2011) 'How many steps/day are enough? for adults', *International Journal of Behavioral Nutrition and Physical Activity*. BioMed Central, 8(1), p. 79. doi: 10.1186/1479-5868-8-79.

Tudor-Locke, C. and Bassett, D. R. (2004) 'How Many Steps/Day Are Enough?', *Sports Medicine*. Springer International Publishing, 34(1), pp. 1–8. doi: 10.2165/00007256-200434010-00001.

U.S. Department of Health and Human Services; Center for Disease Control and Prevention (1996) *Physical Activity and Health A Report of the Surgeon General The President's Council on Physical Fitness and Sports*. Available at: https://profiles.nlm.nih.gov/ps/access/NNBBHB.pdf (Accessed: 10 December 2018).

Uldry, M. and Thorens, B. (2004) 'The SLC2 family of facilitated hexose and polyol transporters.', *Pflügers Archiv : European journal of physiology*, 447(5), pp. 480–9. doi: 10.1007/s00424-003-1085-0.

Venckunas, T. *et al.* (2012) 'Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect', *Applied Physiology, Nutrition, and Metabolism*. NRC Research Press, 37(6), pp. 1038–1046. doi: 10.1139/h2012-087.

Verhagen, A. P. *et al.* (1998) 'The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus.', *Journal of Clinical Epidemiology*, 51(12), pp. 1235–1241. doi: 10.1016/S0895-4356(98)00131-0.

Vincent, H. K., Bourguignon, C. and Vincent, K. R. (2006) 'Resistance training lowers exercise-induced oxidative stress and homocysteine levels in overweight and obese older adults', *Obesity*, 14, pp. 1921–1930. doi: 10.1038/oby.2006.224.

Volek, J. S., VanHeest, J. L. and Forsythe, C. E. (2005) 'Diet and Exercise for Weight Loss', *Sports Medicine*, 35(1), pp.

1–9. doi: 10.2165/00007256-200535010-00001.

Wagenmakers, A. J. M. and Wagenmakers, A. (1999) 'Tracers to investigate protein and amino acid metabolism in human subjects Protein turnover: Skeletal muscle: Exercise: Arterio–venous difference: Tracer balance methods', *Proceedings of the Nutrition Society*, 58, pp. 987–1000. doi: 10.1017/S0029665199001305.

Wang, Y. *et al.* (2009) 'Adaptations to exercise training within skeletal muscle in adults with type 2 diabetes or impaired glucose tolerance: a systematic review.', *Diabetes/metabolism research and reviews*, 25(1), pp. 13–40. doi: 10.1002/dmrr.928.

Warburton, D. E. R. *et al.* (2010) 'A systematic review of the evidence for Canada's Physical Activity Guidelines for Adults.', *The International Journal of Behavioral Nutrition and Physical Activity*. Warburton, Darren E. R., Experimental Medicine Program, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada: BioMed Central Limited, 7. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2012-32233-001&site=ehost-live.

Warburton, D. E. R., Nicol, C. W. and Bredin, S. S. D. (2006) 'Health benefits of physical activity: the evidence.', *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*, 174(6), pp. 801–9. doi: 10.1503/cmaj.051351.

Waterlow, J. C. (John C., Garlick, P. J. and Millward, D. J. (1978) *Protein turnover in mammalian tissues and in the whole body*. North-Holland Pub. Co. Available at: https://books.google.co.uk/books/about/Protein_turnover_in_mammalian_tissues_an.html?id=zvxqAAAAMAAJ&redir_esc=y (Accessed: 15 April 2018).

Waterlow, J. C., Golden, M. H. and Garlick, P. J. (1978) 'Protein turnover in man measured with 15N: comparison of end products and dose regimes.', *The American journal of physiology*, 235(2), pp. E165-74. doi: 10.1152/ajpendo.1978.235.2.E165.

Wewege, M. *et al.* (2017) 'The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis', *Obesity Reviews*. doi: 10.1111/obr.12532.

Wilborn, C. D. and Willoughby, D. S. (2004) 'The role of dietary protein intake and resistance training on Myosin heavy chain expression.', *Journal of the International Society of Sports Nutrition*. BioMed Central, 1(2), pp. 27–34. doi: 10.1186/1550-2783-1-2-27.

Wilkinson, D. J. *et al.* (2014) 'A validation of the application of D(2)O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans.', *American journal of physiology. Endocrinology and metabolism*, 306(5), pp. E571-9. doi: 10.1152/ajpendo.00650.2013.

Wolfe, R. R. (2006) 'The underappreciated role of muscle in health and disease', *The American Journal of Clinical Nutrition*. Oxford University Press, 84(3), pp. 475–482. doi: 10.1093/ajcn/84.3.475.

Woolstenhulme, M. T. *et al.* (2005) 'Desmin increases with high-intensity concentric contractions in humans', *Muscle & Nerve*, 31, pp. 20–24. doi: 10.1002/mus.20170.

Workeneh, B. and Bajaj, M. (2013) 'The regulation of muscle protein turnover in diabetes.', *The international journal of biochemistry & cell biology*, 45(10), pp. 2239–44. doi: 10.1016/j.biocel.2013.06.028.

World Health Organisation (2015) 'WHO | Physical activity'. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs385/en/ (Accessed: 9 March 2016).

World Health Organisation (WHO) (2018) *Global Action Plan on Physical Activity 2018-2030: More Active People For A Healthier World*. Available at: http://apps.who.int/iris/bitstream/handle/10665/272722/9789241514187-eng.pdf?ua=1 (Accessed: 10 December 2018).

World Health Organization (2016) *GLOBAL REPORT ON DIABETES WHO Library Cataloguing-in-Publication Data Global report on diabetes*. Available at: http://www.who.int/about/licensing/copyright_form/index.html (Accessed: 6 December 2018).

World Health Organization (2018) *The top 10 causes of death*, *Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 200-2016*. Available at: http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (Accessed: 23 August 2018).

World Health Organization and International Diabetes Federation (2006) *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia*. Geneva, Switzerland: WHO Press, World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/43588/1/9241594934_eng.pdf.

Xiao, G. G. *et al.* (2008) 'Determination of protein synthesis in vivo using labeling from deuterated water and analysis of MALDI-TOF spectrum.', *Journal of applied physiology (Bethesda, Md. : 1985)*, 104(3), pp. 828–36. doi: 10.1152/japplphysiol.00976.2007.

Yang, N., Garton, F. and North, K. (2009) 'α-Actinin-3 and Performance', in *Genetics and Sports*. Basel: KARGER, pp. 88–101. doi: 10.1159/000235698.

Yarasheski, K. E. *et al.* (1992) 'Measurement of muscle protein fractional synthetic rate by capillary gas chromatography/combustion isotope ratio mass spectrometry.', *Biological Mass Spectrometry*. Metabolism Division, Washington University School of Medicine, St Louis, Missouri 63110.: Wiley, 21(10), pp. 486–490. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=1420371&site=ehost-live.

Yuenyongchaiwat, K. (2016) 'Effects of 10,000 steps a day on physical and mental health in overweight participants in a community setting: a preliminary study.', *Brazilian journal of physical therapy*. Associação Brasileira de Pesquisa e Pós-Graduação em Fisioterapia, 20(4), pp. 367–73. doi: 10.1590/bjpt-rbf.2014.0160.

Appendices

Appendix 1 Summarises the main content (PICO; RQ1)

Summarises the main content (RQ1) of each article according to the PICO principle, which includes Population, Indicator, Comparison, and Outcome measures. The earliest reported study meeting the eligibility criteria was HØjlund et al. published in 2003(Hojlund et al. 2003) therefore the current review considers literature over a 12-year study period from 2003 to 2016. *presented significant difference at *p*-value <0.05 or under

Appendix 2 Summarises the main content (PICO; RQ2)

Summarises the main content (RQ2) of each article according to the PICO principle, which includes Population, Intervention, Comparison, and Outcome measures. The earliest reported study meeting the eligibility criteria was that of Lanza et al (Lanza et al. 2008); therefore, the current review considers literature over an 8-year study period from 2008 to 2015. *presented significant difference at *p*-value < 0.05 or under

Appendix 3 The 82 identified proteins

The 82 proteins that were categorised by molecular function and biological process following UniProtKB.

Appendix 4 The 82 identified proteins profiled in the current work.

The 82 identified proteins profiled in the current work. ANOVA discovered 2 proteins (AT2A2 and FABPH) differed significantly (p<0.05) in abundance between LE, OB, T2DM muscle.

Appendix 5 The 231 proteins (OB vs TR)

The 231 proteins that were categorised by molecular function and biological process following UniProtKB. (OB vs TR)

Appendix 1 summarises the main content (RQ1) of each article according to the PICO principle, which includes Population, Indicator, Comparison, and Outcome measures. The earliest reported study meeting the eligibility criteria was Højlund et al. published in 2003(Hojlund et al. 2003) therefore the current review considers literature over a 12-year study period from 2003 to 2016.
***presented significant difference at p-value <0.05 or under**

Reference	Participants (M/F)	Study groups			Outcome measures		
		Case	Control	Method in proteomics	Uniprot accession number		Protein name (gene name)*
Højlund et al. 2003	N=15 (8/7) Vastus lateralis (biopsy & cell)	T2DM n = 9 (5/4) age 45 ± 2 y BMI 33.3± 1.9 kg.m^{-2}	OWHLT n = 6 (3/3) age 46 ± 2 y BMI 25.7 ± 1.2 kg.m^{-2}	2D-DIGE pH 4-7 MALDI-TOF (P-32)	Up-regulation (6)		
					P08238	Heat shock protein HSP 90-beta	HSP90AB1 (HSP90AB1 HSP90B, HSPC2, HSPCB)
					P11021	78 kDa glucose-regulated protein	GRP78 (HSPA5 GRP78)
					P12109	Collagen alpha-1(VI) chain	COL6A1 (COL6A1)
					P12277	Creatine kinase B-type	KCRB (CKB CKBB)
					P36871	Phosphoglucomutase-1	PGM1 (PGM1)
					Q96A32	Myosin regulatory light chain 2, skeletal muscle isoform	MLRS (MYLPF)
					Down-regulation (2)		
					P06576	ATP synthase subunit beta, mitochondrial	ATPB (ATP5B)
					P10916	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	MLRV
Hittel et al. 2005	N=18 (0/18) Rectus abdominus	OW/OB n = 6 (0/6) age 44 ± 3 y BMI 30.2 ± 0.81 kg.m^{-2} MOB n = 6 (0/6) age 38 ± 3 y BMI 53.8 ± 3.5 kg.m^{-2}	LEHLT n = 6 age 45 ± 3 y BMI 23.8 ± 0.58 kg.m^{-2}	2D-DIGE pH 3-10 11 cm MALDI-TOF	Up-regulation (3)		
					P00568	Adenylate kinase isoenzyme 1	KAD1 (AK1)
					P04075	Fructose-bisphosphate aldolase A	ALDOA (ALDOA ALDA)
					P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P (GAPDH GAPD, CDABP0047, OK/SW-cl.12)
					Down-regulation (0)		
Lefort et al. 2010	N = 34 (18/16) Vastus lateralis	OB (with pre-HT/pre-DM) n = 14 (7/7) age 38 ± 4 y BMI 36 ± 1 kg.m^{-2}	LEOW (with pre-HT) n = 20 (11/9) age 38 ± 3 y BMI 25 ± 1 kg.m^{-2}	1D-SDS-PAGE (12%) HPLC-MS/MS	Up-regulation (10)		
					A6NNS2	Dehydrogenase/reductase SDR family member 7C	DRS7C (DHRS7C SDR32C2)
					O14983	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	AT2A1 (SERCA1)
					P02647	Apolipoprotein A-I	APOA1 (APOA1)
					P11217	Glycogen phosphorylase, muscle form	PYGM (PYGM)
					P12882	Myosin-1	MYH1 (MYH1)
					P27105	Erythrocyte band 7 integral membrane protein	STOM (STOM)
					P32119	Peroxiredoxin-2	PRDX2 (Prdx2 Tdpx1, Tpx)
					P54289	Voltage-dependent calcium channel subunit alpha-2/delta-1	CA2D1 (CACNA2D1 CACNL2A, CCHL2A, MHS3)
					Q9UHG3	Prenylcysteine oxidase 1	PCYOX (PCYOX1 KIAA0908, PCL1, UNQ597/PRO1183)
					Q9UKX2	Myosin-2	MYH2 (MYH2 MYHSA2)
					Down-regulation (11)		
					E9PQ53	NADH dehydrogenase [ubiquinone] 1 subunit C2, isoform 2	NDUCR (NDUFC2-KCTD14)
					O14958	Calsequestrin-2	CASQ2 (CASQ2)
					O75306	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	NDUS2 (NDUFS2)
					O95169	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial	NDUB8 (NDUFB8)
					P05166	Propionyl-CoA carboxylase beta chain, mitochondrial	PCCB (PCCB)
					P10606	Cytochrome c oxidase subunit 5B, mitochondrial	COX5B (COX5B)
					P51970	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	NDUA8 (NDUFA8)
					Q02252	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	MMSA (ALDH6A1 MMSDH)
					Q02252	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	MMSA (ALDH6A1 MMSDH)
					Q86Y39	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11	NDUAB (NDUFA11)
					Q92523	Carnitine O-palmitoyltransferase 1, muscle isoform	CPT1B
Hwang et al. 2010	N = 24 (12/12) Vastus lateralis	OB (with pre-DM) n = 8 (4/4) age 44 ± 3 y BMI 31.6 ± 0.8 kg.m^{-2} T2DM n= 8 (4/4) age 48 ± 3 y BMI 29.0 ± 1.1 kg.m^{-2}	LEHLT n= 8 (4/4) age 37 ± 4 y BMI 24.3 ± 0.8 kg.m^{-2}	1D-SDS-PAGE (4-20%) HPLC-MS/MS	Up-regulation (9)		
					O94760	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	DDAH1 (DDAH1 DDAH)
					P30101	Protein disulfide-isomerase A3	PDIA3 (PDIA3 ERP57, ERP60, GRP58)
					P47897	Glutamine--tRNA ligase	SYQ (QARS)
					P49720	Proteasome subunit beta type-3	PSB3 (PSMB3)
					P50990	T-complex protein 1 subunit theta	TCPQ (CCT8 C21orf112, CCTQ, KIAA0002)
					P50991	T-complex protein 1 subunit delta	TCPD (CCT4 CCTD, SRB)
					Q16543	Hsp90 co-chaperone Cdc37	CDC37 (CDC37 CDC37A)
					Q93034	Cullin-5	CUL5 (CUL5 VACM1)
					Q9Y2K3	Myosin-15	MYH15 (MYH15 KIAA1000)
					Down-regulation (5)		
						Cytochrome c oxidase subunit 6C (Cytochrome c oxidase polypeptide VIc)	COX6C (COX6C)
					P14927	Cytochrome b-c1 complex subunit 7	QCR7 (UQCRB UQBP)
					P35609	Alpha-actinin-2	ACTN2 (ACTN2)
					Q9NP98	Myozenin-1	MYOZ1
					Q9NX63	MICOS complex subunit MIC19	MIC19 (CHCHD3 MIC19, MINOS3)
Thingholm et al 2011	N=30 Vastus lateralis (myotube cell)	OB n =10 age 49 ± 1 y BMI 33.7 ± 1.4 kg.m^{-2} OBT2DM n=10 age 50 ± 1 y BMI 33.5 ± 1.1 kg.m^{-2}	LE n=10 age 51 ± 1 y BMI 24.2 ± 0.5 kg.m^{-2}	 nLC-MS/MS (iTRAQ)	Up-regulation (6)		
					P05413	Fatty acid-binding protein, heart	FABPH (FABP3 FABP11, MDGI)
					P11055	Myosin-3	MYH3 (MYH3)
					P13535	Myosin-8	MYH8 (MYH8)
					P24821	Tenascin	TENA (TNC)
					P60842	Eukaryotic initiation factor 4A-I	IF4A1 (EIF4A1)
					Q9UKX2	Myosin-2	MYH2 (MYH2 MYHSA2)
					Down-regulation (6)		
					O14558	Heat shock protein beta-6	HSPB6 (HSPB6)
					P00325	Alcohol dehydrogenase 1B	ADHB1 (ADH1B ADH2)
					P00813	Adenosine deaminase	ADA (ADA ADA1)
					P02647	Apolipoprotein A-I	APOA1 (APOA1)
					P40121	Macrophage-capping protein	CAPG (CAPG AFCP, MCP)
					P69905	Hemoglobin subunit alpha	HBA (HBA1 HBA2)
Giebelstein et al 2012	N=31 (17/14) Vastus lateralis	OB (pre-DM) n=11 (6/5) age 49 ± 1 y BMI 33.7 ± 1.4 kg.m^{-2}	LEHLT n=10 (5/5) age 51 ± 1 y BMI 24.2 ± 0.5 kg.m^{-2}	2D-DIGE LC-MS/MS (CyDye)	Up-regulation (20)		
					O75323	Protein NipSnap homolog 2	NIPS2 (GBAS NIPSNAP2)
					P02679	Fibrinogen gamma chain	FIBG (FGG PRO2061)
					P04406	Glyceraldehyde-3-phosphate dehydrogenase	G3P (GAPDH GAPD, CDABP0047, OK/SW-cl.12)

		OBT2DM n=10 (6/4) age 50 ± 1 y BMI 33.5 ± 1.1 kg.m^{-2}			P05976	Myosin light chain 1/3, skeletal muscle isoform	MYL1
					P06732	Creatine kinase M-type	KCRM (KCRM)
					P11217	Glycogen phosphorylase, muscle form	PYGM (PYGM)
					P13929	Beta-enolase	ENOB (ENO3)
					P14618	Pyruvate kinase PKM	KYPM (PKM OIP3, PK2, PK3, PKM2)
					P15259	Phosphoglycerate mutase 2	PGAM2 (PGAM2 PGAMM)
					P40925	Malate dehydrogenase, cytoplasmic	MDHC (MDH1, MDHA)
					P45378	Troponin T, fast skeletal muscle	TNNT3 (TNNT3)
					P54652	Heat shock-related 70 kDa protein 2	HSP72 (HSPA2)
					P55084	Trifunctional enzyme subunit beta, mitochondrial	ECHB (HADHB MSTP029)
					P63267	Actin, gamma-enteric smooth muscle	ACTH (ACTG2 ACTA3, ACTL3, ACTSG)
					P69905	Hemoglobin subunit alpha	HBA (HBA1 HBA2)
					Q13011	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	ECH1
					Q14469	Transcription factor HES-1	HES1 (HES1 BHLHB39, HL, HRY)
					Q96A32	Myosin regulatory light chain 2, skeletal muscle isoform	MLRS (MYLPF)
					Q9H7C9	Mth938 domain-containing protein	AAMDC (AAMDC C11orf67, PTD015)
					Q9Y281	Cofilin-2	COF2 (CFL2)
					Down-regulation (6)		
					P08590	Myosin light chain 3	MYL3 (MYL3)
					P10916	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	MLRV
					P13805	Troponin T, slow skeletal muscle	TNNT1 (TNNT1 TNT)
					P17661	Desmin	DESM (DES)
					P40123	Adenylyl cyclase-associated protein 2	CAP2 (CAP2)
					P62736	Actin, aortic smooth muscle	ACTA (ACTA2 ACTSA, ACTVS, GIG46)
Al-Khalili et al 2014a	N=20 (na/na)	T2DM n=10	NGT n=10	2D-DIGE pH 3-11 24cm LC-MS/MS (CyDye)	Up-regulation (8)		
	Vastus lateralis (cell line)				P04792	Heat shock protein beta-1	HSPB1 (HSPB1 HSP27, HSP28)
					P25786	Proteasome subunit alpha type-1	PSA1 (PSMA1 HC2, NU, PROS30, PSC2)
					P25789	Proteasome subunit alpha type-4	PSA4 (PSMA4 HC9, PSC9)
					P49721	Proteasome subunit beta type-2	PSB2 (PSMB2)
					P60900	Proteasome subunit alpha type-6	PSA6 (PSMA6)
					P62195	26S protease regulatory subunit 8	PRS8 (PSMC5 SUG1)
					Q9BTZ2	Dehydrogenase/reductase SDR family member 4	DHRS4 (DHRS4 SDR25C2, UNQ851/PRO1800)
					Q9UBQ0	Vacuolar protein sorting-associated protein 29	VPS29 (VPS29 DC15, DC7, MDS007)
					Down-regulation (3)		
					O75083	WD repeat-containing protein 1	WDR1 (WDR1)
					P14866	Heterogeneous nuclear ribonucleoprotein L	HNRPL (HNRNPL HNRPL, P/OKcl.14)
					Q13838	Spliceosome RNA helicase DDX39B	DX39B (DDX39B BAT1, UAP56)
Al-Khalili et al 2014b	N=20 (20/0)	T2DM (10/0)	NGT (10/0)	2D-DIGE pH 3-11 24cm LC-MS/MS (CyDye)	Up-regulation (29)		
	Vastus lateralis (cell line)				O75390	Citrate synthase, mitochondrial	CISY (CS)
					O75534	Cold shock domain-containing protein E1	CSDE1 (CSDE1 D1S155E, KIAA0885, NRU, UNR)
					O96008	Mitochondrial import receptor subunit TOM40 homolog	TOM40 (TOMM40 C19orf1, PEREC1, TOM40)
					P13010	X-ray repair cross-complementing protein 5	XRCC5 (XRCC5 G22P2)
					P14061	Estradiol 17-beta-dehydrogenase 1	DHB1 (HSD17B1 E17KSR, EDH17B1, EDH17B2, EDHB17, SDR28C1)
					P14866	Heterogeneous nuclear ribonucleoprotein L	HNRPL (HNRNPL HNRPL, P/OKcl.14)
					P23786	Carnitine O-palmitoyltransferase 2, mitochondrial	CPT2 (CPT2 CPT1)
					P25705	ATP synthase subunit alpha, mitochondrial	ATPA (ATP5A1 ATP5A, ATP5AL2, ATPM)
					P30044	Peroxiredoxin-5, mitochondrial	PRDX5 (PRDX5 ACR1, SBBI10)
					P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA
					P34897	Serine hydroxymethyltransferase, mitochondrial	GLYM (SHMT2)
					P38117	Electron transfer flavoprotein subunit beta	ETFB (ETFB FP585)
					P40926	Malate dehydrogenase, mitochondrial	MDHM (MDH2)
					P42765	3-ketoacyl-CoA thiolase, mitochondrial	THIM (ACAA2)
					P47897	Glutamine--tRNA ligase	SYQ (QARS)
					P49411	Elongation factor Tu, mitochondria	EFTU (TUFM)
					P49748	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADV (ACADVL)
					P50213	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	IDH3A (IDH3A)
					P83111	Serine beta-lactamase-like protein LACTB, mitochondrial	LACTB (LACTB MRPL56, UNQ843/PRO1781)
					Q02809	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	PLOD1 (PLOD1 LLH, PLOD)
					Q12906	Interleukin enhancer-binding factor 3	ILF3 (ILF3 DRBF, MPHOSPH4, NF90)
					Q12931	Heat shock protein 75 kDa, mitochondrial	TRAP1 (TRAP1 HSP75)
					Q13263	Transcription intermediary factor 1-beta	TIF1B (TRIM28 KAP1, RNF96, TIF1B)
					Q14191	Werner syndrome ATP-dependent helicase	WRN (WRN RECQ3, RECQL2)
					Q92499	ATP-dependent RNA helicase DDX1	DDX1 (DDX1)
					Q92945	Far upstream element-binding protein 2	FUBP2 (KHSRP FUBP2)
					Q99798	Aconitate hydratase, mitochondrial	ACON (ACO2)
					Q9HCC0	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	MCCB (MCCC2)
					Q9NSD9	Phenylalanine--tRNA ligase beta subunit	SYFB (FARSB FARSLB, FRSB, HSPC173)
					Down-regulation (18)		
					P04792	Heat shock protein beta-1	HSPB1 (HSPB1 HSP27, HSP28)
					P06733	Alpha-enolase	ENOA (ENO1 ENO1L1, MBPB1, MPB1)
					P07900	Heat shock protein HSP 90-alpha	HSP90A (HSP90AA1 HSP90A, HSPC1, HSPCA)
					P09211	Glutathione S-transferase P	GSTP1 (GSTP1 FAEES3, GST3)
					P13797	Plastin-3	PLST (PLS3)
					P16152	Carbonyl reductase [NADPH] 1	CBR1 (CBR1)
					P28161	Glutathione S-transferase Mu 2	GSTM2 (GSTM2 GST4)
					P30084	Enoyl-CoA hydratase, mitochondrial	ECHM (ECHS1)
					P30711	Glutathione S-transferase theta-1	GSTT1 (GSTT1)
					P35080	Profilin-2	PROF2 (PFN2)
					P60981	Destrin	DEST (DSTN ACTDP, DSN)
					P61086	Ubiquitin-conjugating enzyme E2 K	UBE2K (UBE2K HIP2, LIG)
					P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA (PPIA CYPA)
					P82279	Protein crumbs homolog 1	CRUM1 (CRB1)

					Q16555	Dihydropyrimidinase-related protein 2	DPYL2 (DPYSL2 CRMP2, ULIP2)
					Q99497	Protein deglycase DJ-1	PARK7
					Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	SH3L3 (SH3BGRL3 P1725)
					Q9Y617	Phosphoserine aminotransferase	SERC (PSAT1 PSA)
Caruso et al 2014	N=22 (14/8) Vastus lateralis biopsy	OCs (pre-DM) n=7 (5/2) age 44 ± 4 y BMI 32.5 ± 1.4 $kg.m^{-2}$ T2DM n=7 (4/3) age 53 ± 4 y BMI 32.8 ± 1.5 $kg.m^{-2}$	LCs n=8 (5/3) age 46 ± 4 y BMI 23.2 ± 0.5 $kg.m^{-2}$	1D-SDS-PAGE HPLC-MS/MS	Up-regulation (9)		
					O14732	Inositol monophosphatase 2	IMPA2 (IMPA2 IMP.18P)
					P19474	E3 ubiquitin-protein ligase TRIM21	RO52 (TRIM21 RNF81, RO52, SSA1)
					P21980	Protein-glutamine gamma-glutamyltransferase 2	TGM2 (TGM2)
					P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha	P85A (PIK3R1 GRB1)
					P30153	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	2AAA (PPP2R1A)
					P62753	40S ribosomal protein S6	RS6 (RPS6 OK/SW-cl.2)
					P62993	Growth factor receptor-bound protein 2	GRB2 (GRB2 ASH)
					P67775	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	PP2AA (PPP2CA/B)
					Q13061	Triadin	TRDN (TRDN)
					Down-regulation (4)		
					O75083	WD repeat-containing protein 1	WDR1 (WDR1)
					P05091	Aldehyde dehydrogenase, mitochondrial	ALDH2 (ALDH2 ALDM)
					P33176	Kinesin-1 heavy chain	KINH (KIF5B KNS, KNS1)
					Q92901	60S ribosomal protein L3-like	RL3L (RPL3L)
Hussey 2013	N = 12 (8/4) at rest Vastus lateralis	T2DM n = 6 (4/2) age 54 ± 4 y BMI 29 ± 2 $kg.m^{-2}$	Control n = 6 (4/2) age 48 ± 2 y BMI 28 ± 3 $kg.m^{-2}$	1D-SDS-PAGE (4-20%) HPLC-MS/MS	Up-regulation (2)		
					P02765	Alpha-2-HS-glycoprotein	FETUA (AHSG FETUA, PRO2743)
					P02787	Serotransferrin	TF (TRFE)
					P12882	Myosin-1	MYH1 (MYH1)
					Down-regulation (13)		
					P00403	Cytochrome c oxidase subunit 2	COX2 (MT-CO2 COII, COXII, MTCO2)
					P00505	Aspartate aminotransferase, mitochondrial	AATM (GOT2)
					P15121	Aldose reductase	ALDR (AKR1B1 ALDR1)
					P17174	Aspartate aminotransferase, cytoplasmic	AATC (GOT1)
					P24539	ATP synthase F(0) complex subunit B1, mitochondrial	ATP5F1 (ATP5F1)
					P25705	ATP synthase subunit alpha, mitochondrial	ATPA (ATP5A1 ATP5A, ATP5AL2, ATPM)
					P40926	Malate dehydrogenase, mitochondrial	MDHM (MDH2)
					Q13011	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	ECH1
					Q7Z4W1	L-xylulose reductase	DCXR (DCXR SDR20C1)
					Q8N335	Glycerol-3-phosphate dehydrogenase 1-like protein	GPD1L (GPD1L KIAA0089)
					Q99623	Prohibitin-2	PHB2 (PHB2 BAP, REA)
					Q9UDW1	Cytochrome b-c1 complex subunit 9	UCR9 (UQCR10 UCRC, HSPC119)

Appendix 2 Summarises the main content (PICO; RQ2)

Summarises the main content (RQ2) of each article according to the PICO principle, which includes Population, Intervention, Comparison, and Outcome measures. The earliest reported study meeting the eligibility criteria was that of Lanza et al (Lanza et al. 2008); therefore, the current review considers literature over an 8-year study period from 2008 to 2015. *presented significant difference at *p*-value < 0.05 or under

Appendix 2 summarises the main content (RQ2) of each article according to the PICO principle, which includes Population, Intervention, Comparison, and Outcome measures. The earliest reported study meeting the eligibility criteria was that of Lanza et al (Lanza et al. 2008); therefore the current review considers literature over an 8 year study period from 2008 to 2015.*presented significant difference at p-value <0.05 or under

0	Participants (M/F)	Study groups		Intervention					Outcome measure		
		Case	Control	Modality	Intensity	Volume (min/session)	Frequency (times/week)	Duration (week)	Protein description		
Lanza et al 2008	N = 42 (24/18)	Trained	SED	Endurance (cycling or running)	na	≥1h	6	≥4 y	OT/OS		YT/YS
	n_{Young} = 22 (12/10)	YT=11	YS=11	(self-reported)					Up-regulation (18)		Up-regulation (16)
		Age							Cytochrome c oxidase subunit II		Cytochrome C oxidase subunit II
		26 ± 1 y	23 ± 1 y						Cytochrome c oxidase subunit VIIc		Pyruvate dehydrogenase
	n_{Old} = 20 (12/8)	BMI	BMI						Citrate synthase		Mitochondrial creatine kinase
		22.7±0.7 $kg.m^{-2}$	23.1±0.9 $kg.m^{-2}$						Mitochondrial creatine kinase		Cytochrome c oxidase subunit IV isoform 1
									Ubiquinol-cytochrome c reductase core protein II		Citrate synthas e
									ATP synthase		Cytochrome c oxidase subunit Vb
									Oxoglutarate dehydrogenase		ATP synthase
	Vastus lateralis (biopsy)	OT=10	OS						E2 component of pyruvate dehydrogenase complex		Ubiquinol-cytochrome c reductase core protein II
		Age	Age						Cytochrome c oxidase subunit Vb		Cytochrome c oxidase subunit VIIc
		65 ± 2	65 ± 2						Cytochrome c oxidase subunit IV isoform 1		Aspartate aminotransferase 2
	LC-MS/MS	BMI	BMI						Isocitrate dehydrogenase 2 (NADP+)		Oxoglutarate dehydrogenase
		24.4±1.0 $kg.m^{-2}$	24.4±0.7 $kg.m^{-2}$						Cytochrome c oxidase subunit Va		Cytochrome c oxidase subunit Va
									Aspartate aminotransferase 2		Isocitrate dehydrogenase 2 (NADP+)
									Malate dehydrogenase 2		Malate dehydrogenase 2
									Pyruvate dehydrogenase		Aspartate aminotransferase 1
									Aspartate aminotransferase 1		Aconitase 2
									Aconitase 2		
									Triosephosphate isomerase 1		
Holloway et al 2009	N = 5 (5/0)	One group		Endurance (motorised threadmill)	6x1 min 90-100% V_{02max}	30	3	6	Up-regulation (4)		
		Pre-post							P25705	ATP synthase subunit alpha, mitochondrial	ATPA (ATP5A1 ATP5A, ATP5AL2, ATPM)
	age 21 ± 2 y								P06576	ATP synthase subunit beta, mitochondrial	ATPB (ATP5B)
				Vastus lateralis	4 min 50% V_{02max}				P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA
	Vastus lateralis (biopsy)								P40939	Trifunctional enzyme subunit alpha, mitochondrial	ECHA (HADHA HADH)
	1D-SDS-PAGE LC-MS/MS										
Egan et al 2011	N = 8 (8/0)	One group		Endurance training (cycling)	80% V_{02max}	60	everyday	2 (14 d)	Up-regulation (21)		
	Age 23 ± 2 y	Pre-post							P03915	NADH-ubiquinone oxidoreductase chain 5	NU5M (MT-ND5 MTND5, NADH5, ND5)
	BMI 23.6 ± 0.9 $kg.m^{-2}$								P51970	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	NDUA8 (NDUFA8)
									Q9P0J0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 13	NDUFA13 (NDUFA13 GRIM19, CDA016, CGI-39)
	Vastus lateralis (biopsy)								P25705	ATP synthase subunit alpha, mitochondrial	ATPA (ATP5A1 ATP5A, ATP5AL2, ATPM)
									P00505	Aspartate aminotransferase. mitochondrial	AATM (GOT2)
	2DIGE LC-MS/MS								P02144	Myoglobin	MYG (MB)
									P04075	Fructose-bisphosphate aldolase A	ALDOA (ALDOA ALDA)
									P04179	Superoxide dismutase [Mn], mitochondrial	SODM (SOD2)
									P06576	ATP synthase subunit beta, mitochondrial	ATPB (ATP5B)
									P07954	Fumarate hydratase, mitochondrial	FUMH (FH)
									P09622	Dihydrolipoyl dehydrogenase, mitochondrial	DLDH (DLD GCSL. LAD. PHE3)
									P15259	Phosphoglycerate mutase 2	PGAM2 (PGAM2 PGAMM)
									P17540	Creatine kinase S-type, mitochondrial	KCRS (CKMT2)
									P29590	Protein PML (Promyelocytic leukemia protein) (RING finger protein 71) (Tripartite motif-containing protein 19)	PML (PML MYL, PP8675, RNF71, TRIM19)
									P30086	Phosphatidylethanolamine-binding protein 1	PEBP1 (PEBP1 PBP, PEBP)
									P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	ODO2 (DLST)
									P40926	Malate dehydrogenase, mitochondrial	MDHM (MDH2)
									P49411	Elongation factor Tu, mitochondria	EFTU (TUFM)
									P60174	Triosephosphate isomerase	TPIS (TPI1, TPI)
									P61604	10 kDa heat shock protein, mitochondrial	CH10 (HSPE1)
									Q9UIJ7	GTP:AMP phosphotransferase AK3, mitochondrial	KAD3 (AK3)
									Down-regulation (5)		
									P20674	Cytochrome c oxidase subunit 5A, mitochondrial	COX5A (COX5A)
									P02511	Alpha-crystallin B chain	CRYAB (CRYAB CRYA2, HSPB5)
									O14983	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	AT2A1 (SERCA1)
									Q96A32	Myosin regulatory light chain 2, skeletal muscle isoform	MLRS (MYLPF)
									P11762	Galectin-1	LEG1 (LGALS1)
Husssey et al 2013	n = 6 (4/2)	One group		Endurance	60 min 55% V_{02max}	60	3 d/wk	4	Up-regulation (3)		
		Pre-post							P02765	AHSG cDNA FLJ55606, higly similar to Alpha-2-HS-glycoprotein	FETUA (AHSG, FETUA, PRO2743)
	T2DM										
									O95169	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial	NDUB8 (NDUFB8)
	(OW/OB)										
	n = 6 (4/2)				6x5 min 70% V_{02max}	30	2 d/wk		P12882	Myosin-1	MYH1 (MYH1)
									Down-regulation (1)		
	age 54 ± 4 y								P02511	Alpha-crystallin B chain	CRYAB (CRYAB)
									P15121	Aldose reductase	ALDR (AKR1B1 ALDR1)
	BMI 29 ± 2 $kg.m^{-2}$								Q8N335	Glycerol-3-phosphate dehydrogenase 1-like protein	GPD1L (GPD1L KIAA0089)
									P04075	Fructose-bisphosphate aldolase A	ALDOA (ALDOA ALDA)
	Vastus lateralis (biopsy)								O75112	LIM domain-binding protein 3	LDB3 (LDB3 KIAA0613, ZASP)
	1D-SDS-PAGE LC-MS/MS										
Schild et al 2015	N = 5	ET & LE	UT & LE/OW/OB	Endurance exercice	na	5h per week		>5 y	Up-regulation (43)		
									O00330	Pyruvate dehydrogenase protein X component, mitochondrial	ODPX (PDHX PDX1)
	Vastus leteralis (biopsy)		n = 5						O43181	NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial	NDUS4 (NDUFS4)
		n = 5	Age 24 ± 5 y						O75306	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	NDUS2 (NDUFS2)
		Age 27 ± 2 y							O75489	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	NDUS3 (NDUFS3)
			BMI 25.7 ± 5.0						O75746	Calcium-binding mitochondrial carrier protein Aralar1	CMC1 (SLC25A12 ARALAR1)
	LC-MS/MS	BMI 23.4 ± 1.6 $kg.m^{-2}$	$kg.m^{-2}$						O75947	ATP synthase subunit d, mitochondrial	ATP5H (ATP5H My032)
									P00403	Cytochrome c oxidase subunit 2	COX2 (MT-CO2 COII, COXII, MTCO2)
									P03915	NADH-ubiquinone oxidoreductase chain 5	NU5M (MT-ND5 MTND5, NADH5. ND5)
									P06576	ATP synthase subunit beta, mitochondrial	ATPB (ATP5B)
									P11177	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	ODPB (PDHB PHE1B)
									P12235	ADP/ATP translocase 1	ADT1 (SLC25A4 ANT1)
									P14854	Cytochrome c oxidase subunit 6B1	COX6B1 (COX6B1 COX6B)
									P17540	Creatine kinase S-type, mitochondrial	KCRS (CKMT2)
									P21912	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	SDHB (SDHB SDH, SDH1)
									P24310	Cytochrome c oxidase subunit 7A1, mitochondrial	COX7A1 (COX7A1 COX7AH)
									P24539	ATP synthase F(0) complex subunit B1, mitochondrial	ATP5F1 (ATP5F1)
									P28331	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	NDUS1 (NDUFS1)
									P30038	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	AL4A1 (ALDH4A1 ALDH4, P5CDH)
									P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA
									P36542	ATP synthase subunit gamma, mitochondrial (F-ATPase gamma subunit)	ATPG (ATP5C1 ATP5C, ATP5CL1)
									P48735	Isocitrate dehydrogenase [NADP], mitochondrial	IDHP (IDH2)
									P49748	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADV (ACADVL)
									P49753	Acyl-coenzyme A thioesterase 2, mitochondrial	ACOT2
									P54819	Adenylate kinase 2, mitochondrial	KAD2 (AK2)
									Q00325	Phosphate carrier protein, mitochondrial	MPCP (SLC25A3 PHC, OK/SW-cl.48)
									Q02218	2-oxoglutarate dehydrogenase, mitochondrial	ODO1 (OGDH)
									Q02978	Mitochondrial 2-oxoglutarate/malate carrier protein	M2OM (SLC25A11 SLC20A4)
									Q16795	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	NDUA9 (NDUFA9 NDUFS2L)
									Q96IX5	Up-regulated during skeletal muscle growth protein 5	USMG5 (USMG5 DAPIT. HCVFTP2. PD04912)
									Q99643	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial	C560 (SDHC CYB560, SDH3)
									Q9HCC0	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial	MCCB (MCCC2)
									Q9P2R7	Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCB1 (SUCLA2)
									Q9Y2Z9	Ubiquinone biosynthesis monooxygenase COQ6, mitochondrial	COQ6 (COQ6 CGI-10)
									Q9Y6C9	Mitochondrial carrier homolog 2	MTCH2 (MTCH2 MIMP, HSPC032)
									Q9Y6M9	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9	NDUB9 (NDUFB9 LYRM3. UOOR22)
									P31930	Cytochrome b-c1 complex subunit 1, mitochondrial	QCR1 (UQCRC1)
									O75964	ATP synthase subunit g, mitochondrial	ATP5L (ATP5L)
									O75390	Citrate synthase, mitochondrial	CISY (CS)
									Q99798	Aconitate hydratase, mitochondrial	ACON (ACO2)
									P42126	Enoyl-CoA delta isomerase 1, mitochondrial	ECI1 (ECI1 DCI)
									Q13011	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	ECH1
									Q53EW7	Branched-chain-amino-acid aminotransferase	Q53EW7 (BCAT2)
									O75208	Ubiquinone biosynthesis protein COQ9, mitochondrial	COQ9 (COQ9)

Appendix 3 The 82 identified proteins

The 82 proteins that were categorised by molecular function and biological process following UniProtKB.

Molecular function

Translocase	AT2A1	AT2A2	AT2A3	ATPB										
Membrane protein	MFSD6													
Ribonucleoprotein	RL40	CPEB1												
RNA-binding	ANXA2	CPEB1												
DNA-binding	ENOA	ZN644												
Protease inhibitor	PEBP1													
Hypotensive agent	HBB													
Cytokine	G6PI													
Oxidoreductase	ACADV	G3P	IDHP	LDHB	LDHC	MDHC	MDHM	PRDX1	PRDX6	SODC				
Transferase	AATC	AATM	G3P	K6PF	KAD1	KCRM	KCRS	KPYM	PGK1	PYGM	RN145			
Isomerase	G6PI	PGAM2	PGAM4	PGM1	TPIS									
Kinase	K6PF	KAD1	KCRM	KCRS	KPYM	PGK1								
Antioxidant	PRDX1	PRDX6	SODC											
Chaperone	GRP78	HSP7C	HSPB1	HSPB6	HSP72									
Lyase	ACON	ALDOA	ALDOC	CAH3	ENOA	ENOB	ENOG	TPIS						
Aminotransferase	AATC	AATM												
Hydrolase	GRP78	PGAM2	PGAM4	PRDX6										
Activator	CPEB1													
Allosteric enzyme	K6PF	KPYM	PYGM											
Actin-binding	FLNC	MYH1	MYH13	MYH3	MYH4	MYH6	MYH7	TNNI1	TPM2					
Muscle protein	ACTC	CASQ1	MYG	MYH1	MYH13	MYH3	MYH4	MYH6	MYH7	MYL1	MYL3	MYOM1	TNNI1	TPM2
Developmental protein	FHL1													
Motor protein	MYH1	MYH13	MYH3	MYH4	MYH6	MYH7	MYL1	MYL3						
Calmodulin-binding	MYH1	MYH13	MYH3	MYH4	MYH6	MYH7								
Ribosomal protein	RL40													
Myosin	MYH1	MYH13	MYH3	MYH4	MYH6	MYH7	MYL1	MYL3						
Peroxidase	PRDX1	PRDX6												
Repressor	CPEB1	ENOA	HSP7C											
Multifunctional enzyme	PRDX6													
Vasoactive	HBB													
Glycosyltransferase	PYGM													
Serine protease inhibitor	PEBP1													
Growth factor	G6PI													

Biological function

Carbohydrate metabolism	PGM1	PYGM											
Glucose metabolism	PGM1												
Glycogen metabolism	PYGM												
Gluconeogenesis	G6PI	TPIS											
Glycolysis	ALDOA	ALDOC	ENOA	ENOB	ENOG	G3P	G6PI	K6PF	KPYM	PGAM2	PGAM4	PGK1	TPIS
Lipid metabolism	ACADV	PRDX6											
Fatty acid metabolism	ACADV												
Lipid degradation	PRDX6												
Stress response	HSP72	HSP76	HSP7C	HSPB6	HSPB1								
Transport	AT2A3	AT2A1	AT2A2	ATPB	FABPH	AATM	ATPA	HBA	HBB	HEMO	MYG		
Ion transport	AT2A1	AT2A2	AT2A3	ATPA	ATPB								
Calcium transport	AT2A1	AT2A2	AT2A3										
Oxygen transport	HBA	HBB	MYG										
Lipid transport	AATM												
Hydrogen ion transport	ATPA	ATPB											
ATP synthesis	ATPA	ATPB											
TCA	ACON	MDHC	MDHM	IDHP									
Transcription regulation	ENOA	HSP7C	ZN644										
Transcription	ENOA	HSP7C	ZN644										
mRNA processing	CPEB1	HSP7C											
mRNA splicing	HSP7C												
Translation regulation	CPEB1	G3P											
Spermatogenesis	HSP72												
Host-virus interaction	HEMO	HSP7C	VIME										
Plasminogen activation	ENOA												
Apoptosis	G3P												
Blood coagulation	ANXA5												
Hemostasis	ANXA5												
Glyoxylate bypass	IDHP												
Cellular component	MFSD6												
Amino-acid biosynthesis	AATC												
Ubl conjugation pathway	RN145												
Differentiation	FHL1	HSP72											

Appendix 4 The 82 identified proteins profiled in the current work.

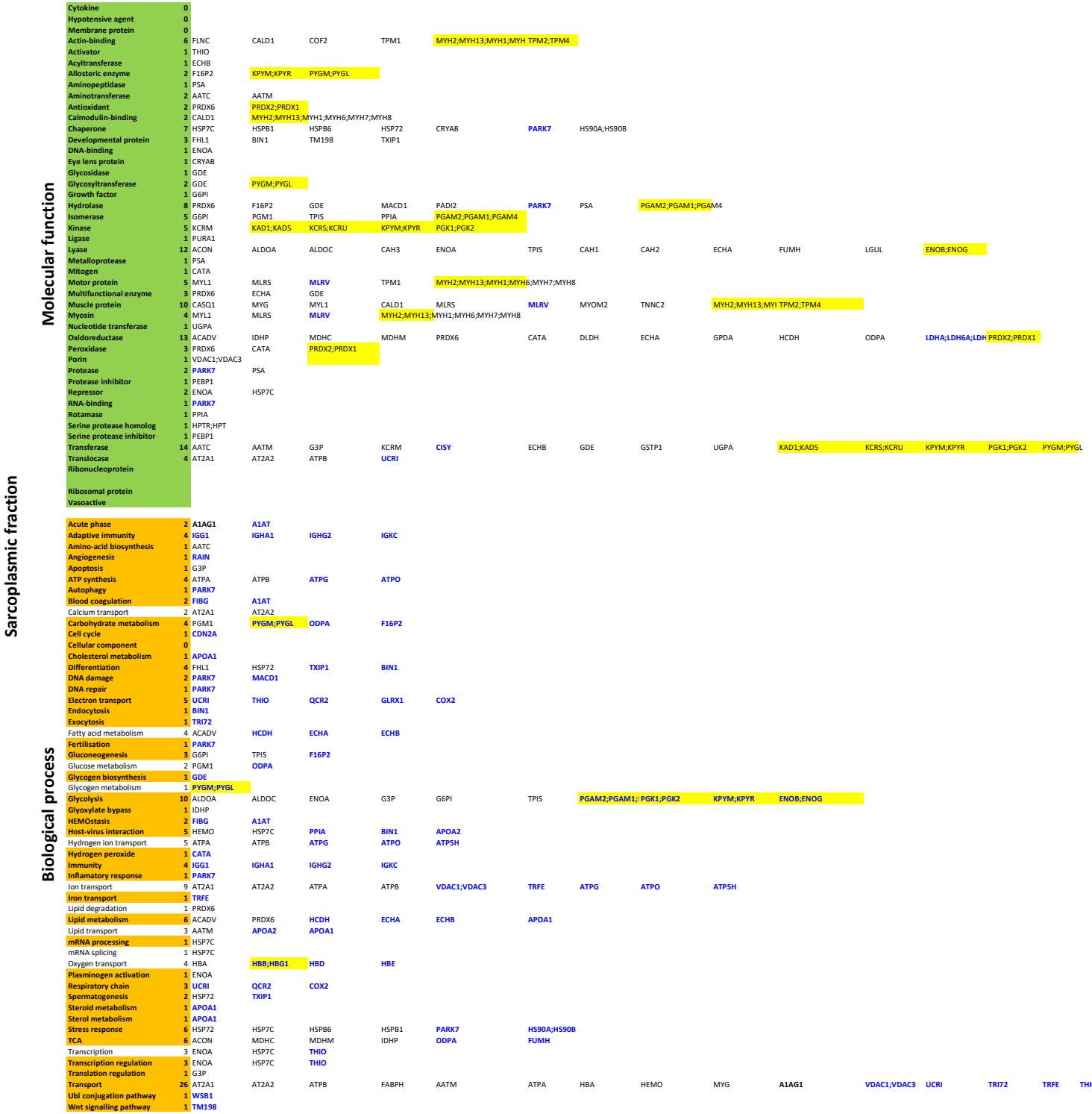
The 82 identified proteins profiled in the current work. ANOVA discovered 2 proteins (AT2A2 and FABPH) differed significantly (p<0.05) in abundance between LE, OB, T2DM muscle.

Appendix 4 The 82 identified proteins profiled in the current work. ANOVA discovered 2 proteins (AT2A2 and FABPH) differed significantly ($p<0.05$) in abundance between LE, OB, T2DM muscle.

Accession	Description	Peptide count	Unique peptides	Confidence score	Anova (p)	FDR	Maximum fold change	Highest mean condition	Lowest mean condition
AT2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	8	2	272.83	0.0146*	0.05	2.18	T2DM	LE
FABPH	Fatty acid-binding protein, heart	1	1	66.19	0.0371*	0.05	1.74	T2DM	LE
AT2A1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	13	5	518.35	0.07	0.05	1.22	LE	T2DM
FHL1	Four and a half LIM domains protein 1	7	7	285.76	0.07	0.05	1.37	T2DM	LE
ZN644	Zinc finger protein 644	4	2	32.99	0.07	0.05	1.79	T2DM	OB
AT2A3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	7	1	170.34	0.07	0.05	6.30	LE	OB
CAH3	Carbonic anhydrase 3	6	3	284.05	0.11	0.05	1.64	T2DM	LE
PGAM4	Probable phosphoglycerate mutase 4	3	1	59.23	0.11	0.05	1.51	LE	T2DM
MYL3	Myosin light chain 3	1	1	16.89	0.15	0.05	2.01	T2DM	LE
VIME	Vimentin	2	2	45.79	0.16	0.05	1.16	T2DM	LE
MYH3	Myosin-3	4	1	64.24	0.17	0.05	1.65	OB	T2DM
RL40;	Ubiquitin-60S ribosomal protein L40	2	2	84.58	0.18	0.05	1.44	T2DM	LE
RN145	RING finger protein 145	1	1	37.15	0.18	0.05	1.55	T2DM	LE
MYL1	Myosin light chain 1/3, skeletal muscle isoform	3	3	136.74	0.19	0.05	1.78	T2DM	OB
HSPB1	Heat shock protein beta-1	4	4	137.63	0.22	0.05	1.60	T2DM	LE
LDHC	L-lactate dehydrogenase C chain	2	1	81.36	0.23	0.05	1.78	T2DM	LE
AATC	Aspartate aminotransferase, cytoplasmic	2	2	49.25	0.25	0.05	1.41	OB	T2DM
SODC	Superoxide dismutase [Cu-Zn]	1	1	31.4	0.28	0.05	1.64	OB	LE
MDHC	Malate dehydrogenase, cytoplasmic	4	4	128.51	0.28	0.05	1.30	T2DM	LE
ALDOA	Fructose-bisphosphate aldolase A	11	11	482.45	0.29	0.05	1.20	OB	T2DM
K22E	Keratin, type II cytoskeletal 2 epidermal	5	3	147.34	0.29	0.05	1.75	T2DM	LE
MFSD6	Major facilitator superfamily domain-containing protein 6	1	1	14.34	0.30	0.05	1.76	LE	T2DM
PRDX1	Peroxiredoxin-1	4	3	58.12	0.31	0.05	1.42	T2DM	LE
TNNI1	Troponin I, slow skeletal muscle	1	1	4.22	0.31	0.05	2.42	OB	T2DM
PRDX6	Peroxiredoxin-6	4	3	101.9	0.33	0.05	1.15	OB	T2DM
HSP7C	Heat shock cognate 71 kDa protein	5	1	89.9	0.34	0.05	2.84	LE	T2DM
G3P	Glyceraldehyde-3-phosphate dehydrogenase	9	8	494.51	0.37	0.05	1.35	OB	LE
MDHM	Malate dehydrogenase, mitochondrial	8	8	113.31	0.39	0.05	1.52	T2DM	LE
HEMO	Hemopexin	2	2	39.72	0.40	0.05	1.42	LE	T2DM
ENOA	Alpha-enolase	6	2	369.22	0.40	0.05	3.29	OB	LE
PGM1	Phosphoglucomutase-1	5	5	133.27	0.41	0.05	1.22	LE	T2DM
POTEF;	POTE ankyrin domain family member F	5	1	128.6	0.42	0.05	2.00	T2DM	LE
MYH6	Myosin-6	5	2	54.26	0.44	0.05	1.46	T2DM	LE
ACADV	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	4	4	70.76	0.44	0.05	1.22	OB	LE
ACTBL	Beta-actin-like protein 2	5	2	85.38	0.45	0.05	1.47	LE	T2DM
ANXA2	Annexin A2	2	2	45.05	0.46	0.05	1.14	OB	LE
MYH13	Myosin-13	7	1	74.7	0.47	0.05	145.12	OB	T2DM
HSPB6	Heat shock protein beta-6	3	3	85.09	0.47	0.05	1.94	OB	LE
KAD1	Adenylate kinase isoenzyme 1	4	3	147.09	0.47	0.05	1.38	OB	LE
TPM2;	Tropomyosin beta chain	2	1	47.55	0.48	0.05	1.77	OB	LE
HSP72	Heat shock-related 70 kDa protein 2	4	1	82.82	0.49	0.05	3.71	T2DM	OB
WDR11	WD repeat-containing protein 11	3	2	17.91	0.50	0.05	1.41	LE	T2DM
HBB;	Hemoglobin subunit beta	3	2	144.34	0.50	0.05	4.60	T2DM	OB
HSP76;	Heat shock 70 kDa protein 6	2	1	73.76	0.51	0.05	1.44	LE	T2DM
PGK1	Phosphoglycerate kinase 1	6	6	163.09	0.51	0.05	1.14	LE	T2DM
ACTC;	Actin, alpha cardiac muscle 1	9	4	162.71	0.52	0.05	2.24	T2DM	LE
KCRM	Creatine kinase M-type	13	12	733.38	0.53	0.05	1.31	T2DM	OB
PYGM	Glycogen phosphorylase, muscle form	13	13	291.82	0.55	0.05	1.08	T2DM	OB
ACON	Aconitate hydratase, mitochondrial	3	3	59.12	0.57	0.05	1.57	OB	LE
ENOG	Gamma-enolase	4	1	172.64	0.57	0.05	16.94	OB	LE
K1C9	Keratin, type I cytoskeletal 9	1	1	39.94	0.57	0.05	250.33	T2DM	OB
PEBP1	Phosphatidylethanolamine-binding protein 1	2	2	84.56	0.57	0.05	1.63	OB	LE
ATPA	ATP synthase subunit alpha, mitochondrial	7	7	149.94	0.58	0.05	1.12	T2DM	OB
TBA4A	Tubulin alpha-4A chain	3	3	109.74	0.59	0.05	1.34	LE	OB
FLNC	Filamin-C	12	10	240.59	0.59	0.05	1.36	T2DM	LE
HBA	Hemoglobin subunit alpha	5	4	201.8	0.60	0.05	3.98	T2DM	OB
K6PF	6-phosphofructokinase, muscle type	2	2	40.34	0.62	0.05	1.27	LE	OB
MYH7	Myosin-7	2	1	41.9	0.65	0.05	48.52	OB	LE
CASQ1	Calsequestrin-1	4	3	116.06	0.66	0.05	1.18	LE	T2DM
TPIS	Triosephosphate isomerase	12	11	484.54	0.67	0.05	1.33	LE	T2DM
G6PI	Glucose-6-phosphate isomerase	3	3	38.87	0.68	0.05	1.28	T2DM	OB
ATPB	ATP synthase subunit beta, mitochondrial	7	7	189.45	0.70	0.05	2.11	T2DM	OB
K2C1B	Keratin, type II cytoskeletal 1b	2	1	70.77	0.73	0.05	1.92	OB	T2DM
MYH1	Myosin-1	6	1	86.34	0.74	0.05	1.19	OB	LE
AATM	Aspartate aminotransferase, mitochondrial	3	1	45.44	0.74	0.05	1.51	LE	T2DM
GRP78	78 kDa glucose-regulated protein	3	2	57.55	0.77	0.05	2.18	LE	OB
MYH4	Myosin-4	5	1	55.84	0.77	0.05	1.39	OB	T2DM
ENOB	Beta-enolase	10	6	400.11	0.78	0.05	1.16	LE	T2DM
ALDOC	Fructose-bisphosphate aldolase C	3	3	41.86	0.78	0.05	1.11	LE	T2DM
KPYM	Pyruvate kinase isozymes M1/M2	14	12	440.44	0.78	0.05	1.07	OB	T2DM
MYG	Myoglobin	4	4	316.51	0.80	0.05	1.08	OB	T2DM
KCRS	Creatine kinase S-type, mitochondrial	3	3	92.02	0.83	0.05	1.15	LE	OB
K2C1	Keratin, type II cytoskeletal 1	5	4	206.13	0.83	0.05	4.73	T2DM	LE
ALBU	Serum albumin	24	24	848.97	0.85	0.05	1.32	OB	LE
LDHB	L-lactate dehydrogenase B chain	2	1	80.27	0.86	0.05	1.18	LE	T2DM
PDLI3	PDZ and LIM domain protein 3	2	2	112.81	0.88	0.05	1.08	T2DM	LE
PGAM2	Phosphoglycerate mutase 2	6	4	161.55	0.89	0.05	1.16	LE	OB
IDHP	Isocitrate dehydrogenase [NADP], mitochondrial	4	4	67.43	0.92	0.05	1.05	T2DM	LE
LDHA;LDH6A	L-lactate dehydrogenase A chain	5	4	164.8	0.93	0.05	1.11	OB	T2DM
ANXA5	Annexin A5	3	3	36.42	0.95	0.05	1.03	LE	T2DM
CPEB1	Cytoplasmic polyadenylation element-binding protein 1	3	2	36.24	0.97	0.05	1.27	T2DM	LE
MYOM1	Myomesin-1	1	1	5.35	1.00	0.05	1.27	LE	OB

Appendix 5 The 231 proteins (OB vs TR)

The 231 proteins that were categorised by molecular function and biological process following UniProtKB. (OB vs TR)


Sarcoplasmic fraction
Molecular function
Biological process


Myofibrilla fraction
Molecular function
Actin-binding 25 FLNC MYH1 MYH3 MYH4 MYH6 MYH7 TNNI1 ACTBL ACTN2 ACTN3 DYST KLHL5 MICA3 MYH8 MYH9 MYO15 MYOTI MYPC1 MYPC2 NEBU TNNI2 TPM1 TPM3 MYH2;MY TPM2;TPM4
Allosteric enzyme 1 PYGM;PYGL
Calmodulin-binding 9 MYH1 MYH3 MYH4 MYH6 MYH7 MYH8 MYH9 MYO15 TITIN
Chromatin regulator 1 BRE1A
Developmental protein 3 FHL1 CENPE IF122
DNA-binding 4 ENOA H2B1B KIF22 TRI33
Glycosyltransferase 1 PYGM;PYGL
Guanine-nucleotide releasing factor 1 HERC1
Hydrolase 3 IIGP5 MRP1 PDP1
Hypotensive agent 1 HBB
Initiation factor 1 EIF3J
Kinase 4 KCRM STK31 TAOK2 TITIN
Lyase 3 ALDOA ENOA ENOG;ENOB
Monooxygenase 1 MICA3
Motor protein 17 MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 DYH7 CENPE KIF22 MLRS MLRV MYH8 MYH9 MYL6B MYO15 MYH2;MYH7B
Muscle protein 34 ACTC CASQ1 MYG MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 MYOM1 TNNI1 ACTC ACTS;ACTE DESM DYST MLRS MLRV MYH8 MYL6B MYOM2 MYOTI MYPC1 MYPC2 NEBU TNNC1 TNNC2 TNNT1 TNNT3 TNNI2 TPM1 TPM3 MYH2;MY TPM2;TPM4
Myosin 12 MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 MLRS MLRV MYL6B MYO15 MYH2;MYH7B
Oxidoreductase 4 G3P MICA3 MYH8 MYH9
Protein phsphatase 1 PDP1
Repressor 2 ENOA TRI33
Ribonucleoprotein 1 RS27A
Ribosomal protein 1 RS27A
RNA-binding 2 CO052 TNR6C
Serine/threonine-protein kinase 3 STK31 TAOK2 TITIN
Transferase 9 G3P KCRM BRE1A HERC1 STK31 TAOK2 TITIN TRI33 PYGM;PYGL
Translocase 1 AT2A1;AT2A2
Vasoactive 1 HBB
Activator
Aminotransferase
Antioxidant
Chaperone
Cytokine
Growth factor
Isomerase
Membrane protein
Multifunctional enzyme
Peroxidase
Protease inhibitor
Serine protease inhibitor
Biological process
ATP synthesis 0
Blood coagulation 0
Cellular component 0
Fatty acid metabolism 0
Gluconeogenesis 0
Glucose metabolism 0
Glyoxylate bypass 0
HEMOstasis 0
Hydrogen ion transport 0
Lipid degradation 0
Lipid transport 0
mRNA processing 0
mRNA splicing 0
Spermatogenesis 0
Stress response 0
TCA 0
Apoptosis 1 G3P
Calcium transport 1 AT2A1;AT2A2
Carbohydrate metabolism 1 PYGM;PYGL
Cell adhesion 4 MYPC1 MYPC2 MYH9 DYST
Cell cycle 5 LRCC1 MICA3 CNTRL;E CEP63 CENPE
Cell division 5 LRCC1 MICA3 CNTRL;E CEP63 CENPE
Cell shape 1 MYH9
Cholesterol metabolism 1 NPCL1
Cilium biogenesis/degradation 1 IF122
Differentiation 1 FHL1
DNA damage 1 CEP63
Electron transport 1 NDUA4
Endocytosis 1 EHBP1
Exocytosis 1 MICA3
Glycogen metabolism 1 PYGM;PYGL
Glycolysis 4 ALDOA ENOA G3P ENOG;ENOB
Hearing 1 MYO15
Host-virus interaction 1 ADT1
Ion transport 1 AT2A1;AT2A2
Lipid metabolism 1 NPCL1
Mitosis 1 LRCC1
Mitosis 2 CEP63 CENPE
Oxygen transport 3 HBA HBB MYG
Plasminogen activation 1 ENOA
Protein biosynthesis 1 EIF3J
Protein transport 1 EHBP1
Respiratory chain 1 NDUA4
RNA-mediated gene silencing 1 TNR6C
Steroid metabolism 1 NPCL1
Sterol metabolism 1 NPCL1
Transcription 2 ENOA TRI33
Transcription regulation 2 ENOA TRI33
Translation regulation 2 G3P TNR6C
Transport 9 HBA HBB MYG NDUA4 MRP1 HERC1 EHBP1 AT2A1;AT ADT1
Ubl conjugation pathway 3 TRI33 HERC1 BRE1A

Appendix 6 List of significant proteins (25/18) OB vs TR

One-way ANOVA reported 25 and 18 proteins from sarcoplasm and myofibrillar fractions demonstrated significantly different in abundance between OB and TR.

	Accession	Description	Function	Peptide count	Unique peptides	Confidence score	ANOVA (*p*)	FDR (*q*-value)	mum fold ch	Regulation in OB
Sarcoplasm										
1	ACTS;ACTB;POTEF; ACTBM;POTEI;ACTBL	Actin, alpha s	Muscle contraction	11	11	864.73	0.01	0.05	0.83	Down
2	ADT1;ADT3;ADT4	ADP/ATP tra	Regulation of insulin secretion	2	2	79.28	0.01	0.05	0.71	Down
3	ALDOA	Fructose-bis	Glucose metabolic process	18	16	1329	0.02	0.05	1.22	Up
4	ALDOC	Fructose-bis	Glucose metabolic process	3	1	207.84	0.02	0.05	1.48	Up
5	APOA1	Apolipoprote	Lipid metabolic process	6	5	382.61	0.01	0.05	0.7	Down
6	AT2A1	Sarcoplasmic	Muscle contraction	25	13	1870.96	0.02	0.05	1.27	Up
7	CAH3	Carbonic anh	Carbonate dehydratase activity	9	9	708.86	0	0.05	1.75	Up
8	CISY	Citrate synth	TCA	1	1	71.31	0	0.05	0.82	Down
9	COX2	Cytochrome	OXPHOS	2	2	89.23	0	0.05	0.55	Down
10	ENOB;ENOG	Beta-enolase	Glucose metabolic process	18	13	1651.11	0.04	0.05	1.21	Up
11	G3P	Glyceraldehy	Glucose metabolic process	13	13	1285.17	0	0.05	1.44	Up
12	GLRX1	Glutaredoxin	Transport	1	1	55.32	0	0.05	1.22	Up
13	HSPB1	Heat shock p	Stress response	3	3	299.32	0.01	0.05	1.18	Up
14	KCRM	Creatine kina	Muscle contraction	16	16	1507.69	0	0.05	1.48	Up
15	KLH41	Kelch-like pr	Cytoskeleton/structural protein	1	1	54.84	0	0.05	0.54	Down
16	KPYM;KPYR	Pyruvate kin	Muscle contraction	20	20	1513.93	0.04	0.05	1.23	Up
17	LDHA;LDH6A;LDH6B	L-lactate deh	TCA	13	12	958.92	0	0.05	1.49	Up
18	MLRV	Myosin regul	Muscle contraction	4	4	211.31	0	0.05	0.72	Down
19	PARK7	Protein/nucl	Stress response	3	3	198.73	0.02	0.05	1.32	Up
20	PFKAM;PFKAP	ATP-depende	Glucose metabolic process	10	8	705.31	0.03	0.05	1.21	Up
21	PGK1;PGK2	Phosphoglyc	Glucose metabolic process	13	12	998.57	0.02	0.05	1.25	Up
22	RUND1	RUN domain-	Transport	1	1	38.71	0	0.05	1.63	Up
23	TPIS	Triosephosp	Glucose metabolic process	12	12	969.09	0	0.05	1.34	Up
24	TRI72	Tripartite mo	Transport	2	2	153.44	0.04	0.05	0.66	Down
25	UCRI	Cytochrome	OXPHOS	1	1	35.03	0	0.05	0.56	Down
Myofibrils										
1	ACTN2	Alpha-actinin	Muscle contraction	43	23	2972.93	0	0.05	0.84	Down
2	AT2A1;AT2A2	Sarcoplasmic	Muscle contraction	2	2	69.18	0	0.05	1.58	Up
3	CASQ1	Calsequestrin	Cytoskeleton/structural protein	4	4	315.34	0.01	0.05	1.54	Up
4	CENPE	Centromere-a	Chromosome	3	1	38.42	0.02	**0.05**	0.79	Down
5	CENPJ	Centromere p	Chromosome	1	1	15.03	0	0.05	0.71	Down
6	EIF3J	Eukaryotic tr	Cytoskeleton/structural protein	1	1	13.74	0.01	**0.05**	0.7	Down
7	ENOA	Alpha-enolas	Glucose metabolic process	2	1	103.51	0.02	0.05	1.46	Up
8	FHL1	Four and a ha	Cytoskeleton/structural protein	2	2	93.85	0	**0.05**	1.62	Up
9	FLNC	Filamin-C	Cytoskeleton/structural protein	8	5	209.6	0	0.05	0.83	Down
10	LRCC1	Leucine-rich	Chromosome	2	1	39.01	0.03	0.05	0.62	Down
11	MRP1	Multidrug res	Transport	1	1	5.31	0.02	**0.05**	1.34	Up
12	MYG	Myoglobin	Muscle contraction	4	3	191.27	0.03	**0.05**	1.42	Up
13	MYH1	Myosin-1	Muscle contraction	103	13	9070.18	0.01	**0.05**	1.59	Up
14	MYH3	Myosin-3	Muscle contraction	36	1	2545.96	0.01	0.05	2.37	Up
15	MYL6B	Myosin light	Muscle contraction	2	1	101.17	0.01	0.05	0.7	Down
16	MYPC2	Myosin-bind	Muscle contraction	8	7	426.51	0.03	0.05	1.18	Up
17	PDP1	[Pyruvate de	Lipid metabolic process	2	1	52.53	0	0.05	0.82	Down
18	TNNT1	Troponin T, s	Muscle contraction	4	3	193.91	0.01	**0.05**	0.76	Down

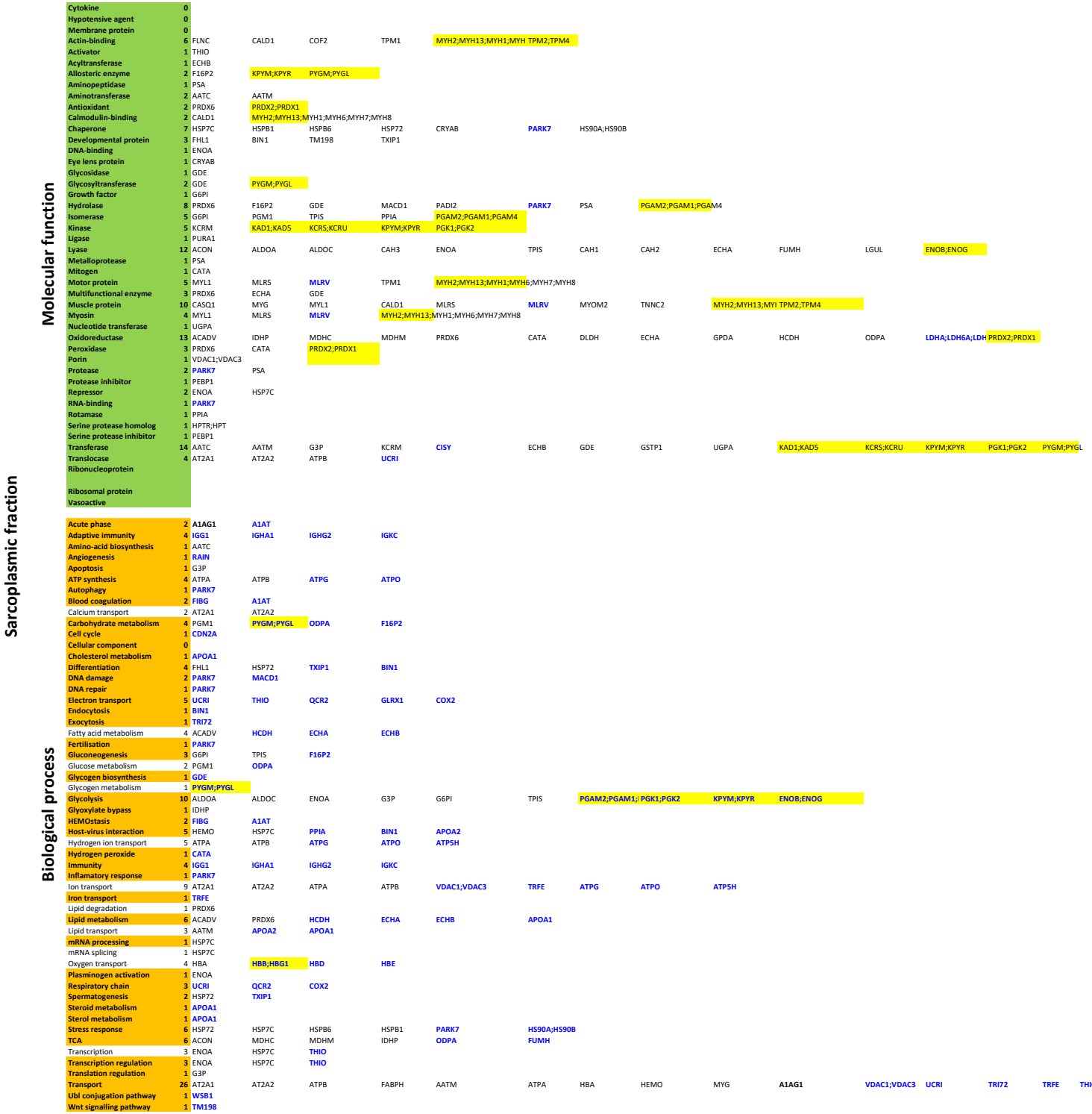
Appendix 7 FSR of non-significant proteins OB vs TR

FSR of proteins that are able to measure but do not significant in abundance.

	Protein accession	Function	abundance change		Newly fractional synthesis rate (FSR)							Fraction
			Fold change of OB/TR	*p*	OB			TR			*p*	
					FSR±SD (%/day)			FSR±SD (%/day)				
Reported statistically significant in FSR only												
					Increased FSR in OB							
1	PGM1	Glucose metabolic process	1.20	0.06	6.20	±	0.00	2.03	±	0.00	0.01	Sarcoplasm
2	MYL1	Muscle contraction	1.13	0.07	0.65	±	0.00	0.58	±	0.00	0.00	Myofibrils
3	ALDOA	Glucose metabolic process	1.00	0.88	4.48	±	0.00	2.39	±	0.00	0.00	Myofibrils
4	APOA2	Lipid metabolic process	0.98	0.68	7.41	±	0.00	6.77	±	0.00	0.01	Sarcoplasm
5	TNNI1	Muscle contraction	0.92	0.41	6.94	±	0.01	5.34	±	0.01	0.00	Myofibrils
6	MLRV	Muscle contraction	0.92	0.42	18.34	±	0.02	2.22	±	0.01	0.00	Myofibrils
7	TITIN	Cytoskeleton/structure	0.91	0.27	2.91	±	0.00	2.61	±	0.00	0.00	Myofibrils
8	HEMO	Transport	0.88	0.48	5.29	±	0.00	5.01	±	0.00	0.01	Sarcoplasm
9	MYH7	Muscle contraction	0.82	0.07	4.59	±	0.00	1.18	±	0.00	0.00	Myofibrils
10	IGKC	Cytoskeleton/structure	0.80	0.50	2.93	±	0.00	2.36	±	0.00	0.00	Sarcoplasm
11	ALBU	Transport	0.79	0.14	3.75	±	0.00	3.09	±	0.00	0.00	Sarcoplasm
12	TPM3	Muscle contraction	0.77	0.07	1.77	±	0.00	1.21	±	0.00	0.00	Myofibrils
13	ENOB	Glucose metabolic process			40.91	±	0.04	0.61	±	0.00	0.00	Sarcoplasm
14	PYGM	Glucose metabolic process			1.95	±	0.00	1.39	±	0.00	0.00	Sarcoplasm
					Decreased FSR in OB							
15	MYG	Muscle contraction	1.30	0.05	1.17	±	0.00	10.62	±	0.02	0.00	Sarcoplasm
16	FHL1	Cytoskeleton/structure	1.29	0.08	1.56	±	0.00	16.46	±	0.07	0.00	Sarcoplasm
17	TNNI2	Muscle contraction	1.18	0.11	0.93	±	0.00	1.25	±	0.00	0.05	Myofibrils
18	TNNT3	Muscle contraction	1.15	0.09	0.77	±	0.00	1.05	±	0.00	0.00	Myofibrils
19	HBB	Transport	1.15	0.48	2.14	±	0.00	12.61	±	0.01	0.00	Myofibrils
20	TPM1	Muscle contraction	1.15	0.18	1.20	±	0.00	1.45	±	0.00	0.00	Myofibrils
21	MLRS	Muscle contraction	1.12	0.13	0.99	±	0.00	2.21	±	0.00	0.00	Myofibrils
22	TNNC2	Muscle contraction	1.06	0.35	1.66	±	0.00	2.39	±	0.00	0.00	Myofibrils
23	TPM2	Muscle contraction	1.06	0.16	1.44	±	0.00	1.37	±	0.00	0.00	Myofibrils
24	MYOM2	Muscle contraction	1.05	0.17	0.63	±	0.00	34.88	±	0.04	0.00	Myofibrils
25	LDB3	Cytoskeleton/structure	1.05	0.36	1.23	±	0.00	1.68	±	0.00	0.01	Myofibrils
26	PRDX6	Anti-oxidant	1.05	0.36	1.50	±	0.00	7.17	±	0.01	0.00	Sarcoplasm
27	DESM	Cytoskeleton/structure	1.04	0.54	1.68	±	0.00	2.94	±	0.00	0.00	Myofibrils
28	IDHP	TCA	1.02	0.93	5.45	±	0.00	22.01	±	0.03	0.00	Sarcoplasm
29	PDLI5	Cytoskeleton/structure	0.97	0.47	0.92	±	0.00	1.54	±	0.00	0.00	Myofibrils
30	NEBU	Muscle contraction	0.96	0.82	2.86	±	0.00	32.27	±	0.00	0.00	Myofibrils
31	MYH2	Muscle contraction	0.95	0.52	0.95	±	0.00	1.39	±	0.00	0.00	Myofibrils
32	AATM	Lipid metabolic process	0.94	0.42	2.93	±	0.00	36.48	±	0.03	0.00	Sarcoplasm
33	KCRM	Muscle contraction	0.93	0.94	3.59	±	0.00	1.75	±	0.00	0.00	Myofibrils
34	MDHM	TCA	0.92	0.50	4.47	±	0.00	10.11	±	0.01	0.00	Sarcoplasm
35	MYL3	Muscle contraction	0.88	0.52	0.91	±	0.00	0.43	±	0.00	0.00	Myofibrils
36	ATPB	OXPHOS	0.86	0.27	0.91	±	0.00	4.71	±	0.01	0.00	Sarcoplasm
37	TRFE	Transport	0.86	0.38	5.74	±	0.00	17.00	±	0.00	0.00	Sarcoplasm
38	FLNC	Cytoskeleton/structure	0.85	0.07	2.36	±	0.00	8.30	±	0.01	0.00	Sarcoplasm
39	PFKAM	Glucose metabolic process			5.79	±	0.00	7.17	±	0.00	0.00	Sarcoplasm
40	HS71A	Cell death			1.35	±	0.00	5.19	±	0.00	0.00	Sarcoplasm
41	KPYM	Muscle contraction			1.43	±	0.00	2.05	±	0.00	0.00	Sarcoplasm
42	PGK1	Glucose metabolic process			1.18	±	0.00	0.89	±	0.00	0.00	Sarcoplasm
43	PRDX2	Anti-oxidant			0.65	±	0.00	10.98	±	0.00	0.00	Sarcoplasm
					No change							
1	A1AT	Stress response			**7.80**	±	0.00	7.80	±	0.00	NA	Sarcoplasm
2	HBA	Transport	0.80	0.69	0.86	±	0.00	0.86	±	0.00	0.00	Sarcoplasm
Reported FSR in one group only												
1	AT2A1	Muscle contraction			1.56		0.00					Myofibrils
2	FLNC	Cytoskeleton/structure			4.59		0.00					Myofibrils

Appendix 8 The 231 proteins (OB; pre-HIT vs post-HIT)

The 231 proteins that were categorised by
molecular function and biological process following UniProtKB.


Sarcoplasmic fraction
Molecular function
Biological process


Myofibrilla fraction
Molecular function
Actin-binding 25 FLNC MYH1 MYH3 MYH4 MYH6 MYH7 TNNI1 ACTBL ACTN2 ACTN3 DYST KLHL5 MICA3 MYH8 MYH9 MYO15 MYOTI MYPC1 MYPC2 NEBU TNNI2 TPM1 TPM3 MYH2;MY TPM2;TPM4
Allosteric enzyme 1 PYGM;PYGL
Calmodulin-binding 9 MYH1 MYH3 MYH4 MYH6 MYH7 MYH8 MYH9 MYO15 TITIN
Chromatin regulator 1 BRE1A
Developmental protein 3 FHL1 CENPE IF122
DNA-binding 4 ENOA H2B1B KIF22 TRI33
Glycosyltransferase 1 PYGM;PYGL
Guanine-nucleotide releasing factor 1 HERC1
Hydrolase 3 IIGP5 MRP1 PDP1
Hypotensive agent 1 HBB
Initiation factor 1 EIF3J
Kinase 4 KCRM STK31 TAOK2 TITIN
Lyase 3 ALDOA ENOA ENOG;ENOB
Monooxygenase 1 MICA3
Motor protein 17 MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 DYH7 CENPE KIF22 MLRS MLRV MYH8 MYH9 MYL6B MYO15 MYH2;MYH7B
Muscle protein 34 ACTC CASQ1 MYG MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 MYOM1 TNNI1 ACTC ACTS;ACTE DESM DYST MLRS MLRV MYH8 MYL6B MYOM2 MYOTI MYPC1 MYPC2 NEBU TNNC1 TNNC2 TNNT1 TNNT3 TNNI2 TPM1 TPM3 MYH2;MY TPM2;TPM4
Myosin 12 MYH1 MYH3 MYH4 MYH6 MYH7 MYL1 MYL3 MLRS MLRV MYL6B MYO15 MYH2;MYH7B
Oxidoreductase 4 G3P MICA3 MYH8 MYH9
Protein phsphatase 1 PDP1
Repressor 2 ENOA TRI33
Ribonucleoprotein 1 RS27A
Ribosomal protein 1 RS27A
RNA-binding 2 CO052 TNR6C
Serine/threonine-protein kinase 3 STK31 TAOK2 TITIN
Transferase 9 G3P KCRM BRE1A HERC1 STK31 TAOK2 TITIN TRI33 PYGM;PYGL
Translocase 1 AT2A1;AT2A2
Vasoactive 1 HBB
Activator
Aminotransferase
Antioxidant
Chaperone
Cytokine
Growth factor
Isomerase
Membrane protein
Multifunctional enzyme
Peroxidase
Protease inhibitor
Serine protease inhibitor
Biological process
ATP synthesis 0
Blood coagulation 0
Cellular component 0
Fatty acid metabolism 0
Gluconeogenesis 0
Glucose metabolism 0
Glyoxylate bypass 0
HEMOstasis 0
Hydrogen ion transport 0
Lipid degradation 0
Lipid transport 0
mRNA processing 0
mRNA splicing 0
Spermatogenesis 0
Stress response 0
TCA 0
Apoptosis 1 G3P
Calcium transport 1 AT2A1;AT2A2
Carbohydrate metabolism 1 PYGM;PYGL
Cell adhesion 4 MYPC1 MYPC2 MYH9 DYST
Cell cycle 5 LRCC1 MICA3 CNTRL;E CEP63 CENPE
Cell division 5 LRCC1 MICA3 CNTRL;E CEP63 CENPE
Cell shape 1 MYH9
Cholesterol metabolism 1 NPCL1
Cilium biogenesis/degradation 1 IF122
Differentiation 1 FHL1
DNA damage 1 CEP63
Electron transport 1 NDUA4
Endocytosis 1 EHBP1
Exocytosis 1 MICA3
Glycogen metabolism 1 PYGM;PYGL
Glycolysis 4 ALDOA ENOA G3P ENOG;ENOB
Hearing 1 MYO15
Host-virus interaction 1 ADT1
Ion transport 1 AT2A1;AT2A2
Lipid metabolism 1 NPCL1
Mitosis 1 LRCC1
Mitosis 2 CEP63 CENPE
Oxygen transport 3 HBA HBB MYG
Plasminogen activation 1 ENOA
Protein biosynthesis 1 EIF3J
Protein transport 1 EHBP1
Respiratory chain 1 NDUA4
RNA-mediated gene silencing 1 TNR6C
Steroid metabolism 1 NPCL1
Sterol metabolism 1 NPCL1
Transcription 2 ENOA TRI33
Transcription regulation 2 ENOA TRI33
Translation regulation 2 G3P TNR6C
Transport 9 HBA HBB MYG NDUA4 MRP1 HERC1 EHBP1 AT2A1;AT ADT1
Ubl conjugation pathway 3 TRI33 HERC1 BRE1A

Appendix 9 List of significant proteins (16/25) OB; pre-HIT vs post-HIT

One-way ANOVA reported 16 and 25 proteins from sarcoplasm and myofibrillar fractions demonstrated significantly different in abundance in response to 10-week-HIT

	Accession	Description	Function	Peptide count	Unique peptides	Confidence score	Anova (*p*)	FDR (q-value)	Maximum fold change	abundance post-HIT
			Sarcoplasm							
1	AATM	Aspartate aminotransferase, mitochondrial	Lipid metabolic process	6	6	345.53	0.01	0.05	1.21	Up
2	ACTS;ACTB;POTEF; ACTBM;POTEI;ACTBL	Actin, alpha skeletal muscle	Muscle contraction	11	11	864.73	0.04	0.05	1.15	Up
3	ADT1;ADT3;ADT4	ADP/ATP translocase 1	Regulation of insulin secretion	2	2	79.28	0	0.05	1.57	Up
4	COX2	Cytochrome c oxidase subunit 2	OXPHOS	2	2	89.23	0	0.05	1.54	Up
5	COX41	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	OXPHOS	1	1	40.21	0.01	0.05	1.46	Up
6	EF1A2;EF1A1	Elongation factor 1-alpha 2	Lipid metabolic process	2	2	113.88	0.05	0.05	1.16	Up
7	FIBG	Fibrinogen gamma chain	Extracellular region	1	1	39.34	0.04	0.05	0.84	Down
8	HS90A;HS90B	Heat shock protein HSP 90-alpha	Cell death	1	1	54.38	0.01	0.05	1.3	Up
9	HSP7C	Heat shock cognate 71 kDa protein	Cell death	3	1	172.73	0.04	0.05	0.72	Down
10	KCRS;KCRU	Creatine kinase S-type, mitochondrial	Muscle contraction	8	8	546.22	0.02	0.05	1.33	Up
11	LGUL	Lactoylglutathione lyase	Extracellular region	1	1	48.14	0.04	0.05	0.54	Down
12	TBA8	Tubulin alpha-8 chain	IFCO	1	1	49.49	0.01	0.05	2.09	Up
13	THIO	Thioredoxin	Transport	1	1	89.65	0.05	0.05	0.81	Down
14	TPIS	Triosephosphate isomerase	Transport	12	12	969.09	0.03	0.05	0.82	Down
15	TPM2;TPM4	Tropomyosin beta chain	Muscle contraction	3	1	170.51	0	0.05	1.36	Up
16	WSB1	WD repeat and SOCS box-containing protein 1	IFCO	1	1	37.75	0.01	0.05	1.94	Up
			Myofibrils							
1	ACTN3	Alpha-actinin-3	Muscle contraction	16	6	953.32	0.01	0.05	1.73	Up
2	ADH1_YEAST	Alcohol dehydrogenase 1	Glucose metabolic process	12	9	425.1	0.02	0.05	1.13	Up
3	AKAP9	A-kinase anchor protein 9	IFCO	5	1	60.78	0.03	0.05	0.63	Down
4	BRE1A	E3 ubiquitin-protein ligase BRE1A	OXPHOS	1	1	5.56	0.01	0.05	1.2	Up
5	CE295	Centrosomal protein of 295 kDa	Chromosome	1	1	18.85	0	0.05	1.61	Up
6	CNTRL;ECT2	Centriolin	Chromosome	3	1	47.84	0.01	0.05	0.58	Down
7	CO052	Uncharacterized protein C15orf52	Other	1	1	34.69	0.01	0.05	1.47	Down
8	DESM	Desmin	IFCO	8	7	569.46	0.01	0.05	1.22	Up
9	DYH7	Dynein heavy chain 7, axonemal	IFCO	3	2	63.6	0	0.05	0.64	Down
10	DYST	Dystonin	Other	7	2	94.57	0.03	0.05	1.21	Up
11	ERC2	ERC protein 2	IFCO	2	1	29.27	0.03	0.05	0.75	Down
12	GOGB1	Golgin subfamily B member 1	Other	2	1	21.55	0.03	0.05	1.08	Up
13	IF122	Intraflagellar transport protein 122 homolog	IFCO	1	1	38.78	0.02	0.05	0.56	Down
14	IIGP5	Interferon-inducible GTPase 5	Other	1	1	17.32	0.01	0.05	0.68	Down
15	KLHL5	Kelch-like protein 5	IFCO	1	1	16.79	0.02	0.05	0.14	Down
16	MICA3	Protein-methionine sulfoxide oxidase MICAL3	IFCO	2	2	38.59	0.02	0.05	0.75	Down
17	MPP9	M-phase phosphoprotein 9	IFCO	2	1	66.11	0.01	0.05	0.64	Down
18	MRP1	Multidrug resistance-associated protein 1	Transport	1	1	5.31	0	0.05	0.68	Down
19	MYH15	Myosin 15	Muscle contraction	7	2	169.09	0.03	0.05	1.15	Up
20	MYOM1	Myomesin-1	Muscle contraction	4	2	107.51	0.01	0.05	0.67	Down
21	PDP1	[Pyruvate dehydrogenase [acetyl-transferring]]-phosphatase 1, mitochondrial	Lipid metabolic process	2	1	52.53	0.02	0.05	0.89	Down
22	RS27A	Ubiquitin-40S ribosomal protein S27a	Cell death	1	1	36.39	0	0.05	1.43	Up
23	STK31	Serine/threonine-protein kinase 31	Chromosome	1	1	16.16	0.01	0.05	0.58	Down
24	TAOK2	Serine/threonine-protein kinase TAO2	IFCO	1	1	33.39	0.02	0.05	0.9	Down
25	TRI33	E3 ubiquitin-protein ligase TRIM33	OXPHOS	3	1	25.53	0.04	0.05	0.61	Down

Appendix 10 FSR of measurability proteins (OB; pre-HIT vs post-HIT)

FSR of proteins that are able to measure (97 proteins) in response to 10-week-HIT

	Protein accession	Function	abundance change		Newly fractional synthesis rate (FSR)							Fraction
			Fold change of OB/TR	*p*	OB			TR			*p*	
					FSR±SD (%/day)			FSR±SD (%/day)				
	Reported statistially significnat in FSR but not significant in abundance											
1	KCRM	Muscle contraction	1.07	0.11	2.27	±	0.00	0.85	±	0.00	0.00	Myofibrils
2	TITIN	Cytoskeleton/structure	1.04	0.16	1.38	±	0.00	2.14	±	0.00	0.00	Myofibrils
3	MYG	Muscle contraction	0.89	0.19	1.17	±	0.00	0.89	±	0.00	0.00	Sarcoplasm
4	TNNT3	Muscle contraction	0.93	0.26	0.77	±	0.00	2.56	±	0.00	0.00	Myofibrils
5	MYOZ1	Cytoskeleton/structure	0.96	0.28	1.52	±	0.00	2.44	±	0.00	0.00	Myofibrils
6	ALBU	Transport	1.08	0.32	3.45	±	0.00	4.41	±	0.01	0.00	Sarcoplasm
7	MYH7	Muscle contraction	1.15	0.40	3.71	±	0.01	2.09	±	0.00	0.00	Myofibrils
8	MYOM2	Muscle contraction	0.98	0.42	0.74	±	0.00	2.11	±	0.00	0.00	Myofibrils
9	LDB3	Cytoskeleton/structure	0.96	0.42	1.26	±	0.00	3.17	±	0.00	0.00	Myofibrils
10	MYPC1	Cytoskeleton/structure	1.02	0.49	2.29	±	0.00	3.23	±	0.01	0.00	Myofibrils
11	TNNI2	Muscle contraction	1.15	0.51	0.96	±	0.00	1.46	±	0.00	0.00	Myofibrils
12	MYL1	Muscle contraction	0.97	0.56	0.64	±	0.00	1.88	±	0.00	0.00	Myofibrils
13	PGM1	Glucose metabolism	0.96	0.69	6.20	±	0.00	1.12	±	0.00	0.00	Sarcoplasm
14	NEBU	Muscle contraction	1.01	0.74	4.05	±	0.01	2.70	±	0.00	0.00	Myofibrils
15	IGKC	Glucose metabolism	0.99	0.80	2.91	±	0.00	3.15	±	0.00	0.01	Sarcoplasm
16	TPM1	Muscle contraction	0.99	0.83	0.98	±	0.00	0.92	±	0.00	0.00	Myofibrils
17	FLNC	Cytoskeleton/structure	0.99	0.84	2.36	±	0.00	4.52	±	0.01	0.00	Sarcoplasm
18	TNNI1	Muscle contraction	1.02	0.86	1.28	±	0.00	6.66	±	0.01	0.00	Myofibrils
19	MLRS	Muscle contraction	1.02	0.97	0.66	±	0.00	0.52	±	0.00	0.01	Myofibrils
20	HBB	Transport	0.89	0.98	1.57	±	0.00	2.67	±	0.01	0.02	Myofibrils
21	ACTS	Muscle contraction			0.39	±	0.00	2.09	±	0.00	0.00	Myofibrils
22	MYH2	Muscle contraction			0.98	±	0.00	1.51	±	0.00	0.00	Myofibrils
23	TPM2	Muscle contraction			0.94	±	0.00	9.70	±	0.04	0.00	Myofibrils
24	KAD1	Extracellular region			0.61	±	0.00	50.49	±	0.06	0.00	Sarcoplasm
25	KPYM	Muscle contraction			1.43	±	0.00	0.93	±	0.00	0.00	Sarcoplasm
26	LDHA	TCA			1.36	±	0.00	50.91	±	0.06	0.00	Sarcoplasm
27	PYGM	Glucose metabolism			1.95	±	0.00	1.78	±	0.00	0.00	Sarcoplasm
28	ATPB	OXPHOS	1.12	0.13	0.91	±	0.00	0.83	±	0.00	0.80	Sarcoplasm
29	MYL3	Muscle contraction	1.18	0.37	0.72	±	0.00	1.15	±	0.00	0.57	Myofibrils
30	TNNC2	Muscle contraction	1.02	0.86	2.05	±	0.00	1.72	±	0.00	0.08	Myofibrils
31	TRFE	Transport	0.87	0.51	5.74	±	0.00	3.57	±	0.01	0.29	Sarcoplasm
32	IGG1	Extracellular region	0.97	0.84	3.68	±	0.00	3.81	±	0.00	0.75	Sarcoplasm
		Report FSR in one group only										
1	AATC	TCA			0.01	±	0.00					Sarcoplasm
2	AATM	TCA			0.03	±	0.00					Sarcoplasm
3	ALDOA	Glucose metabolism			0.02	±	0.00					Sarcoplasm
4	ADT1	ADP/ATP translocase 1			0.02	±	0.00					Sarcoplasm
5	APOA1	Lipid metabolism			0.04	±	0.00					Sarcoplasm
6	AT2A2	Muscle contraction			0.04	±	0.00					Sarcoplasm
7	CAH2	Carbonate dehydratase activity			0.03	±	0.00					Sarcoplasm
8	CASQ1	Muscle contraction			0.01	±	0.00					Sarcoplasm
9	F16P2	Glucose metabolism			0.03	±	0.00					Sarcoplasm
10	COX5A	OXPHOS			0.01	±	0.00					Sarcoplasm
11	FHL1	Cytoskeleton/structure			0.02	±	0.00					Sarcoplasm
12	G6PI	Glucose metabolism			0.98	±	0.00					Sarcoplasm
13	GDE	Lipid metabolism			0.01	±	0.00					Sarcoplasm
14	HSPB1	Cell death			0.01	±	0.00					Sarcoplasm
15	IDHP	TCA			0.05	±	0.00					Sarcoplasm
16	IGHG2	Cytoskeleton/structure			0.02	±	0.00					Sarcoplasm
17	MDHC	TCA			0.05	±	0.01					Sarcoplasm
18	PADI2	Cytoskeleton/structure			0.02	±	0.00					Sarcoplasm
19	PDLI3	Cytoskeleton/structure			0.01	±	0.00					Sarcoplasm
20	PEBP1	Cytoskeleton/structure			0.02	±	0.00					Sarcoplasm
21	PRDX2	Antioxidant			0.01	±	0.00					Sarcoplasm
22	SYPL2	Transport			0.01	±	0.00					Sarcoplasm
23	TM198	Transport			0.52	±	0.09					Sarcoplasm
24	TPIS	Glucose metabolism			0.01	±	0.00					Sarcoplasm
25	TRI72	Transport			0.03	±	0.00					Sarcoplasm
26	AT2A1	Muscle contraction			1.93%	±	0.00					Myofibrils
27	ENOA	Glucose metabolism			1.50%	±	0.00					Myofibrils
28	FHL1	Cytoskeleton/structure			3.70%	±	0.00					Myofibrils
29	HBA	Transport			1.55%	±	0.00					Myofibrils
30	MYG	Muscle contraction			0.73%	±	0.00					Myofibrils
31	PDLI3	Cytoskeleton/structure			2.70%	±	0.00					Myofibrils
32	TNNC1	Muscle contraction			1.08%	±	0.00					Myofibrils

Appendix 11 National Research Ethic Service (NRES documents)

West Midlands - Black Country Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

05 August 2016

Professor Jatin G Burniston
Research Institute for Sport & Exercise Sciences
Tom Reilly Building
Byrom Street
L3 3AF

Dear Professor Burniston

Study title:	**Muscle protein turnover: the effects of physical inactivity and exercise training**
REC reference:	**16/WM/0296**
IRAS project ID:	**208784**

Thank you for your submission responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair together with another Committee member.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Georgia Copeland, nrescommittee.westmidlands-blackcountry@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see

"Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Copies of advertisement materials for research participants [Recruitment poster]	v2	13 July 2016
Covering letter on headed paper [Response to committee comments]		13 July 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)	v1	07 June 2016
GP/consultant information sheets or letters [Letter to GP]	v1	07 June 2016
Letters of invitation to participant [Recruitment email]	v2	13 July 2016
Other [CV - Katie Hesketh]		
Participant consent form [Consent form - Part A]	v2	13 July 2016
Participant consent form [Consent form - Part B]	v2	13 July 2016
Participant information sheet (PIS) [PIS - Part A]	v2	13 July 2016
Participant information sheet (PIS) [PIS - Part B]	v2	13 July 2016
REC Application Form [REC_Form_14072016]		14 July 2016
Research protocol or project proposal [Research Protocol]	v2	22 July 2016
Summary CV for Chief Investigator (CI) [Jatin Burniston - CV]	v1	07 June 2016
Summary CV for student [Kanchana Srisawat - CV]	v1	07 June 2016
Summary CV for supervisor (student research) [Sam Shepherd - CV]	v1	07 June 2016
Validated questionnaire [Framingham CVD risk questionnaire]	v1	07 June 2016
Validated questionnaire [Readiness to Exercise questionnaire]	v1	07 June 2016
Validated questionnaire [Paffenbarger Physical Activity questionnaire]	v1	07 June 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators

- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/WM/0296	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

PP

Dr Hilary Paniagua
Chair

Email:nrescommittee.westmidlands-blackcountry@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Dave Harriss*

LIVERPOOL JOHN MOORES UNIVERSITY

How does cycling protect you from Type 2 diabetes?

Help us find out by taking part in a research study

We are looking for male participants who are either well-trained endurance athletes or physically inactive individuals who are overweight.

To be eligible, you must be:

- Aged 30-45 years
- Endurance athletes should have been training more than 3 h/week for at least a year
- Physically inactive individuals should usually undertake less than 2 x 30 min sessions of vigorous activity each week.

The study will involve health and fitness assessments, including body composition and maximum exercise capacity. In addition you will be asked to consume heavy water and we will also collect blood and muscle samples

For more details, please contact:

Kanchana Srisawat

Tel: 0151 904 6233

Email:
k.srisawat@2015.jmu.ac.uk

Katie Hesketh

Tel: 01519046233

Email:
k.hesketh@2012.jmu.ac.uk

Research Institute for Sport & Exercise Sciences

Version 2: 13.07.16

dream plan achieve

LIVERPOOL JOHN MOORES UNIVERSITY

Participant Information Sheet

Muscle protein turnover: the effects of physical inactivity and exercise training

Part A

Location(s):

Research Institute for Sport & Exercise Sciences, Liverpool John Moores University

Investigators:

Miss Kanchana Srisawat, Miss Katie Hesketh, Dr Sam Shepherd, Dr Lynne Boddy, Dr Graeme Close, Dr Matthew Cocks and Professor Jatin Burniston

You are being invited to participate in a research project. However, before you give consent to participate in this study, it is important that you completely understand why this research is being conducted and what will be required of you. Please ensure that you take time to read through this information sheet. If there are any areas that are not clear, or that you would like more information on, feel free to contact the researchers who will be happy to provide this information for you.

What is the purpose of the study?

Obesity and physical inactivity increase the risk of type 2 diabetes and cardiovascular disease. Regular exercise, however, is an effective means of reducing the risk for type 2 diabetes, but the mechanisms responsible for this benefit are unclear. Muscle is a major target for the positive benefits of exercise because it has an important role in whole-body metabolism and therefore weight control. Muscle itself is made of thousands of different proteins that play a role in metabolising fats and sugars, as well as in generating force to produce movement. All proteins exist in a continuous cycle of breakdown and renewal (i.e. protein turnover) and this is essential to prevent the accumulation of damaged or unwanted proteins. An accumulation of damaged proteins is one of the hallmarks of ageing, and it may be that modern lifestyles are accelerating this process and a loss of muscle protein quality is responsible for earlier onset of disease such as type 2 diabetes. By measuring the turnover of all proteins in muscle, Part A of this study aims to identify which proteins are affected by being sedentary and overweight.

Am I eligible for this study?

You are likely to be eligible for this study if you fulfil the following criteria (group 1):

- Male, aged 30-45 years
- BMI ≥28 $kg.m^{-2}$
- Healthy (no known cardiovascular or metabolic disorders)
- Not involved in regular structured physical activity (i.e. engaged in less than 2 sessions of 30 min per week for at least 1 year)

Or (group 2):

- Male, aged 30-45 years
- BMI ≤25 $kg.m^{-2}$
- Healthy (no known cardiovascular or metabolic disorders)
- Involved in regular exercise training (i.e. engaged in aerobic exercise training more than 3 sessions of 60 minutes per week for at least 2 years)

Do I have to take part?

No. Taking part in this study is entirely voluntary. You will be given at least 24 hours to consider taking part in the study. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

May I be excluded from the study?

Yes. You can be excluded from the study at any time point if you do not adhere to the study conditions.

Benefits:

We will first test indicators of health and your fitness on an exercise bike. The fitness and health checks (including facility access) comes free of charge, and would otherwise come at a considerable cost at a private gym. Once the study has finished, you will be provided with the opportunity to take part in 10 weeks of exercise training (Part B – information on request). You will also be compensated for lost time, travel, parking, etc. to the sum of £200 on completion of the experimental procedures (see protocol overview). This payment should not be used as a motivation to take part in this study.

What will happen to me if I take part?

If you agree to take part in the study, you will first be asked to attend an initial meeting and a pre-screening visit. If you are eligible for the study, you then be asked to attend the laboratory on seven separate occasions over a 15 day experimental period. All visits will take place at the School of Sport & Exercise Sciences at Liverpool John Moores University.

Pre-experimental procedures:

Initial meeting (~30 mins):

If you are interested in taking part you will be asked to attend an initial meeting at Liverpool John Moores University. In this meeting you will be able to ask any questions that you may have about the study, and if you would still like to participate you will be asked to complete a consent form and undergo a medical screening assessment, which includes questions about your medical history, diet and physical activity levels as well as recording your heart beat (i.e. electrocardiogram; ECG) and measuring your blood pressure.

Pre-screening visit (~3 hours):

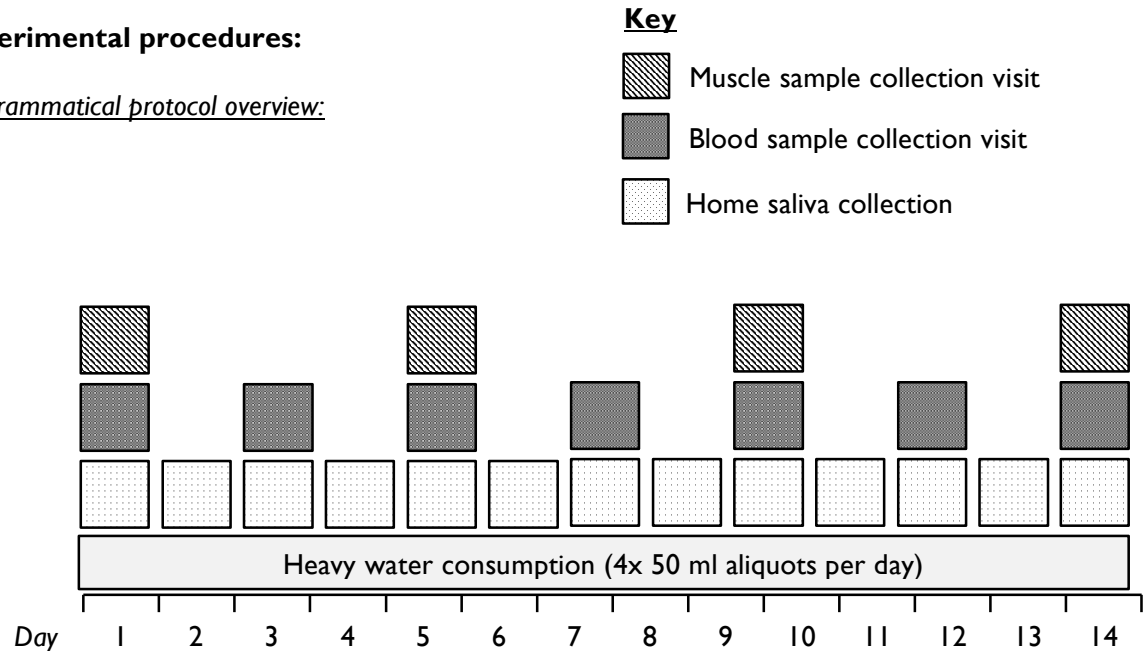
On a separate occasion you will be asked to report to the laboratory having completed an overnight fast, and having abstained from caffeine, alcohol and vigorous exercise the day before. We will then conduct a number of tests to assess your health.

1. Body composition – This is will be measured by a whole body 'DXA' scan, which uses low-power X-rays to measure the amount and location of your body fat.
2. Oral glucose tolerance test (OGTT) – This test measures your blood sugar levels. A cannula (small flexible tube) will be placed into a forearm vein of one arm to allow us to obtain blood samples at various time-points. After the first blood sample (25 ml – about the size of an espresso) you will be asked to drink a sugary drink. Following this, further small blood samples (5 ml – a teaspoon) will be collected after 15, 30, 45, 60, 90 and 120 minutes. The cannula will then be removed.
3. Fitness test – This test will involve cycling through increasing levels of resistance on a stationary bicycle. The test will compromise an initial 3 minute period of easy cycling, and the resistance will increase every 3 minutes until fatigue. Throughout the test, breath samples will be measured to determine the volume of oxygen consumed.

If you are eligible, you will then take part in the experimental procedures (detailed below). The first visit of the experimental procedures will take place at least 3 days after the pre-screening visit.

Experimental procedures:

Diagrammatical protocol overview:



Muscle sample collection visits (~1 hour):

On 4 occasions you will attend the laboratory after an overnight fast, and having abstained from caffeine, alcohol and vigorous exercise the day before. We will then conduct three assessments as follows:

1. Blood sample – A blood sample (5 ml – a teaspoon) will be obtained from the forearm of one arm by an experienced phlebotomist.
2. Muscle biopsy – A small muscle sample (about the size of a ¼ of a pea) will be taken from the outside of your thigh of one leg following administration of local anaesthetic. The procedure is a standard tool and has been used successfully on a regular basis by experienced staff in our laboratory. The information gained by analysing muscle samples is crucial to our scientific objectives and may discover new information about the health risks of physical inactivity or the protective effects of exercise training. We may also retain your muscle samples after the current investigation has been completed and re-analyse them as part of different experiments, for example to test new analytical techniques or draw comparisons against different population cohorts.

Blood sample collection visits (~1 hour):

On 3 occasions you will visit the laboratory to provide a small blood sample (5 ml – a teaspoon) from the forearm of one arm (obtained by a trained phlebotomist). You will also be asked to recall the food you have eaten in the preceding 24 hours. This is important as what you eat can affect the measurements that we are making in the study.

Home saliva collection (~5 mins):

Each morning you will be required to collect a sample of your own saliva (by passively drooling into a collection tube) at home.

Heavy water consumption:

You will be asked to consume 4 aliquots (50 ml/aliquot – about the size of a spirit shot-glass) of heavy water distributed evenly throughout the day during the entire experimental period.

Physical activity monitoring:

You will be asked to wear an activity monitor during each day of the experimental period.

At the conclusion of Part A of the study, participants that are part of group 1 will be invited to take part in Part B of the study.

Risks:

The most obvious risks to you involve blood sampling, muscle sample collection, heavy water consumption and high intensity cycling.

Blood sampling:

Blood samples will be taken on 8 occasions**.** You will feel a sharp pain when the needle is inserted but this will be short-lived. The researchers are experienced in this technique so the pain experienced will be minimal. You may also develop a small bruise on your arm which can be prevented by applying pressure on the arm when the cannula or needle has been taken out (the researcher will remind/instruct you to do this, as is good practice). A total of 70 ml of blood will be taken during the study (equivalent to a small glass of wine).

Muscle biopsy:

During the whole experiment you will have a total of 4 muscle biopsies taken from the outside of each thigh. This procedure is a standard tool which has been used in research for the last 40 years, and has been used successfully on many occasions by the research team. Trained staff will perform the muscle biopsies and have extensive experience with this procedure. The muscle biopsies will be performed under local anaesthetic. You may experience a small amount of pain while the local anaesthetic is administered. Following the local anaesthetic a small incision will be made (approx. 0.5-1cm) through the skin (a new incision will be made for each biopsy); you will not feel any pain or discomfort during this as a result of the local anaesthetic. Thereafter the biopsy may be associated with a feeling of deep pressure and/or mild discomfort, but only for a short time (approx. 30 seconds per biopsy). After the experiment your leg may still feel a bit stiff or sore but this should not last longer than 2-3 days. Sometimes a small scar where the incision was made (approx. 0.5-1cm) will remain visible for a longer period of time. Complications such as bruising, infection and pain have been reported but this is very rare (less than 2% chance). There is a minimal chance of infection of the wound, but we will use strict sterile techniques to minimize this risk and we advise you not to swim during the 48 hours after the biopsy has been taken. There is also a minimal chance of permanent loss of sensation or soreness, and keloid scaring in non-Caucasians at the biopsy site. We will give you all the instructions you need to reduce any risks to an absolute minimum. In the unlikely event that you experience any of the above symptoms, or any other symptoms related to the muscle biopsy following the study, you are welcome to contact the study physician who will be happy to discuss the problem with you. In the rare event that they, or a medically trained member of their team are unavailable, our advice would be to visit your GP.

DXA scanning:

The amount of X-ray radiation from a whole body DXA scan is extremely low – it is less than would be received during a chest X-ray and similar to the level received by passengers during a 5 hour aeroplane flight.

Heavy water consumption:

Heavy water is a non-hazardous clear liquid that is made using the heavy isotope of hydrogen (chemical symbol H), which is nicknamed 'deuterium' and has the chemical symbol 'D'. Accordingly heavy water is abbreviated as D_2O rather than H_2O. Because deuterium (D) is the stable isotope of hydrogen (H), heavy water does not emit harmful ionising radiation and it is not classified as dangerous. No long-term adverse effects of heavy water have been reported in humans in the substantial body of clinical research literature. However, acute side effects including nausea and vertigo may occur when heavy water is consumed in a large (i.e. >150 ml) single dose. This is why we have given instructions for you to consume small (50 ml) doses at 4 regular intervals throughout the day.

Fitness test:

You will experience fatigue during the fitness tests and training. This is normal and will be short-lived and you should fully recover within hours of the process. However, during such vigorous exercise there is a very minimal risk of unforeseen heart failure. The risk in healthy people equates to 1 death every 2.6 million workouts. Even in patients with heart disease, who are recognised as high risk, the risk equates to 1 death every 176,000 exercise training hours. As such, the risk is deemed extremely small and these procedures are regularly conducted within the laboratory. You are also free to stop exercising at any point if you feel uncomfortable. People trained in emergency first aid will always be on hand if there is a problem.

Confidentiality:

All data and samples will be labeled with a code, not with your name. Only the research team which is in contact with you has access to the link between code and name of the subject. The results of this study are expected to be published in a scientific journal, but names of participants will never be published.

Rights:

It is your choice whether or not you wish to take part in this study. If you wish to take part in this study, you will be given this information sheet to read and be asked to sign a consent form. You are reminded that if you decide to take part in the study, you are still free to withdraw from the study at any time without giving a reason. If you withdraw from the study, the data and blood samples already collected will be retained, unless you request for them to be removed within 12 weeks of the first visit.

What happens if something goes wrong on the day of the trials?

If you have any concerns regarding your involvement in this research, please discuss these with the research team in the first instance. If you wish to make a complaint, please email: researchethics@ljmu.ac.uk or telephone the secretary of the Research Ethics Committee on 0151 904 6467 and your communication will be re-directed to an independent person as appropriate.

All procedures have been included within Liverpool John Moores University Liability insurances and if you are harmed in any way by taking part in this research project your normal rights apply and you may have ground for legal action.

What happens now?

You will be asked to complete an informed consent form to confirm that you are happy to participate in this study. You will be asked to keep a copy of this information sheet and the signed consent forms. We will then inform your GP that you have decided to take part in this study.

Thank you for your time, and if you want to participate in the study or have any further questions, please feel free to contact any of the investigators listed below.

Miss Kanchana Srisawat
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

Office: 0151 904 6233
E-mail: k.srisawat@2015.ljmu.ac.uk

Miss Katie Hesketh
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

Office: 0151 904 6233
E-mail: k.hesketh@2012.ljmu.ac.uk

LIVERPOOL JOHN MOORES UNIVERSITY

Participant Information Sheet

Muscle protein turnover: the effects of physical inactivity and exercise training

Part B

Location(s):

Research Institute for Sport & Exercise Sciences, Liverpool John Moores University

Investigators:

Miss Kanchana Srisawat, Miss Katie Hesketh, Dr Sam Shepherd, Dr Lynne Boddy, Dr Graeme Close, Dr Matthew Cocks and Professor Jatin Burniston

You are being invited to participate in a research project. However, before you give consent to participate in this study, it is important that you completely understand why this research is being completed and what will be required of you. Please ensure that you take time to read through this information sheet. If there are any areas that are not clear, or that you would like more information on, feel free to contact the researchers who will be happy to provide this information for you.

What is the purpose of the study?

Part A of the study aimed to identify which proteins in muscle are different between sedentary overweight people compared to endurance-trained athletes. Recently, there has been interest in the use of high-intensity interval training to improve health and fitness in previously sedentary people. Indeed, using this exercise mode, studies have shown that similar health and fitness benefits can be gained but with a much smaller time commitment compared to aerobic exercise training (e.g. jogging, cycling etc. on 5 days per week). Part B of this study aims to investigate how-high intensity interval training effects the turnover of muscle proteins.

Am I eligible for this study?

You are likely to be eligible for this study if you fulfil the following criteria:

- *Having undertaken Part A of the study*
- Male, aged 30-45 years
- BMI ≥28 $kg.m^{-2}$
- Healthy (no known cardiovascular or metabolic disorders)
- Not involved in regular structured physical activity (i.e. engaged in less than 2 sessions of 30 min per week for at least 1 year)

Do I have to take part?

No. Taking part in this study is entirely voluntary. You will be given at least 24 hours to consider taking part in the study. If you would like to participate, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

May I be excluded from the study?

Yes. You can be excluded from the study at any time point if you do not adhere to the study conditions.

Benefits:

You will take part in 10 weeks of exercise training, supervised by an investigator. Following the training we will re-test your fitness and a number of health indicators to see how much you have improved

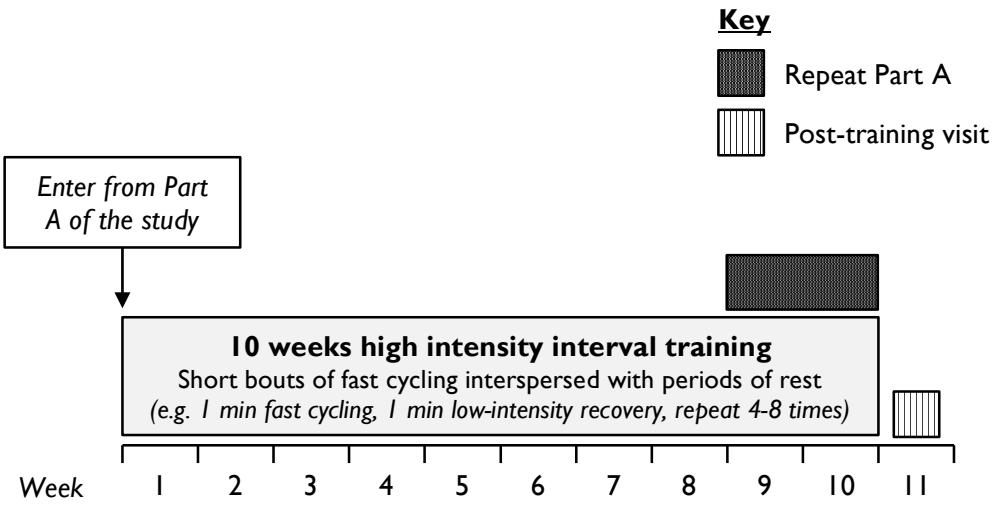
after the training. You will receive detailed feedback on what changes you have achieved in response to the training. The fitness and health checks and supervised training (including facility access) comes free of charge, and would otherwise come at a considerable cost at a private gym. After the study has finished, we will provide advice on options for you to continue exercising using simple equipment typically available at a commercial gym. You will also be compensated for lost time, travel, parking, etc. to the sum of £200 on completion of the experimental procedures (see protocol overview). This payment should not be used as a motivation to take part in this study.

What will happen to me if I take part?

If you agree to take part in the study, you will complete 10 weeks of supervised training. During the final 2 weeks of the training period you will be required to repeat the "Experimental procedures" portion of Part A of the study (identical in all respects).

Study procedures:

Diagrammatical protocol overview:



Training intervention:

You will attend the Research Institute for Sport & Exercise Sciences (Liverpool John Moores University) for 3 supervised cycling sessions per week for a period of 10 weeks (a total of 30 training sessions). Each session will begin with a short warm up of cycling at low intensity (~3 min). Thereafter, you will perform repeated bouts of fast cycling (1 min) interspersed with periods of rest (1 min). A short cool down will be performed at the end of each session. During weeks 1 and 2 you will complete 4 bouts of fast cycling, which will increase to 5 bouts during weeks 3 and 4, 6 bouts during weeks 5 and 6, 7 bouts during weeks 7 and 8, and 8 bouts during weeks 9 and 10. Each session will last no longer than 30 minutes in total.

Repeat of Part A:

During the final 2 weeks of the training intervention (i.e. weeks 9 and 10) you will also repeat the schedule of activities described in detail in Part A, including: (i) daily deuterium consumption, saliva collection and physical activity monitoring; (ii) bi-daily collection of blood samples and dietary information; and (iii) a total of 4 muscle biopsies.

Post-training visit (~3 hours):

Three days following the conclusion of the experimental procedures you will be asked to report to the laboratory having completed an overnight fast, and having abstained from caffeine, alcohol and vigorous exercise the day before. We will then conduct a number of tests to assess changes in your health following the training.

1. Body composition – This is will be measured by a whole body 'DXA' scan, which uses low-power X-rays to measure the amount and location of your body fat

2. Oral glucose tolerance test (OGTT) – This test measures your blood sugar levels. A cannula (small flexible tube) will be placed into a forearm vein of one arm to allow us to obtain blood samples at various time-points. After the first blood sample (25 ml – about the size of an espresso) you will be asked to drink a sugary drink. Following this, further small blood samples (5 ml – a teaspoon) will be collected after 15, 30, 45, 60, 90 and 120 minutes. The cannula will then be removed.
3. Fitness test – This test will involve cycling through increasing levels of resistance on a stationary bicycle. The test will compromise an initial 3 minute period of easy cycling, and the resistance will increase every 3 minutes until fatigue. Throughout the test, breath samples will be measured to determine the volume of oxygen consumed.

Risks:

The most obvious risks to you involve blood sampling, muscle sample collection, heavy water consumption and high-intensity cycling.

Blood sampling:

Blood samples will be taken on 8 occasions**.** You will feel a sharp pain when the needle is inserted but this will be short-lived. The researchers are experienced in this technique so the pain experienced will be minimal. You may also develop a small bruise on your arm which can be prevented by applying pressure on the arm when the cannula has been taken out (the researcher will remind/instruct you to do this, as is good practice). A total of 70 ml of blood will be taken during the study (equivalent to a small glass of wine).

Muscle biopsy:

During the whole experiment you will have a total of 4 muscle biopsies taken from the outside of each thigh. This procedure is a standard tool which has been used in research for the last 40 years, and has been used successfully on many occasions by the research team. Trained staff will perform the muscle biopsies and have extensive experience with this procedure. The muscle biopsies will be performed under local anaesthetic. You may experience a small amount of pain while the local anaesthetic is administered. Following the local anaesthetic a small incision will be made (approx. 0.5-1cm) through the skin (a new incision will be made for each biopsy); you will not feel any pain or discomfort during this as a result of the local anaesthetic. Thereafter the biopsy may be associated with a feeling of deep pressure and/or mild discomfort, but only for a short time (approx. 30 seconds per biopsy). After the experiment your leg may still feel a bit stiff or sore but this should not last longer than 2-3 days. Sometimes a small scar where the incision was made (approx. 0.5-1cm) will remain visible for a longer period of time. Complications such as bruising, infection and pain have been reported but this is very rare (less than 2% chance). There is a minimal chance of infection of the wound, but we will use strict sterile techniques to minimize this risk and we advise you not to swim in the 48 hours after the biopsy has been taken. There is also a minimal chance of permanent loss of sensation or soreness, and keloid scaring in non-Caucasians at the biopsy site. We will give you all the instructions you need to reduce any risks to an absolute minimum. In the unlikely event that you experience any of the above symptoms, or any other symptoms related to the muscle biopsy following the study, you are welcome to contact the study physician who will be happy to discuss the problem with you. In the rare event that they, or a medically trained member of their team are unavailable, our advice would be to visit your GP.

DXA scanning:

The amount of X-ray radiation from a whole body DXA scan is extremely low – it is less than would be received during a chest X-ray and similar to the level received by passengers during a 5 hour aeroplane flight.

Heavy water consumption:

Heavy water is a non-hazardous clear liquid that is made using the heavy isotope of hydrogen (chemical symbol H), which is nicknamed 'deuterium' and has the chemical symbol 'D'. Accordingly heavy water is abbreviated as D_2O rather than H_2O. Because deuterium (D) is the stable isotope of hydrogen (H), heavy water does not emit harmful ionising radiation and it is not classified as dangerous. No long-term adverse effects of heavy water have been reported in humans in the substantial body of clinical research literature. However, acute side effects including nausea and vertigo may occur when heavy water is consumed in a large (i.e. >150 ml) single dose. This is why we have given instructions for you to consume small (50 ml) doses at 4 regular intervals throughout the day.

Exercise:

You will experience fatigue during the fitness tests and training. This is normal and will be short-lived and you should fully recover within hours of the process. However, during such vigorous exercise there is a very minimal risk of unforeseen heart failure. The risk in healthy people equates to 1 death every 2.6 million workouts. Even in patients with heart disease, who are recognised as high risk, the risk equates to 1 death every 176,000 exercise training hours. As such, the risk is deemed extremely small and these procedures are regularly conducted within the laboratory. You are also free to stop exercising at any point if you feel uncomfortable. People trained in emergency first aid will always be on hand if there is a problem.

Confidentiality:

All data and samples will be labeled with a code, not with your name. Only the research team which is in contact with you has access to the link between code and name of the subject. The results of this study are expected to be published in a scientific journal, but names of participants will never be published.

Rights:

It is your choice whether or not you wish to take part in this study. If you wish to take part in this study, you will be given this information sheet to read and be asked to sign a consent form. You are reminded that if you decide to take part in the study, you are still free to withdraw from the study at any time without giving a reason. If you withdraw from the study, the data and blood samples already collected will be retained, unless you request for them to be removed within 12 weeks of the first visit.

What happens if something goes wrong on the day of the trials?

If you have any concerns regarding your involvement in this research, please discuss these with the research team in the first instance. If you wish to make a complaint, please email: researchethics@ljmu.ac.uk or telephone the secretary of the Research Ethics Committee on 0151 904 6467 and your communication will be re-directed to an independent person as appropriate.

All procedures have been included within Liverpool John Moores University Liability insurances and if you are harmed in any way by taking part in this research project your normal rights apply and you may have ground for legal action.

What happens now?

You will be asked to complete an informed consent form to confirm that you are happy to participate in this study. You will be asked to keep a copy of this information sheet and the signed consent forms. We will then inform your GP that you have decided to take part in this study.

Thank you for your time, and if you want to participate in the study or have any further questions, please feel free to contact any of the investigators listed below.

Miss Kanchana Srisawat
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

Office: 0151 904 6233
E-mail: k.srisawat@2015.ljmu.ac.uk

Miss Katie Hesketh
Research Institute for Sport & Exercise Sciences
Liverpool John Moores University
Byrom Street
Liverpool
L3 3AF

Office: 0151 904 6233
E-mail: k.hesketh@2012.ljmu.ac.uk

LIVERPOOL JOHN MOORES UNIVERSITY

Consent Form

Title of Project: **Muscle protein turnover: the effects of physical inactivity and exercise training**

Name of Researchers: Miss Kanchana Srisawat, Miss Katie Hesketh, Dr Sam Shepherd, Dr Lynne Boddy, Dr Graeme Close, Dr Matthew Cocks, and Professor Jatin Burniston

Part A

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated 13.07.16 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. I also understand that if I withdraw from the study the data already collected will be retained, unless I request for it to be removed within 12 weeks of the first visit. ☐
3. I agree that tissue samples collected during this study may be retained and used in future research. ☐
4. I agree to my GP being informed of my participation in the study. ☐
5. I agree to take part in Part A of the above study. ☐

Name of Participant	Date	Signature

Name of Person taking consent	Date	Signature

When completed: 1 copy for participants, 1 for research site file, 1 (original) to be kept in notes.

LIVERPOOL JOHN MOORES UNIVERSITY

Consent Form

Title of Project: **Muscle protein turnover: the effects of physical inactivity and exercise training**

Name of Researchers: Miss Kanchana Srisawat, Miss Katie Hesketh, Dr Sam Shepherd, Dr Lynne Boddy, Dr Graeme Close, Dr Matthew Cocks, and Professor Jatin Burniston

Part B

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated 13.07.16 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. I also understand that if I withdraw from the study the data already collected will be retained, unless I request for it to be removed within 12 weeks of the first visit. ☐
3. I agree that tissue samples collected during this study may be retained and used in future research. ☐
4. I agree to my GP being informed of my participation in the study. ☐
5. I agree to take part in Part A of the above study. ☐

Name of Participant	Date	Signature

Name of Person taking consent	Date	Signature

When completed: 1 copy for participants, 1 for research site file, 1 (original) to be kept in notes.

Appendix 12 MPhil transfer to PhD confirmation

Srisawat, Kanchana

From: McKeon, Jo
Sent: 16 January 2017 11:11
To: Srisawat, Kanchana
Cc: Burniston, Jatin; Shepherd, Sam; Lisboa, Paulo
Subject: Approval of Application to Transfer from MPhil to PhD

Dear Kanchana,

I am very pleased to confirm that the Chair of the University's Research Degrees Committee has approved your application for transfer of registration from MPhil to PhD. This will be reported to RDC at its next scheduled meeting.

The Chair approved the application on behalf of RDC subject to the Independent Assessor's comments. These have been forwarded to your Director of Studies to enable you to receive feedback on your application.

Congratulations on your successful transfer from MPhil to PhD.

Kind regards,

LIVERPOOL JOHN MOORES UNIVERSITY

Jo McKeon
Research Student Support Officer,
Graduate School, Academic Registry,
1st Floor, Aquinas Building, Maryland Street, Liverpool L1 9DE
Tel: 0151 904 6375 Fax: 0151 904 6462
Web: http://www2.ljmu.ac.uk/ Email: j.m.mckeon@ljmu.ac.uk

How to find us

Please enter the building via the Aldham Robarts Library, go up to the first floor by the lift or the stairs and across the walkway into the Aquinas Wing. For further details on how to find us please use the following link: https://www.ljmu.ac.uk/contact-us/find-us

Please note that the Graduate School now operates a drop-in service between the hours of 2pm and 4pm on weekdays. Outside of these hours staff are available by appointment either in person or for a telephone consultation on weekdays. If you wish to arrange an appointment or telephone consultation please contact the relevant member of staff either by telephone or email with 'Appointment Request' in the subject bar.

Appendix 13 Chart of thesis summary

Dynamic proteome profiling of human muscle responses to high-intensity interval training

Gaps:
- Most research in –omic area reported protein abundance change
- Molecular basis is unknown esp. muscle protein turnover.
- Require further evidence to support fundamental knowledge
- Protein synthesis would appointed underlying mechanism which effect on muscle deterioration

Main Research Questions
RQ1: What changes occur in the skeletal muscle proteome associated with sedentary lifestyle and obesity?
RQ2: What is the dynamic proteome response of human muscle to high-intensity interval training.

Key messages:
1. 'chronic inflammatory in muscle of OB/T2DM; intermediate filament lead change in extracellular matrix which effected by mechanical transduction from change in intermediate filament.'
2. Muscle fibre remodeling regarding chronic obesity but can be improved by sustainable HIT.
3. Current findings support the above phenomena and reported HIT improve health status such as insulin sensitivity, fitness test, and at molecular level (improve cytoskeleton and muscle contraction proteins)

Chapter I: General Introduction

- Obesity and sedentary behaviour as risk factors for T2DM
- The contribution of skeletal muscle to whole body metabolism and insulin sensitivity
- Endurance exercise as a preventative countermeasure against T2DM risk

Chapter II: Evidence synthesis

If Obese is a sign of metabolic syndrome as a risk factor of T2DM and exercise is an effective medication, what are the hypothesis of underlying proteome in which associate with skeletal muscle decline and adaptation?

- Systematic review
- PRISMA, PICO, quality assessment (AMSTAR and the Delphi list)
- Meata-analysis

Main findings

- Greater proportion of muscle fibre type II under the effect of OB/T2DM e.g. over expression of MYH1 SERCA1 MYH2
- Chronic inflammatory lead OB/T2DM e.g. over expression of Tenascin C
- Endurance exercise (esp. HIT) improve muscle and mitochondrial protein e.g. NDUA8, NDUFA13, ATPB

Chapter III: Method development

If a protein becomes more or less abundance regarding pathophysiology among LE, OB, and T2DM individuals, what is the most effective method to investigate the underlying mechanism regarding this change?

Methods
- Human protein profile (LC-MS/MS)
- Mass Isotopomer Distribution Analysis (MIDA)
- Deuterium oxide incorporation in vivo labelling

Main findings

- Athlete's paradox (Greater abundance of FABPH)
- Up-regulation in muscle type I protein (SERCA2)
- FSR methods are accurate and can be used in human

Created by Kanchana Srisawat

Chapter IV: Cross-sectional analysis Of Obese vs Trained

What changes occur in the skeletal muscle proteome of humans that are OB, exhibit signs of the metabolic syndrome?

Method development from Chapter III	N = 8 (TR = 4, OB =4)	14 days deuterium labelled	4 muscle biopsies (at day 0, 4, 9, 14)	LC-MS/MS (protein profile and FSR measurement)

Main findings

- Greater abundance but reduce protein quality in CAH3, HSPB1, SERCA1, G3P, MYH1, KCRM
- Lesser both protein abundance and FSR in ACTN2
- Greater both abundance and FSR in ALDOA

Chapter V: Longitudinal Analysis of Muscle Proteome Responses to High Intensity Interval Training (HIT) in Obese Individuals

What changes occur in the skeletal muscle proteome of humans that partake in sustainable time –efficient high intensity interval exercise intervention?

Method development from Chapter III	N = 4 (OB only)	10-week-HIT (4-8 bouts)	LC-MS/MS (protein profile and FSR measurement)

Main findings

- Positive outcome in 5 proteins that impaired in OB in Chapter IV i.e. COX2, TPIS, ALDOA, ACTN2, MYH1, CAH3, G3P, AT2A1, KCRM
- Discover 2 proteins
  - Desmin – never report in shorter period of HIT
  - Alpha-actinin 3 – response to muscle development and mostly found in athlete

Hypothesis based on obesity outcome

- Muscle inflammation by reducing Muscle-Cytoskeleton-protein (low abundance but high FSR in **ACTN2**) indicate loss of protein homeostasis
- OB skeletal muscle dysfunction suggests defect of protein quality in ATPase proteome in which responsible for H+ in NADH electron transfer. (SERCA1 showed greater abundance but decreased FSR)
- Higher Reactive Oxygen Species (ROS) in OB (**COX2**) would associated with higher thermodynamic driving forces, which is a result from low metabolic turnover rate (low abundance and FSR in **COX2** protein).

Hypothesis based on 10-week-HIT outcome

- HIT remodelling muscle protein associated with intermediate filament and mitochondrial proteome, and increase high velocity in muscle detect from **ACTN3** which is found only during muscle development or resistance training interventions,
- Increase newly synthesis of mitochondrial protein and mitochondrial muscle protein remodelling is enhanced by endurance training. This suggests the interaction mechanism between mitochondrial and cytoskeletal protein (**AATM, COX2, ACTN3, DESM**)
- HIT promote glucose uptake or glycogen storage in muscles by increasing insulin sensitivity but reveals negative impact on carbohydrate synthesis (**TPIS**).