School of Medicine

The heterogeneity of the functional, metabolic, and molecular responses of skeletal muscle and clinical adaptations to exercise training in pre-clinical and human models

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Abstract

Introduction: Exercise training triggers numerous physiological adaptations that promote individual general health, which were found to be mediated through myokines. Exercise training stimulates myokines expression from skeletal muscle and released into the circulation. However, individuals respond very differently to exercise training i.e. high responders to low/non-responders. The aims of the thesis were i) to explore the impact of Fndc5/irisin (a myokine) on skeletal muscle metabolism and fat remodelling, ii) to investigate how trainability, acute and chronic exercise effect the release of myokines, and lastly, iii) to test the existence of low/non-responder to all exercise training modes and the impact on health benefits. **Methods:** Fndc5 was locally overexpressed in rat hind-limb via *in vivo* electroporation technique. Molecular analysis were performed in control and overexpressed muscle and also adipose tissue. Inbred animal model, low responder (LRT) and high responder (HRT) trainers, was used to investigate trainability on myokines' profiles, which were examined at baseline, following acute exercise, and after 3-weeks of training. Finally a novel human clinical intervention study was conducted wherein participants were studied following, 4-weeks of either endurance or resistance training, then crossed over following a 6-week washout. **Findings:** Fndc5 overexpression resulted in physiological levels of circulating irisin, which had minimal impact on skeletal metabolism and browning adipose tissue. Exercise training caused an acute elevation of myokines, while 3-weeks training increased/decreased myokines baseline concentrations and temporal responses area under the curve. In general, 'myokine' profiles were not able to clearly distinguish between LRT/HRT animals and explain their trainability. In the human cross-over study, the 6-weeks of detraining were enough to washout the enhanced mode specific adaptions. The adaptive responses varied from high to low/non responder with suggested mode preference for individual. Finally, the magnitude of fitness adaptive responses to training modes were not link to the extent of health benefits responses.

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List of Abbreviation

Acetyl-CoA Carboxylase	ACC	Endurance exercise training	EET
Adenosine triphosphate	ATP	Enzyme-linked immune assay	ELISA
Alkali-soluble protein	ASP	Eukaryotic initiation factor 4E	eIF4E
Amino acids	AA	Fascicle length	Lf
AMP-activated protein kinase	AMPK	Fat mass	FM
Anaerobic threshold	AT	Fat-free mass	FFM
Angiopoitien1	ANGPT1	Fibronectin type III domain containing 5	Fndc5
Are under the curve	AUC	Fibroblast growth factor-21	FGF21
Baseline	BL	Fluorodeoxyglucose	FDG
BAX pro-apoptosis factor	BAX	Fms related tyrosine kinase 1	FLT1
B cell lymphoma-	BCL-2	Fractalkine	FKN
Body mass index	BMI	Fractional synthetic rate	FSR
Carbon dioxide	CO_2	Glucose transporter 4	GLUT4
Cardiopulmonary exercise testing	CPET	Glucose-6-phosphate	G6P
Cell death activator	CIDEA	Glycogen synthase	GS
Citrate synthase	CS	Glycogen synthase 1	GYS1
Control	Ctrl	Glycogen phosphorylase	GP

Complementary DNA	cDNA	Glyceraldehyde 3-phosphate dehydrogenase	GAPDH
Creatine kinase	CK	Heat shock protein 60	HSP60
Cross section area	CSA	Hexokinase	HK
Cysteine-aspartic acid protease	caspase3	Hexokinase 2	HK2
Deuterium	D_2O	Myogenic regulatory factors	MRFs
Diastolic	DBP	High density lipoprotein	HDL
high aerobic response to training	HRT	In vivo electroporation	IVE
Dual-energy X-ray absorptiometry	DXA	Insulin-like growth factor-1	IGF-1
Electrocardiogram	ECG	Interleukin-6	IL-6
Intraperitoneal glucose tolerance test	IPGTT	Overexpression	OE
Kinase domain insert receptor	KDR	Oxygen	O_2
Lactate threshold	LT	Oxygen saturation	SpO_2
Leg blood flow	LBF	Pennation angle	PA
Light chain 3B	LC3B	Perchloric Acid	PCA
Lipid lipoprotein	LDL	Phosphocreatine	Pcr
Low response trainers	LRT	Phosphatidic acid	PA
Mammalian target of rapamycin	mTOR	Phospholipase D	PLD
Maximum voluntary contraction	MVC	Phosphoinositide 3-kinase	PI3K

Mean arterial blood pressure	MAP	Polyvinylidene difluoride membrane	PVDF
Metabolic syndrome	MS	Proline-rich Akt substrate of 40 kDa	PRAS40
Mechano-growth factor	MGF	Pyruvate Kinase	PK
Methylchloroformate	MCF	Rate of perceived exertion	RPE
Mile meter of mercury	mmHg	Rapamycin-insensitive companion	RICTOR
Mitochondrial transcription factor A	TFAM	Rectus femurs	RF
Muscle protein break down	MPB	Red blood cell	RBC
Muscle protein synthesis	MPS	Regulator-associated protein of mTOR	RAPTRO
Muscle ring finger 1	Murf-1	Regulated in development and DNA damage response1	REDD1
Muscle-tendon units	MTU	Respiratory exchange ratio	RER
Muscle thickness	MT	Ribosomal nucleic acid	RNA
Myogenin	MyoG	Succinate dehydrogenase enzyme A	SDHA
Revolutions per minute	RPM	Systolic	SBP
Ribosomal protein L13a	RPL13a	Tibialis Cranialis	TC
Myostatin	MSTN	Total body water	TBW
N-methoxycarbonyl methyl esters	MCME	Total creatine	Tcr
Non-communicable disease	NCDs	Tumour suppressor 2	TSC2
Nuclear respiratory factors	NRF	Two diabetes mellitus	T2DM

Oral glucose tolerance test	OGTT	Tyrosine-protein kinase receptor	TIE2
Uncoupling proteins	UCP	Voltage-dependent anion channel	VDAC
Vastus lateralis	VL	White adipose	WAT
Vastus medialis	VM	1-Repetion max	1-RM
Vascular endothelial growth factor A	VEGFA	2-Deoxy-D-glucose	2DG
Ventilation	VE	4E- Binding protein 1	4E-BP1
Ventilatory anaerobic threshold	VAT	70-kDa ribosomal protein S6 kinase	p70S6K

List of conference presentations and published abstracts

- **Chapter 3**. An oral presentation of “The effect of rat hind-limb Fndc5 overexpression upon skeletal muscle metabolism, in vivo” at 23rd annual Congress of the EUROPEAN COLLEGE of SPORT SCIENCE (ECSS), Dublin, 2018. Published abstract.

- **Chapter 4**. An oral presentation of “The impact of acute exercise upon myokine secretion in rat models outbred for either low or high aerobic capacity in response to treadmill run training” at Europhysiology conference, London, 2018. Published abstract.

- **Chapter 5**. A poster of “The plasticity of “exercise adaptability” in relation to resistance vs. endurance training” at physiology society conference, Aberdeen, 2019. Published abstract.

1 General introduction

1.1 Skeletal muscle physiology

Skeletal muscles are voluntary muscles that make up most of the muscle tissue. The 700 individual skeletal muscles creat the largest organ in the human body that comprises ~40% of total body mass. Skeletal muscle mainly consists of 75% water, 20% protein, and other elements as inorganic salts, mineral, fats, and carbohydrate. 50-75% of total body proteins are found in skeletal muscles, where 30-50% of whole-body protein turnover (synthesis and breakdown) occurs[1]. Skeletal muscles primary function are maintaining body posture and locomotion, where chemical energy converts to mechanical power and force. Body-posture is maintained by applying constant muscular tension. While muscle contraction pulls on the tendon to move skeleton bones and limbs. Skeletal muscles also provide support to soft tissues like in the abdominal wall and pelvic cavity to protect and shield internal organs from injury and guard the entrance and exits to digestive and urinary tract openings, which facilitate a voluntary control over swallowing, defecation, and urination. Finally, skeletal muscles participate in regulating whole-body metabolism[2,3].

Skeletal muscles store glucose in the form of glycogen, fat as intramuscular fat droplets, and amino acids (AA) as proteins. In fact, skeletal muscle is considered as the main reservoir for AAs in the human body, flowing against their concentration gradients to be released into the bloodstream in times of need. For instance, during starvation, AAs maintain the blood glucose level through gluconeogenesis or contribute to the synthesis of organ-specific proteins as needed from various tissues such as the heart, brain, and skin. Furthermore, skeletal muscles maintain core-body temperature through heat production and consumption of oxygen and fuel

during contraction[2,4]. Skeletal muscles and heart consume up to 30% of energy during rest, which could rise to 100% of the consumption, during exercise training[5].

Skeletal muscle is made off well-arranged muscle fibers (myofibers) that are surrounded by connective tissue. Each myofiber consists of the myofibril, which has thick (Myosin) and thin (Actin) filaments. The muscle fiber cytoplasm (sarcoplasm) contains a transverse tubular system (T tubule), sarcoplasmic reticulum, and mitochondria, which help in defining the muscle fiber type[3,6]. Mainly, there are two types of muscle fibers. Type I are slow-twitch fibers that primarily depend on oxidative metabolism for energy production. Consequently, they fire long and weak muscle contractions and resist fatigue. Whereas type II are fast-twitch fibers that mainly rely on non-oxidative (glycolytic) metabolism for energy production. They ignite short and powerful contractions, but are more susceptible to fatigue. Type II fibers are sub-classified into IIa, and IIx fibers. Type IIa has the ability to use oxidative and non-oxidative sources of energy[7,8].

1.1.1 Energy Metabolism

Skeletal muscles are designed to contract. In every single contraction, energy is required in the form of Adenosine triphosphate (ATP), which can be produced through three basic energy systems; ATP-phosphocreatine (ATP-PCr) system, glycolytic system, and oxidative system[9]. In the ATP-PCr system, energy is generated from two energetic molecules (ATP, and PCr). ATP hydrolysis generates energy and adenosine diphosphate (ADP). However, the intracellular storage of ATP is very limited. With full muscle activation, stored ATP lasts for 2 seconds. PCr is a high-energy phosphate molecule that reconstitutes ATP from ADP molecule in the presence of Creatine kinase (CK). This reversible reaction of CK creates a storing

system that is 10 times more efficient than stored ATP. Nonetheless , this system only supports high intensity exercise for a short time (10-20 seconds)[10].

$(Pcr + ADP \leftrightarrow Cr + ATP)$

The glycolytic system depends on the anaerobic production of ATP from glucose molecules with absence of oxygen. The glucose may be derived from glycogen storage or from plasma glucose. This energy system generates small amount of ATP rapidly, maintaining muscle contraction for a couple of minutes. However, its ends product (lactate, H^+) lowers the intracellular and blood pH that alongside other metabolites accumulation impair enzyme activity. Consequently, it then impairs ATP production and muscle function, known as muscle fatigue[1].

The oxidative energy system relies on the availability of oxygen and mitochondrial content since oxidative phosphorylation occurs within the mitochondrial network. This energy system is characterized by low rate production of ATP that can be sustained for a long time, up to hours, by consuming free fatty acids as source for most of the ATP production[1]. Typically, carbohydrate as plasma glucose or intracellular glycogen, and fat as free fatty acids or intracellular triglycerides are the main sources of energy in skeletal muscle under most conditions. Although, proteins as AAs have a very limited contribution (5-10%) in rest and exercise. Shifting between sources is dependent upon the contraction intensity; low intensity would mainly use free fatty acids whereas high intensity initially depletes glycogen content[11].

1.1.2 Glucose metabolism

Since glucose is a source of energy during contraction, skeletal muscle facilitates its uptake and utilization which emphasizes skeletal muscle fundamental role in maintaining normal glycaemic (blood glucose) levels, in tandem with the liver and pancreatic β-cells (Insulin). Skeletal muscle, in addition to adipose, is the primary peripheral tissue that insulin targets. It is well known that in the postprandial state, insulin is secreted by the pancreas to lower plasma glucose level by enhancing its uptake and utilization in peripheral tissues, which accounts for ~90% of the disposal. Therefore, skeletal muscle is considered as important as both an insulin-dependent and non-insulin-dependent tissue for glucose uptake. In both situations, transportation across the cell membrane is a rate-limiting step for glucose metabolism in skeletal muscle[12].

Glucose transportation across the cell membrane is via a specific family of glucose transporter proteins (GLUT), which is a family of 13 members. GLUT4 is one of the most abundant in adipose, cardiac, and skeletal muscle. Insulin and muscle contraction stimulate GLUT4 translocation from the intracellular pool, the inactive form, to the plasma membrane, the active form, to facilitate glucose transportation across the cell membrane[13]. GLUT4 is critical in increasing glucose uptake into contracting skeletal muscle. Zisman et al (2000), found that genetic disruption of GLUT4 in mice reduced contraction-induced glucose uptake[14]. Whereas, contraction had a negligible effect on glucose uptake in GLUT4 knock out mice[15].

The intracellular glucose, glycogen, can be located in three distinctive sites in the muscle fibres. Small intramyofibrillar and intermyofibrillar depots exist inside the myofibril. While ~80% is stored between myofibrils, close to the sarcoplasmic reticulum and mitochondria[16]. Glycogen content is regulated by the balance between glycogen breakdown (Glycogenolysis)

and glycogen production (Glycogenesis). Glycogen is the main source of carbohydrate in skeletal muscle during intermediate and high-intensity activity. Glycogenolysis provides source of energy (glucose) for ATP resynthesizes, where the glycogen degradation rate is proportional to the level of physical stress intensity[17]. In fact, the amount of glycogen content could define the training ability[18]. After glycogen depletion during physical activity, glycogen replenishment is the main priority in a contracted muscle. Therefore, glucose transportation and glycogen synthesis increase dramatically, to fulfil the muscle requirement of glycogen, which could last for 48 hours post exercise[19]. The process of glycogen synthesis is under the enzymatic control of Glycogen synthase (GS) activity while glycogen phosphorylase (GP) controls glycogen breaking down. More of the regulatory molecules of glucose uptake and metabolism will be discussed in (section 1.2.5.1).

1.1.3 Protein metabolism

Skeletal muscle is the largest pool of protein, where 30-50% of whole body protein turnover occurs, with a rate of ~1.2-1.4 %/day (synthesis and breakdown are equivalent in weight maintaining individuals) in a healthy active individual. The muscle mass of the healthy individual is maintained by a dynamic equilibrium between the rate of muscle protein break down (MPB) and the rate of muscle protein synthesis (MPS). Net MPB overcomes the MPS in situations of muscle wasting as in fasting, bed rest, organ failure, and critical illness. On the other hand, net MPS exceeds MPB in muscle gain situations as in fed status and in response to exercise training[20]. The key mediator of the MPS signalling pathway is mechanistic target of rapamycin (mTOR)[21]. It's up and down stream would be discussed in more details with the molecular regulations of protein synthesis (section 1.2.5.2).

1.2 Physical activity as medicine

Non-infectious chronic diseases, i.e. non-communicable diseases (NCDs), are one of the top ten causes of death in the world (WHO 2016). Cardiovascular diseases, type 2 diabetes (T2D), respiratory diseases, and osteoarthritis are the most common chronic diseases. In the past, these diseases were associated with aging and were more common in elderly people. Recently, they have become more prevalent within younger generations. The early onset of chronic diseases is probably a result of the shift in lifestyle, excess energy intake and more predominantly physical inactivity[22]. Low physical activity in middle-age women (less than one hour of exercise per week) increased the rate of mortality by 52% in comparison to physically active women[23]. In contrast, studies have shown that increased physical activity and fitness levels in both men and women reduced the risk of death by 20-35%, due to improvements in cardiovascular and respiratory systems[24,25]. The beneficial effects of exercise training on preventing and treating diseases are undeniable, observations from randomized trials have reported that regular physical activity contributed significantly and positively to the treatment of chronic diseases[26].

Therefore, exercise training has been frequently prescribed as therapeutic treatment for patients with chronic obstructive pulmonary disease, hypertension, metabolic disorder (T2DM, obesity, and insulin resistance), muscle and bone disease, cancer, and depression[27]. Like any other therapeutic agent, dose (exercise intensity and duration), frequency (session per week), types must be taken into account. In addition to the rising phenomena of “high/low and non-responders” to exercise that will be discussed in detail in the heterogeneity of adaptive response to exercise (section 1.4).

1.2.1 Type of exercise training “endurance vs resistance”

Exercise training can be classified into two classes, endurance exercise training (EET), and resistance exercise training (RET). EET is structured to include prolonged aerobic activity like running and cycling. Where RET, as in weight training, is mainly structured to involve overcoming the resistive forces of an external load. The different natures of both exercise training modes lead to distinctive mode-specific adaptions. EET mainly leads to improvement in aerobic fitness, resulting from central cardiorespiratory improvement[28], and peripheral enhancement in muscular angiogenesis[29] and mitochondrial biogenesis[30]. While in contrast, RET primarily relates to muscle hypertrophy that consequently results in the enhancement in muscle strength[31,32].

The mode-specific adaptions for either type of exercise promote general health benefits on individuals. For instance, the improvement of exercise capacity (aerobic fitness) with EET decreases the mortality rate due to cardiorespiratory diseases[33]. Similarly, the gain in muscle mass and strength reverses/slows physical frailty in the elderly[34]. Alongside those beneficial effects of the primary mode adaptions, they have an impact on whole-body health by improving insulin sensitivity[34,35], lowering low-density lipoprotein (LDL)-cholesterol[36] and blood pressure[37]. Accordingly, participation in exercise training was promoted by the American College of Sports Medicine (ACSM) to help maintain a healthy life-style. They recommended individuals undertake ~150 min per week of EET and ~3 times a week of RET of moderate intensity[28].

In summary, any challenging physical task will initiate a series of integrated physiological changes involving all or most of the body systems/organs. The musculoskeletal system will contract and control movement, while cardiovascular and respiratory systems would help in

sustaining endurance, via prolonged substrate/O_2 delivery. Therefore, each of these physiological systems will undergo specific adaptations to enhance the body's efficiency and response to engaging in an exercise training intervention. However, cessation of exercise training will lead to detraining, which means losing the efficiency and capacity that was increased through training-induced adaptations. EET and RET initiate a variety of physiological responses in the musculoskeletal, cardiovascular, respiratory, endocrine, and immune systems[38]. It is well established that the primary adaption to EET is aerobic capacity and to RET are muscle mass and strength, nonetheless, both modes training shared health benefits adaptive response on body composition and metabolic health parameters. Next table summarises the adaptive outcomes of both EET and RET (Table 1.1).

Table 1.1 Summary of endurance exercise training (EET) and resistance exercise training (RET) adaptive responses. ↑ Increase, ↓ Decrease, ↔ Unchanged.

Adaptive outcome / Mode of training	EET	RET
Aerobic capacity (VO_2max)	↑[39,40]	↔[40]
Muscle mass	↔[41]	↑[41,42]
Muscle strength	↔[43]	↑[44,41,45]
Angiogenesis (Muscle)	↑[46]	↑[47,48]
Mitochondrial biogenesis (Muscle)	↑[49,50,51,52,53,54]	↔[55,56,57]
Body Fat (%)	↓[58,59]	↓[59,41]
Insulin resistance	↓[60]	↓[60,41]
Fasting insulin	↓[58]	↓[41]
Fasting glucose	↓[40,39,58]	↓[41]
Blood pressure	↓[61,62,63,40,39]	↓[36,63,64,41]
Resting Heart rate	↓[65]	↔[65]
HDL-cholesterol	↑[66,40]	↑[41]
LDL-cholesterol	↓[66,39]	↓[41]

1.2.2 Cardiorespiratory fitness

As the one of the most adaptive outcome to EET, it is important to understand the underlying features that drive changing in aerobic capacity. The main function of cardiovascular and respiratory systems is supplying the body with oxygen (O_2) and nutrients and removing carbon dioxide (CO_2) and metabolic waste. Muscle activity increases the oxygen demand primarily to the working muscle, where cardiopulmonary systems respond by enhancing the rate of blood flow and extent of tissue perfusion. Eventually, with a continued increase in the intensity of physical activity, cardiovascular and respiratory systems may reach to their maximum capacity. The oxygen uptake attained during maximal exercise intensity is defined as the maximal oxygen uptake (VO_2max, litres of O_2 consumed per unit time, and unit body mass)[67].

In exercise physiology, VO_2max is defined as the highest rate of oxygen uptake and utilization during severe/intense exercise. It is considered as a primary measure to demonstrate the cardiorespiratory (aerobic) fitness of an individual. For instance, the VO_2max in well-trained athletes (80 ml/kg/min) can be two times higher than in sedentary counterparts (35 ml/kg/min)[68]. A low-level VO_2max can also indicate the risk of premature mortality and susceptibility to metabolic and cardiovascular diseases[33].

Aerobic capacity is a multifactorial index, since O_2 delivery goes through series of physiological processes that may set limitations on VO_2max. This could be either at a central physiological level, as pulmonary diffusion capacity, cardiac output (CO), and O_2 carrying capacity or at a peripheral level as skeletal muscle vascular diffusion/perfusion and metabolism[69]. According to Fick equation, VO_2max = (SVmax × HRmax) × (CaO_2max - CvO_2max). Where maximal (limiting) values in the equation are for stroke volume (SV), heart rate (HR), arterial oxygen content (CaO_2), and mixed venous oxygen content (CvO_2). From the equation, VO_2max

depends on CO, which is the total volume of blood pumped by the heart / minute (SV × HR), and difference of oxygen content between arterial and mixed venous blood (A-V O_2). A reduction in one or more of HR, SV, and CaO_2, or increase in CvO_2 would compromise VO_2max[70], similarly increases in these may induce benefits in maximal aerobic capacity.

The difference in VO_2max between sedentary individuals and athletes is mainly accounted for the maximal CO. During exercise, HR and SV increase to meet the metabolic demand on the working muscle. CO raised from ~5 L/min at rest to ~20 L/min in young males[71], which reached ~35 L/min during maximal exercise in elite athletes[72]. The increase in CO during exercise is mainly a consequence of HR augmentation. Although, exercise training doesn't change the maximal HR, which indicates that the increased maximal CO is due to enhancement in SV. In aerobic-athletes with higher CO, left ventricular hypertrophy has been observed[73], which could help in explaining the expansion of left ventricular end-diastolic volume that induces the blood pumping capability. At rest, the CO is similar between trained and untrained individuals despite the greater SV. This because of a decrease in resting HR in trained individuals[74]. An expansion in blood volume also facilitates the increase in SV, which may also include an increase in plasma volume and red blood cell content[75].

Another factor may cause a limitation of VO_2max is the blood flow to working skeletal muscle. Exercise training changes dramatically the pattern of blood flow and more blood would be deliver to the active skeletal muscles and skin, which usually receive ~20% of CO during rest. This would be accomplished by elevating CO and redirecting the blood flow away from low demands area i.e. splanchnic organs, to high demand areas i.e. skeletal muscles. At the peripheral level, the expanded difference of A-V O_2 with increasing work rate indicates an elevation in O_2 extraction in skeletal muscle[76]. This is due to an expansion in capillary density

in skeletal muscles, which facilitates O_2 extraction across skeletal muscle and vascular bed[77]. More on the angiogenesis would be discussed in (section 1.2.5.4).

VO_2max for an individual was also suggested to be regulated by the mitochondrial content and activity of skeletal muscle. A linear relationship between muscle mitochondrial volume and VO_2max was suggested by Hoppeler (1990). Although, it appears that humans have an excess volume of mitochondria i.e. excess capacity, beyond that required to meet their VO_2max[78]. However, a study in rodents reached to the conclusion that mitochondrial impairment is not associated with training capacity and VO_2max, when endurance training improved mitochondrial content and function without impacting aerobic capacity[58]. Similarly, the 46% improvement of mitochondrial density of 6-weeks EET was not showing an influence on VO_2peak in human trials[54]. This draw a picture that mitochondrial capacity appears not to be a limiting factor for VO_2max but could affect other facets of exercise performance. The level of a defect in a mitochondrial myopathy patients was correlated to the level of exercise intolerance[81]. However, EET training was able to enhance the oxidative capacity and mitochondrial content[82], which led to other metabolic benefits i.e. increasing fat oxidation[83], reducing lactate production and consequently improving fatigue resistance[84]. All of which contributed in improving exercise performance. In section (1.2.5.3), the mitochondrial biogenesis would be discussed in details.

Cardiopulmonary exercise testing (CPET) is the most widely used system to measure aerobic capacity i.e. VO_2max. It utilises breath-by-breath analysis techniques to monitor O_2 utilization and CO_2 production during rest and then exercise to provide an accurate assessment of cardiopulmonary functional capacity[85]. During the CPET, VO_2max appears as a plateau in the diagram of exercise intensity against VO_2, which represents the disassociation between the

increments in exercise intensity with VO_2 as in (Fig 1.1). However, practically 50% of the participants, in a clinical setting, will not reach the plateau phase (VO_2max)[86]. Therefore, secondary criteria have been used to recognize VO_2max such as respiratory exchange ratio (RER), HRmax, and blood lactate concentration[87]. Due to these limitations, the use of VO_2peak was also suggested to be a more realistic index. VO_2peak is the average of VO_2 over 20-30 seconds, at expected maximal effort during the incremental exercise test[67].

During incremental exercise, the interaction between cardiovascular, respiratory, and peripheral metabolism is assessed from a number of CPET parameters. Ventilation (VE), VCO_2, VO_2, and HR are expected to increase in a linear pattern with the increase in working load until a set point, where the relationship starts to shift. Aerobic glycolysis is favourable when sufficient O_2 is supplied to working skeletal muscle. However, the body switches to anaerobic glycolysis at a set point when O_2 delivery is falling behind demand. The level of VO_2 at the switch point is defined as the anaerobic threshold (AT) index. Anaerobic glycolysis produces lactic acid and in skeletal muscle that diffuses into the circulation. The VO_2 level at accumulated lactate in blood is known as the lactate threshold (LT). Buffering lactate acidity through bicarbonate reaction would yield VCO_2, which consequently elevates VE to help expel excess VCO_2. Ventilatory anaerobic threshold (VAT) occurs when the VE exponentially increases relative to VO_2 increase, which in fact reflect AT (Fig 1.2). The RER is known as the ratio of VCO_2/ VO_2. It is measured by the gas exchange in the mouth-piece and indicates the metabolic fuel utilization. Increased RER usually results from an increase in lactic acid or hyperventilation. A peak of >1.1 in RER is a good sign of subjects achieving maximal effort. AT is widely used as a submaximal index of exercise capacity since achieving a true VO_2max is unlikely. AT in untrained individuals occurs at ~45-60% of VO_2max or VO2peaK[70,88]. In

contrast, it is significantly lower in heart failure patients[89], which highlights the role of AT in cardiorespiratory health.

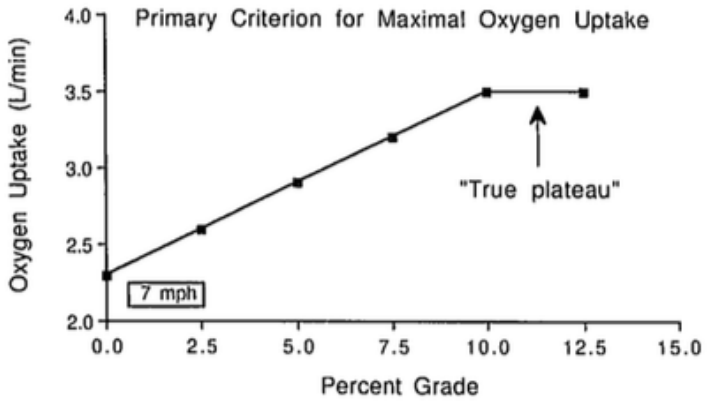

Figure 1.1 Theoretical representation of "true" VO_2max. Shows a plateau in VO_2 (oxygen uptake) as a percentage grade continues increase. Image from[86].

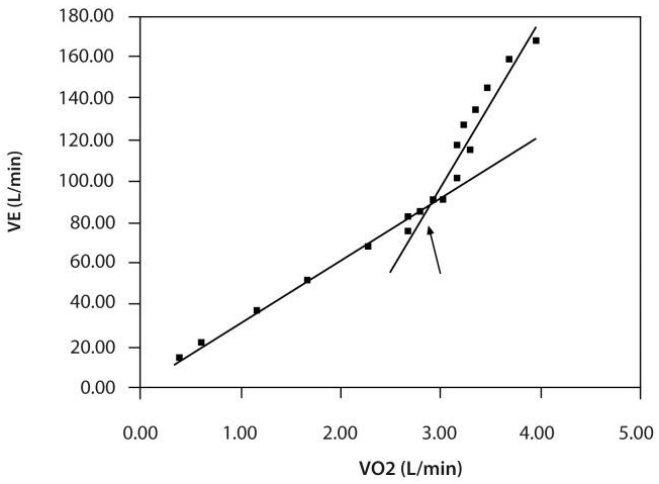

Figure 1.2 Determination of the anaerobic threshold in cardiopulmonary exercise test. Anaerobic threshold indicated by the arrow through a change in ventilation (VE) with increasing oxygen uptake (VO2). Image from[90].

1.2.3 Muscle mass and strength

Muscle mass and strength are important in many aspects of health and quality of life since skeletal muscles are primarily involved in many activities of daily life. Losing muscle mass and strength due to decrease in fiber size and number, which occurs with age (sarcopenia) or disease (cachexia), reduce the mobility of an individual. Such limitations in mobility would clearly affect daily activities and impair quality of life[91]. The importance of maintaining muscle mass (and function) in health is undeniable, and often underappreciated. As resting energy expenditure varies according to muscle mass, greater muscle mass reduces the risk of obesity[4]. In contrast, a decline in skeletal muscle mass increases the risk of cardiovascular and metabolic disease or even death in severe cases[92]. Therefore, low muscle mass can be used as an independent predictor of hospitalization, disability, and mortality[93]. . Muscle mass also has a remarkable role during illness. In acute stress situations i.e. sepsis, advanced cancer, and traumatic injury, the demand for AAs is higher than during the postabsorptive/fasted state, due to the requirement for the synthesis of new acute phase proteins e.g. immune and wound healing proteins[94]. Major factor of preventing muscle wasting with age or other muscle wasting conditions is physical activity/exercise training. Exercise training triggers an adaptive response in skeletal muscle that may modify its mass, architecture, and strength. The extent of those adaptations are driven by the exercise mode (EET or RET), intensity, duration, and also nutritional support[95]. However, gain in muscle mass and strength are manly driven by RET (Table 1.1)

It is generally accepted that the accumulation of muscle mass in response to acute bouts of RET during prolong intervention results from sustained periods where MPS is greater than MPB, which leads to muscle hypertrophy and characterized by an increase in fiber size and

cross-sectional area (CSA)[50]. The hypertrophic response will be affected by the intensity, duration, and mode of contraction. A study by Kumar et al (2012) found that MPS was maximally elevated with exercise intensities >60% of 1-repetition maximum (1-RM)[96]. However, exercising at high intensity is not a prerequisite for a hypertrophic response. As low intensity high volume exercise (to failure), is more effective in prolonging the duration and magnitude of increased MPS; even though a greater volume was required[50]. Both the nutritional and training status of an individual, in addition to intensity and volume, impact the time course of MPS in individuals. MPS is blunted during and immediately after an exercise session due to the competing energetic demands of muscle during exercise[97]. MPS increases from around 45 to 150 min post exercise, and remains acutely responsive to feeding up to ~48 hours after an exercise bout. However, the availability of protein/AA is important in promoting MPS over MPB, during this time[98,99]. On its own exercise is ultimately catabolic in nature.

The progressive effect of the RET and training status of individuals regulate the long-term temporal response of MPS. MPS in untrained individuals responds more (~50%) to exercise than trained individuals[100]. This is supported by chronic progressive RET studies which show that MPS increases over 3-weeks, then returns to baseline levels from 3-6 weeks, indicating that muscle gain is not linear[101] and doesn't simply continue uncontrolled i.e. muscle gains are limited. Despite the positive net protein balance that RET promotes, RET enhances MBP by up to 30-50%, lasting for ~24 hours[102], likely due to tissue remodelling. In the fasting situation, the negative net protein balance prior to exercise bout would be sustained, in the absence of AAs substrate availability, after the RET bout by the increased MPB thereby facilitating a catabolic effect on skeletal muscle[103]. Post-exercise nutrition and availability of proteins/AAs is extremely important to inhibit MPB and support MPS, and ensure the positive net balance,

resulting in a robust hypertrophic response. In addition, the feeding status would promote hyperinsulinemia suppressing MPB. As with MPS, the magnitude of MPB suppression is lower in trained individuals than untrained, which explains the hypertrophic response in trained people despite the decrease in MPS[100].

The usage of stable isotope approaches, gold standard methodologies, enlighten us with protein turnover rates and behaviour of MPS during different condition[104]. The technique depends on the presence of a heavy isotope (labelled) being incorporated into AA, then into protein. Replacing hydrogen with the natural occurring heavy isotope ^{2}H (Deuterium) e.g. ring-D_5-phenylalanine[105], the labelled Phe behaves in the same way as the endogenous Phe, which allows us to follow (trace) it's metabolism, and calculate its turnover rate i.e. incorporation into tissue protein. By sampling muscle tissue and measuring the amount of labelled Phe incorporated into muscle, using mass spectrometric techniques, it is possible to calculate the rate of muscle protein synthesis, when comparing that to the labelling of the free AA in the precursor pool of choice (i.e. intramuscular pool), permits calculation of the fractional synthesis rate (FSR)[106]. Fractional breakdown rate (FBR) can also be determined by applying principle of precursor-products as FSR but with reversing the pools. The movement of AA from free intercellular pool to protein-bound pool represents protein synthesis, while protein breakdown is the movement of AA from protein-bound to free intercellular if the AA is not synthesized in the tissue[107].

In addition to change in the protein turnover synthesis rate, exercise training (RET) impacts the muscle architecture, which describes the three-dimensional arrangement of fibres within muscle. The parallel or pennation of muscle fibers arrangement pattern define fascicle length (Lf), pennation angle (PA), and the CSA, which are major determinants of force production

and velocity[91]. The muscle architecture is malleable whereupon mechanical stimuli such as exercise training triggers a structural changes in healthy and unhealthy individuals, across all ages[108]. 4-5 weeks of RET can induce modest changes in PA and Lf in muscle architecture, which appear to precede the increase in muscle size in young healthy individuals[42], for example PA was higher in hypertrophied muscle[109]. However, the morphological adaptations depend on the contraction mode. Franchi, et al (2014) found that PA was greater with concentric (+12%) training than eccentric (+5%). While %change of Lf was higher after eccentric than concentric (+30% vs 5%)[31]. Ultrasound technique provides reliable, non-invasive approaches to assess muscle architecture i.e. the PA, Lf, and muscle thickness (MT) [31]. *Vastus lateralis* (VL) is considered a good representative of quadriceps muscle architecture, it is superficial, and so is easy to access, with clear structure and uniform architecture in comparison to other muscles in the quadriceps[110].

Assonating with change in muscle mass and size, muscle strength (force) depends on the number of sarcomere acting in parallel, which could be effected by muscle characteristics for instance, muscle length, contraction velocity, joint angle, and PA of arranged fibres. In addition, maximal force production is positively related to CSA. In fact, the improvement in muscle strength with RET, was related to enhanced of neural function (greater recruitment rate of discharge), increase in muscle CSA, change in muscle architecture, and possible, increase adaption in metabolites (ACSM 2009). This could indicate the clinical relevant of muscle strength. It's well known that muscle strength is required in all types of daily activities. Further, higher level of strength was linked with fewer cardiovascular disease incidences, lower risk of non-communicable disease, and better metabolic profile[28].

One of the most commonly used measures to describe RET intensity is the percentage of 1-repetition maximum (1-RM). 1-RM is the maximum external load that can be lifted for one complete repetition. Despite the 1-RM test reliability across populations[112], it is quite challenging to undertake, due to the need to limit the number of attempts so the subject is not fatigued. In addition, care is required to avoid injuries during the 1-RM test, especially in elderly[113]. Therefore, the use of multiple repetitions technique, as 5-RM, and 8-RM has been suggested to help better predict the 1-RM. Basing RET programmes on a percentage of 1-RM value, i.e. 60-80% is able to elicit improvements in muscle strength, and induce hypertrophy[112]. Further, moderate to higher intensity RET (65-85%) were showing greater impact on strength than lower intensities, even when increasing number of repetitions to match workload[114].

Maximal voluntary contraction (MVC) is also a common measure for muscle strength, which is performed on an isokinetic Dynamometer. MVC is a static measurement of isometric contraction of the limb (as in knee extension) which assesses different muscle-tendon units (MTU). MVC is recognized as the maximal exertion force that can be produced around joint. A reduction in MTU is indicative of low muscle strength[43]. 4-weeks of high intensity RET (volume & intensity) led to improvements in muscle strength as indicated by %change in 1-RM and MVC, additionally, a linear relation between changes in 1-RM and MVC was also found[115]. Periods as short as one week of RET are able to induced muscle strength[45]. In contrast to RET, EET led to no improvement in muscle strength nor MTUs, as runners had similar MTU properties to inactive individuals[43].

1.2.4 Metabolic health

Impairment in metabolic health as in obesity may lead to hypertension, diabetes, dyslipidaemia, and insulin resistance. Clustering two or more of these complications with obesity indicates a metabolic syndrome (MS/MetS). The incidence of MS is associated with a low levels of physical activity in young individuals[116] with strong evidence that exercise training decrease the risk of MS. For instance, 'The Studies of a Targeted Risk Reduction Intervention through Defined Exercise' (STRRIDE) reveal that both low amounts of moderate and high volumes of vigorous exercise training improve MS in comparison to control[117].

Since obesity has major role in metabolic health, studying body composition and its impact on individual health and fitness helps in understanding the adaptive response to exercise training. Body composition are divided into numerous compartments: fat-free mass (FFM), fat mass (FM), total body water (TBW), and bone mineral density (BMD). The two main components that associated with physical performance and health in individuals are the FM and FFM (muscle mass)[118]. An excessive accumulation of fat in adipose tissue to the level that causes deterioration in individual health is termed obesity (>30 in BMI). Obese individuals are at a greater risk of developing cardiovascular disease, T2DM, and non-fatal health conditions i.e. osteoarthritis, and infertility[119]. The distribution of excess fat deposition is important. Central adiposity, in particular, visceral fat, is strongly related to a higher risk of cardiovascular and T2DM disease. However, abdominal subcutaneous fat is also correlated to increased metabolic risk factors albeit to a lesser extent[120,121]. Low physical activity in a sedentary lifestyle is considered one of the main causes of a high percentage of body fat. Slentz et al (2005) showed that low physical activity and a sedentary lifestyle resulted in elevated visceral fat in overweight middle-aged adults. However, participation in low-intensity exercise training

inhibited the accumulation of visceral fat and participation in high intensity exercise significantly decreases both visceral and subcutaneous fat[122]. Trained athletes exhibit a lower percentage of body fat than the general population, with typical body fat in competitive runners of 4-10% in men and 7-15% in women. EET reduces the body fat composition by promoting lipolysis of fat tissue to release free fatty acids (FFA), which is main a source of energy in prolong physical activity. Skeletal muscle mitochondria oxidase FFA to produce energy and spare muscle glycogen. The enhancement in skeletal muscle mitochondria and capillaries density with EET improve FFA oxidation. In addition, to the increase in the protein expression of transportation proteins, carnitine and FFA binding proteins[83]. On the other side, RET increases the muscle mass in minimum of 1-2 kg, which subsequently elevates the resting metabolic rate[123] (7% increase[95]). As in (Table 1.1), both modes of training promote reduction in body fat[124,122].

Body composition can be assessed through many methods that vary in sensitivity, cost, and accessibility. For instance, body fat percentage can be measured simply by manual calipers, densitometry, bioelectrical impedance analysis, and dual-energy X-ray absorptiometry (DXA). Currently, the most accessible and widely used method to measure whole-body composition is DXA, providing estimates of BMD, body fat percentage/FM, and lean muscle mass/FFM. In addition, it provides regional analysis for the area of interest such as trunk and limbs. Such properties provide the researcher with the ability to study changes in body composition in response to chronic exercise and dietary interventions, albeit over relatively long periods (3-6 months). As well as, the ability to investigate the impact of aging and chronic diseases on body composition e.g sarcopenia, T2DM, and Obesity, and specifically lean mass or bone mineral content and density in osteoporosis[125].

Excessive body fat, obesity, elevates the risk of hyperlipidaemia (dyslipidaemia), which is common with MS as a result of dysregulation in lipid metabolism. These undesirable elevations of lipid profiles increase the risk of cardiovascular disease[116]. However, not all lipids are bad, increases in High-density lipoprotein (HDL) levels are deemed good. HDL is a protective factor against cardiovascular disease and helps in reducing blood glucose[126]. In contrast, an increase in low-density lipid lipoprotein (LDL) is bad as it associates with high total cholesterol, which had been linked to a greater incidence of coronary heart disease[127,128]. Exercise training improves lipid profile by reducing plasma level of triglyceride, total cholesterol, and LDL and increasing HDL[95]. This positive effect is recognized with both modes of training, i.e. EET and RET. A review by Gordon et al (2014) found that EET combined with weight loss resulted in reduced LDL, total cholesterol, and triglyceride and increased HDL levels[129]. Similar findings were observed for RET, by Wane et al (2012) when they indicated that RET may improve cardiovascular health through reductions in total cholesterol, triglyceride, and LDL and increase in HDL[95] (Table 1.1).

With the escalating prevalence of obesity, T2D and insulin resistance increase, which was suggested as a major link between low physical activity and MS. It is well established that insulin sensitivity is greater in trained and highly active subjects than sedentary people, and exercise training, either EET or RET, also improved insulin action[26] (Table 1.1).

Finally, the impact of exercise on the cardiovascular system could be detected on individual blood pressure (BP), which is the force that circulating blood applies to the walls of all blood vessels. Hypertension (high blood pressure) is a common complication and risk factor for cardiovascular disease i.e. coronary heart disease, stroke, and heart failure[130]. Exercise training improved systolic blood pressure (SBP), which is the peak of arterial BP at the

beginning of the cardiac cycle, and diastolic blood pressure (DBP), the pressure at the resting phase of the cardiac cycle. Improvement in DBP and SBP lead to a reduction of mean arterial blood pressure (MAP), represents average blood pressure in the artery over a full cardiac cycle[131]. However, the magnitude of exercise training on blood pressure improvement is higher with hypertensive individuals (SBP >140 and DBP >90 mmHg). An endurance, dynamic resistance, conventional resistance, and isometric resistance all decreased SBP and DBP as in (Table 1.1) However, EET was the most effective, especially with hypertensive males[63,65]. Another meta-analysis study on the exercise effect on resting HR reached to the conclusion that EET was also effective in lowering HR, but not RET[65].

1.2.5 Skeletal muscle molecular adaptation to exercise

The clinical impact of exercise training is a result from changes that occur at the molecular level within skeletal muscle. Studying these molecular changes will help in understanding the underlying mechanisms and identifying the key regulators of the health benefits induced by exercise training.

1.2.5.1 Molecular regulation of glucose uptake

GLUT4 activation is a rate limiting step for glycolysis in skeletal muscle since it is responsible of crossing the glucose into the cell to be used as fuel (glucose uptake). The signalling pathway of GLUT4 translocation which occurs during exercise (contraction) has been found to be distinct from insulin dependent GLUT4 pathway[132]. However, both pathways involve the activation of number of signalling molecules that involved in GLUT4 translocation i.e. TBC1D1 and TBC1D4 (also known as AS-160)[133]. During contraction, the translocation relies on feed-forward Ca^{2+} release from sarcoplasm reticulum or a feedback signal from Ca^{2+}-activated contraction, as consequence of mechanical stretch, metabolic, and energy stress[13,134].

Changes in Ca^{2+} and the AMP/ATP ratio activates AMP-activated protein kinase (AMPK), which phosphorylates AS-160 molecules to increase the GLUT4 translocation[135]. At rest and following insulin release, the activation of the insulin receptor activates phosphoinositide 3-kinase (PI3K) and its downstream protein kinase P (PKB also known as Akt) to facilitate the translocation through AS-160 phosphorylation[136,137]. Regular exercise training improves both insulin and contraction-stimulated glucose uptake (transport capacity). The repeated exercise bouts lead to a chronic increase in GLUT4 expression, which subsequently improve insulin sensitivity of working skeletal muscle. Insulin receptor substrate-1 (IRS-1) and IRS-2, are other molecules that regulates glucose uptake through binding to insulin tyrosine kinase to phosphorylate Akt. On obese and insulin resistance subjects, a reduction in phosphorylated-IRS-1^{Ser612} was reported, which could explain insulin resistance. Acute exercise training increase the phosphorylation of IRS-1, suggesting an activation of IRS-1 and improvement in insulin sensitivity. Another factor that have been proposed to improve insulin sensitivity is exercise-induced glycogen synthesis in skeletal muscle[138].

Glycogen synthesis is under the enzymatic control of Glycogen synthase (GS) activity, which requires de-phosphorylation in different sites[134]. The de-phosphorylation through protein phosphatase 1 (PP1) and the allosteric activation of glucose-6-phosphate (G6P), which is a product of the first step of glycolysis by Hexokinase (HK), are essentials for glycogen synthesis. A study by Bouskila et al (2010) found that knocked out mice of GS sensitivity to G6P expressed an 80% reduction in glycogen content[139]. Insulin would stimulate glycogen synthesis by increasing the glucose uptake and de-phosphorylation of GS through the Akt/PI3K pathway[140] (Fig 1.3). Exercise training is capable of enhancing the de-

phosphorylation of GS more than insulin, which means a higher activation in active skeletal muscle[141].

Glycogen synthesis with exercise is regulated predominantly by the cellular energy sensor AMPK. Acutely, AMPK inhibits glycogen synthesis by directly phosphorylating GS at Ser641 thereby providing glucose as substrate for ATP production, in tandem with the increase in GLUT4 translocation[142]. As a result, AMPK activation coupled with GLUT4 translocation will improve insulin sensitivity at the cellular level, increasing post-exercise glucose influx and subsequently intracellular G6P[143] independently of insulin. The increase in insulin sensitivity is correlated with the amount of glycogen depletion during exercise[134]. Glycogen particles also bind to AMPK β-subunits and inhibits its activity, which activate GS and subsequently promotes glycogen synthesis. In fact, a higher AMPK activity is linked to lower muscle glycogen content[144].

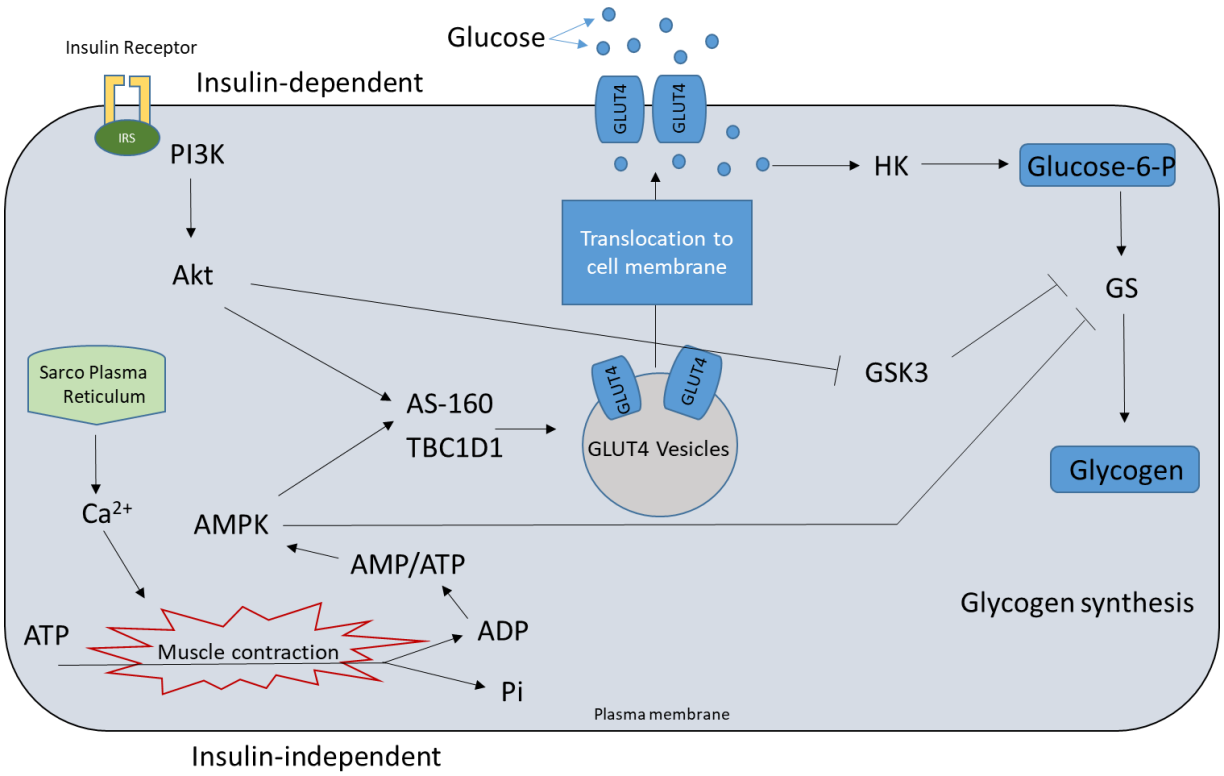

Figure 1.3 Molecular signalling for glucose uptake and glycogen synthesis. Phosphoinositide 3-kinases (PI3K), Adenosine tri-phosphate (ATP), Adenosine di-phosphate (ADP), Phopspho (Pi), Calcium (Ca^{2+}), hexokinase (HK), Glucose transport 4 (GLUT4), Glycogen synthase kinase 3 (GSK3), Glycogen synthase (GS), glucose-6-phosphate (Glucose-6-P).

1.2.5.2 Molecular regulation of muscle mass

The molecular regulation of protein synthesis is a highly integrated system involving two intracellular biological processes, translation efficiency and/or translation capacity. The increased rate of protein synthesis per unit amount of mRNA is a measure of the translation efficiency. While translation capacity (ribosomal biogenesis) is a measure of total ribosomal RNA content per unit of tissue[145]. mTORC, plays a central role in regulation protein synthesis to different stimuli, and can regulate both these processes. There are two mTORC complexes,

mTORC1 and mTORC2. TORC1 is identified by the presence of regulator-associated protein of mTOR (RAPTOR), while TORC2 is identified by the binding to rapamycin-insensitive companion (RICTOR)[146]. However, mTORC1 is more involved and sensitive to exercise and energy related stimuli than mTORC2. In general, mTOR is also stimulated by hormones and growth factors, mechanotransduction, amino acids, and the energetic state of the cell[147].

The activation of mTORC1 phosphorylates translation initiating factor substrates i.e. 70-p70S6K and 4E-BP1. The active form of p70S6K in turn phosphorylates ribosomal S6 to up-regulate mRNA translation initiation. Whereas, the phosphorylation of 4E-BP1 disassociates its binding to eukaryotic translation initiation factors 4E (eIF4E). The free eIF4E, the amount of which is correlated to phosphorylated 4E-BP1, binds instead to eIF4A and eIF4G to form the multi-subunits complex eIF4F, which is key regulator of translation initiation, along with the formation of eIF3F scaffolding which recruits the 48S pre-initiation complex[21,146]. Insulin-like-growth factor-1 (IGF-1) or splice variant like mechano-growth factor (MGF) production both stimulate mTORC1 via canonical pathway IGF1-PI3K-Akt/PKB-mTOR. Additionally, phosphatidic acid (PA)/ phospholipase D (PLD) are lipid second messengers which also target mTORC1 activation as part of an intrinsic signalling pathway[148] as illustrated in (Fig 1.4).

On the other side, the breakdown of protein within cells into smaller peptides is mainly regulated though the ubiquitin-proteasome system (UPS), involving the 26S proteasome. Four ubiquitin molecules are required to label the targeted protein before the 26S proteasome is activated, this is an ATP-dependent process controlled by three enzymes, Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase enzyme (E3)[149,150]. RET induces an adaptive response, involving an increase in mRNA of UPS molecules as E3, Murf-1, and MAFBx/Atrogin-1 along with their upstream transcription factor FOXO1[151]. Murf-1 shows a

temporal mode of expression peaking ~1-2 hours post-exercise and returning to baseline around 8 hours. In line with decreases in MPB observed following long term RET (10-weeks), at which time the increase in activity of the post-exercise UPS becomes diminished[152].

While proteolysis following RET relies on the UPS mainly, following EET breakdown is controlled by combination of UPS and autophagy-lysosomal pathways. With Ultra-EET increases in mRNA expression of Murf-1 and Atrogin-1 are observed, in addition to increases in key factors of autophagy i.e. ATG4B, Cathepsin L, and LC3B[153]. However, increased energy demand and metabolic stress also have a central role in the increasing autophagy genes. As demonstrated in a rat study that reported a higher autophagy gene expression in fasted rats than fed, which was mediated through AMPK[50].

1.2.5.3 Mitochondrial biogenesis

The mitochondria are dynamic organelles that have a central role in skeletal muscle metabolic plasticity and flexibility; where mitochondrial content is regulated by the functional energy requirements of the tissue. Mitochondria adapt to the new demands via processes of biogenesis, mitophagy, fission and fusion. So far the most studied feature with physical activity is the mitochondrial biogenesis[154], which the process of growth and division of pre-existing mitochondria. The biogenesis increases not only the number of mitochondria but also enhances its size and mass[155], which can evaluated by the mitochondrial content (volume density). There are different approaches to estimate mitochondrial content (density). Electron microscopic is the golden technique for direct estimation of morphological organelle volume in relation to the cellular volume. However, indirect measurements, relying on the estimate activity of typical marker enzyme such as citrate synthase (CS) or the expression of mitochondrial protein as cytochrome-c, can also assess the mitochondrial content[156]. A study

by Larsen et al (2012) in evaluating the best biomarkers for mitochondrial content had listed CS activity as the second best choice after Cardiolipin[157].

Exercise training promotes mitochondrial adaptation relying on its type, intensity, frequency, and duration[158]. The enchantment in mitochondrial proteins (enzymes, and electron transport system), respiratory capacity[53], and density[54] are more predominant to EET. Different modalities of EET such as walking, running, and swimming increase mixed MPS as a consequence of increases in sarcoplasmic and mitochondrial MPS instead of myofibrillar protein, as with RET[50]. In fact, mitochondrial hypertrophy have been suggested as the primary reason of exercise-induced mitochondrial density[49]. Despite the unchanged mitochondrial content with RET (Table 1.1), couple of study reported increase in mitochondrial function[56,57].

Exercise training triggers the mitochondrial biogenesis via multiple molecular events, which starts with signalling kinase that triggers the biogenesis and ends with incorporation of mitochondrial and nuclear gene products to expand organelle[155]. Mitochondrial biogenesis is a complicated process that needs the coordination between expression and assembly of ~1500 proteins of nuclear and mitochondrial genomes[159]. The process is crucially regulated by PGC-1α, which co-activates and enhances the expression of several transcriptional factors that stimulate nuclear-encoded mitochondrial genes[160]. In addition, PGC-1α indirectly, regulates the mitochondrial DNA (mtDNA) transcription by activating nuclear receptor factor 1 (NRF1), co-activator that increases the expression of mitochondrial transcription factor A (TFAM)[161]. The upstream activators are factors that are sensitive to the mechanical stress and energy-depletion imposed by prolonged exercise. P38 MAPK (the mechanical stress factor) is thought to be activated through the elevation of cystolic Ca^{2+} following muscle contraction and to regulate exercise related PGC-1α expression and activation[162]. AMPK is implicated as

an energetic stress factor that also regulates exercise related PGC-1α. The depletion of ATP and increase in local ADP and AMP leads to AMPK activation, which is critical for exercise capacity and mitochondrial dysfunction. Exercise-induced AMPK activation is linked to increased PGC-1α protein[161]. Another candidate of regulating exercise related increases in PGC-1α is P53, known as tumour suppressor gene. Genetic deletion of P53 reduces exercise capacity and oxidative phosphorylation in skeletal muscle, via de-activation of TFAM and mtDNA transcription[163] as in (Fig 1.4).

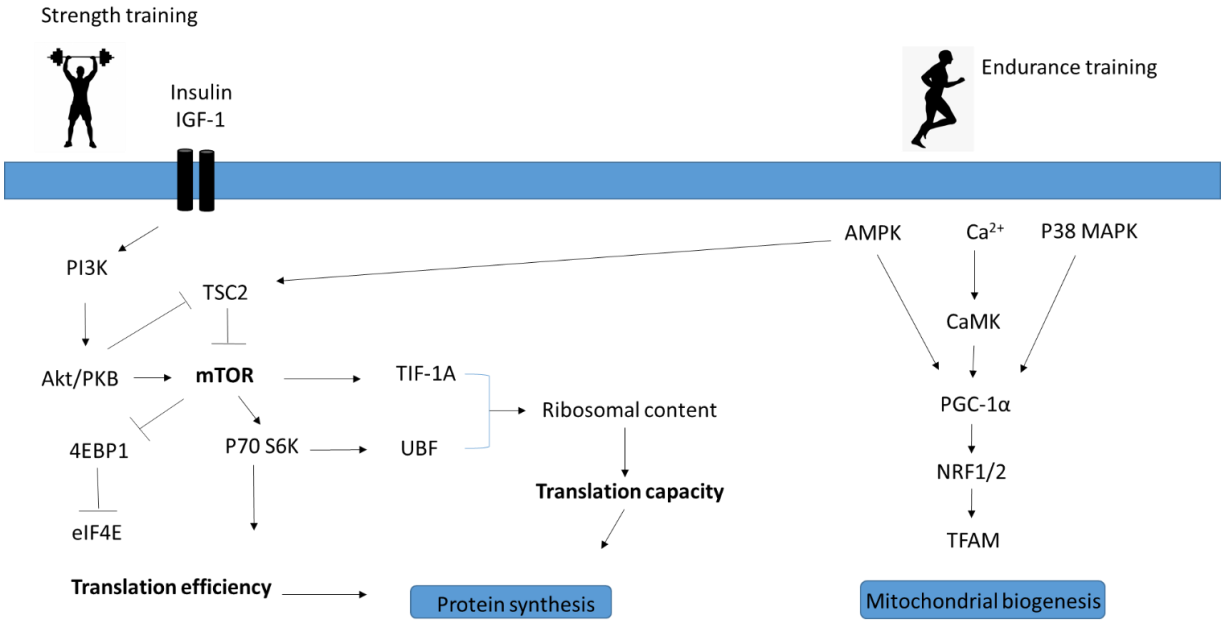

Figure 1.4 Molecular signalling for muscle gain and mitochondrial biogenesis. Phosphoinositide 3-kinases (PI3K), protein kinase B (PKB), mammalian target of Rapamycine (mTOR), tumour suppressor 2 (TSC2), eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), Eukaryotic initiation factor 4E (eIF4E), 70-kDa ribosomal protein S6 kinase (P70 S6K). Upstream binding factor (UBF), 5-AMP-activated protein kinase (AMPK), P-P38 MAPK, Calcium (Ca^{2+}), Ca^{2+}/calmodulin-dependent protein kinase (CaMK). Peroxisome proliferator-activated receptor-gamma co-activator-1-alpha (PGC-1α), nuclear respiratory factors (NRF-1/2), and mitochondrial transcription factor A (TFAM).

1.2.5.4 Angiogenesis

The aerobic capacity was suggested to be limited by volume density of the peripheral blood vessels in skeletal muscle with increase of oxygen demand[164]. Therefore, the impact of exercise training on the processes of forming a new blood vessels from pre-existing vascular network, angiogenesis, was investigated as an adaptive response to improve physical performance. The remodelling and expansion of existing network during angiogenesis requires mature activated endothelial cells to degrade from basement membrane, migrate, and proliferate to form new capillaries[165].

The angiogenesis is under the balance of endogenous activators e.g. vascular endothelia growth factor (VEGF) and inhibitory factors as Agiostatin. Blood vessels growth would be favoured if the activators level exceeds the inhibitors. In vis versa, the process stops if the inhibitors transcend the activators, which is predominant situation under most of the physiological condition[166]. The remarkable plasticity and dynamic of skeletal muscle vascular bed allow for expanding and regression according to the stimulus. The metabolic stress (hypoxia, low pH, and hypocalcaemia), physical stress (pressure), and inflammation produce pro-angiogenic factors to promote angiogenesis[165]. It is well established that exercise training enhances the VEGF expression and capillaries density (capillary to fiber ratio) as an adaptive response to local mechanical and metabolic stress in working skeletal muscle with both modes of training (Table 1.1)[46,47,48,167]. Conversely, detraining or reduction in muscle contractile activity leads to capillary regression[168].

At molecular level, angiogenesis is consider as a complex and highly regulated physiological process that relies on numerous factors, “it requires a complete programmatic orchestration”[169], ~30 pro-angiogenic (on switchers) and anti-angiogenic (off switchers). One

of the most studied pro-angiogenic factors is VEGF family, comprising 7 factors (VEGF A to F, and PIGF). They act through specific tyrosine kinase receptors i.e. VEGFR-1 and VEGFR-2[165]. VEGF is the predominant regulator of basal muscle capillarization and exercise-induced angiogenesis[170,171], via the regulation of the vascular permeability. A single exercise bout increased VEGF mRNA immediately in both animals[172] and humans[173]. Further, exercise training induces the gene expression of VEGFR-1 (FLT1), though the impact on VEGFR-2 (KDR) was not determined[174].

Other factors that are involved in angiogenesis are the angiopoietins, angiopoietin-1 (ANGPT1) and angiopoietin-2 (ANGPT2). They are protein growth factors that stimulate angiogenesis with high selectivity for endothelial cells[165]. ANGPT1 is a receptor agonist that stabilizes existing vasculature, whereas ANGPT2 is a receptor antagonist that destabilizes vasculature and facilitates new vessel growth (capillary sprouting)[174]. Both angiopoietins act through same receptor (Tie-2). Therefore, changes in ANGPT2/ANGPT1 ratio have been used as indicators of active angiogenesis (proangiogenic) in the presence of VEGF[175]. 2 hours after acute treadmill exercise with ischemia, mRNA ANGPT2 and the ANGPT2/ANGPT1 ratio have been demonstrated to be increased, with no change in ANGPT1 or Tie-2 gene expression[174]. However, chronic (6-weeks) aerobic exercise training has been associated with increased gene expression of ANGPT1 and Tie-2 on skeletal muscle[176]. Suggesting the role of training duration (acute vs chronic) on angiogenesis factors.

1.3 Skeletal muscle as an endocrine organ, “role in adaption”

After introducing the skeletal muscle physiology and physical activity (exercise training) impact on fitness, general health, and skeletal muscle regulatory molecules, it is time to explore a new aspect of skeletal muscle, which may help in understanding the force behind exercise adaptive responses. In addition to maintaining body posture and facilitating locomotion/physical activity, and providing substrate for energy production and other metabolic processes that impact on whole-body energy metabolism, skeletal muscle has, more recently been considered to be an endocrine organ. Skeletal muscle is known to facilitate this by secreting specific “peptide, cytokines” that communicate systemically with remote tissues and organs e.g. adipose, liver, brain, bone and pancreas[177]. The hypothesis that skeletal muscle possesses hormonal factors that would be released in response to muscle contraction and glucose demand, was in fact proposed more than 50 years ago by Goldstein[178]. These cytokines were first named as “the work factors”, “work stimulus”, and “exercise factors”. However, it was not until the new millennium, that researchers confirmed that the cytokine (IL-6) was actively secreted from contracting human skeletal muscle into the blood circulation and that its metabolic role was to increase hepatic glucose production[179]. Skeletal muscle, and myoblasts and myocytes, were then identified as highly secretory cells, with hundreds of secretory factors being identified. Later, those cytokines were termed “myokines”[180,181].

1.3.1 Myokines

Myokines are defined as cytokines or peptides that are expressed, produced, and released by skeletal muscle into the blood circulation to exert paracrine or endocrine effect on other cells, tissue, and organs[182]. The autocrine effect of myokines in the skeletal muscle was later included in this definition[183]. Since then, the list of putative myokines has grown continuously, and skeletal muscle has been shown to produce several myokines i.e. IL-6, IL-15, BDNF, LIF, FGF21, FSTL-1, SPARC, Irisin, and Myostatin. Many of these myokines are contraction induced factors, being released in response to exercise then promoting physiological adaptions on both skeletal muscle through autocrine/paracrine effects and remote tissues in an endocrine manner[184,185]. Myokines influence muscle metabolism by stimulating glucose uptake, glycogen synthesis, lipolysis, and mitochondrial biogenesis. In addition to anti-inflammatory and anti-oxidative effects, these myokines are also believed to stimulate muscle hypertrophy, angiogenesis, and improve insulin sensitivity. Myokines are also known to enhance browning and lipolysis in adipose tissue, increase hepatic glucose production, pancreatic insulin release, promote bone formation, improve cognitive ability, and increase revascularization[181,186].

1.3.1.1 FGF21

Fibroblast growth factor-21 (FGF21) is an endocrine hormone, of the FGF family, known to modulate cell growth, proliferation, and metabolism[187]. FGF21 is predominantly expressed in liver, although has been identified in adipose tissue, pancreases, brain, heart, gastrointestinal tract, and finally skeletal muscle[188]. It was consequently considered a myokine when hyperinsulinemia elevated FGF21 expression in human skeletal muscle and Akt overexpression in C2C12 increased FGF21 secretion[180,189]. Later, this idea was further

supported by data showing an acute increase (~100%) in circulating FGF21 after exercise in humans and mice[190]. 2-weeks of endurance training led to increase in FGF21 serum level in human[191]. However, 3-weeks of sprint interval training[192] and 3 months of combined resistance training in obese women[193] resulted in decreased serum levels of FGF21. Despite the variability in FGF21 plasma levels, data suggest that exercise training (endurance) would enhance FGF21 acutely, but lower its baseline chronically.

Numerous studies have observed that FGF21 plays a role in the regulation of glucose and lipid metabolism. Administration of FGF21 to diabetic rodents decreased plasma glucose and triglycerides, and improved insulin sensitivity in vivo[194]. In liver transgenic mice, FGF21 offered protection from diet-induced obesity[180], which raised the possibility of FGF21 as a therapeutic agent preventing obesity and diabetes. In fact, circulating FGF21 was found to be higher in metabolic diseases such as T2D and obesity[195], which could be a compensating action. Elevated FGF21 has also been reported in mitochondrial myopathy, and metabolic stress condition, therefore, FGF21 is considered a stress hormone[196].

1.3.1.2 SPARC

Secreted protein acidic and rich in cysteine (SPARC) is a glycoprotein. First identified in bone as osteonectin, then subsequently recognized in a skeletal muscle (myoblasts and myotubes), where it was increased during development and regeneration[186]. It is a matricellular protein that facilitates the interaction between cells and extracellular proteins, but has no obvious structural function[189]. Norheim et al (2011), recognized SPARC as myokine when they found that resistance exercise increased the mRNA expression of SPARC In human skeletal muscle, after identifying the SPARC as one of the secreted protein in the media of cultured primary human skeletal muscle cells[197].This was supported later by Aoi et al (2013) when they found

that a single bout of exercise increased protein levels of SPARC, in all muscle fractions, although it was enriched in the plasma membrane of mice[198]. In addition, the plasma levels increased immediately up to 50-100% after exercise for 30 min at 70% of their VO_2max and then returned to baseline, in both mice and humans. Interestingly, 4-weeks of training did not alter the baseline levels of SPARC, but enhanced the exercise-induced response[198].

SPARC elicited an autocrine effect on skeletal muscle, by facilitating muscle remodelling through binding specifically to actin[199], and furthermore enhancing glucose uptake through interaction with AMPK and GLUT4 translocation[200]. SPARC also has endocrine effects on adipocyte turnover, inhibiting adipogenesis and reducing fat accumulation *in vivo*[201]. SPARC, initially known as osteonectin peptide, is positively associated with bone mineralization[202]. Further, SPARC has been demonstrated to initiate colon cell apoptosis and inhibit colon tumorigenesis[198]. Epidemiological studies reveal that fasting plasma SPARC is higher in T2D than healthy control groups. In addition, plasma SPARC was correlated positively with BMI, fasting insulin, HOMA-IR, triglyceride, and percentage of fat[203,204].

1.3.1.3 Osteocrine/Musclin

A member of the natriuretic peptide family with molecular weight of 11.4 kDa. In 2003, It was first identified as bone-secreted protein, Osteocrine, which modulates bone formation and metabolism[205]. A year later, same peptide was independently identified as a novel myokine, musclin. Skeletal muscle expresses the musclin gene, Osten, and its protein. The mRNA expression of musclin is regulated by both nutritional and hormonal interventions. For instance, its expression on skeletal muscle is affected by feeding status, decreases during fasting and increases after a meal. The skeletal muscle gene expression is also mediated by insulin via the PI3K pathway[206,207], and can also be modulated by palmitate via PERK in C2C12

myotubes[208]. However, musclin negatively feeds back on insulin action. Nishizawa et al (2004) reported that recombinant musclin treatment inhibited insulin effects on glucose uptake and glycogen synthesis in myocytes[206], measured using ^{3}H-2-DG, and also decreased the expression of GLUT4[209]. The inhibition of insulin-mediated glucose uptake by musclin treatment occurred through blocking the Akt signalling pathway[210]. Data shows that musclin induces insulin-resistance that predisposes to T2D. The gene expression of musclin was augmented in mice with insulin-resistance[206], and rats fed a high fat diet also show increased plasma and protein levels in skeletal muscle[209]. Circulating plasma of musclin is higher in untreated TD2, and overweight/obese subjects compared to lean individuals. Musclin was also positively correlated to fasting glucose, serum insulin, HOMA-IR, and triglyceride, which may indicate a role in obesity and T2D[209,211].

Exercise training increased musclin protein expression in *gastrocnemius* muscle and increased mRNA expression in *tibialis anterior* muscle, but not in other muscles in the lower limb. Increased musclin was also seen in plasma of exercised mice in comparison to controls. Musclin was reported to be an exercise tolerance factor, since Osten-knockout mice showed lower aerobic capacity, they ran for less time, distance, and their overall workload was lower. Interestingly, the training capacity was restored with musclin administration via an osmotic pump. They suggested the difference in aerobic exercise was due to the level of ANP-dependent PGC-1α regulation of mitochondrial biogenesis[212]. A contrary finding revealed that swim exercise training decreased musclin expression in rats on a high fat diet in comparison to a non-exercised high fat diet control group. The decrease in musclin was following the exercise-induced insulin sensitivity[213].

1.3.1.4 Fractalkine

Fractalkine (FKN) is a chemokine, also known as CX3CLI or neurotactin. It is a unique chemokine in having two chemical forms, membrane-bound or soluble. FKN is expressed in neurons, endothelial cell, hepatocytes, adipocyte, vascular smooth muscle, and skeletal muscle. CX3CR1 is a G-protein-couple receptor for FKN signalling, which is expressed on monocyte, T-cells, and NK cells. As a membrane-bound protein, FKN, promotes cell to cell adhesion in leukocytes, which aids vascular recruitment, activation, and survival. In hepatocytes, FKN is an anti-inflammatory and suppresses tissue fibrosis. While in brain, it regulates the interaction between neurons and glial cells. The soluble extra-cellular form of FKN is a result of proteolytic cleavage of the membrane bound FKN that may act in both a paracrine and autocrine fashion[214–216]. FKN may also exert an endocrine effect[217].

FKN Knockout mice showed a defect of glucose-induced insulin secretion, which was restored with FKN administration. *In vitro* studies reveal that FKN controls the intracellular Ca^{2+} in islets in the pancreas to impact insulin secretion[215]. Circulating levels of FKN are positively associated with T2D, obesity, and insulin-resistance[214], which is more likely because of the high level of insulin in these situations. In an autocrine fashion, FKN exerts a similar action as in the islets by protecting the myotubes from insulin-resistance that induced through TNFα, via blocking the NF_KB signalling pathway[217]**.**

A single bout of exercise increases both mRNA and protein expression of FKN in skeletal muscle[218,219], and plasma concentrations are increased in humans following 1 hour cycling, reaching a peak after 3 hours[216]. In addition to exercise, TNF-α infusion, which increase in the circulation after vigorous exercise[220], elevated FKN in plasma after 4 hours[219]. However, muscle may not be the primary source of circulating FKN, since FKN from femoral vein of an

exercised leg was not different from the rested leg[219]. Furthermore, FKN was not detected in conditioned media from myotubes treated with TNF-α[217].

1.3.1.5 BDNF

Brain-derived neurotrophic factor, is a well-known member of neurotrophin family, a group of structurally related growth factors that modulate various neuronal processes. BDNF acts through the tropomyosin-related kinase B receptor, which is expressed abundantly in brain[185]. BDNF is also expressed in skeletal muscle, and its expression is induced by exercise and electrical stimulation[181]. Intense contraction of normal and diabetic rats induced BDNF expression in the soleus muscle[221]. Physical activity seems to be essential for the induction of BDNF expression in skeletal muscle. Gomez-Pinilla et al (2002), showed that exercise-induced mRNA and protein expression of BDNF in soleus muscle, while paralysis reduced the levels of expression[222]. Meta-analysis studies concluded that acute aerobic[223] or resistance[224] training are able to elevate BDNF plasma level from 1.5-2 fold change. However, chronic increase was only seen with aerobic training (60%)[186,224,225]. Although, it still not clear if this is muscle-derived. Matthews et al (2009) found that exercise upregulated mRNA and protein expression in skeletal muscle, but not muscle-derived BDNF secretion[226]. Circulating BDNF may primarily (70-80%) derive from brain after exercise[227].

Elevated BDNF mRNA and protein in muscle indicate the potential for a paracrine/autocrine effect in regulating skeletal muscle metabolism. BDNF phosphorylates AMPK and ACC and enhances fat oxidation and glucose utilization *in vivo* and *in vitro*[228]. In addition, BDNF improves insulin-resistance[186], and chronic treatment decreases circulating free fatty acids and total cholesterol in several diabetic animal models[229]. However, obese and non-obese subjects show similar exercise-induced plasma levels of BDNF, after acute aerobic exercise,

which is not linked to elevated glucose nor fatty acids[230]. In central nervous system, BDNF plays role in neuron survival and growth, and in the hypothalamus, it is considered a key element in the control of body mass and energy metabolism. BDNF also enhances cognitive ability, especially learning and memory[187], therefore the importance of its role as a myokine requires further investigation.

1.3.1.6 IL-16

Interleukin-6 (IL-6) was the first cytokine to be identified in blood in response to contracting skeletal muscle. Initially classified as a pro-inflammatory cytokine, but its consistent appearance in the circulation following exercise training suggested an anti-inflammatory role. Indeed IL-6, has been shown to enhance the levels of anti-inflammatory factors such as IL-1ar and IL-10 and inhibit the production of pro-inflammatory factors such as TNFα and IL-1β[186,231]. In addition to skeletal muscle, IL-6 is produced by monocytes/macrophages, fibroblasts, and vascular endothelial cells. However, IL-6 is proto-typical myokine, which exerts its effect on both skeletal muscle in paracrine/autocrine fashion and on distal organs and tissues in endocrine fashion[182].

Locally, in skeletal muscle, IL-6 binds to its homodimer receptor gp130Rβ/IL-6Ra to activate AMPK, which enhances glycogenolysis and lipolysis[232]. Again through AMPK activation, IL-6 is suggested to increase GLUT4 expression and glucose uptake resulting in improved insulin sensitivity[233]. Moreover, IL-6 had been found to enhance insulin-dependent glucose uptake through the PI3K signalling pathway[187]. Further, IL-6 has been shown to increase protein synthesis and muscle growth[234].

The wider endocrine action of IL-6 has been reported in various tissues, such as adipose, liver, pancreas, gut, and immune cells. In adipose, IL-6 enhances lipolysis and fatty acid oxidation in humans. Through elevation of the exercise-induced UCP1, a role in browning white adipose tissue was suggested for IL-6[186]. In liver, IL-6 augments gluconeogenesis, glycogenolysis, and glucose release[187]. While in the gut and pancreas IL-6 enhances GLP1 and insulin secretion, and activates macrophage and apoptosis on tumour cells[202].

Consistently in literature it is demonstrated that muscle contraction and exercise increase the plasma levels of IL-6, and this increase may reach ~100 fold above baseline[235]. The peak IL-6 in plasma is at the end of the exercise training or shortly thereafter, and is followed by rapid decrease to pre-exercise levels[236]. Mode of training, Intensity, duration all determine the magnitude of IL-6 exercise-induced rise[235]. Other factors that believed to influence IL-6 concentrations post-exercise are the number of muscles utilised in the exercise training[181], since training only the upper limbs is insufficient to increase IL-6 above pre-exercise levels[237,238]. Furthermore, the IL-6 plasma response to exercise is determined by the pre-exercise glycogen content, which defines the cells energy status, the higher the glycogen the lower the circulating IL-6[239].

Basal circulating IL-6 concentrations are negatively correlated to the amount of physical activity, the higher the physical activity the lower the IL-6 plasma level[240,241]. This may explain the decrease in basal IL-6 plasma level observed after a training intervention, in the elderly[242], and obese subjects[243]. However, others have observed no change in basal levels[244,245]. The decrease of IL-6 after training intervention was accompanied by an increase in its receptor gene expression, suggesting an increased sensitivity of IL-6. Small increases in fasting rested IL-6 have been found in obesity, insulin resistance, T2D, and cardiovascular disease[235].

1.3.1.7 Fndc5/Irisin

Fibronectin type III domain containing 5 (Fndc5) is a type I transmembrane glycoprotein consisting of 212 AAs in humans and 209 in mice and rats[246]. It is coded through the Fndc5 gene and composed of a signal peptide, two fibronectin type III domains, and a membrane-bound hydrophobic C-terminus[247]. Fndc5 was first discovered in 2002, as a receptor on skeletal muscle, heart, and brain, which plays a role in the differentiation of myoblasts and neurons[248]. In 2012 Bostrom et al (2012) proposed for the first time that Fndc5 cleaves to secret irisin into the blood circulation. The irisin peptide is a proteolytic cleavage of 112 AAs with a molecular weight of 12 kDa[247]. The proposed main function of Fndc5/irisin is browning white adipose tissue by augmenting the overexpression of UCP-1 which increases non-shivering thermogenesis and energy expenditure in sub-cutaneous fat tissues[247].

When Fndc5/irisin was proposed as a protective agent against weight gain[247], this led to great excitement in the field with many researchers investigating the link between Fndc5/irisin and obesity, however it also led to a host of contradicting results. Some postulated that obesity would decrease plasma irisin level since irisin was low in obese animals, and following a high fat diet[249–251]. In addition to the reduction with obesity, Moreno-Navarrete et al (2013), found that irisin is negatively associated with BMI and fat % in a subgroup of non-diabetic subjects[252]. Similar negative associations with BMI were reported in healthy and T2D subjects[253]. However, in other animal studies there was no effect of induced obesity on Fndc5 gene[254] and protein expression or irisin plasma concentrations[255], which were further supported by similar finding in human studies[256,257]. The link to BMI was also questioned when Sanchis-Gomar et al (2014) reported no association between circulating irisin and BMI[258].

A number of researchers proposed that the increase of Fndc5/irisin in obesity, is a protective response to compensate for the abnormal metabolism and insulin sensitivity. Irisin concentration has been reported to be higher in obese compared to healthy and anorexic subjects[259,260]. In addition, irisin concentrations are positively correlated with BMI of healthy middle-aged women[261], subjects with a wide range of body weights[259], and in non-diabetic subjects[262,263]. The positive correlation was also noticed with body weight[259], fat mass, and waist circumference[263].

The debate continues around the relationship of plasma irisin to insulin resistance, with conflicting results. Some studies found that subjects with T2D had low plasma irisin, which indicated a negative correlation with insulin-resistance and suggested the possibility to use irisin as therapeutic agent[252,264,265]. However, similar positive correlations of irisin with fasting glucose and HOMA-IR in non-diabetic subjects (both sedentary, obese) has been found[266,267], suggesting the link may be more complicated than first thought. However, elevated level of irisin may also be due to the existence of irisin resistance (akin to insulin resistance) and irisin is trying to counterbalancing the risk of chronic disease in inactive-life style[266].

The effect of exercise on Fndc5/irisin expression and secretion is a controversial topic. Bostrom et al (2012) found that endurance exercise training for 3-weeks in mice and 10-weeks in human increased Fndc5 gene expression and plasma irisin (1.5-2 fold change)[247]. Huh et al (2012) however demonstrated that although exercise enhanced plasma irisin in humans acutely (18%), there was no change after 8-weeks of training[261]. Other studies in humans report an increase in Fndc5/irisin after training, but only in very active elderly[268] also in children with obesity, following a 1-year physical activity program[269]. Norheim et al (2014) found that although circulating irisin increased immediately after the first and last endurance exercise

bout (cycling) in control and pre-diabetic subjects there was no evidence of a chronic effect i.e. baseline levels were unchanged. Conversely, Fndc5 gene expression was elevated chronically after 12-weeks of training in control and pre-diabetic, and was significantly higher in pre-diabetic group than control[270].

Yet other studies found no change in Fndc5/irisin with exercise training either acutely or chronically[265,271]. Pekkala et al (2013) demonstrated neither different training modes i.e. acute aerobic, chronic endurance training, nor combined endurance and resistance training altered Fndc5/irisin expression significantly, even though the changes in irisin was not consistent with Fndc5 changes[257].

The inconsistency of findings may be related to many factors such as age, training mode, intensity, duration and clinical presentation. However, the field has been beset by technical difficulties in measuring irisin accurately, consequently these difficulties with ELISA kits may explain the confusion within the field. Despite those problems, a meta-analysis, which included ten articles, appeared to confirm the acute elevation of irisin immediately after exercise in adult subjects, in addition it was stated that the pre-existing level of fitness was the only predictive variable of post-exercise irisin levels[272].

Table 1.2: Summary of acute and chronic myokines responses to exercise training.

Myokine	Plasma level in response to exercise training	
	Acute	**Chronic**
FGF21:	• ↔ in human trial (endurance)[191]. • ↑ ~2 fold change in mice and human trials, peaking at1h post (treadmill)[190]. • ↑ ~2 fold change in mice and human trials (endurance)[273].	• ↑ ~2 fold change in human trial (2-wk, endurance)[191]. • ↓ ~1.5 fold change in human trial (3-wk, sprint training)[192]. • ↓ ~1.5 fold change in human trial (3-mo, combined exercise training)[193].
SPARC	• ↑ 1.5-2 fold change immediately after (endurance) in mice and human[198].	• ↔ after (4-wk) of endurance training[198].
Musclin	• ↑~2 fold change in mice (5 days treadmill)	• NA
Fractalkine	• ↑~1.5 fold change at 3h post (one leg cycling for one hour)[216]	• NA
BDNF	• ↑~1.5 fold change post (endurance)[223]. • ↑~1.5 fold change post (resistance)[224].	• ↑ up to 1.5-2 fold change (2-4 wks, endurance not resistance)[274]
IL-6	• ↑ ~100 fold change (according to type, intensity, number of activated muscles)[235].	• ↓ ~ 2 fold change (elderly and obese) [242,243]. • ↔ [244,245].
Irisin	• ↑ ~1.2 fold change at 30 min post (endurance)[261]. • ↑ 1.2 fold change immediately post (endurance)[270].	• ↔ (endurance, 8-wks)[261]. • ↓ (endurance, 12-wks)[270]. • ↑~ 2 fold change (endurance, 3-wks and 10-wks)[247]. • ↑~ 2 fold change (endurance,1 year)[269].

1.4 Heterogeneity in exercise training response:

In the previous section, the adaptive response to exercise training was introduced and discussed. However, it is important to know that the extent of physiological adaptations to exercise training are variant among individuals. It is well known that exercise type, intensity, duration, frequency, and load lead to different adaptive responses. To ensure individuals receive the same relative stimulus, exercise training is standardized and fully supervised in most training intervention. Normally, the data is principally presented as a mean of change and any inter-individual variation is hidden and not reported[275]. However, there is clear heterogeneity in inter-individual responses upon closer inspection. For instance, Raue et al (2012) found that the responses in CSA of VL muscle to 12-weeks RET varied greatly from 1.2 to 10.4 cm^2, with similar variant range in gaining strength from 5.7 to 41.3 kg (1-RM assessment)[276]. Further others reported the percentage change in lean body mass ranged from 3 to 28% after 20-weeks of RET[277]. This variation was also seen with EET, as in Kohrt et al (1991) study, where elderly men and women who trained for 9 months, showed a wide range of VO_2max responses, ranging from 0 to 58% with a mean of around 25%[278]. Similarly, a mean change in VO_2max increase of 25%, with a range from 0% to 50% was reported in young men and women[279]. This has led to the concept of individuals being classified as “high responders” when improvements are large or “low responders” when little or no change is observed[280].

The heterogeneity of individual adaptive responses to standardized exercise training was first addressed back on 1983 with conclusion that the mean response to exercise training could be very misleading. Later, the HERITAGE Family Study was conducted to explore the causes and magnitude of heterogenic response to regular exercise. The study included 720 subjects

from 90 Caucasian families and 40 African American families, who had an examination of cardiovascular and metabolic response to 20-weeks of EET[281]. The variation of VO_2max (Fig 1.3) was ~50% driven by heritability, which includes shared genetic (21 single nucleotide polymorphism) and familial environment. The hereditary also explained the adaptive response to other factor such as HR (~30%) and SBP (~20%)[275]. The magnitudes of VO_2max training response was showing a weak relation to pre-training value (baseline) ~1%. However, other measurements showed a stronger relation to baseline value. For instance, the HR baseline explained ~40% of the HR heterogenic response to exercise training at 50W. Similarly, the baseline of SBP accounted for 32% in exercise-induced change in SBP at 50W[275]. These relation was suggesting a “celling effect”, which means that the exercise induced-response depends on the capacity of improvement. An individual with bad baseline value had more potential to show high favourable response. Factors as sex and age had lower contributions ~5.4% and 4% respectively, while race/ethnicity had minimum impact, accounting for ~1% of the on VO_2max heterogeneity[275]. Similarly neither age nor sex were responsible for the heterogeneity of the RET hypertrophic response in smaller study (278 participants)[282].

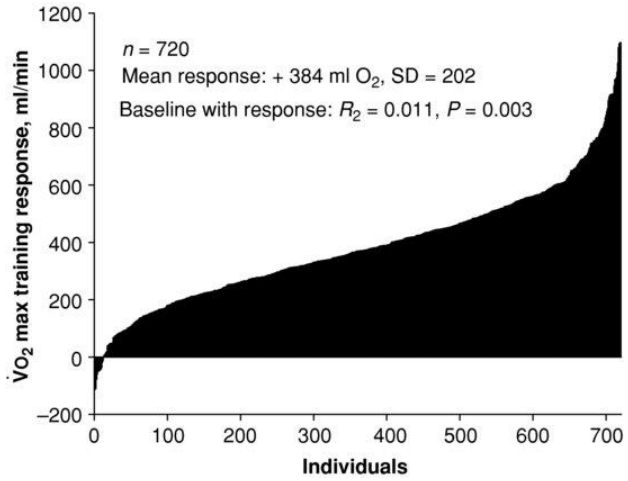

Figure 1.5 Heterogeneity of maximum oxygen uptake (VO_2max) in response to endurance training from the HERITAGE Family Study[275].

Other than genetics and pre-deposit individual features, the homeostatic stress of each exercise bout is a determine force of the adaptive response. Therefore, not following a very strict prescriptive protocols, in term of intensity, fixed duration, and frequency with supervision could lead to heterogenic response. Recovery, return back to baseline homeostasis, is also associated with training induced-homeostatic stress. Therefore, unbalanced training sessions could cause a different stimulus and subsequently heterogenic response. In addition, the ability to recover depends on the life style e.g. sleeping cycle and physiological status of individual[283]. The availability of endogenous and exogenous substrate has a grown evidence of modulating the training response, which indicates the role nutritional status on heterogeneity[281].

At the low end of heterogenic response to exercise training, there are small portion of individuals who are recognized as low/non-responders. The definition of low responder or non-responder is “the lack of difference between a control and treatment condition with respect to a specific variable”[284]. The presence of some non-responders in aerobic capacity was

consistence in endurance training intervention[275,278]. Timmons et al (2010) found that ~20% of participants had very limited increase in their aerobic capacity after months of supervised training[285]. However, a non-responder to VO_2max is not necessarily a non- responder to all measurement. After a year of endurance training, 4 out of 18 participants improved their AT and retesting HR without improving their VO_2max. On the other hand, 3 participants enhanced their VO_2max and AT but not their HR[286]. The concern was raised about the low/non-responders since certain individuals may fail in achieving the health benefits of exercise training. In fact, the improvement in insulin sensitivity after aerobic exercise training ranged from ~10 to 56%. Although Boule et al (2005) explained this variation was down to the small sample size, the HERITAGE family study, ~42% of the participants showed either no or adverse changes in insulin sensitivity, i.e. they were low or non-responders[287]. The prevalence of subjects who showed one metabolic adverse effect (more than typical error) was 31% in collective study of 1,687 subjects, and 6% for two adverse responses, and 0.8% for three or more[288]. The difficulty of conducting a clinical trial specific in identifying low/non-responders leave us with no study so far. However, an animal model was generated with two distinctive response that will be discussed with more details in chapter (4).

1.5 Aims and objectives:

The overall aim was to have a better understanding of myokines contributions in metabolism, trainability, and heterogeneity of the adaptive response to exercise training via a combination of animals' models and human clinical trial.

The first investigation was aimed to explore the Fndc5/irisin autocrine and endocrine impact on skeletal muscle metabolism and fat tissue remolding respectively, with the hypothesis that Fndc5/irisin overexpression would enhance the mitochondrial biogenesis, glucose metabolism, and muscle growth. The Fndc5 gene was overexpressed in rat's hind-limb via *in vivo* electroporation techniques and the impact of physiological irisin on skeletal muscle (autocrine) and adipose tissue (endocrine) metabolism was investigated through different molecular analysis.

In the second investigation, the aim was to identify the role of myokines plasma concertation in trainability with hypothesis that plasma myokine profiles would be favorable according to the myokine action in high response trainers (HRT) animals than low response trainers (LRT). Therefore, the plasma of (LRT/HRT) animals were studied after acute exercise training and 3-weeks EET. The model is the 24^{th} generation of a selective inbreeding of the highest and lowest responders to exercise training. As results, both animal groups exert similar exercise capacity at baseline. However, HRT exercise capacity increases after 3-weeks of EET without improvement in LRT exercise capacity.

After partial understanding of myokines' role in trainability in rat's model and because myokines are proposed to induce the health benefits in other organs, and they derive from exercising muscle, we were keen to investigate the impact of different modes of exercise on

health. In addition, we were keen to explore a potential role of myokines in the adaptive response, the so called responder – non responder hypothesis. Therefore, we decided to screen the myokines' profiles in plasma of same individual pre and post two distinctive types of exercise training, EET and RET. Aiming to explore their role in intra-individual and inter-individuals heterogenic adaptive response. Unfortunately, for some technical issue and shortage of time, we could not achieve our aim.

However, we had the opportunity in the last investigation to examine the intra-individual responses to two distinct types of exercise training, EET and RET in a cross-over design in human volunteers. The main hypothesis was that the high responders to one exercise mode would also be high responders to the other mode, but all would acquire some health benefits. The Human subjects were recruited for a 14-weeks training intervention, where participants undertook 4-weeks of each EET and RET, with a 6-weeks wash out period in a randomized order. Responder status was determined in relation to the primary expected outcome i.e. improved aerobic capacity (EET) or muscle mass/functional gains (RET)

2 Materials and Methods

2.1 Immune blotting (western blot)

To evaluate the protein expression of interested factors, western blot was used on the sarcoplasmic fractions. The protein concentration was measured first by using NanoDrop, then normalized to 1µg/µl with homogenization buffer and Laemmli loading buffer (1M Tris-HCL with pH 6.8, 20% Sodium dodecyl sulphate, Glycerol, 2-Mercatoethanole, and Bromophenol blue). Samples were then heated for 95°C for 5 min using heat block before they were loaded to (Bio-Rad) Criterion XT Bis-Tris-12% SDS-PAGE gel with kaleidoscope protein standard (ladder) by gel loading tip in a tank full of 1X (Bio-rad xt MOPS) running buffer. At the end of the electrophoresis (200V for 50-60 min), the gel was rinsed with ddH_2O and then washed face down with transfer buffer (Tris Base, Glycine, and Methanol) for 5 min in-tray. The electro-transfer was then performed by using the sandwich technique, where activated 0.2µM polyvinylidene difluoride (PVDF) membrane (with 100% ethanol for 1 min) placed on the top of the gel between blotting papers and fibre pads. The sandwich was then placed in a (Bio-rad) transfer tank with pre-chilled transfer buffer and run for 45 min at 100V. The PVDF membrane was blocked with 2.5% skim milk for 60 min with gentle agitation and was then incubated with primary antibody (Ab) (1:2000) at the fridge (4°C) for overnight. All primary Abs were listed in (Table 2.2). Before been incubated with the secondary Ab (HRP-conjugated, 1:2000) for 60 min at room temperature with gentle agitation, the membrane was washed 3x for 5 min each with Tris-buffered saline with tween-20 (TBST; Sodium chloride, Tris base, and 0.1% Tween-20). The membrane was washed from 2^{ND} Ab excess before being incubated with Millipore HRP reagent to be analysed at (Bio-rad) Chemidoc machine[289].

Table 2.1 Western blotting antibodies.

Antibody Name	Manufactured company, Lot No.	Primary Ab	Secondary Ab
Fndc5	Abcam, ab54481	1:2000	1:2000
PGC-1α	Abcam, ab110413	1:2000	1:2000
GLUT4	Abcam, ab33780	1:2000	1:2000
OXPHOS cocktail	Abcam, ab110413	1:50,000	1:10,000
Phospho-Akt Ser473	Cell signalling, #9272	1:2000	1:2000
Phospho-PRAS40 Thr246	Cell signalling, #2640	1:2000	1:2000
Phospho-mTOR Ser2448	Cell signalling, #2971	1:2000	1:2000
Phospho-P70S6 Thr389	Cell signalling, #9234	1:2000	1:2000
Phospho-4EBP1 Ser65/Thr70	Cell signalling, #945	1:2000	1:2000
Phospho-AS160 Thr642	Cell signalling, #8881	1:2000	1:2000
Phospho-GSK3α/β Ser235/236	Cell signalling, #9331	1:2000	1:2000
Phospho- AMPK Thr172	Cell signalling, #2535	1:2000	1:2000
PI3K class III	Cell signalling, #3358	1:2000	1:2000
Beclin-1	Cell signalling, #3495	1:2000	1:2000
LC3B	Cell signalling, #2775	1:2000	1:2000
Atrogin-1	Cell signalling,	1:2000	1:2000
Murf-1	Cell signalling,	1:2000	1:2000
Phospho-GS	Cell signalling, #3891	1:2000	1:2000

GS	Cell signalling, #3893	1:2000	1:2000
Phospho-P38 MAPK	Cell signalling, # 4511	1:2000	1:2000
Cytochrome C	Cell signalling, #5674	1:2000	1:2000
SDHA	Cell signalling. #5674	1:2000	1:2000
HSP60	Cell signalling, #5674	1:2000	1:2000
VDAC	Cell signalling, #5674	1:2000	1:2000
Phospho-ERK	Cell signalling, #9101	1:2000	1:2000
Phospho-TSC2	Cell signalling, #3617	1:2000	1:2000

2.2 RNA extraction

For Ribosomal nucleic acid (RNA) extraction, TRizol Reagent (Life Technologies/Thermo Fisher Scientific) method was used with 5-10mg of muscles or adipose tissue. 500µl of Trizol was added to the tissue in 2ml Eppendorf tube with one sterile stainless-steel bead. The tissue was then homogenized in a shaker (Tissue Lyser II, Qiagen, UK) at 30 frequency for 30 seconds. Homogenate was incubated for 10 min at room temperature and transferred to new Eppendorf tubes. 100µl of Chloroform was added and vortexed for 15 sec, then incubated for another 10 min before being centrifuged for 15 min at 12000g at 4°C, which separated the sample into three layers. 60% of the aqueous layer (upper) was transferred into new nuclease-free Eppendorf tubes by using gel loading tips to avoid any DNA contamination from the intermediate layer. RNA volume was measured by pipette and an equal volume of 100% isopropanol was added with 1µl of Glyco-blue (to visualize the RNA pellet) then inverted 3-5 times before being incubated for 30 min at the fridge to precipitate the RNA. Later, samples were centrifuged at 7000 g at 4°C for 10 min. The RNA was washed twice by adding 500µl of

80% ethanol and spun for 5 min at 7000 g without disturbing the RNA pellet. Before re-suspending, the RNA pellet with 20µl of nucleases free water (DEPC), samples were air-dried for a few mins. The RNA yield was measured by the Nanodrop, 260/280 and 260/230 ratio were used to assess the RNA purity[290].

2.3 cDNA synthesis

The complementary DNA (cDNA) was synthesized from normalized RNA concentration, 500ng/µl, by using High-Capacity cDNA Reverse Transcription kit (Life Technologies) with RNAase inhibitor. The kit protocol was followed, and 10µl of the master mix was made for each sample as showed in the table below. Samples were then placed in iCycler IQ Real-Time PCR with the following thermal cycle, 25°C for 5 min, 37°C for 60 min, 37°C for 60 min, 85°C for 5 min, and then 4°C forever[290].

Table 2.2 cDNA synthesis (Master Mix content)

	1x reation (µl)	25x reactions (µl)
10x RT Buffer	2	50
10x Random Primers	2	50
dNTP mix (100mM)	0.8	20
Reverse transcriptase (50U/ µl)	1	25
RNAase inhibitor	1	25
DPEC water	3.2	80

2.4 Real-Time qPCR

1µl of cDNA was added to each well of the 384-optical well plates (Life Technologies) after being diluted with RNAse-free water 1:4. Each sample was run in triplicate with primers in SYBER Green Master Mix (Life Technologies). The thermo-cycling condition on ViiA 7 Real-

Time PCR System (Life Technologies) were 2 min at 50°c followed by 2 min at 95°C and 40 cycles of 15 seconds at 95°C with 60 seconds at 60°C[290]. The gene expression was presented as fold of change from Ctrl or baseline in animals and human studies respectively. ΔΔ Ct was calculated from gene Ct and Ct of the housekeeping gene, which was glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for rat and Ribosomal protein L13a (RPL13a) for human, according to the following formula:

$$\Delta\Delta Ct = 2^{-(\text{Target gene Ct} - \text{Housekeeping gene Ct})}$$

2.5 Primers design and validation

Primers were designed through Primer-BLAST NCBI website with taking into account the GC content to be 50-60%, avoiding secondary structure, maintaining melting temperature 50-65°c. Primers were then validated in serial dilutions of pooled cDNA (1:4, 1:8, 1:16, 1:32, 1:64). CT values were plotted against the log of dilution to give a standard curve with its slope degree. The efficiency of primers was calculated from slope degree by using the following formula:

E = (10^ (-1/slope)-1)*100

Primers with an efficiency of 85% - 120% were accepted.

Table 2.3 Gene's primers of all studies.

Gene	Forward 5'- 3'	Reverse 5'- 3'
	Rat's primers	
Fndc5	CTTCATGTGGGCAGGTGTCAT	ATTGGGCTCGTTGTCCTTGAT
PGC-1α	CATGTGCAGCCAAGACTCTG	AAAGCTGTCTGTGTCCAGGT
TFAM	GACTTCTGCCCACTGAATGC	AAGCAAACGGCAGAACTCGT
NRF	GACCATCAGCAAAGCCGTGA	ACGTAAGCTCTGCCTGGTTG
UCP1	GCTCCTCCACAAATAGCCCTG	CGGAAGTTGTCGAACTCACCA
UCP2	AGCAGTTCTACACCAAGGGC	TGGAAGCGGACCTTTACCAC
UCP3	CGCCTGGAACAGAACAAAGC	TAACAGTGCAGGGTTCCGTC
ATP5J2	CGAATTTCTGCGGACAACAGG	CTGCAATACCACTGGGGGTG
ATP2A3	TGCTGTTTTCTGGCACCAAT	CAAATTCATCCAGCTTGCGCT
MyoD	CTGCTCTGATGGCATGATGG	CTCCACTATGCTGGACAGGC
MyoG	GTGAATGCAACTCCCACAGC	CGAGCAAATGATCTCCTGGGT
Mrf4	TAAGGAAGGAGGAGCAAGCG	GGGAGTTTGCGTTCCTCTGA
IGF-1	CAGAGCAGATAGAGCCTGCG	TGGGCAGGAATAATGAGGCA
MSTN	ACCATGCCTACCGAGTCTGA	ATCCACAGCTGGGCCTTTAC
Follistatin	GTGTGCCATGAAGGAAGCTG	TCCGAGATGGAGTTGCAAGA
REDD1	GTCTAGTGCCCACCTTTCAGTT	GCTCGGAGCTGTAGAGTTTCTT
BCL-2	TTCATCACAACCCACCTCTCTG	TGCAAGTCCCAACACTCGGG
BAX	CCAGGATCGAGCAGAGAGGAT	TGTTGTTGTCCAGTTCATCGC
Atg7	CAGCCTGTTCATCCAAAGTTCTTG	CTGTGGTTGCTCAGACGGT
Atg5	CAGAAGCTGTTCCGTCCTGT	CCGTGAATCATCACCTGGCT
CstL	CTATCGCCACCAGAAGCACA	ACCACACTGGCCCTGATTCT
Murf-1	CCAAGGACAGAAGACTGAACTGA	CTCCTGCTCCTGAGTGATCC

Atrogen-1	CCAAAACTCAGTATTTCCATCAG	GACTTTGCTATCAGCTCCAACA
Caspase-3	CGGACCTGTGGACCTGAAAA	CGGCCTCCACTGGTATCTTC
m-Calpain	TCGGCATCTATGAGGTCCCA	ATTCTTGTGGGGCTCGAAGG
GLUT4	GGCCGGGACACTATACCCTAT	TCCCCATCTTCAGAGCCGAT
HK2	TGGTTTCAAAGCGGTCGAACT	TCGAGTAGAGAAACCGAGGC
GYS1	CCTGCTCAGAGTGAATGGCA	TTGGCTGTGTCCCATAGCTG
GAPDH	CTCTCTGCTCCTCCCTGTTC	CGATACGGCCAAATCCGTTC
VEGFA	CACGACAGAAGGGGAGCAG	AGATGTCCACCAGGGTCTCA
FLT1	GGGTGTCTATAGGTGCCGAG	AGGGTGATCAGCTCCAGGTT
KDR	ATCCGAAGGGTGAGGAAGGA	CCTTCATTGGCCCGCTTAAC
ANGPT1	GCTGAACGGTTACACAGAGAG	ACGCTCTCCCCGTTAAAGAAA
ANGPT2	CCCCTACATGTCTAACGCCG	TCATCACAGCCGTCTGGTTC
TIE2/TEK	GATGGATAGGGCTCACTCTGC	CCACTCGCATGGTCTACTCG
FGF2	GTGTCCATCAAGGGAGTGTGT	AGTGCCACATACCAACTGGAG
	Human Primers	
PGC-1α	GAGTACTACTTGCTCTTGGTG	GATGATGGAGACAGCTATGGT
NRF	ACGTTTGCTTCGGAAACTTCGAGC	GCCAGAGCAGACTCCAGGTCTT
TFAM	CTGCGCTCCCCCTTCAGTTTTGT	GAAGTTCCCTCCAACGCTGGG
VEGFA	CTTGCTGCTCTACCTCCACCAT	ATGATTCTGCCCTCCTCCTTCT
FLT1	CGGACTGTGGCTGTGAAAATG	TGGCCAATGTGGGTCAAGAT
ANGPT1	GCAGCAGATAGGGTAGAGGA	TGTTTCCTCTCTGTGTGACCG
ANGPT2	GGGTCCTGCAGCTACACTTT	ACAGCATTGGACACGTAGGG
IGF-1Ea	TAAGGAGGCTGGAGATGTATTGC	TCAAATGTACTTCCTTCTGGGTC
MSTN	GCTGCGCCTGGAAACAGCTC	ATCAGTTCCCGGAGTGGAGGC
MyoD	CGACGGCATGATGGACTACA	TAGTAGGCGCCTTCGTAGCA

MyoG	CCAGGGGATCATCTGCTCA	GGTTTCATCTGGGAAGGCCA
UBF	AAGAAGCCTCCATGAACGG	CGGCCAGCTTTTTGTAGTGC
TIF-IA	GTATTGGCATGAGAAACCACGG	CATTTTGTGCCTCCCGAGT
C-MYC	GGTAGTGGAAAACCAGCAGCC	TCTCCTCCTCGTCGCAGTA
Myf5	GATGGCATGCCCGATTGTAAC	GCAATCCAAGCTGGATAAGGA
RPL13a	TAAACAGGTACTGCTGGGC	CTCGGGAAGGGTTGGTGTTC

2.6 Muscle protein, DNA, and RNA concentrations

To determine muscle alkali-soluble protein (ASP), DNA and RNA concentration, ~15mg of wet muscle was homogenized in 1ml of 0.2M Perchloric Acid (PCA) and centrifuged at 4°C at 11,000 rpm for 8 min. The supernatant was discarded, and the pellet was washed twice with 1ml 0.2M PCA, vortexed and centrifuged at 4°C at 11,000 rpm for 8 min. The protein pellet was dissolved in 800µl 0.3M NaOH at 37°C for 30 min. ASP was then quantified by spectrophotometry at 280nm (NanoDrop Lite, Thermo Scientific). The protein was precipitated with 400µl 1M PCA before being centrifuged at 4°C at 5000 rpm for 5 min. The 1500µl supernatant, containing RNA, was quantified by spectrophotometry after another wash to the pellet with 300µl 1M PCA, where supernatant re-joined. The pellet was re-suspended in 1ml 2M PCA and incubated for 60 min on a hot block at 70°C, vortex mixed and centrifuged at 5000 rpm for 5 min and the DNA in supernatant were quantified in total volume of 1300µl. Nanodrop was blank with 0.3 M NaOH for Protein quantification, 0.2 M PCA for RNA, and 2 M PCA for DNA[291].

2.7 Glycogen content in skeletal muscles

Glycogen was extracted and quantified as described previously[292]. Briefly, TC (21.5-39.5mg) were digested in 1M KOH. Na_2SO_4 and ethanol were used to precipitate glycogen, with 0.3mg/ml amyloglucosidase in 0.25 M Acetate buffer (pH 4.75) used to digest the glycogen pellet overnight at 37°C. Samples were then incubated for 25 min at 37°C in phosphate buffer containing 0.5mg/ml 4-aminoantipyrine, 1.6U/ml peroxidase, and 10U/ml glucose oxidase with a pH of 7.0. Glucose was quantified at 490nm against a standard curve. Concentrations were normalised to muscle weight.

2.8 Intraperitoneal glucose tolerance test

During intraperitoneal glucose tolerance test (IPGTT), the glucose and 2-deoxyglucose (2DG) were administered together (2g/kg of glucose and 6mg/kg 2DG (Amersham Biosciences)) as an i.p injection into overnight fasted rats. Blood from the tail vein was collected at 0, 15, 30, 60 and 90-min post-injection. Blood glucose was measured directly after collection on an Accu-Check Advantage meter (Roche Diagnostics, Castle Hill, NSW, Australia)[293].

2.9 Determination of glucose uptake using 2-deoxyglucose

Briefly, 100µl of plasma was aliquoted into fresh Eppendorf tubes with 10µl of fluorodeoxyglucose (FDG) as the internal standard and mixed. 1ml of ice-cold absolute ethanol was then added to each tube. Samples were then incubated in the fridge for 20 min, then spun for 2 min at 10,000 rpm. The supernatant was removed into test tubes and dried completely on a Techne Block® at 90°C for 10 min. 100µl of oxime reagent (20mg hydroxylamine HCl per 1ml pyridine) was then added to each tube which were vortex-mixed and incubated at 85°C for 30 min on a Techne Block®. Samples were cooled for 5 min, then

70µl of BSTFA was added, mixed, and incubated at 85°C for 30 min on a Techne Block®. 70µl was transferred carefully into auto-sampler vials and capped. 2DG was quantified by GC-MS (ISQ, Thermo, Hemel Hempstead, UK) with reference to a standard curve of known concentration[294].

2.10 Muscle 2-deoxyglucose-6-phosphate accumulation

Approximately 20mg of powdered muscle tissue was homogenised 3x with metallic beads for 2 min (30 Hz). During the first homogenization phase, 125µl of ddH_2O with 20µl of internal standard (^{13}C 2-deoxyglucose-6-phospate (2DG6P)) was added then centrifuged for 2 min at 15,000 g at 4°C. In the second phase, 375µl of chilled methanol was added followed by centrifugation for 10 min at 15,000 g at 4°C. The supernatant was then transferred to auto-sampler vials. Finally, the pellet was washed with 500µl ice-cold methanol and all supernatants combined prior to the next step.

All samples were dried under nitrogen for 30 min at 50°C, before a two-step derivatisation. 75µl of methoxyamine solution (20mg/ml of methoxylamine hydrochloride in Pyridine) was added before samples were vortexed and then incubated for 60 min at 90°C. The mixture was cooled for 10 min, then 75µl of BSTFA was added before samples were vortexed and again incubated at 90°C for 60 min[295]. Samples were transferred to autosampler vials ready for GCMS analysis. A standard curve of known 2DG6P concentration was prepared alongside each batch for quantitation.

2.11 Muscle protein synthesis - Plasma enrichment of D_2O

The enrichment of body water was measured as previously described[92]. Briefly, 2µl of 10M NaOH and 1µl of acetone were added to 50µl of plasma and incubated for 24 hours at room temperature to exchange deuterium from the water with hydrogen positions on the acetone under high pH. Acetone was extracted into 200µl of n-heptane, then 0.5µl of heptane was injected into the GC-MS. D_2O Enrichment was calculated from the known standard curve run alongside the plasma samples.

2.12 Isolation of muscle protein fractions

30-50mg of TC muscle was homogenised in 10µl/µg of ice-cold homogenization buffer (Tris-HCL pH 7.4, 50 mM NaF, 10 mM b-glycerophosphate disodium, 1 mM, EDTA, 1mM EGTA, and 1mM activated Na_3VO (Sigma-Aldrich)) with complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). The supernatant (sarcoplasm fraction) was collected from homogenates after 10 min of rotating followed by 5 min centrifugation at 13,000 g at 4°C. The pellet was washed twice from any sarcoplasmic excess by 500µl of homogenisation buffer before it was further homogenised in 500µl mitochondrial extraction buffer (pH 7.5, 20 mM MOPS, 110 mM KCL, and 1 mM EGTA) and centrifuged at 1000 g at 4°C for 5 min to isolate mitochondrial fraction, which was pelleted by spinning at 11,000 g at 4°C for 15 min. The myofibrillar pellet was separated from insoluble collagen by centrifugation and suspended in 0.3M NaOH. 1M PCA was used to precipitate myofibrillar proteins. Sarcoplasmic proteins were precipitated from the main homogenate in 1M PCA and separated by centrifugation. The insoluble collagen was washed with 0.3 M NaOH, followed by 70% ethanol and isolated by centrifugation. All pellets from different fractions were re-suspended in boiling tubes with 1ml 0.1 M HCL and 1ml of Dowex slurry and incubated for overnight (approx.16 hours) at 110°C[92].

2.13 Proteins hydrolysis

To release protein-bound AAs from the myofibrillar and sarcoplasmic protein fractions, acid hydrolysis was carried in column (5ml pipette tip) with filter disk and 2ml of Dowex H^+ resin slurry, which was washed with 6ml of 2 M NH_4OH, then neutralized by 9ml of ddH_2O, and finally primed with 6ml 1 M HCL. The samples were transferred completely to the column by glass pipette. The original sample tube was rinsed with 1ml of HCL and transferred to the column to get all Dowex H^+ from the boiling tube. 2ml of HCL was loaded to the column (1ml a time) until liquid dropped off. Then to separate the HCL and NH_4OH fraction, 1ml of ddH_2O was added twice followed by another 3ml of ddH_2O. Every lot was added after the previous one was gone through. To hydrolysate proteins, 1ml of 2 M NH_4OH was loaded first to go thought. Then 3ml of NH_4OH was loaded with precaution to collect AAs into glass tubes. Finally, samples were dried in a Techne Block® at 90°C for 30 min to evaporate and dry[92].

2.14 Amino acids derivatization

AAs were then derivatized as their *n*-methoxycarbonyl methyl esters (MCME). Dried samples were resuspended in 60µl of ddH_2O and 32µl of methanol, and following vortex, 10µl of pyridine and 8µl of methylchloroformate were added. Samples were then vortexed for 30 seconds and incubated at room temperature for 5 min to react. 100µl of chloroform was used to extract the newly formed MCME AAs, and a molecular sieve was added for ~20 seconds to remove any remaining water by size exclusion before being transported to new clean auto-sampler vials. Incorporation of D_2O into protein-bound alanine was determined by TSQ GS-MS (ThermoFisher Scientific, Hemel Hempstead, UK) alongside a standard curve of know L-alanine-2,3,3,3-d4 enrichment to validate the measurement accuracy of the instrument[92].

2.15 Fractional synthetic rate of protein

MPS in the myofibrillar and sarcoplasmic fractions were determined from the incorporation of deuterium-labelled alanine into protein, using the enrichment of body water, which was corrected for the mean number of D_2O moieties incorporated per alanine as the precursor. The standard equation used is:

$$FSR\ (\%/day) = [(APE_{Ala})] / [(APE_P) \times t] \times 100$$

Where APE_{Ala} is deuterium enrichment of protein-bound alanine, APE_P is mean precursor enrichment over the time period, and t is the time between the D_2O injection and muscle harvesting[92].

2.16 Citrate synthase activity

The enzymatic activity of citrate synthase (CS) was estimated after incubating a mitochondrial suspension of muscle with CS buffer, which contains reaction substrates, Acetyl CoA and Oxaloacetate. As described previously[296], 5-10mg of frozen muscle was homogenized in ice-cold homogenization buffer 100µl/mg (50 mM KH_2PO_4, 1 mM EDTA, and 1% Triton X-100) and centrifuged for 30 min at 22,000 g at 4°C. 300µl of CS master mix was used in a 96-well plate as blank reading before 20µl of mitochondrial supernatant to the well were added and incubated for 3 min at 25°C then read at 412nm again. Delta optical density (sample OD – blank OD) was calculated for each sample. Protein concentration was measured using the Pierce™ BCA Protein assay. CS master mix recipe for 25 reactions is listed on (Table 2.4).

Table 2.4 Citrate synthase Master Mix

Chemical/water	Volume
Tris buffer	2025 µl
DNTP	100 µl
Acetyl CoA. (1.36 mg/ml) with Tris buffer	525 µl
Oxaloacetate. (9.81 mg.ml) with Tris buffer	60 µl
Water	4770 µl

2.17 Adenosine triphosphate, phosphocreatine and creatine assay

As described previously[297], 25mg of wet muscle was frozen dried overnight before being powder. Agar mortar with a pair of tweezers and scalpel were used to gently break up the muscle and remove any connective tissue. Fine powder tissues were then transferred to a fresh Eppendorf tube and weighed before they were stored at -80 in a bag with silica to keep them dry. For samples extraction, an appropriate volume of PCA (0.5 M PCA/1 mM EDTA) was added followed by shacking for 10 min at 4°C then centrifugation for 3 min at 13,000 g at 4°C. A fixed volume of PCA extract was recovered to a new Eppendorf tube without disturbing the pellet. 2.1 M KHCO3 was added (¼ to the recovered volume of PCA) and incubated on ice with an open lid for 10 min. Samples were flicked during the incubation to release air bubbles, whenever it was needed. Centrifugation for 10 min at 13,000 g at 4°C was performed before transferring supernatant to new Eppendorf tubes. Samples were stored at -80 until required for the assay.

2.17.1 ATP/Pcr Assay

Samples were defrosted and gently mixed before placed on ice, while the master mix was prepared according to the following; 500µl of D1 buffer (TEA 100 mM, 10 mM Mg acetate ($Mg(AC)_2.4H2O$,), and 1 mM EDTA, PH 7.5), 100µl DTT, 200µl 1 mM NADP, 20µl 0.04 mM APD, 200µl 5 mM Glucose, 400µl ddH2O, and 20µl Glu6DH were mixed for 20 reactions. 25µl of the sample was added to corresponding well after adding 225µl of master mix to 96 wells flat bottomed. 3 blank wells in each plate were added, where ddH2O substituted sample. The plate was gently mixed in a plate reader and read at 430nm of wavelength as the background value. 4µl of Hexokinase was added and read at same wavelength after gently mix and incubation in dark for 15 min as ATP level. Then, 4µl of Creatine kinase was dissolved in NaHCO3 with 0.05% BSA and added to each well. The plate was then mixed and incubated in dark before reading at 340nm for 35 min.

2.17.2 Creatine Assay

Samples were defrosted and gently mixed before placed in ice, while the master mix was prepared according to the following; 2000µl D4 (100 mM Glycine, and 5 mM Mg $(AC)_2.4H2O$, PH 9.0), 100µl 30 Mm KCL, 400µl 1.5 mM ATP, 300µl 1 mM Phosphoenolpyruvate (PEP), 100µl 0.15 mM NADH, 10µl L-lactate dehydrogenase (L-LDH), and 10µl Pyruvate Kinase (PK) were mixed for 20 reactions. 300µl of the master mix was carefully added to flat bottomed 96 wells, followed by 20µl of samples and 20µl of ddH2O in blank wells. The plate was then read at 340nm as the background value. Then, 10µl of dissolved CK in 0.5% NaHCO3 with 0.05% BSA was added. The plate was then mixed on plat reader and incubated in dark for 35 min to be read at 340nm.

2.17.3 ATP/Pcr and Creatine Calculation

The following equation: {(V2 × (Post Abss - Post AbsBl) - V1 (Pre Abss- Pre AbsB) × EF} / (SV × CF)

V1: Total volume in well before adding the enzyme.

V2: Total volume in well after adding the enzyme.

EF: extraction factor = (Volume of PCA retrieved × 1.25/mg dry muscle weight)

SV: sample volume in the assay.

CF: NADP Coefficient factor = 6.22

2.18 Enzyme-linked immune assay:

Different ELISA kit's protocols were followed to measure the concentration of irisin and IL-6 in rat's plasma and insulin in human's plasma. The kits were based on either a direct sandwich technique (IL-6 and Insulin) or competitive enzyme immunoassay (Irisin). IL-6 and insulin plates are coated with primary Ab that target IL-6 and insulin molecules respectively. The secondary Ab (Biotin-conjugate/enzyme-conjugate) binds to the captured IL-6/insulin, which then binds to streptavidin-HRP to visualize the concentration with the substrate. In the other hand, irisin plate is coated with secondary Ab that has binding site to the primary Ab. Irisin molecules, in standards and unknown sample, would compete with Biotinylated peptide to bind primary Ab. Biotinylated peptide interacts with Streptavidin-HRP that catalyse the substrate. The resulting yellow colour is indirect proportion to irisin concertation.

2.18.1 Irisin ELISA kit

The concentrations of plasma irisin were measured using the most validated ELISA kit (EK-067-29, Phoenix Pharmaceutical)[298]. The kit based on the principle of competitive enzyme immunoassay with detection range of (0.1-1000 ng/ml). Manufacture protocol was followed, in brief, 50µl of samples and standard were added with 25µl of primary Ab and biotinylated peptide in each well. The plate was then incubated at room temperatures for 2 hours before it was washed 4x with 350µl of 1x assay buffer. Prior to 1 hour incubation at room temperature 100µl of Streptavidin-HRP was added. Plate was washed 4x again with 1x assay buffer to remove any excess. The plate was then incubated for the last time (1 hour) with 100µl of TMB substrate. The reaction was stopped by 100µl of 2 N HCL O.D. was read at 450nm. Concentrations were calculated from a generated standard curve.

2.18.2 IL-6 ELISA kit:

Following the Kit protocol (BMS625, ThermoFisher), 1x washing buffer, assay buffer, and standards were prepared and reconstituted with ddH_2O. 7 serial dilutions of stander curve were externally made with assay buffer. The micro-wells were washed with 400µl of washing buffer per well. The plate was firmly tapped on an absorbent paper towel to remove any excess.100µl of standards were added into their wells (in duplicate), while 100µl of assay buffer was added to blank wells and 50µl to all samples wells. 50µl of samples were then loaded to the appropriate well followed by 50µl of Biotin-conjugate to all wells before covering the plate with adhesive film to be incubated at room temperature for 2 hours on a shaker (400 rpm). The plate was then washed 6x, and 100µl of streptavidin–HRP was added to each well. The plate was then re-incubated at room temperature for 1 hour on a shaker with adhesive film followed by another washing step. 100µl of TMB substrate was pipetted to each well, and

the plate was incubated at room temperature for 10 min away from direct light exposure. When the highest standard turned to dark blue, 100µl of stopping solution was pipetted to each well. O.D. was then read on spectrophotometry at 450nm. Results were calculated according to the best-fit standard curve.

2.18.3 Insulin Ultrasensitive kit:

Plasma was defrosted completely, and mixed, then spun at 12,000 rpm for 5-10 min to precipitate any debris. Plasma was then diluted in 1:13 with calibrator 0.1x Enzyme conjugate and 1x washing buffer were made according to the kit protocol (Mercodia, Sweden). 25µl of each calibrator and sample were added into the appropriate pre-designed well. 100µl of 1x enzyme conjugate was added using a multichannel pipette to each well. The plate was then incubated at room temperature for 60 min on a plate shaker (700 rpm). The plate was then inverted to discard the solution and washed with 350µl of 1x washing buffer by using a multichannel pipette. The wash was repeated 5x with firmly tapping against absorbent paper to remove any excess. By using the multichannel pipette, 200µl of TMB substrate was added to each well and incubated at room temperature for 30 min. The reaction was then stopped with 50µl of stopping reagent. The plate was mixed shortly (10 seconds) before reading the O.D. at 450nm on the plate reader. Concertation was calculated from a linear standard curve.

2.19 Multiplex ELISA

Multiplex ELISA was used to detect multi-analytes in the same sample. It is based on immunoassays reaction. The primary Ab are coated on the surface of fluorescent-coded magnetic beads, which would react to Biotinylated detection Ab, and then visualizes through Streptavidin-HRP and substrate solution. The analyser (MAGPIX) uses flow cytometry technique and the results of the bioassay are based on fluorescent reported signal, by the use

of (Luminex xPONENT) software. By following the Kit protocol, antibodies-immobilized beads of different analyte were sonicated, vortexed, and then transferred together to beads mix bottles. Quality controls, washing buffer, serum matrix, and standards were prepared with ddH_2O. 7 points standard curve were diluted. The plate was washed with 200µl of washing buffer per well, then decanted. 25µl of standards, controls, and assay buffer (as blank) were added to appropriate wells, followed by pipetting 25µl of serum matrix into blank, standard, and controls. 25µl of samples were added followed by 25µl of beads mix on all wells. The plate was incubated in dark-cold room (4°C) for 16 hours with gentle agitation. Wells content was removed, and the plate was washed 3x with 200µl of washing buffer with the help of a handheld magnet, which keeps the beads on. 25µl of detection Ab was added per well and incubated for 1 hour, and then 25µl of streptavidin was pipetted as well and incubated for 30 min. Contents were removed and the plate was washed 3x before 150µl of sheath fluid was pipetted to each well. The plat was read on (Luminex, MAGPIX) instrument.

2.20 Subject recruitment

The study was approved by the University of Nottingham, Faculty of Medicine and Health Science Research Ethics Committee, and was conducted according to Declaration of Helsinki. Participants were recruited via advertisement poster (Appendix 1) and word of mouth. Subjects were healthy individual's males with no metabolic disease, such as diabetes, hypertension, asthma, heart disease, stroke, or epilepsy and all were recreationally physically active. All candidates received a copy of the information sheet with all study details (Appendix 2) before they were invited for a screening session. A verbal explanation of all study protocols was given before consent was taken and a health check-up was undertaken. The screening included measurement of height and weight for Body mass index calculation (BMI), blood

pressure, and heart rate. Finally, an Electrocardiogram (ECG) was performed and blood samples were taken. All blood samples were sent to the Pathology Department at Royal Derby Hospital with request for full blood count, coagulation, urea and electrolytes, liver function, thyroid function, and fasting glucose and insulin. Blood and ECG results were assessed by a qualified clinician, prior to the subject being enrolled in the study. Subjects with high BMI >30 or hypertension (blood pressure >140/90 mmHg) were excluded. In addition, subjects currently involved in a regular, structured exercise training program were excluded.

Table 2.5 Subject characteristics. Values are mean ± SD

Age (years)	23.4 ± 4.6
Height (m)	174.4 ± 9.9
Body weight (kg)	73.48 ± 12.59
BMI (kg/m^2)	24.2 ± 3.20
Systolic BP (mmHg)	124.4 ± 7.1
Diastolic BP (mmHg)	74.14 ± 7.8
Resting HR (mmHg)	65.52 ± 8.45

2.21 Endurance exercise training

EET was performed three times a week for 4 consecutive weeks at a percentage of the maximum wattage that was achieved at VO_2max during the cardiopulmonary exercise test (CPET). Lode cycle ergometer was programmed with the wattage and constant rate of pedal revolutions per minute (RPM) was maintained during the training session. EET sessions lasted for 45 min of cycling in the 1ST week, and 60 min in the following 3-weeks. The training intensity was at 60% during the 1st and 2nd week, then increased to 65% and 70% in 3rd and 4th week respectively, to maintain the intensity of exercise to maximize the potential performance results in 4-weeks training period[30].

2.22 Resistance exercise training

RET consisted of six different exercises, three upper and three lower body exercises with no emphasis on either concentric (shortening) or eccentric (lengthening) motion since each of these may induce distinctive morphological adaption in skeletal muscle[31]. Each repetition was a full range of motion of the individual for each apparatus with a moderate velocity of contraction (1-2second CON; 1-2second ECC). RET was performed at 70% of the 1-RM, which was assessed prior to the first training session and after 2-weeks to maintain the training intensity for maximum exercise potential results. RET included two sets of 12 repetitions with 2-min rest between sets for each of the six exercises; leg press, chest press, leg extension, pull-down, leg curl, and seated row. Order of the exercise training was maintained through the training sessions, 3x/week for 4 consecutive weeks. The design (12 rep at 70% of 1-RM for 2 sets, 3 days/week) was used to ensure heavy loading. This design was also recommended by ACSM to achieve hypertrophy and strength gains in our cohort[299].

2.23 “Wash-out” phase

The wash-out period included 6-weeks, when participants were requested to return to their normal physical activity levels and were not required to attend our facility. Even though the diet was not controlled, subjects were asked to maintain their normal diet throughout the course of the study. In addition, the subjects were asked to keep constant habitual physical activity levels during the training and washout period. In two of the participants, the wash-out period was extended due to personal circumstance for 8 and 12-weeks.

2.24 Acute Study Days

Acute study days were undertaken at the beginning (wk-0) and at the end (wk-4) of each training block (EET and RET), 4 acute studies in total. On the acute study day, participants attended the facility fasted overnight or a minimum of 6 hours based on their acute study starting time, this was maintained constant for the individual for each of the 4 visits. The acute studies consisted of 6-hours series of clinical and metabolic tests, including a blood test, an oral glucose tolerance test (OGTT), DXA, HR and BP measurements, ultrasound of leg blood flow (LBF) of femoral artery, muscle structural ultrasound, and CPET. Maximal strength was assessed on a separate day before and after the training period, when the MVC measurement was made to assess unilateral isometric strength, in addition to the 1-RM assessment.

2.24.1 Blood tests

Blood samples were taken from the subjects in evacuated anticoagulant tubes, EDTA, lithium heparin, and potassium oxalate (3ml each). Red cells and plasma were separated by centrifugation at 4^{o}C for 20 min at 3200 rpm and serum was transferred to a new labelled Eppendorf tube and stored at -80^{o}C for later analysis. In addition, other blood samples were sent to the pathology department of Royal Derby Hospital to measure fasting blood glucose and insulin, lipid profile (e.g. triglyceride, total cholesterol, HDL-C, non-HDL-C), and inflammatory profile (e.g. CRP). The result of fasting glucose and insulin were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR): HOMA-IR= (Glucose × Insulin) / 22.5.

2.24.2 Oral glucose tolerance test

2 hour OGTT was performed by taking venous blood via a retrograde cannula in the hand, which was maintained heated at 57^{o}C throughout. A shunt was created by the hot box to allow arterialised blood to be sampled from a vein near the capillary bed in the hand[300]. A saline bag was connected to the cannula on constant drip setting with the cannula port flushed after each withdraw. The first sample was to measure fasting blood glucose after overnight fasting or >6 hours when water was only allowed. Then 75g of anhydrous glucose (Dextrose, PURE SERIES) in ~250ml of water was taken orally, recorded as time 0. Blood samples were then collected every 15 min for the next 120 min. The blood was obtained via a three-way cannula. First syringe to draw off waste, which contains saline with mixed blood, ~2ml. Then ~3ml sample syringe was collected followed by flushing syringe. Whole blood glucose was measured using YSI 2300 STAT PLUS glucose analyser throughout. The remaining of the blood sample was then transferred and gently mixed in an EDTA tube and placed on ice until centrifugation at 4^{o}C for 20 min at 3200 rpm. The aliquoted plasma was stored at -80^{o}C.

2.24.3 Dual-energy X-ray absorptiometry

To have an accurate measurement of training-induced changes in whole-body composition, a whole-body DXA scan was performed on all subjects with an empty bladder. The DXA scan measures whole-body lean tissue mass, fat mass, and bone mineral density (GE Healthcare, LUNAR Prodigy). Scans were analysed via integrated DXA software, with all-regions manually checked by a trained DXA operator for accurate demarcation.

2.24.4 Blood pressure and heart rate

Blood pressure was taken after a prolonged period of rest in the supine position (~2 hours, but >30 min) via non-invasive blood pressure cuff. The result is the mean of three measurements, which were required to be within 10% of each other to be valid. Heart rate was also recorded at this time.

2.24.5 Nutrient provision

To ensure that individuals perform at maximum physical effort during CPET, subjects were given an oral mixed macronutrient feed (Fortisip, Nutridrinks) as a liquid drink – (composed of 16% protein, 49% carbohydrate, and 35% fat), provided as a single bolus ~1 hour prior to CPET testing. The consumed amount and flavour were decided by the participant, however, they were replicated on all subsequent acute studies for each individual.

2.24.6 Cardiopulmonary exercise test

A Lode Corival cycle ergometer with inline gas analysis system (ZAN 600 USB OXI, nSpire Health) was used to assess AT and VO_2max, A mask was fitted to ensure that no air escaped from the mask during the test. An appropriate exercise protocol (watt/min) was determined for each subject according to age, weight, and habitual level of physical activity to ensure that the test lasted for 8-12 min. Participants were provided with details of the test protocol prior to the beginning of the test. There were also verbally encouraged to continue cycling until reaching maximum effort (volitional exhaustion). In addition, a Borg scale (1-10) was used every two min to monitor the rate of perceived exertion (RPE) throughout.

During the test course, heart electrical activity was monitored with a 12-lead ECG system, connected to the CPET. In addition, peripheral capillary oxygen saturation (SpO_2) was monitored with finger clip pulse oximeter. Measurement of resting HR, breathing rate, ECG, and BP, were undertaken during the 3 min rest phase, the 2 min unloaded cycling at 60 rpm (warm-up), and the testing phase when the pre-determined load and incremental protocol was applied.

The observation of a true VO_2 plateau, i.e. when VO_2 does not increase despite the increasing work load, was essential to determine a true VO_2max during the test. In addition, VO_2max was only considered as being achieved if 3 out of the 4 following criteria were met; HR>95% of maximum, respiratory exchange ratio (RER) >1.1, RPE of >9 (modified Borg scale), and failure to maintain 60 rpm at the end of test despite continued verbal encouragement. The average of VO_2 during the last 30 seconds of maximum cycling was taken to represent the VO_2peak measurement. For the AT measurement, an average of three methods, as previously reported[301], was used i.e. conventional V-slope method, respiratory equivalents, and $PETO_2/CO_2$[70]. Those were manually determined by two independent assessors independently of the CPET test.

2.24.7 Muscle architecture

VL muscle was chosen to study the muscle architecture since it is a superficial muscle with clear identifiable fascicles and tendon aponeuroses, which facilitate the measurement of MT, LF, and PA. A standardized *in vivo* image ultrasound protocol as previously described[31] was used to visualise these muscle architecture at the mid-belly of VL. The mid-belly was located as a result of overlapping two marks on the participant's skin. The first mark was 50% of VL length, beginning from the greater trochanter of the femur to the mid-patella (50% of femur

length). The second mark indicated 50% of VL width, which was measured by using 100mm (10-15 MHz) linear-array probe (LA923) from VL's borders. For accurate measurement of the three parameters, the identification of the deep and superficial aponeurosis with clearly visible fibres was necessary. Three images were taken for each of the parameters to improve reliability. Mid-belly MT results were an average of three measurements between the aponeuroses on the same image (Fig 2.1).

Images were collected by the same operator for all of the muscle scans for an individual, i.e. Pre and Post following modes of exercise (EET and RET). The depth that was used in the first scan, was maintained the same in all consecutive scans. Three images were taken for every scan visit to improve reliability.

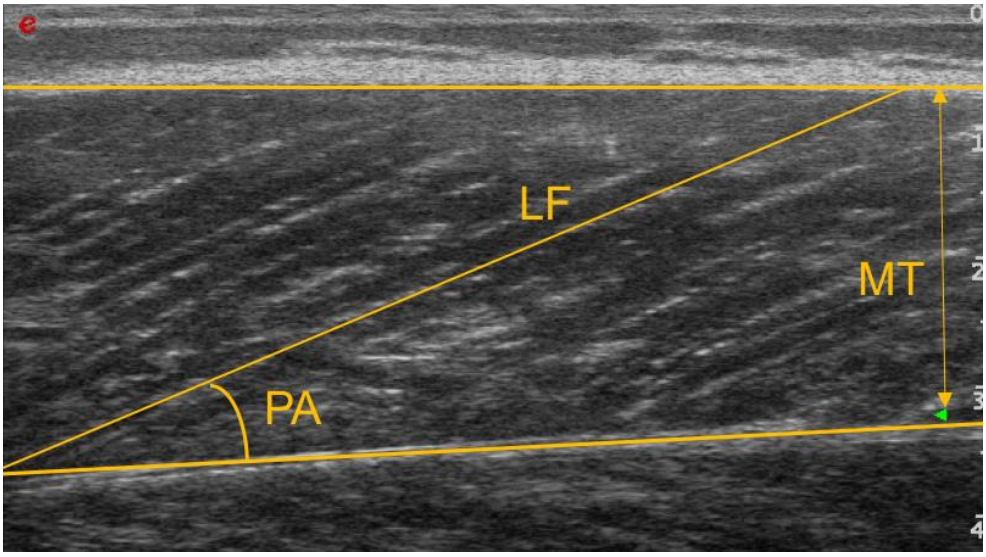

Figure 2.1 Muscle architecture. Ultrasound picture at Vastus lateralis mid-belly. Representative lines show pennation angle (PA), fascicle length (Lf), and muscle thickness (MT).

2.24.8 Dynamometry

Muscle function tests were performed prior to and after 4-weeks of each training mode to assess the change in isometric extensor strength. A Cybex Norm dynamometer, which records the maximum voluntary contraction of the quadriceps, was used to determine the produced torque via an analogue to digital acquisition system (Biopac, System, Inc., Aero Camino Goleta, CA, USA). The dynamometer chair was set up to line the mid-patella (knee pivot) with the dynamometry arm. The position settings were recorded, to be used in the post-training test, before setting the range of motion and weighting the leg. A pre-set program was used, where unilateral MVC measurements were repeated twice for each of the three angles (60^{o}, 70^{o}, and 80^{o}). There was a single repetition to perform before each of the angles to accommodate the task motion. This was followed by the two test repetitions with a 30-second rest, and 1.5 min rest in between angles. Participants were verbally encouraged to give their maximum effort while they were able to visualize their scores on the screen. The highest

(peak) value was obtained from the two repetitions for each angle. 70° MVC results were used as representative of peak isometric strength of the quadriceps[302].

2.24.9 One repetition max

1-RM test was performed at the beginning and at the end of each exercise mode for each of the six exercises. Prior to the 1-RM test, the subjects were familiarised with the apparatus movement by doing 5 repetitions at ~30-40% of their predicted 1-RM. Then according to their rated effort from Borg scale of tested weight, the subsequent load was determined. Subject 1-RM is the maximum weight that participant was able to perform in full range of motion with moderate velocity (1-2 seconds concentric; 1-2 second eccentric), that was achieved in 3-4 attempts with 2-3 min of rest between attempts. Subjects were verbally encouraged to push to their maximum effort in all attempts. Whole-body strength data were presented as Newtons after been calculated by the sum of the 1-RMs multiplied by 0.9807[303].

2.25 Statistical analysis

The data are quoted as means±SD. All data were checked for normal distribution using kolmogorov-Smirnov test of the descriptive analysis. Multiple statistical analysis were performed as appropriate. Unpaired and paired Student's t-test were used to compare Ctrl vs OE and Pre vs Post respectively. Two-way ANOVA was used for time and response with post *hoc test (Sidak's)*. Linear correlation and linear discriminate analysis were used to link fitness to health benefits. In addition, mixed logistics model to calculate odds rates. The analyses were conducted using GraphPad Prism 7 software. A *P* value <0.05 was considered significant.

3 Impacts of rat hind-limb muscle Fndc5/Irisin overexpression on muscle and adipose tissue metabolism

Abstract:

Objective: Myokines - such as irisin - have been purported to exert physiological effects on skeletal muscle in an autocrine/paracrine fashion. In this study, we aimed to investigate the mechanistic role of Fndc5/irisin upregulation in skeletal muscle.

Methods: Overexpression (OE) of Fndc5 in rat hind-limb skeletal muscle was achieved by *in vivo* electro-transfer i.e. bilateral injections of Fndc5 harbouring vectors for OE rats (n=8) and empty vector for control rats (n=8). Seven days later, a bolus of D_2O (7.2ml/kg) was administered via oral gavage to measure muscle protein synthesis. After overnight fast, on day 9, 2-deoxyglucose (2-DG 6mg/kg) was provided during an intraperitoneal glucose tolerance test (IPGTT, 2g/kg) to assess glucose uptake. Animals were euthanized and the *m. tibialis cranialis* (TC) muscle and subcutaneous fat (inguinal) harvested and the metabolic and molecular effects were evaluated.

Results: Muscle Fndc5 gene expression increased with OE (~2-fold; P=0.014), leading to increased circulating irisin (1.5±0.9 to 3.5±1.2 ng/ml, P=0.049). OE had no effect on protein anabolism or mitochondrial biogenesis, however, muscle glycogen was increased, along with GYS1 gene expression (P=0.04 and 0.02, respectively). In addition to an increase in glycogen synthase (GS) activation in OE (P=0.03), there was a trend towards increased GLUT4 protein expression (P=0.09). However, glucose uptake (accumulation of 2DG-6P) was not different. Finally, irisin showed no endocrine effect on mitochondrial biogenesis or uncoupling proteins (UCP) in white adipose tissue.

Conclusion: Our model of dual hind-limb overexpression led to a significant physiological increase in Fndc5/irisin (to levels reported following exercise). Nonetheless, our data indicate limited short-term metabolic impacts of irisin in relation muscle anabolism, mitochondrial biogenesis, glucose uptake or white adipose tissue remodelling. Nonetheless, it cannot be discounted that increases in glycogen may point to some effects in relation to substrate metabolism (longer than IPGTT 90-min).

3.1 Introduction

Skeletal muscle myokines such as BDNF, IL-6, FGF2 and the more recently identified irisin, are purported to exert their actions in an endocrine, paracrine, and autocrine fashion. irisin in particular is a myokine that has received much attention. Irisin is a proteolytic cleavage product of 112 amino acids, with a molecular weight of 12 kDa, derived from its precursor, Fibronectin type III domain containing 5 (Fndc5)[304,305]. Fndc5 is a type I transmembrane glycoprotein consisting of 212 amino acids in humans, and 209 in mice and rats. It is coded by the Fndc5 gene, composed of a signal peptide, two fibronectin type III domains, and a membrane-bound hydrophobic C-terminus. The cleavage product of Fndc5 - irisin - has been associated with promoting weight loss, via increased expression of uncoupling protein 1 (UCP1) in white adipose tissue, by increases non-shivering thermogenesis and energy expenditure in subcutaneous adipose tissue[247]. Moreover, upregulation of Fndc5 in muscle has been linked to the activation of peroxisome proliferator-activated receptor-gamma co-activator-1-alpha (PGC-1α)[246] activation; a regulator of mitochondrial biogenesis. This led to the notion that irisin may underlie certain health promoting and adaptive responses to exercise.

Despite this, the role for irisin underlying the health benefits of exercise and the effect of exercise on Fndc5/irisin expression remains contentious. Huh et al, (2012) showed that exercise training-induced Fndc5 muscle gene expression and increased plasma irisin in humans[5], while a study following the impact of 1-year of physical activity in obese children demonstrated increased irisin concentrations ~12% over baseline[269]. In another study, increased circulating irisin was only transiently observed with high-intensity exercise training (80% of VO_2max), 6 and 19 h post-exercise, whereas, low-intensity exercise (40% of VO_2max) induced a fall in Irisin[306]. In other studies, 8-weeks endurance training elicited an increase in

circulatory irisin in middle-aged and elderly participants but not younger[307]. Similarly, Timmons et al, (2012), showed an increase in Fndc5 gene expression only in elderly subjects undergoing a variety of exercise training regimens[268]. In contrast, others have been unable to demonstrate an effect of exercise training on Fndc5/Irisin[265,271]. Pekkala et al, (2013) showed that neither acute or chronic endurance training, nor combined endurance and resistance training enhanced Fndc5/irisin expression[257]. Data from animal studies are also equivocal, in one study, 6-weeks of free-running enhanced Fndc5 expression on mice skeletal muscle[254], another reported increased irisin levels in normal[250] and obese rats following 8-weeks swim training[250,251], however, another resulted in elevated irisin acutely but not chronically[308]. In contrast 8-weeks of free-running failed to show increased Fndc5 expression; while endurance show increased Fndc5 protein expression muscle without the expected increase irisin secretion[255] and even an acute reduction in Fndc5/irisin levels has been reported after exercise training[309]. Despite these inconsistencies, Fndc5/irisin has been linked to the beneficial effects of exercise including improving lipid profiles[250] and muscle strength[310]. Irisin is also considered to be protective against weight gain, and several studies report a positive correlation to BMI or obesity[259–261,264], however, other studies have observed either negative[252,253] or no correlation[259] to BMI and obesity. Therefore, the regulation of Fndc5/irisin by exercise, and its links to health outcomes including obesity remains contentious and wholly unclear.

Whether Fndc5/irisin exerts its physiological effects in an endocrine or autocrine fashion[304,305], in skeletal muscle is not known. However, *in vitro*, recombinant irisin treatment of muscle cells has been shown to enhance mitochondrial content and oxidative metabolism[311], to induce growth regulatory genes such as insulin-like growth factor-1 (IGF-1)[312], and to augment

glucose uptake and glycogen synthesis[313]. Moreover, *in vivo* treatment of mice with irisin resulted in increases in muscle mass and strength[314]. At the molecular level, increased AMPK phosphorylation with irisin treatment appeared to mediate enhanced glucose uptake[313], while Akt and Erk1/2 pathways were involved in the hypertrophic response, as was reduced Atrogin-1 and MuRF-1 expression[314]. In contrast, systemic elevation of irisin after Fndc5 hepatic adenoviral induction, failed to exert any effects on muscle metabolism[247]. This raises questions as to the specificity of the effects of muscle Fndc5 derived irisin when compared to other tissue derived irisin products and recombinant irisin i.e. which may not reflect muscle Fndc5 expression.

In this study we investigated the potential autocrine role of Fndc5/irisin on skeletal muscle metabolism and the endocrine role on white adipose tissue using a novel approach. We developed an *in vivo* electroporation (IVE) technique to overexpress Fndc5 in dual rat hind-limbs, to determine the impacts on endogenous Irisin, and resulting autocrine/endocrine responses in skeletal muscle and adipose tissue.

3.2 Methods

3.2.1 In vivo electroporation

The transgenic electroporation procedure was performed as previously described[293]. Briefly, *m. tibialis cranialis* (TC) muscles were injected with six separate 50-µl aliquots of Fndc5 DNA prepared in an endotoxin-free sterile saline solution (Qiagen Maxi/Mega-Prep kits, Doncaster, Victoria, Australia). Injections were followed by 80V/cm 100-msec pulses at 1 Hz sequentially via a tweezer electrode attached to an ECM-830 electroporator (BTX Technologies, Inc., Holliston, MA).

3.2.2 Experimental design and ethics

17 Wistar male rats (280-340g) were obtained from Charles River (Margate, UK) and maintained at 22±0.5°C under a 12 hours light/ 12 hours dark cycle on standard chow diet and left for 1 week to acclimatise to their new environment[292]. They were then divided into two groups. The control group (Ctrl, n=8) had bilateral IVE with an empty vector, while the overexpressed group (OE, n=8) had bilateral IVE with Fndc5 vector. In addition, a single control rat was used to provide background enrichment measures for both blood and muscle alanine deuterium labelling prior to the D_2O tracer administration. IVE was performed under isoflurane anaesthesia. 7 days after IVE, a bolus of D_2O was administered by gavage (7.2ml/kg) to both groups. 30 min later, a blood sample was taken to determine peak body water deuterium enrichment. On day 9, all animals were fasted overnight. The following day an intraperitoneal (i.p) glucose tolerance test (IPGTT) was initiated (2g/kg of glucose), along with 2-Deoxy-D-glucose (2DG, 6mg/kg) to assess muscle glucose uptake[292]. Blood and muscle tissues were collected at the end of the procedure after euthanasia (Fig 7). The transfection was performed at the Royal Veterinary College (RVC), University of London. The

procedure was approved by the RVC Ethics and Welfare Committee and was carried out under the UK Home Office license to comply with the Animals Act 1986.

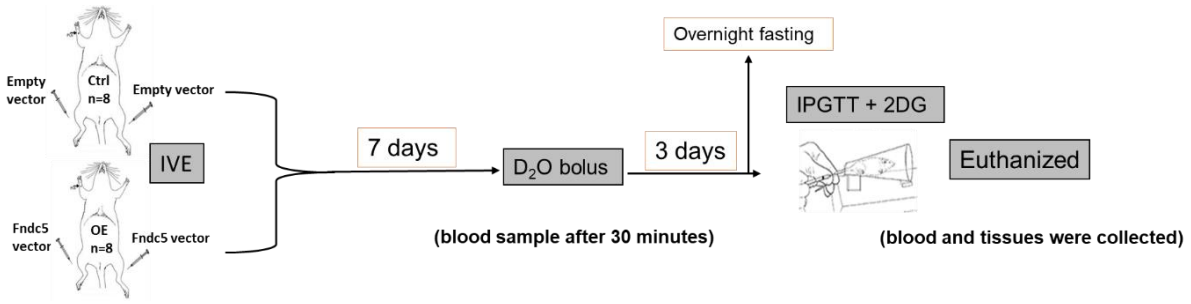

Figure 3.1 Study schematic. Abbreviations: In vivo Electroporation, IVE; Control group, Ctrl; Overexpressed group, OE; Deuterium, D_2O (7.2 ml/kg); Intraperitoneal glucose tolerance test, IPGTT (2 g/kg); 2-deoxy-glucose, 2DG (6 mg/kg).

3.2.3 Molecular analysis

The IVE injections were inserted into the TC muscle, which made it the most effected muscle. Therefore it was chosen as the targets muscle for the overexpression and further analysis. In this study, qPCR technique was used for gene expression on TC and white adipose tissue; immune-blotting was performed on TC muscle homogenate for protein expression. TC muscle was also used in estimating the glycogen content, 2DG6P, mitochondrial density through enzyme assay (CS activity), measuring ATP/Pcr levels, measuring muscle protein, RNA, and DNA. TC was finally used for the isolating, hydrolysing, and derivatizing muscle proteins for FSR calculation. The TC muscle was chosen for the molecular analysis because it was the most effected muscle since it was the site of IVE injections. Rats' plasma were used to measure irisin concentration, 2DG uptake, and D_2O enrichment. The details of previous methods are described in method and materials chapter (2).

3.2.4 Statistical analysis

The data are quoted as means±SD. Comparisons between treated and control muscles were made using either unpaired Student's t-test or two-way ANOVA as appropriate. The analyses were conducted using GraphPad Prism 7 software. A *P* value <0.05 was considered significant.

3.3 Results

3.3.1 Fndc5 gene and protein expression in TC muscle and plasma Irisin concentration

The expression of Fndc5 gene was determined in TC muscles from both groups to determine the success and specificity of the electroporation procedure. A significant increase in Fndc5 expression (2-fold, *P=0.014*, Fig 3.2A) was observed in the TC muscle of OE animals. Additionally, Fndc5 protein (~ 24 kDa) was significantly increased (1.4 fold, *P=0.036*, Fig 3.2B) in the TC muscle of OE animals. Finally, plasma irisin concentration was augmented in OE animals (3.5±1.2 ng/ml) in comparison to control animals (1.5±0.9 ng/ml, *P=0.049*),

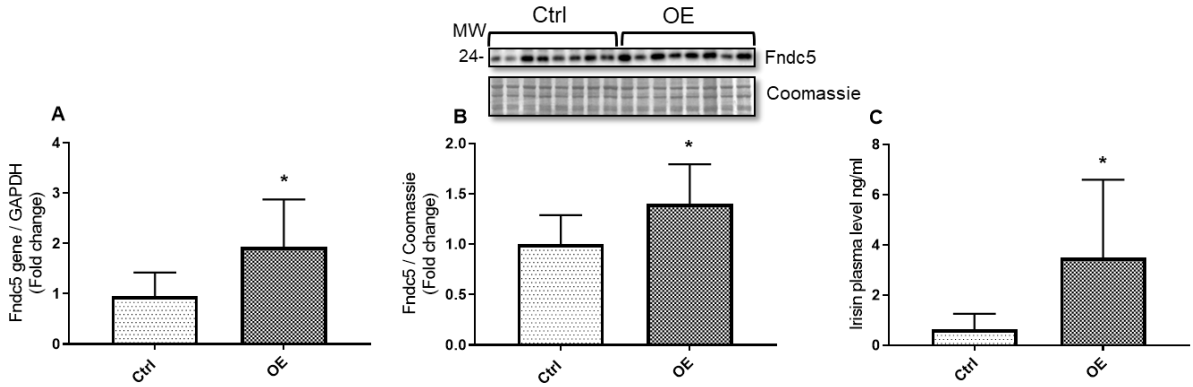

Figure 3.2 Fndc5 expression and Irisin levels in control (Ctrl; n=8) and Fndc5 over expression (OE; n=8) animals. A. Fndc5 mRNA in Tibialis Cranialis (TC) muscle (P=0.014); B. Fndc5 protein expression in TC (P=0.036); C. Plasma irisin concentration (P=0.049). Values are means±SD. Statisitical analysis via unpaired t-test; *=P<0.05 vs Ctrl.

3.3.2 Measurement of glycogen content and factors regulating glycogen levels in muscle

Glycogen content in TC muscle was significantly increased in OE animals (OE 38.5±1.8 vs Ctrl 31.7±2.4 nmol/mg wet weight, *P=0.04*, Fig 3.3A). The gene expression of GYS1 was significantly elevated (20%, *P=0.007*) in OE TC muscle, as was the phosphorylation status of GSK3 Ser21/19 protein (30%, *P=0.039*, Fig 3.3B). Finally, the phospho-GS protein expression by total GS protein expression was decreased in OE animals by (~30%, *P=0.039*, Fig 3.3C), which indicted an increase in GS activity.

3.3.3 Glucose uptake and markers of increased glucose uptake in muscle

Following an IPGTT, the temporal profile (AUC) of plasma glucose was not significantly different between Ctrl and OE animals (Fig 3.3D). Circulating plasma 2DG following IP injection was not different between the groups, nor was the accumulation of 2DG6P in TC muscle (Fig 3.3G). Calculation of fractional extraction was also identical in both groups, suggesting glucose uptake was not different (Fig 3.3H). In support of this there was no difference in the gene expression of GLUT4 or HK2. Finally, no significant change was seen in GLUT4 protein expression or in its up regulators (Fig 3.3F).

3.3.4 Mitochondrial biogenesis in TC muscle

Fndc5 overexpression had no significant effect on the gene expression of PGC-1α, nor its down-stream regulatory genes, NRF and TFAM (Fig 3.4A). There were also no significant changes in muscle UCP2 and UCP3 nor ATP synthesis genes (ATP5J2 and ATP32a) (Fig 3.4B&C). The expression of PGC-1α protein and mitochondrial protein markers such as cytochrome C, SDHA, HSP60, and VDAC were not different between animals (Fig 3.4E). In addition, the electron transport chain complexes (I-IV) were also found to be unaffected by Fndc5 overexpression (Fig 3.4F). Similarly, there was no significant difference in mitochondrial density, assessed by CS activity (Fig 3.4D).

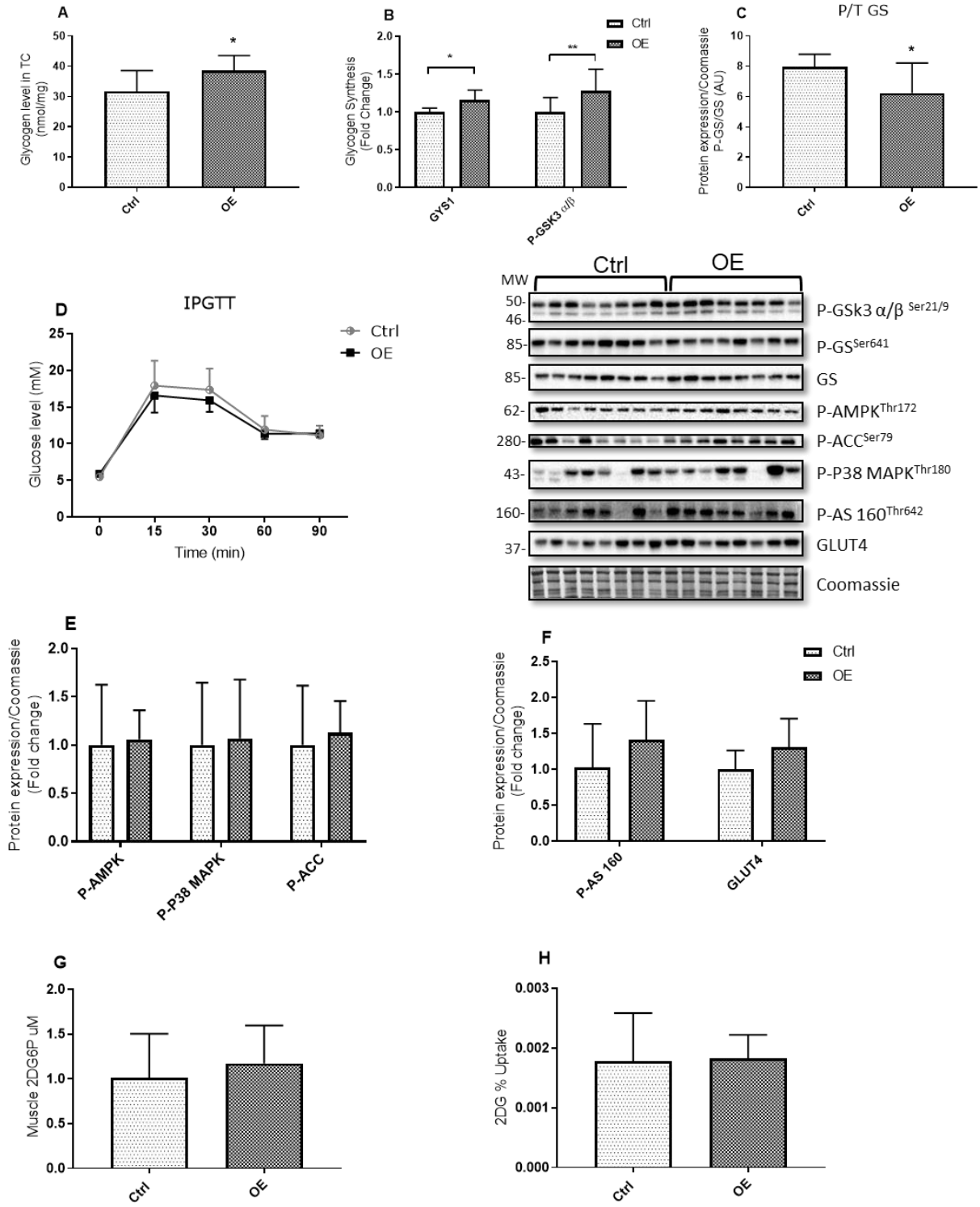
A
Glycogen level in TC (mmol/mg)
Ctrl
OE
B
Glycogen Synthesis (Fold Change)
Ctrl
OE
GYS1
P-GSK3 α/β
C
P/T GS
Protein expression/Coomassie P-GS/GS (AU)
D
IPGTT
Glucose level (mM)
Time (min)
MW
P-GSk3 α/β Ser21/9
P-GS Ser641
GS
P-AMPK Thr172
P-ACC Ser79
P-P38 MAPK Thr180
P-AS 160 Thr642
GLUT4
Coomassie
E
Protein expression/Coomassie (Fold change)
P-AMPK
P-P38 MAPK
P-ACC
F
Protein expression/Coomassie (Fold change)
P-AS 160
GLUT4
G
Muscle 2DG6P uM
H
2DG % Uptake

Figure 3.3 Glycogen content and Glucose uptake in control (Ctrl; n=8) and Fndc5 over expression (OE; n=8) animals. A. Tibialis cranialis (TC) muscle glycogen content; B. Glycogen synthesis 1 (GYS1) and phosphorylated-glycogen synthase kinase 3 α/β (P-GSK3 α/β) gene expression; C. Glycogen synthase (GS) activation (i.e. P-GS divided by GS protein expression); D. Intraperitoneal glucose tolerance test (IPGTT); E. Phosphorylated 5-AMP-activated protein kinase (P-AMPK)^{Thr172}, P-P38 MAPK and P-Acetyl-CoA Carboxylase (P-ACC)^{Ser79}; F. Phosphorylated AS160^{Thr642} and glucose transporter 4 (GLUT4) protein expression; G. TC muscle 2-deoxy-D-glucose 6-phosphate (2DG6P); H. 2DG Fractional uptake (i.e. 2DG6P divided by area under the curve of 2DG plasma). Values are means±SD. Statistical analysis via unpaired t-test; *=P<0.05; **=P<0.01 vs Ctrl.

3.3.5 ATP and phosphocreatine level on TC muscle

No significant differences in ATP or PCr were observed between the two groups, even when normalizing to total creatine (TCr), nor in the ratio of PCr/ ATP (Table 3.1).

3.3.6 Rates of integrated myofibrillar and sarcoplasmic protein synthesis, protein, RNA and DNA content in TC muscle

Integrated protein synthesis of myofibrillar (15.5±2.0%/d in ctrl, 14.4±1.7%/d in OE) and sarcoplasmic (15.7±1.4%/d in Ctrl, 13.9±1.4%/d in OE) fractions from TC muscle, was not different between the groups (Fig 3.5B). In line with this, no differences in alkali soluble protein or nucleic acid content, ASP (87.7±4.9 in Ctrl, 84.2±5.4 in OE), DNA (2.6±0.1 in Ctrl, 2.5±0.1 in OE) and RNA (15.02±0.2 in Ctrl, 15.4±0.2 in OE), all µg mg^{-1} wet weight, were observed. In addition, there were no differences in cell size, estimated from the ratio of total ASP: DNA (33.8±1.31 in Ctrl, 33.5±2.0 in OE), ribosomal capacity from RNA: DNA (0.76±0.04 in Ctrl, 0.75±0.05 in OE) or measures ribosomal efficiency from ratio of RNA: ASP (22.9±1.8 in Ctrl, 23.3±2.5 in OE).

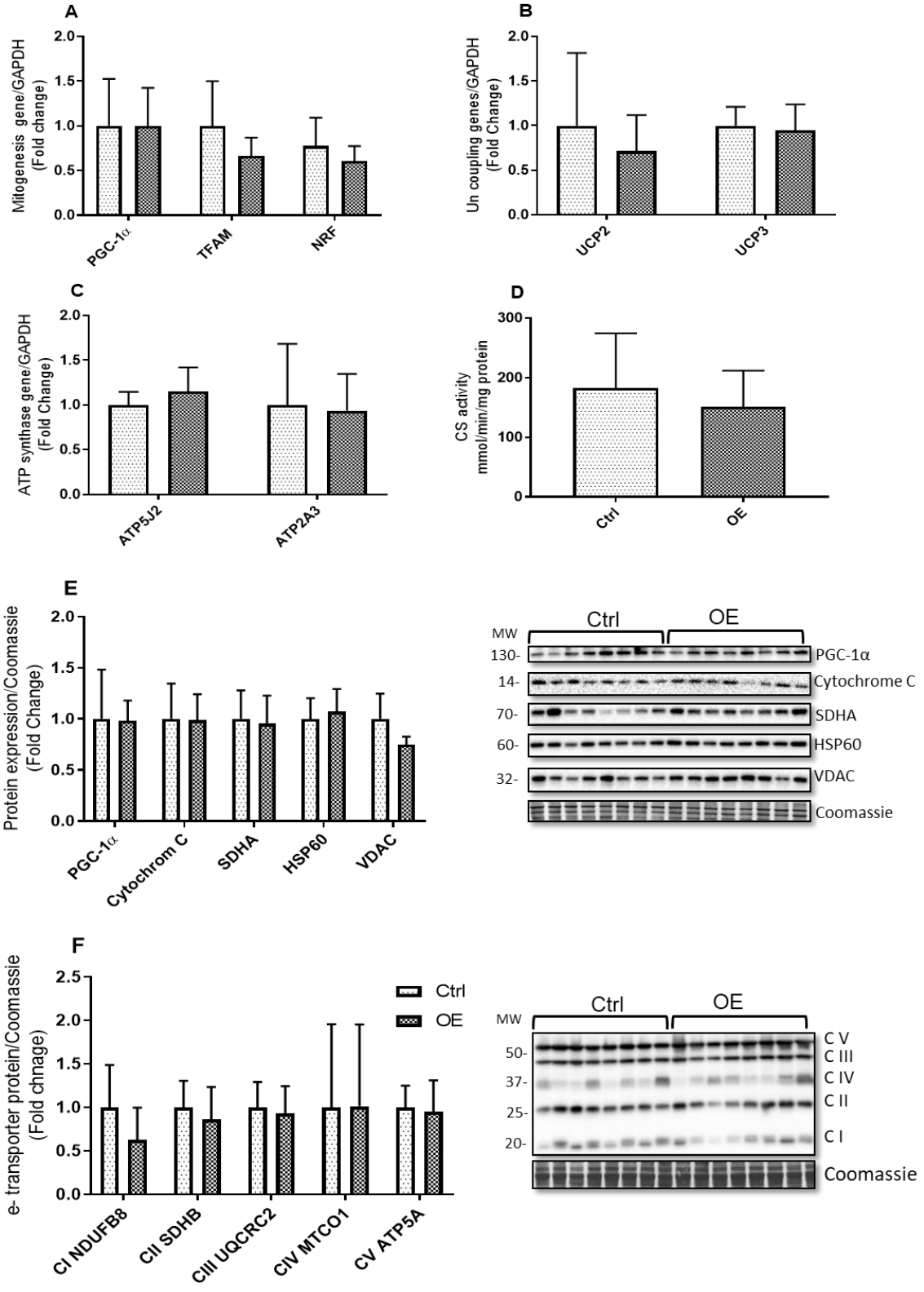
A
Mitogenesis gene/GAPDH (Fold change)
2.0
1.5
1.0
0.5
0.0
PGC-1α
TFAM
NRF
B
Un coupling genes/GAPDH (Fold Change)
UCP2
UCP3
C
ATP synthase gene/GAPDH (Fold Change)
ATP5J2
ATP2A3
D
CS activity mmol/min/mg protein
300
200
100
0
Ctrl
OE
E
Protein expression/Coomassie (Fold Change)
PGC-1α
Cytochrom C
SDHA
HSP60
VDAC
MW
Ctrl
OE
130-
14-
70-
60-
32-
PGC-1α
Cytochrome C
SDHA
HSP60
VDAC
Coomassie
F
e- transporter protein/Coomassie (Fold chnage)
2.5
Ctrl
OE
CI NDUFB8
CII SDHB
CIII UQCRC2
CIV MTCO1
CV ATP5A
MW
50-
37-
25-
20-
C V
C III
C IV
C II
C I
Coomassie

Figure 3.4 Markers of Mitochondrial biogenesis and density in tibialis cranialis (TC) muscle. A. Mitochondrial biogenesis regulatory genes: peroxisome Proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α), nuclear respiratory factors (NRF-1), and mitochondrial transcription factor A (TFAM); B. Gene expression of un-coupling proteins 2 and 3 (UCP2/3); C. ATP synthase genes ATP5J2 and ATP32a; D. Mitochondrial density represented by citrate synthase (CS) activity; E. Protein levels of mitochondrial biogenesis markers: PGC-1α, cytochrome C, succinate dehydrogenase enzyme A (SDHA), heat shock protein 60 (HSP60), and voltage dependent anion channel (VDAC); F. Electron-transporter complexes of mitochondria: CI NADH dehydrogenase ubiquinone 1 beat subunit 8 (NDUFB8), CII Succinate dehydrogenase subunit B (SDHB), CIII ubiquinone cytochrome C reductase complex (UQCRC2), CIV mitochondria encoded cytochrome C oxidase (MTCO1) and CV mitochondria membrane ATP synthases (ATP5A). Values are means±SD. Statisitical analysis via unpaired t-test. No statistically significant differences.

3.3.7 Markers of myogenesis and anabolic cell signalling

The gene expression of MRFs such as MyoD, MyoG, and Mrf4 were not significantly altered by Fndc5 OE. Whilst we observed a significant increase (2.6-fold change) in IGF-1 in the OE animals (P=0.0002, Fig 3.5A). In addition, no significant differences were detected in the Akt-mTOR pathway, or their down-stream signalling proteins, e.g. PRAS40, P70 S6K1, 4E-BP1, and eIF4E (Fig 3.5C&D).

Table 3.1 ATP, phosphocreatine (PCr) and total creatine (TCr) levels in tibialis cranialis (TC) muscle. Values are means±SD. Statistical analysis via unpaired t-test. No statistically significant differences.

	Ctrl	***OE***	***Units***	***P value***
TCr	97.5±8.1	102.2±15.7	mM/mg dry muscle weight	0.49
ATP	19.9±3.6	18.7±4.8	mM/mg dry muscle weight	0.59
PCr	32.9±10.1	32.2±17.4	mM/mg dry muscle weight	0.91
ATP:TCr	0.20±0.03	0.18±0.02	Arbitrary unit	0.19
PCr:TCr	0.33±0.1	0.30±0.1	Arbitrary unit	0.57
PCr:ATP	1.68±0.5	1.62±0.7	Arbitrary unit	0.87

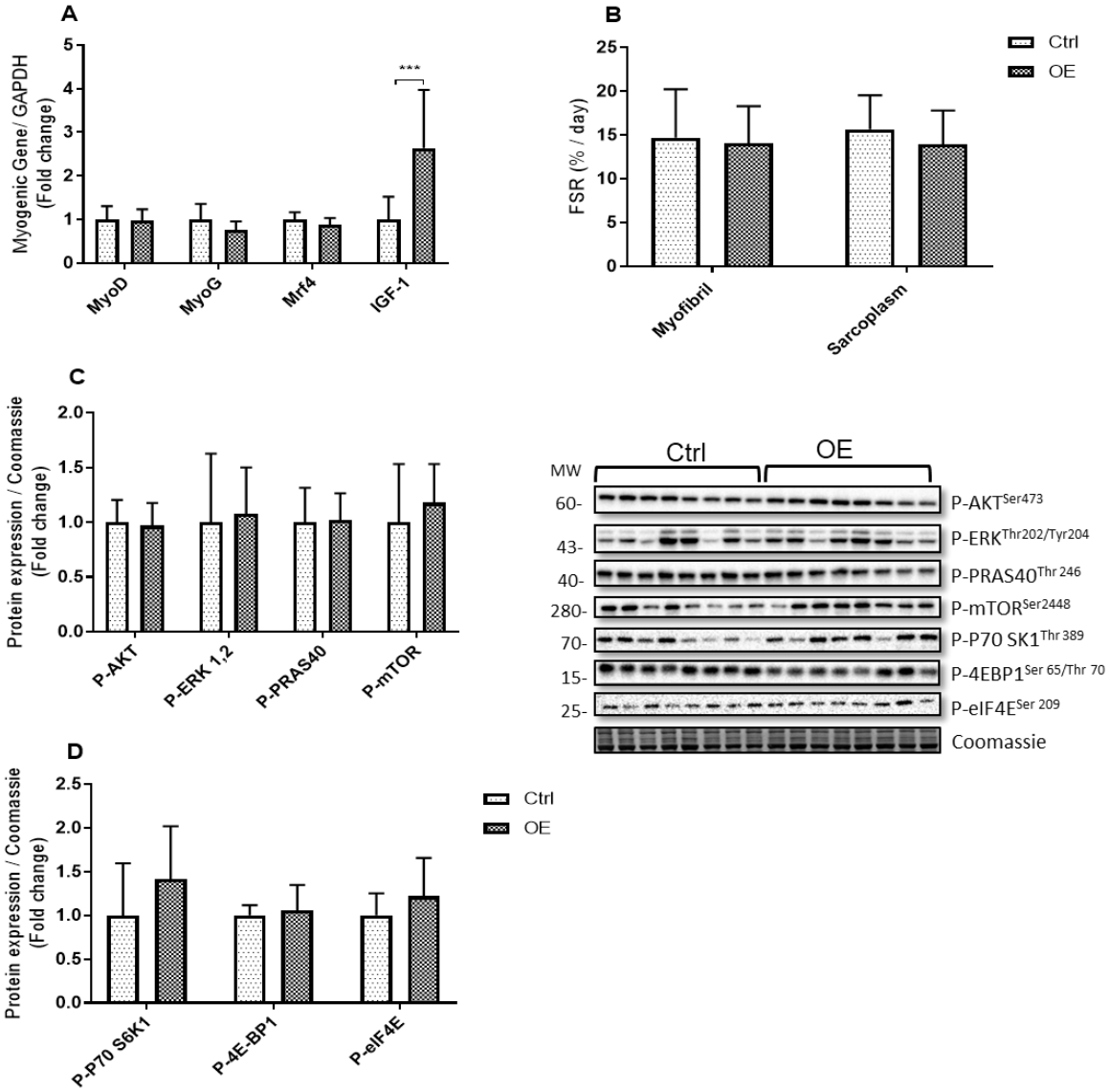

Figure 3.5 Gene expression of muscle regulatory growth factors and insulin like growth factor-1 (IGF-1) in tibialis cranialis (TC) muscle; B. Fractional synthesis rate (FSR) in myofibrillar and sarcoplasmic muscle fractions; C and D. Protein expression of proteins involved in anabolic signalling. Values are means±SD. Statistical analysis via unpaired t-test; ***=P<0.001 vs Ctrl.

3.3.8 Markers of muscle protein breakdown

Fndc5 overexpression had limited impact on markers of muscle catabolism. Gene expression of negative regulators of muscle mass such as Myostatin (MSTN), Follistatin, and regulated in development and DNA damage response 1 (REDD1), apoptosis factors e.g. B cell lymphoma-2 (BCL-2), BAX pro-apoptosis factor (BAX), nor autophagy factors as Atg5 and Atg7, calpain genes like, cysteine-aspartic acid protease (caspase 3) and m-calpain were different between control and OE muscle (Fig 3.6A-D). Finally, the expression of proteins involved in muscle protein catabolism were not different between animals. There were no changes in the phosphorylation of proteins in the autophagy signalling pathway i.e. tumour suppressor 2 (P-TSC2), or protein expression of Beclin-1, light chain 3B (LC3B), Atrogen-1, and muscle ring finger 1(Murf-1) (Fig 3.6E&F).

3.3.9 Markers of mitochondrial biogenesis and fat browning in subcutaneous fat

There were no significant differences observed in genes involved in mitochondrial biogenesis and markers of fat browning, e.g. PGC-1α, NRF, nor TFAM. In addition, there were no significant differences in UCP1 or cell death activator (CIDEA) (Fig 3.7).

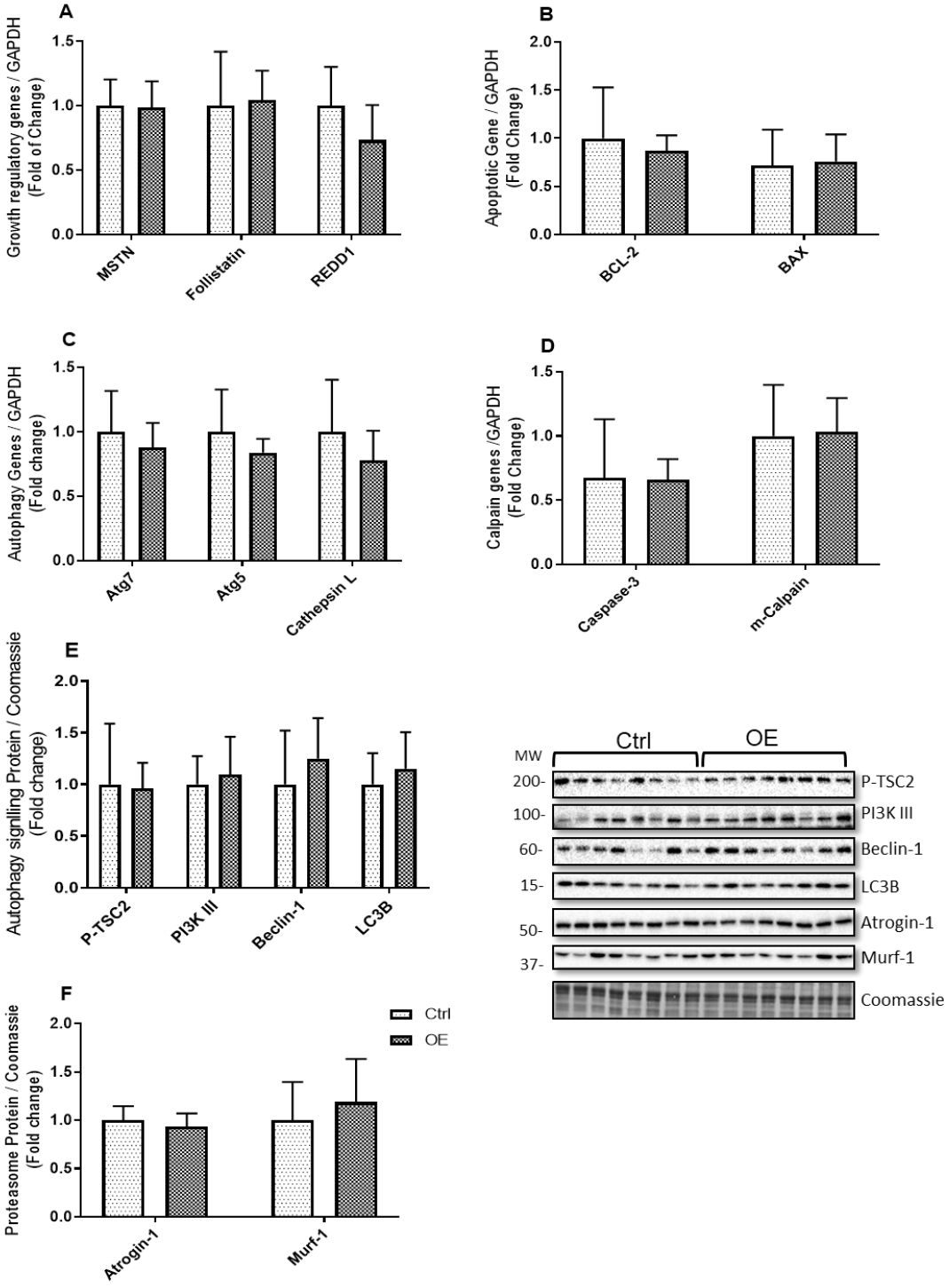
A
Growth regulatory genes / GAPDH (Fold of Change)
1.5
1.0
0.5
0.0
MSTN
Follistatin
REDD1
B
Apoptotic Gene / GAPDH (Fold Change)
2.0
1.5
1.0
0.5
0.0
BCL-2
BAX
C
Autophagy Genes / GAPDH (Fold change)
1.5
1.0
0.5
0.0
Atg7
Atg5
Cathepsin L
D
Calpain genes /GAPDH (Fold Change)
1.5
1.0
0.5
0.0
Caspase-3
m-Calpain
E
Autophagy signlling Protein / Coomassie (Fold change)
2.0
1.5
1.0
0.5
0.0
P-TSC2
PI3K III
Beclin-1
LC3B
Ctrl
OE
MW
200-
100-
60-
15-
50-
37-
P-TSC2
PI3K III
Beclin-1
LC3B
Atrogin-1
Murf-1
Coomassie
F
Proteasome Protein / Coomassie (Fold change)
2.0
1.5
1.0
0.5
0.0
Ctrl
OE
Atrogin-1
Murf-1

Figure 3.6 Gene expression levels in tibialis cranialis (TC) muscle of: A. negative growth regulatory factors: myostatin (MSTN), follistin and regulated in development and DNA damage response 1 (REDD1); B. apoptosis factors: B cell lymphoma 2 (BCL-2) apoptosis suppressor and BAX pro-apoptosis factor (BAX); C. autophagy-related genes: Atg7, Atg5 and cathepsin L; D. calpain factors: cysteine-aspartic acid protease (caspase 3) and m-calpain. E and F. Protein expression of autophagy signalling pathway components: phosphorylated tuberin or tumour suppressor 2 (P-TSC2), phosphoinositide 3-kinase class III (PI3K III), beclin-1, autophagy marker light chain 3 (LC3B), atrogen-1, and muscle ring finger protein-1 (Murf-1). Values are means±SD. Statistical analysis via unpaired t-test. No statistically significant differences.

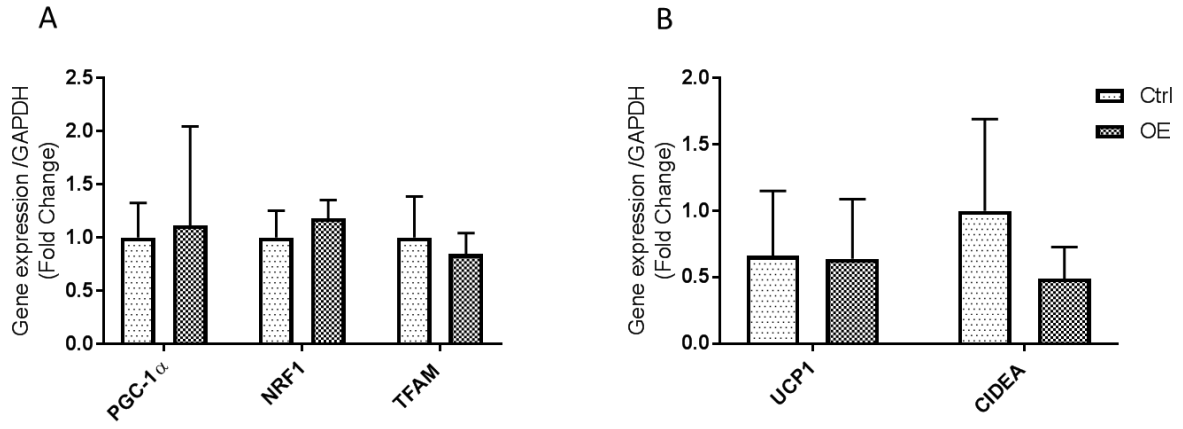

Figure 3.7 Markers of mitochondrial biogenesis in subcautenous fat: A. Proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α), nuclear respiratory factors (NRF), and mitochondrial transcription factor A (TFAM); B. uncoupling protein 1 (UCP1) and cell death activator A (CIDEA). Values are means±SD. Statisitical analysis via unpaired t-test. No statistically significant differences.

3.4 Discussion

Our model of overexpression increased Fndc5 gene expression (~2 fold) to a similar extent reported in humans[257,270,315] and mice[315] following an exercise intervention i.e. a physiologically relevant increase. For example, 8-weeks of treadmill exercise[316] increased Fndc5 expression in mouse skeletal muscle ~1.6 fold. In addition, circulating plasma irisin (3.5ng/ml) in the OE group was similar to concentrations measured (by mass spectrometry) following an exercise intervention (3.6-4.3ng/ml)[317]. Although others have reported irisin concentrations ranging from 0.01-2000 ng/ml in human[246] and 6-1900 ng/ml in rats[249,250,318,319], these are likely the result of technical issues surrounding previously used ELISA kits[246], and perhaps the timing of the irisin measurement[308]. In sum, our model achieved physiologically relevant increases in Fndc5/Irisin, justifying our aims to investigate underlying changes in muscle and adipose tissue.

We report a significant increase in glycogen content (~20%) in skeletal muscle 10 days following Fndc5 OE. Treatment with recombinant irisin has been shown to increased glycogen storage in livers of diabetic mice[140]. Glycogen content is regulated by glycogen synthesis or glycogenolysis[16]. The expression of GYS1 indicates an increase in glycogen synthesis in our OE model, as in Huh et al (2014)[313]. Despite the complexity of the glycogen synthesis signalling pathway, GS is the central regulator and is deactivated by phosphorylation at Ser641. Therefore a decrease in the ratio of phospho-GS/GS suggests GS is activated, as observed our model, and also with irisin treatment in HepG2 cells[140]. GSK3α/β down-regulates GS activation, however phosphorylation at Ser21/9 (P-GSK3α/β) inhibits this effect[320] that falls in line with our observations and also those of Liu et al (2015), thereby supporting the increase in GS activity in our OE model, or with irisin treatment. However, we observed no sign of

insulin (Akt/PI3K) involvement as Liu et al (2015), reported[140]. Crucially, the accumulation of 2-DG6P was identical in both groups, indicating that glucose uptake was similar during the IPGTT in both groups, at least during the 90 min IPGTT, suggesting increased glucose uptake was not responsible for the increased TC muscle glycogen content. Similarly, systemic glucose disposal/handling was also similar in both groups, in line with other studies where an improvement in systemic glucose disposal has been observed only in diabetic animals with both irisin recombinant-treatment and Fndc5 viral induction overexpression[140,315,321]. This may indicate that Fndc5/irisin (within the physiological range) has no/limited impacts on muscle glucose uptake; nonetheless, the increase in glycogen may point to some effects in relation to substrate metabolism.

The next area of investigation related to mitochondrial biogenesis. For example, it was previously reported that recombinant irisin treatment (5nM) of C2C12 cells induced expression of PGC-1α, NRF, TFAM, and UCP3 after 24 hours increasing mitochondrial content[311]. In contrast, irisin treatment (10 and 50nM) in human primary skeletal muscle cells did not impact PGC-1α expression[92]. Similarly, in our study, we observed no difference in PGC-1α or any of its down-stream targets, with no difference in CS activity (mitochondrial content proxy[322]) or electron-transport chain complexes. Our data reveal that local Fndc5 overexpression in skeletal muscle, as in our model, or systemically as in Bostrom et al, with an increase in circulating irisin level, has no impact on muscle mitochondrial biogenesis using *in vivo* models of Fndc5 induction[247].

Another area purported to be under Fndc5/irisin's regulation is the control of muscle mass. For instance, Irisin, has been shown to increase muscle weight, strength, and cross-sectional area in mice[314]. On a molecular level, irisin treatment of primary human skeletal muscle cells

was shown to increase mRNA expression of IGF-1, a growth factor that stimulates cellular proliferation and hypertrophy[323], while decreasing mRNA expression of myostatin, a negative regulator of muscle growth[312]. Yet despite our observation of increased IGF-1 mRNA with Fndc5 OE, there was no effect on myostatin, nor on growth myogenic regulatory factors e.g. MyoD and MyoG. The lack of an irisin effect on MyoD and MyoG was also reported *in vitro*, where irisin treatment increased myotube size but not the expression of myogenic factors[314]. Similalry, our end-points (MPS and muscle protein and nucleic acid content) do not infer a role for Fndc5/Irisin; integrated MPS, measured over the 10 days following electroporation, was identical in control and OE TC muscle, with protein: DNA, RNA: protein, RNA:DNA ratios not different between groups. Despite reports of irisin treatment having an effect on both ERK1/2[312,314] and Akt[314] signalling we did not observe this. Similarly, although, supra-physiological recombinant irisin (1000ng/ml) treatment for 48 hours decreased expression of MPB-related factors, Atrogin-1, and Murf-1 in C2C12 cells[314], the physiological levels of Fndc5/irisin expression, used in this study, had limited impact on markers of muscle proteolysis. Overall these data do not support the notion of anabolic properties of Fndc5/irisin when at physiological concentrations.

Finally, given the proposed endocrine function of irisin in browning white adipose (WAT) tissue, we investigated the effects of muscle hind-limb OE of Fndc5 and increases in circulating irisin in relation to WAT biomarkers of browning. Bostrom et al (2012) reported that increased circulating irisin after adenoviral injection of Fndc5 gene enhanced UCP1 and CIDEA in subcutaneous adipose tissue with significant effects also seen on PGC-1α[247]; we saw no evidence of this in our model. Increases in UCP1 were also reported in another *in vivo* study with evidence of enhancing lipolysis[324]; again our data do not support this. However,

both studies used systemic induction of Fndc5, which may raise irisin levels beyond those found physiologically.

3.5 Conclusion

A dual hind-limb OE of Fndc5 in muscle led to an increase in circulating irisin (similar to that after exercise training), with had no evidence of effects on mitochondrial biogenesis or protein anabolism. Moderate effects noted on glycogen content could not be explained by acute insulin-stimulated glucose uptake. In addition, short-term induction of plasma irisin (~10 days) had no significant impact on browning biomarkers in WAT. Our data collectively indicate that physiological increases in Fndc5/irisin may not be sufficient to induce gross metabolic effects, but this data does not preclude a role for irisin in combination with other exercise factors/myokines, or in relation to more prolonged OE approaches.

4 Myokine responses to acute and chronic exercise in rats selectively inbred for polygenic traits yielding low and high aerobic capacity gains

Abstract:

Objective: To determine the potential role of myokines in adaptive responses to exercise, we adopted the 'low responder trainer' (LRT) and 'high responder trainer' (HRT) model. We hypothesised that HRT would exhibit more pronounced plasma myokine responses to LRT animals, representing greater aerobic adaptive capacity.

Methods: LRT and HRT (N=8) rats were selectively inbred for adaptive capacity to running training. Blood was collected at baseline (BL), immediately (IM), 1h, and 3h after a bout of exercise, and this was repeated after 3-weeks training. Plasma was analysed by multiplex (BDNF, Fractalkine, SPARC, Irisin, FGF21 and Musclin) and (IL-6) ELISA. Data were analysed by two-way repeated measure ANOVA with multi-comparison analysis by post hoc (Sidak's tests); $P<0.05$ was considered significant.

Results: At baseline concentrations of musclin (LRT 83.5±24 vs HRT 25.8±2.5 pg/ml P=0.05) and FGF2 (LRT 133±34 vs HRT 63.5±13 pg/ml P=0.08) were higher in LRT than HRT animals. The training intervention increased musclin baseline concentration in HRT animals (Post-training 53.6±9.2 pg/ml P=0.01) and decreased FGF21 baseline level in LRT animals (Post-training 60±28 pg/ml P=0.02). In addition, training intervention augmented SPARC (LRT Pre-training 0.83±0.09 vs Post-training 2.1±0.6 ng/ml P=0.03, HRT Pre-training 0.70±0.06 vs Post-training 1.8±0.3 ng/ml P=0.06) and irisin (LRT Pre-training 0.62±0.1 vs Post-training 2.6±0.4 ng/ml P=0.008, HRT Pre-training 0.53±0.1 vs Post-training 2.8±0.7 ng/ml) baseline level in both groups and caused generalised decreased in BDNF (LRT Pre-training 2747±293 vs Post-training 1081±330 pg/ml P=0.003, HRT, Pre-training 1976±328 vs Post-training 797±160 pg/ml P=0.02). Musclin acute temporal response expressed as area under the curve (AUC),

was higher in LRT animals pre-training in compare to HRT (LRT Pre-training 306±74 vs HRT Pre-training 88±12 pg/ml×3h^{-1} P=0.009), but HRT post-training AUC increased (Post-training 221±31 pg/ml×3h^{-1} P=0.003). The training intervention also elevated the AUC of SPARC (LRT Pre-training 2.4±0.1 vs Post-training 7.7±1.3 ng/ml×3h^{-1} P=0.02, HRT Pre-training 2.5 ± 0.13 vs Post-training 11.2 ± 2.2 ng/ml×3h^{-1} P=0.0009) and irisin (LRT Pre-training 1.34±0.3 vs 9.6±1.7 ng/ml×3h^{-1} P=0.0002, HRT Pre-training 1.5±0.5 vs 12.1±1.9 ng/ml×3h^{-1} P<0.0001) in both groups.

Conclusion: Exercise training caused an acute and chronic changes on the myokines plasma concentration. In general, myokines response could not be linked to gross adaptive potential in aerobic capacity, making them an unlikely biomarker of performance. However, the baseline levels of myokines could define the metabolic health of individuals as in FGF21 and musclin. Also, musclin was suggesting a possible role in recognizing individual training capacity.

4.1 Introduction

In the previous chapter, we focused on a single myokine, Fndc5, and its autocrine and endocrine effect/lack of effect on local skeletal muscle and adipose tissue respectively. Although, this simplifies the notion there are many myokines had been reported to be realised with contractile activity. The basis of this chapter is to explore more broadly the response of myokines release to exercise training in unique model that with highly distinctive response to exercise.

The physiological adaptations to standardized exercise training are highly variable within individuals. Whereas, some individuals show high improvements in health indices, others show a continuum of responses, from moderate, no change, or even adverse effects. In most studies, the average value is used to represent the overall beneficial outcome of exercise training, which covers the heterogeneity among individual responses. For instance, a ~25% increase in maximal oxygen uptake (VO_2max) was reported in two different studies after endurance training intervention. However, individual responses varied from no change to 50%[279] and 58%[278] of improvement in young and elderly respectively. At the two extremes, there are always individuals who show large improvements, “high responders”, and individuals who show no improvement or even a reduced performance, “low responder”[280].

It is difficult to elucidate the factors that regulates the responses to exercise training as they are determined by a complex interaction of genes and environment. Empirically, exercise capacity is under the control of two types of genetics factors. Intrinsic factors that operate in the sedentary state and extrinsic factors that develop as result of an adaptive response to any physical activity above the sedentary level[325].

The intrinsic factors are well studied through the HERITAGE family studies[275]. On the other hand, a little is known about the extrinsic elements role in exercise capacity. Therefore, an animal model was developed, via artificial selective breeding, wherein rats were elected according to their performance, or lack of, i.e. maximal running distance, during a running to exhaustion treadmill test, to develop low and high trainability in offspring from a heterogeneous rat population (N/NIH). LRT/HRT animals exhibited similar exercise capacities, by running a similar distance, at baseline. However, after 8-weeks of endurance training, HRT animals were able to run 200 meters more, while LRT animals showed a decline in running capacity by -65 meters[325].

Exercise capacity through VO_2max is a strong indicator of morbidity and survivability[326]. Indeed the improvement in VO_2max following aerobic exercise training is linked with the mean life span in animals[327,328] and humans[329,330]. Consequently, LRT animals were prone to increased metabolic dysfunction e.g. insulin resistance, obesity and reduced muscle angiogenesis. Although, these metabolic dysfunctions were not associated with the mitochondrial adaptive response[79], but could be influenced by other factors as chemokines.

It is well known that exercise training initiates a plethora of beneficial adaptive responses. In muscle, for example, aerobic and resistance exercise training improves skeletal muscle endurance and strength respectively, and both mode training enhance skeletal muscle metabolism of glucose and fat[331,95]. Thus, exercise training reduces the risk of chronic diseases such as obesity, type 2 diabetes (T2D), and cardiovascular diseases[305]. Whilst the impact of exercise locally on muscle is perhaps obvious, in terms of increased mass, improved strength and metabolic regulation i.e. glucose and lipid metabolism, how exercise training induces improvements at the whole-body level resulting in benefits to overall health and

wellbeing, is poorly understood. One hypothesis, that has gained a lot of interest, is that muscle produces “exercise factors” after a bout/bouts of exercise - these myokines are released from muscle and act upon tissues locally, in an auto/paracrine fashion, or distally via the bloodstream in and endocrine fashion. Later, a large numbers of myokines have been found to be expressed in, and secreted from skeletal muscle in response to exercise[180,181], though it is not clear which of these myokines can be thought of a true “exercise factors” and which act positively on which tissue.

The implication is that myokines help to build a communication axis between skeletal muscle and remote tissues and organs like liver, adipose tissue, heart, brain, and vascular to exert their effects through their endocrine fashion. For instance, increased circulated irisin enhances browning of subcutaneous white adipose tissue[315], circulated IL-6 improves hepatic glucose release[187], SPARC increases bone formation and density, and BDNF improves cognitive ability[222]. In addition, myokines as IL-6, irisin, SPARC, and BDNF also demonstrate paracrine/autocrine effects on skeletal muscle, improving glucose uptake, and mitochondrial respiration which consequently lead to improved insulin sensitivity. Furthermore, irisin, SPARC, IL-15, and leukaemia inhibitory factors have been suggested to induces muscle hypertrophy[181,186,185]. Nonetheless, impact of training capacity on myokine’s plasma concentration is ill defined. Aiming to further define their role, we adopted a rat model of extreme spectrums in adaptive responses to exercise. We hypothesised HRT animals would display a favourable (according to its role) circulating plasma myokine profile in responses to exercise training that would be sustained following 3-weeks of intervention, possibly reflecting greater adaptive potential and improved health status at baseline.

4.2 Methods

4.2.1 Experiment design and ethics

The LRT/HRT animals were studied; these animals were developed by selective breeding and maintained by Koch and Britton at the University of Michigan[325]. After 2-weeks of acclimatization period, total of 16 female rats from the 24th generation (n=8 LRT, and n=8 HRT) went to an acute bout of exercise, included 30 min of treadmill running (15% incline) at moderated speed (17m/min) and targeted 60~70% of VO_2max. Blood samples were collected prior to exercise training bout (Pre-Training (baseline)) and immediately after, then again at 1h and 3h (Pre-training). Animals went then through daily exercise session of 3-weeks training intervention (exercise bout as described above). During the last exercise session, blood samples were collected in similar pattern to the first acute bout of exercise above (Post-training) (Fig 4.1). Animals had an exercise capacity test before and after the training intervention to calculate exercise response (Fig 4.2). All procedure were carried in accordance with University Committee on Use and Care of Animals at University of Michigan.

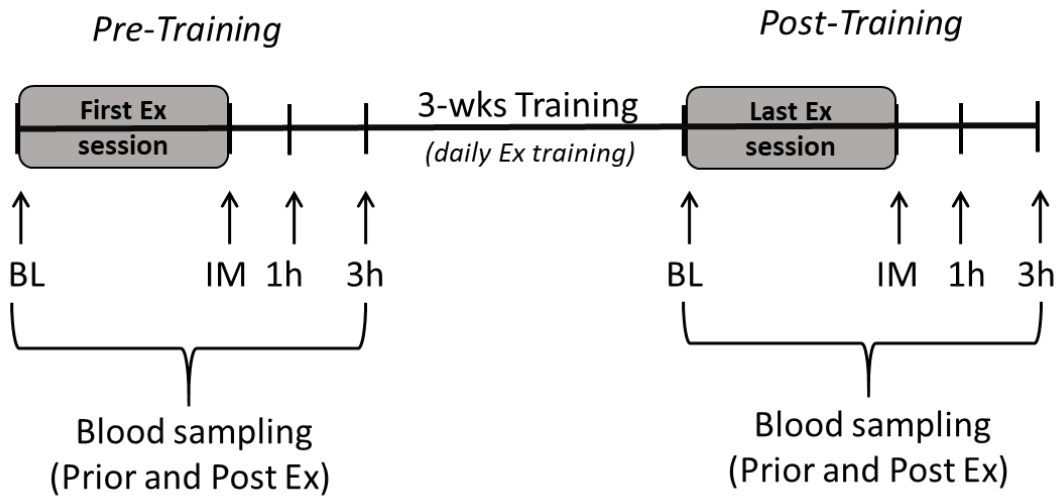

Figure 4.1 Experiment design of myokines concentration to acute and chronic treadmill training of low and high response trainers (LRT-HRT) animals. Baseline (BL) blood samples.

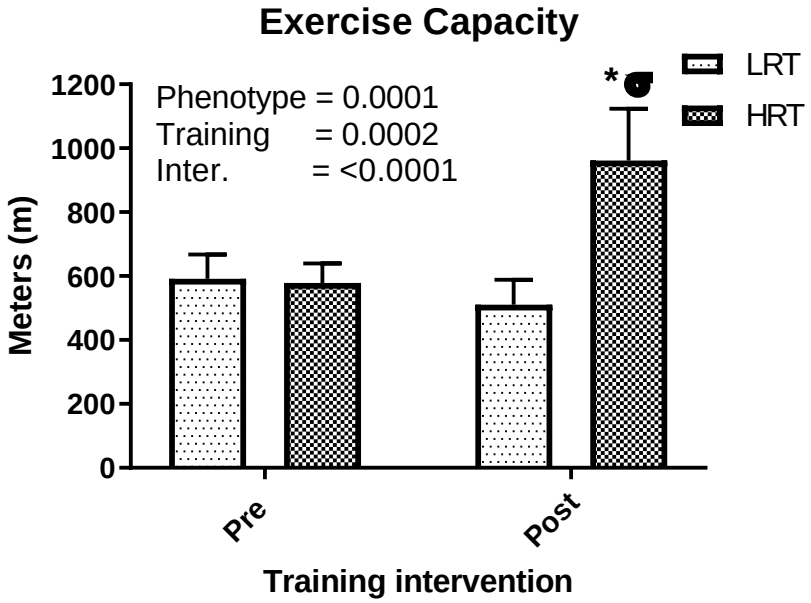

Figure 4.2 Change in exercise capacity (meters) in low response trainers (LRT) and High response trainers (HRT). *****=Significant difference between animal groups. **σ**= significant change from the (Pre) training level. Values are means±SD. P<0.05 is considered significant. Two way ANOVA was used with post hoc test (Sidak's).

4.2.2 Enzyme linked immune sensitive assay

The Multiplex ELISA (Rat Myokine Magnetic Beads Panel, cat # RMYOMAG-88K) was used to detect a multiple-analytes in the same sample. However, for detection range limitation, IL-6 ELISA kit was used (BMS625, ThermoFisher). More details of ELISA protocols are in materials and methods chapter (2).

4.3 Results

4.3.1 Plasma Myokine concentrations

Of the 12 Myokines included in the Multiplex ELISA kit, only 6 were detectable in plasma from pre-clinical models, i.e. irisin, BDNF, SPARC, Fractalkine, FGF21, and musclin (Rat Myokine Magnetic Beads Panel, cat # RMYOMAG-88K). The following were below the limits of detection (Myostatin, IL-15, FSTL, Erythropoietin, and LIF). IL-6 was measured separately using an ELISA kit (BMS625, ThermoFisher).

BDNF concentrations at baseline, Pre and Post training, were similar between the LRT and HRT groups. However, the training intervention reduced the baseline concentrations significantly in both animals. (LRT Pre-training 2747±293 vs Post-training 1081±330 pg/ml *P=0.003*, HRT Pre-training 1976±328 vs Post-training 797±160 pg/ml *P=0.02*) (Fig 4.3A). Similarly, the acute temporal response to exercise training did not show any significant differences between the groups at pre and post training intervention when expressed as AUC. However, the total AUC dropped in both groups after the training intervention. (LRT Pre-training 7127±1015 vs Post-training 3028±681 pg/ml×3h^{-1} *P=0.0002*, HRT Pre-training 5918±872 vs 3429±448 pg/ml×3h^{-1} *P=0.012*) (Fig 4.4A, A).

Fractalkine baseline concentration were not different between animals Pre or Post training and there was no impact of training on these concentrations. Regarding the acute temporal response post the first training session, Fractalkine concentrations dropped at three hours in both groups (LRT BL 63±7.7 vs 3h 35.4±2.7 pg/ml *P=0.01*, HRT BL 72.5±6.7 vs 3h 40.8±2.3 pg/ml *P=0.001*). Although, the temporal response for the last training session showed an immediate increase in HRT animals (BL 70±7.5 vs IM 100±15.4 pg/ml *P=0.02*) that returned to baseline at (1h 74±5.8 vs IM 100±15.4 pg/ml *P=0.05*) and (3h 73.6±13.6 vs IM 100±15.4 pg/ml *P=0.05*), no changes were observed in the LRT group. AUC data suggested no phenotyping nor training effect on the temporal response despite the greater post training AUC in HRT animals (Post-training LRT 166±25 vs HRT Post-training 234±26 pg/ml×$3h^{-1}$ *P=0.05*) (Fig 4.4A, B).

IL-6 baseline concentrations were similar between animals Pre and Post training with no effect of training on baseline levels. The temporal response for the first training session showed an immediate increase (LRT BL 161.8±28 vs IM 350±23 pg/ml *P<0.0001*) which was sustained at (1h 283±47 vs BL 161.8±28 pg/ml *P=0.01*) from BL in LRT but not HRT animals, this was followed by a decrease at (3h 237±48 vs IM 350±23 pg/ml *P=0.02*). The post-training session demonstrated an initial increase (HRT BL 138±25 vs IM 230±37 pg/ml *P=0.04*) in HRT animals, this returned to baseline at (3h 140±37.6 vs IM 230±37.5 pg/ml *P=0.05*). However, AUC data suggest no effect of phenotype nor training on temporal response (Fig 4.4A, C).

SPARC baseline concentrations were similar across animals but significant increase were observed post training on the baseline level in both groups (LRT Pre-training 0.83±0.09 vs Post-training 2.1±0.6 ng/ml *P=0.03*, HRT Pre-training 0.70±0.06 vs Post-training 1.8±0.3 ng/ml *P=0.06*) (Fig 4.3B). Parallel to the increase in baseline after training, the total AUC increased

in both groups (LRT Pre-training 2.4±0.1 vs Post-training 7.7±1.3 ng/ml×3h^{-1} *P=0.02*, HRT Pre-training 2.5±0.13 vs Post-training 11.2 ± 2.2 ng/ml×3h^{-1} *P=0.0009*). Regardless the similarity in AUC between groups, in HRT animals SPARC was elevated immediately post exercise during both the first training session (HRT BL 0.7±0.06 vs IM 1.0±0.07 ng/ml *P=0.01*) and last training session (HRT BL 1.8±0.3 vs IM 5±0.9 ng/ml *P=0.007*), and remained higher in the last training session at 3h (3h 4.0±1.2 vs BL 1.8±0.3 ng/ml *P=0.02*) (Fig 4.4A, D).

Irisin baseline concentrations were not different between animals at either Pre or Post training. Also, the training had a similar impact on the baseline concentrations in both groups (LRT Pre-training 0.62±0.1 vs Post-training 2.6±0.4 ng/ml *P=0.008*, HRT Pre-training 0.53±0.1 vs Post-training 2.8±0.7 ng/ml *P=0.003*) (Fig 4.3C). The temporal pattern was similar in both groups. Although there was a clear increase after training in AUC in both groups (LRT Pre-training 1.34±0.3 vs 9.6±1.7 ng/ml×3h^{-1} *P=0.0002*, HRT Pre-training 1.5±0.5 vs 12.1±1.9 ng/ml×3h^{-1} *P<0.0001*). Surprisingly irisin declined significantly in HRT animals at 1h during the pre-training acute bout of exercise (HRT IM 1.2±0.1 vs 0.26±0.1 ng/ml *P=0.01*), but not significant in LRT (Fig 4.4B, E).

FGF21 baseline concentrations tended to be higher in LRT than HRT animals (LRT Pre-training 133±34 vs HRT Pre-training 63.5±13 pg/ml, *P=0.08*). However, baseline levels after training were similar between animals. The training impacted FGF21 in the LRT group by decreasing the baseline concentration (Pre-training 133±34 vs Post-training 60±28 pg/ml, *P=0.02*) (Fig 4.3D). The temporal changes were similar across animals at Pre and Post the training intervention except for a single point during the first training session, at 1h the LRT had a higher concentration than HRT (LRT 1h 254±90 vs HRT 1h 110±21 pg/ml *P=0.03*). While in the last training session, both groups showed immediate elevation from baseline (LRT

BL 60±28 vs IM 235±43 pg/ml *P<0.0001*, HRT BL 48±7 vs IM 177±41 pg/ml *P=0.003*), and total AUC was identical (Fig 4.4B, F).

Musclin concentrations at baseline were significantly different between animals (LRT Pre-training 83.5±24 vs HRT Pre-training 25.8±2.5 pg/ml *P=0.05*). In addition, the training exerted a significant effect on baseline concentrations in HRT animals (Pre-training 25.8±2.5 vs Post-training 53.6±9.2 pg/ml *P=0.01*) (Fig 4.3E). The Pre-training acute temporal response was higher in LRT animals than HRT (LRT Pre-training 306±74 vs HRT Pre-training 88±12 pg/ml×3h^{-1} *P=0.009*). Further, a significant impact of training was clear in HRT animals. HRT AUC increased (Pre-training 88±12 vs Post-training 221±31 pg/ml×3h^{-1} *P=0.003*). During both training sessions, a significant immediate increase was noticed only with LRT animals (Pre-training BL 83.5±24 vs IM 192±51 pg/ml *P<0.0001*, Post-training BL 95.9±22 vs IM 159±32 pg/ml *P<0.0001*), where significant different between the groups was seen as well. (Pre-training LRT IM 192±51 vs Pre-training HRT IM 41.6±6.5 pg/ml *P<0.0001*, Post-training LRT IM 159±32 vs Post-training HRT IM 77.2±6.9 pg/ml *P=0.007*) (Fig 4.4B, G).

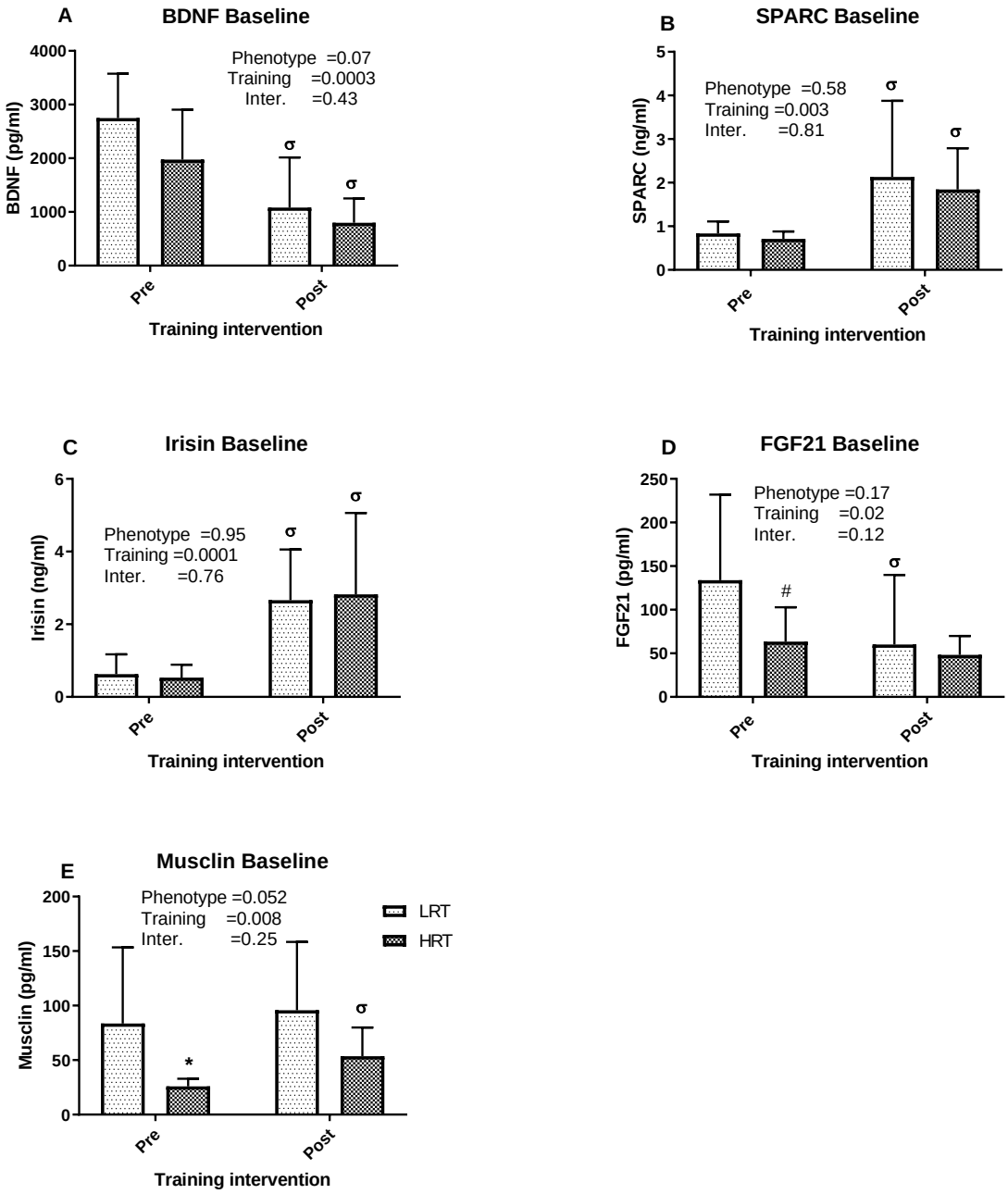

Figure 4.3 Baseline concentration of plasma myokines pre and post training intervention. A. Brain-derived neutrophic factor (BDNF). B. Secreted protein acidic and rich in cysteine (SPARC). C. Irisin. D. Fibroblast growth factors 21 (FGF21). E. Musclin. *= Significant difference between animal groups. σ= significant change from the (Pre) training level. # <0.1. Values are means±SEM. P<0.05 is considered significant. Two-way ANOVA was used with post hoc test (Sidak's).

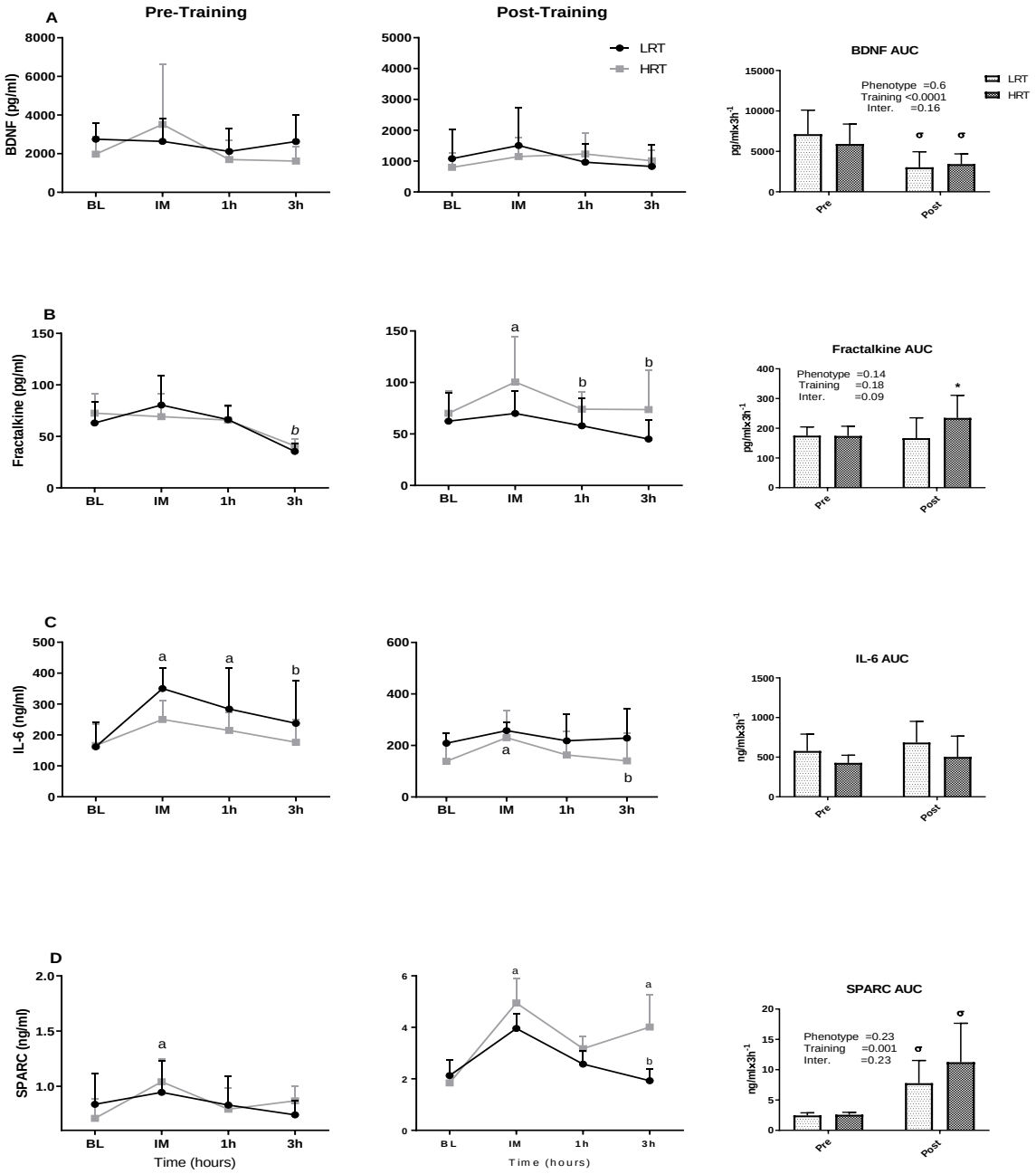

Figure 4.4A Temporal change in concentration of plasma myokines pre and post training intervention and area under the curve (AUC) A. Brain-derived neutrophic factor (BDNF). B. Fractalkine. C. Interlukine-6 (IL-6). D. Secreted protein acidic and rich in cysteine (SPARC). Baseline (BL), Immediately after (IM). *= Significant difference between animal groups. σ= significant change from the (Pre) training level. a= significant change from BL. b= significant change from IM. Values are means±SEM. P<0.05 is considered significant. Two-way ANOVA was used with post hoc test (Sidak's).

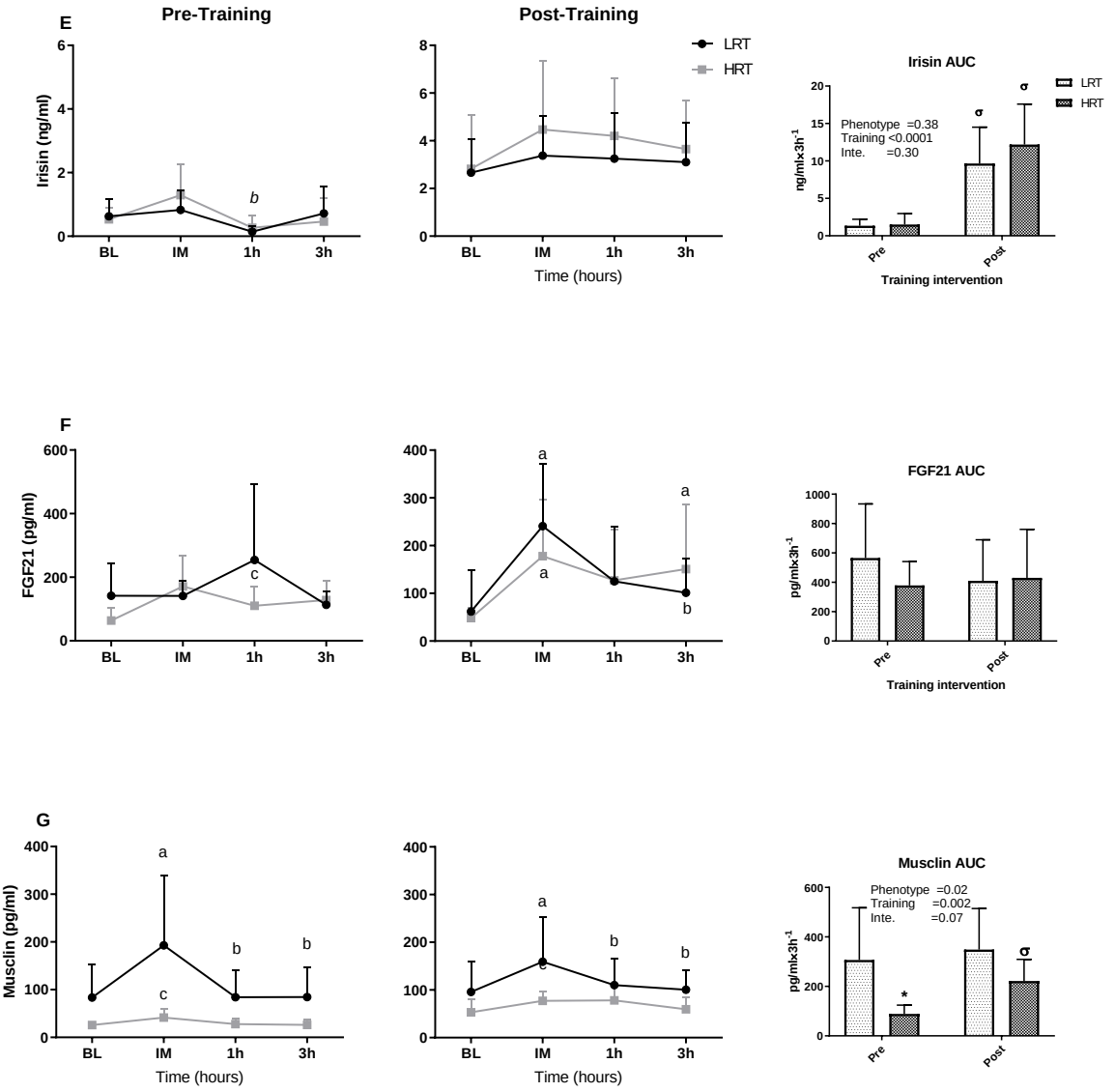

Figure 4.4B temporal change in concentration of plasma myokines pre and post training intervention represented in area under the curve (AUC). E. Irisin F. Fibroblast growth factors 21 (FGF21). G. Musclin. Baseline (BL), Immediately after (IM). *= Significant difference between animal groups. σ= significant change from the (Pre) training level. a= significant change from BL. b= significant change from IM. c= significant between animals type. Values are means±SEM. P<0.05 is considered significant. Two-way ANOVA was used with post hoc test (Sidak's).

4.3.2 Statistical Analysis

To determine the difference between groups, two-way repeated measures ANOVA was used. Further, we used Sidak's post hoc test for multiple comparisons between pre vs post (in same group), and pre vs pre and post vs post (across groups). The multiple comparison P value was reported if the P value of training or phenotype was significant with or without significant interaction. Data are means±SD. P value <0.05 was considered significant.

4.4 Discussion

To gain further understanding of the potential role of myokines in underlying aspects of variation in adaptive response to exercise training (training capacity), we employed an experimental rat model of two divergent traits (LRT/HRT). LRT and HRT share a similar exercise capacity (running distance) at baseline, but HRT animals show a 54% improvement

in running distance in response to treadmill training whereas LRT animals failed to respond[325]. In addition, LRT animals were prone to developing metabolic disorders such as insulin resistance and adiposity without exercise training at a young age[79]. However, while we show that exercise-induces, and training alters myokine responses, few consistent links to phenotype manifested.

4.4.1 Baseline differences in resting plasma mokine levels

Baseline myokine levels have been linked to human health and body composition. For example, FGF21 is elevated in obese and T2D individuals[195] as is the case in mitochondrial myopathy and metabolic stress conditions[196]. Similarly, BMI, fasting insulin, HOMA-IR, triglyceride, and percentage of fat (to whole-body mass) were positively correlated to plasma level of SPARC in human study[203,204]. Therefore, given the fact that the metaboilc health of LRT animals is considered “poorer” than HRT in term of insulin resistance and obesity[79], we hypothesied that this would manifest in the basal concentration of plasma myokines. In line with this, elevated FGF21 and musclin in LRT animals at baseline (i.e. pre-training) somewhat supports previous reports of higher concentrations of FGF21[195] and musclin[209,211] in diabetic and obese subjects[195,209,211]. Elevated musclin was previously positively linked to high fasting glucose, triglyceride, HOMA-IR, and serum insulin[209,211], concurrent with the LRT condition[79]. High baseline levels may indicate a protective role of myokines against metabolic stress as observed with FGF21 administration improving insulin sensitivity, plasma glucose, and triglyceride in diabetic mice[194]. However, the increase may also indicate the development of body resistance similar to what happens with insulin, which has been noted with FGF21 in liver and fat tissue[332].

Since we know these animals produce distinctive training responses, we next investigated the impact of 3-wks of running training on baseline myokines profiles. However, in general, adaptive response of baseline circulating myokines to the training intervention did not distinguish gross differences in trainability, as we observed increaseed (Irisin and SPARC), decreased (BDNF), and no changes (Fractalkine and IL-6) concentration. The reduction of FGF21 in LRT animals was in line with previous studies when a 3-wks of sprint interval training[192] and 3 months of combined resistance training in obese woman[193] decreased FGF21 levels. However, musclin remained elevated in LRT perhaps due to the low trainability of LRT and the lack of change in aerobic and metabolic status. Whereas increases HRT musclin level fits with its suggested role in improving exercise capacity[212].

4.4.2 Impact of Acute exercise on circulating myokines

A single (namely, *acute*) bout of exercise induces specific changes in circulating myokine concentrations[236] e.g. immediately as with IL-6[236] or temporally as with the acute falls in irisin that have been described previously[306]. However, the magnitude of the acute response of IL-6 for example, has been linked to the intensity and duration of exercise, the number of activated muscles, and also the levels of muscle glycogen[182]. LRT/HRT animals were bred to show distinct adaptive responses, which is an accumulation of acute signalling and transcriptional events following each exercise bout[333]. A single exercise bout of similar intensity, confirmed by oxygen consumption and muscle glycogen concentrations, revealed contrasting transcriptional responses to exercise in LRT/HRT[79]. However, nothing is known about the acute myokine response to a standardized exercise bout in these animals. Nonetheless, acute myokine responses were not distinctily different between the groups, which could indicate a limited role of training capacity on the contractile-induced released of

myokines. Indeed, only musclin showed a potential link to training capacity; in line with this, knocking out the musclin gene leads to a decrease in aerobic capacity which was subsequently restored by recombinant Musclin administration[212]. This may indicate a compensatory role of musclin to improve LRT low aerobic capacity.

There was also limited evidence of an impact of the training intervention on the pattern of temporal responses of myokines to an acute bout of exercise (when compared to those observed in pre-training), however there were some clear differences with regard to the AUC of a number of myokines. Although training significantly increased SPARC and Irisin concentrations in both groups, and reduced BDNF, both Fractalkine and musclin responses also yielded group differences. The observation of an elevation of Fractalkine in HRT animals was unexpected since elevated Fractalkine has been linked to obesity, insulin resistance and T2D[214]. Nonetheless, Fractalkine is also proposed to block downstream TNF-α signalling (NF_KB) and thus prevent insulin resistance in myotubes[217], indicative of a positive role of muscle. Clearly, further work is needed to establish a firm role for Fractalkine. The improved musclin AUC in the HRT animals, which was unchanged in LRT animals, support the role of musclin on enhancing aerobic capacity[212].

Despite similarities in responses across the groups, significant increases in circulatory SPARC and irisin following training were notable. Irisin and SPARC are proposed to have parallel physiological functions, i.e. enhanced glucose uptake and lipolysis[200,201,313] and demonstrate positive correlation to both BMI and fasting insulin[260,204]. Although herein we report an increase in irisin concentrations, which is supported in the literature[261,269,315], as well as not[257,265,271].

Likewise, SPARC has been shown to remain unchanged following 4-wks endurance training[198], in contrast to our findings. Another unexpected change with training was the decrease in basal BDNF and temporal response since previously aerobic[225] and resistance[224] exercise increased circulating BDNF. These inconsistencies indicates the need for further research on the role of myokines in promoting health through exercise, with more standardized protocol since factors such as time of sampling, species, exercise mode, and training intensity are potential confounders to interpretation. In addition, trainability (as assessed by running capacity) may rely more on the blood supply and substrate delivery. Improvement in the central (heart output) and peripheral (skeletal muscle blood vessels) cardiovascular system had more contribution than the solely within the skeletal muscle improvement[79]. This may explain the limited impact of trainability on systemic myokine profiles.

4.4.3 Limitation and conclusion

As with studies of this nature, we may have missed temporal aspects e.g. the peak rise in some myokines such as SPARC, Fractalkine, irisin and BDNF which have been shown to peak ~30 min after acute exercise training[224,334]. Nonetheless, our data are novel in demonstrating patterns of acute, chronic or both changes in plasma myokines in LRT/HRT

rats. This model develops gross heterogeneity in adaption response to exercise training that facilitates an understanding of extrinsic molecular networks responsible for such variation[325]. Even though our data suggest a limited influence of individual training capacity in relation to myokines, their resting (baseline) level may be influenced by metabolic traits induced by the outbreeding regime e.g. FGF21 and musclin. We conclude i) baseline myokine abundance was linked to outbreeding polygenic genetic traits i.e. may be inherited, ii) myokines respond with temporal and quantitative specificity both to a single bout of exercise and to sustained exercise training, iii) there is limited evidence myokines could explain the marked differences in trainability of the LRT and HRT model. In sum, it is unlikely myokine responses can explain gross differences in trainability. Studies are needed to experimentally assess the physiological veracity of myokines role in adaptation to exercise.

5 The plasticity of “exercise adaptability” in relation to resistance vs. endurance training in human

Abstract

Introduction: EET and RET initiate mode specific physiological adaptions, EET improves aerobic capacity[30] and RET increases muscle mass and strength[303], and both promote general health benefits e.g. improved insulin sensitivity[37]. However, the magnitude of the adaptive responses range among individuals from high responders to non-responders regardless of mode[335,277]. It has not been answered before if the same individual would response similarly to different modes of training or not.

Aim: To explore 'within individual' heterogeneity of adaptation to RET and EET using a cross-over design.

Methods: The study was conducted as a 14-week randomised cross-over intervention (vis-à-vis: 4-weeks RET or EET; 6-week wash-out; then 4-weeks EET or RET) on (n=16) young healthy male subjects. Pre- and post-analyses were held for each training mode in relation to measures of aerobic capacity (VO_2max/AT) and muscle hypertrophy and strength (muscle mass and size). In addition, muscle biopsy was taken pre- and post-each exercise training mode for molecular analysis. Paired and unpaired t-tests were used to test the primary adaptive response and detraining effect. Repeated measure two-way ANOVA was used for clinical health changes with *Sidak's post hoc* test. Linear correlation and linear discriminate analysis were used to link changes in fitness with health benefits and to find a marker that predict the training response respectively. Data are means±SD. *P* value <0.05 was considered significant.

Results: 4-weeks of EET improved VO_2max (42.8±10.4 vs. 45.9±9.4 ml/kg/min; *P*=0.004) and AT (24.9±8.1 vs. 28.7±8.9 ml/kg/min; *P<0.0001*)), while RET increased appendicular lean body mass (ALBM) (26.8±4.3 vs. 27.4±4.1 kg; *P=0.014*) and lower body muscle strength (630±129 vs. 883±158 N, *P<0.0001*). By testing results of (8 subjects) of each group ((1) EET-RET, (2) RET-EET), the 6-week washout was sufficient to return these adaptive values to baseline. i.e. VO_2max pre-EET (44.1±7.7 ml/kg/min) was identical to pre-RET (44.6 ± 5.7 ml/kg/min; P=0.89). The same was true for AT (*P=0.88*) in group (1). Similarly, ALBM pre-RET (26.8±2.2 kg) was not different to ALBM pre-EET (27.2±2.6 kg; *P=0.40*) and pre-RET strength (655.1±94) was close to pre-EET (700.4±55, *P=0.2*) in group (2). The percentage change (%Δ) of VO_2max was significantly negatively correlated to ALBM (r=-0.52, *P=0.04*) and trended with %Δ strength (r=-0.43, *P=0.09*). The molecular analysis confirmed the impact of EET on the mitochondrial biogenesis via increasing expression of TFAM gene (~30%, *P=0.02)*, phospho-AMPKα (~50%, *P=0.03)*, and OXPHOS complexes (CI ~30%, *P=0.007)*, CII ~30%, *P=0.0006*, and CIV 40%, *P=0.008*), and on angiogenesis through enhancing VEGFA gene expression (50%, *P=0.001)*. On the other hand molecular analysis after RET showed only a decrease in MSTN gene expression (30%, *P=0.04*) and an increase in RNA: DNA ratio (from 0.25±0.14 vs Post 0.30±0.18 AU, *P=0.02)*.

Conclusion: 4-weeks of exercise training was sufficient to elicit anticipated exercise-mode specific adaptations i.e. aerobic capacity gains with EET and lean mass/function gains with RET. A 6-week washout was sufficient to reverse these gains. Finally, as anticipated, responses to EET and RET within groups was highly variable; however, across exercise modes, the responses suggested a mode specific preference for individuals i.e. high responder for EET was more likely to be a low responder to RET and vice-versa. In addition, some individuals exerted modest improvements to both modes.

5.1 Introduction

In the previous two chapters we studied the impact of individual myokine on skeletal muscle metabolism and the effect of trainability on myokines release. Because exercised skeletal muscle release myokines that are proposed to induce health benefits in skeletal muscle and other organs, we were keen to investigate the impact of different training modes of exercise training on health. In addition, we were keen to explore a potential role of myokines in the adaptive response, the so called responder – non responder hypothesis. Therefore in this chapter, we were interested to screen the myokines profiles and their adaptive response to two types of exercise training (EET, RET) in same individual in a novel study design. As the nature of research, we faced some technical problems and challenges that we could not overcome to explore the myokines profiles within the time frame of my PhD studies. However, the human clinical trial provided novel data that help us to understand the heterogeneity of the adaptive response to exercise training.

Low physical activity has both direct and indirect (by provoking other risk factors as obesity, diabetes, and hypertension) effects on mortality and morbidity associated with NCDs, being linked with ~16% of all deaths[336,22,336]. In fact, studies showed undertaking regular physical activity reduced the mortality rate in men and women by 20-35%[24,25]. Therefore, exercise training has been promoted as a therapeutic treatment for some patient groups[26]. However, structured exercise regimes mainly split into two modes, EET, involves prolonged/intense aerobic training, or RET, contractile activity designed to overcome resistive force of external loads. The very different natures of EET and RET, initiate mode-specific physiological adaptive

responses. EET primarily results in improvements in cardiorespiratory function[28] as result of enhanced microvascular and microvascular angiogenesis[29], and muscular mitochondrial content[30]. In contrast, RET primarily induces increases in muscle mass i.e. hypertrophy, and consequently strength and power[31,32]. The improvement in aerobic capacity and the enhancement of muscle mass and strength decreases the mortality rate in cardiorespiratory disease[33] and the physical frailty[34] of elderly respectively. In addition, both training modes promote similar whole body beneficial adaptive outcomes as outlined in (Table 1.1)[34,35,37,36].

Inter-individual heterogeneity of adaptive response to standardized exercise training regimes has been reported with both exercise training modes. A fully supervised RET induced a wide variation in hypertrophic response in CSA of VL muscle[276], as well as in lean body mass[277]. Similar variations have been observed with fully-supervised EET in VO_2max old[278] and young[279]. “low responder” or “non-responder” accounting for ~20% of participants in aerobic capacity after weeks/months of supervised EET training[285]. The phenomena of “exercise resistance” was not restricted to the primary mode-specific outcome but was also observed with other positive health benefits associated with the adaptive response to exercise. As an example, Boule et al (2005) reported that ~42% of participants in an exercise training programme showed no improvement in insulin sensitivity[287]. In a practical context, this suggests that approximately 20 to 30% of people would not get fitter/improve (i.e. either in terms of aerobic capacity or muscle mass/strength) and therefore exercise training would

perhaps be ineffective, in improving other aspects of health, i.e. blood pressure, cholesterol levels or insulin sensitivity.

Some attempts have been made to understand the nature of ‘exercise resistance’, using a selective breeding rat model, Koch and Britton have developed (LRT/HRT) model, however, clearly this is not easily resolved in humans. The exercise resistant LRT rats demonstrated greater metabolic dysfunction e.g. insulin resistance and adiposity, while the HRT rats were protected[79]. Meaning that resistance to the benefits of exercise may also prevent the improvement in health and fitness that are normally associated with exercise training.

Our primary aim was to have a better understanding of “exercise resistance” by first comparing the magnitude of the adaptive response in the same individual for different modes of exercise training (EET or RET). In other words, would a low responder to EET be a high responder for RET and vice versa, or would they be a low responder for both modes? To answer this, we used a cross over design within the same individuals, undertaken in a random manner to EET or RET then switched after a 6-week washout period to the other mode.

We also investigated the relationship between “fitness” and positive health adaptions to exercise training. We aimed to determine if the magnitude of physiological mode-specific adaption (aerobic capacity and muscle hypertrophy) was related to the magnitude of the positive health adaptation (lowering blood pressure, improving glycaemic status etc.).

5.2 Methods

5.2.1 Subjects recruitment

The study was approved by the University of Nottingham, Faculty of Medicine and Health Science Research Ethics Committee, and was conducted according to Declaration of Helsinki. All Subjects had medical questionnaire, physical examination, and resting electrocardiogram. 16 healthy males with no metabolic, respiratory, and cardiovascular disorders and not involved in structured physically activities were recruited. Participant characteristics are shown in (Table 2.5), for more details please refer to general methods chapter (2.20).

5.2.2 Study design

Subjects were randomized into one of the two groups i.e. 4-weeks of EET, 6-weeks wash-out period (no training), then 4-weeks of RET for group (1) or vice versa as in group (2) (RET - washout - EET). Subjects were asked to fast for 2 hours (water ad libitum) before each training session. In two of the participants, the wash-out period was extended due to personal circumstances to 8 and 12-weeks (Fig 5.1). 4 acute study days were undertaken at the beginning (wk 0) and at the end (wk 4) of each training block (EET and RET). Participants attended the facility fasted overnight for a minimum of 6 hours. On the acute study day, baseline saliva sample was collected prior to obtaining muscle biopsy from the VL, which was followed by collecting blood for biochemical tests, performing a 2 hour oral OGTT[300], measuring resting BP and HR, and having DXA scan prior to the lunch break on empty bladder. The lunch was maintained the same for each participant through the 4 visits i.e. Fortisip (Nutridrinks) flavour and quantity consumed. The acute study day ended with a CPET, to assess respiratory fitness, after the muscle ultrasound. Muscle function was assessed on a separate day before and after the training period, when MVC measurement was made to

assess unilateral isometric strength, in addition to the 1-RM assessment (Fig 5.2). Further details of training protocol and acute study day protocols are provided in the general method chapter (2.24).

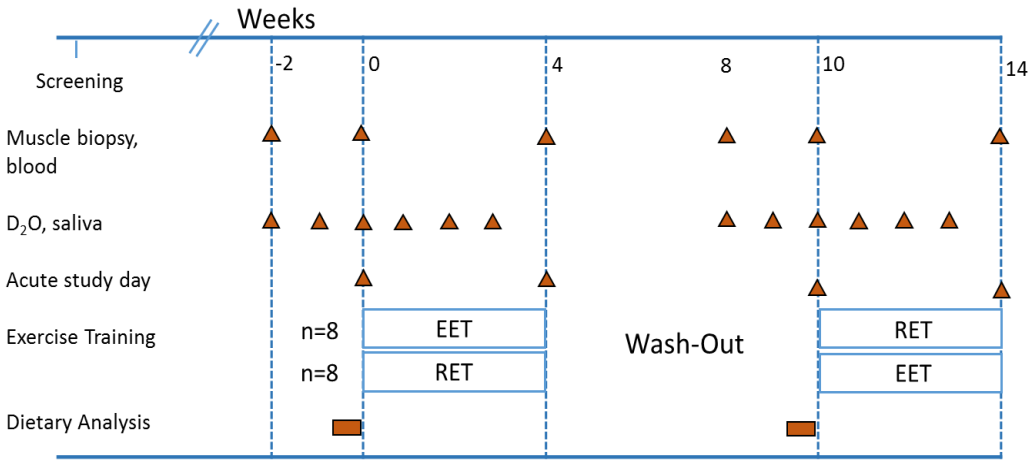

Figure 5.1 Study overview scheme. Endurance exercise training (EET). Resistance exercise training (RET). Deuterium (D_2O) was given to assess muscle protein synthesis, however time and instrument capacity issues mean the data was not available for this thesis.

5.3 Molecular analysis

The genes expression were investigated on VL muscles using qPCR technique and immune-blotting for proteins expression. VL muscle was also used to estimate mitochondrial density through OXPHOS and also measure muscle ASP, RNA, and DNA.

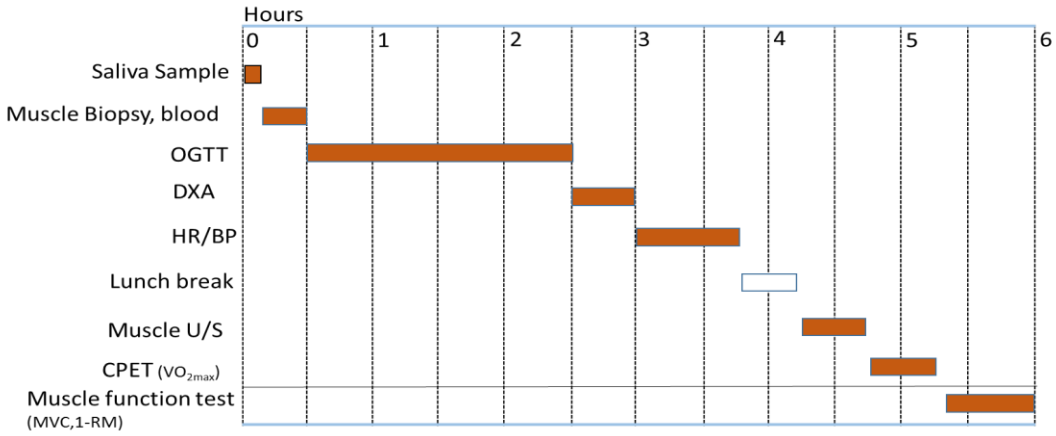

Figure 5.2 Acute study day scheme. Oral glucose tolerance test (OGTT). Dual x-ray absorptiometry (DXA). Heart rate (HR). Blood pressure (BP). Ultrasound (U/S). Cardio-pulmonary exercise test (CPET). Maximum voluntary contraction (MVC). 1-repetition maximum (1-RM).

5.4 Statistical analysis

All data were checked for normal distribution using kolmogorov-Smirnov test of the descriptive analysis. Data are expressed as means±SD. Student's 2-tailed paired and unpaired t-test were used to examine changes in the mode-primary adaptive response and washout effect respectively. However, to study the impact of EET and RET on clinical adaptations, repeated-measures two-way ANOVA with *Sidak's post hoc* test was used. Linear correlations of fitness against health benefits were assessed using Pearson's product moment correlation coefficient. While linear discriminate analysis (LDA) was used to find the best indicators of the adaptive response to each mode of exercise training. LDA nominated the best factors that can separate between responder and non-responder to exercise training. Finally, a mixed-logistic regression was applied to calculate the odds of high-responder to EET to be high or low-

responder to RET. P<0.05 was consider significant. All of the statistical analysis were performed using GraphPad Prism 7.0 (La Jolla, CA, UAS).

5.5 Results

4-weeks of either EET or RET was deemed sufficient to generate expected improvements in mode specific adaptations, i.e. VO_2max and AT with EET and muscle mass/hypertrophy and strength gains with RET.

5.5.1 Cardiorespiratory fitness

As expected, 4-weeks of EET enhanced VO_2max (43.2±9.7 – 46.2±9.2 ml/kg/min, *P=0.0005*), and AT (25.4±7.3 – 28.8±8.0 ml/kg/min, *P<0.0001*) (Fig 5.3A &B). Although the mean difference for VO_2max was 3.0 ml/kg/min (7.9 %Δ), individuals' response range from -1.2 to +8.8 ml/kg/min (-2.5 to 33 %Δ). Similarly, the mean difference of AT was 3.4 ml/kg/min (~14 %Δ) with range of individuals' responses from 0.1 to 5.8 ml/kg/min (0.4 to 29 %Δ) (Fig 5.3C & D). VO_2max and AT were unchanged after RET (Fig 5.3E & F).

5.5.2 Muscle mass

After 4-weeks of RET, DXA showed small, but significant, increases in total lean body mass (TLBM) (54.35±7.7 – 55.33±7.7 kg, *P*=0.019), and ALBM (26.1±4.4 – 26.8±4.2 kg, *P=0.005*) (Fig 5.4A & B). The heterogeneity of the response to RET was also apparent (Fig 5.4C & D). The change of TLBM ranged from -2 to 3.2 kg (-4 to 5.8 %Δ) with mean increase of ~1.0 kg (1.9 %Δ). Similarly, the mean difference in ALBM was 0.63 kg (2.6 %Δ) with individual responses from -0.3 to 2.5 kg (-1 to 9.1 %Δ). EET did not result in changes in either total body lean mass or ALBM (Fig 5.4E & F).

5.5.3 Muscle size

Ultrasound analysis of the VL muscle at mid belly showed a significant increase in MT after RET (26.0±4.1 - 27.3±4.3 mm, *P=0.02*), a mean increase of 1.2±1.8 mm (4.77 %∆). In addition, the PA of the muscle fibers was also significantly increased following RET (22.6±3.9 - 24.9±3.7◦, *P=0.01*), a mean increase of 2.4±3.2◦ (11 %∆) (Fig 5.5A & B). However, the individuals' responses were widely variable (Fig 5.5C & D), from -2.61 to 3.65 mm (-9.5 to 13 %∆) and -3.6 to 6.6◦ (-11.5 to 38 %∆) in MT and PA respectively. There was a strong correlation between ΔMT and ΔPA (r=0.71, P<0.0001), suggesting that the increase in MT and PA are associated. Further, the ∆ in leg lean mass also correlated with changes in MT (r=0.37, P=0.036). With EET there were no changes in MT, PA (Fig 5.5E & F).

5.5.4 Muscle function

Changes in muscle strength were restricted to RET (Fig 5.6E & F). Lower body strength i.e. the sum of leg exercises was increased with RET only (630±129 - 883±158 N, *P<0.0001*). Individuals' responses were highly variable, ranging from 69 to 478 N (11.4 – 69.3 %∆) with a mean increase of 184±107 N (27.3 %∆) (Fig 5.6A & C). MVC was increased at 70° angle after RET (246±46 - 264±47 Nm, *P=0.04*). The change in individuals' responses however ranged from -22 to 84 Nm (-8.8 – 44.1 %∆) with mean of 18.2± Nm (8.8 %∆) as in (Fig 5.6B & D). The improvement in lower body strength was correlating with muscle gain in leg (r=0.55. *P=0.03*) and appendicular (ALBM) (r=0.57, *P=0.024*).

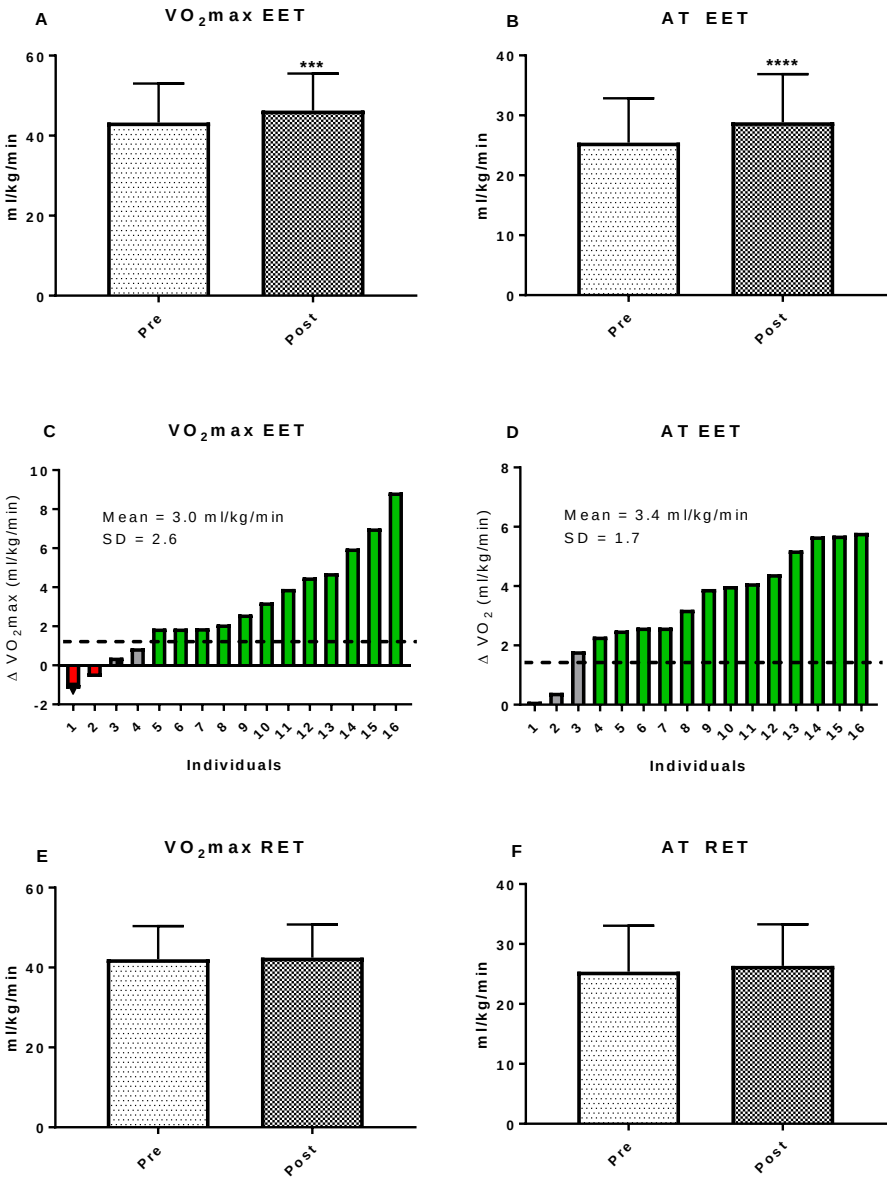

Figure 5.3 Change in cardiorespiratory parameters of EET and RET. A-B. The maximum of oxygen consumption (VO_2max) and anaerobic threshold (AT) with EET respectively. C-D. Individual's response to EET. Green: positive change up to the level that show clinical enhancement on individual (~1.9 ml/kg/min). Grey: positive change without reaching the clinical improvement level. Red: negative change. E-F. VO_2max and AT with RET respectively. Paired t-test and. Data are mean±SD. P value<0.05 considered significant. ***<0.001. ****<0.0001.

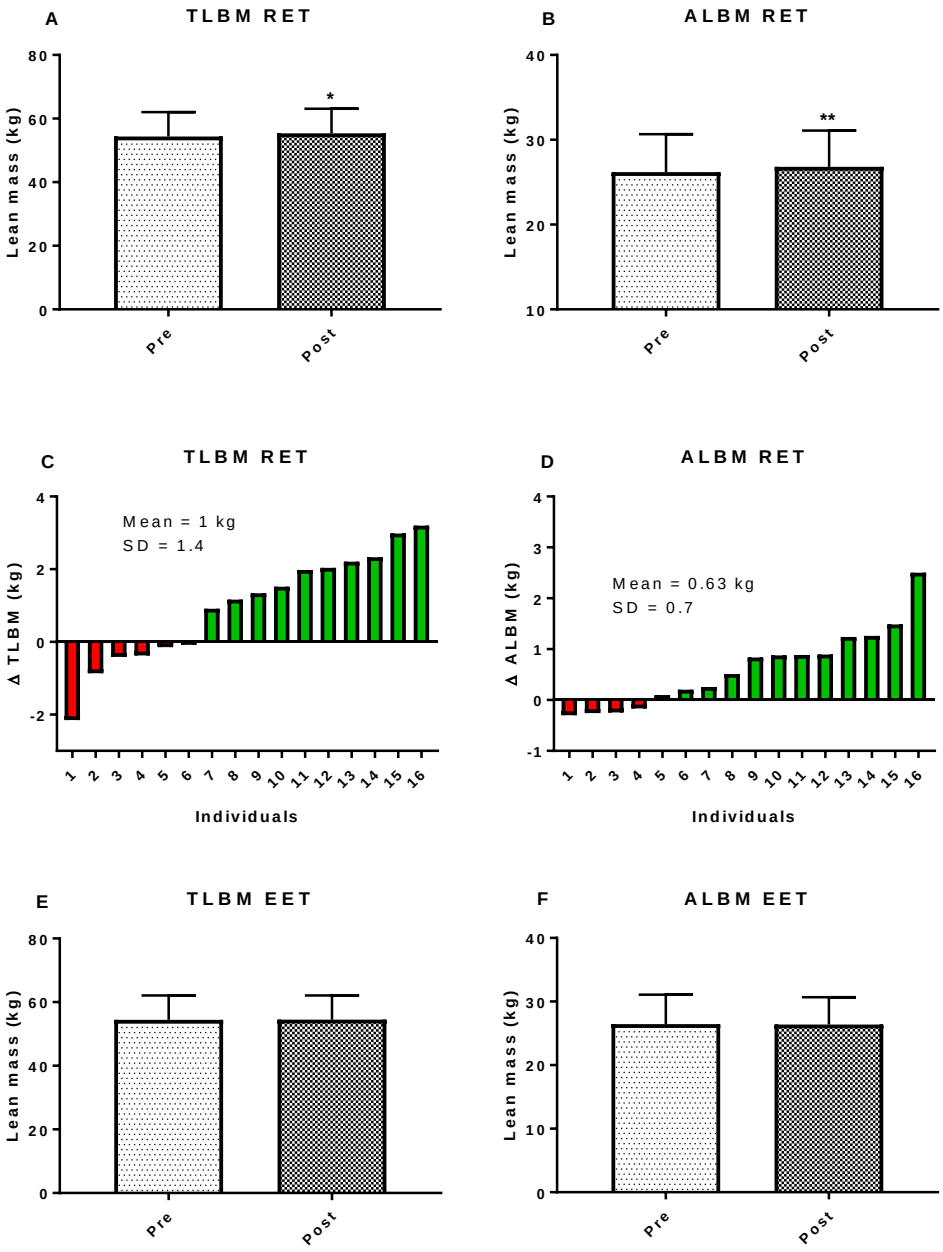

Figure 5.4 Change in Lean body mass parameters for RET and EET. A-B. Total lean body mass (TLBM) and appendicular lean body mass (ALBM) respectively with RET. C-D. Individual's response on TLBM and ALBM with RET. Green: positive change. Red: negative change. E-F. TLBM and ALBM respectively with EET. Paired t-test was used. Data are mean±SD. P value<0.05 considered significant. * P<0.05, and **<0.01.

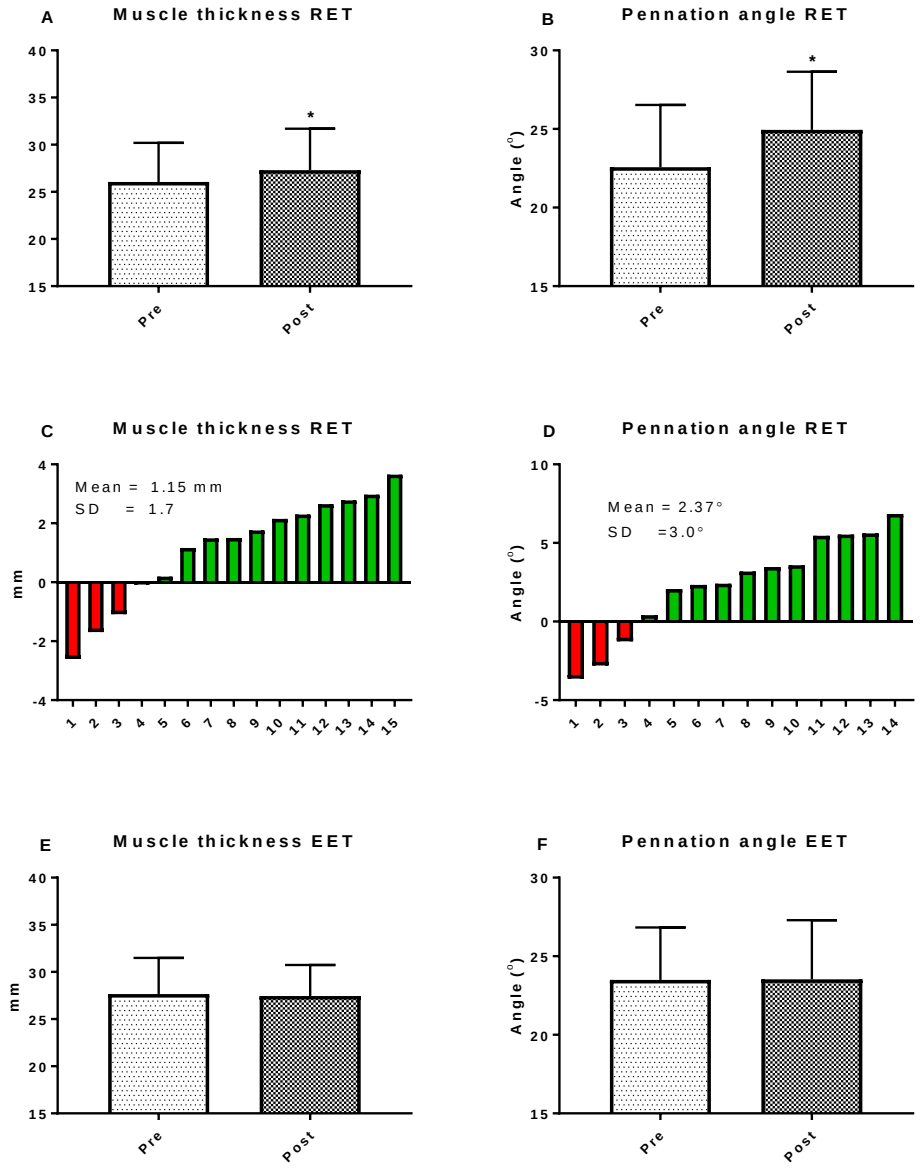

Figure 5.5 Leg muscle size with EET and RET. A-B Muscle thickness (MT) and Pennation angle of muscle fiber (PA) at mid billy *vastus lateralis* muscle respectively. C-D. Individual's response in MT and PA to RET. Green: positive change Red: negative change E-F. MT and PA with EET respectively. Paired t-test was used. Data are mean±SD. P value<0.05 considered significant. * P<0.05.

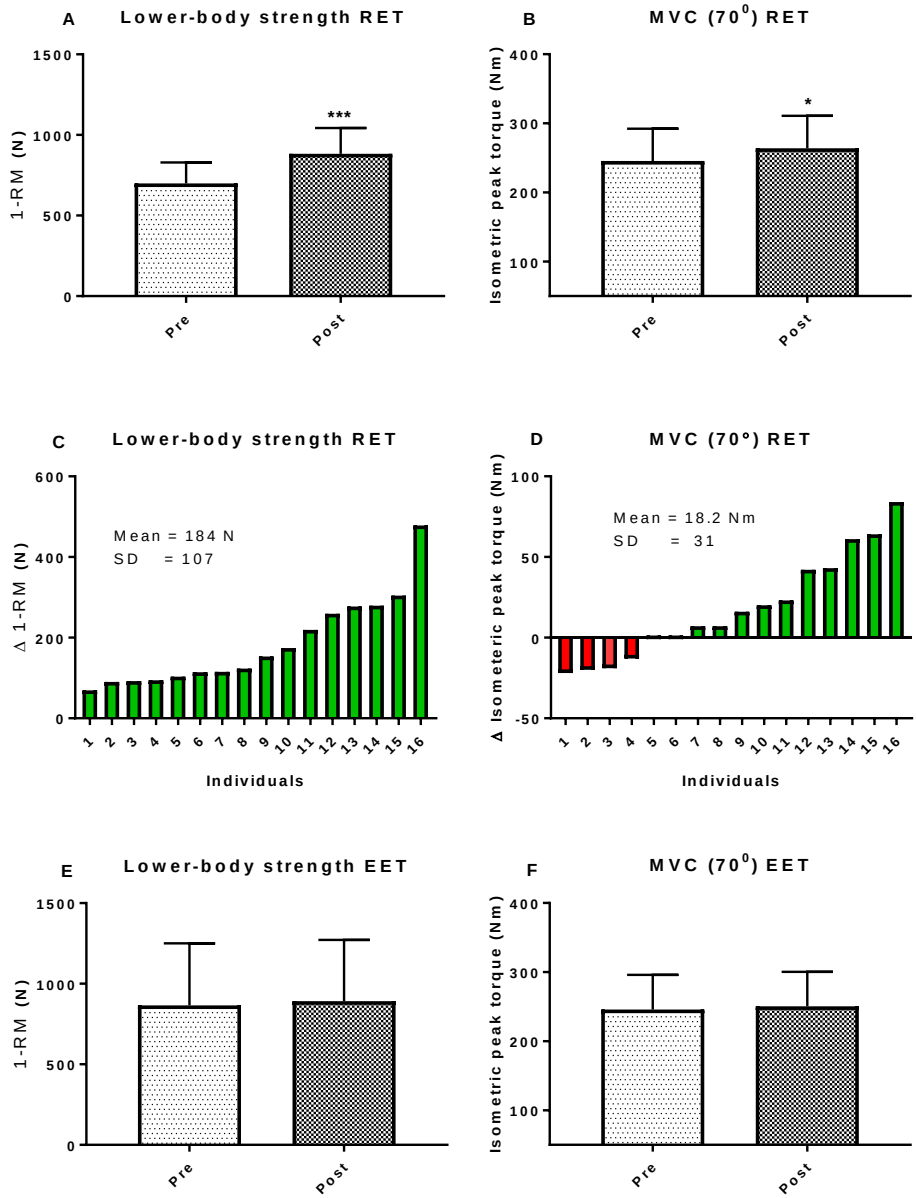

Figure 5.6 Muscle strength. A-B. Lower body strength with RET though 1 max repetition (1-RM) assessment and maximum voluntary contraction (MCV) at 70^0 with RET. C-D. Individual's response to RET. Green: positive change. Red: negative change. E-F. Paired t-test was used. Data are mean±SD. P value<0.05 considered significant. * P<0.05, and ***<0.001.

5.5.5 "Washout" period

In both groups the 6-week washout period resulted in a return to the baseline level of the mode specific adaptations i.e. VO_2max/AT and muscle mass/function. In the participants who started with EET then RET (n=8), the VO_2max was increased after 4-weeks EET (Pre-EET 44.31±7.1 vs Post-EET 46.9±7.3 ml/min/kg, *P=0.0008*). After the washout period VO_2max returned to baseline (Pre-EET 44.31±7.1 vs Pre-RET 44.4±5.3 ml/min/kg, *P=0.9*) (Fig 5.7A). Similarly, with AT, (Pre-EET 27.6±7.3 vs Post-EET 31.5±7.6 ml/min/kg, *P=0.0009*) and after the washout period returned close to baseline level (Pre-EET 27.6±7.3 vs Pre-RET 29.5±5.4 ml/min/kg, *P=0.5*) (Fig 5.7B).

For individuals who started RET then EET (n=8), the TLBM mean was elevated by 1 kg but not statically significant after 4-weeks RET (Pre-RET 54.35±5.4 vs Post-RET 55.3±5.8 kg, *P=0.1*). After the washout period there was no different among the baselines (Pre-RET 54.35±5.4 vs Pre-EET 55.05±5.8 kg, *P=0.8*) (Fig 5.7C). The ALBM after RET was trending to increase (Pre-RET 25.5±2.9 vs Post-RET 26.42±3.1 kg, *P=0.07*), and the two baselines measures were not different (Pre-RET 25.5±2.9 vs Pre-EET 25.9±3.4 kg, *P=0.8*) (Fig 5.7D). MVC at 70° was unchanged throughout (Pre-RET 241±35 vs Post-RET 260±42 N, *P= 0.1*) (Pre-RET 241±35 vs Pre-EET 248±62, *P=0.7*) (Fig 5.7F). However, lower body strength increased after 4-weeks of RET (Pre-RET 655.1±94 vs Post-RET 822.7±99 N, *P= 0.0006*) and return to baseline (Pre-RET 655.1±94 vs Pre-EET 700.4±55, *P=0.2*) (Fig 5.7E).

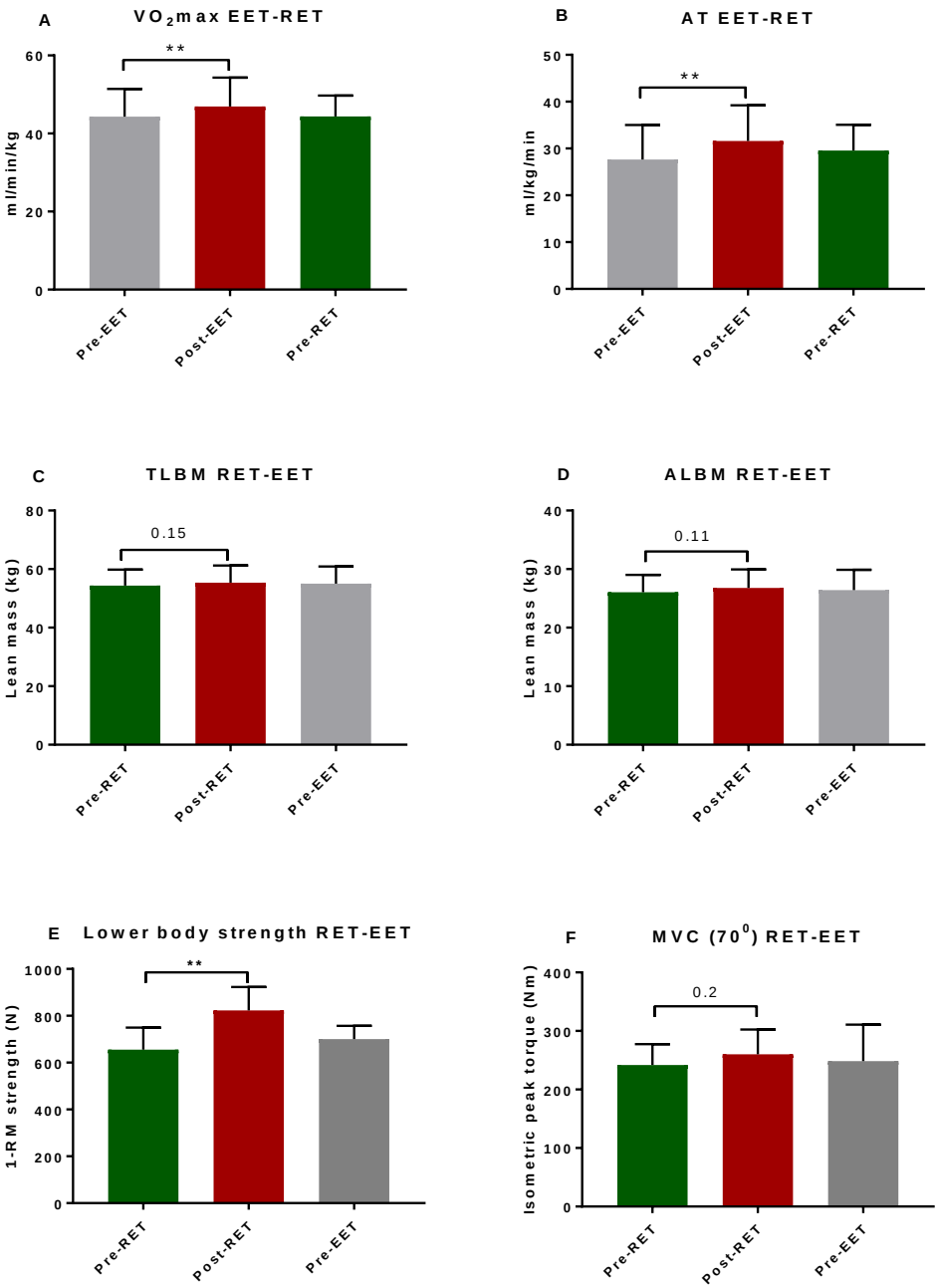
A
VO2max EET-RET
**
60
40
20
0
ml/min/kg
Pre-EET
Post-EET
Pre-RET
B
AT EET-RET
**
50
40
30
20
10
0
ml/kg/min
Pre-EET
Post-EET
Pre-RET
C
TLBM RET-EET
0.15
80
60
40
20
0
Lean mass (kg)
Pre-RET
Post-RET
Pre-EET
D
ALBM RET-EET
0.11
40
30
20
10
0
Lean mass (kg)
Pre-RET
Post-RET
Pre-EET
E
Lower body strength RET-EET
**
1000
800
600
400
200
0
1-RM strength (N)
Pre-RET
Post-RET
Pre-EET
F
MVC (70⁰) RET-EET
0.2
400
300
200
100
0
Isometric peak torque (Nm)
Pre-RET
Post-RET
Pre-EET

Figure 5.7 Washout period effect on clinical variables. A-B. Washout in group (1), maximum oxygen uptake (VO_2max) and anaerobic threshold (AT) respectively. C-F. Washout in group (2) total lean body mass (TLBM), appendicular lean body mass (ALBM), lower body strength, and maximum voluntary contraction (MVC). Paired and unpaired t-test were used in comparing pre vs post and baselines respectively. Data are mean±SD. P value<0.05 considered significant. * P<0.05. **<0.01.

5.5.6 Health benefits to exercise training

5.5.6.1 Haemodynamic indexes

The effect of exercise on haemodynamic parameters were only observed with DBP and MAP measures following EET. DBP was decreased with EET by -5.4±7.4 mmHg (-7.6%) (70±7.7 - 65.3±10.1 mmHg, *P=0.04*). Similarly, the MAP improved after EET by -4.5±6.3 (-5.3%) (85.6±7.0 - 81.0±8.8 mmHg, *P=0.03*). No change in SBP or HR was observed with either training mode. (Table 5.1).

5.5.6.2 Body fat composition

Body fat composition was reduced in general with both training modes (Table 5.1). EET decreased the percentage of total body fat by -0.66±0.9% (22.5±6.2 - 21.8±6.2 %, *P=0.01*), while RET caused a reduction by -0.84±1.1% (22.7±6.0 - 21.8±5.9 %, *P=0.004*). Further, the reduction in abdominal fat with EET was by -1.4±2% (23.0±10.2 - 21.6±10 %, *P=0.02*), and by -1.4±1.9 with RET (23.7±9.5 - 22.3±10 %, *P=0.03*). However, gynoid fat decreased by 0.81±1.6% with EET (23.8±6.7 - 23.0±6.4 %, *P=0.072*), and was unchanged with RET (23.5±6.1 - 22.7±6 %, *P=0.12*). Finally, the ratio of abdominal fat over gynoid (A/G ratio) tended to change only after RET (0.99±0.2 - 0.94±0.24, *P=0.09*). Despite the significant decrease in total and abdominal fat with both modes of training, no correlation of intra-individual response to EET and RET.

5.5.6.3 Glycaemic indices

There was no significant effect of either exercise training mode on systemic glucose disposal. The AUC in both modes were similar pre and post training. In support of this the insulin AUC during the OGTT was not affected by EET or RET. HOMA-IR, which is used to represent insulin resistance was also unchanged in response to both EET and RET. Similarly, insulin sensitivity as estimated using the Cederholm index was unaffected by exercise (Table 5.1).

5.5.6.4 Biochemical indices

The cholesterol profile and C-reactive protein (CRP) were unchanged with both EET and RET (Table 5.1).

5.5.7 Skeletal molecular adaption with EET and RET

5.5.7.1 Mitochondrial biogenesis markers and content

As expected, EET had an impact on both the mitochondrial content and mediators of mitochondrial biogenesis. Although gene expression of PGC-1α increased ~40%, this was not significant (*P=0.1*). However, transcription factor A mitochondrial (TFAM) was significantly elevated after EET (*P=0.02*) (Fig 5.8A). The protein level, expression of PGC-1α was trending to increase (*P=0.06*) with significant elevation of P-AMPK (~50%, *P=0.03*) (Fig 5.8B). The mitochondrial content estimated by the proteins expression of the 5 complexes involved in mitochondrial oxidative phosphorylation (OXPHOS) was also increased. EET had an impact on three of the five complexes. Expression of complexes I (*P=0.007*) and II (*P=0.0006*) were increased to ~30%, and complex IV (*P=0.008*) by 40%. Complex V increased insignificantly

~10% (*P=0.08*) and there was no change in complex III protein expression (Fig 5.8C). RET did not impact any of these markers.

5.5.7.2 Angiogenesis markers

Factors known to be involved in angiogenesis were increased with EET, as expected, but not with RET. VEGFA increased by up to 50% following EET (*P*=0.001). In addition, Fms related tyrosine kinase 1 (FLT1), which is a known VEGFA receptor, was elevated (*P*=0.07) (Fig 5.8D). However, the gene expression of Angiopoiten1, and 2 (ANGPT1, ANGPT2), and the ratio (ANPGT2/1) were unchanged.

5.5.7.3 Markers of Hypertrophy, anabolic cell signalling and ribosomal biogenesis

The impact of EET on the hypertrophic markers was very limited, therefore, we focused on RET effect. Despite the trending increase in muscle mass positive regulatory factors such as IGF-1Ea (*P=0.09*), other myogenic regulatory factors were unchanged, MyoD and MyoG. However, the most common negative regulator of muscle mass, MSTN, was 30% decreased with RET (*P=0.043*) (Fig 5.9A). Similarly, the markers involved in anabolic signalling pathways were unchanged in response to 4-weeks of either EET and RET (Fig 5.9B).

In addition, the gene expression of ribosomal markers as UBF, TIF-IA, and C-Myc did not show significant change in response to either mode of training. However, the ratio of RNA: DNA was increased with RET (Pre 0.25±0.14 vs Post 0.30±0.18 AU, *P=0.02*) (Fig 5.9C).

Table 5.1 Clinical adaptations to EET and RET. Body fat %: android over gynoid (A/G). Glycaemic: area under the curve (AUC). Haemodynamic indexes: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR). Body fat%: total, abdominal, gynoid, and abdominal/gynoid (A/G ratio). Glycaemic index: glucose and insulin area under the curve (AUC), HOMA-IR, Cederholm (SI). Biochemical indexes: Total blood cholesterol (Total Chole), high density lipoprotein cholesterol (HDLC), non-HDL-C, ratio of chole/HDL, and triglyceride. Inflammatory factor: C-reactive protein (CRP). 2-way ANOVA with multiple comparison (post *hoc test Sidak's*) was used. Data are mean±SD. P value<0.05 considered significant. ↔ unchanged, ↓ significant decreased.

Clinical Variable	EET	%Δ	RET	%Δ	Exercise type	Training
Haemodynamic indexes (mmHg)						
SBP	↔	-1.8%	↔	-0.3%	*P=0.57*	*P=0.22*
DBP	↓	-7.6% *P=0.04*	↔	-3.8%	*P=0.55*	*P=0.002*
MAP	↓	-5.3% *P=0.03*	↔	-2.4%	*P=0.54*	*P=0.002*
HR Beats/min	↔	-1.5%	↔	-1.4%	*P=0.35*	*P=0.87*
Body fat (%)						
Total body	↓	-3.2% *P=0.018*	↓	-3.4% *P=0.004*	*P=0.9*	*P=0.003*
Abdominal	↓	-6.0% *P=0.02*	↓	-1.5% *P=0.03*	*P=0.41*	*P=0.001*
Gynoid	↓	-3.2% *P=0.07*	↔	-2.6%	*P=0.63*	*P=0.023*
A/G ratio	↔	-2.9%	↓	-5.0% *P=0.09*	*P=0.017*	*P=0.027*
Glycaemic indexes						
Glucose AUC (mmol/L/120 mins)	↔	-3.4%	↔	-1.0%	*P=0.23*	*P=0.93*
Insulin AUC (µIU/ml/120 mins)	↔	-2%	↔	-4%	*P=0.47*	*P=0.029*
HOMA-IR	↔	-0.73%	↔	3.8%	*P=0.70*	*P=0.27*
SI	↔	9.8%	↔	7.5%	*P=0.53*	*P=0.12*
Biochemical indexes (mmol/L)						
Total Chole	↔	-0.16%	↔	-4.7%	*P=0.47*	*P=0.24*
HDLC	↔	-0.98%	↔	-4.7%	*P=0.21*	*P=0.31*
Non-HDL-C	↔	-0.07%	↔	-5.4%	*P=0.54*	*P=0.15*
Chole/HDL ratio	↔	-0.96%	↔	-0.14%	*P=0.68*	*P=0.63*
Triglyceride	↔	-0.77%	↔	16%	*P=0.60*	*P=0.84*
CRP (mg/L)	↔	40%	↔	33%	*P=0.07*	*P=0.68*

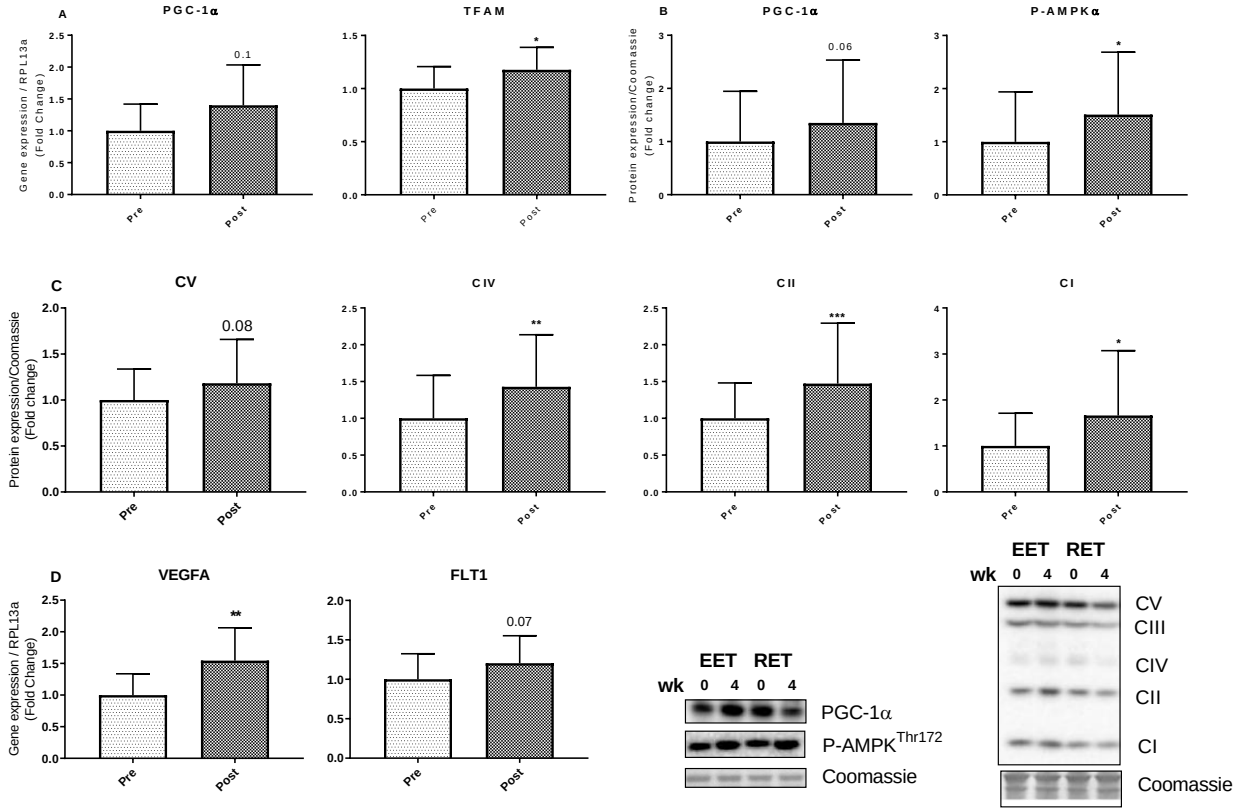

Figure 5.8 Molecular adaptions with EET. A. gene expression of mitochondrial biogenesis markers, Peroxisome Proliferator-activated receptor-gamma coactivator-1-alpha (PGC-1α) and mitochondrial transcription factor A (TFAM). B. protein expression of PGC-1α and Phospho- AMP-activated protein kinase (P-AMPK^{Thr172}). C. proteins expression of OXPHOS complexes, CI NADH dehydrogenase ubiquinone 1 beat subunit 8 (NDUFB8), CII Succinate dehydrogenase subunit B (SDHB), CIV mitochondria encoded cytochrome C oxidase (MTCO1), CV mitochondria membrane ATP synthases (ATP5A). D. Protein expression of angiogenesis markers, vascular endothelial growth factor A (VEGFA), and its receptor Fms related tyrosine kinase 1 (FLT1). Paired t-test was used. Data are mean ±SD. P value<0.05 considered significant. * P<0.05, **<0.01, and ***<0.001.

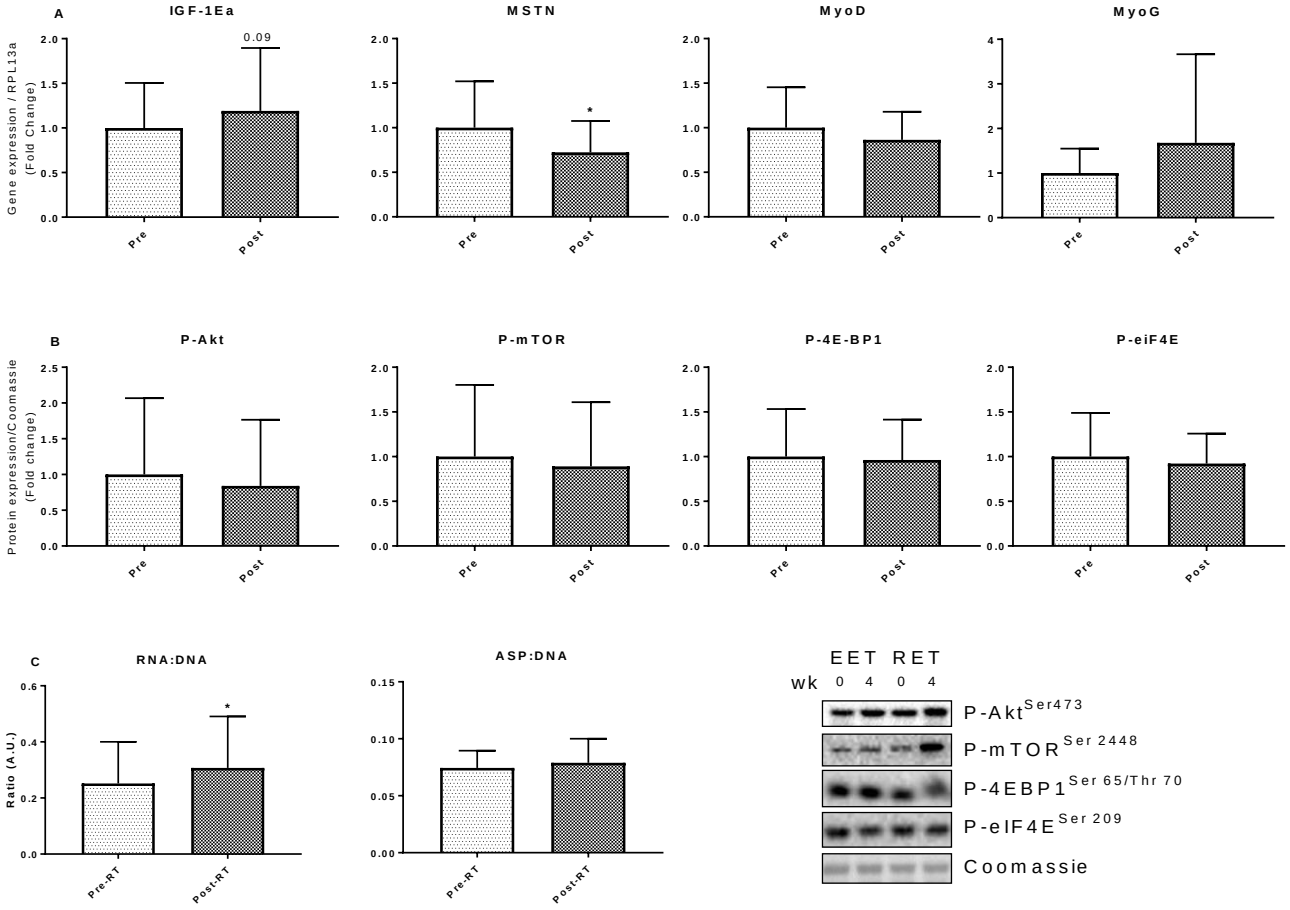

Figure 5.9 Molecular adaptations to RET. A. gene expression of muscle growth factor and myogenic regulatory factors, insulin-like growth factor-1 Ea (IGF-1Ea), myostatin (MSTN), MyoD, myogenin (MyoG). B. proteins expression of anabolic signalling pathway. Phospho-protein kinase B (P-Akt), phospho-mechanistic target of rapamycin (P-mTOR), phospho-eukaryotic translation initiation factor 4E-binding protein 1 (P-4E-BP1), and phospho-Eukaryotic initiation factor 4E (P-eiF4E). C. ratios of RNA: DNA content and Alkaline soluble protein (ASP): DNA. Paired t-test was used. Data are mean±SD. P value<0.05 considered significant. * P<0.05.

5.5.8 Fitness vs health benefits:

It is well known that cardiorespiratory and muscle mass define the fitness level of individuals, and their improvement depend on the type of exercise training (VO_2max with EET, muscle mass with RET). Therefore, we consider the changes in VO_2max and muscle mass as indicators of individual's fitness level. To explore the links between individual responses "fitness" and health benefits, correlation analyses were performed between percentage change (%Δ) in mode specific primary outcomes (VO_2max and AT with EET / TLBM and ALBM with RET) and %Δ in health adaptive responses (Table 5.2). For glycaemic indexes, the %Δ in glucose AUC was negatively correlated to TLBM and ALBM (r= -0.66, *P=0.008*). While %Δ of insulin AUC was negatively correlating to %Δ of AT (*r= -0.56, P=0.02*). The %Δ of insulin sensitivity correlated with ALBM (r=0.53, *P=0.03*) and trending with %Δ of AT (r=0.46, *P=0.07*). Regarding body fat composition, %Δ in total body fat showed a strong negative correlation to TLBM (r= -0.70, *P=0.002*). TLBM specifically correlated with %Δ gynoid fat (r= -0.48, *P=0.05*), whereas, VO_2max was trending to correlate with %Δ abdominal fat (r=0.44, *P=0.09*). No correlation was found between the primary outcomes and indices of blood pressure or blood lipid profiles or inflammatory factor, CRP.

To investigate which of the adaptive outcomes would best classify and separate between the responders and non-responders to EET and RET, LDA was applied on the Δ change of the health benefits measurements with binominal values of the primary outcomes (responders were coded by 1, non-responders were coded by 0). A cut-off value (≥ 2.56 ml/kg/min for VO_2max, ≥ 420 g for muscle mass[337]) were used for EET and RET respectively to categorized individual's response into responders and non-responders. The cut-off values represent the typical error in measurements, which were calculated from the two baseline tests, and were

similar to what have been reported in literature. The analysis for each training mode was performed separately with their corresponding response. The analysis showed that changes in changes in total cholesterol and abdominal fat are the most defining markers for the response status to EET (Fig 5.10A &B). While changes in Cederholm and total body fat were the most two important variables in recognizing responders and non-responders of RET (Fig 5.10C &D).

Table 5.2 Correlation between percentage of change (%Δ) of clinical adaptations and primary outcomes: anaerobic threshold (AT) and maximum oxygen consumption (VO_2max) for EET. Total lean body mass (TLBM) and appendicular body mass (ALBM) for RET. Pearson's coefficient was used. P<0.05 was consider significant. *<0.05, **<0.01, # <0.1.

Variables	**%Δ AT**	**%Δ VO_2max**	**%Δ TLBM**	**%Δ ALBM**
	EET		RET	
Glycaemic index:	r	r	r	r
Glucose AUC	-0.01	0.03	-0.66**	-0.66**
Insulin AUC	-0.56*	-0.07	0.08	-0.41
HOMA-IR	-0.35	-0.37	0.27	-0.16
Cerderholm	0.46#	0.14	0.31	0.53*
Haemodynamic index:				
SBP	0.03	-0.13	-0.09	-0.06
DBP	-0.11	-0.09	-0.34	-0.24
MAP	-0.07	-0.11	-0.30	-0.22
HR	0.21	0.15	-0.21	-0.34
Body Fat index:				
Total body fat	-0.11	-0.40	-0.70**	-0.19
Abdominal fat	-0.24	-0.44#	-0.25	-0.14
Gynoid fat	-0.35	-0.28	-0.48*	-0.21
A/G ratio	-0.02	-0.35	0.025	0.29
Blood Cholesterol index:				
Total Chol	-0.08	0.20	0.006	0.26
HDL-C	-0.06	0.30	-0.14	0.08
Non-HDL-C	-0.18	0.15	0.05	0.17
Chol/HDL ratio	0.04	-0.01	0.05	0.10
Triglyceride	-0.05	0.04	0.32	0.23

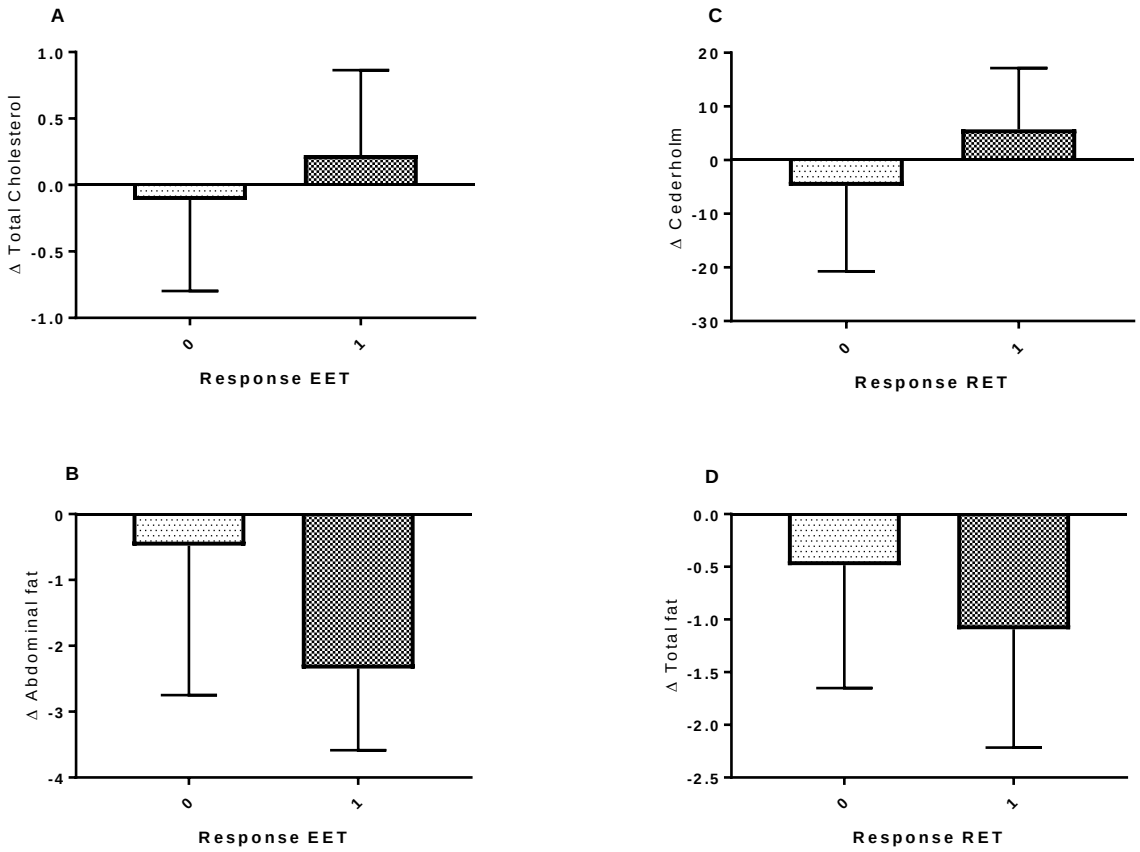

Figure 5.10 Health benefits as markers of training response. Response to endurance exercise training (EET) via maximum oxygen consumption (VO_2max) and resistance exercise training (RET) via appendicular lean body mass (ALBM) were divided into (0 = non-responder) or (1 = responder). A-B. Change in total cholesterol and abdominal fat respectively with EET. C-D. Change in Cederholm index and total body fat with RET.

Table 5.3 Subject's responses to training modes in primary outcomes and metabolic health benefits. Each subject responses to mode training primary outcomes (VO_2max with EET, ALBM with RET) were plotted, followed by the corresponding metabolic health benefit response (Diastolic blood pressure (DBP), HOMA-IR, abdominal fat %). Cut-off value for each of the parameters were used to reorganise responses into responder (green colour) or non-responder (red colour). Endurance exercise training (E), resistance exercise training (R). The score is the sum of the numbers of positive response of health benefits.

Subjects	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16	
VO_2max/ALBM	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R	E	R
DBP																																
HOMA-IR																																
Abdominal fat																																
Score out of 3	3	0	3	0	2	0	1	0	1	0	1	2	1	1	1	0	0	2	0	2	2	2	1	2	1	2	0	1	2	1	1	0

Individual responses were also plotted in heat map to help in visualizing the impact of primary outcome adaption and health benefits. Three of the health benefits, which highly linked to metabolic health, were selected (DBP, HOMA-IR, and abdominal fat). A score was given to each training mode of each participant according to number of amended measurements (Table 5.3). From the table, 8 subjects (50%) scored higher with EET than RET and 6 (37.5%) scored better with RET. Although, 2 subjects (12.5%) had equal scores across modes. The most frequent score in the subjects who showed improvements in VO_2max was 1 (5 subjects). While 2 was the most frequent score among responders to RET (5 subjects). For the non-responders to EET (8 subjects), 5 of them improved 2 (2 subjects) or at least 1 (3 subjects) health parameter. Alternatively, 6 out of 7 non-responders to RET, showed no improvement in any of the three health benefits.

5.5.9 Intra-Individual responses:

The expected mode-specific primary physiological outcomes for each of training mode were used to compare the level and specificity of individual responses. VO_2max and AT represented the EET response while ALBM and lower body strength were chosen for RET. Each Individual's response %Δ was plotted, and the plot was divided into sections indicative of high responses, according to mean+1SD of %Δ. Interestingly, there was a significant negative correlation between %Δ ALBM and %Δ VO_2max (r=-0.52, *P=0.04*) (Fig 5.10). In addition, %Δ strength correlated with %Δ VO_2max (r=-0.43, *P=0.09*). Conversely, there was no correlation of muscle gain or strength with AT.

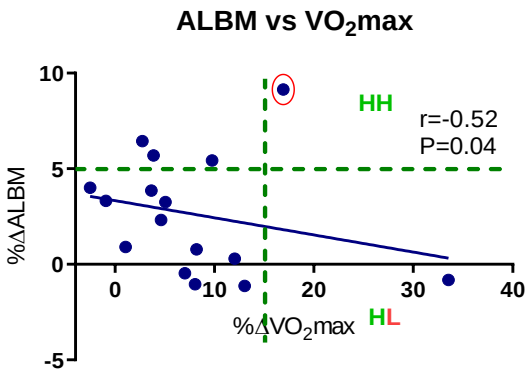

Figure 5.11 Individual's responses to mode-specific physiological primary outcome during both training modes. Maximum oxygen consumption (VO_2max) and appendicular lean body mass (ALBM) were used for EET and RET respectively. Subjects' response levels were plotted as percentage change (%Δ). Pearson's coefficient was used. P<0.05 was consider significant.

After sorting individuals' responses into responder and non-responder for the mode specific-primary adaptions according to the previous cut-off value, the probability of a non-responder to RET being a responder to EET is ~85%. In addition, high responders to RET have low probability of being a high responder to EET (22%). The intercept from the mixed model logistic regression analysis was (-3.0). The odds ratio of individuals being responders to both modes of training is less than 1:1. (0.046, *P=0.024*) (Table 5.4).

Table 5.4 Fixed Effects Parameter Estimates.

					95% Exp(B) Confidence Interval			
Names	**Effect**	**Estimate**	**SE**	**exp(B)**	**Lower**	**Upper**	**z**	**p**
(Intercept)	(Intercept)	0.269	0.673	1.3093	0.35037	4.893	0.401	0.689
ALBM1	1 - 0	-3.045	1.345	0.0476	0.00341	0.665	-2.263	0.024

5.6 Discussion

Exercise training triggers a number of physiological adaptions that usually lead to improvements in at least some aspects of an individual's fitness and overall health. However, the adaptive responses (fitness or health) vary widely amongst individuals, wherein some individuals have a significant positive response (high-responder) others show low, no, or even a negative response (low/non-responder)[275,276,280]. The presence of low/non-responder status is undeniable, and the mechanisms underlying this are as yet unexplained. It is also not yet clear, if the low/non-responder is specific to a particular exercise mode or if this exercise-resistance can be overcome with different mode, intensity, or duration of exercise training; i.e. would a low/non-responder to one mode of training would be a high-responder to another? Further, does the magnitude of this adaptive response also define the level of health benefit that may be achieved? Herein, we tried to assess the response of the same individual to two standardised but distinct training programs, EET and RET, and explore relationship between any adaptive response (fitness) and potential health benefits e.g. blood pressure, HR, insulin sensitivity etc.

5.6.1 The cross-over study design

4-weeks of exercise training successfully induced an improvement in the primary training mode outcomes (VO_2max and AT for EET, ALBM and muscle strength for RET), at least for some of the individuals, which have previously been validated in short term training interventions, producing a robust response in aerobic capacity with EET[30] and muscle mass and strength with RET[338]. In addition, the 6-weeks of "washout" were deemed sufficient to return the primary mode adaptation to baseline, again this was based on previous evidence showing that VO_2max declined rapidly after 3-4 weeks of detraining[339]. In another recent study,

4-weeks of cessation exercise training were able to return VO_2max and health benefit beneficial adaptations to baseline levels[340].

5.6.2 The mode-specific adaptions to EET and RET and their heterogenetic responses

In line with previous literature[275], we noticed inter-individual response variation in both VO_2max and AT gains following 4-weeks EET. The HERITAGE Family study showed that ~50% of heterogeneity in VO_2max response was driven by hereditary factors[341], primarily genotype[342]. Herein, we did not assess the hereditary effect on training response, but were we able to minimize the effect of other factors such as age and gender.

In the HERITAGE Family study, of 720 subjects, a limited role of age on the heterogeneity of the response was reported, accounting for only 4%. However, the predictive role of age was eliminated with 125 male and female in the age range 17-29[275], so this is unlikely to be a factor in our study since our subjects were young and of similar age (18-33). Subject sex is not determining factor in our study since our study was limited to male subjects only even though it accounted for 5.4% of the prediction response[275]. Our subjects were from different ethnic but according to HERITAGE Family race accounted for <1% of the heterogeneity in VO_2max. Finally, the role of initial (baseline) VO_2max accounted for 1% in the HERITAGE Family[275], which increased significantly in cohorts with smaller sample sizes[343,344]. Despite our limited sample size, the correlation between VO_2max and Δ, was not significant, but it was significant with $\%\Delta$, as in HERITAGE Family of Skinner et al (2001). However, they also concluded that initial VO_2max had no significant contribution to subsequent response[345].

Another explanation for the variation in VO_2max gain could be the difficulty in determining the VO_2max plateau, as some participants may not push themselves to their maximal effort, which could be due to physiological or psychological factors. In addition, reaching the "true plateau" of VO_2max is quite challenging, such that a number of participants failed to reach a true maximum[346]. Edvardsen et al (2014) reported that only ~50% of participants could reach a true plateau, this could also due to the existence of a submaximal plateau during CPET[87].

In this study, the training protocol was standardized, of fixed duration and intensity, aiming to minimize the homeostatic stress of exercise training on the heterogeneity of response. However, prescribing the EET as a percentage of VO_2max may contribute to inter-individual variation since it may not be truly representative, or contribute a fixed proportion of the metabolic response[281]. Other physiological factors that may contribute to the overall training response include sleep, psychological stress, habitual physical activity and dietary intake[281]. Since we did not collect records of sleeping patterns, rest, or habitual physical activity, imbalance in between stress and recovery may lead to maladaptation and subsequently produce heterogenic response[347].

In the HERITAGE Family study, CPET was performed two times pre and two times post training intervention to ensure reliable measurements were taken. They reported the mean of less than 5% difference or chose the highest reading if the difference was higher than 5%[275]. Due to study restrictions and as limitation of this study, CPET was only performed once pre and post training intervention, therefore, a possible contribution of technical and day-to-day variations in the measurements of aerobic capacity cannot be excluded in this study. However, we tried to control this variation by exposing the volunteers to same conditions pre the test. In addition, our CPET was monitored by trained clinicians to make sure that individual would give

his best performance during the test, and CPET was accepted if VO_2max plateau appeared, or 3 out of 4 conditions were met (HR>95% of maximum, RER>1.1, RPE of >9, and failure to maintain 60 rpm at the end of test despite continued verbal encouragement). The 'Familiarization effect' may also lead to improvement in CPET performance as the subjects become more familiar with the procedures. It could be no a major flaw of the response variability, but still a factor.

To overcome the limitation of not reaching the VO_2max in some participants, we also focused on the AT response to EET as a submaximal parameter. The AT response showed less heterogeneity with positive change in all individuals. As in our study, AT improved regardless of improvement in VO_2max[348]. Therefore, AT may be a more informative indicator of individual responses to EET.

The aim with RET was to induce a response in both hypertrophy and muscle function/strength, which we achieved. As with EET, RET resulted in inter-individuals heterogeneity of responses. Even though 4-weeks of training may not be enough to exert maximal hypertrophic responses in younger adults, increases in muscle CSA have been observed at week 4 in 12-weeks of a training intervention[349], however, we have recently reported that the gain in muscle hypertrophy, to a similar exercise regime, peaked after the 3rd week of RET, at least in terms of both MPS gains and *vastus lateralis* muscle thickness increases, a further 3-weeks of RET resulted in no further gains[338].

The heterogenic response in muscle mass gain, thickness, and strength parameters (1-RM, and MVC) were consistent with previous studies, ranging from no gain to twice the average gain[276,277]. For instance, the change in leg muscle mass by DXA ranged from -7 to 14%, and

changes in VL MT from 2 to 30%. Further, changes in muscle strength also varied from -8 to 60% in a retrospective analysis of 20-24 weeks of RET[282] as in our study. The variation in muscle gain is complex and not effected by the pre-existing characteristics such as age, gender, initial muscle mass, physical activity level, or diet[277,282]. As we controlled age and sex herein, we also saw no correlation between initial levels of muscle mass or strength before and after RET.

Gains in muscle mass have been linked to changes in muscle architecture, i.e. the PA of the fibres. Kawakami et al (1993), had seen greater PA in hypertrophied human *triceps brachii* in comparison to normal muscle[109]. Herein PA increased in response to RET and was positively correlated to the change in MT, which indicated that increase in MT and thus VL hypertrophy is more likely influenced by PA increase.

5.6.3 The Health benefits

The health responses assessed for each of the training modes (EET, RET) included body composition (lean and fat mass), metabolic health (glycaemia), vascular health (blood pressure, blood lipid profile). Overall, some improvements were noted in response to both modes of training e.g. body composition. While EET was more effective and significant than RET in improving blood pressure.

5.6.3.1 Blood pressure

Exercise training is recommended for the prevention and treatment of hypertension[350]. Different effect of EET and RET are expected on blood pressure because of different mechanical characteristics and physiological adaptations. In this study we observed an improvement on DBP and MAP after EET only. A meta-analysis concluded that EET

decreased SBP (-3.8 mmHg) and DBP (-2.58 mmHg) in both normotensive and hypertensive people[61]. Our reported reduction in DBP was similar to previously reported reductions in DBP (3-4 mmHg), which is clinically relevant and associated with reduced cardiac morbidity (5%), and all-cause mortality (4%)[351]. However, this reduction was not correlated to any of the mode-specific adaptive response.

Even though, both EET and RET have resulted in improved SBP and DBP[63,61,352], a study fall in line with our findings reported a limited effect of RET in lowering blood pressure, concluded that EET is the recommended mode of exercise training to cause blood pressure reduction[62]. However, a very recent comparative study reported that neither EET nor RET was able to provide significant reduction in SBP or DBP, and only combined exercise training (RET and EET in same session) reduce DBP[351]. A common finding in studies that report an impact on blood pressure, with either mode, is the reduction in DBP, which is less common in SBP. The contradiction regarding the lack of effect on SBP could be a reflection of the intervention duration, as in most of the studies, the effect on SBP was observed only after 12-weeks on training[353,354]. Therefore, 4-weeks of training could not be a sufficient for significant reduction on SBP. In addition, the blood pressure reduction was more pronounced within hypertensive population than non-hypertensive[355].

5.6.3.2 Body composition

Increased adiposity is considered as a risk factor for cardiovascular disease, either as total body or regional fat deposition. In fact, abdominal fat deposition carries greater risk than total body fat[356]. Despite the different nature of training modes, both showed a positive impact on total body and regional fat. Previous literature studied the impact of EET and RET on adiposity on healthy and obese subjects. In Studies Targeting Risk Reduction Interventions Through

Defined Exercise-Aerobic Training and/or Resistance Training (STRRIDE/AT/RT), both EET and RET induced a reduction in body fat. However, EET was more efficient in lowering abdominal fat (visceral)[357], which in line with our findings, since the reduction was 6% with EET and 1% with RET. In another study where obese subjects were divided into EET or RET, authors concluded that RET was not as effective in reducing body fat as EET, and even questioned the RET role in improving adiposity. They speculated that the lack of change (%) with RET, was linked to the increase in muscle mass since the total fat mass was unchanged[358]. Another study on young healthy participants supports the limited impact of RET on total and abdominal fat, whereas EET reduced both[356]. Our findings however, support a role of RET on adiposity, since total fat mass was decreased as well as the (%). The discrepancy observed in the literature could be a result of different aspects of the training regime, e.g. intensity and volume. We employed six exercises (3 upper, 3 lower) at 70% of 1-RM that was re-estimated after 2-weeks of training to keep the intensity through whole intervention, while Perez-Gomez et al (2013) used 5 exercises with intensity of 50-90% of 1-RM[356]. In addition, inter-individual heterogeneity to exercise training makes it even harder to interpret these data.

It is well known that RET consumes less energy than EET for same training duration. There is ~67% greater calorie expenditure with EET compared to RET, which may explain the different impact on body fat[357]. RET will likely reduce body fat through increasing of resting metabolic rate as consequence of increased lean body mass, i.e. a greater number of cells[359]. The correlation between %Δ in TLBM with %Δ total body fat (%), i.e muscle gain and fat loss is supportive of previous hypotheses. Similarly, the correlation between, %Δ VO_2max %Δ abdominal fat, strengthen the calorie expenditure hypothesis with EET.

5.6.3.3 Glycaemic Index

The beneficial impact of exercise training on metabolic health is well recognized. Regular physical activity improves insulin sensitivity to mediate glucose uptake in peripheral tissue (e.g. skeletal muscle), which occurs through multiple adaptations in glucose and lipid metabolism[360]. In particular, EET is well recognized for its positive effect on insulin sensitivity through intrinsic changes in glucose handling[361]

Despite, the non-significant change of EET on glucose/insulin tolerance, or insulin sensitivity, the %Δ in AT had correlated to the %Δ in insulin response and %Δ in insulin sensitivity. However, these glycaemic indices did not correlate with the %Δ of VO_2max as reported before[361,348]. The crucial role of mitochondrial content and function (oxidative capacity) to insulin resistance was suggested before. A decline with age or inherited defect in mitochondrial function was associated to insulin resistance[362,363], which could explain the correlation of improved insulin sensitivity of EET to improved AT, rather than VO_2max.

Likewise, the RET has been found to improve glucose tolerance and insulin sensitivity[123]. However, a lesser impact was reported with RET when compared to EET, in healthy women[361], in line with our observations. Interestingly, the %Δ in ALBM correlated to %Δ in glucose disappearance and consequently insulin sensitivity. RET is suggested to improve glucose metabolism and insulin resistance, not simply as a result of the increase in muscle mass as storage/disposable tissue, but also as the quality of trained muscle, thereby enhancing the resting metabolic rate, and the mobilization/utilization of visceral and subcutaneous fat[123].

5.6.4 Molecular adaptations associated with improvements in aerobic capacity or mass/function:

As discussed, the specific training mode will trigger a unique sequence of the adaptations, from the molecular level to the macro-level. Although, there are overlapping interactions between the two training modes, the phenotypic adaptations are quite distinct. Herein we report on the impact of EET on mitochondrial biogenesis and angiogenesis and RET on anabolic regulators of muscle protein synthesis and ribosomal biogenesis.

5.6.4.1 Mitochondrial biogenesis

EET is known to enhance aerobic capacity via improving the cardiorespiratory system in delivering O_2 to working muscle (O2 extraction and transport) and the boosting the ability of working muscle aerobic energy. Skeletal muscle aerobic energy depends on the number (density) and activity/function of the mitochondria. Mitochondrial biogenesis is a complex process that is crucially mediated by PGC-1α, which could be considered as a central regulator of the endurance/aerobic exercise adaptive response[364,159]. PGC-1α stimulates nuclear-encoded mitochondrial genes, as NRF1 to increase expression of TFAM for mitochondrial DNA (mtDNA) transcription[160,161]. In our study, we reported a trend towards increases in PGC-1α gene and protein expression post-EET, in line with previous literature from animal[365] and human[366] studies. Consequently, TFAM increased significantly with EET, indicating the successful induction of mitochondrial biogenesis. EET leads to energy stress in skeletal muscle that would increase the phosphorylation of AMPK, as in our study, which was concurrent with increases in PGC-1α protein expression[161]. AMPK is also essential for mitochondrial biogenesis since deleting its gene *in vivo* leads to mitochondrial dysfunction[367]. Finally, we assessed the mitochondrial content through the expression of the proteins in the mitochondrial respiratory transfer chain complexes and demonstrated substantial increases in

3 of the 5 complexes with EET, confirming the increased efficiency of mitochondrial biogenesis.

5.6.4.2 Angiogenesis:

Herein, we also investigated the impact of EET and RET on angiogenesis in muscle. Activation of angiogenesis, expansion of capillary networks, is considered important element of the EET response. We focused our investigation on two angiogenesis regulators families in skeletal muscle, VEGF with its receptor (FLT1), and the ANGPTs. The binding of VEGF to its endothelial receptor facilitates endothelial proliferation, migration, and formation of new functional capillaries. Acute EET increases gene expression of VEGF in skeletal muscle of human[368], rats[369], and mice[370]. Acute EET was also able to increase FLT1 in rats[371]. In contrast to our finding, 6-weeks of EET did not show increases in the gene expression of VEGF nor its receptor, in either high and low responders in human skeletal muscle[176], however in line with our findings, chronic EET (8-weeks) increased mRNA of VEGF[167]. A study in rats following 25 days of EET, also reported an increase in gene expression of angiogenetic factors, which declined thereafter to baseline levels. VEGF peaked after 1-3 days after training, whereas VEGF receptors (FLT1, KDR) peaked between day 3-8 and declined rapidly after[174]. This rapid loss, which is either a result of a short-half life or reduced transcription, may explain the lack of a response on FLT1 and KDR in our study.

Angiopoietins act synergistically with VEGF to promote angiogenesis, and are suggested to be crucial for angiogenesis. Both angiopoietins, ANGPT1 and ANGPT2, bind to the same tyrosine kinase receptor Tie-2, which create agonist-antagonist competition. ANGPT1 stabilizes existing vasculature, whereas ANGPT2 destabilizes and promotes new vasculature. Therefore, the ratio of ANGPT2/ANGPT1 is used as indicator of the pro- anti-angiogenic

direction. Lloyd et al (2003) reported elevated level of ANGPT2 mRNA expression with increase in ANGPT2/ANGPT1 ratio and Tie-2 in rats after intense EET[174]. In contrast, Timmons et al (2005) reported an increase in gene expression of ANGPT1 rather than ANGPT2 in human after 6-weeks of EET. However, the increase was restricted to the high responders group[176]. Despite the discrepancy, no significant changes were seen in our results for either angiopoietins, which could be the result of sampling time, as our tissue was collected 48 hours after last exercise session, instead of 24 hours.

5.6.4.3 Anabolic signalling and ribosomal markers:

Muscle mass and the RET induced hypertrophic response are regulated by, amongst other compounds, growth factors such as IGF-1 and MSTN[291]. IGF-1 has been implicated in many anabolic pathways, since it has centre role in muscle growth, differentiation, and the regeneration of skeletal muscle. IGE-1Ea and IGF-1Eb are isoforms of IGF-1 in adult mammals, which may perform slight different functions[372]. The dominant isoform, IGF-1Ea, promotes satellite cell differentiation and protein synthesis. Overexpression of IGF-1Ea increased muscle hypertrophy in young mice[373]. Therefore, the trend towards increase in IGF-1Ea gene expression, we observe after 48 hours of last exercise bout, may indicate that the hypertrophic response in our study was driven by the IGF-1Ea acutely following the bout of exercise.

We also observed a significant decrease in MSTN gene expression, which has been reported previously with an acute bout of RET[374]. MSTN is a negative regulator of muscle mass, via inhibition of MPS and possibly opposing mTORC1 signalling[375]. The reduction in MSTN with RET may influence muscle mass gains, as we found a significant negative correlation to ALBM. In line with our finding, MSTN inhibition led to an increase in muscle mass and strength

in adult mice[376]. In contrast however, we previously found no change in MSTN gene expression in skeletal muscle of young and old participants following 6-weeks of RET[291]. Further, gene expression of MSTN has been reported to be elevated after 12-weeks of RET[377]. However, Bergen et al (2015) concluded that MSTN is homeostatic regulator rather than a cause of sarcopenia in males[378]. Despite the trend for an increase in IGF-1Ea and significant decrease in MSTN, none of the other myogenic factor we measured showed any change i.e. MyoD and MyoG. However, %Δ in MyoD proportionally correlated to %Δ ALBM, which may indicate that MyoD was acutely involved in driving muscle growth.

Muscle hypertrophy has two facets key process, translation efficacy and translation capacity. We found no change in the mTORC1 nor any upstream/downstream regulators despite its purported centre role in translation efficiency and MPS[379]. This anabolic signalling pathway is upregulated in hours after the RET and lasts only for a short time after the last exercise session. A limitation in our protocol, may be that the muscle biopsy was taken 48 hours after last exercise session. Regarding the translation capacity, none of the markers of ribosomal biogenesis markers, i.e. c-Myc, TIF- IA, and UBF were significantly altered. However, the total RNA increased with RET, as has been reported previously[338]. Total RNA content can be enhanced after few bouts[380]of RET and after several weeks[381] in human studies. Subsequently, we noted an increase in the RNA: DNA ratio, which is an indicator of the capacity for protein synthesis[338], and this change in RNA: DNA correlated with changes in TLBM.

5.6.5 Fitness vs health

The significant correlation between VO_2max and muscle mass Δ change with health benefits were discussed previously in body composition and glycaemic sections. However, the lack of any correlation also provides us with important messages. The presence of low/non-responders to EET for instance, raises a concern about these individuals having no health benefits of exercise training, as low aerobic capacity is linked to low survivability[325]. Therefore, it is important to know that changes in VO_2max are not the only link to health improvements. The data herein, showed that 50% of VO_2max low/non-responders had improvements in at least one metabolic health parameter. In addition, more than 50% of the responders for VO_2max showed an improvement in only one metabolic/health marker (DBP, HOMA-IR, and abdominal fat).

On the other hand, the response to RET via muscle mass gains suggested a strong link to improved health parameters. 6 of 7 low/non-responders had no clinically significant improvement in the selected three metabolic health measurements. This perhaps demonstrates the importance of muscle mass and its impact on other health benefits; this can be seen with high responders to RET, where more than 50% of them showed improvements in at least 2 metabolic/health factors. Previous observations indicate that the improvement in VO_2max is a subsequent of enhancement in cardiorespiratory and metabolic health. While the improvement in cardiorespiratory and metabolic health are the subsequent benefits of muscle gain. These again emphasize the importance of muscle in health.

5.6.6 Intra-Individual responses to different exercise modes

It is was also imperative to investigate how would the low/non-responder adapt to different exercise training modes. By comparing the primary mode-adaptions (VO_2max - EET and ALBM/strength - RET) in same individuals, we noted that in general there was a negative correlation. Suggesting that a high-responder to EET would be a low-responder to RET and vice versa, with a gradation of responses; i.e. intermediate responders, responding to both training mode. This would support the hypothesis of preference mode of training among individuals.

However, defining the low/non-responders is a challenging process, especially from statistical prospective. By applying the basic definition of low/non-responders on our participants, as individual who show no measurable change or even an adverse response, the prevalence of low-responder in VO_2max was in line with previous literature (~20%) following EET[285]. Others have considered the clinical meaningful of changes[382]; i.e. changes that are not clinically significant does not count, and the technical error of any measurements[288], which when applied in this study reveal that 50% (8 subjects) of our participants were non-responders with EET and 43.7% (7 subjects) showed no response with RET. The calculated probability herein, showed that low/non-responders to RET have 85% (6 out of 7 subjects) chance to be responders and 15% to be low/non-responders with EET.

5.6.7 Conclusion

The small sample size, number of participants in this cohort is an obvious limitation. Even though we were able to detect mode specific adaptive responses, a larger cohort would produce more powerful statistical analysis to our finding herein. In fact, the correlation that we reported here could be impacted by the sample size, and a larger sample size could strengthen or weaken the relationships. However, this is to our knowledge the first undertaking of its kind and represents a significant body of work and effort. We believe that the results presented here reflect what is occurring in response to different exercise modes, despite the low number.

4-weeks of training intervention was able to improve both primary mode outcomes, AT and VO_2max with EET and muscle mass, size, and strength with RET. Further, detraining for 6-weeks washed out the mode specific changes. A heterogeneity in Inter-individual response was seen in both EET and RET. More importantly, the intra-individual response to both training mode suggested a mode preference for some individuals. Exercise training health benefits were not restricted to one exercise mode, but occurred through a different pathways, as with the impact on insulin sensitivity. The magnitude of VO_2max response with EET was not a defining factor for health benefits. Gaining muscle mass with RET was linked to health benefits. Finally, the molecular changes appeared to broadly support the clinical adaptions observed, EET enhanced angiogenesis markers and mitochondrial biogenesis, while RET increased growth factors and ribosomal capacity for protein synthesis.

6 General Discussion

Skeletal muscles secrete myokines into the circulation as an adaptive response to exercise bout and exercise training, which then act in an autocrine, paracrine, and/or endocrine fashion to promote fitness and health benefits in the body. Since the concept of myokines was established, with IL-6, the list of putative myokines has continued to grow, with many researchers striving to uncover the underlying mechanisms of their expression, secretion, and physiological actions.

Herein, the role of a recently proposed myokine, Fndc5/irisin, on the metabolism of skeletal muscle and adipose tissue was investigated in an animal model, wherein the Fndc5 gene was successfully overexpressed in rat hind-limb muscles using an IVE technique. The local overexpression of Fndc5 gene, to the level that was reported after exercise training (~2 fold change)[257,270,315], led to an increase in systemic circulating irisin that was again similar to the reported exercise induced level (3.5 ng/ml)[317]. The physiological increase in circulating irisin and FNDC5 expression in the electroporated muscle, when compared to the control animals, meant we had achieved the stated aim and permitted further investigation of the metabolic role of physiological Fndc5/irisin in skeletal muscle and adipose tissue. However, the increase had a limited impact on skeletal muscle and adipose tissue metabolism, which does not support the positive effect of previous literature, where irisin recombinant treatment, i.e. supraphysiological levels, were used i.e. the significant impact of irisin treatment was dose and time dependent. The main effect of irisin on muscle, in our model, appeared to be with regard to glucose metabolism, as we see increased glycogen levels, upregulated GYS1 and a reduced P-GS/GS ratio, which were supported by other studies in muscle and liver cell lines[313,140], but this was not due to an increased rate of glucose uptake. However, this may

partially explain the benefits on glucose homeostasis and insulin sensitivity observed in response to exercise training. Moreover, we saw no changes in mitochondrial content or activity, suggesting increased mitochondrial biogenesis was not regulated through irisin.

Myokines were first proposed as "exercise factors", as exercise training increases the circulating levels of these compounds. However, the myokine response to exercise training varies amongst individuals with some showing no or very low responses, high responses, and a continuum of responses in between. It is not known if the magnitude of the myokine release is related to the level of the adaptive response i.e. do high levels of myokines predominate in high responders or vice versa? Fortunately, we had access to LRT/HRT rat model[325] and were able to conduct a study in which we measured baseline, acutely post exercise, and chronically after training intervention myokines responses. These animals inbred for either low responder trainability or high responder trainability. The myokine profile at baseline reflected the metabolic status of rats, particularly for FGF21 and musclin. Both are reported high in diabetic and obese subjects, similar to the levels/metabolic status of LRT animals at baseline[195,209,211,79]. Regarding the acute response to exercise session, the profiles for most of the measured myokines, were not defined by the level of trainability, except for musclin, which was higher in LRT animals. In previous literature, musclin demonstrated an ability to improve aerobic capacity in mice[212], which could explain the LRT elevated musclin level i.e. as compensation for their low aerobic capacity. However, following 3-weeks of endurance training musclin baseline levels were unchanged to pre-training baseline in LRT. Perhaps as a result of sustained metabolic disorder and low aerobic capacity of these animals. Conversely, the observed increased in musclin baseline level in HRT animals following training intervention goes along with the improvement in their aerobic capacity. Therefore, more

detailed research are required to uncover the underlying mechanisms of exercise factors (myokines). In addition, studies to explore myokines role in the human adaptive response to exercise training are requisite. However, we need to understand the human heterogenic adaption to exercise training first.

To explore the phenomenon of low/non-responders and its impact on health benefits, in a human model, we undertook a study using a novel approach. Same participants were crossed over the two distinctive 4-weeks exercise training (EET and RET) in a random fashion. In previous clinical studies, low/non-responders were identified to each of the training mode EET[278,279] and RET[276,277]. However, it is well known that exercise type, intensity, and duration define the adaptive response. This led to our research question, 'Would a low responder to EET be a low responder to RET as well?" First of all, we confirmed that the 4-weeks of training initiated the mode-specific adaptive response (VO_2max and AT for EET, and hypertrophy and strength for RET) as had been previously validated in training intervention of EET, induced aerobic capacity[30], and RET, increase muscle and strength[338]. Relying on the rapid decline in VO_2max (3-4 weeks of detraining[339]), the 6-weeks of "washout" returned the mode-specific adaptation to baseline. This allowed us to go then explore the individual's responses to the other mode of training. A particularly interesting finding in this study was that a low responder in RET in muscle mass was most likely (85%) a high responder in EET in VO_2max, i.e., an individual would respond better in one training mode of the other. However, intermediate responders were also existed, who appear to respond to both modes to a similar magnitude.

We were also interested to know if the magnitude of response to mode-specific outcomes would define the health benefits of exercise training. Simple correlation analysis indicated that %Δ VO_2max or AT (for EET) and lean mass (for RET) do not closely reflect the clinical

outcomes in general. However, insulin sensitivity correlated to %Δ AT of EET and ALBM of RET, supporting physiological roles for both EET and RET in enhancing insulin sensitivity. Similarly, the increase in total lean mass would reduce the % of total body fat as in our results[358]. Although, the negative correlation and heat map showed that being a low/non-responder to VO_2max would not restrict the improvement for health benefits. Our finding suggests that enhancement of cardiorespiratory and metabolic health are key for improved VO_2max. On the other hand, gaining muscle mass seems to be the key for improving insulin sensitivity, body fat, and blood pressure, which accentuate the role of muscle tissue in maintain health.

Final Conclusion

In this thesis, physiological Fndc5/irisin, has limited effects on the metabolism of skeletal muscle and adipose tissue after successful local transgenic of Fndc5 on hind-limb of rats and increase of circulated irisin.

In addition, in a rat trainability model, circulating myokines plasma concentrations were not related to the trainability of rats. However, musclin showed the potential to either define low responders or improvements in aerobic capacity. Furthermore, some myokines could be indicators for metabolic health, as FGF21 and musclin.

Finally, a novel approach exposing the same subject to different modes of exercise training indicated that low/non-responders in any of the two exercise training modes (EET or RET), would have high chance to respond better to the other mode of training (RET or EET). However, some participants response similarly to both modes (intermediated responders). In

general, the extent of the health benefits were not related to the magnitude of adaptive response in fitness.

Scope of future work

The plasma concentration of myokines in the pre-clinical study of LRT-HRT rat's model (chapter 4) revealed Musclin's potential to identify the aerobic capacity of an individual. In addition to its proposed ability to enhance aerobic capacity. However, little is known about its role in angiogenesis, which suggests further research is needed. *In vitro* studies on C2C12 cells or HCSs may reveal if the Musclin has any effect on skeletal muscle angiogenesis. Also, given the many problems measuring cytokines accurately, using mass spectrometric techniques, as gold standard approaches, for analysing plasma myokines would provide more accurate and robust results.

In the present human clinical study (chapter 5), MPS was not assessed due to time and capacity limitations and access to the mass spectrometric instrumentation. Measuring MPS would help provide information on the rate of synthesis of both myofibrillar and mitochondrial compartments, thereby providing mechanistic information which may support the differential adaptive response between EET and RET. In addition, MPS may help to distinguish the low from high responders, and a hypothesis that low/non-responders may show a blunted MPS. Further, sophisticated statistical analysis of the mode-specific outcomes with health benefits is ongoing, taking the technical error into consideration, to then divide individuals into responders or non-responders. This may also reveal clinical markers that report on responder status to EET and RET. Finally, recruiting more participants would produce a more robust statistical analysis following the protocol outlined in this thesis.

7 References

1. Frontera WR, Ochala J. Skeletal Muscle: A Brief Review of Structure and Function. *Behav Genet*. 2015;45(2):183-195. doi:10.1007/s00223-014-9915-y

2. Smith K, Rennie MJ. 4 Protein turnover and amino acid metabolism in human skeletal muscle. *Baillieres Clin Endocrinol Metab*. 1990;4(3):461-498. doi:10.1016/S0950-351X(05)80065-5

3. Martini B, Bartholomew EF. *Essentials of Anatomy and Physiology, Ed*. Second. New Jersey: Prentice-Hall; 1997.

4. Wolfe RR. The underappreciated role of muscle in health and disease. *Am J Clin Nutr*. 2006;84(3):475-482. doi:10.1093/ajcn/84.3.475

5. Gallagher D, Belmonte D, Deurenberg P, et al. Organ-tissue mass measurement allows modeling of ree and metabolically active tissue mass. *Am J Physiol - Endocrinol Metab*. 1998;275(2 38-2):E249-E258. doi:10.1152/ajpendo.1998.275.2.e249

6. Berne RM, Levy MN. *Physiology*. Second. ST. Louis: The C.V. Mosby Company; 1988.

7. Zierath JR, Hawley JA. Skeletal muscle fiber type: Influence on contractile and metabolic properties. *PLoS Biol*. 2004;2(10). doi:10.1371/journal.pbio.0020348

8. Schiaffino S, Reggiani C. Fiber types in Mammalian skeletal muscles. *Physiol Rev*. 2011;91(4):1447-1531. doi:10.1152/physrev.00031.2010

9. Westerblad H, Bruton JD, Katz A. Skeletal muscle: Energy metabolism, fiber types,

fatigue and adaptability. *Exp Cell Res*. 2010;316(18):3093-3099. doi:10.1016/j.yexcr.2010.05.019

10. Wallimann T, Tokarska-Schlattner M, Schlattner U. The creatine kinase system and pleiotropic effects of creatine. *Amino Acids*. 2011;40(5):1271-1296. doi:10.1007/s00726-011-0877-3

11. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol - Endocrinol Metab*. 1993;265(3 28-3). doi:10.1152/ajpendo.1993.265.3.e380

12. Sinacore DR, Gulve E a. The role of skeletal muscle in glucose transport, glucose homeostasis, and insulin resistance: Implications for physical therapy. *Phys Ther*. 1993;73(12):878-891. doi:10.1093/ptj/73.12.878

13. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev*. 2013;93(3):993-1017. doi:10.1152/physrev.00038.2012

14. Zisman A, ODILE D. PERONI, E. DALE ABEL, et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med*. 2000;6(8):4-8. doi:10.1038/78693

15. RYDER JW, KAWANO Y, GALUSKA D, et al. Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. *FASEB J*. 1999;13(15):2246-2256. doi:10.1096/fasebj.13.15.2246

16. Prats C, Gómez-Cabello A, Hansen A V. Intracellular compartmentalization of skeletal

muscle glycogen metabolism and insulin signalling. *Exp Physiol*. 2011;96(4):385-390. doi:10.1113/expphysiol.2010.052860

17. Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab*. 2013;17(2):162-184. doi:10.1016/j.cmet.2012.12.012

18. Knuiman P, Hopman MTE, Mensink M. Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. *Nutr Metab*. 2015;12(1). doi:10.1186/s12986-015-0055-9

19. Burke LM, Van Loon LJC, Hawley JA. Postexercise muscle glycogen resynthesis in humans. *J Appl Physiol*. 2017;122(5):1055-1067. doi:10.1152/japplphysiol.00860.2016

20. Atherton PJ, Wilkinson DJ, Smith K. Feeding Modulation of Amino Acid Utilization. *Mol Nutr Amin Acids Proteins*. 2016:109-124. doi:10.1016/b978-0-12-802167-5.00009-8

21. Atherton PJ, Smith K. Muscle protein synthesis in response to nutrition and exercise. *J Physiol*. 2012;590(5):1049-1057. doi:10.1113/jphysiol.2011.225003

22. Durstine JL, Gordon B, Wang Z, Luo X. Chronic disease and the link to physical activity. *J Sport Heal Sci*. 2013;2(1):3-11. doi:10.1016/j.jshs.2012.07.009

23. Hu FB, Willett WC, Li T, Stampfer MJ, Colditz GA, Manson JAE. Adiposity as compared with physical activity in predicting mortality among women. *N Engl J Med*. 2004;351(26):2694-2703. doi:10.1056/NEJMoa042135

24. Macera CA, Hootman JM, Sniezek JE. Major public health benefits of physical activity. *Arthritis Rheum*. 2003;49(1):122-128. doi:10.1002/art.10907

25. Blair SN, Kohl HW, Paffenbarger RS, Clark DG, Cooper KH, Gibbons LW. Physical Fitness and All-Cause Mortality: A Prospective Study of Healthy Men and Women. *JAMA J Am Med Assoc*. 1989;262(17):2395-2401. doi:10.1001/jama.1989.03430170057028

26. Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: The evidence. *CMAJ*. 2006;174(6):801-809. doi:10.1503/cmaj.051351

27. Pedersen BK, Saltin B. Evidence for prescribing exercise as therapy in chronic disease. *Scand J Med Sci Sport*. 2006;16(SUPPL. 1):3-63. doi:10.1111/j.1600-0838.2006.00520.x

28. Garber CE, Blissmer B, Deschenes MR, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Med Sci Sports Exerc*. 2011;43(7):1334-1359. doi:10.1249/MSS.0b013e318213fefb

29. Keller P, Vollaard NBJ, Gustafsson T, et al. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. *J Appl Physiol*. 2011;110(1):46-59. doi:10.1152/japplphysiol.00634.2010

30. Hoier B, Passos M, Bangsbo J, Hellsten Y. Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Exp Physiol*. 2013;98(2):585-597. doi:10.1113/expphysiol.2012.067967

31. Franchi M V., Atherton PJ, Reeves ND, et al. Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta Physiol*.

2014;210(3):642-654. doi:10.1111/apha.12225

32. Erskine RM, Fletcher G, Folland JP. The contribution of muscle hypertrophy to strength changes following resistance training. *Eur J Appl Physiol*. 2014;114(6):1239-1249. doi:10.1007/s00421-014-2855-4

33. Kokkinos P, Jonathan Myers, John Peter Kokkinos, et al. Exercise capacity and mortality in black and white men. *Circulation*. 2008;117(5):614-622. doi:10.1161/CIRCULATIONAHA.107.734764

34. Evans WJ. Effects of exercise on body composition and functional capacity of the elderly. In: *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*. Vol 50. ; 1995:147-150. doi:10.1093/gerona/50a.special_issue.147

35. Henriksen EJ. Invited review: Effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol*. 2002;93(2):788-796. doi:10.1152/japplphysiol.01219.2001

36. Kelley GA, Kelley KS, Vu Tran Z. Aerobic exercise, lipids and lipoproteins in overweight and obese adults: A meta-analysis of randomized controlled trials. *Int J Obes*. 2005;29(8):881-893. doi:10.1038/sj.ijo.0802959

37. Green DJ, Eijsvogels T, Bouts YM, et al. Exercise training and artery function in humans: Nonresponse and its relationship to cardiovascular risk factors. *J Appl Physiol*. 2014;117(4):345-352. doi:10.1152/japplphysiol.00354.2014

38. Rivera-Brown AM, Frontera WR. Principles of exercise physiology: Responses to acute exercise and long-term adaptations to training. *PM R*. 2012;4(11):797-804.

doi:10.1016/j.pmrj.2012.10.007

39. Ostman C, Smart NA, Morcos D, Duller A, Ridley W, Jewiss D. The effect of exercise training on clinical outcomes in patients with the metabolic syndrome: A systematic review and meta-analysis. *Cardiovasc Diabetol*. 2017;16(1):1-11. doi:10.1186/s12933-017-0590-y

40. Wewege MA, Thom JM, Rye KA, Parmenter BJ. Aerobic, resistance or combined training: A systematic review and meta-analysis of exercise to reduce cardiovascular risk in adults with metabolic syndrome. *Atherosclerosis*. 2018;274:162-171. doi:10.1016/j.atherosclerosis.2018.05.002

41. Acosta-Manzano P, Rodriguez-Ayllon M, Acosta FM, Niederseer D, Niebauer J. Beyond general resistance training. Hypertrophy versus muscular endurance training as therapeutic interventions in adults with type 2 diabetes mellitus: A systematic review and meta-analysis. *Obes Rev*. 2020;(November 2019). doi:10.1111/obr.13007

42. Seynnes OR, De Boer M, Narici M V. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J Appl Physiol*. 2007;102(1):368-373. doi:10.1152/japplphysiol.00789.2006

43. Karamanidis K, Arampatzis A. Mechanical and morphological properties of different muscle-tendon units in the lower extremity and running mechanics: Effect of aging and physical activity. *J Exp Biol*. 2005;208(20):3907-3923. doi:10.1242/jeb.01830

44. Lee JH, Kim DH, Kim CK. Resistance Training for Glycemic Control, Muscular Strength, and Lean Body Mass in Old Type 2 Diabetic Patients: A Meta-Analysis. *Diabetes Ther*.

2017;8(3):459-473. doi:10.1007/s13300-017-0258-3

45. Coburn JW, Housh TJ, Malek MH, et al. Neuromuscular responses to three days of velocity-specific isokinetic training. *J Strength Cond Res*. 2006;20(4):892-898. doi:10.1519/R-18745.1

46. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. *Physiol Rev*. 1992;72(2):369-417. doi:10.1152/physrev.1992.72.2.369

47. Gavin TP, Drew JL, Kubik CJ, Pofahl WE, Hickner RC. Acute resistance exercise increases skeletal muscle angiogenic growth factor expression. *Acta Physiol*. 2007;191(2):139-146. doi:10.1111/j.1748-1716.2007.01723.x

48. Holloway TM, Snijders T, Van Kranenburg J, Van Loon LJC, Verdijk LB. Temporal Response of Angiogenesis and Hypertrophy to Resistance Training in Young Men. *Med Sci Sports Exerc*. 2018;50(1):36-45. doi:10.1249/MSS.0000000000001409

49. Lundby C, Jacobs RA. Adaptations of skeletal muscle mitochondria to exercise training. *Exp Physiol*. 2016;101(1):17-22. doi:10.1113/EP085319

50. Atherton PJ, Phillips BE, Wilkinson DJ. Exercise and Regulation of Protein Metabolism. In: *Progress in Molecular Biology and Translational Science*. Vol 135. ; 2015:75-98. doi:10.1016/bs.pmbts.2015.06.015

51. Jacobs RA, Lundby C. Mitochondria express enhanced quality as well as quantity in association with aerobic fitness across recreationally active individuals up to elite athletes. *J Appl Physiol*. 2013;114(3):344-350. doi:10.1152/japplphysiol.01081.2012

52. Hoppeler H, Howald H, Conley K, et al. Endurance training in humans: Aerobic capacity and structure of skeletal muscle. *J Appl Physiol*. 1985;59(2):320-327. doi:10.1152/jappl.1985.59.2.320

53. Jacobs RA, Flück D, Bonne TC, et al. Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. *J Appl Physiol*. 2013;115(6):785-793. doi:10.1152/japplphysiol.00445.2013

54. Montero D, Cathomen A, Jacobs RA, et al. Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake induced by moderate endurance training. *J Physiol*. 2015;593(20):4677-4688. doi:10.1113/JP270250

55. Wilkinson SB, Phillips SM, Atherton PJ, et al. Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J Physiol*. 2008;586(15):3701-3717. doi:10.1113/jphysiol.2008.153916

56. Porter C, Reidy PT, Bhattarai N, Sidossis LS, Rasmussen BB. Resistance Exercise Training Alters Mitochondrial Function in Human Skeletal Muscle. *Med Sci Sports Exerc*. 2015;47(9):1922-1931. doi:10.1249/MSS.0000000000000605

57. Pesta D, Hoppel F, Macek C, et al. Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. *Am J Physiol - Regul Integr Comp Physiol*. 2011;301(4). doi:10.1152/ajpregu.00285.2011

58. García-Hermoso A, Saavedra JM, Escalante Y, Sánchez-López M, Martínez-Vizcaíno V. Endocrinology and adolescence: Aerobic exercise reduces insulin resistance markers in obese youth: A meta-analysis of randomized controlled trials. *Eur J Endocrinol*. 2014;171(4):R163-R171. doi:10.1530/EJE-14-0291

59. Ismail I, Keating SE, Baker MK, Johnson NA. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. *Obes Rev*. 2012;13(1):68-91. doi:10.1111/j.1467-789X.2011.00931.x

60. Ahmadizad S, Haghighi AH, Hamedinia MR. Effects of resistance versus endurance training on serum adiponectin and insulin resistance index. *Eur J Endocrinol*. 2007;157(5):625-631. doi:10.1530/EJE-07-0223

61. Whelton SP, Chin A, Xin X, He J. Effect of aerobic exercise on blood pressure: A meta-analysis of randomized, controlled trials. *Ann Intern Med*. 2002. doi:10.7326/0003-4819-136-7-200204020-00006

62. Cardoso CG, Gomides RS, Queiroz ACC, et al. Acute and chronic effects of aerobic and resistance exercise on ambulatory blood pressure. *Clinics*. 2010;65(3):317-325. doi:10.1590/s1807-59322010000300013

63. Cornelissen VA, Smart NA. Exercise training for blood pressure: a systematic review and meta-analysis. *J Am Heart Assoc*. 2013;2(1):1-9. doi:10.1161/JAHA.112.004473

64. Lemes ÍR, Ferreira PH, Linares SN, MacHado AF, Pastre CM, Netto J. Resistance training reduces systolic blood pressure in metabolic syndrome: A systematic review and meta-analysis of randomised controlled trials. *Br J Sports Med*. 2016;50(23):1438-

1442. doi:10.1136/bjsports-2015-094715

65. Reimers A, Knapp G, Reimers C-D. Effects of Exercise on the Resting Heart Rate: A Systematic Review and Meta-Analysis of Interventional Studies. *J Clin Med*. 2018;7(12):503. doi:10.3390/jcm7120503

66. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. In: *Medicine and Science in Sports and Exercise*. Vol 33. ; 2001:502-515. doi:10.1097/00005768-200106001-00021

67. Mezzani A, Agostoni P, Cohen-Solal A, et al. Standards for the use of cardiopulmonary exercise testing for the functional evaluation of cardiac patients: A report from the Exercise Physiology Section of the European Association for Cardiovascular Prevention and Rehabilitation. *Eur J Prev Cardiol*. 2009;16(3):249-267. doi:10.1097/HJR.0b013e32832914c8

68. Herdy AH, Uhlendorf D. Reference values for cardiopulmonary exercise testing for sedentary and active men and women. *Arq Bras Cardiol*. 2011;96(1):54-59. doi:10.1590/S0066-782X2010005000155

69. Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc*. 2000;32(1):70-84. doi:10.1097/00005768-200001000-00012

70. Albouaini K, Egred M, Alahmar A, Wright DJ. Cardiopulmonary exercise testing and its application. *Postgrad Med J*. 2007;83(985):675-682. doi:10.1136/hrt.2007.121558

71. Mortensen SP, Damsgaard R, Dawson EA, Secher NH, González-Alonso J. Restrictions in systemic and locomotor skeletal muscle perfusion, oxygen supply and V̇O2 during high-intensity whole-body exercise in humans. *J Physiol*. 2008;586(10):2621-2635. doi:10.1113/jphysiol.2007.149401

72. Ekblom B, Hermansen L. Cardiac output in athletes. *J Appl Physiol*. 1968;25(5):619-625. doi:10.1152/jappl.1968.25.5.619

73. Osborne G, Wolfe LA, Burggraf GW, Norman R. Relationship between cardiac dimensions, anthropometric characteristics and maximal aerobic power (V̇O2max) in young men. *Int J Sports Med*. 1992;13(3):219-224. doi:10.1055/s-2007-1021257

74. Hellsten Y, Nyberg M. Cardiovascular adaptations to exercise training. *Compr Physiol*. 2016;6(1):1-32. doi:10.1002/cphy.c140080

75. Convertino VA. Blood volume response to physical activity and inactivity. In: *American Journal of the Medical Sciences*. Vol 334. ; 2007:72-79. doi:10.1097/MAJ.0b013e318063c6e4

76. Joyner MJ, Casey DP. Regulation of increased blood flow (Hyperemia) to muscles during exercise: A hierarchy of competing physiological needs. *Physiol Rev*. 2015;95(2):549-601. doi:10.1152/physrev.00035.2013

77. McGuire BJ, Secomb TW. Estimation of capillary density in human skeletal muscle based on maximal oxygen consumption rates. *Am J Physiol - Hear Circ Physiol*. 2003;285(6 54-6). doi:10.1152/ajpheart.00559.2003

78. Hoppeler H. The different relationship of V̇O2 to muscle mitochondria in humans and quadrupedal animals. *Respir Physiol*. 1990;80(2-3):137-145. doi:10.1016/0034-5687(90)90077-C

79. Lessard SJ, Rivas DA, Alves-Wagner AB, et al. Resistance to aerobic exercise training causes metabolic dysfunction and reveals novel exercise-regulated signaling networks. *Diabetes*. 2013;62(8):2717-2727. doi:10.2337/db13-0062

80. Davies KJA, Packer L, Brooks GA. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch Biochem Biophys*. 1981;209(2):539-554. doi:10.1016/0003-9861(81)90312-X

81. Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, Haller RG. The spectrum of exercise tolerance in mitochondrial myopathies: A study of 40 patients. *Brain*. 2003;126(2):413-423. doi:10.1093/brain/awg028

82. Menshikova E V., Ritov VB, Fairfull L, Ferrell RE, Kelley DE, Goodpaster BH. Effects of exercise on mitochondrial content and function in aging human skeletal muscle. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2006;61(6):534-540. doi:10.1093/gerona/61.6.534

83. Horowitz JF, Klein S. Lipid metabolism during endurance exercise. In: *American Journal of Clinical Nutrition*. Vol 72. ; 2000:558-563. doi:10.1093/ajcn/72.2.558s

84. Cho Y, Hazen BC, Gandra PG, et al. Perm1 enhances mitochondrial biogenesis, oxidative capacity, and fatigue resistance in adult skeletal muscle. *FASEB J*. 2016;30(2):674-687. doi:10.1096/fj.15-276360

85. Taylor C, Nichols S, Ingle L. A clinician's guide to cardiopulmonary exercise testing 1: An introduction. *Br J Hosp Med*. 2015;76(4):192-195. doi:10.12968/hmed.2015.76.4.192

86. Howley ET, Bassett DR, Welch HG. Criteria for maximal oxygen uptake: Review and commentary. *Med Sci Sports Exerc*. 1995;27(9):1292-1301. doi:10.1249/00005768-199509000-00009

87. Edvardsen E, Hem E, Anderssen SA. End criteria for reaching maximal oxygen uptake must be strict and adjusted to sex and age: A cross-sectional study. *PLoS One*. 2014;9(1):18-20. doi:10.1371/journal.pone.0085276

88. Balady GJ, Arena R, Sietsema K, et al. Clinician's guide to cardiopulmonary exercise testing in adults: A scientific statement from the American heart association. *Circulation*. 2010;122(2):191-225. doi:10.1161/CIR.0b013e3181e52e69

89. Sullivan MJ, Higginbotham MB, Cobb FR. Exercise training in patients with chronic heart failure delays ventilatory anaerobic threshold and improves submaximal exercise performance. *Circulation*. 1989;79(2):324-329. doi:10.1161/01.CIR.79.2.324

90. Ghosh AK. Anaerobic threshold: Its concept and role in endurance sport. *Malaysian J Med Sci*. 2004;11(1):24-36.

91. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M. Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol*. 2012;3 JUL(July):1-18. doi:10.3389/fphys.2012.00260

92. Wilkinson DJ, Franchi M V., Brook MS, et al. A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol - Endocrinol Metab*. 2014;306(5):571-579. doi:10.1152/ajpendo.00650.2013

93. Newman AB, Kupelian V, Visser M, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2006;61(1):72-77. doi:10.1093/gerona/61.1.72

94. Biolo G, Declan Fleming RY, Maggi SP, Nguyen TT, Herndon DN, Wolfe RR. Inverse regulation of protein turnover and amino acid transport in skeletal muscle of hypercatabolic patients. *J Clin Endocrinol Metab*. 2002;87(7):3378-3384. doi:10.1210/jcem.87.7.8699

95. Westcott WL. Resistance training is medicine: Effects of strength training on health. *Curr Sports Med Rep*. 2012;11(4):209-216. doi:10.1249/JSR.0b013e31825dabb8

96. Kumar V, Atherton PJ, Selby A, et al. Muscle protein synthetic responses to exercise: Effects of age, volume, and intensity. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2012;67(11):1170-1177. doi:10.1093/gerona/gls141

97. Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol - Endocrinol Metab*. 1999. doi:10.1152/ajpendo.1999.276.4.e628

98. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J*

Physiol. 2009. doi:10.1113/jphysiol.2008.164483

99. McKay BR, O'Reilly CE, Phillips SM, Tarnopolsky MA, Parise G. Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. *J Physiol*. 2008;586(22):5549-5560. doi:10.1113/jphysiol.2008.160176

100. Phillips SM, Tipton KD, Ferrando AA, Wolfe RR. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol - Endocrinol Metab*. 1999. doi:10.1152/ajpendo.1999.276.1.e118

101. DeFreitas JM, Beck TW, Stock MS, Dillon MA, Kasishke PR. An examination of the time course of training-induced skeletal muscle hypertrophy. *Eur J Appl Physiol*. 2011. doi:10.1007/s00421-011-1905-4

102. Biolo G, Maggi SP, Williams BD, Tipton KD, Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol - Endocrinol Metab*. 1995. doi:10.1152/ajpendo.1995.268.3.e514

103. Phillips SM, Tipton KD, Aarsland A, Wolf SE, Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol - Endocrinol Metab*. 1997. doi:10.1152/ajpendo.1997.273.1.e99

104. Smith K, Rennie MJ. The measurement of tissue protein turnover. *Baillieres Clin Endocrinol Metab*. 1996. doi:10.1016/S0950-351X(96)80651-3

105. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids

on incorporation of labeled amino acids into human muscle protein. *Am J Physiol - Endocrinol Metab*. 1998. doi:10.1152/ajpendo.1998.275.1.e73

106. Rennie MJ, Smith K, Watt PW. Measurement of human tissue protein synthesis: An optimal approach. *Am J Physiol - Endocrinol Metab*. 1994. doi:10.1152/ajpendo.1994.266.3.e298

107. Zhang X, Chinkes DL, Wolfe RR, Robert R. Breakdown Rates From a Pulse Tracer Injection. *Am J Physiol Endocrinol Metab*. 2002;283:753-764.

108. Narici M, Franchi M, Maganaris C. Muscle structural assembly and functional consequences. *J Exp Biol*. 2016;219(2):276-284. doi:10.1242/jeb.128017

109. Kawakami Y, Abe T, Fukunaga T. Muscle-fiber pennation angles are greater in hypertrophied than in normal muscles. *J Appl Physiol*. 1993;74(6):2740-2744. doi:10.1152/jappl.1993.74.6.2740

110. Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat*. 2006;209(3):289-310. doi:10.1111/j.1469-7580.2006.00619.x

111. American College of Sports Medicine. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc*. 2009;41(3):687-708. doi:10.1249/MSS.0b013e3181915670

112. Taylor JD, Fletcher JP. Reliability of the 8-repetition maximum test in men and women. *J Sci Med Sport*. 2012;15(1):69-73. doi:10.1016/j.jsams.2011.07.002

113. Shaw CE, McCully KK, Posner JD. Injuries during the one repetition maximum assessment in the elderly. *J Cardiopulm Rehabil*. 1995;15(4):283-287. doi:10.1097/00008483-199507000-00005

114. Steib S, Schoene D, Pfeifer K. Dose-response relationship of resistance training in older adults: A meta-analysis. *Med Sci Sports Exerc*. 2010;42(5):902-914. doi:10.1249/MSS.0b013e3181c34465

115. Laidlaw DH, Kornatz KW, Keen DA, Suzuki S, Enoka RM. Strength training improves the steadiness of slow lengthening contractions performed by old adults. *J Appl Physiol*. 1999;87(5):1786-1795. doi:10.1152/jappl.1999.87.5.1786

116. Golbidi S, Mesdaghinia A, Laher I. Exercise in the metabolic syndrome. *Oxid Med Cell Longev*. 2012;2012. doi:10.1155/2012/349710

117. Johnson JL, Slentz CA, Houmard JA, et al. Exercise Training Amount and Intensity Effects on Metabolic Syndrome (from Studies of a Targeted Risk Reduction Intervention through Defined Exercise). *Am J Cardiol*. 2007;100(12):1759-1766. doi:10.1016/j.amjcard.2007.07.027

118. Malina RM, Geithner CA. Body Composition of Young Athletes. *Am J Lifestyle Med*. 2011;5(3):262-278. doi:10.1177/1559827610392493

119. Ofei F. Obesity - a preventable disease. *Ghana Med J*. 2005;39(3):98-101. http://www.ncbi.nlm.nih.gov/pubmed/17299552%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1790820.

120. Patel P, Abate N. Role of subcutaneous adipose tissue in the pathogenesis of insulin resistance. *J Obes*. 2013;2013. doi:10.1155/2013/489187

121. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest*. 1995;96(1):88-98. doi:10.1172/JCI118083

122. Slentz CA, Aiken LB, Houmard JA, et al. Inactivity, exercise, and visceral fat. STRRIDE: A randomized, controlled study of exercise intensity and amount. *J Appl Physiol*. 2005;99(4):1613-1618. doi:10.1152/japplphysiol.00124.2005

123. Tresierras MA, Balady GJ. Resistance training in the treatment of diabetes and obesity: Mechanisms and outcomes. *J Cardiopulm Rehabil Prev*. 2009. doi:10.1097/HCR.0b013e318199ff69

124. Nunes PRP, Barcelos LC, Oliveira AA, et al. Effect of resistance training on muscular strength and indicators of abdominal adiposity, metabolic risk, and inflammation in postmenopausal women: controlled and randomized clinical trial of efficacy of training volume. *Age (Omaha)*. 2016;38(2). doi:10.1007/s11357-016-9901-6

125. Imboden MT, Swartz AM, Finch HW, Harber MP, Kaminsky LA. Reference standards for lean mass measures using GE dual energy x-ray absorptiometry in Caucasian adults. *PLoS One*. 2017;12(4):1-12. doi:10.1371/journal.pone.0176161

126. Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA. The emerging role of HDL in glucose metabolism. *Nat Rev Endocrinol*. 2012;8(4):237-245. doi:10.1038/nrendo.2011.235

127. Vergès B. Lipid modification in type 2 diabetes: The role of LDL and HDL. *Fundam Clin Pharmacol*. 2009;23(6):681-685. doi:10.1111/j.1472-8206.2009.00739.x

128. Talayero BG, Sacks FM. The role of triglycerides in atherosclerosis. *Curr Cardiol Rep*. 2011;13(6):544-552. doi:10.1007/s11886-011-0220-3

129. Gordon B, Chen S, Durstine JL. The effects of exercise training on the traditional lipid profile and beyond. *Curr Sports Med Rep*. 2014;13(4):253-259. doi:10.1249/JSR.0000000000000073

130. Heidenreich PA, Albert NM, Allen LA, et al. Forecasting the impact of heart failure in the united states a policy statement from the american heart association. *Circ Hear Fail*. 2013;6(3):606-619. doi:10.1161/HHF.0b013e318291329a

131. DeMers D, Wachs D. *Physiology, Mean Arterial Pressure*.; 2019.

132. Lund S, Holman GD, Schmitz O, Pedersen O. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc Natl Acad Sci U S A*. 1995;92(13):5817-5821. doi:10.1073/pnas.92.13.5817

133. Kramer HF, Witczak CA, Taylor EB, Fujii N, Hirshman MF, Goodyear LJ. AS160 regulates insulin- and contraction-stimulated glucose uptake in mouse skeletal muscle. *J Biol Chem*. 2006;281(42):31478-31485. doi:10.1074/jbc.M605461200

134. Jensen TE, Richter EA. Regulation of glucose and glycogen metabolism during and after exercise. *J Physiol*. 2012;590(5):1069-1076. doi:10.1113/jphysiol.2011.224972

135. Mackenzie RWA, Elliott BT. Akt/PKB activation and insulin signaling: A novel insulin signaling pathway in the treatment of type 2 diabetes. *Diabetes, Metab Syndr Obes Targets Ther*. 2014;7:55-64. doi:10.2147/DMSO.S48260

136. Mora A, Komander D, Van Aalten DMF, Alessi DR. PDK1, the master regulator of AGC kinase signal transduction. *Semin Cell Dev Biol*. 2004;15(2):161-170. doi:10.1016/j.semcdb.2003.12.022

137. Sakamoto K, Holman GD. Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *Am J Physiol - Endocrinol Metab*. 2008;295(1):29-37. doi:10.1152/ajpendo.90331.2008

138. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med*. 2017;2(1):1-26. doi:10.1136/bmjsem-2016-000143

139. Bouskila M, Hunter RW, Ibrahim AFM, et al. Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. *Cell Metab*. 2010;12(5):456-466. doi:10.1016/j.cmet.2010.10.006

140. Liu TY, Shi CX, Gao R, et al. Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes. *Clin Sci*. 2015;129(10):839-850. doi:10.1042/CS20150009

141. Hingst JR, Bruhn L, Hansen MB, et al. Exercise-induced molecular mechanisms promoting glycogen supercompensation in human skeletal muscle. *Mol Metab*. 2018;16(July):24-34. doi:10.1016/j.molmet.2018.07.001

142. Carling D, Grahame Hardie D. The substrate and sequence specificity of the AMP-activated protein kinase. Phosphorylation of glycogen synthase and phosphorylase kinase. *BBA - Mol Cell Res*. 1989;1012(1):81-86. doi:10.1016/0167-4889(89)90014-1

143. Janzen NR, Whitfield J, Hoffman NJ. Interactive roles for ampk and glycogen from cellular energy sensing to exercise metabolism. *Int J Mol Sci*. 2018;19(11). doi:10.3390/ijms19113344

144. Jørgensen SB, Nielsen JN, Birk JB, et al. The α2-5′ AMP-activated protein kinase is a site 2 glycogen synthase kinase in skeletal muscle and is responsive to glucose loading. *Diabetes*. 2004;53(12):3074-3081. doi:10.2337/diabetes.53.12.3074

145. McCarthy JJ, Esser KA. Anabolic and catabolic pathways regulating skeletal muscle mass. *Curr Opin Clin Nutr Metab Care*. 2010;13(3):230-235. doi:10.1097/MCO.0b013e32833781b5

146. Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. *Crit Rev Biochem Mol Biol*. 2014;49(1):59-68. doi:10.3109/10409238.2013.857291

147. Università di Padova. Dipartimento di Scienze Biomediche. M, Ludwig Boltzmann Institut für Elektrostimulation und Physikalische Rehabilitation. A, Marini M, Veicsteinas A. The exercised skeletal muscle: a review. *Eur J Transl Myol*. 2010;20(3):105. doi:10.4081/ejtm.2010.1807

148. Brook MS, Wilkinson DJ, Phillips BE, et al. Skeletal muscle homeostasis and plasticity in youth and ageing: Impact of nutrition and exercise. *Acta Physiol*. 2016;216(1):15-41. doi:10.1111/apha.12532

149. Pasiakos SM, Carbone JW. Assessment of skeletal muscle proteolysis and the regulatory response to nutrition and exercise. *IUBMB Life*. 2014. doi:10.1002/iub.1291

150. Passmore LA, Barford D. Getting into position: The catalytic mechanisms of protein of protein ubiquitylation. *Biochem J*. 2004. doi:10.1042/BJ20040198

151. Nedergaard A, Vissing K, Overgaard K, Kjaer M, Schjerling P. Expression patterns of atrogenic and ubiquitin proteasome component genes with exercise: Effect of different loading patterns and repeated exercise bouts. *J Appl Physiol*. 2007. doi:10.1152/japplphysiol.01445.2006

152. Stefanetti RJ, Lamon S, Wallace M, Vendelbo MH, Russell AP, Vissing K. Regulation of ubiquitin proteasome pathway molecular markers in response to endurance and resistance exercise and training. *Pflugers Arch Eur J Physiol*. 2015. doi:10.1007/s00424-014-1587-y

153. Jamart C, Benoit N, Raymackers JM, Kim HJ, Kim CK, Francaux M. Autophagy-related and autophagy-regulatory genes are induced in human muscle after ultraendurance exercise. *Eur J Appl Physiol*. 2012. doi:10.1007/s00421-011-2287-3

154. Groennebaek T, Vissing K. Impact of resistance training on skeletal muscle mitochondrial biogenesis, content, and function. *Front Physiol*. 2017;8(SEP). doi:10.3389/fphys.2017.00713

155. Jornayvaz FR. and SGI. Regulation of mitochondrial biogenesis. *Essays Biochem*. 2014;47:1-15. doi:10.1042/bse0470069.Regulation

156. Hood DA. Plasticity in Skeletal, Cardiac, and Smooth Muscle Invited Review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol*. 2001;90(90):1137-1157.

157. Larsen S, Nielsen J, Hansen CN, et al. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol*. 2012;590(14):3349-3360. doi:10.1113/jphysiol.2012.230185

158. Ljubicic V, Joseph AM, Saleem A, et al. Transcriptional and post-transcriptional regulation of mitochondrial biogenesis in skeletal muscle: Effects of exercise and aging. *Biochim Biophys Acta - Gen Subj*. 2010;1800(3):223-234. doi:10.1016/j.bbagen.2009.07.031

159. Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev*. 2008;88(2):611-638. doi:10.1152/physrev.00025.2007

160. Handschin C, Spiegelman BM. The role of exercise and PGC1α in inflammation and chronic disease. *Nature*. 2008. doi:10.1038/nature07206

161. Drake JC, Wilson RJ, Yan Z. Molecular mechanisms for mitochondrial adaptation to exercise training in skeletal muscle. *FASEB J*. 2016;30(1):13-22. doi:10.1096/fj.15-276337

162. Wright DC, Han DH, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1α expression. *J Biol Chem*. 2007;282(1):194-199. doi:10.1074/jbc.M606116200

163. Daniel S. Herman, Ph.D., Lien Lam, Ph.D., Matthew R.G. Taylor, M.D., Ph.D., Libin Wang, M.D., Ph.D., Polakit Teekakirikul, M.D., Danos Christodoulou, B.S., Lauren Conner, B.S., Steven R. DePalma, Ph.D., Barbara McDonough, R.N., Elizabeth Sparks, R.N.P., D MD. p53 Improves Aerobic Exercise Capacity and Augments Skeletal Muscle Mitochondrial DNA Content. *Bone*. 2010;23(1):1-7. doi:10.1038/jid.2014.371

164. Jones AM, Carter H. Oxygen uptake-work rate relationship during two consecutive ramp exercise tests. *Int J Sports Med*. 2004. doi:10.1055/s-2004-820960

165. Korivi M, Hou C, Chen C, Lee J, Kesireddy SR, Kuo C. Angiogenesis: Role of Exercise Training and Aging. *Adapt Med*. 2010;(April). doi:10.4247/am.2010.aba005

166. Roskoski R. Vascular endothelial growth factor (VEGF) signaling in tumor progression. *Crit Rev Oncol Hematol*. 2007;62(3):179-213. doi:10.1016/j.critrevonc.2007.01.006

167. Gavin TP, Ruster RS, Carrithers JA, et al. No difference in the skeletal muscle angiogenic response to aerobic exercise training between young and aged men. *J Physiol*. 2007;585(1):231-239. doi:10.1113/jphysiol.2007.143198

168. Olfert IM, Birot O. Importance of Anti-angiogenic Factors in the Regulation of Skeletal Muscle Angiogenesis. *Microcirculation*. 2011;18(4):316-330. doi:10.1111/j.1549-8719.2011.00092.x

169. Chinsomboon J, Ruas J, Gupta RK, et al. The transcriptional coactivator PGC-1α mediates exercise-induced angiogenesis in skeletal muscle. *Proc Natl Acad Sci U S A*. 2009;106(50):21401-21406. doi:10.1073/pnas.0909131106

170. Wagner PD, Olfert IM, Tang K, Breen EC. Muscle-targeted deletion of VEGF and exercise capacity in mice. *Respir Physiol Neurobiol*. 2006. doi:10.1016/j.resp.2005.09.007

171. Delavar H, Nogueira L, Wagner PD, Hogan MC, Metzger D, Breen EC. Skeletal myofiber VEGF is essential for the exercise training response in adult mice. *Am J Physiol - Regul Integr Comp Physiol*. 2014;306(8):586-595. doi:10.1152/ajpregu.00522.2013

172. Breen EC, Johnson EC, Wagner H, Tseng HM, Sung LA, Wagner PD. Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. *J Appl Physiol*. 1996. doi:10.1152/jappl.1996.81.1.355

173. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ. Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol - Hear Circ Physiol*. 1999;276(2 45-2). doi:10.1152/ajpheart.1999.276.2.h679

174. Lloyd PG, Prior BM, Yang HT, Terjung RL. Angiogenic growth factor expression in rat skeletal muscle in response to exercise training. *Am J Physiol - Hear Circ Physiol*. 2003;284(5 53-5):1668-1678. doi:10.1152/ajpheart.00743.2002

175. Holash J, Maisonpierre PC, Compton D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science (80-)*. 1999. doi:10.1126/science.284.5422.1994

176. Timmons JA, Jansson E, Fischer H, et al. Modulation of extracellular matrix genes

reflects the magnitude of physiological adaptation to aerobic exercise training in humans. *BMC Biol*. 2005;3:1-10. doi:10.1186/1741-7007-3-19

177. Iizuka K, Machida T, Hirafuji M. Skeletal muscle is an endocrine organ. *J Pharmacol Sci*. 2014;125(2):125-131. doi:10.1254/jphs.14R02CP

178. GOLDSTEIN MS. Humoral nature of the hypoglycemic factor of muscular work. *Diabetes*. 1961. doi:10.2337/diab.10.3.232

179. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes*. 2004. doi:10.2337/diabetes.53.7.1643

180. Whitham M, Febbraio MA. The ever-expanding myokinome: Discovery challenges and therapeutic implications. *Nat Rev Drug Discov*. 2016;15(10):719-729. doi:10.1038/nrd.2016.153

181. Pedersen BK. Muscle as a secretory organ. *Compr Physiol*. 2013;3(3):1337-1362. doi:10.1002/cphy.c120033

182. Pedersen BK, Åkerström TCA, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol*. 2007;103(3):1093-1098. doi:10.1152/japplphysiol.00080.2007

183. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: Skeletal muscle as a secretory organ. *Nat Rev Endocrinol*. 2012;8(8):457-465. doi:10.1038/nrendo.2012.49

184. Pedersen BK, Brandt C. The role of exercise-induced myokines in muscle homeostasis

and the defense against chronic diseases. *J Biomed Biotechnol*. 2010;2010. doi:10.1155/2010/520258

185. So B, Kim H-J, Kim J, Song W. Exercise-induced myokines in health and metabolic diseases. *Integr Med Res*. 2014;3(4):172-179. doi:10.1016/j.imr.2014.09.007

186. Huh JY. The role of exercise-induced myokines in regulating metabolism. *Arch Pharm Res*. 2018;41(1):14-29. doi:10.1007/s12272-017-0994-y

187. Schnyder S, Handschin C. Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. *Bone*. 2015;80:115-125. doi:10.1016/j.bone.2015.02.008

188. Tezze C, Romanello V, Sandri M. FGF21 as modulator of metabolism in health and disease. *Front Physiol*. 2019;10(MAR):1-9. doi:10.3389/fphys.2019.00419

189. Ost M, Coleman V, Kasch J, Klaus S. Regulation of myokine expression: Role of exercise and cellular stress. *Free Radic Biol Med*. 2016;98:78-89. doi:10.1016/j.freeradbiomed.2016.02.018

190. Kim KH, Kim SH, Min YK, Yang HM, Lee JB, Lee MS. Acute Exercise Induces FGF21 Expression in Mice and in Healthy Humans. *PLoS One*. 2013;8(5):2-8. doi:10.1371/journal.pone.0063517

191. Cuevas-Ramos D, Almeda-Valdés P, Meza-Arana CE, et al. Exercise increases serum fibroblast growth factor 21 (FGF21) levels. *PLoS One*. 2012;7(5):1-8. doi:10.1371/journal.pone.0038022

192. Scalzo RL, Peltonen GL, Giordano GR, et al. Regulators of human white adipose

browning: Evidence for sympathetic control and sexual dimorphic responses to sprint interval training. *PLoS One*. 2014;9(3). doi:10.1371/journal.pone.0090696

193. Yang SJ, Hong HC, Choi HY, et al. Effects of a three-month combined exercise programme on fibroblast growth factor 21 and fetuin-A levels and arterial stiffness in obese women. *Clin Endocrinol (Oxf)*. 2011;75(4):464-469. doi:10.1111/j.1365-2265.2011.04078.x

194. Xu J, Stanislaus S, Chinookoswong N, et al. Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models - Association with liver and adipose tissue effects. *Am J Physiol - Endocrinol Metab*. 2009;297(5):1105-1114. doi:10.1152/ajpendo.00348.2009

195. Chavez AO, Molina-Carrion M, Abdul-Ghani MA, Folli F, DeFronzo RA, Tripathy D. Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. *Diabetes Care*. 2009;32(8):1542-1546. doi:10.2337/dc09-0684

196. Kim KH, Lee MS. FGF21 as a mediator of adaptive responses to stress and metabolic benefits of anti-diabetic drugs. *J Endocrinol*. 2015;226(1):R1-R16. doi:10.1530/JOE-15-0160

197. Norheim F, Raastad T, Thiede B, Rustan AC, Drevon CA, Haugen F. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. *Am J Physiol - Endocrinol Metab*. 2011;301(5):1013-1021. doi:10.1152/ajpendo.00326.2011

198. Aoi W, Naito Y, Takagi T, et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut*. 2013;62(6):882-889. doi:10.1136/gutjnl-2011-300776

199. Jørgensen LH, Jepsen PL, Boysen A, et al. SPARC Interacts with Actin in Skeletal Muscle in Vitro and in Vivo. *Am J Pathol*. 2017;187(2):457-474. doi:10.1016/j.ajpath.2016.10.013

200. Song H, Guan Y, Zhang L, Li K, Dong C. SPARC interacts with AMPK and regulates GLUT4 expression. *Biochem Biophys Res Commun*. 2010;396(4):961-966. doi:10.1016/j.bbrc.2010.05.033

201. Nie J, Helene Sage E. SPARC inhibits adipogenesis By its enhancement of β-catenin signaling. *J Biol Chem*. 2009;284(2):1279-1290. doi:10.1074/jbc.M808285200

202. Hoffmann C, Weigert C, C. H, C. W. Skeletal muscle as an endocrine organ: The role of myokines in exercise adaptations. *Cold Spring Harb Perspect Med*. 2017;7(11). doi:10.1101/cshperspect.a029793

203. Wu D, Li N, Yang M, Liu H, Yang G. Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. *Eur J Endocrinol*. 2011;165(4):597-601. doi:10.1530/EJE-11-0131

204. Wang Z, Song HY, An MM, Zhu LL. Association of serum SPARC level with severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease. *Int J Clin Exp Med*. 2015;8(10):19290-19296.

205. Thomas G, Moffatt P, Salois P, et al. Osteocrin, a Novel Bone-specific Secreted Protein That Modulates the Osteoblast Phenotype. *J Biol Chem*. 2003;278(50):50563-50571. doi:10.1074/jbc.M307310200

206. Nishizawa H, Matsuda M, Yamada Y, et al. Musclin, a Novel Skeletal Muscle-derived Secretory Factor. *J Biol Chem*. 2004;279(19):19391-19395. doi:10.1074/jbc.C400066200

207. Yasui A, Nishizawa H, Okuno Y, et al. Foxo1 represses expression of musclin, a skeletal muscle-derived secretory factor. *Biochem Biophys Res Commun*. 2007;364(2):358-365. doi:10.1016/j.bbrc.2007.10.013

208. Gu N, Guo Q, Mao K, et al. Palmitate increases musclin gene expression through activation of PERK signaling pathway in C2C12 myotubes. *Biochem Biophys Res Commun*. 2015;467(3):521-526. doi:10.1016/j.bbrc.2015.10.005

209. Chen WJ, Liu Y, Sui Y Bin, et al. Positive association between musclin and insulin resistance in obesity: Evidence of a human study and an animal experiment. *Nutr Metab*. 2017;14(1):1-12. doi:10.1186/s12986-017-0199-x

210. Liu Y, Huo X, Pang XF, Zong ZH, Meng X, Liu GL. Musclin inhibits insulin activation of Akt/protein kinase B in rat skeletal muscle. *J Int Med Res*. 2008;36(3):496-504. doi:10.1177/147323000803600314

211. Chen WJ, Liu Y, Sui Y Bin, Zhang B, Zhang XH, Yin XH. Increased circulating levels of musclin in newly diagnosed type 2 diabetic patients. *Diabetes Vasc Dis Res*. 2017;14(2):116-121. doi:10.1177/1479164116675493

212. Subbotina E, Sierra A, Zhu Z, et al. Musclin is an activity-stimulated myokine that enhances physical endurance. *Proc Natl Acad Sci U S A*. 2015;112(52):16042-16047. doi:10.1073/pnas.1514250112

213. Yu J, Zheng J, Liu XF, et al. Exercise improved lipid metabolism and insulin sensitivity in rats fed a high-fat diet by regulating glucose transporter 4 (GLUT4) and musclin expression. *Brazilian J Med Biol Res*. 2016;49(5):1-6. doi:10.1590/1414-431X20165129

214. Shah R, Hinkle CC, Ferguson JF, et al. Fractalkine is a novel human adipochemokine associated with type 2 diabetes. *Diabetes*. 2011;60(5):1512-1518. doi:10.2337/db10-0956

215. Lee YS, Morinaga H, Kim JJ, et al. The fractalkine/CX3CR1 system regulates β cell function and insulin secretion. *Cell*. 2013;153(2):413-425. doi:10.1016/j.cell.2013.03.001

216. Catoire M, Mensink M, Kalkhoven E, Schrauwen P, Kersten S. Identification of human exercise-induced myokines using secretome analysis. *Physiol Genomics*. 2014;46(7):256-267. doi:10.1152/physiolgenomics.00174.2013

217. Eckardt K, Görgens SW, Raschke S, Eckel J. Myokines in insulin resistance and type 2 diabetes. *Diabetologia*. 2014;57(6):1087-1099. doi:10.1007/s00125-014-3224-x

218. Strömberg A, Olsson K, Dijksterhuis JP, Rullman E, Schulte G, Gustafsson T. CX3CL1—a macrophage chemoattractant induced by a single bout of exercise in human skeletal muscle. *Am J Physiol - Regul Integr Comp Physiol*. 2016;310(3):R297-

R304. doi:10.1152/ajpregu.00236.2015

219. P. P, J. H, V. B, B.K. P. Abstracts of the 49th Annual Meeting of the EASD. *Diabetologia*. 2013;56(S1):1-566. doi:10.1007/s00125-013-3012-z

220. Bernecker C, Scherr J, Schinner S, Braun S, Scherbaum WA, Halle M. Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scand J Med Sci Sport*. 2013. doi:10.1111/j.1600-0838.2011.01372.x

221. Copray S, Liem R, Brouwer N, Greenhaff P, Habens F, Fernyhough P. Contraction-induced muscle fiber damage is increased in soleus muscle of streptozotocin-diabetic rats and is associated with elevated expression of brain-derived neurotrophic factor mRNA in muscle fibers and activated satellite cells. *Exp Neurol*. 2000;161(2):597-608. doi:10.1006/exnr.1999.7306

222. Gómez-Pinilla F, Ying Z, Roy RR, Molteni R, Reggie Edgerton V. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol*. 2002;88(5):2187-2195. doi:10.1152/jn.00152.2002

223. Dinoff A, Herrmann N, Swardfager W, Lanctôt KL. The effect of acute exercise on blood concentrations of brain-derived neurotrophic factor in healthy adults: a meta-analysis. *Eur J Neurosci*. 2017;46(1):1635-1646. doi:10.1111/ejn.13603

224. Yarrow JF, White LJ, McCoy SC, Borst SE. Training augments resistance exercise induced elevation of circulating brain derived neurotrophic factor (BDNF). *Neurosci Lett*. 2010;479(2):161-165. doi:10.1016/j.neulet.2010.05.058

225. Coelho FG de M, Gobbi S, Andreatto CAA, Corazza DI, Pedroso RV, Santos-Galduróz RF. Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): A systematic review of experimental studies in the elderly. *Arch Gerontol Geriatr*. 2013;56(1):10-15. doi:10.1016/j.archger.2012.06.003

226. Matthews VB, Åström MB, Chan MHS, et al. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia*. 2009. doi:10.1007/s00125-009-1364-1

227. Rasmussen P, Brassard P, Adser H, et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol*. 2009;94(10):1062-1069. doi:10.1113/expphysiol.2009.048512

228. Pedersen BK, Pedersen M, Krabbe KS, Bruunsgaard H, Matthews VB, Febbraio MA. Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals: Experimental Physiology-Hot Topic Review. *Exp Physiol*. 2009;94(12):1153-1160. doi:10.1113/expphysiol.2009.048561

229. Carson BP. The potential role of contraction-induced myokines in the regulation of metabolic function for the prevention and treatment of type 2 diabetes. *Front Endocrinol (Lausanne)*. 2017;8(MAY):1-8. doi:10.3389/fendo.2017.00097

230. Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj A, Huang CJ. Brain-derived neurotrophic factor and substrate utilization following acute aerobic exercise in obese individuals. *J Neuroendocrinol*. 2015;27(5):370-376. doi:10.1111/jne.12275

231. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiol Rev*. 2008;88(4):1379-1406. doi:10.1152/physrev.90100.2007

232. Kelly M, Gauthier MS, Saha AK, Ruderman NB. Activation of AMP-activated protein kinase by interleukin-6 in rat skeletal muscle: Association with changes in cAMP, energy state, and endogenous fuel mobilization. *Diabetes*. 2009;58(9):1953-1960. doi:10.2337/db08-1293

233. Ikeda SI, Tamura Y, Kakehi S, Sanada H, Kawamori R, Watada H. Exercise-induced increase in IL-6 level enhances GLUT4 expression and insulin sensitivity in mouse skeletal muscle. *Biochem Biophys Res Commun*. 2016;473(4):947-952. doi:10.1016/j.bbrc.2016.03.159

234. Gao S, Durstine JL, Koh HJ, Carver WE, Frizzell N, Carson JA. Acute myotube protein synthesis regulation by IL-6-related cytokines. *Am J Physiol - Cell Physiol*. 2017;313(5):C487-C500. doi:10.1152/ajpcell.00112.2017

235. Volek JS, Freidenreich DJ, Saenz C, et al. Metabolic characteristics of keto-adapted ultra-endurance runners. *Metabolism*. 2016;65(3):100-110. doi:10.1016/j.metabol.2015.10.028

236. Ostrowski K, Hermann C, Bangash A, Schjerling P, Nielsen JN, Pedersen BK. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol*. 1998;513(3):889-894. doi:10.1111/j.1469-7793.1998.889ba.x

237. Hirose L, Nosaka K, Newton M, et al. Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev*. 2004;10:75-90.

238. Bergfors M, Barnekow-Bergkvist M, Kalezic N, Lyskov E, Eriksson JW. Short-term effects of repetitive arm work and dynamic exercise on glucose metabolism and insulin sensitivity. *Acta Physiol Scand*. 2005;183(4):345-356. doi:10.1111/j.1365-201X.2005.01407.x

239. Steensberg A, Febbraio MA, Osada T, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol*. 2001;537(2):633-639. doi:10.1111/j.1469-7793.2001.00633.x

240. Cesari M, Penninx BWJH, Pahor M, et al. Inflammatory Markers and Physical Performance in Older Persons: The InCHIANTI Study. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2004;59(3):242-248. doi:10.1093/gerona/59.3.m242

241. Colbert LH, Visser M, Simonsick EM, et al. Physical activity, exercise, and inflammatory markers in older adults: Findings from the health, aging and body composition study. *J Am Geriatr Soc*. 2004;52(7):1098-1104. doi:10.1111/j.1532-5415.2004.52307.x

242. Kohut ML, McCann DA, Russell DW, et al. Aerobic exercise, but not flexibility/resistance exercise, reduces serum IL-18, CRP, and IL-6 independent of β-blockers, BMI, and psychosocial factors in older adults. *Brain Behav Immun*. 2006;20(3):201-209. doi:10.1016/j.bbi.2005.12.002

243. Bruun JM, Helge JW, Richelsen B, Stallknecht B. Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. *Am J Physiol - Endocrinol Metab*. 2006;290(5). doi:10.1152/ajpendo.00506.2005

244. Bruunsgaard H, Bjerregaard E, Schroll M, Pedersen BK. Muscle Strength after Resistance Training Is Inversely Correlated with Baseline Levels of Soluble Tumor Necrosis Factor Receptors in the Oldest Old. *J Am Geriatr Soc*. 2004;52(2):237-241. doi:10.1111/j.1532-5415.2004.52061.x

245. Larsen AI, Aukrust P, Aarsland T, Dickstein K. Effect of aerobic exercise training on plasma levels of tumor necrosis factor alpha in patients with heart failure. *Am J Cardiol*. 2001;88(7):805-808. doi:10.1016/S0002-9149(01)01859-8

246. Perakakis N, Triantafyllou GA, Fernández-Real JM, et al. Physiology and role of irisin in glucose homeostasis. *Nat Rev Endocrinol*. 2017;13(6):324-337. doi:10.1038/nrendo.2016.221

247. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481(7382):463-468. doi:10.1038/nature10777

248. Erickson HP. Irisin and FNDC5 in retrospect. *Adipocyte*. 2013;2(4):289-293. doi:10.4161/adip.26082

249. Yang Z, Chen X, Chen Y, Zhao Q. Decreased irisin secretion contributes to muscle insulin resistance in high-fat diet mice. *Int J Clin Exp Pathol*. 2015;8(6):6490-6497.

250. Lu Y, Li H, Shen SW, et al. Swimming exercise increases serum irisin level and reduces body fat mass in highfat-diet fed Wistar rats. *Lipids Health Dis*. 2016;15(1). doi:10.1186/s12944-016-0263-y

251. Yang XQ, Yuan H, Li J, et al. Swimming intervention mitigates HFD-induced obesity of rats through PGC-1?alpha;-irisin pathway. *Eur Rev Med Pharmacol Sci*. 2016;20(10):2123-2130.

252. Moreno-Navarrete JM, Ortega F, Serrano M, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab*. 2013;98(4):E769-78. doi:10.1210/jc.2012-2749

253. Choi YK, Kim MK, Bae KH, et al. Serum irisin levels in new-onset type 2 diabetes. *Diabetes Res Clin Pract*. 2013;100(1):96-101. doi:10.1016/j.diabres.2013.01.007

254. Morton TL, Galior K, McGrath C, et al. Exercise Increases and Browns Muscle Lipid in High-Fat Diet-Fed Mice. *Front Endocrinol (Lausanne)*. 2016;7(June):1-8. doi:10.3389/fendo.2016.00080

255. Rocha-Rodrigues S, Rodríguez A, Gouveia AM, et al. Effects of physical exercise on myokines expression and brown adipose-like phenotype modulation in rats fed a high-fat diet. *Life Sci*. 2016;165:100-108. doi:10.1016/j.lfs.2016.09.023

256. Peterson JM, Mart R, Bond CE. Effect of obesity and exercise on the expression of the novelmyokines, Myonectin and Fibronectin type III domain containing 5. *PeerJ*. 2014;2014(1):1-16. doi:10.7717/peerj.605

257. Pekkala S, Wiklund PK, Hulmi JJ, et al. Are skeletal muscle FNDC5 gene expression and irisin release regulated by exercise and related to health? *J Physiol*. 2013;591(21):5393-5400. doi:10.1113/jphysiol.2013.263707

258. Sanchis-Gomar F, Alis R, Pareja-Galeano H, et al. Circulating irisin levels are not correlated with BMI, age, and other biological parameters in obese and diabetic patients. *Endocrine*. 2014;46(3):674-677. doi:10.1007/s12020-014-0170-9

259. Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity-Correlation with body mass index. *Peptides*. 2013;39(1):125-130. doi:10.1016/j.peptides.2012.11.014

260. Pardo M, Crujeiras AB, Amil M, et al. Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. *Int J Endocrinol*. 2014;2014. doi:10.1155/2014/857270

261. Huh JY, Panagiotou G, Mougios V, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism*. 2012;61(12):1725-1738. doi:10.1016/j.metabol.2012.09.002

262. Schiebel J, Kapilashrami K, Fekete A, et al. Structural basis for the recognition of mycolic acid precursors by KasA, a condensing enzyme and drug target from Mycobacterium tuberculosis. *J Biol Chem*. 2013;288(47):34190-34204. doi:10.1074/jbc.M113.511436

263. Park KH, Zaichenko L, Peter P, Davis CR, Crowell JA, Mantzoros CS. Diet quality is associated with circulating C-reactive protein but not irisin levels in humans. *Metabolism*. 2014;63(2):233-241. doi:10.1016/j.metabol.2013.10.011

264. Liu JJ, Wong MDS, Toy WC, et al. Lower circulating irisin is associated with type 2 diabetes mellitus. *J Diabetes Complications*. 2013;27(4):365-369. doi:10.1016/j.jdiacomp.2013.03.002

265. Kurdiova T, Balaz M, Vician M, et al. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: In vivo and in vitro studies. *J Physiol*. 2014;592(5):1091-1107. doi:10.1113/jphysiol.2013.264655

266. Moreno M, Moreno-Navarrete JM, Serrano M, et al. Circulating irisin levels are positively associated with metabolic risk factors in sedentary subjects. *PLoS One*. 2015;10(4):1-11. doi:10.1371/journal.pone.0124100

267. Huerta AE, Prieto-Hontoria PL, Fernández-Galilea M, et al. Circulating irisin and glucose metabolism in overweight/obese women: effects of α-lipoic acid and eicosapentaenoic acid. *J Physiol Biochem*. 2015;71(3):547-558. doi:10.1007/s13105-015-0400-5

268. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? *Nature*. 2012;488(7413):E9-E10. doi:10.1038/nature11364

269. Blüher S, Panagiotou G, Petroff D, et al. Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. *Obesity*. 2014;22(7):1701-1708. doi:10.1002/oby.20739

270. Norheim F, Langleite TM, Hjorth M, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. *FEBS J*.

2014;281(3):739-749. doi:10.1111/febs.12619

271. Ellefsen S, Vikmoen O, Slettaløkken G, et al. Irisin and FNDC5: effects of 12-week strength training, and relations to muscle phenotype and body mass composition in untrained women. *Eur J Appl Physiol*. 2014;114(9):1875-1888. doi:10.1007/s00421-014-2922-x

272. Fox J, Rioux B V., Goulet EDB, et al. Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: A meta-analysis. *Scand J Med Sci Sport*. 2018;28(1):16-28. doi:10.1111/sms.12904

273. Tanimura Y, Aoi W, Takanami Y, et al. Acute exercise increases fibroblast growth factor 21 in metabolic organs and circulation. *Physiol Rep*. 2016;4(12):1-8. doi:10.14814/phy2.12828

274. Dinoff A, Herrmann N, Swardfager W, et al. The Effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): A meta-analysis. *PLoS One*. 2016;11(9):1-21. doi:10.1371/journal.pone.0163037

275. Bouchard C, Rankinen T. Individual differences in response to regular physical activity. In: *Medicine and Science in Sports and Exercise*. Vol 33. ; 2001:446-451. doi:10.1097/00005768-200106001-00013

276. Raue U, Trappe TA, Estrem ST, et al. Transcriptome signature of resistance exercise adaptations: Mixed muscle and fiber type specific profiles in young and old adults. *J Appl Physiol*. 2012;112(10):1625-1636. doi:10.1152/japplphysiol.00435.2011

277. Phillips BE, Williams JP, Gustafsson T, et al. Molecular Networks of Human Muscle Adaptation to Exercise and Age. *PLoS Genet*. 2013;9(3). doi:10.1371/journal.pgen.1003389

278. Kohrt WM, Malley MT, Coggan AR, et al. Effects of gender, age, and fitness level on response of V{O(2max)) to training in 60-71 yr olds. *J Appl Physiol*. 1991;71(5):2004-2011. doi:10.1152/jappl.1991.71.5.2004

279. Bouchard C. Individual differences in the response to regular exercise. In: *International Journal of Obesity*. Vol 19. ; 1995.

280. Phillips BE, Kelly BM, Lilja M, et al. A practical and time-efficient high-intensity interval training program modifies cardio-metabolic risk factors in adults with risk factors for type II diabetes. *Front Endocrinol (Lausanne)*. 2017;8(SEP):1-11. doi:10.3389/fendo.2017.00229

281. Mann TN, Lamberts RP, Lambert MI. High responders and low responders: Factors associated with individual variation in response to standardized training. *Sport Med*. 2014;44(8):1113-1124. doi:10.1007/s40279-014-0197-3

282. Ahtiainen JP, Walker S, Peltonen H, et al. Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages. *Age (Omaha)*. 2016;38(1):1-13. doi:10.1007/s11357-015-9870-1

283. Kenttä G, Hassmén P. Overtraining and recovery. A conceptual model. *Sport Med*. 1998. doi:10.2165/00007256-199826010-00001

284. Sparks LM. Exercise training response heterogeneity: physiological and molecular insights. *Diabetologia*. 2017;60(12):2329-2336. doi:10.1007/s00125-017-4461-6

285. Timmons JA, Knudsen S, Rankinen T, et al. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J Appl Physiol*. 2010;108(6):1487-1496. doi:10.1152/japplphysiol.01295.2009

286. Scharhag-Rosenberger F, Walitzek S, Kindermann W, Meyer T. Differences in adaptations to 1 year of aerobic endurance training: Individual patterns of nonresponse. *Scand J Med Sci Sport*. 2012. doi:10.1111/j.1600-0838.2010.01139.x

287. Boule NG, Weisnagel SJ, Lakka TA, et al. Effects of exercise training on glucose homeostasis. *Diabetes Care*. 2005;28(1):108-114.

288. Bouchard C, Blair SN, Church TS, et al. Adverse metabolic response to regular exercise: Is it a rare or common occurrence? *PLoS One*. 2012;7(5). doi:10.1371/journal.pone.0037887

289. Beltran Valls MR, Wilkinson DJ, Narici M V., et al. Protein Carbonylation and heat shock proteins in human skeletal muscle: Relationships to age and sarcopenia. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2015;70(2):174-181. doi:10.1093/gerona/glu007

290. Nakhuda A, Josse AR, Gburcik V, et al. Biomarkers of browning of white adipose tissue and their regulation during exercise- And diet-induced weight loss. *Am J Clin Nutr*. 2016;104(3):557-565. doi:10.3945/ajcn.116.132563

291. Brook MS, Wilkinson DJ, Mitchell WK, et al. Synchronous deficits in cumulative muscle

protein synthesis and ribosomal biogenesis underlie age-related anabolic resistance to exercise in humans. *J Physiol*. 2016;594(24):7399-7417. doi:10.1113/JP272857

292. Roustit MM, Vaughan JM, Jamieson PM, Cleasby ME. Urocortin 3 activates AMPK and AKT pathways and enhances glucose disposal in rat skeletal muscle. *J Endocrinol*. 2014;223(2):143-154. doi:10.1530/JOE-14-0181

293. Cleasby ME, Reinten TA, Cooney GJ, James DE, Kraegen EW. Functional studies of Akt isoform specificity in skeletal muscle in vivo; maintained insulin sensitivity despite reduced insulin receptor substrate-1 expression. *Mol Endocrinol*. 2007;21(1):215-228. doi:10.1210/me.2006-0154

294. Chee CSCEBASALWDSKGPLSFB, Shannon CE, Burns A, et al. Relative contribution of intramyocellular lipid to whole-body fat oxidation is reduced with age but subsarcolemmal lipid accumulation and insulin. *Diabetes (New York); 2016*. 2016;65(4):840-850. doi:10.2337/db15-1383.The

295. Cuthbertson DJ, Babraj JA, Mustard KJW, et al. 5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside acutely stimulates skeletal muscle 2-deoxyglucose uptake in healthy men. *Diabetes*. 2007;56(8):2078-2084. doi:10.2337/db06-1716

296. Baker DJ, Constantin-Teodosiu D, Jones SW, Timmons JA, Greenhaff PL. Chronic treatment with the β2-adrenoceptor agonist prodrug BRL-47672 impairs rat skeletal muscle function by inducing a comprehensive shift to a faster muscle phenotype. *J Pharmacol Exp Ther*. 2006;319(1):439-446. doi:10.1124/jpet.106.107045

297. Wibom R, Hultman E, Johansson M, Matherei K, Constantin-Teodosiu D, Schantz PG.

Adaptation of mitochondrial ATP production in human skeletal muscle to endurance training and detraining. *J Appl Physiol*. 1992;73(5):2004-2010. doi:10.1152/jappl.1992.73.5.2004

298. Polyzos SA, Mantzoros CS. An update on the validity of irisin assays and the link between irisin and hepatic metabolism. *Metabolism*. 2015;64(9):937-942. doi:10.1016/j.metabol.2015.06.005

299. Chakraborty A, Ghosh S, Mukhopadhyay P, et al. Trapping effect analysis of AlGaN/InGaN/GaN Heterostructure by conductance frequency measurement. In: *MRS Proceedings*. Vol XXXIII. ; 2014:81-87. doi:10.1007/s13398-014-0173-7.2

300. Moore MC, Cherrington AD, Mann SL, Davis SN. Acute fructose administration decreases the glycemic response to an oral glucose tolerance test in normal adults. *J Clin Endocrinol Metab*. 2000;85(12):4515-4519. doi:10.1210/jc.85.12.4515

301. Boereboom CL, Phillips BE, Williams JP, Lund JN. A 31-day time to surgery compliant exercise training programme improves aerobic health in the elderly. *Tech Coloproctol*. 2016;20(6):375-382. doi:10.1007/s10151-016-1455-1

302. Gharahdaghi N, Rudrappa S, Brook MS, et al. Testosterone therapy induces molecular programming augmenting physiological adaptations to resistance exercise in older men. *J Cachexia Sarcopenia Muscle*. 2019. doi:10.1002/jcsm.12472

303. Phillips B, Williams J, Atherton P, et al. Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise. *J Appl Physiol*. 2012;112(3):347-353.

doi:10.1152/japplphysiol.01031.2011

304. Keller C, Steensberg A, Pilegaard H, et al. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB J*. 2001;15(14):2748-2750. doi:10.1096/fj.01-0507fje

305. Pedersen BK. Muscles and their myokines. *J Exp Biol*. 2011;214(2):337-346. doi:10.1242/jeb.048074

306. Tsuchiya Y, Ando D, Goto K, Kiuchi M, Yamakita M, Koyama K. High-intensity exercise causes greater irisin response compared with low-intensity exercise under similar energy consumption. *Tohoku J Exp Med*. 2014;233(2):135-140. doi:10.1620/tjem.233.135

307. Miyamoto-Mikami E, Sato K, Kurihara T, et al. Endurance training-induced increase in circulating irisin levels is associated with reduction of abdominal visceral fat in middle-aged and older adults. *PLoS One*. 2015;10(3):2-13. doi:10.1371/journal.pone.0120354

308. Samy DM, Ismail CA, Nassra RA. Circulating irisin concentrations in rat models of thyroid dysfunction - Effect of exercise. *Metabolism*. 2015;64(7):804-813. doi:10.1016/j.metabol.2015.01.001

309. Czarkowska-Paczek B, Zendzian-Piotrowska M, Gala K, Sobol M, Paczek L. One session of exercise or endurance training does not influence serum levels of Irisin in rats. *J Physiol Pharmacol*. 2014;65(3):449-454.

310. Kim HJ, So B, Choi M, Kang D, Song W. Resistance exercise training increases the

expression of irisin concomitant with improvement of muscle function in aging mice and humans. *Exp Gerontol*. 2015;70:11-17. doi:10.1016/j.exger.2015.07.006

311. Vaughan RA, Gannon NP, Barberena MA, et al. Characterization of the metabolic effects of irisin on skeletal muscle in vitro. *Diabetes, Obes Metab*. 2014;16(8):711-718. doi:10.1111/dom.12268

312. Huh JY, Dincer F, Mesfum E, Mantzoros CS. Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and metabolism in humans. *Int J Obes*. 2014;38(12):1538-1544. doi:10.1038/ijo.2014.42

313. Huh JY, Mougios V, Kabasakalis A, et al. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. *J Clin Endocrinol Metab*. 2014;99(11):E2154-E2161. doi:10.1210/jc.2014-1437

314. Reza MM, Subramaniyam N, Sim CM, et al. Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy. *Nat Commun*. 2017;8(1):1-17. doi:10.1038/s41467-017-01131-0

315. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481(7382):463-468. doi:10.1038/nature10777

316. Abedpoor N, Taghian F, Ghaedi K, et al. PPARγ/Pgc-1α-Fndc5 pathway up-regulation in gastrocnemius and heart muscle of exercised, branched chain amino acid diet fed mice. *Nutr Metab*. 2018;15(1):1-15. doi:10.1186/s12986-018-0298-3

317. Jedrychowski MP, Wrann CD, Paulo JA, et al. Detection and quantitation of circulating human irisin by tandem mass spectrometry. *Cell Metab*. 2015;22(4):734-740. doi:10.1016/j.cmet.2015.08.001

318. Samy DM, Ismail CA, Nassra RA. Circulating irisin concentrations in rat models of thyroid dysfunction - Effect of exercise. *Metabolism*. 2015;64(7):804-813. doi:10.1016/j.metabol.2015.01.001

319. Varela-Rodríguez BM, Pena-Bello L, Juiz-Valiña P, Vidal-Bretal B, Cordido F, Sangiao-Alvarellos S. FNDC5 expression and circulating irisin levels are modified by diet and hormonal conditions in hypothalamus, adipose tissue and muscle. *Sci Rep*. 2016;6. doi:10.1038/srep29898

320. Lee J, Kim MS. The role of GSK3 in glucose homeostasis and the development of insulin resistance. *Diabetes Res Clin Pract*. 2007;77(3 SUPPL.). doi:10.1016/j.diabres.2007.01.033

321. Xin C, Liu J, Zhang J, et al. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. *Int J Obes*. 2016;40(3):443-451. doi:10.1038/ijo.2015.199

322. Galgani JE, Johannsen NM, Bajpeyi S, et al. Role of skeletal muscle mitochondrial density on exercise-stimulated lipid oxidation. *Obesity*. 2012;20(7):1387-1393. doi:10.1038/oby.2011.166

323. Florini JR, Ewton DZ, Coolican SA. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev*. 1996;17(5):481-517. doi:10.1210/er.17.5.481

324. Xiong XQ, Chen D, Sun HJ, et al. FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. *Biochim Biophys Acta - Mol Basis Dis*. 2015;1852(9):1867-1875. doi:10.1016/j.bbadis.2015.06.017

325. Koch LG, Pollott GE, Britton SL. Selectively bred rat model system for low and high response to exercise training. *Physiol Genomics*. 2013;45(14):606-614. doi:10.1152/physiolgenomics.00021.2013

326. Marton O, Koltai E, Takeda M, et al. Mitochondrial biogenesis-associated factors underlie the magnitude of response to aerobic endurance training in rats. *Pflugers Arch Eur J Physiol*. 2015;467(4):779-788. doi:10.1007/s00424-014-1554-7

327. Goodrick CL. Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, body weight, and metabolic rate. *Gerontology*. 1980;26(1):22-33. doi:10.1159/000212390

328. Holloszy JO. Exercise increases average longevity of female rats despite increased food intake and no growth retardation. *Journals Gerontol*. 1993;48(3). doi:10.1093/geronj/48.3.B97

329. Nakamura E. Effects of habitual physical exercise on physiological age in men aged 20-85 years as estimated using principal component analysis. *Eur J Appl Physiol Occup Physiol*. 1996;73(5):410-418. doi:10.1007/BF00334417

330. Sarna S, Sahi T, Koskenvuo M, Kaprio J. Increased life expectancy of world class male athletes. *Med Sci Sports Exerc*. 1993;25(2):237-244. doi:10.1249/00005768-

199302000-00013

331. Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J Appl Physiol*. 2016;121(6):831-838. doi:10.1152/jappl.1984.56.4.831

332. Fisher FM, Chui PC, Antonellis PJ, et al. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. *Diabetes*. 2010;59(11):2781-2789. doi:10.2337/db10-0193

333. Pilegaard H, Ordway GA, Saltin B, Neufer PD. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol - Endocrinol Metab*. 2000;279(4 42-4). doi:10.1152/ajpendo.2000.279.4.e806

334. Laske C, Banschbach S, Stransky E, et al. Exercise-induced normalization of decreased BDNF serum concentration in elderly women with remitted major depression. *Int J Neuropsychopharmacol*. 2010;13(5):595-602. doi:10.1017/S1461145709991234

335. Davidsen PK, Gallagher IJ, Hartman JW, et al. High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. *J Appl Physiol*. 2011;110(2):309-317. doi:10.1152/japplphysiol.00901.2010

336. Blair SN, Sallis RE, Hutber A, Archer E. Exercise therapy - the public health message. *Scand J Med Sci Sport*. 2012. doi:10.1111/j.1600-0838.2012.01462.x

337. Vickers RR. Measurement Error in Maximal Oxygen Uptake Tests. 2003;(04).

338. Brook MS, Wilkinson DJ, Mitchell WK, et al. Skeletal muscle hypertrophy adaptations

predominate in the early stages of resistance exercise training, matching deuterium oxide-derived measures of muscle protein synthesis and mechanistic target of rapamycin complex 1 signaling. *FASEB J*. 2015;29(11):4485-4496. doi:10.1096/fj.15-273755

339. Coyle EF, Martin WH, Bloomfield SA, Lowry OH, Holloszy JO. Effects of detraining on responses to submaximal exercise. *J Appl Physiol*. 1985. doi:10.1152/jappl.1985.59.3.853

340. Nolan PB, Keeling SM, Robitaille CA, Buchanan CA, Dalleck LC. The effect of detraining after a period of training on cardiometabolic health in previously sedentary individuals. *Int J Environ Res Public Health*. 2018. doi:10.3390/ijerph15102303

341. Bouchard C, An P, Rice T, et al. Familial aggregation of V̇O(2max) response to exercise training: Results from the HERITAGE family study. *J Appl Physiol*. 1999;87(3):1003-1008. doi:10.1152/jappl.1999.87.3.1003

342. Bouchard C, Sarzynski MA, Rice TK, et al. Genomic predictors of the maximal O2 uptake response to standardized exercise training programs. *J Appl Physiol*. 2011;110(5):1160-1170. doi:10.1152/japplphysiol.00973.2010

343. Saltin B, Hartley LH, Kilbom Å, Åstrand I. Physical training in sedentary middle-aged and older men II. Oxygen uptake, heart rate, and blood lactate concentration at submaximal and maximal exercise. *Scand J Clin Lab Invest*. 1969;24(4):323-334. doi:10.3109/00365516909080169

344. Hautala AJ, Kiviniemi AM, Mäkikallio TH, et al. Individual differences in the responses

to endurance and resistance training. *Eur J Appl Physiol*. 2006;96(5):535-542. doi:10.1007/s00421-005-0116-2

345. Skinner JS, Jaskólski A, Jaskólska A, et al. Age, sex, race, initial fitness, and response to training: The HERITAGE Family Study. *J Appl Physiol*. 2001;90(5):1770-1776. doi:10.1152/jappl.2001.90.5.1770

346. Poole DC, Wilkerson DP, Jones AM. Validity of criteria for establishing maximal O 2 uptake during ramp exercise tests. *Eur J Appl Physiol*. 2008;102(4):403-410. doi:10.1007/s00421-007-0596-3

347. Kreher JB, Schwartz JB. Overtraining Syndrome: A Practical Guide. *Sports Health*. 2012;4(2):128-138. doi:10.1177/1941738111434406

348. Vollaard NBJ, Constantin-Teodosiu D, Fredriksson K, et al. Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. *J Appl Physiol*. 2009;106(5):1479-1486. doi:10.1152/japplphysiol.91453.2008

349. Green H, Goreham C, Ouyang J, Ball-Burnett M, Ranney D. Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *Am J Physiol - Regul Integr Comp Physiol*. 1999;276(2 45-2):591-596. doi:10.1152/ajpregu.1999.276.2.r591

350. Pescatello LS, Franklin BA, Fagard R, et al. American College of Sports Medicine position stand. Exercise and hypertension. *Med Sci Sports Exerc*. 2004.

351. Schroeder EC, Franke WD, Sharp RL, Lee D chul. Comparative effectiveness of aerobic, resistance, and combined training on cardiovascular disease risk factors: A randomized controlled trial. *PLoS One*. 2019;14(1):1-14. doi:10.1371/journal.pone.0210292

352. Kelley GA, Kelley KS. Progressive resistance exercise and resting blood pressure: A meta- analysis of randomized controlled trials. *Hypertension*. 2000. doi:10.1161/01.HYP.35.3.838

353. Calders P, Elmahgoub S, De Mettelinge TR, et al. Effect of combined exercise training on physical and metabolic fitness in adults with intellectual disability: A controlled trial. *Clin Rehabil*. 2011. doi:10.1177/0269215511407221

354. Ho SS, Dhaliwal SS, Hills AP, Pal S. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. *BMC Public Health*. 2012. doi:10.1186/1471-2458-12-704

355. Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension*. 2005;46(4):667-675. doi:10.1161/01.HYP.0000184225.05629.51

356. Perez-Gomez J, Vicente-Rodríguez G, Royo IA, et al. Effect of endurance and resistance training on regional fat mass and lipid profile. *Nutr Hosp*. 2013;28(2):340-346. doi:10.3305/nh.2013.28.2.6200

357. Slentz CA, Bateman LA, Willis LH, et al. Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by HOMA in

overweight adults from STRRIDE AT/RT. *Am J Physiol - Endocrinol Metab*. 2011;301(5):1033-1039. doi:10.1152/ajpendo.00291.2011

358. Willis LH, Slentz CA, Bateman LA, et al. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. *J Appl Physiol*. 2012;113(12):1831-1837. doi:10.1152/japplphysiol.01370.2011

359. Lemmer JT, Ivey FM, Ryan AS, et al. Effect of strength training on resting metabolic rate and physical activity: Age and gender comparisons. *Med Sci Sports Exerc*. 2001;33(4):532-541. doi:10.1097/00005768-200104000-00005

360. Borghouts LB, Keizer HA. Exercise and insulin sensitivity: A review. *Int J Sports Med*. 2000. doi:10.1055/s-2000-8847

361. Poehlman ET, Dvorak R V., DeNino WF, Brochu M, Ades PA. Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: A controlled randomized trial. *J Clin Endocrinol Metab*. 2000;85(7):2463-2468. doi:10.1210/jc.85.7.2463

362. Petersen KF, Befroy D, Dufour S, et al. Mitochondrial dysfunction in the elderly: Possible role in insulin resistance. *Science (80-)*. 2003. doi:10.1126/science.1082889

363. Szendroedi J, Schmid AI, Meyerspeer M, et al. Impaired mitochondrial function and insulin resistance of skeletal muscle in mitochondrial diabetes. *Diabetes Care*. 2009;32(4):677-679. doi:10.2337/dc08-2078

364. Safdar A, Little JP, Stokl AJ, Hettinga BP, Akhtar M, Tarnopolsky MA. Exercise

increases mitochondrial PGC-1α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *J Biol Chem*. 2011;286(12):10605-10617. doi:10.1074/jbc.M110.211466

365. Baar K, Wende AR, Jones TE, et al. Adaptations of skeletal muscle to exercise: Rapid increase in the transcriptional coactivator PGC-1. *FASEB J*. 2002. doi:10.1096/fj.02-0367com

366. Pilegaard H, Saltin B, Neufer DP. Exercise induces transient transcriptional activation of the PGC-1α gene in human skeletal muscle. *J Physiol*. 2003. doi:10.1113/jphysiol.2002.034850

367. Lantier L, Fentz J, Mounier R, et al. AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity. *FASEB J*. 2014. doi:10.1096/fj.14-250449

368. Gavin TP, Stallings HW, Zwetsloot KA, et al. Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. *J Appl Physiol*. 2005;98(1):315-321. doi:10.1152/japplphysiol.00353.2004

369. Olfert IM, Breen EC, Mathieu-Costello O, Wagner PD. Skeletal muscle capillarity and angiogenic mRNA levels after exercise training in normoxia and chronic hypoxia. *J Appl Physiol*. 2001. doi:10.1152/jappl.2001.91.3.1176

370. Gavin TP, Westerkamp LM, Zwetsloot KA. Soleus, plantaris and gastrocnemius VEGF mRNA responses to hypoxia and exercise are preserved in aged compared with young female C57BL/6 mice. *Acta Physiol*. 2006. doi:10.1111/j.1748-1716.2006.01609.x

371. Gavin TP, Wagner PD. Attenuation of the exercise-induced increase in skeletal muscle Flt-1 mRNA by nitric oxide synthase inhibition. *Acta Physiol Scand*. 2002. doi:10.1046/j.1365-201X.2002.00987.x

372. Scicchitano BM, Rizzuto E, Musarò A. Counteracting muscle wasting in aging and neuromuscular diseases: the critical role of IGF-1. *Aging (Albany NY)*. 2009. doi:10.18632/aging.100050

373. Ascenzi F, Barberi L, Dobrowolny G, et al. Effects of IGF-1 isoforms on muscle growth and sarcopenia. *Aging Cell*. 2019;18(3):1-11. doi:10.1111/acel.12954

374. Hulmi JJ, Tannerstedt J, Selänne H, Kainulainen H, Kovanen V, Mero AA. Resistance exercise with whey protein ingestion affects mTOR signaling pathway and myostatin in men. *J Appl Physiol*. 2009;106(5):1720-1729. doi:10.1152/japplphysiol.00087.2009

375. Amirouche A, Durieux AC, Banzet S, et al. Down-regulation of Akt/mammalian target of rapamycin signaling pathway in response to myostatin overexpression in skeletal muscle. *Endocrinology*. 2009;150(1):286-294. doi:10.1210/en.2008-0959

376. Whittemore LA, Song K, Li X, et al. Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. *Biochem Biophys Res Commun*. 2003;300(4):965-971. doi:10.1016/S0006-291X(02)02953-4

377. Willoughby DS. Effects of Heavy Resistance Training on Myostatin mRNA and Protein Expression. *Med Sci Sports Exerc*. 2004;36(4):574-582. doi:10.1249/01.MSS.0000121952.71533.EA

378. Bergen HR, Farr JN, Vanderboom PM, et al. Myostatin as a mediator of sarcopenia versus homeostatic regulator of muscle mass: Insights using a new mass spectrometry-based assay. *Skelet Muscle*. 2015;5(1):1-16. doi:10.1186/s13395-015-0047-5

379. West DWD, Baehr LM, Marcotte GR, et al. Acute resistance exercise activates rapamycin-sensitive and -insensitive mechanisms that control translational activity and capacity in skeletal muscle. *J Physiol*. 2016;594(2):453-468. doi:10.1113/JP271365

380. Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA, Adams GR. Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. *J Appl Physiol*. 2005. doi:10.1152/japplphysiol.00895.2004

381. Haddad F, Baldwin KM, Tesch PA. Pretranslational markers of contractile protein expression in human skeletal muscle: Effect of limb unloading plus resistance exercise. *J Appl Physiol*. 2005. doi:10.1152/japplphysiol.00553.2004

382. Hecksteden A, Kraushaar J, Scharhag-Rosenberger F, Theisen D, Senn S, Meyer T. Individual response to exercise training - A statistical perspective. *J Appl Physiol*. 2015;118(12):1450-1459. doi:10.1152/japplphysiol.00714.2014

Appendix 1

PEDAL or PUSH?

Which type of exercise is best for you...

Researchers from The University of Nottingham at the Royal Derby Hospital are looking for Healthy *Male* Volunteers between 18-40 years of age

Exploring why different people respond differently to different types of exercise

- Does not involve drug therapy
- Involves 2 periods of 4-weeks exercise training
- Involves blood, muscle & saliva samples
- An inconvenience allowance will be paid

For more information please contact:

Mr Wesam Farrash:

mzxwf@nottingham.ac.uk

Tel: 01332 724685

Text: "STUDY X1" to 07575777918

Appendix 2

Clinical, Metabolic and Molecular Physiology Research Group

Healthy Volunteer's Information Sheet

Title of Project: Exploring the mechanistic basis, and plasticity of "exercise resistance" to improve human health (Exercise X-over).

Names of Investigators:
Dr Bethan Phillips, Assistant Professor
Dr Phil Atherton, Associate Professor
Dr Ken Smith, Principal Research Fellow
Mr Wesam Farrash MSc, PhD Student
Dr Dan Wilkinson, Assistant Professor

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of your consent form and this leaflet.

Background

The health of our body and our muscles is very much linked to our level of physical inactivity (sedentary behaviour) or activity (exercise). For example, physical inactivity causes our muscles to shrink in size and weaken and, if sustained, inactivity is linked to poor health outcomes. In contrast, exercise is linked with improved health e.g., stronger muscles and a healthier heart. Therefore it would seem that it is straightforward that we should be promoting exercise to everyone to improve health. Indeed, if you asked anybody they would almost certainly tell you that exercise is "good for you". In addition, most people would expect that if you were to cycle, lift weights, or go jogging regularly, you would expect to get fitter and stronger. However, some interesting and unexpected findings have been reported in recent years… In very large exercise training studies with hundreds of participants, it has been demonstrated that some individuals fail to get fitter or stronger (1/3 people). Even more alarmingly, some people don't improve in expected health parameters (blood pressure, blood sugar control etc). These results reveal that while exercise, on the whole, generates positive health benefits, one size does not fit all. So it seems that people claiming they weren't making progress at the gym in terms of, say, running ability or muscle gains may have been right all along. Therefore, this project aims to investigate this phenomenon of "exercise resistance"

by exploring multiple potential mechanisms behind "exercise resistance" and then trying to understand if this is a 'global' phenomenon or if someone who is exercise resistant for one type of exercise (e.g. cycling) may be responsive to a different training mode (e.g. weight training). In addition, this study will aim to assess if 'performance' gains (i.e. muscle mass, cycling ability) correspond with health parameters (i.e. insulin sensitivity). Overall this project could have large implications for understanding the control of exercise adaptation and health outcomes in relation to "exercise resistance", and moreover, will form the basis for pre-identifying "non-responders"; paving the way for personalised medicine approaches in the future.

What does the study involve?

Screening Visit

Before you formally enter the study you will be asked to attend our facility (the Medical School, Royal Derby Hospital) for a screening visit to ensure that you are eligible for participation in this study. During this visit (approximately 60 minutes), we will explain the study in full and answer any questions you may have, then (i) ask you to sign a consent form for the study, (ii) take a blood sample (10-20ml), and (iii) conduct a health assessment - including measuring your height and weight and a trace of your hearts electrical activity (an ECG).

Study Overview

Once we have received the results of your blood tests from screening we will contact you to inform you of your results and confirm your inclusion into the study. If you are happy to proceed with the study we will organise a mutually convenient day on which you will attend our facility to begin the study. The study period will last a total of 14 weeks in duration- although 6 of these weeks are "wash-out" between different exercise training types and thus have little involvement. Over the first exercise period, you will need to visit the facility 3x each week for 4 weeks to perform "Exercise 1" (either cycling or weight training). You will then have a 6-week "wash-out" period where we will ask you to return to pre-study physical activity levels. After this you will have another 4 weeks where you will be asked to train at the facility 3x each week performing "Exercise 2"- the alternate exercise type to that performed in the first 4-weeks. Before and after each type of exercise training (weeks 0, 4, 10 and 14) you will be asked to attend our unit for an acute study, which will last approximately 6 hours (see details below). In addition, at weeks -2, and 8 (2 weeks before you start each type of exercise) you will be asked to attend our unit for a short visit so that we can take small blood, muscle and saliva samples. You will also drink some 'heavy water' that is slightly altered from normal drinking water so that we can use it to assess muscle-building capacity (see Acute Studies section below). These visits allow us look at how your muscle is changing over the course of the exercise training regimes. Before your first exercise regime and at one point during each training period and the "wash-out" phase we will ask you to fill in a physical activity questionnaire and complete a 4-day diet diary, this is so we can gain information about your habitual diet and physical activity behaviours and try to ensure you return to them during the "wash-out" phase.

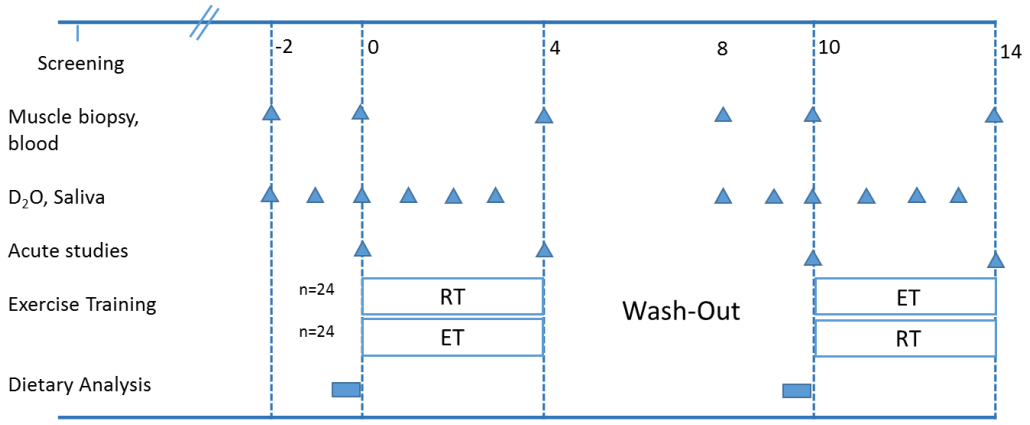

Figure 1. Study Overview Scheme; RT = resistance (weight) training, ET = endurance (cycling) training

Acute Studies

You will need to attend for the acute studies at ~0900h fasted from midnight with water *ad lib*. The acute studies will begin with small blood, muscle and saliva samples, with the blood draw from an arm vein and the muscle biopsy from your *m.vastus lateralis*- a muscle at the front of your thigh. At this time you will also be asked to drink some 'heavy water'. This water contains a stable isotope tracer (deuterium in place of hydrogen, but is not radioactive), which is slightly different from normal water, but it is perfectly safe. The muscle biopsy will be taken after an injection of local anaesthetic to numb the area. After the local anaesthetic, a trained clinician will make a small (0.5 cm) cut through the skin and underlying muscle sheath to take the biopsy (a small piece about the size of an orange pip) with a special set of forceps. With the local anaesthetic you may feel pushing during this procedure but you should feel no pain. A stitch is placed in the skin after the biopsy to aid healing. A tight bandage will be placed around the thigh after the biopsy and should remain in place for ~12-hours to speed healing and minimise bruising.

After this we will perform an oral glucose tolerance test (OGTT) which tests how sensitive your body is to sugar. A small cannula (thin plastic tube) will be placed into one of the veins in the back of your hand to allow us to take multiple blood samples. This hand will then be placed into a warm box for 2-hours. After we have taken an initial blood sample, we will ask you to drink 75g of glucose (sugar) dissolved in 250mL water. We will then take a blood sample from the cannula every 15-minutes for 2-hours.

A DXA (dual-energy x-ray absorptiometry) scan will then measure your body composition and leg muscle mass, and an ultrasound scan will look at your muscle architecture (fibre length and thickness). The ultrasound scan is similar to the scan carried out to image babies during pregnancy and involves some cool jelly and a probe being placed on your thigh. A leaflet describing the DXA scan in more detail is attached. After this your resting heart rate and blood pressure will be recorded. We will then feed you a standard light lunch before giving you 30-min of rest.

After the rest period you will perform a cardio-pulmonary exercise test (CPET). This test involves cycling on a static bike with the intensity getting progressively harder until you cannot cycle anymore. During this test you will wear a facemask so that we can measure the amount of oxygen you are using. This test will give us information to set the intensity of your cycling training. After this you will participate in a battery of simple muscle function tests to assess your strength, power, force, balance and gait speed. These tests will also provide us with information needed to set the intensity of your weight training.

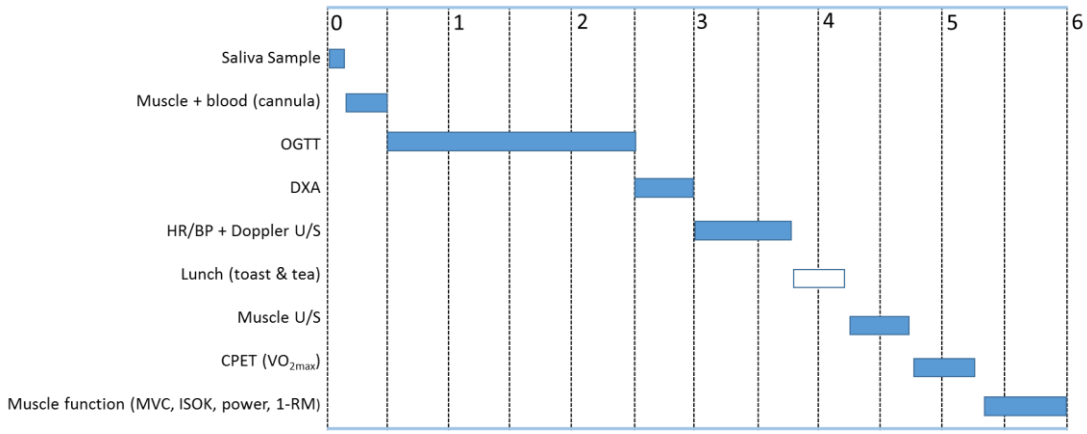

Figure 2. Acute Study Scheme

Exercise Training

Both of the exercise types will be performed for 4 weeks, 3x each week with each session last approximately 1-hour. "Exercise 1" is also known as TREND – traditional endurance training (ET), and will comprise 60-min cycling at a moderate intensity (60% of maximal effort) as determined by the CPET test in your acute studies. "Exercise 2", also known as RET – resistance exercise training, will comprise whole-body weight training using specialised machines. You will perform 2 sets of 12 repetitions across 6 exercises (3 upper and 3 lower body) at 70% 1-RM (repetition maximum) as determined by the strength tests in your acute studies.

In addition, during the exercise training periods you will be asked to drink small weekly doses of the heavy water outlined above. You will need to provide a saliva sample before and 3 hours after this drink. On your first, middle (session 6 or 7) and last exercise session of each type we will take small blood samples before, immediately after, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after the exercise session. We will also take an additional muscle biopsy from your *m.vastus lateralis*, as in the acute study, 6 hours after your first exercise session of each type. These blood and muscle samples will be used to measures changes in gene expression as a result of unaccustomed exercise.

Why have you been chosen?
Primarily because you have expressed an interest in taking part in the study and you have met with the entry criteria and do not have on-going medical problems. We will be recruiting healthy, recreationally active males aged 18-40 years.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

You are requested to fast on the night before your screening and acute study visits, and avoid heavy exercise for 48 hours prior to your acute study visits. Other than attending the sessions outlined above, the only other request is to maintain your habitual diet and exercise for the duration of the study.

What is the drug or procedure that is being tested?

This study is assessing the impact of traditional endurance training (TREND) vs. resistance exercise training (RET) for improving indices of health, fitness and physical function. We are looking to assess if people who do not respond well to one type of exercise for the expected outcome (e.g. muscle mass with resistance exercise training) are able to respond well to a different type of exercise, and if level of response is linked to health benefits.

What are the side effects of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known. In the light of our experience, muscle biopsies are well tolerated by all our volunteers. Scarring is hardly perceptible and fades with time. However, the procedure may cause mild pain in some volunteers, but with appropriate local analgesia this is minimal; indeed data from a previous audit of the procedure demonstrated that 83% of our participants experienced less than or the expected discomfort during the muscle biopsy and all respondents said that they would or may be willing to participate in a similar study in the future. Some discomfort (muscle tenderness and stiffness) may be felt for 2-3 days afterwards but mild painkillers will usually effectively abolish this. There is a slight risk of infection at biopsy sites, however all procedures are performed under sterile conditions to minimize any risk of infection and aftercare advice and dressings are given. Damage to small nerves of the skin during the biopsy is possible; however these nerves grow back with restoration of normal sensation, without difficulty. We will provide you with a short, anonymized questionnaire about your experience of the biopsy procedures during this study should you wish to respond.

The risks involved in venous blood sampling are very small. Occasionally, however there may be bruising due to leakage from a blood vessel on withdrawal of the needle, but this is uncommon with good practice. The amount of blood taken during the acute studies in this project is much less than if you donate blood.

What are the exclusion criteria for this study?

We are recruiting healthy men between the ages of 18 to 40 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid a disturbance allowance or having an invasive procedure (e.g. blood sample >50ml) you would not be eligible to take part.

You would also be unsuitable if you have particular medical conditions or are taking certain medications. If you are interested in this study please discuss these further with the study doctor.

What are the possible disadvantages and risks of taking part?

Healthy volunteers have been chosen for this study; however, it is possible that the routine tests could detect unknown health problems. Should this be the case you will be informed and advised to attend your GP practice for further management. Your GP will also be notified.

What if something goes wrong?

In the unlikely event of a complication following the study or that you should wish to voice grievances, in the first instance please contact the clinician involved in the execution of the study or the lead investigator, Dr Beth Phillips (T: 01332 724731; E: beth.phillips@nottingham.ac.uk). If you are still dissatisfied then you should direct these concerns to the Administrator, University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee, c/o School of Medicine Education Centre, B Floor, Medical School, QMC Campus, Nottingham University Hospitals, NG7 2UH, email: louise.sabir@nottingham.ac.uk .

Will my taking part in this study be kept confidential?

We respect your right to privacy and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement will carry details of your name and address, but no health related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to University staff with direct responsibility for ensuring the study is conducted appropriately.

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you that leaves the University will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it, with the exception of a letter sent that may be sent to your GP advising of your participation in this study if abnormal results need to be disclosed.

If you agree, your personal data (address, telephone number etc.) will be kept for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality and only direct members of the research team will have access to your personal data.

What will happen to any samples I give?

We will use the blood and biopsy samples to see how your muscles respond to different types of exercise. We may also use your muscle samples to generate cell-culture samples so that we can further investigate mechanisms involved in muscle health and disease outside of the human body. We will also seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?

In the current study we will collect blood samples that can be used to obtain information about your genes (RNA and DNA), however this analysis will not give us information on disease prognosis and samples will not be subject to genetic manipulation.

Who is funding the research?

This research is funded by The University of Nottingham.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee.

Contact for Further Information

Thank you for taking the time to read this information. For further information you can contact Mr Wesam Farrash (Research Student) on 01332 724685 or at mzxwf@nottingham.ac.uk, alternatively contactable at Clinical, Metabolic and Molecular Physiology, University of Nottingham, School of Medicine, Division of Graduate Entry Medicine and Health, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT.