MITOCHONDRIAL BIOGENESIS, SIGNALLING PATHWAYS INVOLVED, AND NUTRIENT INTERACTIONS IN RESPONSE TO HIGH INTENSITY INTERVAL RUNNING

Críonna Tobin, BSC, H-Dip Nut, IOC Grad Dip Sports Nut

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Submitted for the award of PhD

Dublin City University

School of Health and Human Performance

Supervisor: Prof Niall M. Moyna

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Volume 1 of 1

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

Study 1: This study investigated the effects of high intensity interval running (HIIR) and diet on the activation of signalling pathways involved in mitochondrial biogenesis. HIIR did not increase AMPK or p38MAPK phosphorylation. Therefore, it was unclear whether feeding carbohydrate (CHO) during recovery from this acute bout of high intensity exercise would blunt the activation of these pathways during a subsequent bout of HIIR. An increase in ACCβ phosphorylation was found after HIIR and was not blunted by CHO feeding during a subsequent bout of HIIR.

Study 2: This study compared the changes in mitochondrial biogenesis and endurance performance in response to two weeks of HIIR or endurance running (ER) in Gaelic games players. Compared to 6 sessions of ER, HIIR induced a similar improvement in endurance performance and a more pronounced increase in mitochondrial biogenesis.

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Abbreviations

ACCβ	Acetyl-CoA carboxylase β
ADP	Adenosine diphosphate
AICAR	5-aminoimidazole-4-carboxamide-1-b-4-ribofuranoside
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate kinase
AS160	RAB/GAPase-activating protein
ATF-1	Activating transcription factor 1
ATP	Adenosine monophosphate
BAT	Brown adipose tissue
Ca^{2+}	Calcium
CaMK II/IV	Calcium/calmodulin dependant protein kinase
CaMKK	Calcium calmodulin kinase kinase
CBS	Cystathionine β-synthase
CHO	Carbohydrate
COX	Cytochorme oxidase
CPT-1	Carnitine palmitoyltransferase I
CRE	CRE binding protein
CRE	Cyclic AMP response element
CREB	CRE binding protein
CS	Citrate synthase
DNA	Deoxyribonucleic acid

ERK	Extracellular signal-related kinase
ERRα	Estrogen related receptor α
ET	Endurance training
ETC	Electron transport chain
FA	Fatty acid
FABPpm	Fatty acid associated binding protein
FADH2	Reduced form of flavin adenine dinucleotide (FAD)
FAT/CD36	Fatty acid translocase
FFA	Free fatty acid
FOXO1	Forkhead box protein O1
GLUT4	Glucose transporter 4
GPAβ	β-guanidinopropionic acid
GS	Glycogen Synthase
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HII	High intensity interval
HIIE	High intensity interval exercise
HIIT	High intensity interval training
HK	Hexokinase
JUN	c-Jun NH2-terminal kinase
LCFA	Long chain fatty acid
LKB1	Liver kinase B1

LPL	Lipoprotein lipase
MCAD	Mediuma chain acyl CoA dehydrogenase
MCD	Malonyl-CoA decarboxylase
MEF	Myoctyte enhancer factor
MKK3K	Mitogen activated protein 3 kinase
MKK6K	Mitogenacitivated protein 6 kinase
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
NAD^+	Oxidised from NADH
NADH	Nicotinamide adenine dinucleotide
NRF-1	Nuclear receptor factor 1
NRF-1	Nuclear receptor factor 2
O_2	Oxygen
OXPHOS	Oxidative phosphorylation
$p160^{MBP}$	p160 myb binding protein
P38MAPK	p38 mitogen activated protein kinase
PCr	Creatine phosphate
PDC	Pyruvate dehydrogenase complex
PDH	Pyruvate dehydrogenase
PDK4	Pyruvate dehydrogenase kinase 4
PGC	PGC-1 related coactivator
PGC-1α	PPARγ coactivator 1α

Pi	Inorganic phosphate
PKC	Protein kinase C
PPARα	Peroxisome proliferator activated alpha
PPARγ	Peroxisome proliferator-activated receptor γ
PPARγ	Peroxisome proliferator activated gamma
PPO	Peak power output
RIP140	Nuclear receptor interacting protein 1
RMR	Basel metabolic rate
SDH	Succinate dehydrogenase
SIRT1	Sirtuin (silent mating type information regulation 2 homolog) 1
SRC-1	Steroid receptor complex 1
TCA	Tricarboxylic acid cycle
Tfam	Transcription factor A
TRAP	Thyroid recptor associated protein
$\dot{V}O_2max$	Maximal oxygen uptake
WAT	White adipose tissue
BAT	Brown adipose tissue
β-HAD	3 hydroylacyl CoA dehydrogenase

Chapter 1

Introduction

Skeletal muscle is a very versatile tissue as is evident by its ability to adapt to distinct patterns of contractile activity [1,2]. Exercise represents a stimulus capable of inducing both acute and chronic cellular adaptations in skeletal muscle [3]. Chronic adaptations are likely to be induced by cumulative bouts of contractile activity leading to an increase in gene and protein expression, and ultimately distinct phenotypic changes [4,5].

Endurance training (ET), which is characterized by prolonged bouts of exercise at submaximal intensities, elicits metabolic and morphological responses that promote a more oxidative phenotype [4,6]. These adaptations are intricately linked to mitochondrial biogenesis [5,7]. Over the past decade our understanding of the molecular events involved in this process has advanced greatly. Signalling pathways activated in response to perturbations' in myocellular calcium (Ca^{2+}) concentrations and metabolic rates, changes in muscle force tension and hormone signalling have culminated in inducing changes in gene and protein expression in the hours after exercise [8].

High intensity interval activity refers to repeated session of brief exercise bouts performed at maximal effort (≥ 90% $\dot{V}O_2$max). Depending on the training intensity, a single session can last from a few seconds (s) to several minutes (min), with multiple bouts separated by passive or active recovery periods. Recent evidence suggests that acute or chronic low volume high intensity interval exercise can induce metabolic adaptations that are similar to endurance training [9–13]. Studies employing high intensity interval training (HIIT) have consistently found an increase in muscle oxidative capacity, indicated by an

increase in mitochondrial enzymes such as citrate synthase (CS), cytochrome c oxidase and 3 hydroylacyl CoA dehydrogenase (β-HAD) [10,12,14,15]. The ability of HIIT to elicit rapid changes in skeletal muscle oxidative capacity is related to its ability to recruit both type 1 and type II fibers.

From a cellular perspective a key regulator of oxidative enzyme expression in a number of cell types, including skeletal muscle is the peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC-1α) [16]. PGC-1α is a transcriptional co-activator which through the activation of both nuclear and mitochondrial transcription factors up-regulates mitochondrial biogenesis [17,18]. Several signalling pathways have also been linked to exercise induced activation of PGC-1α and mitochondrial biogenesis, including adenosine monophsophate activated protein kinase (AMPK) [19], p38 mitogen activated protein kinase (p38MAPK) [20] and calcium-calmodulin dependant protein kinase (CaMK) [21]. A number of HIIT studies have found an increase in PGC-1α, AMPK, p38MAPK, and CaMK with a subsequent increase in oxidative enzyme activity, and in some studies enhanced performance in endurance exercise sessions [11,12,15,22,23]. The majority of the studies which show an increase in mitochondrial biogenesis after HIIT have used cycling protocols and recreationally active and/or sedentary subjects [11,12,15,22].

The effects of HIIT on mitochondrial biogenesis may be influenced by certain nutrient-gene interactions [24–26]. When exercise is undertaken in the presence of low muscle glycogen content, the transcription of a number of genes involved in mitochondrial biogenesis are up-regulated [27,28]. Some of these transcription factors have glycogen binding domains, and when muscle glycogen is low, these factors are discharged to associate with

different proteins, subsequently inducing training adaptations [26]. However, a recent study found that activation of a number of signalling proteins, which play a key role in the activity of several transcription factors may also be sensitive to nutrients and their activation enhanced by CHO ingestion [25].

Study Aims

The following series of studies will i) investigate the activation of signalling pathways induced by high intensity interval running ii) assess whether CHO feeding blunts the activation of these signalling pathways iii) determine the effect of 6 sessions of high intensity training or endurance training on skeletal muscle mitochondrial biogenesis.

Objectives

1. To investigate the effects of an acute bout of HIIR on the activation of AMPK and p38MAPK signalling pathways
2. To compare the effect of feeding either a solution high in CHO or a nonenergetic placebo solution on the activation of HIIR induced signalling molecules
3. To compare the effects of 6 sessions of either HIIR with ER on proposed mediators of mitochondrial biogenesis
4. To determine whether 6 sessions of either HIIR or ER can enhance performance

Hypotheses

1. A bout of high intensity interval running will activate AMPK and p38MAPK signalling pathways
2. Feeding a high CHO diet prior to a HIIR session will blunt the activation of the signalling molecules
3. 6 sessions of both HIIR and ER will induce similar exercise induced skeletal muscle adaptations
4. Performance will be enhanced in both groups after HIIR and ER

Chapter 2

Review of Literature

Mitochondria

Mitochondria play a central role in cellular energy metabolism, providing the majority of the energy for skeletal muscles under aerobic conditions. An increase in mitochondrial volume referred to as mitochondrial biogenesis [29] enhances the capacity of the muscle cell to generate ATP via oxidative phosphorylation [30,31]. In 1967, Hollozsy *et al*., (1967) [30] reported an increase in skeletal muscle mitochondrial number and oxidative capacity in response to endurance training in rats. The increase in mitochondria, were associated with an improvement in endurance exercise performance. The introduction of the needle biopsy technique in the late 1960s, allowed researchers to investigate the changes in mitochondrial structure and function in human skeletal muscle in response to acute and chronic exercise. Mitochondrial density, enzyme number and activity was subsequently found to significantly increase in men and women following ET ranging from 8 to 20 weeks [32–36]. Mitochondrial density varies depending on the energy requirements of the cell. Tissues with a high capacity to perform aerobic metabolic functions such as the kidney and skeletal muscle have a larger number of mitochondria [37]. Intermyofibrillar mitochondria are thought to have a higher activity per unit of mass than sacrolemma mitochondrial and play a major role in maintaining the adenosine monophosphate (ATP) supply during contractile activity [38].

Mitochondrial Function

Mitochondria are oval shaped organelle, about 2 μm in length and 0.5 μm in diameter and exist as both an individual capsule shaped organelle or an interconnected network, much like the sarcoplasmic reticulum [39]. Mitochondria in skeletal and cardiac muscle are located in the sarcolemma, immediately beneath the cell membrane and deeper within the muscle in the intermyofibrillar space. Sarcolemmal mitochondria account for 5-40% of the total mitochondrial volume depending upon muscle fiber type [38]. Dynamic in nature, mitochondria continuously engage in both fusion and fission [40]. Fusion is the process by which mitochondria combine with other mitochondria, while fission is the process by which mitochondria, derived from pre-existing mitochondria, split. The balance of fusion and fission is likely a major determinant of mitochondrial morphology [40].

Mitochondria are composed of an outer and inner phospholipid layer, an intermembrane space and an internal soluble matrix (Figure 2.1). The outer membrane is a relatively simple phospholipid bilayer, containing protein structures called porins. These voltage-dependent anion channels are the most abundant proteins in the mitochondrial outer membrane and are permeable to molecules <10 Kd. ions, nutrient molecules, ATP, adenosine monophosphate (ADP), nicotinamide adenine (NAD) and Coenzyme A can pass through the outer membrane with ease [40].

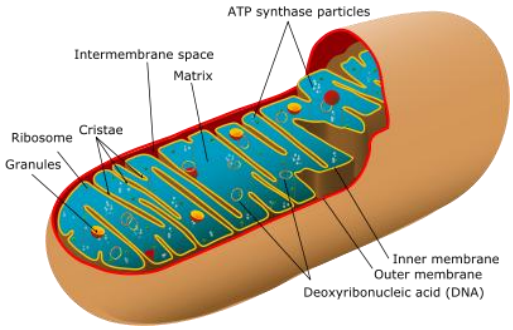

Figure 2.1 Structure of the mitochondria

The inner mitochondrial membrane is a highly complex structure and is the site of oxidative phosphorylation. It includes all of the complexes of the electron transport chain (ETC), the ATP synthase complex, and transport proteins. The membrane is folded into a series of internal ridges known as cristae which increases the surface area for ATP production and contains the proteins responsible for replication of mitochondrial deoxyribonucleic acid (DNA) [37]. The movement of molecules and ions across the inner mitochondrial membrane require specific membrane transporters. This separation of the matrix from the cytosolic environment by the inner membrane is a central feature in the conversion of free energy derived from oxidizable substrates. The mitochondrial matrix contains a range of enzymes, which form parts of major metabolic pathways such as CHO, lipid and amino acid oxidation and urea and haem biosynthesis [41].

Mitochondrial Evolution

Mitochondria appear to have originated from an ancient bacterium that was captured by a primitive eukaryotic cell [42]. Within the cytoplasm of the eukaryotic cell, both the bacterium and the primitive eukaryotic cell developed a symbiotic relationship [42]. The two cells enjoying this relationship evolved into mitochondria and eukaryotic cells.

Similarities in the results of DNA analysis of the genomes of mitochondria and the genomes of the bacterium *Rickettsia Prowazekki* support this theory [42]. Both *Rickettsia Prowazekk*i and mitochondria rely on eukaryotic cells in order to reproduce. The size and structure of ribosomes found within mitochondria are similar to ribosomal sequences found in bacteria. The fact that mature mitochondria are derived from pre-existing mitochondria by fission, which is similar to binary fission, the method by which bacteria multiply suggests that mitochondria and bacteria share a common ancestor. Indeed, mitochondrial ribosomes and transfer ribonucleic acid (RNA) molecules are similar to those of bacteria.

Mitochondrial Biogenesis

Skeletal muscle is a very pliable tissue and repeated exercise sessions elicit specific morphological and metabolic adaptations referred to as mitochondrial biogenesis. These adaptations result in an increase in mitochondrial content per gram of tissue and/or a change in mitochondrial composition with an alteration in the protein content. Exercise-induced mitochondrial biogenesis is a complex process that involves specific signalling processes which are initiated by rapid changes in Ca^{2+} flux, ATP turnover and the increase in oxygen (O_2) consumption [18]. Various signalling cascades initiate replication of DNA, and its transcription. This involves the transcription of a primary RNA transcript which is subsequently spliced to messenger RNA (mRNA) (Figure 2.2). Skeletal muscle contraction generates transient increases in the quantity of mRNA [43–45]. This increase in mRNA induces an increase in protein synthesis leading to a change in the steady state level of specific proteins and a new functional threshold. The increase in transcriptional activity and the subsequent protein synthesis is thought to be due to the cumulative effects of multiple

exercise bouts. However, recent studies have found that a single bout of exercise can induce changes in the transcription of genes, leading to an increase in oxidative muscle phenotype with improved resistance to fatigue [46].

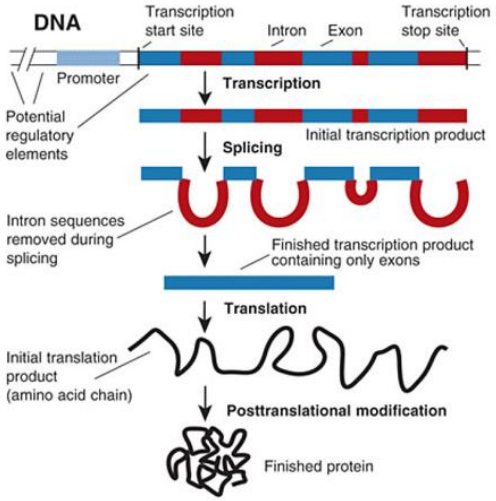

Figure 2.2 Gene expression in eukaryotes

Control of Mitochondrial Biogenesis

The complex process of mitochondrial biogenesis is under the control of several transcriptional co-activators and transcription factors, which are also regulated by exercise and include PGC-1α and the peroxisome proliferator-activated receptor (PPAR) family. PGC-1α acts as a 'master regulator' of mitochondrial biogenesis in skeletal muscle, via the co-activation of a number of mitochondrial transcription factors (Figure 2.3) [17,47].

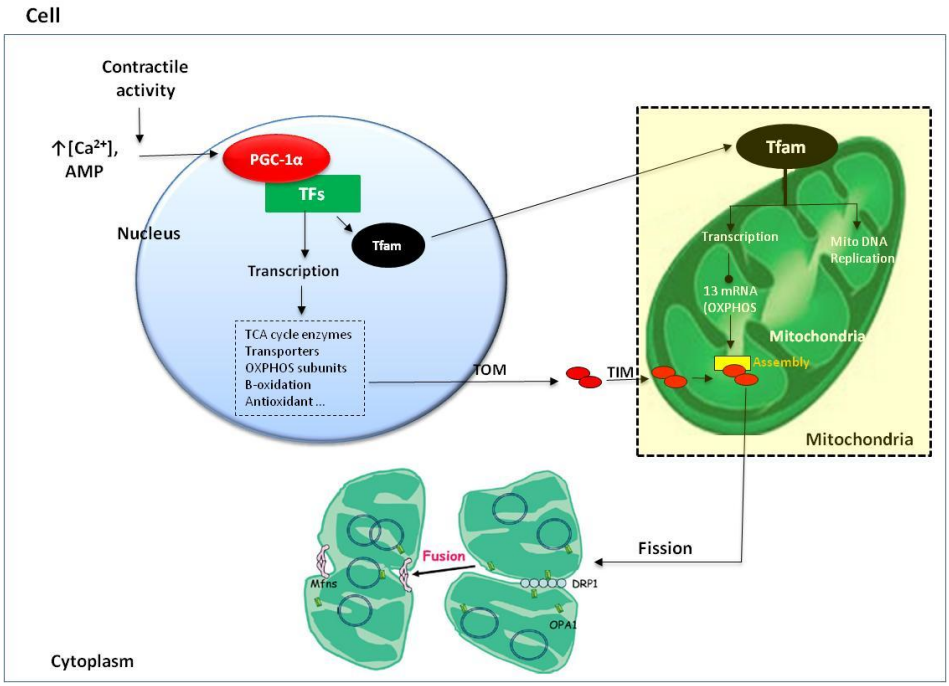

Figure 2.3 Mitochondrial biogenesis induced by contractile activity

Peroxisome proliferator-activated receptor gamma co-activator (PGC-1α) activates transcription factors (NFs) leading to transcription of nuclear- encoded proteins and of the mitochondrial transcription factor A (Tfam). Tfam activates transcription and replication of the mitochondrial genome. Nuclear-encoded proteins are imported into mitochondria through the outer- (TOM) or inner (TIM) membrane transport machinery. Nuclear- and mitochondria-encoded subunits of the respiratory chain are then assembled. Mitochondrial fission through the related protein 1 (DRP1) for the outer membrane and OPA1 for the inner membrane of mitochondria allow mitochondrial division while mitofusins (Mfn) control mitochondrial fusion. Processes of fusion and fission lead to proper organization of the mitochondrial network. OXPHOS: oxidative phosphorylation.

Molecular Mechanisms Regulating Mitochondrial Biogenesis

Signal transduction refers to the activation of a cell surface receptor via intra or extracellular stresses and signalling molecules. Many of the signal transduction pathways are transduced to cytosolic or nuclear targets by the action of kinase or phosphatase enzymes on key regulatory pathways [48]. Skeletal muscle contraction activates signalling pathways by altering a range of primary messengers including calcium flux, ATP turnover, cellular stress, redox potential, O_2 tension and reactive oxygen species production (Figure 2.4). Many of these signalling pathways have been found to be involved in the regulation of

mitochondrial biogenesis [4,49–51]. It is well established that acute and chronic exercise is a significant stimulus for the regulation of multiple metabolic and transcriptional processes in skeletal muscle [8]. These adaptive responses are mediated by specific genes that are up-regulated by complex regulatory pathways. Different homeostatic perturbations, such as those related to metabolic flux, skeletal muscle loading, hormonal and neuronal alterations are sensed by specific sensory molecules that activate specific signalling cascades. These cascades ultimately control muscle fate via the regulation of gene expression.

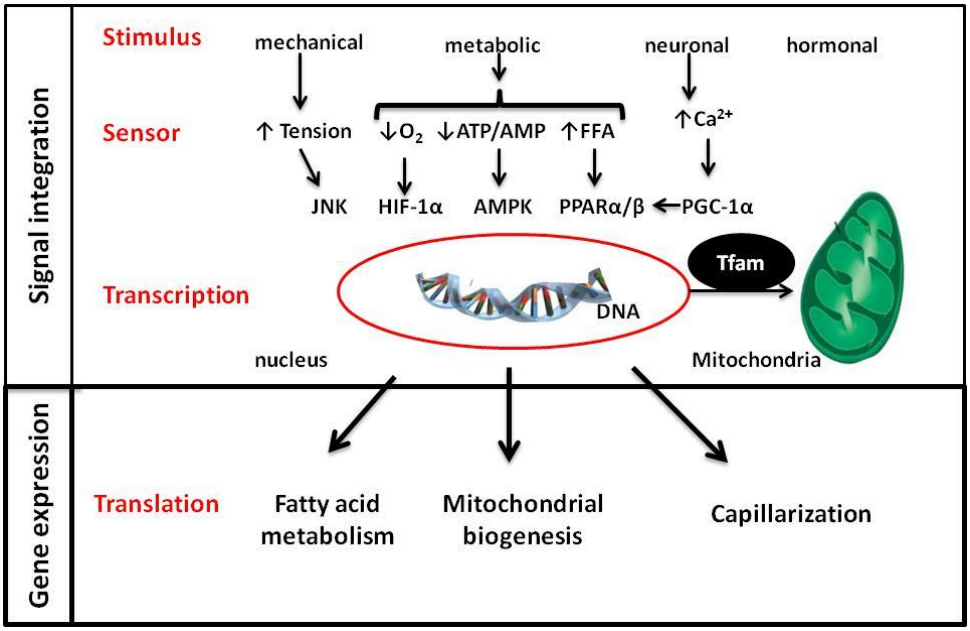

Figure 2.4 The complex stimulus of exercise in recruited muscle

Different homeostatic perturbations, such as those related to metabolic flux, loading, hormonal and neuronal alterations, are converted by specific sensory molecules into the activation of signalling cascades. These ultimately control muscle fate via the regulation of gene expression. Consequently, gene expression represents an important layer of control for the processing of physiological information towards

Adenosine Monophosphate Activated Protein Kinase-Mediated Signalling (AMPK)

Adenosine monophosphate activated protein kinase (AMPK) is a member of the sucrose non-fermentor kinase family that functions as an intracellular fuel sensor (Figure

2.5) [52]. It is a heterotrimeric serine/threonine protein comprising an alpha (α) (63 kDa), beta (β) (38 kDa) and gamma (γ) (38 kDa) subunits. The α subunit is the catalytic subunit and requires phosphorylation for activation. The β and γ subunits are regulatory, although they have been found to interact with the active subunit in liver. Two isoforms of the α and β subunits have been identified and 3 isoforms of the γ subunits making a total of 12 possible heterotrimeric AMPK combinations [53].

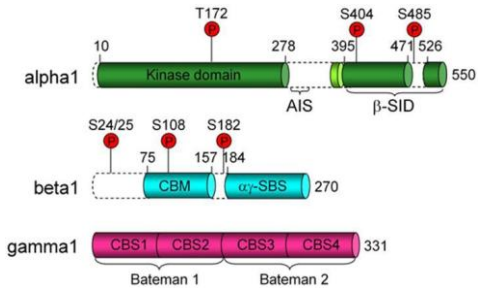

Figure 2.5 The structure of adenosine monophosphate activated protein kinase (AMPK)

AMPK monitors cellular energy status in response to cellular energy deprivation by increasing ATP production while simultaneously decreasing the cellular energy-consuming anabolic processes [54]. It is activated in response to an increase in AMP. A 5-fold increase in the adenosine diphosphate (ADP) to ATP ratio during muscle contraction results in a 25-fold increase in the AMP (adenosine monophosphate) to ATP ratio indicating that the cellular concentrations of AMP change much more dramatically than ATP or ADP [53].

Activated AMPK inhibits cellular growth and fatty acid and sterol biosynthetic pathways and stimulates catabolic pathways [55,56]. The effects of AMPK are mediated primarily by direct substrate phosphorylation that controls enzymatic activity and the transcriptional complexes regulating these processes [57].

Acute exercise [58–61] and electrical stimulation [62] increase phosphorylation of AMPK and its enzymatic activity. AMP moderately activates AMPK by binding to the cystathionine β-synthase (CBS) domain of the γ subunit [63]. Binding to the CBS domain results in a conformational change in the enzymes structure making AMPK a better substrate for up-stream kinase(s) such as LKB1 (liver kinase B1) or calcium calmodulin dependant kinase kinase (CaMKK) [57]. Binding of LKBI and CaMKK phosphorylates the α-subunit on Thr^{172} making it a poorer substrate for inactivating phosphatases [64]. This enhances AMPK activity more than 100-fold [65] (Figure 2.6). The effects of AMP binding on AMPK are antagonized by high ATP concentrations, therefore [ATP]/[AMP] is thought to be the best predictor of AMPK activity [54].

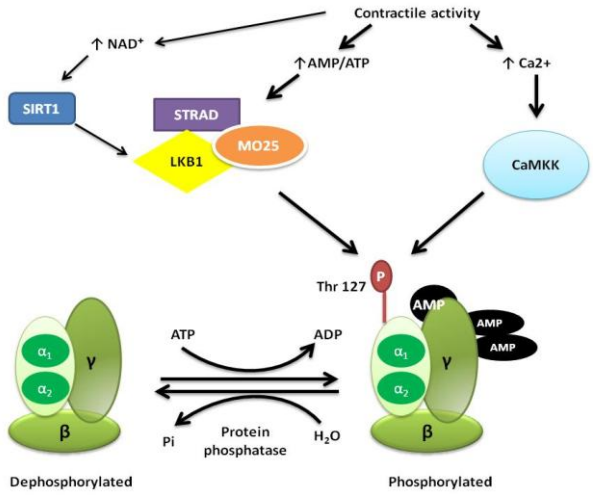

Figure 2.6 Regulation of AMPK activation in response to exercise

AMPK is activated by phosphorylation of Thr^{172} catalysed by LKB1:STRAD:MO25 complex in response to increase in the AMP/ATP ratio and by CaMKK in response to elevated Ca^{2+} levels. SIRT 1 activation leads to deacetylation of Lys48 and possibly other key lysine residues in LKB1. This activated LKB1s kinase activity, which in turn phosphorylates AMPK. Thr^{172} is dephosphorylated by PP2C protein phosphatase switching active AMPK to the inactive form.

AMPK Upstream Protein Kinases

LKB1, CaMKK and more recently SIRT1 (silent mating type information regulation 2 homolog) a NAD^+-dependent histone/protein deacetylase have been identified as important up-steam kinases for AMPK in muscle and most other cells. The compound aminoimidazole-4-carboxymide-1-β-D-ribfuranoside (AICAR) is an analog of AMP that is capable of stimulating AMPK. The effects of AICAR administration in combination with electrical stimulation has been investigated in mice lacking the LKB1 gene [66,67]. These studies found that α1 AMPK activity was only partially activated and α2 AMPK phosphorylation was absent following both *in situ* [68,69] and *ex vivo* [67] stimulation. More recent studies have indicated that LKB1 activity is not increased during exercise but is continually activated in the muscle cell in the resting state, allowing AMPK to be continually phosphorylated and dephosphorylated in a futile cycle [68,70].

SIRT1, has been shown to activate AMPK in rat liver cells *in vivo* under conditions of calorie restriction [71]. The deacetylation of LKB1 by SIRT1 is associated with its movement into the cytoplasm, where it binds to and is activated by STRAD. Studies have found that this mechanism activates AMPK in human embryonic kidney cells [72]. Although studies have found an increase in SIRT1 gene expression after an acute bout of prolonged exercise in rat skeletal muscle [73], this has yet to be linked to the activation of LKB1. In human skeletal muscle, it has been found that exercise induced up-regulation of SIRT1 plays a role in mitochondrial biogenesis [74,75]. However, the mechanism by which SIRT1 induces these adaptations and whether it is associated with the activation of AMPK has yet to be elucidated.

Exercise Activation and regulation of AMPK

Activation of total AMPK in muscle during exercise contraction was first demonstrated in rats [76]. A Six-fold increase in AMPK activity was found in response to 5 min of exercise at treadmill velocity > 20 $m \cdot min^{-1}$. The increase in kinase activity was relatively transient and returned to baseline within 90 min after the cessation of exercise.

It was later shown in human skeletal muscle that the AMPK activation was mediated by activation of both the α_1 and α_2 AMPK containing trimers. The degree to which the two α sub-units were activated was found to be related to both exercise intensity and duration [66,77,78].

Muscle glycogen is thought to partly regulate the activity of AMPK during exercise. At rest, AMPK activity is increased in the presence of low muscle glycogen levels compared to normal. AMPK activity has been found to significantly increase after 20 min of exercise at 80% $\dot{V}O_2$peak in humans when exercise is undertaken in the presence of low muscle glycogen levels [79]. In contrast, Wadley *et al*., (2006) [80] compared the effect of absolute versus relative exercise intensity on AMPK signaling and substrate metabolism under normoxic and hypoxic conditions in untrained men. A higher AMPK activation was found during 30 min of cycling at the same absolute workload under normoxic (51% $\dot{V}O_2$max) than hypoxic (11.5% O_2) (72% $\dot{V}O_2$max) conditions despite the fact that pre-exercise glycogen levels were similar at the start of exercise and the rates of muscle glycogen utilisation were similar in both conditions [80]. There was no significant difference between AMPK α_1 or α_2 at the same absolute exercise intensities (51% Normoxia vs. 72% Hypoxia) despite significantly different levels of muscle glycogen utilisation. The effect of muscle glycogen concentration

on AMPK activity is dependent on the glycogen binding domain on the β-subunit and varies according to the degree of glycogen branching [81].

The Activation of AMPK by High Intensity Interval Training

Recent findings indicate that both the α_1 and the α_2 AMPK subunits are stimulated by intense interval exercise [23,82]. Chen *et al*., (2000) [82] found an increase in both α_1 and α_2 AMPK phosphorylation of approximately two to three fold respectively, in untrained men (n=7) and women (n=4) following a 30 s supramaiximal cycle exercise bout. In a more recent study Gibala *et al*., (2009)[23] also found a significant increase in both α_1 and α_2 AMPK phosphorylation in six untrained men following a single session of HIIT involving 4 x 30 s bouts of cycling at maximal effort, with 4 min of active recovery between exercise bout. Clark *et al*., (2004) [83] found an increase in AMPK phosphorylation at Thr^{172} on both the AMPK α_1 and α_2 subunits in endurance trained cyclists in response to seven HIIT sessions involving cycling over a 3 wk period. Each session consisted of a 20 min warm-up at 65% $\dot{V}O_2$peak followed by 8 x 5 min work bouts at 85% $\dot{V}O_2$peak with 60 s of active recovery.

To date, relatively few studies examining the effect of high intensity interval exercise (HIIE) on AMPK activity have used a running protocol. Bartlette *et al*., (2012) [84] found a 1.9 fold increase in AMPK phosphorylation compared to resting levels in 10 recreationally active men following a single session of HIIE involving 6 x 3 min bouts of treadmill running at 90% $\dot{V}O_2$max interspersed with 3 min of active recovery at 50% $\dot{V}O_2$max. Participants ran for 7 min at 70% VO_2max before and after the interval session. Serpiello *et al*., (2011) [85] only found moderate changes in AMPK phosphorylation following a single session of HIIE and 12 sessions of HIIE undertaken 3 times per week for 4 weeks in recreationally active men (n=7)

and women (n=3). The training program consisted of 3 sets of 5 x 4 s maximal sprints interspersed with 20 s of passive recovery and 4.5 min of a recovery period between sets. It is possible that the duration of the sprint exercise may not have been of sufficient duration to induce a significant increase in AMPK activation.

Recent studies have found that only 3 heterotrimeric AMPK complexes ($\alpha_1/\beta_2/\gamma_1$, $\alpha_2/\beta_2/\gamma_1$, $\alpha_2/\beta_2/\gamma_3$) are expressed in human skeletal muscle and each is activated differently during exercise [86]. The $\alpha_2/\beta_2/\gamma_3$ AMPK subunit was found to be activated during short intense exercise $\leq$ 20 min in duration [86], while the $\alpha_2/\beta_2/\gamma_1$ is activated following $\geq$ 60 min of moderate intensity exercise [87]. Interestingly, increases in the activity of the $\alpha_2/\beta_2/\gamma_3$ complex is correlated with an increase in acetyl-CoA carboxylase (ACCβ) phosphorylation, suggesting that this complex may play a role in the regulation of fatty acid oxidation [86,87]. An increase in ACCβ phosphorylation in response to both endurance and high intensity exercise may therefore indicate the activation of the AMPK $\alpha_2/\beta_2/\gamma_3$ isomer [23,88]. In contrast, the $\alpha_2/\beta_2/\gamma_1$ complex is linked to the activation of AS160 (Rab GAPase-activating protein), a molecule that has been associated with the regulation of glucose uptake [89].

Metabolic Effects of AMPK in Skeletal Muscle During Exercise

Although AMPK is a pleiotropic signalling molecule that is involved in the regulation of glycogen metabolism, fatty acid oxidation and protein synthesis, it plays a major role in skeletal muscle metabolism by activating signaling cascade that stimulates glucose uptake in contracting muscles [90,91] (Figure 2.7). Muscle glucose uptake is increased in perfused rat skeletal muscle in response to chemical activation of AMPK by AICAR [90].

Glucose uptake requires the translocation of the glucose transporter 4 (GLUT4) transporters to the plasma membrane. This process is mediated by the intracellular signalling molecule, AS160 that is activated by AMPK. [92] In contrast, phosphorylation of glycogen synthase by AMPK activation decreased the rate of glycogen synthesis by reducing the activity of glycogen synthase (GS) [93].

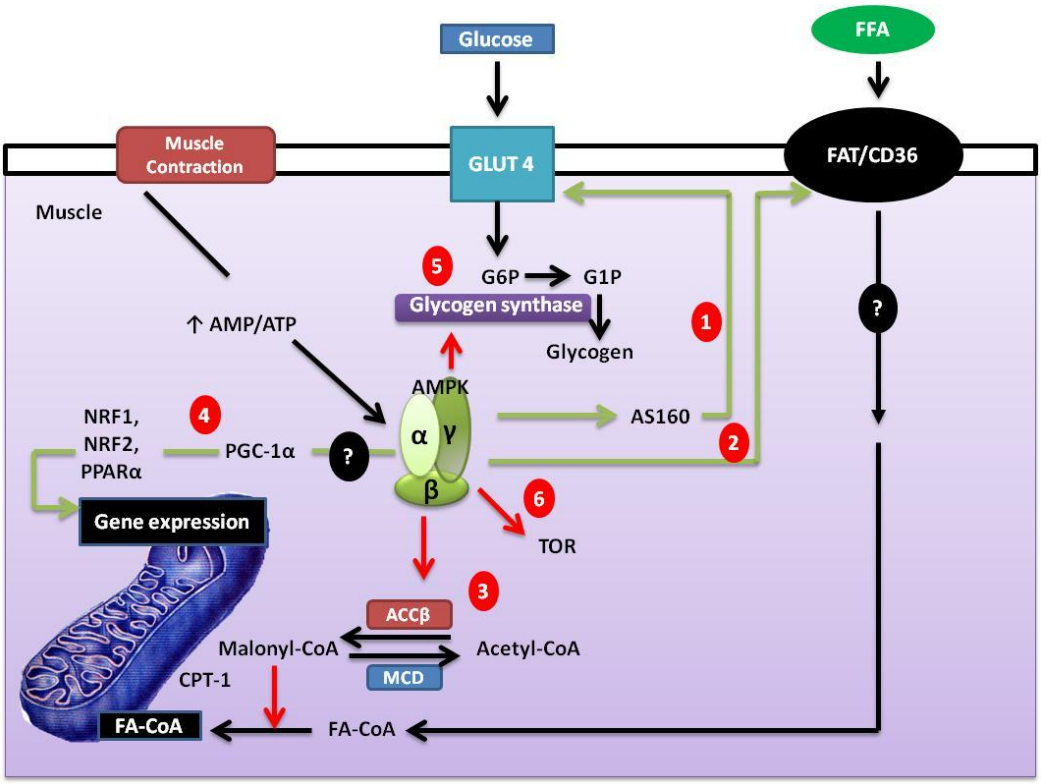

Figure 2.7 Metabolic changes known to be induced by AMPK in skeletal muscle

These including stimulation of glucose (1) and fatty acid uptake (2), fatty acid oxidation (3), mitochondrial biogenesis (4), inhibition of glycogen synthesis (5) and inhibition of TOR, hypertrophy (6). Question marks indicate that the direct target for AMPK responsible for the observed downstream effect is not known. The effect on fatty acid uptake has to date only been observed in cardiac muscle.

The transport of long chain fatty acids (LCFA) into the resting or exercising muscle is essential for the oxidation of free fatty acids (FFA) and subsequent ATP production. The mechanism by which AMPK regulates FFA oxidation is primarily at the level of LCFA entry

into the mitochondria via phosphorylation of the biotin dependant enzyme, ACCβ at Ser^{218}. AMPK deactivates ACCβ and potentially activates malonly-CoA decarboxylase (MCD) activity which decreases the formation of malonly CoA, [94], a potent inhibitor of carnitine palmitoyl transferase-1 (CPT-1) (Figure 2.7). The interaction of AMPK with ACCβ does not necessarily alter the carboxylase activity but desensitizes ACCβ to allosteric activation by citrate [77] and sensitizes ACCβ to inhibition by palmitoyl-CoA [95]. Therefore, a reduction in ACCβ activity will decrease the formation of malonly-CoA, relieving the inhibition of FA entry into the mitochondria and enhance FFA oxidation [96].

AMPK Signalling and Mitochondrial Biogenesis

In addition to the regulation of acute metabolic function, the protein targets of AMPK are also involved in regulating exercise induced alterations in skeletal muscle, including mitochondrial biogenesis. Administration of AICAR to rats for 4 weeks results in a 2.5-fold increase in AMPK activity, with subsequent increases in skeletal muscle mitochondrial enzymes including, hexokinase (HK), CS, and succinate dehydrogenase (SDH), and an increase in cytochrome c and GLUT 4 activity [97]. Incubation of the epitrochlearis muscle with AICAR for 18 h increases GLUT4 protein by 50%, indicating that AMPK activation by AICAR is independent of systemic factors [98]. Fryer *et al*., (2002) [99] found that AMPK activation improves glucose uptake and induces the translation of GLUT4 mRNA, along with an increase in the expression of HK by over expressing a constituently-active AMPK gene in skeletal muscle.

The mechanism by which AMPK induces protein expression and mitochondrial biogenesis, depends on the activation of several transcription factors, and co-activators

(Figure 2.8). It appears that AMPK mediated mitochondrial biogenesis involves the posttranslational modification of the transcriptional co-activator, PGC-1α and subsequent expression of the downstream transcription factors, nuclear transcription factor 1 (NTF-1) and nuclear transcription factor 2 (NTF-2), mitochondrial Tfam (transcription factor A) and peroxisome proliferator activated receptor alpha (PPARα). Bergeron *et al*., (2001) [100] found AMPK activation in rats in response to a 9 week dietary intervention which included the guanidine derivate, *β*-guanadinopropionic acid (*β*-GPA) promoted skeletal muscle mitochondrial biogenesis through PGC-1α and NRFs. AMPK increases GLUT4 via the PGC-1α pathway. Michael *et al*., (2001) [101] found increases in GLUT4 protein and the transcriptional activity of myocyte-specific enhancer factor (MEF) isoforms. These are GLUT4 transcription factors that are activated in response to adenovirus mediated over expression of PGC-1*α* in both animal and cultured muscle cell lines.

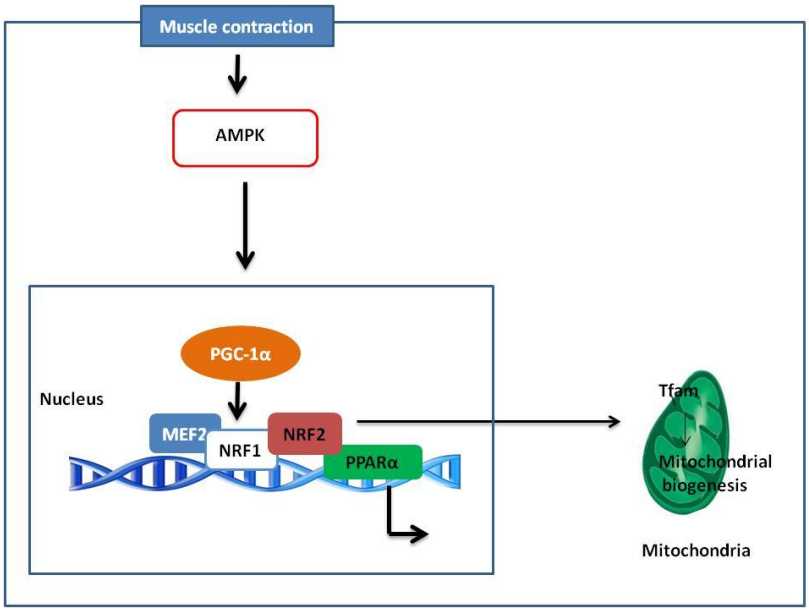

Figure 2.8 AMPK mediates mitochondrial biogenesis

AMPK mediates mitochondrial biogenesis through PGC-1α, NRF 1, NRF 2, Tfam, MEF and PPARα.

Exercise also activates AMPK and promotes mitochondrial biogenesis via the PCG-1α pathway. Terada *et al*., (2002) [102] found an increase in AMPK activation and PGC1α mRNA expression following 6 h of low intensity swimming. An increase in AMPK activation and PGC-1α has also been found after low frequency (10 Hz) electrically stimulating isolated rat muscle to mimic endurance training [62]. However, AMPK may not be essential in inducing mitochondrial biogenesis. In a recent study PGC-1α mRNA expression increased despite a 60% reduction in AMPK signalling at rest and during exercise in α_2 AMPK knockout mice [103]. The significant decrease in AMPK activation may be explained by the absence of a functional copy of the α_2 AMPK isoform. In a subsequent study, they found no reduction in the training-induced increases in HK, GLUT4, cytochrome c oxidase complex IV subunit 1, 3 β-HAD, or CS protein in response to exercise in α_2 AMPK knockout mice [104].

In summary, AMPK plays an important role as an energy sensor in skeletal muscle cells and helps to maintain a state of intracellular homeostasis. Activation of AMPK

enhances the muscle cells ability to produce energy by up-regulating a number of oxidative enzymes. There is evidence linking exercise induced increases in AMPK to mitochondrial biogenesis. However, many other signalling pathways exist such as, MAP kinase, protein kinase C (PKC), CaMK and calcineurin are activated in response to exercise and may overlap with the actions of AMPK.

Calcium Dependent Signaling Pathways and Mitochondrial Biogenesis

The increase in cytosolic Ca^{2+} triggered by contractile activity has also been shown to play a role in mitochondrial biogenesis [85,88,105]. Raising cytosolic Ca^{2+} in myotubes increases gene expression of GLUT4 [98], NRF-1, -2 and PGC-1α [105]. Calcium dependant pathways such as CaMKs, have been implicated as signalling molecules in the response to exercise. Two of these multifunctional kinases, CaMKII and CaMKIV are thought to play an important role in affecting muscle phenotype.

Recent studies have shown an increase in activated CaMK II protein signaling in response to both repeated sprint exercise (10 min running at 50% of $\dot{V}O_2$peak followed by 3.5 min at 100% $\dot{V}O_2$peak) and endurance exercise (2 h cycling at 80% $\dot{V}O_2$max) [85,88]. There was also an increased phosphorylation and subsequent removal of the histone deacetylase (HDAC 5) protein in response to exercise, which allows for its export from the nucleus, removing the inhibition on MEF2 and ultimately allowing PGC-1α transcription to progress [106] (Figure 2.9). Activation of CRE binding protein (CREB) via phosphorylation is also up-regulated by protein kinases such as CaMKII [107].

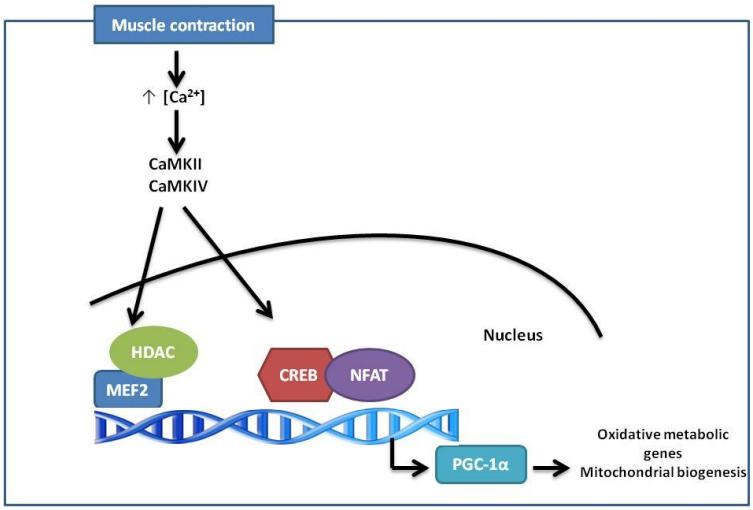

Figure 2.9 The role of calcineurin signalling in PGC-1α up-regulation

Signalling through calcineurin occurs through downstream substrates, the nuclear factor of activated T cells (NFAT) and myocyte enhancer factor (MEF2). Acute exercise leads to phosphorylation of the class II histone deacetylases (HDACs) by Ca^{2+}–calmodulin-dependent protein kinase IV (CaMKIV) and thus contributes to activation of the MEF2 transcriptional activity

In summary, there is strong evidence that contractile mediated activation of CaMKII may play a role in mitochondrial biogenesis in human skeletal muscle, and that its activity is influenced by exercise intensity.

Mitogen activated protein kinases (MAPK)

Mitogen activated protein kinases (MAPK) transduce extracellular signals that lead to a variety of cellular responses including proliferation, differentiation, apoptosis and adaptation to environmental stress [108]. Skeletal muscle MAPK can be divided into four subfamilies; extracellular signal related kinase (ERK) 1/2, p38MAPK, c-Jun NH2-terminal kinases (JNK), and the extracellular signal-related kinase 5 (ERK5). The MAPK's are activated through phosphorylation of specific upstream kinases, which are in turn activated by a diverse range of upstream MAPK kinase kinases (MAP3K) in response to external stimuli such as exercise (Figure 2.10) [20,109–111].

Growth factors, cytokines, hypoxia, changes in intracellular calcium, glycogen depletion and mechanical stress, have all been found to stimulate MAPK. MAPK signalling pathways may also play a role in converting mechanical/biochemical stimuli elicited by skeletal muscle contraction into subsequent molecular responses [112,113] and may also been implicated in the regulation of gene transcription in numerous cell types.

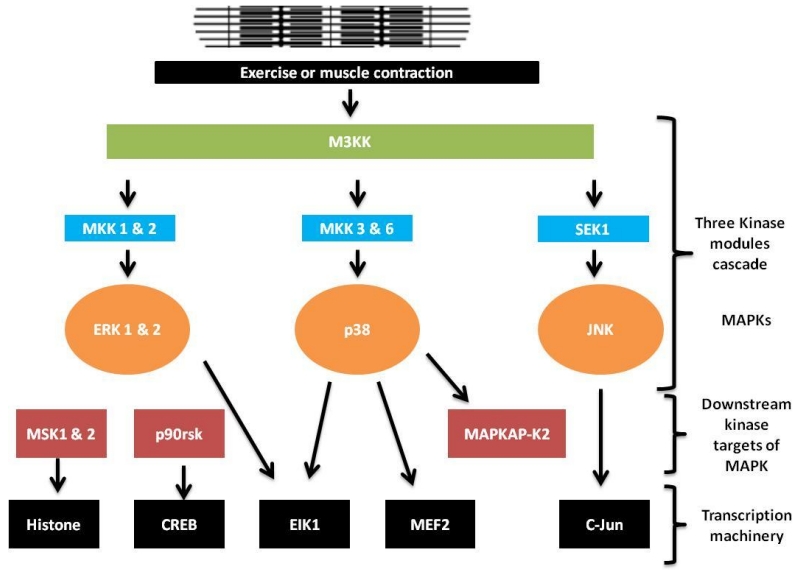

Figure 2.10 Putative exercise-mediated mitogen activated protein kinase (MAPK) signalling cascades in skeletal muscle

Structure and Activation of p38 MAPK

The p38MAPK, is a stress activated protein kinase. It can be activated by environmental stressors, including osmotic gradient change, heat shock, stretch and inflammatory cytokines. In humans, there are 4 isoforms of p38MAPK; p38α, β, γ and δ. The isoforms can be categorised by their Thr-Gly-Tyr (TGY) dual phosphorylation motif. The isoforms have > 60% overall sequence homology and > 90% identity within the kinase domains. Despite this high degree of homology, the isoforms have distinguishable differences in tissue expression, upstream activations and downstream effectors.

The isoform that predominates in skeletal muscle is p38γ. This isoform is phosphorylated and activated by many external stimuli through MAP3K-MAPK kinase kinase (MKK) pathway (Figure 2.10). The protein is rapidly activated by MKK-dependant dual phosphorylation on threonine and tyrosine residues at the sequence Thr-Gly-Tyr, located within the regulatory loop between subunits VII and VIII. This phosphorylation induces a conformational change in the protein allowing ATP and substrate binding. MKK3 and MKK6 can phosphorylate p38MAPK within minutes after exposure to an externally activated stimuli. The fate of the cell is dependent on the duration of phosphorylation, with sustained phosphorylation associated with apoptosis and transient phosphorylation associated with growth factor induced survival. Crosstalk between different signalling pathways also influences the kinetics of p38MAPK signalling and its effects on the cell. This crosstalk makes it difficult to interpret studies examining p38MAPK as the signalling pathways can be activated simultaneously, and as of yet there is a lack of specificity between the pathways. For example, p38MAPK shares common kinase substrates with ERK1/2, including MAPKAP K-2 and -3, MSK1 and 2 and CREB.

Phosphorylated p38MAPK can activate a wide range of substrates that include transcription factors, protein kinases and nuclear proteins. Exercise induced increases in p38MAPK activation have been found to phosphorylate substrates involved in CHO and fat metabolism and mediate the regulation of PGC-1α-dependent gene transcription in skeletal muscle [114] (Figure 2.11).

Exercise Induced p38 AMPK Activation

Cycle exercise [115] and marathon running [113,116] result in phosphorylation of p38MAPK. In an early study Widegren *et al*., (1998) [115] found an increase in p38MAPK phosphorylation in healthy men and women after 1 h of single leg cycle exercise at 70% $\dot{V}O_2$max. There was evidence of phosphorylation 30 min and 60 min after exercise. Interestingly, increased activation p38MAPK was found in both the exercised and non-exercised leg indicating that p38MAPK may be influenced by both local and systemic factors. A 4 fold increase in p38MAPK has also been found in trained men with a $\dot{V}O_2$max of 60 $ml \cdot kg^{-1} \cdot min^{-1}$ after running a marathon [113].

Mei Yu *et al*., (2003) [117] found that activation of p38MAPK was greater in untrained than trained men following 8 x 5 min bouts of cycling at approximately 85% $\dot{V}O_2$peak with 60 s recovery between work bouts. Since both groups exercised at the same relative exercise intensity the findings suggest that trained men may require a greater exercise stimulus to activate p38MAPK than untrained men. Eccentric but not concentric contraction activates p38MAPK [118]. Also an acute bout of high intensity cycling and running increases p38MAPK phosphorylation in both active and sedentary men [13,23,25,119 84] .

The Role of p38 MAPK in Mitochondrial Biogenesis (gene regulation)

Studies in myocyctes have found that p38MAPK directly phosphorylates and activates PGC-1α [112]. It also stimulates down-stream transcription factors of PGC-1α, such as activating transcription factor (ATF-2) and MEF2 [112,120]. Phosphorylating PGC-1α at three sites by p38MAPK stabilises the protein by disrupting its association with repressor protein

p160 myb binding protein ($p160^{mbp}$) and thereby activating its transcriptional activity [121] (Figure 2.11).

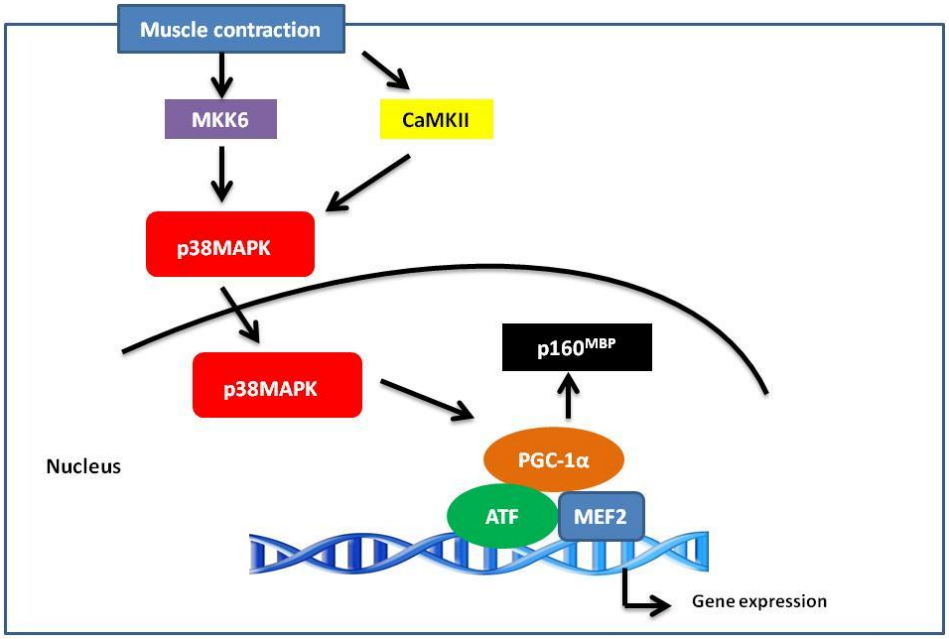

Figure 2.11 Exercise induced activation of p38MAPK and subsequent gene expression

p38 MAPK activation leads to the phosphorylation of transcriptional co-activator PGC-1α, thereby relieving it from the inhibitory action of $p160^{MBP}$ and activating target gene expression. It can also directly phosphorylate PGC- α activating transcription factors AKT-2 and MEF2.

A single bout of running or motor nerve stimulation induces a transient increase in PGC-1α mRNA in mouse plantaris muscle, mediated by activation of the p38MAPK pathway [112]. The p38-mediated increase in PGC-1α activity was enhanced by the increased expression of the downstream transcription factor ATF-2. This is a nuclear cyclic AMP response element (CRE) motif binding transcription factor, which can be bound and activated by ATF-2, which in turn binds to the response element binding site on the PGC-1α promoter site inducing transcription [112]. The p38MAPK/ATF-2 pathway is critical for contractile induced increase in PGC-1α mRNA [112,122]. Similar increases in p38MAPK phosphorylation has recently been found in human skeletal muscle after an acute bout of

endurance exercise at 40% or 80% $\dot{V}O_2$peak [88]. However, an increase in ATF-2 phosphorylation only occurred in response to endurance exercise at 80% $\dot{V}O_2$peak indicating that the activation of ATF-2 during HIT may be stimulated by other factors. The p38MAPK pathway may also be regulated during contractile activity by increasing the cytosolic Ca^{2+} concentration, leading to the expression of PGC-1α [123,124].

Incubation of human skeletal muscle with caffeine increases cytosolic Ca^{2+} concentration, but is insufficient to cause the same activation of CaMK or p38MAPK and subsequent mitochondrial biogenesis as contractile activity [125]. The increases in PCG-1α and mitochondrial biogenesis seem to be dependent on CaMKII and p38MAPK activities via ATF-2 binding. Inhibition of CaMKII or p38MAPK prevents the calcium induced increase in p38MAPK phosphorylation, ATF-2 activation and subsequent mitochondrial biogenesis [21]. It is possible that p38MAPK is downstream from CaMKII in a signalling pathway and that increasing [Ca^{2+}] in the cytosol induces an increase in PGC-1α mRNA and mitochondrial biogenesis in muscle through the activation of this pathway, CaMKII to p38MAPK inducing AFT-2 activation.

Gibala *et al*., (2009) [23] found a significant increase in phosphorylation of AMPK and p38MAPK immediately after an acute session of intense intermittent cycle exercise involving 4 x 30 s maximal cycling with 4 min of active recovery between bouts. PGC-1α mRNA was significantly increased approximately twofold above rest after 3 h of recovery. However, a significant change in the phosphorylation state of CaMKII was not found suggesting that p38MAPK activation after repeated sprint exercise is mediated by pathways other than CaMKII. MAPK kinase 3 and 6 are classical activators of p38 MAPK in response to numerous

stress stimuli. AMPK may also be an activator of MKK3 and p38MAPK [126]. Signalling through AMPK and p38MAPK to PGC-1α may explain in part the metabolic remodelling induced by low-volume intense interval exercise, including mitochondrial biogenesis and an increased capacity for glucose and fatty acid oxidation.

Genetic and Molecular Responses to Exercise

Contractile activity induces adaptations in muscle phenotype at both the metabolic and physiological level. The changes in muscle phenotype are a result of alterations in the protein content of the cell that may be controlled at a transcriptional, translational and/or a post-translational level. Exercise induced adaptations result in qualitative alterations in protein content that are controlled by an increase in mRNA levels. These exercise induced alterations in muscle are related to either an increase in the transcriptional rate of the specific gene or enhanced RNA stabilisation of specific transcripts.

Gene Transcription

Gene transcription is the process of creating a complementary RNA copy of a DNA sequence and is the first step in gene expression. Methylated histones create a highly compact structure containing DNA that is inaccessible to initiators of transcription. Histones and DNA are exposed to transcriptional regulators following post-translational modification of the histone octomer to form a nucleosome core. Histone demethylation and phosphorylation promotes further chromatin remodeling. For example, histone acetylation by histone acetyl transferase (HAT) provides the molecular basis for initiation of gene transcription [127].

Transcription Factors and Co-Regulators

In skeletal muscle and other metabolic tissues, transcription factors and co-regulator proteins have the ability to target and alter the expression of key metabolic enzymes and mitochondrial proteins, and coordinate the process of mitochondrial biogenesis. The most commonly researched of these proteins, which can be described as metabolic regulators in skeletal muscle include, PGC-1α, PPARs, NRF-1, NRF-2, estrogen related recptor α (ERRα), forkhead box protein O1 (FOXO1), MEF2 and nuclear receptor interacting protein 1 (RIP140). These transcription factors and co-regulators co-ordinate the expression of genes involved in CHO metabolism (HKII, pyruvate dehydrogenase kinase 4 (PDK4), GLUT4), lipid metabolism (CPT-1, medium chain acyl CoA dehydrogenase (MCAD), lipoprotein lipase (LPL)), oxidative phosphorylation (cytochrome c, COXIV) and mitochondrial biogenesis (Tfam).

Mitochondrial Transcription Factor A (Tfam)

Tfam coordinates both mitochondrial and nuclear gene transcription and therefore induces mitochondrial biogenesis in skeletal muscle [49]. Following Tfam mRNA translation, the newly synthesised protein translocates to the mitochondria and activates mitochondrial DNA replication and transcription [128] (Figure 2.3). Studies have found that Tfam heterozygous knockout mice have reduced mitochondrial transcription and increased respiratory chain dysfunction [129]. The transcriptional regulation of Tfam is not yet well understood but it seems that NRF-1 is essential in activating transcriptional activity [130]. Tfam has been found to increase in response to aging in human muscle. It is believed that

the age related increase in Tfam is to maintain mitochondrial DNA (mtDNA) stability [131]. Certain forms of contractile activity have been found to induce Tfam expression in humans by binding to its promoter region [132]. An acute bout of HIIE involving 7 x 30 s bouts of cycling at maximal intensity resulted in a 1.5 fold increase in Tfam mRNA in endurance trained male cyclists [133]. In contrast, Tfam mRNA was not altered following high volume interval exercise involving 3 x 20 min at 87% $\dot{V}O_2$peak [133]. It is possible that high exercise intensity may be required to increase the expression of the Tfam gene in endurance trained athletes.

Four weeks of one legged knee extensor training resulted in a 3.4 fold increase in Tfam mRNA following an acute 3 h bout of two legged knee extensor exercise [134]. In contrast, Norrbom *et al*., (2004) [135] found no increase in Tfam at 6 h following 45 min of knee extensor exercise.

A single exercise session involving 10 x 4 min bouts of cycling at 90% $\dot{V}O_2$max with 2 min passive recovery between each high intensity exercise bout, increased Tfam mRNA by 54% [15]. The exercise session was repeated on 6 occasions during the following 2 weeks and Tfam mRNA remained elevated immediately following each session. The Tfam protein content was unchanged whereas mtDNA was increased during the 2 week training program. The increase in mtDNA during the training, suggests that activation of existing Tfam may contribute to mitochondrial biogenesis after training. Electrical stimulation of the tibialis anterior and the extensor digitorum longus muscles in rats (10 Hz, 3 h/day) for 2 weeks increased Tfam mRNA levels by 55% after 4 days, along with an increased the import of Tfam into the mitochondria. [136]. The increase in mitochondrial Tfam was accompanied with

an increase in COX subunit III mRNA and an increase in COX enzyme activity after 7 days of stimulation. A recent study found a 37% increase in Tfam protein content after 2 weeks of HIIT involving 8-12 x 60 s at 100% peak power output with 75 s passive recovery between bouts of exercise [74]. There was also a 29% increase in COX maximal activity and a 35% and 38% increase in protein content of COX subunits, II and IV, respectively. These findings support the role of Tfam in co-ordinating the expression of mitochondrial proteins and subsequent mitochondrial biogenesis.

The Discovery of Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ) co-activator (PGC) 1α

PGC-1α was discovered during research on the science of adaptive thermogenesis, a physiological process through which energy is dissipated as heat in response to environmental stressors such as cold and over-feeding. It has long been known that peroxisome proliferator activated receptor gamma (PPAR-γ) is essential for tissue differentiation such as the conversion of white adipose tissue (WAT) to brown adipose tissue (BAT). The primary physiological function WAT and BAT is energy storage and energy dissipation in the form of heat, respectively. Early studies indicated that PPAR-γ activation was required, but not sufficient to activate brown tissue adipose differentiation. This lead to the discovery of PGC-1α as a co-activator of PPAR-γ, as PGC-1α binds to PPAR-γ, co-activating the transcription of genes involved in BAT differentiation. In this process PGC-1α was instrumental in stimulating mitochondrial biogenesis, increasing fatty acid oxidation and uncoupling oxidative phosphorylation in skeletal muscle and BAT via adaptive thermogenesis.

Expression of PGC-1α in skeletal muscle

The expression of PGC-1α mRNA is prominent in tissues with high energy demands, such as the heart and skeletal muscle [137,138]. In rat skeletal muscle PGC-1α content is related to mitochondrial content [139]. In contrast, Norrbom *et al*., (2004) found no difference in human skeletal muscle PGC-1α mRNA levels between type 1 and type 2 muscle fibres [135]. Others have found PGC-1α protein expression markedly increased in type IIb muscle fibers compared to type IIa [140].

Structure of PGC-1α

The PGC-1α family contains homologues, PGC-1β and PGC-1 related co-activator (PGC), along with PGC-1α. PGC-1α contains approximately 795 amino acids with a molecular mass of 92 kDa. The N-terminal domain contains regions for interacting with transcription factors [141] and the C-terminal domain has an RNA-binding motif (Figure 2.12).

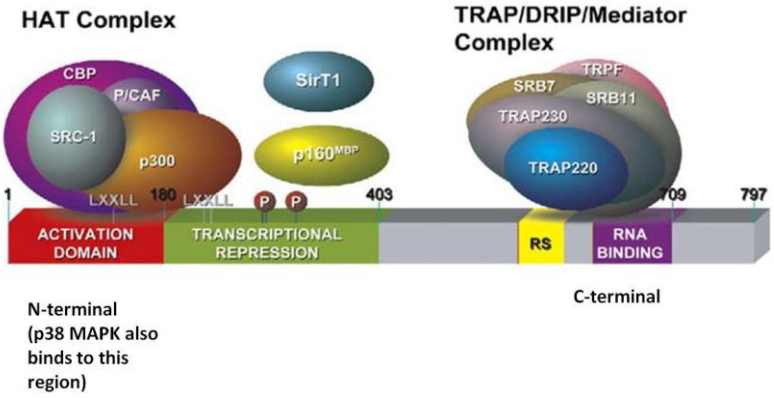

Figure 2.12 The structure of PGC-1α

Protein complexes associated with PGC-1α. PGC-1α binds to the AT and TRAP/DRIP/Mediator complexes at the amino and carboxyl termini, respectively. SIRT1 and p160 bind to the repression domain, which also contains three p38 MAP kinase phosphorylation sites. LXXLL and LLXXL denote nuclear receptor by PGC-1α and β of mitochondrial genes encoded by the two binding sites.

Interaction of PGC-1α with Transcription Factors

PGC-1α is not directly involved in the modification of histones, but instead mediates interactions between transcription factors and RNA polymerases [142]. There is a conformational change in PGC-1α, when it docks with and co-activates a transcription factor. This increases its binding with HAT proteins including steroid receptor coactivator (SRC-1) and CBP/p300, allowing for histone acetylation, subsequent chromatin remodeling and access to transcription factors that increase gene transcription [142]. The additional docking of a thyroid receptor associated protein (TRAP) complex at the C terminus is also required for complete transcriptional activation by recruiting RNA polymerase II [143]. Both of these mechanisms are required for PGC-1α to exert transcriptional control on targeted genes.

PGC-1α Gene Expression and Contractile Activity

MEF2 is a transcription factor with response elements on a variety of genes involved in muscle remodelling [144]. Contractile activity induces the expression of MEF2 and CREB (cAMP-response element binding protein), mediated by AMPK and CaMK (Figure 2.13).

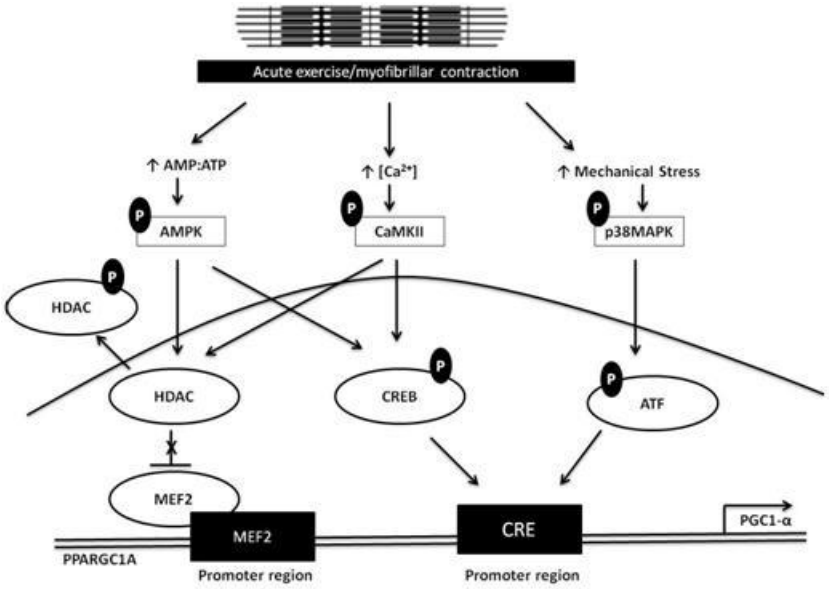

Figure 2.13 The suggested coupling between exercise factors potentially involved in inducing PGC-1α gene expression. Adopted from Egan 2008

Acute myofibrillar contraction results in the phosphorylation (p) and activation of AMPK, CaMKII and p38 MAPK through molecular sensing of increased ATP turnover, increased calcium release from the sarcoplasmic reticulum, and mechanical stress, respectively. AMPK and CaMKII phosphorylate class IIa HDACs, leading to their nuclear exclusion and relieving the inhibitory effect of HDACs on MEF2 transcriptional activity at the MEF2 binding sequence on the PGC-1*α* promoter. AMPK and CaMKII also phosphorylate and activate CREB, resulting in an activating effect on the CRE sequence of the PGC-1*α* promoter. p38 MAPK phosphorylates and activates ATF-2, that in turn acts on the same CRE site resulting in transcriptional activation. These combined effects on the MEF2 and CRE sequences result in increased PGC-1*α* promoter activity and increased PGC-1*α* gene transcription. HDAC, Histone deacetylases

Enhanced expression of MEF2 leads to improved binding to the promoter region of the PGC-1α gene, increasing PGC-1α expression. PGC-1α can then co-activate MEF2 providing a positive feed-forward signal that rapidly induces PGC-1α expression in an autoregulatory loop [107] (Figure 2.13). MEF2 binds to PGC-1α on at least two binding sites and alters its expression through MEF2 interaction with HDAC5 [145]. Recent studies [146,147] in mice, have found that MEF2 and CRE sequence elements on the promoter region play an essential role in PGC-1α induced activity, as inhibition of both sequences in mutant mice completely abolished PGC-1α activity. PGC-1α induces the expression of ERRα, a nuclear receptor which activates the expression of NRF-1, NRF-2 and ERRα itself [148] (Figure 2.14).

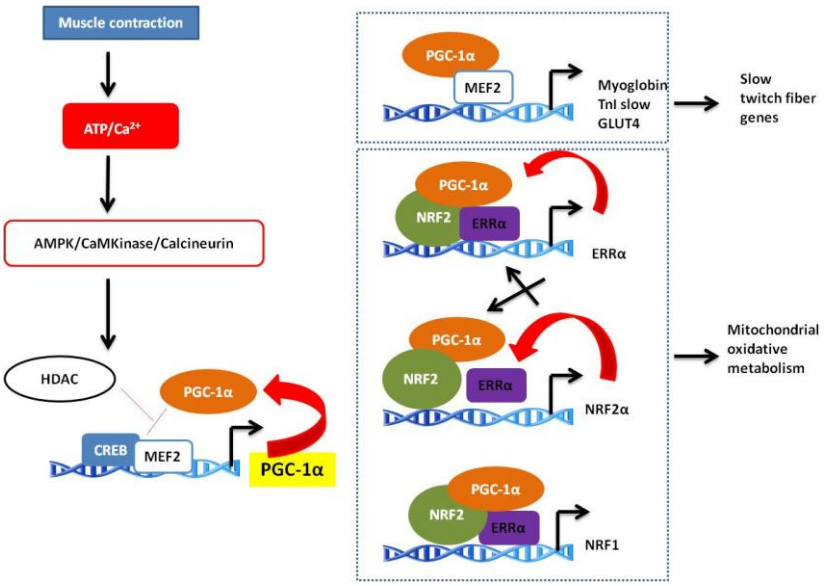

Figure 2.14 Regulation of PGC-1α expression in skeletal muscle and mechanisms by which PGC-1α stimulates mitochondrial gene expression

Co-activation of MEF2 by PGC-1α provides a positive feed-forward signal to rapidly induce PGC-1α expression following muscle contraction. PGC-1α induces the expression of ERRα, which activates the expression of NRF-1, NRF-2, and ERRα itself. These molecular events lead to the stimulation of nuclear-encoded mitochondrial genes. PGC-1α also simultaneously regulates the expression of slow-twitch muscle fiber genes through co-activation of MEF2.

NRF-1 and -2 have been identified as transcription factors which induce the expression a number of mitochondrial genes encoded by the cell nucleus, including β-ATP synthase, cytochrome c, COX subunit IV and mitochondrial Tfam. Co-activation of NRF-1 and -2 facilitates the transcription of the nuclear encoded Tfam. Once the Tfam mRNA is translated, the newly synthesised protein translocates to the mitochondria and activates mitochondrial DNA replication and transcription [128]. This pathway coordinates both mitochondrial and nuclear gene transcription and therefore induces mitochondrial biogenesis in skeletal muscle.

Another mechanism which regulates PGC-1α expression in muscle after exercise involves the activation of p38MAPK (Figure 2.11). P38MAPK can activate both MEF2 and

AFT-2. In rats, a short bout of exhaustive swimming increases the phosphorylation of p38MAPK and ATF-2 in parallel with PGC-1α translocation to the nucleus [122]. These events coincided with an increase in CS and cytochrome c mRNA, which precede an increase in PGC-1α protein. Although limited to rats, this study demonstrated the role p38MAPK in PGC-1α activation, and its translocation to the nucleus. Egan *et al*., (2010) [88] recently found that 2 h of cycling at 80% $\dot{V}O_2$ max increased both p38MAPK and AKT-2 phosphorylation, and PGC-1α mRNA expression.

The Regulation of PGC-1α activity

The activity of PGC1-α is tightly controlled by transcriptional and posttranslational mechanisms. The signalling protein p38MAPK phosphorylates and activates PGC-1α in response to cytokine stimulation in the muscle [110]. Phosphorylation of PGC-1α by p38MAPK enhances PGC-1α activity in two ways. Firstly, it increases the stability and half-life of the PGC-1α protein, along with disrupting the interaction between PGC-1α and $p160^{MBP}$, a repressor protein, which inhibits its activity in myoblasts (Figure 2.15).

AMPK regulated PGC-1α expression through direct and indirect mechanisms. AMPK directly phosphorylates PGC-1α which mediates an increase in PGC-1α protein [47]. AMPK is linked indirectly to the activation of PGC-1α via the direct phosphorylation of HDAC5 resulting in its exportation from the nucleus (Figure 2.15). The exportation of HDAC5 .from the nucleus facilitates the expression of PGC-1α through the recruitment of HAT containing proteins. In this way AMPK not only enhances mitochondrial biogenesis by inducing PGC-1α expression but also by activating the protein.

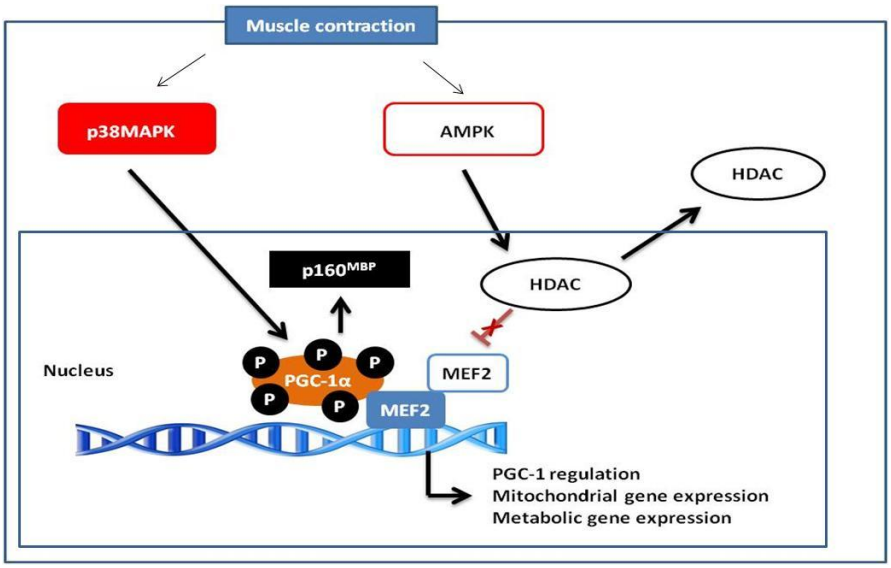

Figure 2.15 The regulation of PGC-1α activity via AMPK and p38AMPK phosphorylation

Class II HDAC regulation of MEF2 activity. MEF2 is a transcription factor and is kept in an inactive state via binding to the Class II HDACs. Phosphorylation of HDAC by a kinase, such as AMPK induce their nuclear export, removing the inhibition on MEF2 and subsequently inducing the expression of PGC-1α. Phosphorylation of PGC-1 α by p38 AMPK disrupting the interaction between PGC-1α and p160Mbp, a repressor protein, which inhibits its activity in myoblasts.

The acetylation status of PGC-1α protein is the most prominent modification in terms of controlling its activity and physiological output [149,150] (Figure 2.16). SIRT1 induces deacetylation of PCG-1α [150]. SIRT1 activity is up-regulated by nutrient deprivation and fasting conditions. SIRT1 is a NAD^+ dependent enzyme that is regulated by fluctuations in the levels of NAD^+ and/or NAD^+/NADH ratio. In muscle and hepatic cells, SIRT1 has been shown to deacetylate and thereby activate PGC-1α and induce transcription of target genes [150]. In a recent study involving nutrient deprived muscle cell lines, up-regulation of SIRT1 enhanced gene expression of mitochondrial and oxidative genes and enhanced fat oxidation [151]. Interestingly AMPK phosphorylation and activation of PGC-1α at Thr^{177} and Ser^{538} [47] is suggested to prime PGC-1α for deacetylation by SIRT1 [150].

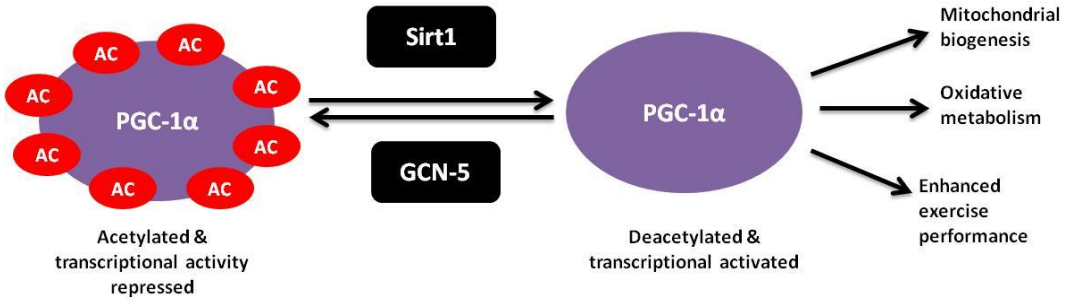

Figure 2.16 Control of PGC-1 activity via acetylation and deacetylation

SIRT1-mediated deacetylation activates PGC-1α, while acetylation by GCN5 inhibits PGC-1α-directed gene expression. SIRT1, sirtuin 2 ortholog 1; Ac, acetyl group; GCN-5,

Studies examining the putative role of the SIRT1 protein in the induction of mitochondrial biogenesis in whole muscle after exercise is less clear. In rats, 14 d of both high intensity (30 $m \cdot min^{-1}$, 18.5% incline, 60 $min \cdot d^{-1}$) and low intensity (20 $m \cdot min^{-1}$, 18.5% incline, 90 $min \cdot d^{-1}$) exercise increased SIRT1 and PGC-1α proteins, HK activity, GLUT4 protein, mitochondrial proteins and enzyme activities, and in the soleus muscle [152]. However, SIRT1 protein concentration in rat skeletal muscle decreased after 7 days of chronic electrical stimulation and in response to chronic administration of AICAR despite increased mitochondrial content [153], suggesting that an increase in SIRT1 protein may not be essential for exercise induced mitochondrial biogenesis. Dumke *et al*., (2009) [154] found an increase in SIRT1 mRNA in trained men after 3 h of cycling at 57% PPO whereas, 6 weeks of HIIT (1 h of 10 x 4 min intervals at 90% $\dot{V}O_2$peak separated by 2 min passive recovery, 3 d per week) decreased SIRT1 protein content in men [155]. This study also found an increase in maximal activities of mitochondrial enzymes in skeletal muscle and PGC-1α protein, along with whole muscle SIRT1 activity. However, the decrease in SIRT1 protein content is not consistent with its role as a major regulator of PGC-1α induced mitochondrial biogenesis.

Further research is needed to determine the role of SIRT1 in PGC-1α induced mitochondrial biogenesis.

In summary, the expression of PGC-1α is controlled by a variety of transcriptional and posttranslational modifications allowing for a system that responds to a number of cellular stimuli. This helps to ensure that PGC-1α co-activates the transcription of the precise protein(s) required to satisfy the energy needs of the cell.

Metabolic Effects of PGC-1α

PGC-1α and Lipid Metabolism

PPARα is co-activated by PGC-1α which subsequently regulates fatty acid uptake and oxidation, via the transcriptional control of several nuclear genes encoding mitochondrial β-oxidation enzymes, including MCAD and muscle CPT-1 [156] (Figure 2.17). The transcription factor ERRα is also known to play a central role in integrating PGC-1α and lipid metabolism. It directly activates the expression of the PPARα gene in cardiac myocytes, skeletal myotubes and primary mouse fibroblasts (Huss 2004). It also binds to the promoter region of the gene encoding MCAD which catalyses the first step in β-oxidation and therefore directly regulates fatty acid oxidation [158].

A recent study by Benton *et al*., (2008) [139] demonstrated a role of PGC-1α in fatty acid metabolism in rat models. A modest over expression of PGC-1α in rat muscle cells resulted in an increase in fatty acid transporters FAT/CD36 and an increase in palmitate oxidation.

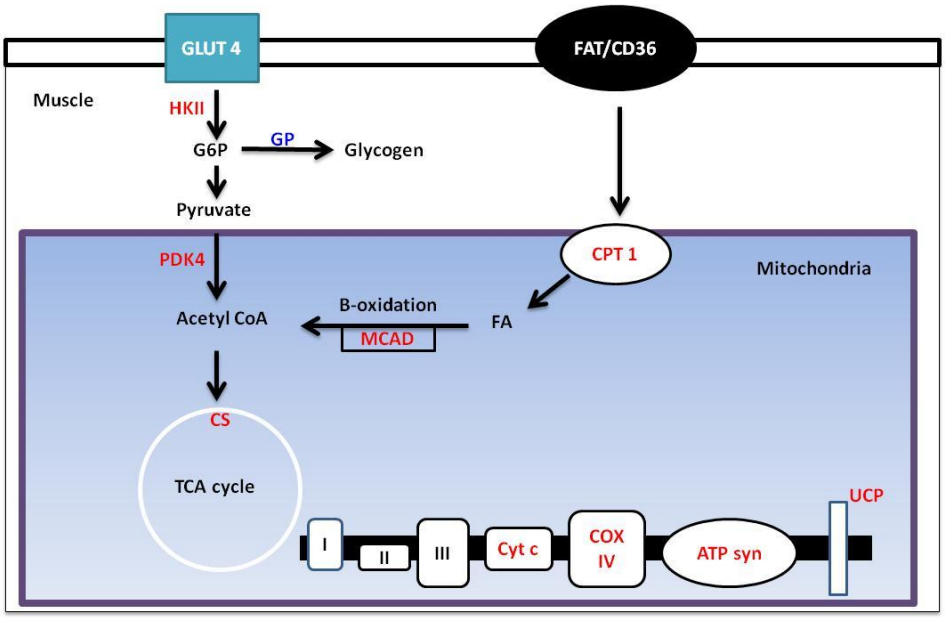

Figure 2.17 Schematic overview of selected metabolic proteins regulated by PGC-1 α in skeletal muscle*

*Proteins in red are induced by PGC1- α, whereas the protein in blue is reduced

PGC-1α and Glucose Uptake

GLUT4 is the key glucose transport protein in skeletal muscle. It is well established that exercise induced PGC-1α stimulates an oxidative phenotype which contains an increased high concentration of GLUT4, making it plausible that PCG-1α may induce the expression of the GLUT4 protein. An over expression of PGC-1α in myotubes results in an increased GLUT4 expression [101]. In contrast, an over expression of PGC-1α in transgenic mice failed to induce an increase in GLUT4 expression [159]. This resulted in a decrease in insulin stimulated muscle glucose uptake [160]. It is important to note that although transgenic animal models are useful to study the function of certain genes, they may not however, be appropriate for investigating the effect of PGC-1α GLUT4 expression and insulin sensitivity.

The effects of GLUT4 gene expression are thought to be partially mediated through PGC-1α binding to and activating MEF2 [101]. Removal of HDAC repression on MEF2 via HDAC phosphorylation allows MEF2 to act on its binding site in the promoter region of the GLUT4 gene and recruit PGC-1α to enhance the transcription process. A single bout of exercise reduces HDAC5 association with MEF2 [161], possibly through phosphorylation of HDAC5 by exercise-induced kinases. In addition, acute exercise increases MEF2/PGC-1α association, which may indicate an exercise-induced enhancement in MEF2 transcriptional function, thereby potentially leading to increased PGC-1α and GLUT4 expression by the mechanisms previously described.

Cellular glucose concentration is not only controlled at the level of entry into the cell via GLUT-4, but also at the level of entry of pyruvate into the citric acid cycle. The pyruvate dehydrogenase complex (PDC), catalyses the irreversible decarboxylation of pyruvate to acetyl CoA. Pyruvate dehydrogenase kinase 4 phosphorylates and deactivates PDC resulting in a glucose sparing effect and enhancing fatty acid oxidation (Figure 2.18). Activators of PPARα, induces the expression of PDK4, although the mechanism is not clearly understood [162]. In myotubes co-activation of ERRα by PGC-1α occurs independently of PPARα activation of the mouse PDK4 gene promoter [163]. These studies indicate that muscle genes activated by PGC-1α enhance fatty acid oxidation which may serve to replenish muscle glycogen concentrations by decreasing glucose utilisation during recovery from exercise.

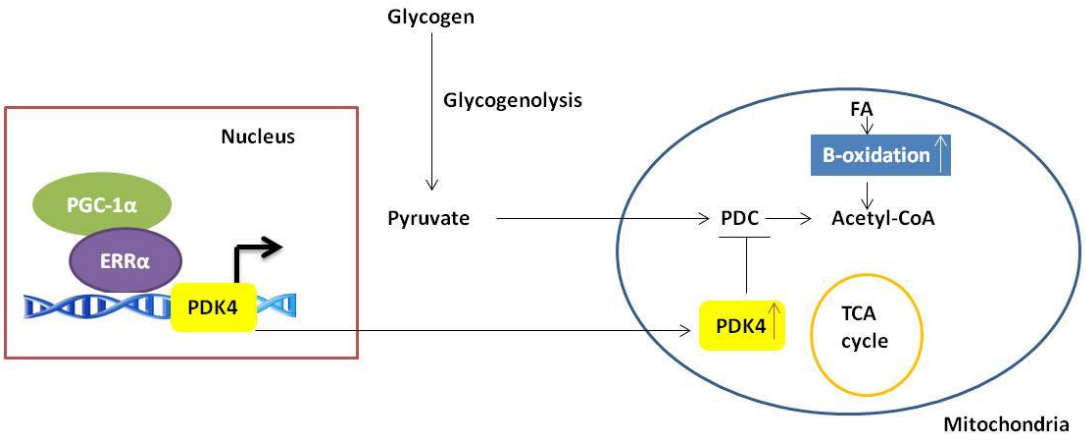

Figure 2.18 PGC-1α mediated up-regulation of pyruvate dehydrogenase kinase (PDK) 4

PCG-1α mediated up-regulation of PDK4 leads to an increase in fat oxidation in skeletal muscle. ERRα, estrogen related receptor α, PDC pyruvate dehydrogenase complex, TCA tricarboxylic acid

In summary, the up-regulation of mitochondrial fatty acid oxidation induced via PGC-1α in conjunction with enhanced glucose uptake and storage, suggests a critical role for PGC-1α in fuel selection in resting muscle.

PGC-1α and Substrate Regulation

An increase in energy expenditure induced by contractile activity alters the substrate environment within the muscle and appears to modulate alterations in PGC-1α expression. In 9 physically active untrained men, Pilegaard *et al.*, (2005) [164] found that 5 d of reduced CHO intake increased PGC-1α mRNA for 24 h after a 75 min exercise bout of cycling at 75% $\dot{V}O_2$max . Other studies have also found that changes in muscle glycogen after exercise are related to the changes in PGC-1α protein [165]. If PGC-1α is influenced by glycogen and CHO availability it makes sense that PGC-1α may also modulate the usage of CHO within the muscle. Overexpression of PGC-1α in mice has been shown to increase the basal rates of

glucose transport with subsequent increases in intracellular glycogen concentration [166] and also prevented muscle glycogen depletion during exercise. The effects of PGC-1α on glycogen metabolism were attributed to an increase in glucose transport, reduced glycolytic flux, and downregulation of glycogen phosphorylase. Palmitoylcarnitine oxidation also increased indicating that the increase in lipid oxidation may have also contributed to the changes in CHO metabolism [166]. In contrast, muscle glycogen repletion rates post-exercise were reduced in PGC-1α-deficient animals.

Since PGC-1α has a substantial effect of lipid metabolism, it may be possible that fatty acid availability may also alter the expression of PGC-1α. Watt *et al*., (2004) [167] found an increase in PGC-1α mRNA in the presence of reduced circulating levels of fatty acids. However, others failed to find a relation between FFA and exercise-induced PGC-1α mRNA expression [168]. More recent studies have found that intramuscular fatty acids or their metabolites and not circulating fatty acids may mediate the changes in PGC-1α expression in skeletal muscle [169,170]

The Role of PGC-1α in Skeletal Muscle Mitochondrial Biogenesis

Contractile activity activates PGC-1α, which induces the expression of a large number of nuclear and mitochondrial genes, thereby activating the coordinated expression of mitochondrial proteins, including increased respiratory chain subunit expression, mitochondrial density and mitochondrial enzyme activity [124,165,171]. The role PGC-1α plays in inducing mitochondrial biogenesis was first demonstrated by expressing PGC-1α in myotubes [17] and in mouse skeletal muscle [172]. This resulted in an increase in the expression of genes involved in oxidation phosphorylation such as cytochrome c and COX IV. Ectopic

expression of PGC-1α in myotubes stimulates GLUT4 expression and mitochondrial oxidation. Transgenic expression of PGC-1α in fast twitch skeletal muscle fibers markedly increased myoglobin protein expression, which was accompanied by the transformation of the muscle fibers to the metabolic characteristics of slow twitch muscle fibers [172]. In contrast, PGC-1α deficient mice have been shown to have reduced mRNA content of genes encoding mitochondrial oxidative proteins and are prone to contraction induced fatigue [172,173].

The regulation of mitochondrial biogenesis through PGC-1α, NRF-1 and ERRα and was described in an above section. In addition to the stimulatory effect of NRFs on Tfam expression, these transcription factors also bind to target sequences on nuclear- and mitochondrial-encoded mitochondrial genes, including cytochrome c and COXIV [174]. Similarly, ERRα in association with NRF-2 has been shown to be related to PGC-1α-induced mitochondrial biogenesis [175,176]. ERRα was shown to be essential for PGC-1α-mediated mitochondrial biogenesis and function upstream of NRF-1 action in the program of oxidative phosphorylation gene expression [176]. This implies that ERRα binding sites are present in the promoters of genes such as cytochrome c and β-ATP synthase [174].

Exercise as a Stimulus for PGC-1α

PGC-1α's involvement in mediating exercise induced mitochondrial biogenesis is supported by both acute endurance exercise [3,134,168,177] and several weeks of endurance training [134,140,178]. Pilegaard *et al*., (2003) [134] were the first to demonstrate the effects of both acute exercise and prolonged exercise training on PGC-1α transcription in human skeletal muscle. After 4 weeks of one legged knee extensor exercise training, PGC-1α mRNA

was increased in the trained leg at rest and after exercise (3 h of two-legged knee extensor exercise). It is interesting to note that PGC-1α mRNA was increased to a greater extent in the trained leg despite the fact that the relative work intensity for the acute bout of exercise was much lower for the trained than the untrained leg. This suggests that the mechanism(s) regulating the exercise-induced activation of the PGC-1α gene in muscle may become more sensitive to acute exercise with endurance training [179].

Cycling for 2 h at 50% PPO has been found to result in a 4.8 fold increase in PGC-1α mRNA immediately after exercise and a 12 fold increase 1 h post-exercise respectively, in 7 untrained men [168]. The muscle levels of PGC-1α mRNA returned to basal levels 4 h post exercise. In contrast, Tunstall et al., (2002) [180] failed to find an increases in the expression of PGC-1α gene in untrained men and women following 9 d of endurance exercise for 1 h a day at an intensity corresponding to 63% $\dot{V}O_2$max. It is possible that the expression of PGC-1α mRNA may require a stimulus from muscle contraction lasting more than 1 h. It is also possible that the accumulative effect of a number of consecutive bouts of acute endurance exercise may be required to increase the expression of PGC-1α mRNA. Russell *et al.,* (2003) [140] found a 2.2 fold increase in PGC-1α mRNA and a 2.8 fold increase in protein concentration in response to 6 weeks of combined interval (1-3 min of 5-6 intervals @70-80% $\dot{V}O_2$max) and submaximal exercise training (40 min running @ 60% $\dot{V}O_2$max) in untrained men. Differences in the acute and chronic responses of PGC-1α mRNA are probably due to the acute and chronic changes in cellular homeostasis with the constant stress of exercise training gradually stimulating a new increased steady state level of PGC-1α gene expression.

High Intensity Interval Exercise as a Stimulus for Mitochondrial Biogenesis

The majority of early studies that have examined the effect of acute and chronic exercise training on PGC-1α induced mitochondrial biogenesis have involved primarily steady state submaximal endurance exercise [135,165,168]. Recently, evidence is accumulating which indicates that both acute and chronic low volume HIIE can also induce mitochondrial biogenesis. Gibala *et al*., (2009) [23] found a twofold increase in PGC-1α mRNA content in human skeletal muscle, 3 h after a single bout of HIIT in college aged men. The exercise session involved 4 x 30 s of maximal cycling with a 4 min active recovery period between each exercise bout. Using a similar study design Burgomaster *et al*., (2008) [11] reported a similar increase in PGC-1α protein in response to 6 weeks of high intensity cycle training (3 $d \cdot week^{-1}$) and 6 weeks of endurance training involving 40-60 min of cycling at 65% $\dot{V}O_2peak$, 5 days per week in untrained men. The similar increase in PGC-1α protein occurred despite the fact that the total training time and energy expenditure was 66 % lower (1.5 h vs. 4.5 h) and 90% (225 versus 2250kj $week^{-1}$) lower in the high intensity training group than the endurance training group. Earlier studies by both Burgomaster *et al.,* (2005) [12] and Gibala *et al.,*(2006) [22] also found an increase in maximal activity and/or protein content of mitochondrial enzymes in response to HIIT. Acute and chronic HII studies involving running have also found an increase in PGC-1α mRNA and protein content [24,84,85]. Interestingly in one of these studies a mere 12 min of HIIR over a 4 week period resulted in a 33% increase in PGC-1α content [85].

The relation between an elevation in mRNA abundance and a subsequent increase in PGC-1α protein content after acute or chronic exercise training is unclear. Animal studies

have reported a 50% increase in PGC-1α protein content at various time points up to 18 h following an acute bout of either prolonged swimming or running [112,122,181–183]. A recent study by Gibala *et al*., (2009) [23] found a twofold increase in PGC-1α mRNA content in human skeletal muscle, 3 h after a single bout of HIIE in college age men. The exercise session involved 4 x 30 s of maximal cycling with a 4 min active recovery period between each interval.

Results from human studies that have measured PGC-1α protein content following acute and chronic exercise are equivocal. Despite an acute increase in PGC-1α mRNA, Gibala *et al*., (2009) [23] found that PGC-1α protein content remained unchanged in response to 4 x 30 s bouts of maximal cycling with a 4 min active recovery period. Psilander *et al*., (2010) [133] compared the effect of two different acute bouts of interval training separated by 7 d on PGC-1α mRNA and PGC-1α protein content in 10 trained endurance cyclists. The first session involved 7 x 30 s bouts of cycling at 120 W with 4 min of active recovery between each high intensity cycle. During the second laboratory visit participants performed 3 x 20 min bouts of cycling at approximately 87% $\dot{V}O_2$ peak. Although, there was a significant increase in PGC-1α mRNA following both exercise sessions, PGC-1α protein content did not significantly change [133]. Similar findings were reported by Watt *et al*., (2004) [167] in recreationally active men during and 3 h following a 3 h bout of moderate intensity cycling at 60% $\dot{V}O_2$max.

In contrast, a recent study by De Filippis *et al*., (2008) [184] found that PGC-1α protein content was increased by 20% and 40% when measured at 30 min and 300 min after an acute bout of exercise involving 4 x 10 min of combined moderate and high intensity cycling

separated by 2 min of active recovery with no resistance. Using college age men, Burgomaster *et al*., (2008) [11] also found an increase in PGC-1α protein content following 6 weeks of either low-volume HIIT or ET involving cycling. Furthermore, Mathai *et al*., (2008) [165] found that a 23% increase in PGC-1α protein immediately following a single exhaustive bout of endurance training at 65% $\dot{V}O_2$ max. PGC-1α protein content remained elevated at 2 h of recovery, while PGC-1α mRNA had returned to resting levels.

PGC-1α mRNA and PGC-1α protein expression were measured 4 and 24 h following the 1st, 3rd, 5th and 7th HIIT exercise bouts in untrained men [15]. Exercise sessions involved 10 x 4 min bouts of cycling at 90% $\dot{V}O_2$max with 2 min passive recovery between each high intensity exercise bout. There was a significant increase in PGC-1α protein expression 24 h after the first session. PGC-1α protein expression continued to increase, attaining a plateau by the 5-7th training session. PGC-1α mRNA increased significantly 4 h after the first session. However, in contrast to the PGC-1α protein expression, PGC-1α mRNA expression was attenuated as training progressed despite a continual increase in training power output. These findings indicate that transcriptional responses to exercise sessions may be blunted with training and that presence of an increase in PGC-1α mRNA, more than one exercise session or the attainment of a specific exercise intensity is required to increase PGC-1α protein.

The rapid up-regulation of PGC-1α mRNA and subsequent protein synthesis during recovery from exercise is consistent with its role in the regulation of exercise-induced gene expression. A number of studies have shown that mitochondrial biogenesis progresses in the absence of an increase in PGC-1α protein content [185]. It is possible therefore, that an

increase in PGC-1α protein may not be necessary to mediate alterations in gene expression in response to an acute bout of exercise and exercise training. [122,185].

The Mechanism of PGC-1α in Mitochondrial Biogenesis

Until recently it was assumed that the exercise induced stimulation of mitochondrial biogenesis was mediated by an increase in PGC-1α protein. [102,186] There is now evidence that the initial phase of the adaptive increase in mitochondrial biogenesis induced by exercise is mediated by the activation of PGC-1α rather than by an increase in PGC-1α protein expression. Wright *et al*., (2007b) [187] found that the increase in protein content of several mitochondrial enzymes immediately after 6 h of swimming in rat preceded an increase in PGC-1α protein. However, the increase in PGC-1α protein content was preceded by rapid translocation of PGC-1α into the nucleus followed by NRF-1 and -2 binding to DNA and increased CS mRNA. In contrast, Hargreaves et al., (2004) [161] did not find an increase in nuclear PGC-1*α* protein immediately following 1 h of cycling at 70% $\dot{V}O_2$max. They did however, find indices of increased nuclear PGC-1*α* activity (co-activation of the transcription factor MEF-2) with no change in total PGC-1*α* protein content. Little et al., (2010) [188] found a 25% and 54% increase in nuclear PGC-1α and protein content PGC-1α after a single bout of high intensity training after a 90 min bout of moderate intensity endurance training, respectively. In a more recent study Little *et al*., (2011) [13] found that HIIT increases nuclear PGC-1α which coincided with increased mRNA expression of several mitochondrial genes.

Collectively, these findings suggest that activation of PGC-1α in response to acute exercise mediates the early stages of mitochondrial biogenesis and that increases in PGC-1α protein expression may serve to enhance the transcriptional response to subsequent

exercise challenges or sustain the greater mitochondrial volume already achieved through training [134]. The role of augmenting nuclear PGC 1α contents via nuclear translocation from the cytoplasmic pool remains equivocal in humans.

Nutrient Interactions on Exercise Induced Skeletal Muscle Adaptations

Carbohydrate availability is vital for performance during both prolonged submaximal exercise (>90 min) and during intermittent high intensity exercise. The benefits of consuming a diet high in CHO to optimise pre-exercise glycogen stores has long been known to prolong time to fatigue during prolonged submaximal exercise [189–193]. Based on observations that training with low glycogen levels can increase training induced gains in skeletal muscle oxidative capacity and performance [194,195], it is now being suggested that athletes should train with low CHO stores but restore fuel availability for competition ("train low, compete high").

Hansen *et al*., (2005) [196] found that training low and competing high significantly improved performance (Figure 2.19). In this study, 7 untrained men participated in a 10 week knee extensor 'kicking' training program. One leg trained twice every second day while the other leg trained daily. A diet high in CHO ($8 g \cdot kg^{-1} \cdot day$) was consumed throughout the study, ensuring that one leg performed each exercise session in a glycogen replete state, whereas the leg that trained twice daily completed the second exercise session glycogen depleted. There was a significant increase in resting muscle glycogen and CS activity at the end of the 10 week training period in the leg that trained twice a day compared with the leg that trained once daily. There was also a 2-fold greater increase in time to fatigue in the 'train low' leg in comparison to the leg that trained in a glycogen replete state.

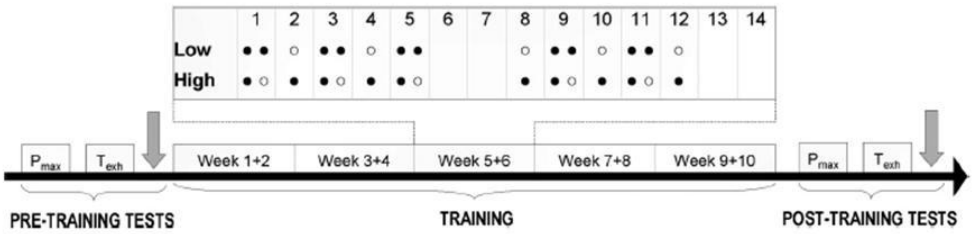

Figure 2.19 Schematic representation of Hansen et al., (2005) [196]

At least 48 h after the last performance test, a blood and muscle biopsy (grey arrows) were obtained during rest and an overnight fast. After the first set of performance tests participants trained both legs for 10 wks., followed by a second post-training set of performance tests. The training consisted of a 14 x 5 day cycle. During one 14-day period, one leg (low) was trained for 5 days by the participants performing 2 bouts of dynamic knee extensor exercise, each bout lasting 1 hr and separated by 2 h rest. Alternatively the other leg (high) was trained every day for 5 d per week, by participants performing 1 bout of dynamic knee extensor exercise for 1 h. Numbers in the boxes demote the number of days. The filled circles demote exercise, while the unfilled are rest.

A study by Yeo *et al*., (2008) [197] also found skeletal muscle adaptations and improvements in performance responses to twice daily training sessions in 14 endurance trained cyclists over a 3 week training period. Participants performed 6 exercise sessions per week divided into three sessions of cycling for 100 min at 70% $\dot{V}O_2$peak, and three sessions of 8 x 5 min at maximal sustained power with 1 min recovery between sets (Figure 2.20). Participants were randomly selected into one group that performed one exercise session per day for 6 days (high group) and a second group (low group) that trained twice every second day. Each interval session was preceded by 60 min of cycling at 70% $\dot{V}O_2$max. Before and after the 3 week training period each participant completed a 60 min time trial to measure performance improvements. There were significant increases in resting muscle glycogen concentrations, rates of whole body fat oxidation during steady state exercise and an increase in CS and β-HAD activities in the low group only. However, despite these clear metabolic enhancements, which should in theory confer performance improvements, there was a similar improvement in performance (10%) in both training groups.

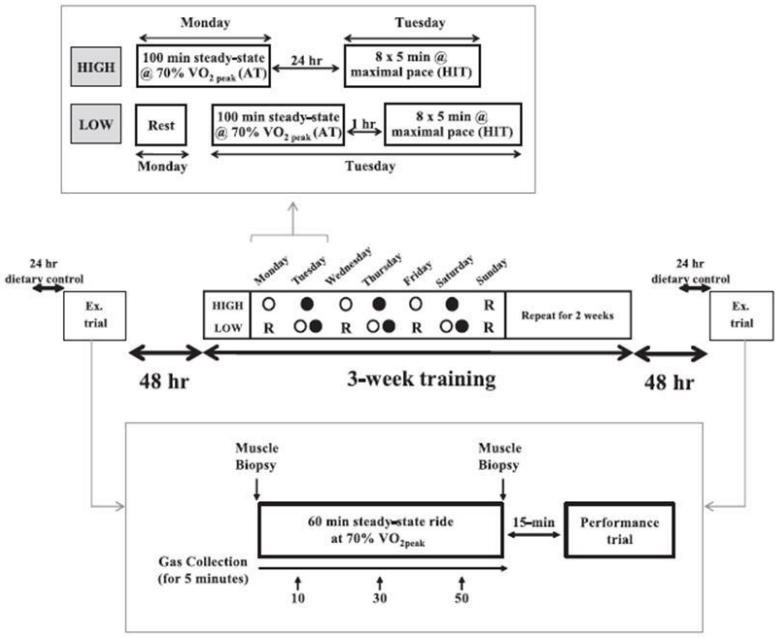

Figure 2.20 Overview of study design, Yeo et al., (2008) [197]

The unfilled circles denote aerobic training (AT)- high daily training. The unfilled circles represent high intensity interval training (HIIT). R= rest.

The mechanisms responsible for these training adaptations under restricted CHO intake remain unclear. Initially, it was found that low glycogen concentrations during exercise stimulated the rate of transcription of a number of genes involved in metabolic adaptations [26,198]. Several of these transcription factors contain glycogen binding domains which are released and targeted toward different proteins when muscle glycogen levels are low. Exercising with low muscle glycogen also enhances the activation of intracellular signalling pathways, such as AMPK and p38MAPK which control the expression of gene transcription and subsequent mitochondrial biogenesis.

AMPK is an important metabolic regulator that decreases anabolic and increases catabolic processes to maintain cellular homeostasis [199]. When muscle glycogen is low at rest, AMPK activity is increased, and its activation is significantly enhanced during exercise

compared to when glycogen levels are high [79,200]. The rate of muscle glycogen utilisation during exercise does not affect AMPK activation when pre-exercise glycogen levels are normal [80].

Exogenous CHO availability has been found to influence metabolic regulation in skeletal muscle and impact gene expression [201,202]. CHO ingestion both before and during exercise blunts training induced adaptations [203,204]. High glucose availability suppresses AMPK activation in rat skeletal muscle in vitro [201]. In humans however, pre-exercise CHO feeding did not augment AMPK phosphorylation [203]. In this cross-over study nine recreationally active men participated in a 2 h constant load cycle exercise at 75% $\dot{V}O_2$peak. Participants exercised in either a fasted or fed state (150g of CHO before and 1 g· kg^{-1}· bw^{-1} h^{-1} during exercise). Although exercise induced AMPK phosphorylation was slightly decreased in the group that exercised under high CHO availability, it was not statistically significant. These findings were confirmed in a more recent study in which 9 recreationally active men completed 2 separate bouts of cycle exercise at 65% $\dot{V}O_2$peak for 120 min. Participants ingested either an 8% CHO solution or an artificially sweetened placebo during exercise. Despite the fact that CHO supplementation significantly increased blood glucose levels there was a similar increase in AMPK phosphorylation in both groups [202].

One of the mechanism by which CHO ingestion during exercise is thought to reduce AMPK activity is via an increase in AMP. In contracting skeletal muscle AMP/ATP ratio is a major regulator of AMPK activity during exercise, with a reduction in AMP decreasing the activity of AMPK. However, in the study by Lee-young *et al*., (2006) [202] there was a disconnect between the changes in skeletal muscle AMP and AMPK activity. AMP and

AMP/ATP ratio increased during both trials, with the decrease in both analytes significantly lower in the CHO than the placebo group. These differences did not translate into a decrease in AMPK activity. McConell et al., (2005) [205] also found that AMPK activity was not increased during prolonged exercise at 65% $\dot{V}O_2$peak despite a 4-8 fold increase in the concentration of AMP during exercise. The activation of AMPK requires a substantial increase in AMP and therefore a relatively small reduction in AMP may not translate into a reduction in AMPK activity [206]. Even though there was a decrease in AMP during exercise in the CHO fed group there was still a 12-fold increase in AMP which may help to explain why AMPK activity was not reduced by CHO availably.

Morton *et al*., (2009) [24] simultaneously examined the effects of decreased muscle glycogen concentrations and the influence of exogenous glucose availability on training adaptations in 23 recreationally active men. Participants undertook a 6 week training program that consisted of 4 high-intensity running sessions at a fixed intensity (Figure 2.21). They were divided into 3 groups and undertook the exercise sessions under either high CHO availability (single day training sessions), train low (2 training sessions per day, with the second session completed under glycogen depleted conditions) or train low + glucose (train twice per day with glucose intake before and during the second exercise session) conditions. Improvements in performance were similar in all groups with 10% and 18% improvements in $\dot{V}O_2$max and distance run during the Yo-Yo intermittent test respectively. However, the group that trained with both low glycogen and low exogenous glucose availability had significantly greater increases in the activity of SDH. It is possible an increase in AMPK and/or p38AMPK due to a reduced CHO availability may have been partly responsible for the increase in SDH. Both of these signalling molecules can phosphorylate and activate PGC-

1α, which could allow for the increased expression of mitochondrial proteins, although this study provides no definite insight into this mechanism. Cochran *et al.,* (2010) [25] found no significant effect of reduced CHO availability on AMPK phosphorylation after a bout of HIT, suggesting that this pathway is not involved in nutrient enhanced metabolic adaptations after an acute bout of exercise training.

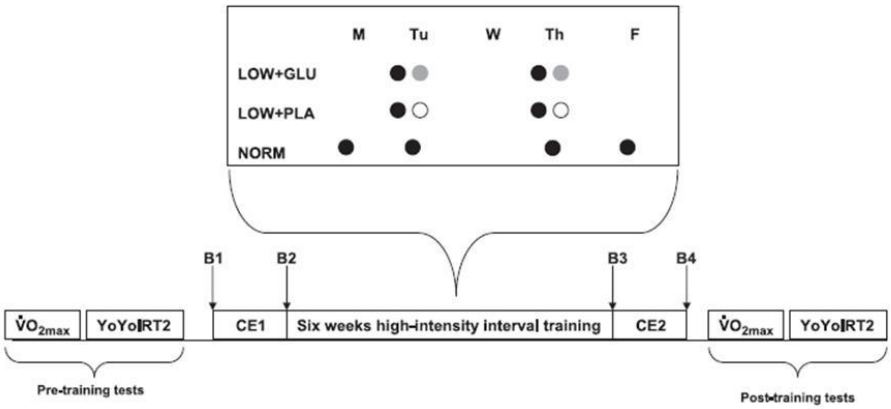

Figure 2.21 Overview of study design from Morton et al., 2009 [24]

B, signifies blood and biopsy sampling. Subjects trained 4 x d·wk, either twice a day on Tuesday (Tu) and Thursday (Th) (Low+ Glu and Low + Pla groups) or once daily on Monday (M), Tu, Th and Friday (F) (Norm groups). Three days after the last training session, participants performed an additional exercise session (CE2) to assess the effects on the physiological and metabolic cost of the exercise bout. Dark circles represent, non-supplemented exercise session, grey, CHO supplemented exercise session and open, placebo-supplemented during training.

The MAPK pathways may also play a role in the interaction of signaling pathways with nutrients to enhance metabolic adaptations after exercise. The p38MAPK protein is activated by cellular stress which couples cellular disturbances to various adaptive responses in skeletal muscle [108]. Chan *et al.,* (2007) [207] found a decrease in skeletal muscle p38MAPK phosphorylation after 60 min of cycle exercise at 70% $\dot{V}O_2$peak in the presence of reduced glycogen content and reduced CHO availability. In contrast, Yeo et al., (2010) [208] found that reduced glycogen content was not related to an enhanced p38MAPK activity.

Cochran *et al*., (2010) [25] investigated the effects of manipulating CHO intake during recovery from HIIT exercise on the skeletal muscle adaptations to a subsequent bout of exercise. Ten recreationally active men participated in two identical sessions of HIT involving 5 x 4 min of cycling at 90-95% heart rate reserve with 2 min of active recovery separated by 3 h of rest (Figure 2.22). During the recovery period participants consumed

either 1.2 g·kg^{-1} h^{-1} (HI-HI) or a sweetened placebo (HI-LO). There was 4-fold increase in p38MAPK phosphorylation after the first HIT session, which returned to normal prior to the second session in both groups. However, after the second session p38MAPK was 50% higher in HI-LO than the HI-HI. Since resting glycogen and glycogen depletion was similar between groups, it is possible that exogenous CHO availability altered the metabolic response to HIIT. ATP and PCr levels were also reportedly lower after the second exercise session in the HI-LO in comparison to the HI-HI group, which may also have contributed to the increase in p38MAPK activity. Exercising with reduced CHO availability increases FFA, and therefore the possible effects of FFA on inducing metabolic adaptations in response to exercise must to taken into account. There was a 2-fold increase in FFA in the CHO restricted condition before the second exercise session compared to the high CHO-supplemented group, which may have influenced the increase in p38MAPK phosphorylation in the HI-LO. However, there is evidence that p38MAPK phosphorylation is increased when FFA levels are suppressed during prolonged exercise in humans [167].

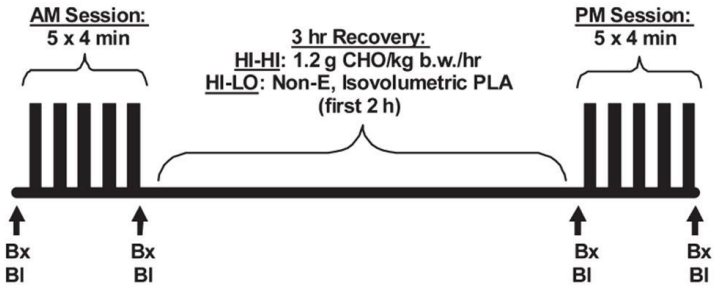

Figure 2.22 Overview of study design, Cochran et al., 2010 [25]

Performance Enhancements Conferred by Nutrient Interactions

Studies have reported an increase in mRNA expression of genes related to fatty acid oxidation when training is undertaken in a fasted state compared to a CHO-fed state. However, there was no difference in performance [24,197]. De Block *et al.*, (2008) [209] also demonstrated the effects of CHO feeding on skeletal muscle adaptations to endurance training. Twenty recreationally active men participated in a 6 week training study consisting of cycling 1-2 h 3 $d \cdot wk^{-1}$ at 75% $\dot{V}O_2$peak. Participants were divided into two groups and trained in either a fasted or fed state. Substrate utilisation was analysed during a 2 h bout of moderate exercise before and after the training period. Although training in a fasted state increased fatty acid binding protein (FABPm), fatty acid oxidation was not increased, compared to the fed state. Changes in peak $\dot{V}O_2$ were similar in the fed and fasted state.

In another study nine recreationally active men undertook a 10 week one legged-knee extensor endurance training study in which they trained their respective legs 2 h per day, on alternative days, 5 days per week [210]. Participants trained one leg while consuming a glucose drink, and the other while consuming a placebo drink. There were similar increases in muscle glycogen concentration, CS and β-HAD activities in both groups. Time to fatigue and power output during the endurance test increased equally in both legs after the training period.

Summary

Significant progress has been made over the past decade to elucidate the regulation of exercise induced mitochondrial biogenesis, with a substantial body of evidence describing a subset of transcriptional factors and transcriptional co-regulators which are involved in

this process. Skeletal muscle contraction activates various signalling pathways involving protein kinases, transcription factors and transcriptional co-regulators which transduce physiological stimuli into transcriptional adaptations. PGC-1α is a key metabolic controller which regulates gene expression by a sequential and dynamic recruitment of different sets of proteins making a functional multiprotein complex that transcribes specific genes. PGC-1α is activated by multiple signalling pathways, although AMPK and p38MAPK seem to be the more dominant activators induced by exercise [23]. Exercise training results in an increase in PGC-1α and a subsequent increase in mitochondrial content, enhanced fatty acid oxidation, increased resting muscle glycogen content and enhanced exercise performance. PGC-1α is also indirectly involved in regulating the expression of mtDNA transcription through the increased expression of Tfam. An increase in PGC-1α protein content may therefore indicate the co-coordinated metabolic expression of both nuclear and mitochondrial genome in response to exercise. An increase in Tfam protein content may further cooborate exercise induced mitochondrial gene expression.

Complexes of the ETC are comprised of multiple subunits which are encoded by both nuclear and mitochondrial genome. Tfam and PGC-1α have been found to induce the expression of mtDNA and nuclear DNA which encodes these respiratory proteins [13,18,136] . An increase in these subunits would therefore indicate the co-ordinated expression of both nuclear and mitochondrial gene expression.

An increase in PGC-1α in response to exercise has also been consistently associated with a subsequent increase in mitochondrial enzyme (CS and/or β-HAD) [11,12,22] activity indicative of mitochondrial biogenesis.

HIIT is a unique model for studying the molecular mechanisms that regulate and control mitochondrial biogenesis. Recent studies have demonstrated the ability of a single HII cycle exercise session to induce similar phenotypic changes that resemble those elicited by endurance training. This research study explores whether HII running can induce the same signalling cascades which activate mitochondrial biogenesis as HII cycle and whether nutrient interactions may blunt these pathways.

Chapter 3

STUDY 1

Introduction

Recent studies have found that HIIT stimulates an increase in mitochondrial volume, known as mitochondrial biogenesis. The molecular events that promote these metabolic adaptations in the muscle cell involve a complex cascade involving the activation of specific signalling pathways which regulate exercise induced gene expression and protein synthesis (Figure 3.1). Two integral components of the signalling pathways in skeletal muscle are AMPK and p38MAPK, which have been implicated as potential mediators in this adaptation process (Figure 2.13). Both of these protein kinases are responsive to cellular stress in the skeletal muscle. AMPK acts to maintain cellular energy homeostasis [57], while p38AMPK couples cellular disturbances to adaptive or maladaptive responses in the muscle [20]. In a recent study, Gibala *et al*., [23] found an increase in phosphorylation of AMPK (subunits α1 and α2) and p38MAPK ($p>0.05$) immediately after an acute session involving 4 x 30 s bouts of maximal cycling with 4 min of passive recovery between bouts. In addition, PGC-1α mRNA, the master regulator of mitochondrial biogenesis was increased approximately twofold above rest after 3 h of recovery ($p>0.05$). The majority of studies that demonstrate an enhanced activation of both AMPK and p38MAPK after HIIT have involved cycle ergometry.

Recent studies indicate that an increase in both endogenous CHO (muscle glycogen) and exogenous CHO (glucose supplementation) before and during exercise blunts the

activation of both AMPK and p38MAPK [24,25]. Morton *et al*., (2009) [24] simultaneously examined the effects of decreased muscle glycogen concentrations and the influence of exogenous glucose availability on training adaptations and found a significant increase in $\dot{V}O_2$max and the distance completed during an intermittent run test. However, the group that trained with both low glycogen and low exogenous glucose availability had a greater increase in SDH activity, indicating training induced mitochondrial biogenesis. The signalling pathways were not investigated in this study.

Signalling pathways and nutrient interactions after HIIE have been examined in only one study that involved cycling. No studies have investigated the activation of signalling pathways in response to CHO feeding or low muscle glycogen content in weight bearing exercise such as running which involves a greater muscle mass involvement than running.

Study Aim

To investigate the effects of high intensity interval running (HIIR) and diet on the activation of signalling pathways involved in mitochondrial biogenesis.

Study Objectives

1. To examine the effects of an acute bout of HIIR on the AMPK, p38MAPK and ACCβ signalling pathways
    1.1 To compare the effect of feeding CHO or placebo during recovery from an acute bout of HIIR on the AMPK, p38MAPK and ACCβ signalling pathways in response to a subsequent bout of HIIR
2. To compare the effect of feeding CHO or placebo during recovery from an acute bout of HIIR on a subsequent bout of HIIR.

Study Hypothesis

1. An acute bout of HIIR will significantly activate the AMPK, p38MAPK and ACCβ signalling pathways
    1.1 Compared to placebo, feeding a CHO solution during recovery following an acute bout of HIIR will blunt the activation of AMPK, p38MAPK and ACCβ activity during a subsequent bout of HIIR
2. Compared to placebo, feeding a CHO solution during recovery following an acute bout of HIIR will result in a significant increase in performance during a subsequent bout of HIIR

Methods

Participants

Eighteen club level Gaelic football players (mean ± SD; age 25.83 ± 5.40 yr; BMI 24.55 ± 1.40 $kg \cdot m^2$; $\dot{V}O_2max$ 55.14 ± 4.90 $ml \cdot min^{-1} \cdot kg^{-1}$) participated in the study. Participants were fully informed of the experimental procedures and possible discomforts associated with the study before giving their written informed consent to participate. The study was approved by the Research Ethics Committee at Dublin City University (DCUREC 148).

Overview of Study Design

The study design is outlined in figure 3.1. Participants made two visits to the High Performance Laboratory in the School of Health and Human Performance in DCU. The first visit was used to measure $\dot{V}O_2max$. During the second visit, participants performed two identical exercise trials separated by a 3 h recovery period (experimental trials). After the first exercise trial participants were randomized to a CHO or placebo (P) group, in which they were fed either a CHO or a placebo solution and a low CHO lunch during the first 2 h of the 3 h recovery period. A muscle biopsy and blood sample was taken before and after each exercise trial.

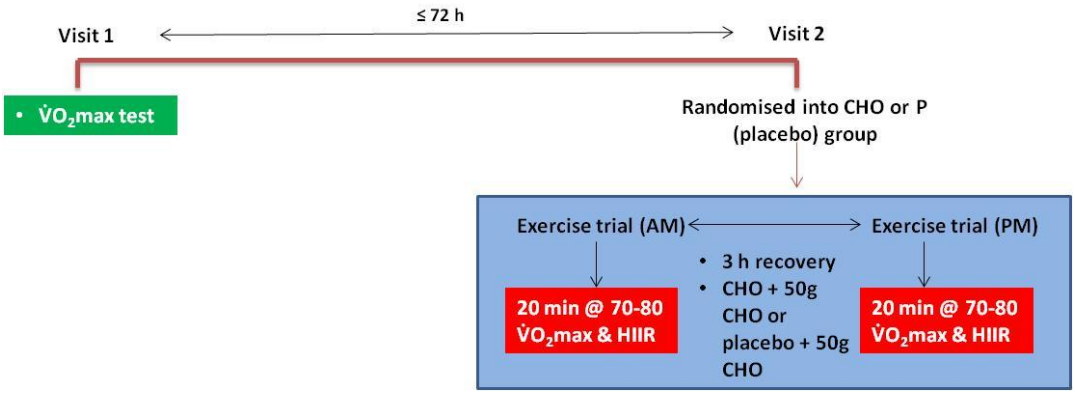

Figure 3.1 Study 1 research design

Experimental Trial

The two experimental trials were identical except for the nutritional manipulation. The experimental protocol is outlined in figure 3.2. Participants arrived in the laboratory 2 h after consuming a standardised breakfast (617 kcal; 60% CHO, 30% fat, 10% protein). They were instructed to refrain from caffeine for 12 h, and alcohol and physical activity beyond activities of daily living for 24 h before the experimental trials.

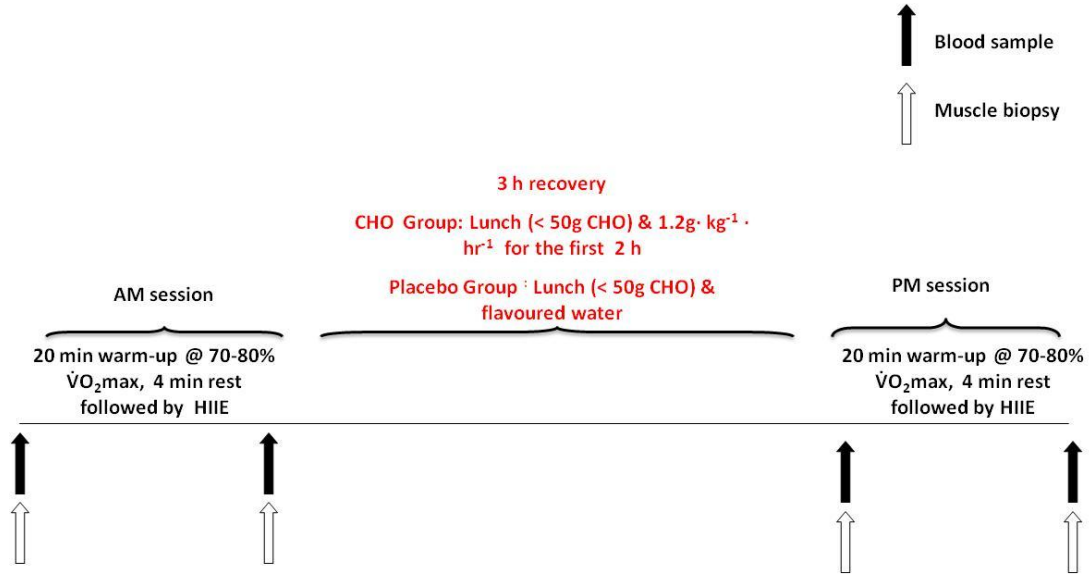

Figure 3.2 Study 1 Experimental protocol

Resting muscle and blood samples were obtained, after which participants completed a 20 min warm-up on a treadmill at an intensity corresponding to 70-80% $\dot{V}O_2$max (endurance run (ER)) followed by a 4 min passive recovery period. This was followed by a HIIR protocol. A second muscle and blood sample was obtained immediately following the bout of HIIR.

Participants then rested for 3 h and consumed either 1.2 g CHO·g· kg $^{-1}$·h^{-1} or a similar volume of flavoured water for the first 2 h. They were provided with 500 ml of the appropriate beverage and instructed to consume it at regular intervals during the 2 h interval. The CHO consisted of maltodextrin, fructose and dextrose (Kinetica Ltd., Carbery, Ireland). Each participant also consumed a standardised low-CHO lunch consisting of 304 kcal: 52% CHO, 39% fat, 9% protein during the first hour of the recovery period. Water was ingested *ad libitum* during the 3 h recovery period. Following the recovery period, participants undertook a second bout of HIIR that was identical to the first session. Muscle and blood samples were obtained before and after exercise.

High Intensity Interval Exercise

Participants performed 3 sets of maximal sprints (Figure 3.3). A set consisted of 3 x 110 m with a 20 s passive recovery period between each run (1 set) and a 4 min passive recovery period between sets. Following each set a blood sample was taken to determine blood lactate concentration. Each sprint was timed using electronic timing gates.

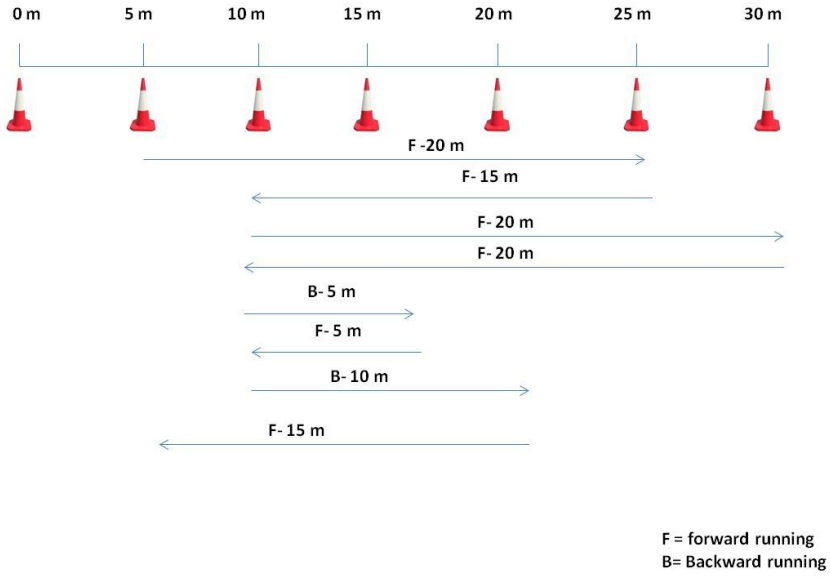

Figure 3.3 High intensity interval running (HIIR) protocol

Anthropometrics

Height and body mass were measured to the nearest 0.1 cm using a portable scale (Seca 707 Balance Scales, GmbH, Hamburg, Germany). Participants removed their shoes and stood with their feet together on the base plate with their arms loosely by their side. Each participant was asked to take a deep breath and to stand with their back as straight as possible against the vertical measuring rods and to look straight ahead. Body mass was measured to the nearest 0.1 kg. Participants were instructed to wear a light top and shorts and to remove their shoes prior to the measurement.

Maximal Aerobic Capacity

Maximal aerobic capacity was determined on a treadmill (Woodway ELG 55, Waukesha, WI) using a ramp protocol. Subjects ran at 8 $km \cdot h^{-1}$, 10 $km \cdot h^{-1}$ and 12 $km \cdot h^{-1}$ for 3 min at 1% gradient. For the remainder of the test, the speed remained constant and the gradient increased by 1% every min until the participant reached volitional fatigue. Heart rate and RPE were recorded during the final 10 sec of each min.

Cardiorespiratory and Metabolic Measures

Respiratory metabolic responses were determined using standard open-circuit spirometry techniques (Sensormedics Vmax 229, SensorMedics Corp., CA). Prior to testing, the gas analysers were calibrated with standard gases of known concentration. A mass flow sensor (Sensormedics, Loma Linda, CA, USA) was used to collect breath-by-breath measurement of ventilation. A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was used to calibrate the mass flow sensor prior to each test.

Mass Flow Sensor Heated Wire Anemometer-Mode of Operation

The mass flow sensor is a low resistance tube with a tapered internal diameter extending from both ends of a laminar flow throat. A cold and hot stainless steel wire electrically heated to -180°C and -240°C respectively, are centered in the flow stream. These wires are elements in a servo-controller bridge circuit that maintain the resistance ratio of the two wires at a constant value. If only the temperature of the inspired gases changes, then both wires lose heat at the same rate and no current change is required to keep the bridge balanced. As air flows across the wires, the hot air loses heat more rapidly than the cold air and current must be added to keep the bridges balanced at a 3:4 ratio. The amount of current required is proportional to the mass flow of the gas. This method ensures that the sensor measures only the heat loss from the molecular convection of the moving gas stream, and not the artifact due to cooling of the gas as it passes through a breathing assembly. The mass flow meter responds to instantaneous flow rates between 0-16 $L.sec^{-1}$ and integrated flow between 0-350 $L \cdot min^{1-}$ with flow resistance <1.5 $cmH_2O \cdot L^{-1} \cdot sec^{-1}$. The mass flow sensor was outputted to the analyser module of the Vmax 229 and was sampled at a rate of 125 Hz.

Mass Flow Sensor Calibration

A 3 L volume syringe (Sensormedics, Loma Linda, CA, USA) was connected to the mass flow sensor, and stroked four times in order to measure inspired and expired volumes. The volumes were calculated by expressing 3 L as a fraction of each measured inspired and expired volume achieved during calibration. An average correction factor was calculated for inspired and expired volumes, and used to fine-tune the volume measurement.

A verification procedure was performed. This involved stroking the 3 L volume syringe four times. Inspired and expired volumes were measured using the newly calculated correction factors. In order to pass the calibration procedure, one of the four strokes had to have an average flow rate <0.5 $L \cdot sec^{-1}$, and at least one of the four strokes had to have an average flow >3.0 $L.sec^{-1}$.

Gas Analysers

The Vmax 229 utilizes a rapid response infrared measurement technique. An O_2 and CO_2 analyser is integrated with the Vmax 229. A small sample of inspired air is drawn through a sample cell, and exposed to an infrared light through an optical that is passed through a band pass filter and the sample cell. An infrared detector responds to the amount of infrared light that passes through the sample cell. The amount of light that passes through the sample cell varies according to the concentration of CO_2 in the sample cell. Based on measured levels of infrared light intensity, the analyser computes the PCO_2 in the gas sample. The CO_2 analyser is linearly scaled across the 0-100% range with a resolution of 0.01% CO_2, and a response time of <130 ms (10-90%) at 500 $ml \cdot min^{-1}$ flow. The O_2 analyser is based on the high paramagnetic susceptibility of O_2. A diamagnetic glass dumbbell suspended in a magnetic field rotates in proportion to the PO_2. The analyser is linearly scaled across the 0-100% range with a resolution of 0.01% O_2 and a response time of <130 ms (10-90%) at 500$ml \cdot min^{-1}$ flow.

Calibration of CO_2 and O_2 Analysers

The gas analysers were calibrated with standard gases of known concentration (BOC gases, Dublin, Ireland). The first calibration gas contained 26.00 ± 0.02% oxygen and the

balance nitrogen (N_2). The second calibration gas contained 4.00 ± 0.02% carbon dioxide, 16.00 ± 0.02% O_2, and the balance N_2. A small bore drying tube connected to the CO_2 and O_2 analysers sampled the calibration gases. The absorption and evaporative properties of the drying tube ensured that the relative humidity of the calibration gas was equilibrated to ambient conditions prior to sampling by the O_2 and CO_2 analysers. The calibration gas was sampled at a rate of 125 Hz. The response time was similar between O_2 and CO_2 analyser.

Ratings of Perceived Exertion

RPE was obtained using the 16-point Borg category RPE scale. Prior to the maximal exercise test participants read a standard set of perceptual scaling instructions. These instructions followed an established format used in previous investigations. Low and high "perceptual anchors" were established during the maximal exercise test. This involved asking participants to assign a rating of 6 (low anchor) to the lowest exercise intensity, and 20 (high anchor) to the highest exercise intensity. Participants were instructed to make their subjective assessments of perceived exertion relative to these minimum and maximum standards (perceptual anchors).

Lactate Measurements

Blood samples were drawn from the earlobe. Prior to each sample, the ear lobe was wiped with alcohol and allowed to dry thoroughly. The base of ear lobe was jabbed with a lancet (Accu-Chek Softclix, UK) and the first drop of blood was wiped away. Pressure was placed on the ear lobe with the thumb and forefinger in order to provide an appropriate sample. A 5 μL sample of whole blood was automatically aspirated into a single use,

enzyme-coated electrode test strip (Lactate Pro Akray, Japan). The reagent strip fills by capillary action directly from the earlobe site.

Blood samples were analysed using and a hand-held portable analyser (Lactate Pro Akray, Japan). The measuring range is 0.8–23 mM. Lactate in the sample reacts with potassium ferricyanide and lactate oxidase to form potassium ferrocyanide and pyruvate. Upon the application of a given voltage, ferrocyanide is oxidised, releasing electrons and creating a current. This current is measured amperometrically and is directly proportional to the lactate concentration of the blood sample. The result is displayed after 60 s. The Lactate Pro is supplied with a check strip and a calibration strip that provide a non-quantitative indication of instrument accuracy.

Performance Measurements

Wireless electronic timing gates (Fusion Sport International) were used to measure the time for each 110 m interval sprint. The timing gates were placed at the starting line. Participants were encouraged to complete each interval sprints in the shortest possible time.

Muscle Biopsy

Approximately 100-150 mg of muscle was obtained from the m. vastus lateralis midway between the upper pole of the patella and the anterior superior iliac spine at the anterior border of the iliotibial band using the percutaneous needle biopsy technique described by Bergstrom with the aid of suction [190]. Two biopsies were obtained from each leg during each trial. Each biopsy was obtained from a separate incision site, with incision sites spaced 2–3 cm apart.

A small area of skin was shaved if necessary and 5.0 ml 1% w/v lidocaine HCl was infiltrated into the skin and subcutaneous tissues as far as the fascia but not into the muscle itself. The biopsy area was further cleansed using povidine-iodine solution and a sterile drape placed around the incision site. A 5 mm skin incision was made through the skin and the deep fascia with a number 11 blade scalpel.

The obturator was removed from the 5 mm Bergstrom biopsy needle. The needle was inserted with window closed (inner needle advanced to its furthest forward position). The needle was advanced, moving easily through skin until resistance was felt from the muscle sheath and then pushed through the sheath. A sample of tissue was guillotined by opening the outer needle window (retracting inner needle) and then closing the window with forward thrust of the inner needle. A 200 ml syringe attached to the proximal port of the needle permitted suction to be applied to draw the sample into the needle port. Closure of the port using a sharp trocar completed the acquisition of the sample. This procedure was repeated approximately 3 times without removing the needle from the thigh. The needle was then removed and the obturator used to evacuate the specimen. Samples were snap-frozen in liquid nitrogen and stored at -80° C until analysis.

After the procedure, direct pressure was applied for approximately 5 min to minimise haematoma formation. Once satisfied of haemostasis, several steristrips were applied to close the incision. A waterproof dressing was placed over the wound. An elastic stockinette was placed over the thigh for further compression for a few hours if required.

Blood and Muscle Analysis

Muscle Metabolites and Glycogen

Two pieces of frozen wet muscle (10–15 mg) were removed from each of the first biopsy samples under liquid nitrogen for glycogen, metabolite and protein analysis. The muscle from each biopsy was freeze dried, dissected free of visible blood and connective tissue, and powdered for metabolite and glycogen analyses. An aliquot of freeze-dried muscle (10–12 mg) was extracted with 0.5 mol·L^{-1} perchloric acid (HClO4), containing 1 mmol·L^{-1} EDTA, and neutralized with 2.2 mol·L^{-1} $KHCO_3$. The supernatant was used to determine creatine, PCr and ATP contents with enzymatic spectrophotometric assays [211]. Muscle glycogen content was determined from 2 aliquots of freeze-dried muscle (each 2–3 mg) from all biopsies, as described elsewhere [211]. Muscle metabolites were corrected for total creatine, as described below.

Muscle calculations

Muscle ADP and free AMP (AMP) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions [212]. Specifically, ADP was calculated using the measured ATP, creatine, and PCr values, an estimated H^+ concentration, and the creatine kinase constant of 1.66 x 109 [213]. AMP was calculated from the estimated ADP and measured ATP content, using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (Pi) was calculated by adding the estimated resting free phosphate of 10.8 mmol·kg^{-1} dry weight [212] to the difference in PCr content (D [PCr]) minus the accumulation of glucose 6-phosphate between rest and selected exercise time points.

Substrate level phosphorylation was calculated between rest and 5 min, using the following equation:

ATP provision rate: 1.5 (Δ[lactate])+ [ΔPCr]

Where Δ is the difference between the rest and 5 min values and brackets indicate concentration [214].

Western Blots Analysis

Muscle Homogenization: Frozen muscle samples (30 mg) were added to ice-cold lysis buffer (50 mM Tris-HCl, 1 mM EDTA, 1 mM EGTA, 50mM NaF, 1% Triton X-100, 10 mM sodium β-glycerol phosphate, 5 mM sodium pyrophosphate, 2 mM DTT, 1 mM PMSF, 0.004% protease inhibitor cocktail (Sigma Aldrich, Oakville, ON), and 1mM Na orthovanadate). Samples were scissor minced and then centrifuged at 1500 g for 15 min at 4°C, and the supernatant was obtained.

Protein concentration of the homogenate was determined using a BCA assay. Muscle homogenate was solubilized in 4 x Laemmli's buffer, resolved by SDS-PAGE, and wet-transferred to nitrocellulose membrane (2 h, 30 V for all except ACCβ membranes which were left overnight at 30V). The membranes were blocked for 1 h and then incubated overnight at 4°C with commercially available primary antibodies to measure, total and phosphorylated AMPK (1:1000), p38MAPK (1:1000) and ACCβ (1:1000) (Cell Signaling Technology, Inc). Secondary antibodies were also purchased from Cell Signaling Technology and were diluted as follows; AMPK (1:1000), p38MAPK (1:1000) and ACCβ

After incubation with appropriate secondary antibody for 1 h, the immune complexes were detected using the enhanced chemiluminescence method (Western

Lightning Plus-ECL; PerkinElmer, Waltham, MA), and quantified with densitometry (Gene Tools software, PerkinElmer). All phospho-proteins were normalised to the corresponding proteins after stripping the phospho antibody for 30 min at 50°C in stripping buffer (65 mM Tris HCl, 2% SDS vol/vol, 0.8% mercaptoethanol vol/vol) and re-probing with the primary antibody for the corresponding total.

Blood Measurements

Venous whole blood was collected in 4 ml vacutainers. The whole blood was analysed for lactate and glucose. The supernatant was stored at –20 °C and analyzed for glycerol and free fatty acids with a colorimetric assay [211] (Randox, UK.), and insulin with a magnetic bead panel (Millipore, Cork, Ireland).

Statistical Analysis

SPSS 19 for Windows statistical software will be used to perform the statistical analysis. Normality and homogeneity of variance were assessed using the Kolmogorov-Smirnov test and Levene's test, respectively. A paired sample T-test was used to compare the changes in muscle and blood parameters in all 18 participants in response to the AM HIIR session. The performance times and blood lactate levels for the 3 HIIR's within each set were averaged and compared to each other using a one way repeated measures ANOVA. A time (pre and post) x group (CHO and P) 2-way repeated measure ANOVA was used to compare the changes in muscle and blood and performance parameters in response to the PM HIIR session. The performance times for the 3 HIIR's within each set were averaged and compared to each other using a two way (group (2) x time (3) 2-way repeated measures ANOVA. Blood lactate levels for the 3 HIIR's within each set were averaged and compared

to each other and baseline using a two way (group (2) x time (4) 2-way repeated measures ANOVA. No significant interactions were found and all the main effects were probed using a Bonferroni for multiple comparisons. Statistical significance will be accepted at the $P < 0.05$ level of confidence.

Results

The anthropometric characteristics of the participants in both the CHO and P group.are shown in table 3.1.

Table 3.1 Anthropometric data

	CHO	Placebo
Age	357.43 ± 115.68	288.24 ± 82.02ł
Weight	18.78 ± 4.86	18.39 ± 3.73
Height	60.15 ± 17.73	60.16 ± 11.30
$\dot{V}O_2$max ($ml \cdot kg^{1} \cdot min^{1}$)	35.96 ± 10.37	43.07 ± 13.10

Blood Lactate Levels

Acute Exercise – AM (N=18)

Blood lactate levels before and after the acute AM bout of ER and HIIR are illustrated in figure 3.4. Circulating blood lactate levels were higher ($p<0.001$) at the end of the 20 min submaximal run than baseline-1. The 20 min ER was followed by 4 min of passive recovery. Blood lactate was still significantly ($p<0.001$) elevated above baseline at the end of the recovery, immediately prior to HIIE. Blood lactate levels were significantly higher than baseline-2 after each of the 3 sets of HIIE. There was a significant difference ($p<0.001$) in blood lactate between each of the HIIR's.

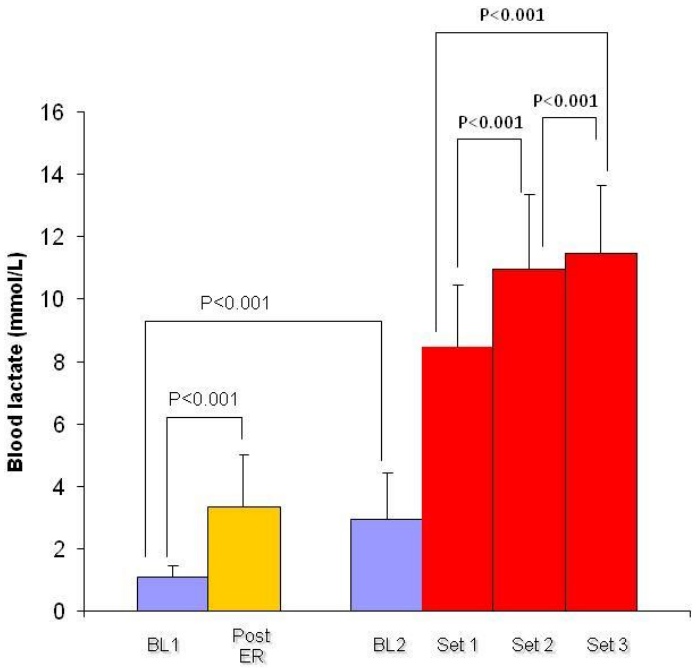

Figure 3.4 Lactate measures before and after the AM exercise session

Acute Exercise – PM (N=9)

There was no significance difference in blood lactate levels between the CHO and P condition during the ER or the sets of HIIE.

Placebo Group (N=9)

PM blood lactate levels in the placebo group before and after the acute bouts of ER and HIIR are illustrated in figure 3.5. Blood lactate levels were higher ($p<0.001$) than baseline-1 following the 20 min submaximal run and remained significantly elevated above baseline following a further 4 min of passive recovery. Blood lactate levels were significantly higher than baseline-2 after each sets of HIIE. In addition, there was a significant difference in blood lactate between set 1 and 2 and between set 1 and 3 of HIIE.

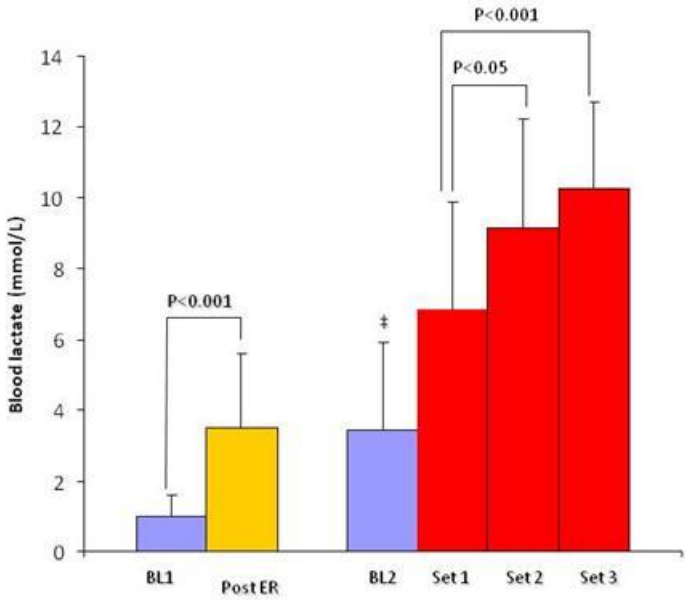

Figure 3.5 PM blood lactate levels in the placebo group before and after the PM exercise bout. ‡p< vs BL1

Carbohydrate Group (N=9)

PM blood lactate levels in the CHO group before and after the acute bouts of ER and HIIR are illustrated in figure 3.6. At the end of the 20 min submaximal run blood lactate levels were higher ($p<0.01$) than baseline 1 and remained significantly elevated above baseline following the 4 min of passive recovery. Blood lactate levels were significantly higher than baseline-2 after each set of HIIE. In addition, there was a significant difference in blood lactate levels between each set of HIIE.

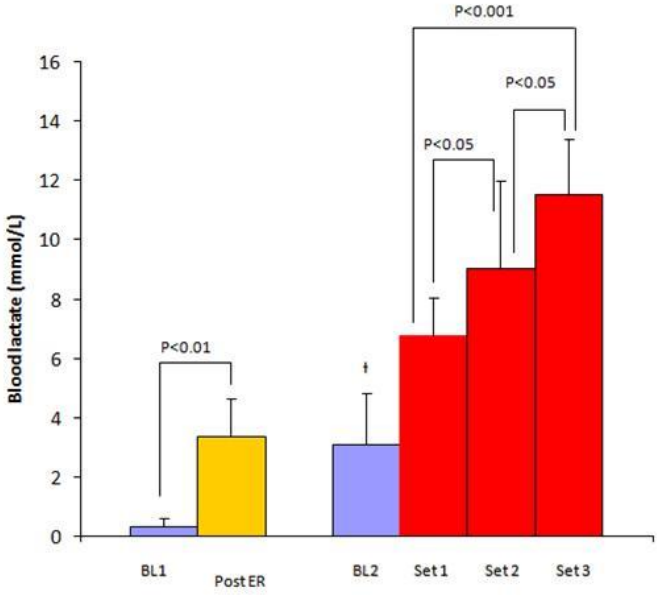

Figure 3.6 PM blood lactate levels in the CHO group before and after the PM exercise bout. †p<0.01 vs BL1

Performance Times During HIIE

Acute Exercise – AM (N=18)

The performance times were averaged across the 3 high intensity exercise bouts within each set and are presented in Figure 3.7. There was no difference in the performance time during the first and second set. The average time was significantly slower during the third set than the second set.

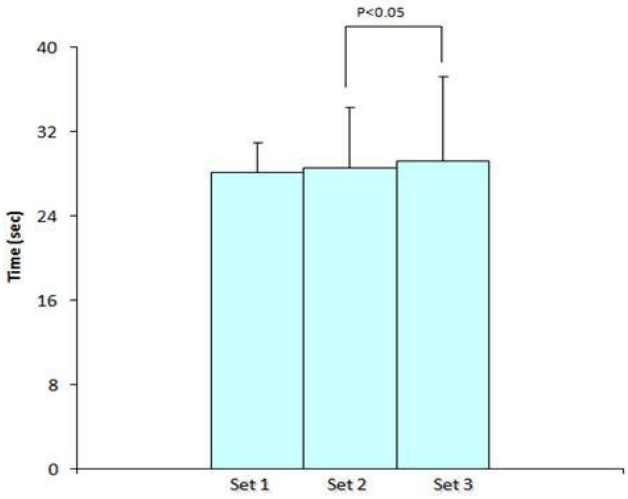

Figure 3.7 Performance times during each set of HIIR during the AM session

Acute Exercise – PM (N=9)

The performance times for the CHO and P group are illustrated in figure 3.8. There was no significant difference in performance times between any of the 3 sets of HIIR within the CHO or P condition. Performance time was significantly faster in the CHO than the P group during the first set of HIIR. Although performance time was faster in the CHO than the P during set 2 and set 3 it did not reach statistical significance ($p<0.08$). The average times in the CHO and P group over the 3 HIIR sets were 27 and 30 s in the AM exercise session and 26 and 31 s in the PM exercise session respectively. These averages were compared using a t-test and the CHO group was statistically faster than the P group in the AM exercise session ($p<0.03$). The CHO group in the PM exercise session was almost statistically faster than the P group ($p<0.056$)

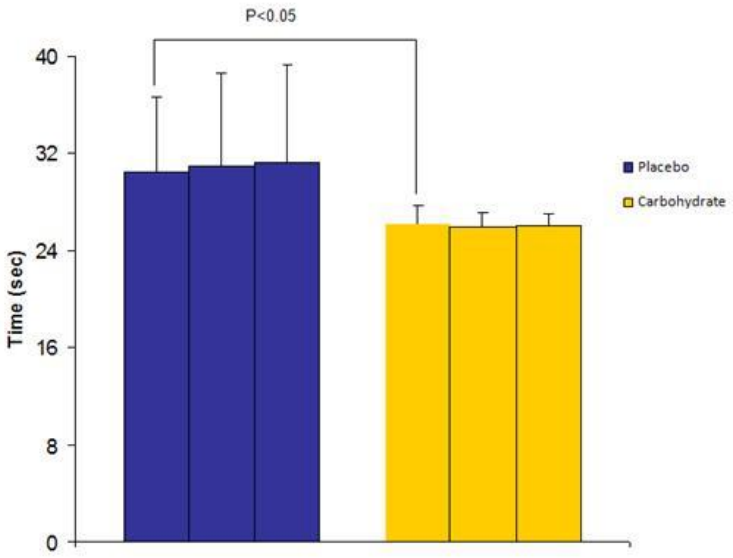

Figure 3.8 The average time taken to complete each set in placebo and CHO group

Muscle Levels of Glycogen, ATP, PCr and Cr Before and After the AM Exercise Session

Table 3.2 summarises the muscle levels of glycogen, ATP, PCr and Cr before and after the AM exercise session. Compared to baseline, muscle glycogen content decreased by 10% ($p<0.01$), and there was no significant change in ATP, PCr or Cr following the combined ER and HIIR session.

Table 3.2 Muscle levels of glycogen, ATP, PCr and Cr before and after the AM exercise session (n=18)

	Pre Exercise	Post Exercise
Glycogen (mmol·kg^{-1}.dw)	357.43 ± 115.68	288.24 ± 82.02†
ATP (mmol.kg^{-1} dw	18.78 ± 4.86	18.39 ± 3.73
PCr (mmol.kg^{-1} dw)	60.15 ± 17.73	60.16 ± 11.30
Cr (mmol.kg^{-1}.dw)	35.96 ± 10.37	43.07 ± 13.10

Values are means ± SD; †p < 0.01 vs. pre-training

Blood Parameters Before and After the AM Exercise Session

Table 3.3 summarises the measured blood parameters before and after the AM exercise session. Compared to baseline, blood levels of glucose, lactate, NEFA and glycerol were decreased (p<0.001) following the combined ER and HIIR session. There was no significant difference in plasma insulin levels before or after the AM exercise session.

Table 3.3 Blood parameters before and after the AM exercise session

	Baseline-1	Post Exercise
Glucose (mmol/L)	3.92 ± 0.65	6.01 ± 1.13‡
Lactate (mmol/L)	0.99 ± 0.38	10.48 ± 2.77‡
Non-esterified fatty acids (mmol/L)	0.08 ± 0.04	0.26 ± 0.14‡
Glycerol (µmol/L)	16.85 ± 6.23	196.83 ± 29.82‡
Insulin (µIU/ml)	71.98 ± 94.03	56.53 ± 45.92

Values are means ± SD; ‡p < 0.001 vs. pre-training

Muscle Levels of Glycogen, ATP, PCr and Cr Before and After the AM Exercise Session

Table 3.4 summarises the muscle levels of glycogen, ATP, PCr and CR before and after the PM exercise session. Compared to baseline, muscle glycogen content decreased significantly (p<0.001), in both CHO and P group (Figure 3.9). Muscle PCr content were also significantly lower after than before exercise in both CHO (p<0.01) and P groups (p<0.05).

Compared to baseline, there was no significant change in muscle levels of ATP or Cr in CHO or P after the exercise session.

Blood Parameters Before and After the PM Exercise Session

Table 3.4 summarises the measured blood parameters before and after the PM exercise session. Compared to baseline, blood levels of glucose, lactate, NEFA and glycerol were significantly decreased following the exercise session. Plasma insulin was lower ($p<0.05$) in P than CHO before the exercise session, and did not change relative to baseline in either condition after exercise.

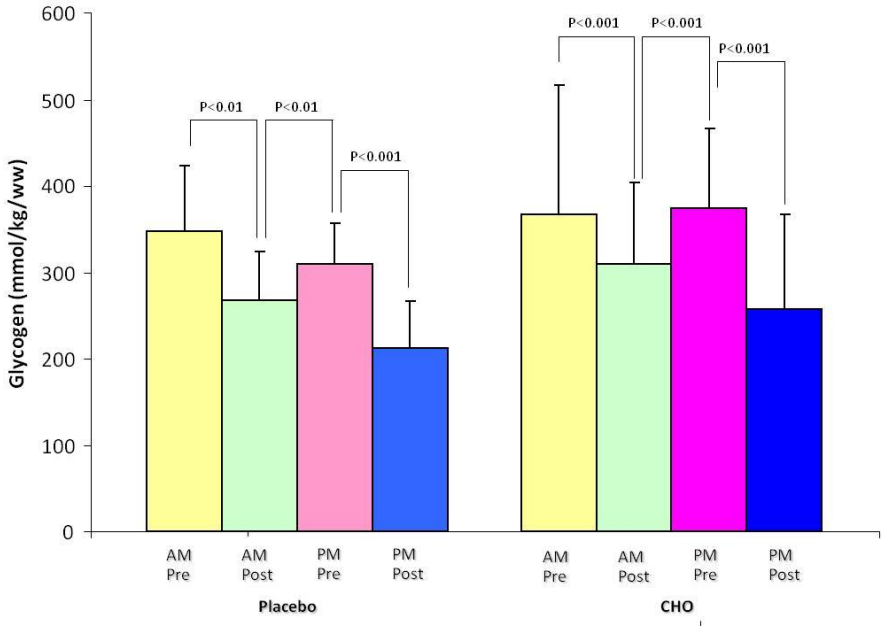

Figure 3.9 Glycogen levels before and after the AM exercise session and before and after the PM exercise session in the placebo and CHO group

Table 3.4 Muscle levels of glycogen, ATP, PCr and Cr before and after the PM exercise session

	Group			
	Placebo (n=9)		CHO (n=9)	
	Pre Exercise	Post Exercise	Pre Exercise	Post Exercise
Glycogen (mmol·kg^{-1}.dw)	309.76 ± 43.73	212.26 ± 50.66‡	374.27 ± 92.36	257.37 ± 110.28‡
ATP (mmol.kg^{-1}.dw	19.51 ± 3.67	20.58 ± 3.45	18.55 ± 2.43	18.23 ± 2.53
PCr (mmol.kg^{-1}.dw)	67.89 ± 8.48	52.49 ± 17.78*	68.47 ± 7.24	48.70 ± 18.90†
Cr (mmol.kg^{-1}.dw)	38.76 ± 6.55	50.77 ± 14.37	41.16 ± 9.45	53.40 ± 16.29

Values are means ± SD; *p < 0.05 vs. pre-training; †p < 0.01 vs. pre-training; ‡p < 0.001 vs. pre-training

Table 3.5 Pre and post exercise blood data before and after PM exercise

	Group			
	Placebo (n=9)		CHO (n=9)	
	Pre Exercise	Post Exercise	Pre Exercise	Post Exercise
Glucose (mmol/L)	4.33 ± 0.33	5.33 ± 1.29*	3.71 ± 0.57	5.35 ± 1.08‡
Lactate (mmol/L)	0.96 ± 0.46	8.38 ± 2.70‡	1.37 ± 0.23	11.72 ± 3.32‡
Non-esterified fatty acids (mmol/L)	0.40 ± 0.18	0.75 ± 0.21‡	0.07 ± 0.04	0.36 ± 0.14‡
Glycerol (µmol/L)	42.81 ± 17.05	221.07 ± 55.92‡	17.12 ± 6.62	208.08 ± 43.75‡
Insulin (µIU/ml)	32.26 ± 18.26 [a]	26.68 ± 18.19	85.86 ± 102.80	63.56 ± 41.04

Values are means ± SD; *p < 0.05 vs. pre-training; ‡p < 0.001 vs. pre-training; a p < 0.05 vs. CHO

Muscle Signalling Proteins

Tables 3.5 and 3.6 summarise the muscle signalling proteins levels before and after the AM and PM exercise session respectively. Maximal activity of ACCβ at baseline and after the AM and PM exercise sessions is illustrated in figures 3.10. Compared to baseline, phosphorylation and maximal activity of ACCβ increased significantly ($p<0.001$) after the first exercise bout. Following the PM exercise session, there was also a significant increase ($p<0.001$) in ACCβ activity in CHO and P compared to baseline.

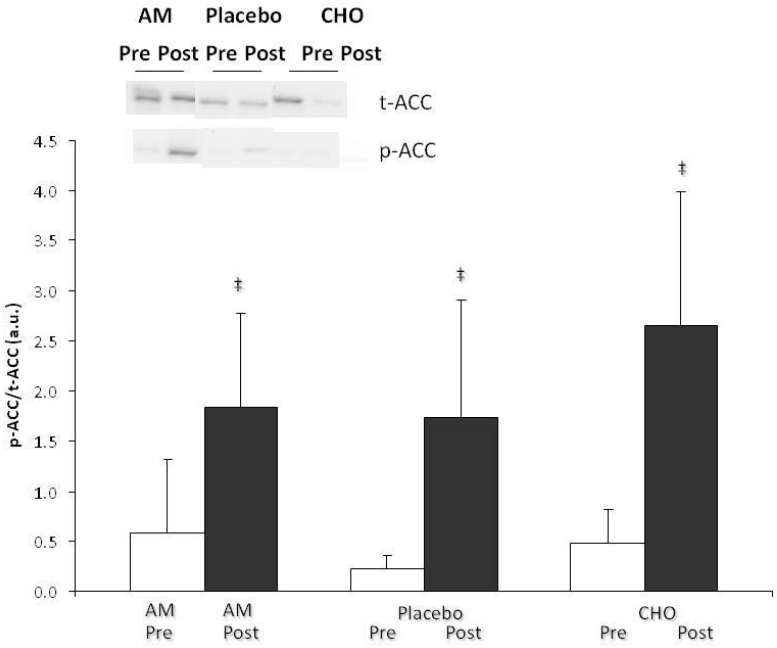

Figure 3.10 Maximal activity of Acetyl-CoA carboxylase β

Maximal activity of AMPK and P38MAPK after the AM and PM exercise sessions are illustrated in figures 3.11 and 3.12. There was no effect on either the AM or PM combined ER and HIIE session on total or phosphorylated AMPK or p38MAPK

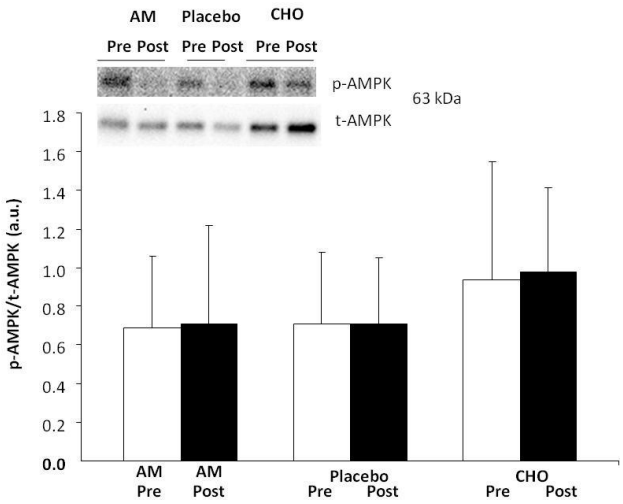

Figure 3.11 Maximal activity of Activated monophosphate protein kinase (AMPK)

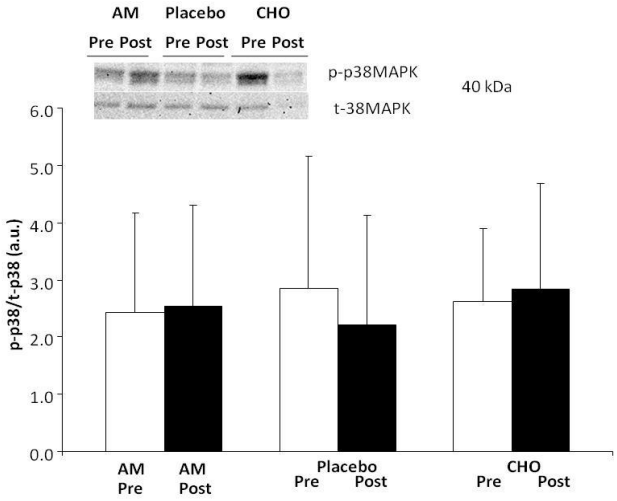

Figure 3.12 Maximal activity of p38 Mitogen activated protein kinase (p38AMPK)

Table 3.6 Signalling proteins before and after AM exercise

	Pre Exercise	**Post Exercise**
Phospho AMPK (A.U.)	0.71± 0.41	0.61 ± 0.38
Total AMPK (A.U.)	1.06 ± 0.54	1.11 ± 0.60
Phospho/Total AMPK	0.69 ± 0.37	0.71 ± 0.51
Phospho ACCβ (A.U.)	0.46 ± 0.43	1.23 ± 1.48*
Total ACCβ (A.U.)	1.12 ± 1.28	1.12 ± 1.96
Phospho/Total ACCβ (A.U.)	0.59 ± 0.72	1.83 ± 0.95‡
Phospho MAPK (A.U.)	3.81 ± 2.90	3.42 ± 2.18
Total MAPK (A.U.)	1.62 ± 0.63	1.41 ± 0.51
Phospho/Total MAPK(A.U.)	2.43 ± 1.74	2.53 ± 1.77

Values are means ± SD; *p < 0.05 vs. pre-training; ‡p < 0.001 vs. pre-training

Table 3.7 Signalling proteins before and after PM exercise

	Group			
	Placebo (n=9)		CHO (n=9)	
	Pre Exercise	Post Exercise	Pre Exercise	Post Exercise
Phospho AMPK (A.U.)	0.48 ± 0.22	0.64 ± 0.04	0.39 ± 0.22	0.63 ± 0.38
Total AMPK (A.U.)	0.71 ± 0.32	1.03 ± 0.71	0.50 ± 0.30	0.67 ± 0.42
Phospho/Total AMPK (A.U.)	0.71 ± 0.37	0.71 ± 0.34	0.94 ± 0.61	0.98 ± 0.43
Phospho ACCβ (A.U.)	0.12 ± 0.08	0.66 ± 0.62ł	0.12 ± 0.12	0.55 ± 0.49‡
Total ACCβ (A.U.)	0.48 ± 0.41	0.63 ± 0.99	0.30 ± 0.24	0.35 ± 0.48
Phospho/Total ACCβ (A.U.)	0.32 ± 0.13	1.74 ± 1.17‡	0.49 ± 0.32	2.65 ± 1.34ł
Phospho MAPK (A.U.)	3.26 ±2.40	2.88 ± 2.68	2.36 ± 1.13	3.18 ± 2.16
Total MAPK (A.U.)	1.22 ± 0.29	1.36 ± 0.51	1.22 ± 0.29	1.36 ± 0.51
Phospho/Total MAPK (A.U.)	2.85 ± 2.32	2.21 ± 1.92	2.61 ± 1.29	2.84 ± 1.85

Values are means ± SD; †p < 0.01 vs. pre-training; ‡ p < 0.001 vs. pre-training

Summary

After the AM exercise session, ACCβ was the only signalling molecule to increase. There was no effect of the exercise session on AMPK or p38MAPK. Although muscle glycogen was significantly decreased after the AM exercise session, the depletion rate was low. In the PM exercise session CHO had no effect on signalling pathways and ACCβ was again up-regulated in both groups. Muscle glycogen decrease significantly in both groups. Performance time was significantly faster in the CHO than the P group during the first set of HIIR during the PM exercise session. Although performance time was faster in the CHO than the P during set 2 and set 3 it did not reach statistical significance ($p<0.08$)

Chapter 4

STUDY 2

Introduction

It is well established that ET induces numerous physiological and metabolic adaptations that improve submaximal endurance capacity [32,215]. Although this type of training offers significant training adaptations it requires a large time commitment. Recent studies have shown that brief repeated sessions of maximal capacity HIIT induces changes in skeletal muscle energy metabolism that resemble endurance type training [12,13,23]. A study by Gibala *et al*., (2006) [22] found that 6 sessions of HIIT over 2 weeks, totaling approximately 15 min of maximal cycling increases the maximal activity of mitochondrial enzymes and improves performance during tasks that rely heavily on aerobic energy provision.

PGC-1α is a nuclear transcriptional co-activator that regulates several important metabolic processes, including mitochondrial biogenesis [137,171]. This transcriptional co-activator has the ability to act as an energy sensor for the cell, exerting its effects through interactions with transcription factors bound to specific DNA elements in the promoter region of genes, translating changes in metabolism into alterations in gene expression. PGC-1α co-activates a number of transcription factors, such as NRF-1, NRF-2 and ERRα, that lead to the transcriptional activation of mitochondrial Tfam and a number of nuclear-encoded respiratory proteins (Figure 4.1). Tfam directs both replication and transcription of the

mitochondrial genome which encodes fundamental respiratory proteins such as COX IV. Subunits comprising the ETC complexes (complex 1-V) are encoded by both nuclear and mitochondrial genome and therefore serve as markers of the co-ordinated expression of nuclear and mitochondrial gene transcription by PGC-1α.

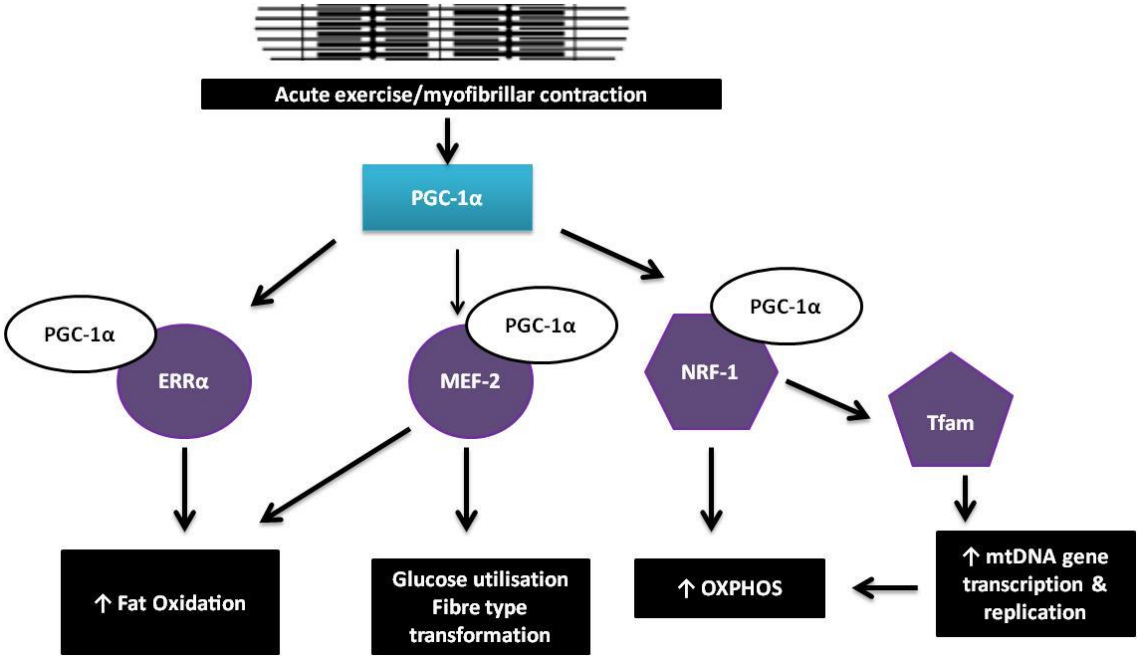

Figure 4.1 PGC-1α co-activation of transcription factors in skeletal muscle

Transcription factors, such as NRF-1, NRF-2 and ERRα, which stimulate the transcriptional activation of mitochondrial Tfam and a number of nuclear-encoded respiratory proteins which induce mitochondrial biogenesis. Peroxisome proliferator-activated receptor γ co-activator α, PGC-1α;NRF1 and 2, nuclear receptor factor 1 and 2; ERRα estrogen related receptor; mitochondrial transcription factor A

Recent HIIT studies have found a significant relation between increases in both AMPK and p38MAPK activities and increases in PGC-1α mRNA and skeletal muscle oxidative capacity. An increase in CS protein content [74], β-HAD maximal activity [11] and COX subunits of II and IV [74] was indicative of the enhanced oxidative capacity of the muscle. This demonstrates the role PGC-1α plays in enhancing mitochondrial biogenesis after HIIT [11,12,23].

With the exception of Bartlett *et al*., (2012) [84] Serpiello *et al*.,(2011) [85] and Morton *et al,* (2009) [24] all other studies on HIIT have involved cycle exercise. Bartlett and Morton used similar HIIR protocols (5 x 3 min at 90% $\dot{V}O_2$max separated by 3 min of active recovery between 25-50% $\dot{V}O_2$max) to determine the activation of mitochondrial biogenesis through p38MAPK and AMPK signalling pathways [84]. Serpiello *et al*.,(2011) [85] is the only study to date to demonstrated both the acute and chronic effects of sprint training on the molecular signalling pathways associated with mitochondrial biogenesis.

Study Aim

To compare the physiological and metabolic responses of two weeks of HIIR or ER and the subsequent effects on endurance exercise performance.

Study Objectives

1. To compare changes in p38MAPK, AMPK, PGC-1α, Tfam protein content, the enzymatic activity of CS and β-HAD and the protein content of oxidative enzymes, complex I-V, in skeletal muscle before and after 6 sessions of either HIIR and ER
2. To compare resting muscle glycogen concentration before and after 6 sessions of either ER and HIIR
3. To compare the physiological and metabolic responses in response to 6 sessions of ER and HIR training
4. To compare the effect of 6 sessions of either ER and HIIR on endurance exercise performance

Study Hypothesis

1. There will be a similar increase in p38 MAPK, AMPK, PGC-1α, Tfam protein content, the enzymatic activity of CS and β-HAD and the protein content of oxidative enzymes, complex I-IV in the ER and HIIR groups after 6 exercise sessions.
2. Resting muscle glycogen concentration will have increased to a similar extent in the HIIR and ER groups after 6 exercise sessions
3. $\dot{V}O_2$ max will not increase in either the HIIR or ER group after 2 weeks of training.

4. There will be a greater performance enhancement in the HIIR group in comparison to the ER group after 6 sessions of training

Methods

Subjects

Fourteen club level Gaelic footballers (mean ± SD; age 21.71 ± 2.28 yr; BMI 24.25 ± 1.25 $kg \cdot m^2$; $\dot{V}O_2max$ 55.14 ± 4.90 $ml \cdot min^{-1} \cdot kg^{-1}$) participated in the study. Participants were fully informed of the experimental procedures and possible discomforts associated with the study before giving their written informed consent to participate. The study was approved by the Ethics Committee at Dublin City University (DCUREC 148).

Overview of Study Design

Participants were randomly assigned to either an ER or a HIIR group. The study design is outlined in figure 4.2 and involved either 6 HIIR or ER sessions over a 2 week period. The experimental procedure consisted of baseline testing, 2 weeks of HIIR or ER, and post-training measurements. A muscle biopsy and blood sample was taken before and after the training period. Participants were instructed to continue their normal dietary practices throughout the study but refrain from alcohol and caffeine 24 h prior to each laboratory visit.

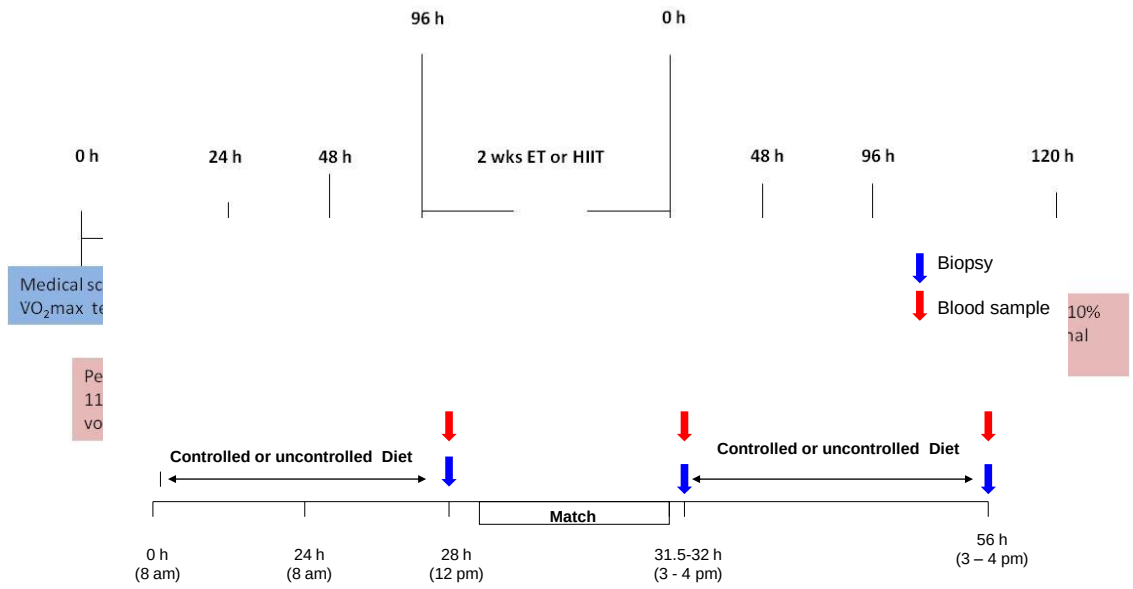

Figure 4.2 Study 2 Research design

Baseline testing

Participants made 3 separate visits to the Human Performance Laboratory at DCU. Each visit was separated by 24 h. The first visit was used to assess anthropometric characteristics and measure maximal aerobic capacity ($\dot{V}O_2max$). During the second visit participants performed an endurance run to exhaustion at 110% v$\dot{V}O_2$ max. A resting needle muscle biopsy sample and a blood sample were taken during the final screening visit. The post-training muscle biopsy was taken 48 h after the last exercise session with additional post training measurements taken after a further 48 h.

Anthropometrics-

(As described in the methods section of chapter 3)

Maximal Aerobic Capacity Assessment/Lactate Threshold

Maximal aerobic capacity was determined on a treadmill (Woodway ELG 55, Waukesha, WI) using a ramp protocol. At baseline and at the end of each 3 min stage a 5 μL blood sample was taken from the earlobe to determine whole blood lactate concentration. Subjects warmed-up at 8 $km \cdot h^{-1}$ for 3 min at 1% gradient. Following the warm-up, the speed was increased by 1 $km \cdot h^{-1}$ every 3 min until blood lactate concentrations reached 4.0 $mmol \cdot L^{-1}$. Treadmill velocity was then kept constant and the gradient increased by 1% every 60 s until the subject reached volitional fatigue. Heart rate and RPE were recorded during the final 10 s of each exercise min.

Performance Test

Participants warmed up for 5 min on a treadmill (Woodway ELG 55, Waukesha, WI) at 50% of their calculated $v\dot{V}O_2$ speed. The speed was then increased to 110% of their calculated $v\dot{V}O_2$ speed and participants ran until volitional fatigue.

Training Program

Participants commenced the training protocol 48 h following the final baseline screening visit. Training involved 3 sessions of HIIR or ER per week for 2 weeks. Endurance training consisted of 50 min of continuous treadmill running at a velocity corresponding to 75% $\dot{V}O_2max$. Before and after each ET session a 5 μL blood sample was taken from the earlobe to determine whole blood lactate concentration. The HIIT protocol involved 3 sets

of high intensity running interspersed with short recovery periods (Figure 4.3). Each interval run was 110 m and involved forward and backward sprints over distances ranging from 10-20 m. A set consisted of 3 x 110 runs with a 20 s recovery period between each run, and a 5 min recovery period between sets. At the end of each run a 5 μL blood sample was taken from the earlobe to determine whole blood lactate concentration.

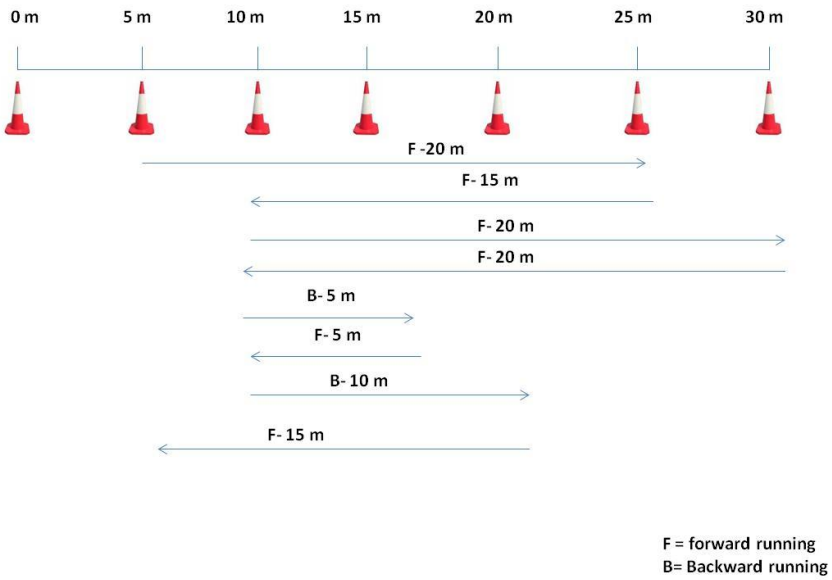

Figure 4.3 High intensity interval running (HIIR) protocol

Post-training measurements

Approximately 48 h following the last training session, a resting muscle biopsy was obtained from the same leg as used for the first biopsy, separated by 2-3 cm from the original incision site. A blood sample was also obtained. Subjects also performed the same pre-training tests after the training protocol, starting with the muscle biopsy, $\dot{V}O_2$ test and performance test (figure 4.2).

Cardiorespiratory and Metabolic Measures

(As described in the methods section of chapter 3).

Mass Flow Sensor Heated Wire Anemometer-Mode of Operation

As described in the methods section of chapter 3.

Mass Flow Sensor Calibration

As described in the methods section of chapter 3.

Gas Analysers

As described in the methods section of chapter 3.

Calibration of CO_2 and O_2 Analysers

As described in the methods section of chapter 3.

Ratings of Perceived Exertion

As described in the methods section of chapter 3.

Lactate Measurements

Blood samples were drawn from the earlobe. Prior to each sample, the ear lobe was wiped with alcohol and allowed to dry thoroughly. The base of the ear lobe was jabbed with a lancet (Accu-Chek Softclix, UK) and the first drop of blood was wiped away. Pressure was placed on ear lobe with the thumb and forefinger in order to provide an appropriate sample. A 5 μL sample of whole blood was automatically aspirated into a single use, enzyme-coated electrode test strip (Lactate Pro Akray, Japan). The reagent strip fills by capillary action directly from the earlobe site.

Blood samples were analysed using and a hand-held portable analyser (Lactate Pro Akray, Japan). The measuring range is 0.8–23 mM. Lactate in the sample reacts with potassium ferricyanide and lactate oxidase to form potassium ferrocyanide and pyruvate. Upon the application of a given voltage, ferrocyanide is oxidised, releasing electrons and creating a current. This current is measured amperometrically and is directly proportional to the lactate concentration of the blood sample. The result is displayed after 60 s. The Lactate Pro is supplied with a check strip and a calibration strip that provide a non-quantitative indication of instrument accuracy.

Muscle Biopsy

As described in the methods section of chapter 3.

Muscle enzyme activity

Two pieces of frozen wet muscle (10–15 mg) was removed from each of the first biopsy samples under liquid nitrogen for western blotting and for the spectrophotometric determination of mitochondrial CS and β-HAD,, maximal activities (37 °C), as described elsewhere [211,216]. An aliquot of each muscle enzyme homogenate (citrate synthase and β-HAD) was then extracted with 0.5 mol·L^{-1} perchloric acid ($HClO_4$), containing 1 mmol·L^{-1} EDTA, and neutralized with 2.2 mol·L^{-1} $KHCO_3$. The supernatant from the extracts was used for the enzymatic spectrophotometric determination of total creatine [211]. Enzyme measurements were normalized to the highest total creatine content measured from all biopsies from each subject.

Muscle metabolites and glycogen

As described in the methods section of chapter 3.

Muscle calculations

As described in the methods section of chapter 3.

Western Blots Analysis

Muscle Homogenization: Frozen muscle samples (30 mg) were added to ice-cold lysis buffer (50 mM Tris-HCl, 1mM EDTA, 1 mM EGTA, 50mM NaF, 1% Triton X-100, 10 mM sodium β-glycerol phosphate, 5 mM sodium pyrophosphate, 2 mM DTT, 1mM PMSF, 0.004% protease inhibitor cocktail (Sigma Aldrich, Oakville, ON), and 1mM Na orthovanadate). Samples were homogenized by machine for 1 min using a FastPrep-24 Instrument (MP Biomedicals, Solon, OH), samples were then centrifuged at 1500 x g for 15 min at 4°C, and the supernatant was obtained. Protein concentration of the homogenate was determined using a BCA assay. Muscle homogenate was solubilized in 4 x Laemmli's buffer, boiled (95°C, 5 minutes), resolved by SDS-PAGE, and wet-transferred to polyvinylidene difluoride membranes [1–1.5 hour, 100 V]. The membranes were blocked for 1 h and then incubated overnight at 4°C with commercially available primary antibodies to measure, AMPKα (1:1000), and p38MAPK (1:1000), (Cell Signaling technology, Danvers, MA), PGC-1α (1:1000), (Calbiochem, La Jolla, CA, USA), Tfam (1:200), (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and complex I-IV (1:1000), (MitoProfile Human Total OXPHOS Human WB Antibody Cocktail, Mitosciences, USA). Secondary antibodies were purchased from Cell Signaling technology, except for those used for Tfam, which were supplied by Santa Cruz. Dilutions for secondary antibodies were as follows: AMPKα (1:2000), and p38MAPK (1:2000), PGC-1α (1:1000), Tfam (1:1000) and complex I-IV (1:1000).

After incubation with appropriate secondary antibody for 1 h, the immune complexes were detected using the enhanced chemiluminescence method (Western Lightning Plus-ECL; PerkinElmer, Waltham, MA), and quantified with densitometry (Gene Tools software, PerkinElmer). Equal loading was confirmed using alpha tubulin (Cell Signaling technology, Danvers, MA).

Blood Measurements

Venous whole blood was collected in 4 ml vacutainers. The supernatant was stored at –20 °C and analyzed for glycerol [211] and non-esterified fatty acids (NEFA) (Randox, UK) with a colorimetric assay and insulin with a magnetic bead panel (Millipore, Cork, Ireland).

Statistical Analysis

SPSS 19 for Windows statistical software was used to perform the statistical analysis. The Kolmogorov-Smirnov test and the Levene's test were used to asses for normality and homogeneity of variance, respectively. Since both tests were significant a paired sample Wilcoxon sign tank test was used to compare within groups, and a Mann Whitney test was used to compare between groups. Statistical significance will be accepted at the $P < 0.05$ level of confidence.

Results

Maximal Aerobic Capacity

Table 4.1 summarises the physiological responses at maximal exercise in the HIIR and ER group before and after training. There was no difference in oxygen uptake, minute ventilation, RPE, or maximal heart rate at maximal exercise in the HIIR or ER group prior to training. There was no significant change in $\dot{V}O_2max$ in either group in response to training. Compared to pre-training, maximal minute ventilation was higher ($p< 0.05$) in the HIIR group after training. Maximal heart rate after training was significantly lower than pre training.

Table 4.1 Physiological responses at maximal exercise in the HIIR and ER group before and after training

	Group			
	HIIT (n=7)		ET (n=7)	
	Pre Training	**Post Training**	**Pre Training**	**Post Training**
Age (yr)	21	-	22	-
Height (cm)	177.11	-	174.82	-
Weight (kg)	74.81 ± 7.88	74.91 ± 7.41	75.14 ± 6.82	75.26 ± 6.64
$\dot{V}O_2$max ($ml \cdot kg^{1} \cdot min^{1}$)	51.48 ± 4.55	55.37 ± 4.34*	51.27 ± 1.88	54.05 ± 4.50
$\dot{V}O_2$max ($L \cdot min^{1}$)	4.03 ± 0.45	4.33 ± 0.50	3.77 ± 0.46	3.98 0.48
Maximal ventilation ($L \cdot min^{1}$)	97.54 ± 18.32	113.21 ± 16.13*	99.5 ± 17.33	105 ± 20.62
RER	1.11 ± 0.08	1.10 ± 0.08	1.10 ± 0.06	1.07 ± 0.07
Maximal heart rate (bpm)	184.24 ± 12.93	189.12 ± 9.62	200.4 ± 12.53	188.8 ± 10.51*
RPE	19.00 ± 1.41	19.71 ± 0.49	19.29 ± 0.76	20.00 ± 0.00

Values are means ± SD; *p < 0.05 vs. pre-training

Performance Test

There was no difference between groups in time to exhaustion at $v\dot{V}O_2$ max at baseline. Time to exhaustion at $v\dot{V}O_2$ max was increased in both the HIIR ($p < 0.05$) and ER group ($p < 0.01$) in response to training (Figure 4.4).

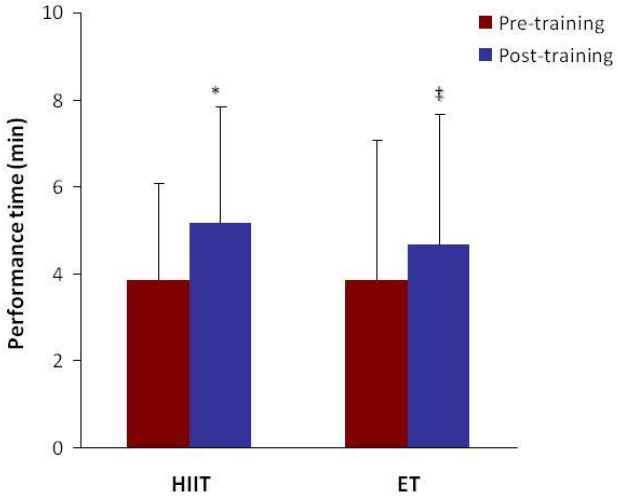

Figure 4.4 Performance increase (min) before and after training in the high intensity interval running (HIIR) and endurance running (ER) group

*$p < 0.05$ vs. pre-exercise; ‡$p < 0.001$ vs. pre-exercise

Blood Lactate Levels During HIIR and ER Training Sessions

Preliminary analysis using a one-way repeated measures ANOVA, compared baseline blood lactate levels in the ER group prior to each session and at the end of each of the 6 training sessions. There was no significant difference in blood lactate levels before or immediately following any of the 6 training sessions. Similarly there was no difference in baseline blood lactate levels or blood lactate levels following set 1, 2 and 3 during any of the 6 training sessions in the HIIR group. Baseline and post exercise blood lactate levels in the ER group were therefore averaged during each training session and compared using a paired sample t-test. A significant difference was then observed between baseline and each set.

Muscle Metabolites

Resting muscle glycogen, PCr, and ATP content was similar in both groups prior to training (table 4.2). Compared to pre-training, resting muscle glycogen content increased significantly after training in the HIIR group, only. There was also a decrease in resting ATP ($p<0.05$) and an increase in PCr ($p<0.05$) content in the HIIR group after training.

Table 4.2 Muscle levels of glycogen, ATP, PCr and Cr in the HIIR and ER groups before and after exercise training

	Group			
	HIIT (n=7)		ET (n=7)	
	Pre Training	Post Training	Pre Training	Post Training
Glycogen ($mmol \cdot kg^{-1} \cdot dw$)	426.71 ± 120.62	575.42 ± 142.13*	426.34 ± 63.63	444.76 ± 97.53
ATP ($mmol \cdot kg^{-1} \cdot dw$)	24 ± 2	21.62 ± 1.52*	24.24 ± 3.74	22.47 ± 1.65
PCr ($mmol \cdot kg^{-1} \cdot dw$)	79.62 ± 4.83	80.45 ± 4.64*	79.63 ± 4.34	77.36 ± 4.44
Cr ($mmol \cdot kg^{-1} \cdot dw$)	38.57 ± 4.23	38.32 ± 3.93	39.22 ± 3.72	41.64 ± 4.93

Values are means ± SD; *p < 0.05 vs. pre-training

Muscle Signalling Proteins

Compared to baseline there was a significant increase in AMPK protein content ($p<0.05$) in the HIIR group (table 4.3) (Figure 4.5). There was no significant difference in p38MAPK before or after training in groups (Figure 4.6).

PCC-1α, ETC Complex Subunits and Tfam Protein

There was no difference in pre-training levels of either complex PGC-1α or any of the subunits of the ETC complexes in the ER or HIIR group. Complex I, II, III, IV, V and PGC-1α protein were significantly increased ($p<0.05$) following 6 sessions of HIIR training. In contrast, ER training significantly increased complex V and PGC-1α ($p< 0.05$) only (table 4.3). There was no significant difference in Tfam in either group. The total level of each protein is illustrated in figure, 4.7- 4.13.

Table 4.3 Signalling and transcription proteins

	Group			
	HIIT (n=7)		ET (n=7)	
	Pre Training	Post Training	Pre Training	Post Training
AMPK (A.U)	737.3 ± 149.41	797 ± 162*	716.67 ± 176.18	879.28 ± 188.74
MAPK (A.U.)	81.72 ± 17.31	89.23 ± 13.91	81.65 ± 16.42	82.23 ± 8.85
Tfam (A.U.)	466.75 ± 148.27	546.45 ± 205.27	571.79 ± 165.12	662.69 ± 264.32
Complex I (A.U.)	336.68 ± 92.77	486.56 ± 194.72*	429.33 ± 127.78	496.81 ± 164.72
Complex II (A.U.)	371.41 ± 243.24	488.67 ± 273.09*	451.36 ± 158	555.09 ±287.81
Complex III (A.U.)	246.74 ±125.32	293.1 ± 113.45*	243.08 ± 86.48	311.2 ±104.37
Complex IV (A.U.)	850.63 ± 428.91	966.65 ± 474.92*	1089.85 ± 412.11	1172.285 ± 414.02
Complex V (A.U.)	505.29 ± 131.28	572 ± 141.17*	472.25 ± 149.31	563.68 ± 166.78*
PGC-1α (A.U.)	150 ± 29.69	182.74 ± 34.16*	123.45 ± 19.13	181.47 ± 47.23*

Values are means ± SD; *p < 0.05 vs. pre-training

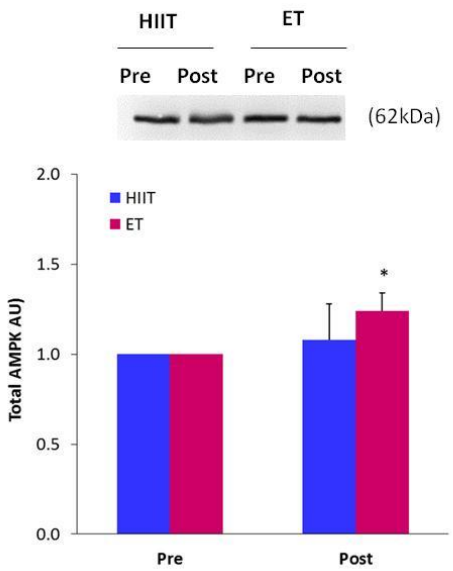

Figure 4.5 Total adenosine monophosphate kinase (AMPK) pre and post training in the HIIR and ER group. *p < 0.05 vs. pre-exercise

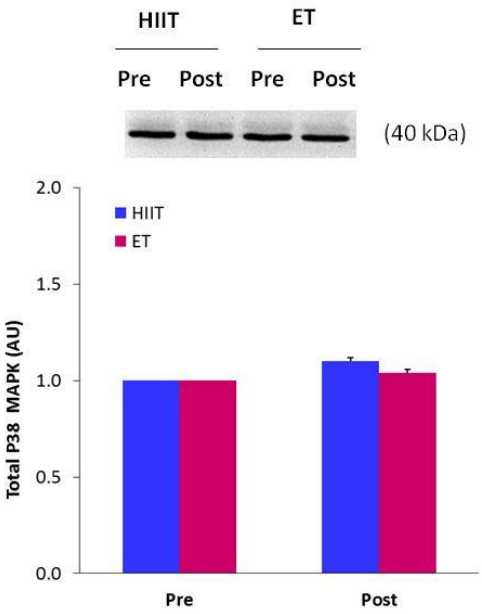

Figure 4.6 p38 mitogen activated protein kinase (p38MAPK) pre and post training in the HIIR and ER group

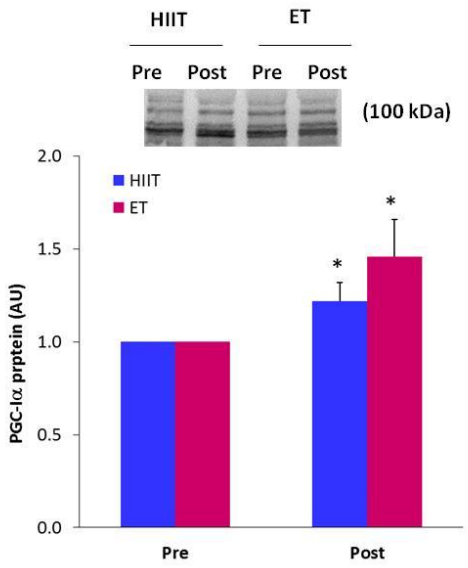

Figure 4.7 Peroxisome proliferator activated receptor γ co-activator 1-α (PGC-1α) pre and post training in the HIIR and ER group. *p < 0.05 vs. pre-exercise

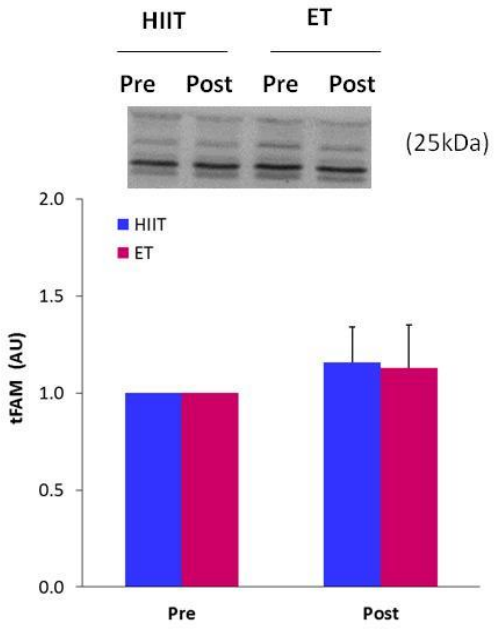

Figure 4.8 Transcription factor A (Tfam) pre and post training in the HIIR and ER group

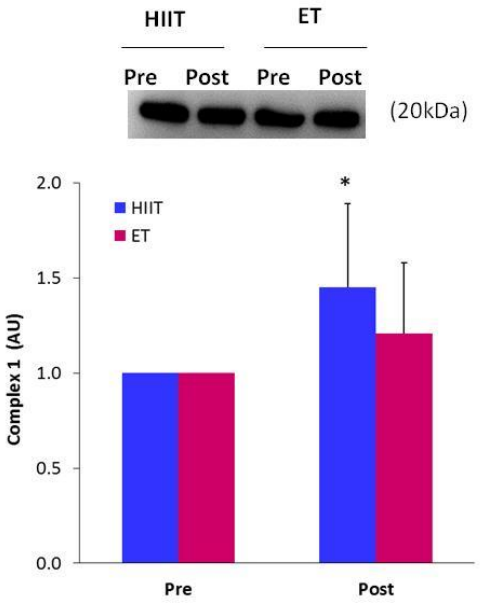

Figure 4.9 Complex I subunit pre and post training in the HIIR and ER group.
*p < 0.05 vs. pre-exercise

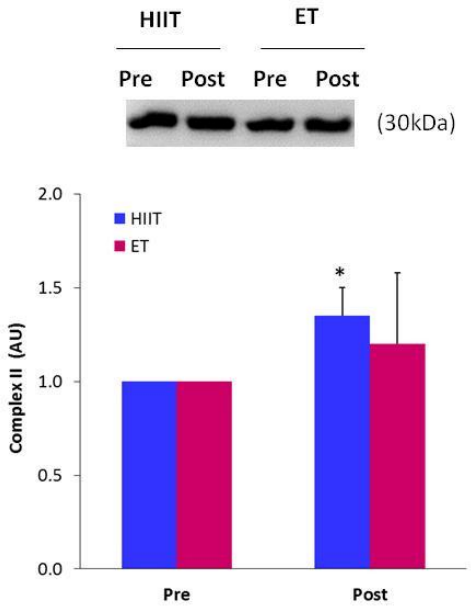

Figure 4.10 Complex II subunit pre and post training in the HIIR and ER group.
*p < 0.05 vs. pre-exercise

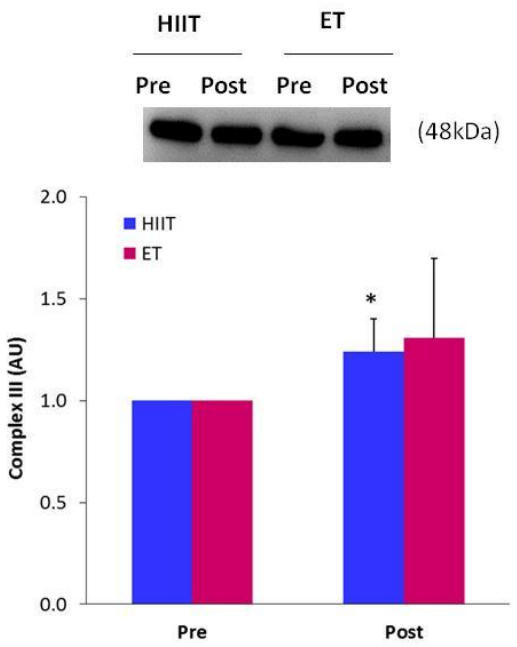

Figure 4.11 Complex III subunit pre and post training in the HIIR and ER group.
*p < 0.05 vs. pre-exercise

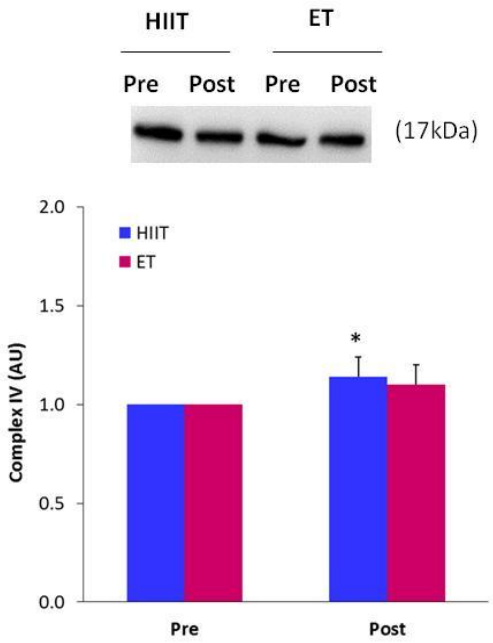

Figure 4.12 Complex IV subunit pre and post training in the HIIR and ER group.
*p < 0.05 vs. pre-exercise

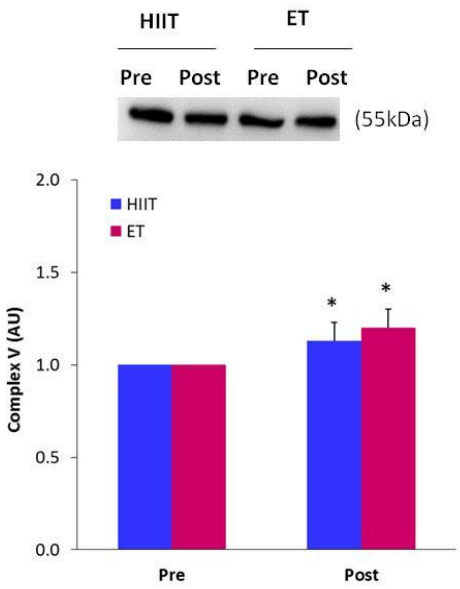

Figure 4.13 Complex V subunit pre and post training in the HIIR and ER group
*p < 0.05 vs. pre-exercise

Skeletal Muscle Mitochondrial Enzymes

There was no difference in the activity of CS or β-HAD in the HIIR or ER group before training. The activity of CS and β-HAD were significant higher ($p < 0.05$) than baseline in the HIIT and ET groups respectively following the 6 training sessions. (Figure 4.14-4.15).

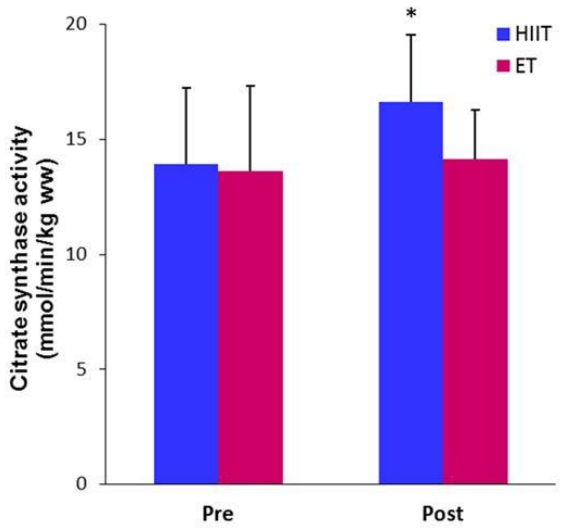

Figure 4.14 Citrate synthase (CS) maximal activity pre and post training in the HIIR and ER group. *$p < 0.05$ vs. pre-exercise

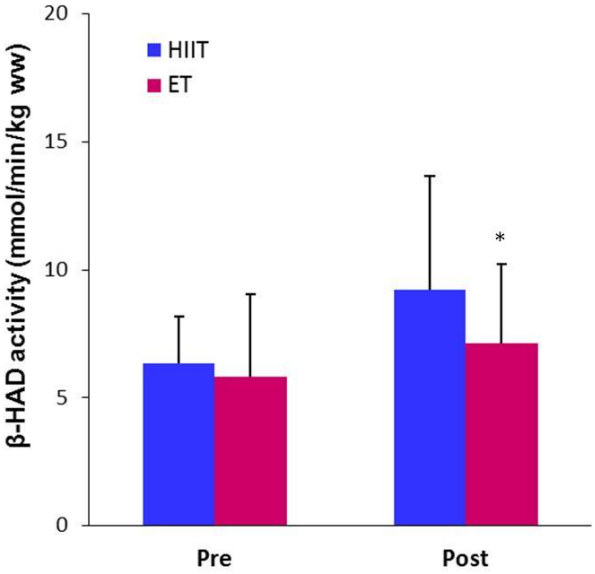

Figure 4.15 3 hydroylacyl-CoA dehydrogenase (β-HAD) maximal activity pre and post training in the HIIR and ER group. *p < 0.05 vs. pre-exercise

Summary

There was a significant increase in resting glycogen levels and AMPK protein levels in the HIIR group only, after training. There was a significant increase in subunits of complex I-V of the ETC in the HIIR group after training, whereas, there was only an increase in the subunit of complex V in the ER group after training. There was no increase in Tfam in either group after training. PGC-1α was significantly elevated in both groups after training. Endurance performance also improved in both the HIIR and ER groups after training. Training also induced a significant increase in the activity of CS and β-HAD in the HIIR and ER groups respectively.

Chapter 5

Discussion

Overview

There is a growing body of evidence to indicate that HIIT has the potential to stimulate skeletal muscle remodeling similar to that associated with ET. Contraction induced signal transduction pathways involved in the initiation of skeletal muscle mitochondrial biogenesis are activated after a single bout of high intensity exercise. Previous studies have found that as little as 6 sessions of maximal high intensity cycling, totaling 15 min of exercise, increases the maximal activity of mitochondrial enzymes and improves submaximal endurance exercise performance. Other adaptations elicited by HIIT and ET include an increase in muscle content of mitochondrial proteins associated and alterations in substrate utilization at the same absolute workload.

Considering that the majority of previous HIIT studies involved untrained individuals, it is perhaps not surprising that there were significant alterations in mitochondrial protein content and function. The present series of studies examined whether acute and chronic exercise of sufficient stimulus would illicit similar alterations in signalling responses and subsequent mitochondrial biogenesis in trained individuals involved in Gaelic football, an invasive team based field sport. The acute session of HIIR was not found to be sufficient to increase the phosphorylation of either AMPK or p38MAPK, although a downstream marker of AMPK activation, ACCβ was increased in response to HIIR, suggesting the possibility of a

transient increase in AMPK signalling. Six sessions of HIIR over a two week period did however, induce a more pronounced increase in mitochondrial biogenesis compared to six sessions of ER.

Study 1

It was hypothesized that a single bout of HIIR would activate specific signalling pathways that regulate gene expression and RNA translation, a key response in early training adaptations to HIIE[57]. AMPK is a key energy sensing enzyme that is activated in skeletal muscle during exercise. It promotes energy production to ensure cellular homeostasis. Activation of AMPK may be related to relative work intensity, with higher workloads related to more pronounced changes in AMPK phosphorylation [88], [217].

A number of recent studies found an enhanced activation of AMPK in response to an acute bout of HII cycling [23,25,82]. Exercise induced phosphorylation of AMPK, a marker of AMPK's activity is well documented in skeletal muscle following HIIE [13,23,25]. In the present study there was no increase in AMPK phosphorylation in response to an acute bout of HIIR. An increase in AMP concentration activates AMPK [57]. Since repeated high intensity sprints induce rapid changes in energy turnover and therefore alter the ATP to AMP ratio, it was expected that AMPK activation would significantly increase after the bout of HIIR. In separate studies muscle ATP levels were found to decrease following 4 Wingate tests interspersed with 4 min recovery periods and after a single 30 s maximal cycle bout [82,218]. Skeletal muscle content of ATP did not significantly change in response to the high intensity interval running session used in the present study, despite the fact that blood lactate levels ranged from 11-14 mmol·L.

To date, only two studies have found an increase in AMPK phosphorylation following an acute bout of HIIR [84,85]. Bartlette *et al*., (2012) [84] had participants perform 6 x 3 min

bouts of HIIR at 90% $\dot{V}O_2$max for a total of 18 min of high intensity activity. In contrast, only 2.5 min of HIIR was undertaken in the present study. It is possible therefore, that a minimum duration of HIIR may be required to induce a significant increase in AMPK activation. Serpiello *et al.,* (2011) [85] examined AMPK activation 1 h following 3 sets of 5 x 4 s sprint runs in recreationally active men. From the published data it is difficult to determine whether AMPK activity was statistically increased after exercise, but an effect size, which the author deemed to be moderate was reported. Furthermore, the change in AMPK was found 1 h after the bout of HII running. This finding contradicts other studies that have found an increase in AMPK immediately after exercise, with a decreased response within 3 h of recovery [23].

The fitness characteristics of the participants may also help to explain the absence of an increase in AMPK activity after the acute bout of HIIR in the present study. AMPK is attenuated in trained muscle compared to untrained at the same relative exercise intensity, but is still significantly elevated above basal levels [117,219]. An increase in skeletal muscle mitochondrial density allows trained individuals to attain the same cellular rate of mitochondrial oxidative phosphorylation with a lower rate per mitochondrion for any absolute workload [30]. ATP and PCr decrease to a smaller degree and free ADP concentrations increase to a lesser extent in cells with elevated mitochondrial contents at the same absolute workload. Better preservation of free ADP lowers the rate of AMP formation and therefore decreases AMPK activation and that of its upstream activators. However, in endurance trained men, cycling for 20 min at 80% $\dot{V}O_2$peak been shown to

increase AMPK phosphorylation despite PCr and ADP concentrations and the AMP/ATP ratio being unchanged [79].

A unique feature of the present investigation is the fact that the participants were trained Gaelic football players. Gaelic football is one of the most popular sports in Ireland and can be described as a hybrid of soccer, rugby and basketball. It involves single or repeated bouts of activity involving high running velocities and muscle power. It is possible, that the capacity of the muscle to sense energy changes in Gaelic football players is therefore more sensitive to that of endurance trained athletes allowing the AMPK response to returned to baseline before the muscle biopsy was taken.

ACCβ enzyme is considered a sensitive indicator of skeletal muscle AMPK activity *in vivo* [82]. Recent HIIT studies have found a large increase in ACCβ phosphorylation coinciding with an increase in AMPK phosphorylation in both running and cycling [23,85]. The fact that the phosphorylation of the downstream enzyme ACCβ increased 3 fold above resting values following the combined ER and HIIR session suggests that there may have been a transient activation of AMPK. Muscle glycogen is also a regulator of AMPK activity [208]. In the present study muscle glycogen content decreased by 69 $mmol^{-1} \cdot kg^{-1} \cdot ww$. Pilegaard *et al*., (2001) suggests that there is a threshold of glycogen depletion between 100-478 $mmol^{-1} \cdot kg^{-1} \cdot ww$ Several exercise studies have found AMPK activity increased when glycogen content was decreased by ≥ 100 $mmol^{-1} \cdot kg^{-1} \cdot ww$ [23–25]. It is therefore possible that the decrease in muscle glycogen levels did not meet the required threshold to enhance AMPK activation.

P38MAPK is a stress activated protein kinase that couples cellular disturbances to various adaptive and maladaptive responses in skeletal muscle [108]. The activation of p38MAPK in skeletal muscle is to some extent dependant on the nature of exercise [3] and/or training status [217]. A number of studies have found an enhanced activation of p38MAPK after an acute bout of HII exercise [13,23,25]. Exercise induced activation of p38MAPK may be mediated by the energetic status of the cell. Similar to the activation of AMPK after HIIT, p38MAPK is also activated in response to an acute bout of HII cycling and lower intensity but higher volume running [23,84]. There was no activation of p38MAPK following the acute bout of combined ER and HII running in the present study, which is consistent with the study undertaken by Serpiello *et al.,* (2011) [85] that involved 3 sets of 5 x 4 s sprint runs in recreationally active men.

In the second part of this study it was hypothesized that compared to feeding a meal low in CHO, feeding a high dose of CHO would blunt the activation of signalling pathways involved in skeletal muscle mitochondrial biogenesis. There was no significant effect of altering CHO feeding during recovery on subsequent exercise induced phosphorylation of AMPK or p38MAPK.

CHO feeding during recovery had no effect on muscle glycogen levels or AMPK activity. AMPK consists of a glycogen binding domain that is allosterically inhibited when bound to glycogen. Previous studies have found that exercise increases AMPK activity when glycogen levels are significantly reduced [79]. In the present it was expected that feeding a large dose of CHO during the recovery period would enhance glycogen stores compared to

the placebo group prior to the PM exercise bout. This increase in glycogen content was expected to reduce AMPK activity. However, there was no significant difference in muscle glycogen levels between the CHO and placebo group after feeding. There was also a similar decrease (32%) in glycogen content in both groups after exercise. In a recent study Coffey *et al.,* (2011) [220] found that feeding a CHO-whey supplement had no effect on AMPK activity following 10 x 6 sprint cycle exercises in healthy men .

Phosphocreatine (PCr) is also a known activator of AMPK [19] and therefore changes in these levels must also be considered when interpreting the lack of AMPK phosphorylation observed after exercise. Phosphocreatine is a high energy compound made up of the amino acid creatine linked to phosphate by a high energy anhydride bond. The PCr concentration in resting muscle is 75-85 mmol·kg dm, which is five to six times greater than ATP [221]. The energy released from the breakdown of PCr is coupled to the synthesis of ATP in a reversible reaction catalyzed by creatine kinase; $PCr + ADP + H^+ \leftrightarrow ATP + Cr$. The forward reaction is favoured during muscle contraction. PCr levels are directly related to power output and the intense nature of sprint running rapidly depletes these levels in order to maintain ATP concentrations. The ability to resynthesis PCr depends on the trained state of the muscle, with trained individual replenishing PCr at a faster rate that untrained individuals and therefore maintaining a greater power output for longer during exercise. It is presumed that the participants in this study were sprint trained from an early age and therefore have a greater ability to replenish PCr levels. Therefore, the decrease in PCr levels observed after the PM exercise session suggests that the muscle was on the cusp of energetic stress. The

addition of another run would have placed further stress on the exercising muscle, decreasing PCr levels further and perhaps activating the AMPK signalling pathway.

Studies have also found that nuclear p38MAPK is phosphorylated in the presence of reduced intramuscular glycogen [222]. It has been speculated that AMPK may be an upstream activator of p38MAPK [223]. A high muscle glycogen content may reduce AMPK phosphorylation and in turn the activation of p38MAPK. However, a recent study found a significant increase in p38MAPK phosphorylation after exercise in the fasted compared to the CHO fed condition despite a similar level of glycogen depletion in both groups [25]. It is possible therefore, that activation of p38MAPK is more sensitive to CHO feeding than glycogen concentration. The effect of the low CHO meal on blood glucose levels must be considered in the present study. Feeding the low CHO meal (50g) to the placebo group could have increased blood glucose levels, preventing a reduced signalling response. However, insulin levels were significantly different between CHO and P, and FFA and glycerol were significantly higher in the P than CHO. This suggests that the low CHO meal did not have a significant impact on blood glucose levels.

It is surprising that feeding 1.2g CHO·kg^{-1}·h^{-1} for 2 h did not result in a measurable increase in glycogen resynthesis between exercise sessions. This is in contrast to studies that have fed 1.0-1.4g CHO·kg^{-1}·h^{-1} post exercise and found glycogen resynthesis rates of 25-45 mmol·kg^{-1} dry wet^{-1}·h^{-1} during recovery [224,225]. In contrast to the present study, these studies performed glycogen depleting exercise protocols before CHO feeding. It is well established that glycogen resynthesis rates are inversely related to pre-feeding glycogen

levels. High muscle glycogen content levels prior to feeding can decrease the activity of glycogen synthase [226]. It is reasonable to speculate that the glycogen levels in the present study which remained high after the AM exercise session may have prevented and/or slowed the rate of glycogen resynthesis during the recovery period. The reduced glycogen resynthesis is consistent with a recent study involving HII cycling [25].

ACCβ plays a role in fatty acid oxidation and is considered a sensitive indicator of skeletal muscle AMPK activity in vivo [82]. The 5 fold increase in ACCβ in both groups was not expected. ACCβ catalyses the reaction that converts acetyl-CoA to malonly-CoA. Insulin and glucose stimulate an increase in ACCβ resulting in an accumulation of malonyl-CoA, which inhibits CATT-1. CAT-I is a major rate limiting step for the transport of LCFA into the mitochondria and is very sensitive to changes in malonyl-CoA concentrations. In fasted mice, ACCβ is inactivated which causes a decrease in malonyl-CoA concentration and an increase in β-oxidation. After re-feeding an increase in malonyl-CoA levels and a decrease in β-oxidation was observed in the rats [227]. Therefore, in the present study it was expected that ACCβ phosphorylation would be reduced in the placebo group, facilitating FFA oxidation.

Performance time was significantly faster in the CHO than the placebo group during the first set of HIIE and the difference almost reached statistical significance ($p<0.08$) when intergroup comparison were made during the second and third set. It is easy to conclude that feeding CHO during the recovery period enhanced HIIR performance, but both muscle glycogen and glucose were similar in both groups after exercise.

Study 2

Skeletal muscle oxidative phenotype in response to HIIT and ET is regulated by PGC-1α, a transcriptional co-activator that serves to coordinate mitochondrial biogenesis. Several signalling pathways have been linked to exercise induced activation of PGC-1α, including AMPK and p38MAPK. Recent acute HIIT studies have found an increase in AMPK phosphorylation which promotes the expression of genes encoding mitochondrial and metabolic proteins [13,23,84,85]. P38MAPK also phosphorylates a variety of substrates including transcription factors and co-activators localised in the cytoplasm or nucleus, and is therefore implicated in the regulation of gene transcription [23,25,84].

In the present study, there was a significant increase in AMPK protein content after HIIR. However, an increase in protein content may not signify an increase in AMPK activity, which would indicate a training induced up-regulation of the pathway. It is interesting to note that in study 1, AMPK and p38MAPK were not activated by an acute bout of high intensity exercise, yet mitochondrial biogenesis was more evident in the HIIR group than the ER group. It is possible that pathways other than those measured may have been activated in response to HIIT which subsequently induced an increased expression of metabolic proteins. Due to the observed increase in the enzymatic activity of ACCβ it may also be that AMPK signalling pathways were increased but the antibody used to detect its activity lacked sensitivity

Numerous studies have found an increase in PGC-1α protein content after 4-6 weeks of HIIT training (Burgomaster, Howarth, & Gibala, 2008; Serpiello et al., 2011). This study is

the first to find a significant increase in PGC-1α protein after 6 sessions of both HIIR and ER. A recent study found an increase in the abundance of nuclear PGC-1α protein, but failed to find any change in total protein content suggesting that the total amount of protein may not be affected by acute or chronic exercise [13,188]. It has been assumed that an increase in PGC-1α protein expression mediates the increase in mitochondrial biogenesis. However, the evidence is equivocal regarding the time course of this adaptive process. Accumulating evidence suggests that PGC-1α's subcellular localisation from the cytosol to the nucleus could allow for its activation and subsequent mitochondrial biogenesis, without the need for an increase in total protein content [13,122,188]. In contrast, Perry *et al,* (2010) [15] found an increase in PGC-1α protein content 24 h after a single high intensity interval cycle session. In this study 10 untrained men participated in 10 x 4 min HII cycle at 90% $\dot{V}O_2$max. The levels of PGC-1α protein content continued to increase and plateaued between the 4th and 7th training session.

Each of the ETC complexes (I-IV) are comprised of multiple subunits. For example, complex IV is made up of 13 subunits, three of which are encoded by mtDNA. The HIIR program in the present study resulted in an increase in protein content of specific subunits of complex I (NADH:ubiquinone oxidoreductase/NADH dehydrogenase), II (succinate dehydrogenase complex); III ((cytochrome bc_1 complex), IV (cytochrome c oxidase (COX)) and complex V (ATP Synthase). Complex I, III and IV of the ETC serve to transfer electrons to a series of carriers of increasing redox potential resulting in an electrochemical gradient across the inner membrane. Complex V is comprised of an ATPase coupled to an inner

membrane protein channel. It couples the proton pumping to ATP hydrolysis. The increase in protein abundance of ETC subunits in response to 2 weeks of HIIR indicated that this mode of training is appropriate for enhancing the capacity for electron flux through the ETC. The increase in the ATP synthase subunit also indicates a greater capacity for ADP phosphorylation. Complex II transfers electrons from $FADH_2$ to ubiquinone. However, unlike complex I, III and IV this complex is not coupled to the pumping of H^+ into the intermembrane space. Previous studies have also found an increase in subunits of complex II, III, IV and V of the ETC after HIIR [13,15,22,25].

An increase in COX maximal activity and ETC complex subunits are well known to increase after endurance training and are commonly used as a representative marker of mitochondrial biogenesis [13,18]. In a recent study, 2 weeks of HIIR was found to increase maximal COX activity with a concomitant increase in the protein content of COX II and COX IV subunits [13]. The subunits of complex I (NDUA8), complex III (UQCRC2) and complex IV (COXII) are mitochondrial encoded [228] (Figure 5.1).

PGC-1α-dependent regulation of the expression of each of these subunits is likely to be mediated by Tfam. Tfam induces the expression of mtDNA that encodes fundamental respiratory proteins [18,136]. PGC-1α has been shown to influence the transcriptional activity of Tfam through NRF activation [18]. It is surprising that Tfam did not increase after HIIR, since there was a significant increase in protein content of subunits of complex, I, III and IV and PGC-1α protein. Although, there is evidence that an increase in mtDNA with no further change in Tfam may indicate that the existing Tfam is sufficient to induce mitochondrial

biogenesis [15,136]. It is also possible that the level of Tfam which is imported from the cytosol to the mitochondria was increased to induce the mitochondrial biogenesis observed after HIIR sessions. This could not be measured with the Bergstrom muscle biopsy technique.

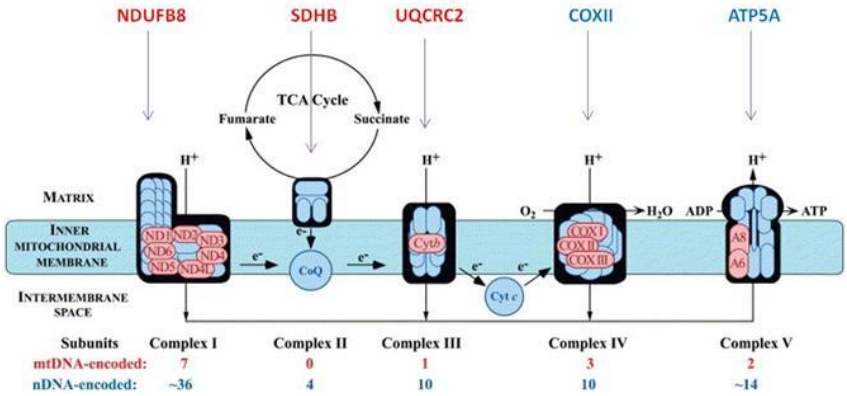

Figure 5.1 Schematic of protein subunits upregulated in complexes of the electron transport chain after high intensity exercise and endurance exercise*

*Proteins in blue are up regulated by both HIIT and ET. Those in red are up-regulated by HIIT only.

The subunits of complex II (SDHB), complex V (ATP5A) are nuclear encoded [229] (Figure 5.1). PGC-1α-dependent regulation of the expression of each of these subunits is likely to be mediated by co-activation of NRF-1, by virtue of the presence of an NRF-1 and -2 binding site on each of the promoter regions of the genes that regulate the transcription of complex II and complex V subunits [229]. In contrast, the subunit in complex V was the only ETC protein that was significantly up-regulated following 6 sessions of ER. This finding suggests that only nuclear encoded mitochondrial proteins are up-regulated after 6 sessions of ET. However, Gibala *et al*., (2006) [22] found an increase in COX subunits II and IV after 6 HII and endurance cycle sessions.

In addition to the increase in skeletal muscle oxidative respiratory markers in response to exercise, there was also significant alterations in both CHO and fat metabolism in response to 2 weeks of HIIR and ER. Previous HIIR studies have consistently found an increase in mitochondrial enzymes [11–13,15]. CS catalyses the condensation of 2 molecules of acetyl CoA and 1 molecule of oxaloacetate to form citrate, and is a key regulatory enzyme in the TCA cycle. It is a common marker of muscle oxidative potential as it exists in constant proportion with other mitochondrial enzymes [230]. Interestingly, CS was increased in the HIIR group only in the present study.

There was no increase in β-HAD activity after HIIR. This is consistent with findings of Burgomaster *et al.*, (2005) [12] in which untrained men participated in 6 HIIR sessions over a 2 week period. In contrast, 7 bouts of HIIR over a 2 week period resulted in a significant increase in the maximal activity of β-HAD and the protein content of the plasma membrane associated fatty acid binding protein (FABPm) [231]. The total training time commitment was 30 times greater (5 h) than the protocol used in the Burgomaster *et al.* (2005) [12] study (10 min). In another study, Burgomaster *et al.,*. (2008) [11] found a significant increase in β-HAD following 6 week HIIR and ER. These findings indicate that an increase in maximal activity of β-HAD requires a minimal volume of intense, interval based exercise training. Resting muscle glycogen was significantly increased in the HIIT only. This is consistent with previous studies that have found an increase in glycogen content after 14 days of sprint training [12,232,233].

Aerobic endurance performance increased by 34% and 21% in HIIR and ER respectively, after training. Numerous studies [12,22] have also found an increase in endurance performance without an increase in $\dot{V}O_2$ max in response to 2 weeks of HIIT. The potential mechanism(s) responsible for the improved performance in endurance running capacity in the HIIR is likely to be related to improvement in respiratory control during exercise as evident by a significant increase in CS activity. There was no increase in CS in the ER group. Although substrate utilization was not measured in the current study it is possible that the increase in endurance performance in the ER group may be related to the changes in β-HAD activity. In addition to changes in the activity of key regulatory enzymes involved in CHO and fat metabolism, other potential mediators of the change in endurance performance may include an increase in skeletal muscle blood flow [234], lactate transport capacity [235], ionic regulation and sarcoplasmic recticulum function [236].

Conclusion

In summary this study found that in trained Gaelic football players an acute bout of HIIR does not activate AMPK or p38MAPK signalling cascades. Although, the significant increase in ACCβ phosphorylation, which was evident in both groups may indirectly indicate an increase in AMPK signalling. The lack of response may be associated with the trained state of the participants whose daily/weekly training sessions involve numerous bouts of HIIR. This could sensitize the muscle to energy changes allowing the signalling responses to return to normal before the muscle biopsy was taken. The intensity and duration of the HIIR session also needs to be considered. The intensity of each session was assessed using blood

lactate measurements which reached between 11-14 mmol·L after each HIIR set. It is therefore likely that the short duration of the HIIE session may also have contributed to the lack of signalling response found after exercise. The decrease in PCr found after the PM exercise session certainly suggests that another HIIR may have induced the activation of signalling pathways. It is difficult to interpret whether CHO feeding had any effect on the signalling pathways without initial activation in the AM exercise session. It may be assumed that CHO feeding had no affect on the AMPK signalling pathways as ACCβ phosphorylation was not affected by CHO consumption. The enhanced performance found in the CHO group during the first set of the PM exercise session should be interpreted with caution as both, muscle glycogen and blood glucose levels were similar in both groups after exercise.

The lack of signalling may also be related to an individual's adaptability which is driven by genotypic variation within a relatively small cluster of genes. Timmons et al.,[237] recently discovered an underlying genetic link to an individual's adaptability for a complex and multifactorial physiological trait (aerobic capacity). However, moderately trained Gaelic games players are a relatively homogenetic cohort, with similar height, weight and endurance capacity. Gaelic games players are also trained from a young age and this conditioning may play a role in epigenetics, inducing a similar genetic make-up and reducing the variability in exercise adaptations.

Despite the lack of signalling after an acute bout of HIIR in the first study, 6 sessions of HIIR coincided with an increase in several classical markers of mitochondrial biogenesis. This increase in both nuclear and mitochondrial gene transcription is clearly due to the

cumulative effect of multiple exercise sessions. The ER training protocol also induced mitochondrial biogenesis signified by an increase in PGC-1α protein content. However, the only other markers of mitochondrial biogenesis upregulated were those encoded by nuclear genome such as the subunit of complex V of the ETC. The increase in activity of β-HAD also links PGC-1α to an enhanced expression of nuclear proteins.

An increase in mitochondrial protein expression occurs following repeated training which induces an increase in mRNA expression of selected gene [5,44,134]. It is suggested that repeated burst of mRNA expression are fundamental to the intracellular adaptive response to exercise training. . Perry *et al*.,(2010) [15] found a 10 fold increase in PGC-1α mRNA, a 53% increase in Tfam mRNA and a 50% increase in PPARβ/δ mRNA 4 h after a HIIE session. An increase in CS and β-HAD 4 and 24 h after the HIIE session was also found. Furthermore, exercise is also suggested to alter the methylation of certain genes in a dose responsive manner, enhancing gene expression [238]. The inclusion of multiple biopsies at more than one time point after the PM exercise session, allowing us to measure mRNA and/or DNA methylation would have allowed for a greater understanding of whether mitochondrial biogenesis was initiated by HIIR. This would help to explain the adaptive increases in protein expression found after HIIR training in the second study.

Interestingly, although HIIR induced a greater increase in mitochondrial biogenesis compared to 6 session of ER, this did not translate into a more enhanced increase in endurance performance.

In conclusion HIIR is a more sufficient stimulus that ER to induced mitochondrial biogenesis in moderately trained Gaelic games players.

Future Directions

1. To compare the effects of HIIT on team sports athletes, endurance athletes and untrained individuals
2. Examine whether there is a threshold of HIIR needed to activate signalling pathways involved in mitochondrial biogenesis
3. Compare the effect of training duration (2, 4 and 6 weeks) on mitochondrial biogenesis
4. To examine the effect of HIIT on mitochondrial biogenesis in sedentary, moderately trained and highly trained women
5. Examine the effect of HIIT of transcription proteins such as NRF-1 and 2, ERRα, MEF2
6. Evaluate the changes in substrate utilisation after 2, 4 and 6 weeks of HIIT

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Appendix A

Study 1

Dublin City University
RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING **HUMAN PARTICIPANTS**

Application No. *(office use only)* DCUREC/2008/ ☐☐☐

Period of Approval *(office use only)* ...../...../..... to ...../...../....

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The **signed original and an electronic copy** of your completed application must be submitted to the DCU Research Ethics Committee.

NB - The hard copy must be signed by the PI. The electronic copy should consist of <u>one file only</u>, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. **Research must <u>not</u> commence until written approval has been received from the Research Ethics Committee.**

PROJECT TITLE	**Effect of nutrients on skeletal muscle signalling pathways in response to high intensity interval training.**
PRINCIPAL INVESTIGATOR(S)	**Prof. Niall Moyna**

Please confirm that **<u>all</u>** supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or interview questions are submitted in draft form, a copy of the final documentation must be submitted for final approval when available.

	INCLUDED	**NOT APPLICABLE**
Bibliography	☒	☐
Recruitment advertisement	☒	☐
Plain language statement/Information Statement	☒	☐

Informed Consent form	☒		☐
Evidence of external approvals related to the research	☐		☒
Questionnaire	☐ draft	☐ final	☒
Interview Schedule	☐ draft	☐ final	☒
Debriefing material	☐		☒
Other	☐		☒

Please note:

1. Any amendments to the original approved proposal must receive prior REC approval.

2. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

Please submit the **signed original, plus the electronic copy** of your completed application to:
Ms. Fiona Brennan, Research Officer, Office of the Vice-President for Research
(fiona.brennan@dcu.ie, Ph. 01-7007816)

Guidelines to Applicants

1.1 PRINCIPAL INVESTIGATOR(S): *The named Principal Investigator is the person with primary responsibility for the research project. Doctoral researchers and Research Masters or their supervisors may be listed as Principal Investigators, depending on the conventions of the discipline and on the individual case. It should be made clear, in subsequent sections of this application, who is carrying out the research procedures. In the case of Taught Masters and undergraduate student projects the supervisors are Principal Investigators.*

2.0 PROJECT OUTLINE: *Provide a brief outline of the project, aims, methods, duration, funding, profile of participants and proposed interaction with them. This description must be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.*

2.1 LAY DESCRIPTION: *Provide a brief outline of the project, including what participants will be required to do. This description must be in everyday language which is free from jargon. Please explain any technical terms or discipline-specific phrases. (No more than 300 words).*

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH: *State the aims and significance of the project (approx. 400 words). Where relevant, state the specific hypothesis to be tested. Also please provide a brief description of current research, a justification as to why this research should proceed and an explanation of any expected benefits to the community.* ***NB – all references cited should be listed in an attached bibliography.***

2.3 PROPOSED METHOD: *Provide an outline of the proposed method, including details of data collection techniques, tasks participants will be asked to do, the estimated time commitment involved, and how data will be analysed. If the project includes any procedure which is beyond already established and accepted techniques please include a description of it. (No more than 400 words.)*

2.4 PARTICIPANT PROFILE: *Provide number, age range and source of participants. Please provide a justification of your proposed sample size. Please provide a justification for selecting a specific gender.*

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED: *Please provide specific details as to how you will be recruiting participants. How will people be told you are doing this research? How will they be approached and asked if they are willing to participate? If you are mailing to or phoning people, please explain how you have obtained their names and contact details. This information will need to be included in the plain language statement. If a recruitment advertisement is to be used, please ensure you attach a copy to this application.*

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES*: Identify, as far as possible, all potential risks to participants (physical, psychological, social, legal or economic etc.), associated with the proposed research. Please explain what risk management procedures will be put in place.*

3.6 ADVERSE/UNEXPECTED OUTCOMES: *Please describe what measures you have in place in the event that there are any unexpected outcomes or adverse effects to participants arising from involvement in the project.*

3.7 MONITORING: *Please explain how you propose to monitor the conduct of the project (especially where several people are involved in recruiting or interviewing, administering procedures) to ensure that it conforms with the procedures set out in this application. In the case of student projects please give details of how the supervisor(s) will monitor the conduct of the project.*

3.8 SUPPORT FOR PARTICIPANTS: *Depending on risks to participants you may need to consider having additional support for participants during/after the study. Consider whether your project would require additional support, e.g., external counselling available to participants. Please advise what support will be available.*

4.0 INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS: *List the academic qualifications and outline the experience and skills relevant to this project that the researchers and any supporting staff have in carrying out the research and in dealing with any emergencies, unexpected outcomes, or contingencies that may arise.*

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED? *Please bear in mind that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity. Participants involved in such projects need to be advised of this limitation.*

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *Participants need to be aware that confidentiality of information provided can only be protected within the limitations of the law - i.e., it is possible for data to be subject to subpoena, freedom of information claim or mandated reporting*

by some professions. Depending on the research proposal you may need to specifically state these limitations.

6.0 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL: *For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.*

8.0 PLAIN LANGUAGE STATEMENT: *Written information in plain language that you will be providing to participants, outlining the phases and nature of their involvement in the project and inviting their participation. Please note that the language used must reflect the participant age group and corresponding comprehension level.*

9.0 INFORMED CONSENT FORM: *This is a very important document that should be addressed by participants to researchers, requiring participants to indicate their consent to specific statements, and give their signature.*

FOR FURTHER INFORMATION AND NOTES ON THE DEVELOPMENT OF PLAIN LANGUAGE STATEMENTS AND INFORMED CONSENT FORMS, PLEASE CONSULT THE DCU REC WEBSITE: WWW.DCU.IE/RESEARCH/ETHICS

1. ADMINISTRATIVE DETAILS

THIS PROJECT IS: *(tick as many as apply)*

- ☐ Research Project
- ☐ Practical Class
- ☒ Student Research Project *(please give details)*
  - ☒ Research Masters
  - ☒ PhD
  - ☐ Taught Masters
  - ☒☐ Undergraduate
- ☐ Funded Consultancy
- ☐ Clinical Trial
- ☐ Other - *Please Describe:*

Project Start Date: **June 2011**

Project End date: **May 2012**

1.1 INVESTIGATOR CONTACT DETAILS

PRINCIPAL INVESTIGATOR(S):

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Prof	Moyna	Niall	7008802	7008888	niall.moyna@dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Dr.	McCaffrey	Noel	0872797597	7008888	noel.mccaffrey@dcu.ie
Dr.	Susta	Davide		7008888	davide.susta@dcu.ie
Ms	Tobin	Crionna	0860705130	7008888	crionna.tobin9@mail.dcu.ie

FACULTY/DEPARTMENT/SCHOOL/ CENTRE: School of Health and Human Performance

1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

☒ YES ☐ NO

1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?*)*

☐ YES ☒ NO

DECLARATION BY INVESTIGATORS

The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research that may arise in conducting this research and acknowledge my obligations and the rights of the participants.

If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.

I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.
Signature(s):

Principal investigator(s): Niall Moyna

Print name(s) in block letters: *Niall Moyna*

Date: *14/4/2011*

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION

Carbohydrate (CHO) is an important fuel source for skeletal muscle during exercise. Athletes are generally advised to ingest a daily diet that is moderate to high in carbohydrate to meet the energy demands of training (1;2). However, recent evidence has suggested however that periodic restriction of carbohydrate before exercise can increase training induced gains in skeletal muscle metabolism and performance (3;4).

Changes in skeletal muscle metabolism in response to training are thought to be the result of the cumulative effects of transient changes in gene activation in response to each exercise bout (5). Acute exercise activates several signaling cascades in muscle which, in turn, stimulate transcription of mitochondrial and metabolic genes to initiate the changes in skeletal muscle metabolism. AMP activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38) have been implicated as potential mediators of this process. Both AMPK and P38 have been shown to activate peroxisome proliferator-activated receptor γ coactivator-α (PGC-1α), the master regulator of mitochondrial gene expression (6;7). Interestingly, the exercise-induced increase in AMPK activity and p38 phosphorylation are augmented when pre-exercise muscle glycogen content is reduced (8, 9). Thus, restricting CHO intake could augment AMPK and p38 activation and subsequently increase the mitochondrial response to training via an increase in PGC-1α activation. Since the restriction of nutrients after exercise is unrealistic in the real world setting we propose that feeding a whey based solution with minimal amounts of carbohydrate will induce similar benefits in muscle metabolism and performance as restricting CHO.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH

Aims of the Research:

To compare the effect of feeding a whey based solution, a carbohydrate based solution or an isocaloric placebo based solution during recovery on the skeletal muscle metabolic responses after high intensity interval training.

Justification:

Restricting the intake of carbohydrate before exercise training has recently been shown to result in larger improvements in skeletal muscle metabolism compared to training with repleted CHO stores (3.4). Previous studies (3,4) have found a greater increase in the activity of metabolic enzymes; citrate synthase (CS) HAD, cytochrome oxidase IV (COXIV) and whole body fat oxidation in response to 3 weeks of training while restricting CHO intake compared to a non CHO restriction protocol. The frequency and volume of training were equal in both groups. However, since the restricted groups trained 2 times/day while the non-restricted trained once per day it was not possible to determine if the greater metabolic adaptations in response to CHO restriction resulted from the fact that the subjects undertook multiple daily exercise sessions or the CHO restriction per se. To answer this question, Morton et al., (10) had 2 groups of endurance trained men perform 6 weeks of high intensity intermittent running on 2 d/week. Group 1 consumed a 6.4% glucose solution and group 2 consumed a placebo solution immediately before every second training session. In addition group 1 and group 2 consumed 3ml/kg CHO solution or placebo respectively during the active recovery periods. There was a training-induced increase in muscle oxidative capacity in the CHO-

restricted condition only. In a more recent study (11) 10 subjects performed two exercise trials in random order separated by > 1 week. Each trial consisted of a morning and afternoon session (5 x 4 min cycling at 90-95% of heart rate reserve) separated by 3 h of recovery during which subjects ingested either a high CHO drink or an isocaloric placebo before the afternoon session. Following the afternoon exercise sessions p38 MAPK phosphorylation was higher in the CHO restricted group. The author concluded that p38 MAPK is a nutrient sensitive signaling molecule which may alter the skeletal muscle adaptive response under CHO-restricted conditions. While the restriction of CHO before an exercise session enhances skeletal muscle oxidative adaptations it is unknown whether feeding a whey protein supplement, which is a non-carbohydrate solution immediately after exercise would convey similar metabolic and signaling responses to a subsequent bout of exercise.

1. *Burke LM (2007) Nutrition strategies for the marathon :fuel for training and racing. Sports Medicine 37: 344-347*
2. *Hawley JA, Gibala MJ (2007) Innovations in athletic performance: role of substrate availability to modify training adaptation and performance. Journal of Sports Science S 1: S115-S124*
3. *Hansen AK, Fischer CP (2005) Skeletal muscle adaptation: training twice every second day vs. training once daily. J Appl.Physiol. 93-99*
4. *Yeo WK, Paton CD (2008) Skeletal muscle adaptation and performance responses to once a day verses twice every second day endurance training regimens. J Appl.Physiol. 1462-1470*
5. *Joesph AM, Pilegaard H (2006) Control of gene expression and mitochondrial biogenesis in muscular adaptation to endurance exercise. Essays Biochem 13-29*
6. *Akimoto T, Pohnert SC (2005) Exercise stimulates PGC-1 alpha transcription in skeletal muscle through actiavtion of the p38 MAPK pathway. J Biol.Chem. 19587-19593*
7. *Jager S, Handschin C (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC1-alpha. Proc Natl Acad Sci USA 12017-12022*
8. *Wojtaszewski JF, MacDonald C (2003) Regulation of 5'-AMP-activated protein kinase activity and substrate utilisation in exercising human skeletal muscle. J Physiol Endocrinol Metab E813-E822*
9. *Chan MH, McGree SL (2004) Altering nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle: association with IL-6 gene transcription during contraction. FASEB J 1785-1787*
10. *Morton JP, Croft L (2009) Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzymes activity in human skeletal muscle. J Appl.Physiol. 1513-1521*
11. *Cochran AJR, Little PJ (2010) Carbohydrate feeding during recovery alters the skeletal muscle metabolic response to repeated sessions of high-intensity interval exercise in humans. J Appl.Physiol. 628-636*

2.3 PROPOSED METHOD

STUDY OVERVIEW

Subjects will make 2 visits to the Human Performance Laboratory in the School of Health and Performance at DCU. The first visit will be used to assess their maximal aerobic capacity (VO_2max). During the second visit they will undertake 2 high intensity interval trials (HIIT) separated by 4 h. Prior to this visit they will be randomly assigned to a whey (W), carbohydrate (C) or placebo (P) group. A muscle biopsy will be taken immediately before and immediately after the first and second HIIT.

STUDY VISITS

Visit 1- This visit will last approximately 1 h, during which subjects will perform an exercise test to assess their maximal aerobic capacity.

Visit 2 – Subjects will complete 2 HIIT on a treadmill, separated by a recovery period of 4 h. Muscle biopsies and blood samples will be taken immediately before and after the first and second high HIIT and 3 h after the second HIIT.

Nutritional Protocol: Prior to visit 2 subjects will be given a standardised breakfast (63% CHO, 25% Fat & 12% protein) to ingest prior to arriving at the laboratory. Following the HIIT, group C will ingest 1.2g/kg^{-1} every hour throughout the recovery period, group W will ingest 0.25g.kg^{-1} of protein every hour throughout the recovery period, and group P will ingest an isocaloric solution every hour throughout the recovery period.

High Intensity Interval Trial (HIIT): This will involve two set of 6 x 2 min runs on a treadmill at 80-90% VO_2max with alternating 2-3 recovery periods. The recovery period between each set will be 4 h.

LABORATORY PROCEDURES

Maximal Aerobic Capacity Assessment: Maximal aerobic capacity will be determined on a treadmill (Woodway ELG 55, Waukesha, WI) using a ramp protocol. The test will be performed ≥ 48 h prior to the first HIIT. Subjects will warm-up at 3.6 mph for 2 min. Following the warm-up, the gradient will be increased at a rate of 0.2% every 12 sec until the subject reaches volitional exhaustion. The test will be deemed to be maximal if it satisfies at least 3 of the following criteria; levelling of oxygen consumption, volitional exhaustion, RER > 1.1 and heart rate within ± 10 beats of the age predicted max. HR will be recorded continuously, and blood lactate will be assessed each minute.

Respiratory Metabolic Measures: Expired oxygen, carbon dioxide, ventilatory volume and respiratory exchange ratios (RER) will be determined using a Sensormedics Vmax 229 metabolic system (SensorMedics Corp., Yorba Linda CA). Prior to testing, the gas analysers will be calibrated with standard gases of known concentration.

Muscle Biopsy: Rationale for muscle biopsy samples

Taken from the vastus lateralis using a sterile 5mm diameter biopsy needle under local anaesthesia.

1. *Little JP. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanism. J Physiol 588.6: 1011-1022, 2010*
2. *Burgomaster KA. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol 586: 151-160, 2008*
3. *Burgomaster KA. Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. J Appl Physiol 100: 2041-2047, 2006*

Statistical Analysis:

SPSS for Windows statistical software will be used to perform the statistical analysis. A 3 x 3 RM ANOVA will be used to compare and within group differences. Statistical significance will be accepted at the $P < 0.05$ level of confidence

2.4 PARTICIPANT PROFILE

Inclusion criteria: Apparently healthy men, currently playing at junior club level or higher level, and between the ages of 18 – 35 years.

Exclusion criteria: Volunteers will be excluded if they smoke, have diabetes, cardiovascular disease or have any other medical conditions that contraindicate exercise participation.

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED

A recruitment advertisement will be emailed to members of the Dublin City University Gaelic games club and all Dublin city Gaelic games clubs. Permission will be sought from each club prior to posting the advertisement. The aim of the study, the rationale for the study, the tests involved, the time commitment and the potential benefits will be explained to the players. Players will be provided with an opportunity to ask questions. If they wish to participate in the study they will have to provide a written informed consent, which will be witnessed on their visit to the School of Health and Human Performance.

2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT*?*

The study findings will be presented at scientific meetings and published in scientific journals. Subjects will be provided with a report, which will summarise the relevant results from their participation in the research project.

2.7 OTHER APPROVALS REQUIRED *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

☐ YES ☐ NO ☒ NOT APPLICABLE

2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?

☐ YES ☒ NO

(If YES, please state both the REC Application Number and Project Title)

3. RISK AND RISK MANAGEMENT

3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?

☒ YES ☐ NO *If YES, this proposal will be subject to full REC review*

If NO, this proposal may be processed by expedited administrative review

3.2 WHAT DOES THE RESEARCH INVOLVE:

	YES	NO
• Use of a questionnaire? (attach copy)?	☐	☒
• interviews (attach interview questions)?	☐	☒
• observation of participants without their knowledge?	☐	☒

• participant observation (provide details in section 2)?	☐	☒
• audio- or video-taping interviewees or events?	☐	☒
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	☐	☒
• administration of any stimuli, tasks, investigations or procedures which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?	☒	☐
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	☐	☒
• investigation of participants involved in illegal activities?	☐	☒
• procedures that involve deception of participants?	☐	☒
• administration of any substance or agent?	☐	☒
• use of non-treatment of placebo control conditions?	☐	☒
• collection of body tissues or fluid samples?	☒	☐
• collection and/or testing of DNA samples?	☐	☒
• participation in a clinical trial?	☐	☒
• administration of ionising radiation to participants?	☐	☒

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES *(see Guidelines)*

The nature and risks involved in the study will be explained prior to starting the study, and a contact number will be provided.

Subjects may experience some muscle soreness in their legs or nausea following the maximal exercise test. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The pre-test likelihood of these risks in asymptomatic men < 55 years of age is very low. There may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. Temporary numbness of the skin near the biopsy site may occur. There may be discomfort when taking blood and the development of a small bruise at the site of puncture.

High intensity may increase the risk for muscle strains and tears. A 5 min warm-up will precede each training session to help reduce the risk of injury.

3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

☐ YES ☒ NO

3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS? *(e.g. risk of infection or where research is undertaken at an off-campus location)*

☒ YES ☐ NO There is a small risk of infection from needle and blood samples. Standard safety procedures will be strictly adhered to.

3.6 ADVERSE/UNEXPECTED OUTCOMES

The School of Health and Human Performance has the facilities to deal with all aspects of this study and an emergency plan is in place for adverse events. All minor injuries will be addressed by an individual trained in first aid (either a member of the research team or the staff). The laboratory is equipped with an emergency crash cart and defibrillator. An individual trained in first aid (or Advanced Cardiac Life Support) will be present during each test. In the unlikely event of a serious adverse outcome, the subject will be brought to the VHI clinic in Airside Retail Park, Swords.

3.7 MONITORING

Weekly meetings will take place between Prof. Niall Moyna (principal investigator) and the other researchers. These meetings will provide opportunities to access progress, give feedback, and monitor development of the research. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers will be familiar with the procedures and the Safety Statement before beginning data collection.

3.8 SUPPORT FOR PARTICIPANTS

It is anticipated that no additional support will be required.

3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?

☐ YES ☒ NO *(If YES, please provide further details.)*

4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS *(Approx. 200 words – see Guidelines)*

Prof. Moyna is an exercise physiologist and has extensive experience with exercise testing

Dr. Noel McCaffrey and Dr. Davide Susta are physicians with extensive experience in the muscle biopsy technique

Crionna Tobin is a PhD student in the School of Health and Human Performance. She has a BSc, a Higher Diploma in Human Nutrition and a Higher Diploma in Sports Nutrition.

5. CONFIDENTIALITY/ANONYMITY

5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?

☒ YES ☐ NO *(If NO, please explain)*

IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?

Confidentiality is an important issue during data collection. Participant's identity, or other personal information, will not be revealed or published. Subjects will be assigned an ID number under which all personal information will be stored in a secure file and saved in

password protected file in a computer at DCU. The investigators alone will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *(Have you included appropriate information in the plain language statement and consent form? See Guidelines)*

☒ YES ☐ NO *(If NO, please advise how participants will be advised.)*

The following statement should be included in the plain language statement.
'Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL *(see Guidelines)*

6.1 HOW WILL THE DATA/SAMPLES BE STORED? *(The REC recommends that all data be stored on campus)*

Stored at DCU ☒
Stored at another site ☐

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

Access by named researchers only ☒
Access by people other than named researcher(s) ☐
Other *:* ☐

6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?

The principal investigator will be responsible for security of the collected data. The data will be kept in locked facilities in the department through which the project is being conducted. Access to the data will only be attainable by the main researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the main researchers, no others will have access to the raw data. Data will be shredded after five years and Prof. Moyna will carry this out.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

The Gaelic Athletic Association

7.2 PROJECT GRANT NUMBER (If relevant and/or known)

7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?

☐ YES ☒ NO

HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?

In the plain language statement

7.5 **DO ANY OF THE RESEARCHERS, SUPERVISORS OR FUNDERS OF THIS PROJECT HAVE A PERSONAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT COMPROMISE THE INDEPENDENCE AND INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR RESULTS OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?**

NO

8. PLAIN LANGUAGE STATEMENT

Plain Language Statement

Dublin City University

Project Title: **Effect of nutrients on skeletal muscle signalling pathways in response to high intensity interval training.**

The Research Study will take place in the School for Human Health and Human Performance DCU and the DCU Sports Grounds

The research study is being funded by the Gaelic Athletic Association.

The principal investigator is: Prof. Niall M. Moyna, (Tel: 7008802 Fax 7008888) EMAIL niall.moyna@dcu.ie

1. Carbohydrate is an important fuel source for skeletal muscle during exercise and athletes are advised to eat a daily diet that is high to moderate in carbohydrate to meet the energy demands of training. There is now evidence that restricting carbohydrate before exercise can increase the fat burning capability of the muscle and improve exercise performance. This study will examine whether a protein, a carbohydrate or a flavoured drink will influence how muscles adapt high intensity exercise.

2. You will make 2 visits to the Human Performance Laboratory in DCU. The first visit to the laboratory will last approximately 1 hour, during which you will undertake a treadmill exercise test to determine your fitness level.

3. The second visit will be 6 hours in duration. You will undertake two high intensity interval trials (HIIT) separated by 4 hours. Each trial will be preceded by a 5 minute warm-up. You will run for 2 min at a high intensity on a treadmill. This will be repeated 5 more times for a total of 6 runs. You will have a 2-3 min recovery between each run. You will then take a 4 hour break and after which you will repeat the 6 high intensity runs. During the 4 hour recovery period you will be fed either; a carbohydrate, protein or flavoured drink every hour. A blood sample and muscle biopsy will be taken immediately before and after the first and second HIIT.

4. Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure file and saved in a password protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

5. The original documentation will be stored for a maximum of 5 years. Thereafter the documentation will be shredded.

6. Your participation in this research project is voluntary and you may withdraw your consent at any time.

If participants have concerns about this study and wish to contact an independent person, please contact:

The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

9. INFORMED CONSENT FORM

INFORMED CONSENT

Title: **Does feeding a nonenergetic placebo solution induce similar muscle signalling responses when compared to a carbohydrate solution after high intensity training**

Principal investigator: Prof Niall M. Moyna

Other investigators: Ms Crionna Tobin, Dr. Noel McCaffrey, Dr. Davide Susta

Purpose: This study will examine whether a carbohydrate solution or an isocaloric placebo solution will influence different pathways in the muscle related to metabolism after high intensity interval exercise.

Participant Requirements

1. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will provide written informed consent for participation in the research project. I will then complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits. I will talk with a medical doctor about the information I have provided and I understand, based on the information provided, the medical doctor may exclude me from participating in the research project. If I agree to participate in the study I will make 2 visits to the Human Performance Laboratory in DCU.
2. For the first visit, I will arrive in the morning to the Human Performance Laboratory in DCU following my normal breakfast. I will take part in a fitness test to measure my fitness level. This will involve me exercising with a mouthpiece (similar to a snorkel) in my mouth to measure the amount of air I breathe in and out.
3. On my second visit to the Human Performance Laboratory I will take part in two high intensity interval trials. Each trial will be separated by 4 hours during which I will ingest either; a protein, carbohydrate or a flavoured drink. I will have a blood sample and muscle biopsy sample taken immediately before and after the first and second high intensity interval trial. The muscle biopsy sample will involve taking a small piece of muscle, about the size of a pea from my thigh with a special biopsy needle. A small area of the leg will be injected with a local anesthetic, then a small incision will be made in the skin and a needle inserted briefly into the muscle. The incision will be closed with sterile strip bandaids, and my leg will be wrapped snugly with an elastic bandage to maintain pressure. Before I leave I will be given supplies to change the dressing around the biopsy sites.
4. I will not drink alcohol or caffeine, or participate in any type of exercise 24 h before my first visit to DCU.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

1. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea

following the maximal exercise test. It should be noted that if the experimental protocol is adhered to, the likelihood of these risks occurring is minimal.

2. I understand that there may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. After the biopsy, my leg may feel stiff and sore. Temporary numbness of the skin near the biopsy may also occur.
3. High intensity may increase the risk for muscle strains and tears. A 5 min warm-up will precede each training session to help reduce the risk of injury.

There may be benefits from my participation in this study. These are:

1. I will receive a copy of my personal results, body fat and fitness measurements
2. I understand that no other benefits have been promised me.

Participant – please complete the following (Circle Yes or No for each question)

I have read the Plain Language Statement	Yes/No
I understand the information provided	Yes/No
I have had an opportunity to ask questions and discuss this study	Yes/No
I have received satisfactory answers to all my questions	Yes/No

<u>My confidentiality will be guarded:</u>

Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Prof Niall Moyna at 01-7008802.

<u>Taking part in this study is my decision.</u>

I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also request that my data and samples be removed from the database or storage and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

<u>Signature:</u>

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

Email to participants:

The school of Health and Human Performance is conducting a study to examine whether feeding either a protein solution, a carbohydrate solution or a placebo solution will influence different pathways in the muscle related to exercise after high intensity exercise. You will make 2 visits to the Human Performance Laboratory in DCU. The first visit will last approximately 1 hour during which you will perform an endurance exercise test. The second visit will be 6 hours in duration, during which you will take part in 2 high intensity exercise trials. Each trial will be separated by 4 hours during which you will ingest either; a protein, carbohydrate or a flavoured drink. A blood sample and muscle biopsy sample will be taken immediately before and after the first and second high intensity interval trial.

We are looking for healthy men between the ages of 18 – 35 years who are currently playing Gaelic football at college or club level.

If you would like to hear more about this study or would consider participating, please contact one of the following;

Crionna Tobin
Email: Crionna.tobin9@mail.dcu.ie
Mobile number: 086-0705130

Thank you,

Niall M. Moyna, PhD

Dublin City University
RESEARCH ETHICS COMMITTEE

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING **HUMAN PARTICIPANTS**

Application No. *(office use only)* DCUREC/2008/ ☐☐☐

Period of Approval *(office use only)* ...../...../..... to .....

This application form is to be used by researchers seeking ethics approval for individual projects and studies. The **signed original and an electronic copy** of your completed application must be submitted to the DCU Research Ethics Committee.

NB - The hard copy must be signed by the PI. The electronic copy should consist of one file only, which incorporates all supplementary documentation. The completed application must be proofread and spellchecked before submission to the REC. All sections of the application form should be completed. Applications which do not adhere to these requirements will not be accepted for review and will be returned directly to the applicant.

Applications must be completed on the form; answers in the form of attachments will not be accepted, except where indicated. No handwritten applications will be accepted. **Research must not commence until written approval has been received from the Research Ethics Committee.**

PROJECT TITLE	**Muscle adaptations in response to low volume high intensity interval training and endurance training in Gaelic games players**
PRINCIPAL INVESTIGATOR(S)	**Prof. Niall Moyna**

Please confirm that **all** supplementary information is included in your application (in both signed original and electronic copy). If questionnaire or interview questions are submitted in draft form, a copy of the final documentation must be submitted for final approval when available.

	INCLUDED		**NOT APPLICABLE**
Bibliography	☒		☐
Recruitment advertisement	☒		☐
Plain language statement/Information Statement	☒		☐
Informed Consent form	☒		☐
Evidence of external approvals related to the research	☐		☒
Questionnaire	☐ draft	☐ final	☒

Interview Schedule	☐ draft	☐ final	☒
Debriefing material	☐		☒
Other	☐		☒

Please note:

3. Any amendments to the original approved proposal must receive prior REC approval.

4. As a condition of approval investigators are required to document and report immediately to the Secretary of the Research Ethics Committee any adverse events, any issues which might negatively impact on the conduct of the research and/or any complaint from a participant relating to their participation in the study

Please submit the **signed original, plus the electronic copy** of your completed application to:
Ms. Fiona Brennan, Research Officer, Office of the Vice-President for Research
(fiona.brennan@dcu.ie, Ph. 01-7007816)

Guidelines to Applicants

1.1 PRINCIPAL INVESTIGATOR(S): *The named Principal Investigator is the person with primary responsibility for the research project. Doctoral researchers and Research Masters or their supervisors may be listed as Principal Investigators, depending on the conventions of the discipline and on the individual case. It should be made clear, in subsequent sections of this application, who is carrying out the research procedures. In the case of Taught Masters and undergraduate student projects the supervisors are Principal Investigators.*

2.0 PROJECT OUTLINE: *Provide a brief outline of the project, aims, methods, duration, funding, profile of participants and proposed interaction with them. This description must be in everyday language that is free from jargon. Please explain any technical terms or discipline-specific phrases.*

2.1 LAY DESCRIPTION: *Provide a brief outline of the project, including what participants will be required to do. This description must be in everyday language which is free from jargon. Please explain any technical terms or discipline-specific phrases. (No more than 300 words).*

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH: *State the aims and significance of the project (approx. 400 words). Where relevant, state the specific hypothesis to be tested. Also please provide a brief description of current research, a justification as to why this research should proceed and an explanation of any expected benefits to the community.* ***NB – all references cited should be listed in an attached bibliography.***

2.3 PROPOSED METHOD: *Provide an outline of the proposed method, including details of data collection techniques, tasks participants will be asked to do, the estimated time commitment involved, and how data will be analysed. If the project includes any procedure which is beyond*

already established and accepted techniques please include a description of it. (No more than 400 words.)

2.4 PARTICIPANT PROFILE: *Provide number, age range and source of participants. Please provide a justification of your proposed sample size. Please provide a justification for selecting a specific gender.*

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED: *Please provide specific details as to how you will be recruiting participants. How will people be told you are doing this research? How will they be approached and asked if they are willing to participate? If you are mailing to or phoning people, please explain how you have obtained their names and contact details. This information will need to be included in the plain language statement. If a recruitment advertisement is to be used, please ensure you attach a copy to this application.*

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES*: Identify, as far as possible, all potential risks to participants (physical, psychological, social, legal or economic etc.), associated with the proposed research. Please explain what risk management procedures will be put in place.*

3.6 ADVERSE/UNEXPECTED OUTCOMES: *Please describe what measures you have in place in the event that there are any unexpected outcomes or adverse effects to participants arising from involvement in the project.*

3.7 MONITORING: *Please explain how you propose to monitor the conduct of the project (especially where several people are involved in recruiting or interviewing, administering procedures) to ensure that it conforms with the procedures set out in this application. In the case of student projects please give details of how the supervisor(s) will monitor the conduct of the project.*

3.8 SUPPORT FOR PARTICIPANTS: *Depending on risks to participants you may need to consider having additional support for participants during/after the study. Consider whether your project would require additional support, e.g., external counselling available to participants. Please advise what support will be available.*

4.0 INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS: *List the academic qualifications and outline the experience and skills relevant to this project that the researchers and any supporting staff have in carrying out the research and in dealing with any emergencies, unexpected outcomes, or contingencies that may arise.*

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED? *Please bear in mind that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity. Participants involved in such projects need to be advised of this limitation.*

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *Participants need to be aware that confidentiality of information provided can only be protected within the limitations of the law - i.e., it is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions. Depending on the research proposal you may need to specifically state these limitations.*

6.0 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL: *For the purpose of this section, "Data" includes that in a raw or processed state (e.g. interview audiotape, transcript or analysis). "Samples" include body fluids or tissue samples.*

8.0 PLAIN LANGUAGE STATEMENT: *Written information in plain language that you will be providing to participants, outlining the phases and nature of their involvement in the project and inviting their participation. Please note that the language used must reflect the participant age group and corresponding comprehension level.*

9.0 INFORMED CONSENT FORM: *This is a very important document that should be addressed by participants to researchers, requiring participants to indicate their consent to specific statements, and give their signature.*

FOR FURTHER INFORMATION AND NOTES ON THE DEVELOPMENT OF PLAIN LANGUAGE STATEMENTS AND INFORMED CONSENT FORMS, PLEASE CONSULT THE DCU REC WEBSITE: WWW.DCU.IE/RESEARCH/ETHICS

1. ADMINISTRATIVE DETAILS

THIS PROJECT IS: *(tick as many as apply)*

- ☐ Research Project
- ☐ Practical Class
- ☐ Funded Consultancy
- ☐ Clinical Trial
- ☒ Student Research Project *(please give details)*
  - ☒ Research Masters
  - ☐ Taught Masters
  - ☒ PhD
  - ☒☐ Undergraduate
- ☐ Other - *Please Describe:*

Project Start Date: **September 2010**

Project End date: **December 2011**

1.1 INVESTIGATOR CONTACT DETAILS

PRINCIPAL INVESTIGATOR(S):

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Prof	Moyna	Niall	7008802	7008888	niall.moyna@dcu.ie

OTHER INVESTIGATORS:

TITLE	*SURNAME*	*FIRST NAME*	*PHONE*	*FAX*	*EMAIL*
Dr.	McCaffrey	Noel	0872797597	7008888	noel.mccaffrey@dcu.ie
Dr.	Susta	Davide		7008888	davide.susta@dcu.ie
Ms	Tobin	Crionna	0860705130	7008888	crionna.tobin9@mail.dcu.ie
Mr.	Cregg	Cathal	0877633021	7008888	cathal.cregg2@mail.dcu.ie
Mr.	Kelly	David	0851618207	7008888	david.kelly59@mail.dcu.ie

FACULTY/DEPARTMENT/SCHOOL/ CENTRE: School of Health and Human Performance

1.2 WILL THE RESEARCH BE UNDERTAKEN ON-SITE AT DUBLIN CITY UNIVERSITY?

☒ YES ☐ NO

1.3 IS THIS PROTOCOL BEING SUBMITTED TO ANOTHER ETHICS COMMITTEE, OR HAS IT BEEN PREVIOUSLY SUBMITTED TO AN ETHICS COMMITTEE?*)*

☐ YES ☒ NO

DECLARATION BY INVESTIGATORS

The information contained herein is, to the best of my knowledge and belief, accurate. I have read the University's current research ethics guidelines, and accept responsibility for the conduct of the procedures set out in the attached application in accordance with the guidelines, the University's policy on Conflict of Interest and any other condition laid down by the Dublin City University Research Ethics Committee or its Sub-Committees. I have attempted to identify all risks related to the research

that may arise in conducting this research and acknowledge my obligations and the rights of the participants.

If there any affiliation or financial interest for researcher(s) in this research or its outcomes or any other circumstances which might represent a perceived, potential or actual conflict of interest this should be declared in accordance with Dublin City University policy on Conflicts of Interest.

I and my co-investigators or supporting staff have the appropriate qualifications, experience and facilities to conduct the research set out in the attached application and to deal with any emergencies and contingencies related to the research that may arise.

Signature(s):

Principal investigator(s): Niall Moyna

Print name(s) in block letters: *Niall Moyna*

Date: *3/9/2010*

2. PROJECT OUTLINE

2.1 LAY DESCRIPTION

Endurance training predominates in intermittent type sports such as Gaelic games in which aerobic fitness is essential (1). Recent studies have shown that brief repeated sessions of 'all-out' high intensity or sprint type interval training (SIT) induce changes in skeletal muscle metabolism that resemble endurance type (ET) training (3;4). Most of the studies which confirm these findings are based on untrained subjects who trained on a stationary bike in a laboratory (4, 5). The purpose of this study is to compare the effect of 6 weeks (3 d/week) of SIT with 6 weeks (3 d/week) of ET on measures of fitness and performance in trained and untrained Gaelic football players. The total training time will be approximately 11 h 33 min and 13.4 min for the ET and SIT respectively. A total of 20 trained (T) and 20 untrained (U) subjects will be randomly assigned to an endurance training group (ET) or a high intensity interval training group (HIT). Before and after the 6 week training program the subjects will have a muscle biopsy and blood sample taken and will undergo a number of tests to measure body composition, speed, power, agility, lactate threshold, aerobic capacity, anaerobic capacity, and endurance performance. Blood lactate levels will be measured before and immediately after the third weekly training session. The results of the study may have significant implications for training guidelines for Gaelic football.

2.2 AIMS OF AND JUSTIFICATION FOR THE RESEARCH

Aims of the Research:

To compare the effect of 6 weeks of SIT and ET on speed, power, agility, maximal aerobic capacity, anaerobic capacity, intermittent endurance capacity, muscle oxidative capacity and selected measures of whole body and skeletal muscle substrate metabolism in trained and untrained Gaelic football players. The total training will be 11 h 33 min and 13.4 min for the SIT and ET group respectively.

Justification:

Endurance training induces numerous physiological and metabolic adaptations that improve endurance capacity (2). Although this type of training offers significant training adaptations it requires a large time commitment. Recent studies have shown that brief repeated sessions of 'all-out' high intensity or sprint type interval training (SIT) induces changes in skeletal muscle energy metabolism that resemble endurance type training (3;4). Gibala et al (4) found similar molecular and cellular adaptations in skeletal muscle following 6 sessions of SIT or endurance training (ET) performed over 2 weeks despite the fact that the total training time commitment and exercise volume were significantly lower in SIT groups.

In a more recent study (5), active but untrained subjects performed a constant-load cycling challenge (1 h at 65% of VO_2max) before and after 6 weeks of SIT or ET. The SIT group trained 3 d/week and each session consisted of four to six repeats of a 30 s 'all out' Wingate Test (mean power output~500W) with 4.5 min recovery between repeats, 3 days per week. The ET group trained 5 d/week and each session consisted of 40 -60 min of continuous cycling at a workload that elicited 65% $\dot{V}O_2$max (mean power output 150W). Despite the large time commitment differences, both protocols induced similar increases ($p<0.05$) in mitochondrial markers for skeletal muscle CHO (pyruvate dehydrogenase E1α protein content) and lipid oxidation (3-hydroxyacyl CoA dehydrogenase activity) and protein content of peroxisome proliferator-activated receptor-γ coactivator-1α. Glycogen and phosphocreatine utilization during exercise were reduced after training, and calculated rates

of whole-body CHO and lipid oxidation were decreased and increased, respectively, with no differences between groups (all main effects, $P < 0.05$). Given the markedly lower training volume in the SIT group, these data suggest that high-intensity interval training is a time-efficient strategy to increase skeletal muscle oxidative capacity and induce specific metabolic adaptations during exercise that are comparable to traditional ET. The majority of training studies (4-7) have used relatively untrained individuals and involved cycling as the mode of exercise.

1. Keane S, Reilly T (1993) Analysis of work rates in Gaelic football. Australian Journal of Sports Science 100-102
2. Gollnick PD AR (1973) Effect of training on enzyme activity and fiber type composition of human skeletal muscle. J. Appl.Physiol. 107-111
3. Henriksson J, Reitman JS (1976) Qualitative measures of enzyme activities in type I and type II muscle fibers of man after training. Acta Physiologica Scandinavica 97: 392-397
4. Gibala MJ, Little PJ (2006) Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. Journal of Physiology 901-911
5. Burgomaster KA, Howarth KR, Gibala MJ (2008) Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. Journal of Physiology 586: 151-160
6. Burgomaster KA, Heigenhauser G J F, Gibala MJ (2006) Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. J Appl.Physiol. 2041-2047
7. Burgomaster KA, Hughes SC, Heigenhauser G J F, Gibala MJ (2005) Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. J Appl.Physiol. 1985-1990

2.3 PROPOSED METHOD

Study Overview

The study will take place in the School of Health and Human Performance at DCU and the DCU Sports Grounds. Subjects will undertake 2 weeks (3d/week) of endurance training (ET) or high intensity interval training (HIT). Subjects will visit DCU on 3 separate occasions (1 screening and 2 study visits) before the study and on 2 separate occasions at the end of the study. They will have a muscle biopsy and blood sample taken and will undergo a number of tests to measure body composition, speed, power, agility, lactate threshold, aerobic capacity, anaerobic capacity, and endurance performance. Heart rate will be recorded during each training session and blood lactate levels will be measured before and immediately after the third weekly training session. Each visit will be separated by at least 24 h.

Screening Visit

The screening visit will last approximately 1 h. Subjects will undergo a brief medical examination and perform a Bangsbo Yo Yo Intermittent Recovery test.

Study Visits

Visit 1 - The visit will last approximately 2 h and will be used to measure body composition, muscle power, speed, agility, lactate threshold and maximal aerobic capacity (VO_2max).

Visit 2 - Subjects will run on the treadmill at 110% v VO_2max until volitional fatigue.

Visit 3 – A blood and a muscle biopsy sample will be taken from each subject

Endurance Training Program

Subjects will run on a treadmill at 70-80% VO_2max. Subjects will run for 50 min during the training sessions.

Sprint-Interval Training Protocol

Subjects will sprint 110 m followed by a 50 metre recovery run. The sprint and recovery run must be completed in 20 sec. This will be repeated 3 more times followed by a 5 min recovery period (1 set).

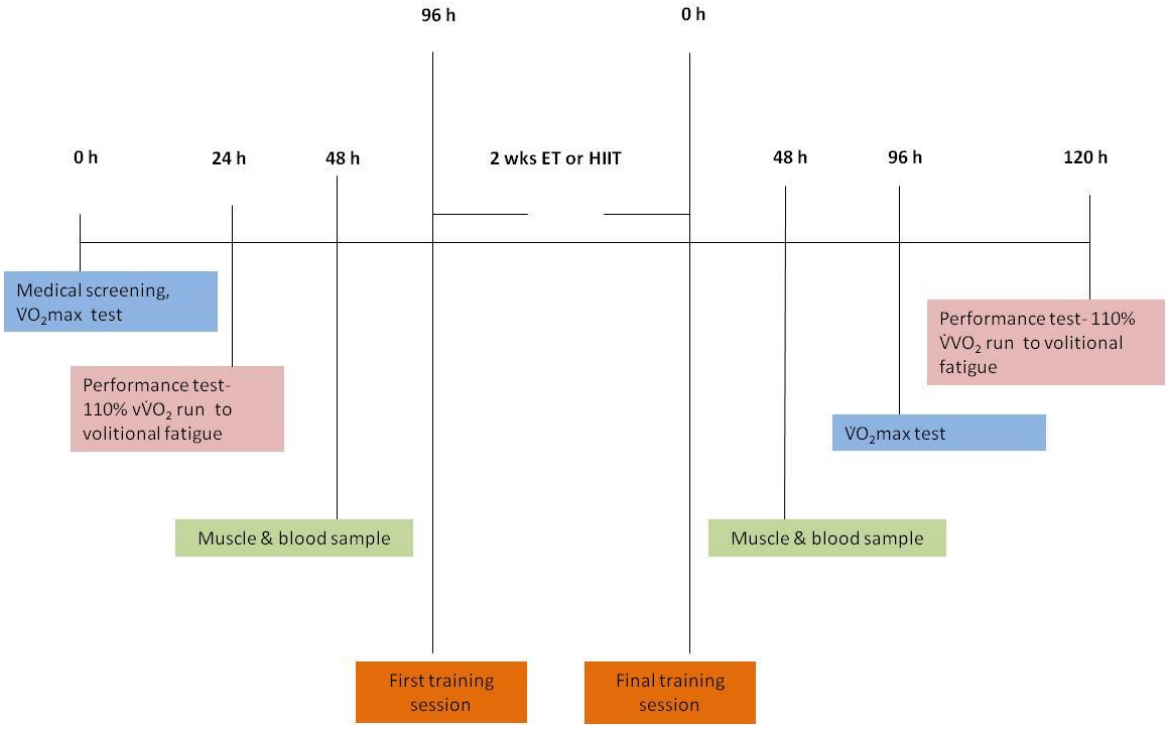

Figure : Training protocol

Laboratory Procedures:

Maximal Aerobic Capacity Assessment/ Lactate Threshold: Maximal aerobic capacity will be determined on a treadmill (Woodway ELG 55, Waukesha, WI) using a ramp protocol. Subjects will warm-up at 8 km/h for 3 min at 1% gradient. Following the warm-up, the speed will be increased 1 km/h every 3 min. At baseline and at the end of each 3 min stage a blood sample will be taken to determine blood lactate concentration. When lactate concentrations reach 4 mmol the speed will remain constant and the gradient will be increased by 1% every 30 sec until the subject reaches volitional exhaustion. The test will be deemed to be maximal if it satisfies at least 3 of the following criteria; levelling of oxygen consumption, volitional exhaustion, RER > 1.1 and heart rate within ± 10 beats of the age predicted max. HR will be recorded continuously, will be assessed each minute.

Respiratory Metabolic Measures: Expired oxygen, carbon dioxide, ventilatory volume and respiratory exchange ratios (RER) will be determined using a Sensormedics Vmax 229

metabolic system (SensorMedics Corp., Yorba Linda CA). Prior to testing, the gas analysers will be calibrated with standard gases of known concentration.

Percent Body Fat: Lange skinfold calliper (Cambridge Scientific Industries, MD) will be used to measure double thickness subcutaneous adipose tissue on the right side of the body. The following anatomical sites will be used: suprailiac, triceps and thigh. A minimum of 2 measurements will be taken at each site. If the measurements vary by more than 1 mm a third measurement will be taken.

Blood Sampling at Rest: A standard venous puncture will be used to collect blood samples at rest before and after the training study. A total volume of 30 ml will be taken.

Blood Sampling During Exercise: Blood samples (5-10 µl) will be taken from the earlobe using capillary tubes. The earlobe will be sterilized with a sterile wipe and then pricked with a lancet (AccuCheck Softclix Pro Lancet, Accu Check, Australia) to promote blood flow.

Lactate Analysis: Whole blood lactate concentration will be measured using a YSI 1500 Sport Lactate Analyzer (YSI UK limited).

Muscle Biopsy: Rationale for muscle biopsy samples

Skeletal muscle biopsies are necessary before and after the training intervention to determine the molecular and cellular adaptations induced by the training intervention in skeletal muscle.

4. *Little JP. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanism. J Physiol 588.6: 1011-1022, 2010*
5. *Burgomaster KA. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol 586: 151-160, 2008*
6. *Burgomaster KA. Effect of short-term sprint interval training on human skeletal muscle carbohydrate metabolism during exercise and time-trial performance. J Appl Physiol 100: 2041-2047, 2006*

Statistical Analysis:

A group (Trained or Untrained) x condition (ET or SIT) x time repeated measures ANOVA will be used to compare the mean differences within and between group. SPSS for Windows statistical software will be used to perform the statistical analysis. Statistical significance will be accepted at the $P < 0.05$ level of confidence

2.4 PARTICIPANT PROFILE

Inclusion criteria: Apparently healthy men, currently playing at junior club level or higher level, and between the ages of 18 – 35 years.

Fitness classification will be based on performance in the Bangsbo Yo-Yo Intermittent Recovery test. Subjects who achieve a level ≤ 16.8 will be classified as untrained. Subjects who achieve a level ≥ 16.8 will be classified as trained

Exclusion criteria: Volunteers will be excluded if they smoke or have any other medical conditions that contraindicate exercise participation.

2.5 MEANS BY WHICH PARTICIPANTS ARE TO BE RECRUITED

A recruitment advertisement will be emailed to members of the Dublin City University Gaelic games club and all Dublin city Gaelic games clubs. Permission will be sought from each club prior to posting the advertisement. The aim of the study, the rationale for the study, the tests involved, the time commitment and the potential benefits will be explained to the players. Players will be provided with an opportunity to ask questions. If they wish to participate in the study they will have to provide a written informed consent, which will be witnessed on their visit to the School of Health and Human Performance.

2.6 PLEASE EXPLAIN WHEN, HOW, WHERE, AND TO WHOM RESULTS WILL BE DISSEMINATED, INCLUDING WHETHER PARTICIPANTS WILL BE PROVIDED WITH ANY INFORMATION AS TO THE FINDINGS OR OUTCOMES OF THE PROJECT*?*

The study findings will be presented at scientific meetings and published in scientific journals. Subjects will be provided with a report, which will summarise the relevant results from their participation in the research project.

2.7 OTHER APPROVALS REQUIRED *Has permission to gain access to another location, organisation etc. been obtained? Copies of letters of approval to be provided when available.*

☐ YES ☐ NO ☒ NOT APPLICABLE

2.8 HAS A SIMILAR PROPOSAL BEEN PREVIOUSLY APPROVED BY THE REC?

☐ YES ☒ NO

(If YES, please state both the REC Application Number and Project Title)

3. RISK AND RISK MANAGEMENT

3.1 ARE THE RISKS TO SUBJECTS AND/OR RESEARCHERS ASSOCIATED WITH YOUR PROJECT GREATER THAN THOSE ENCOUNTERED IN EVERYDAY LIFE?

☒ YES ☐ NO *If YES, this proposal will be subject to full REC review*

If NO, this proposal may be processed by expedited administrative review

3.2 WHAT DOES THE RESEARCH INVOLVE:

	YES	NO
• Use of a questionnaire? (attach copy)?	☐	☒
• interviews (attach interview questions)?	☐	☒
• observation of participants without their knowledge?	☐	☒
• participant observation (provide details in section 2)?	☐	☒
• audio- or video-taping interviewees or events?	☐	☒
• access to personal and/or confidential data (including student, patient or client data) without the participant's specific consent?	☐	☒
• administration of any stimuli, tasks, investigations or procedures	☒	☐

which may be experienced by participants as physically or mentally painful, stressful or unpleasant during or after the research process?		
• performance of any acts which might diminish the self-esteem of participants or cause them to experience embarrassment, regret or depression?	☐	☒
• investigation of participants involved in illegal activities?	☐	☒
• procedures that involve deception of participants?	☐	☒
• administration of any substance or agent?	☐	☒
• use of non-treatment of placebo control conditions?	☐	☒
• collection of body tissues or fluid samples?	☒	☐
• collection and/or testing of DNA samples?	☐	☒
• participation in a clinical trial?	☐	☒
• administration of ionising radiation to participants?	☐	☒

3.3 POTENTIAL RISKS TO PARTICIPANTS AND RISK MANAGEMENT PROCEDURES *(see Guidelines)*

The nature and risks involved in the study will be explained prior to starting the study, and a contact number will be provided.

Subjects may experience some muscle soreness in their legs or nausea following the maximal exercise test. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The pre-test likelihood of these risks in asymptomatic men < 55 years of age is very low. There may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. Temporary numbness of the skin near the biopsy site may occur. There may be discomfort when taking blood and the development of a small bruise at the site of puncture.

3.4 ARE THERE LIKELY TO BE ANY BENEFITS (DIRECT OR INDIRECT) TO PARTICIPANTS FROM THIS RESEARCH?

☒ YES ☐ NO The study will provide information than can be used to develop training programs for Gaelic football.

3.5 ARE THERE ANY SPECIFIC RISKS TO RESEARCHERS? *(e.g. risk of infection or where research is undertaken at an off-campus location)*

☒ YES ☐ NO There is a small risk of infection from needle and blood samples. Standard safety procedures will be strictly adhered to.

3.6 ADVERSE/UNEXPECTED OUTCOMES

The School of Health and Human Performance has the facilities to deal with all aspects of this study and an emergency plan is in place for adverse events. All minor injuries will be addressed by an individual trained in first aid (either a member of the research team or the staff). The laboratory is equipped with an emergency crash cart and defibrillator. An individual trained in first aid (or Advanced Cardiac Life Support) will be present during each

test. In the unlikely event of a serious adverse outcome, the subject will be brought to the VHI clinic on campus.

3.7 MONITORING

Weekly meetings will take place between Prof. N. Moyna (principal investigator) and the other researchers. These meetings will provide opportunities to access progress, give feedback, and monitor development of the research. The School of Health and Human Performance has a detailed list of Standard Operating Procedures for each of the protocols in this study. All researchers will be familiar with the procedures and the Safety Statement before beginning data collection.

3.8 SUPPORT FOR PARTICIPANTS

It is anticipated that no additional support will be required.

3.9 DO YOU PROPOSE TO OFFER PAYMENTS OR INCENTIVES TO PARTICIPANTS?

☐ YES ☒ NO *(If YES, please provide further details.)*

4. INVESTIGATORS' QUALIFICATIONS, EXPERIENCE AND SKILLS *(Approx. 200 words – see Guidelines)*

Prof. Moyna is an exercise physiologist and has extensive experience with exercise testing

Dr. Noel McCaffrey and Dr. Davide Susta are physicians with extensive experience in the muscle biopsy technique

Crionna Tobin is a PhD student at the School of Health and Human Performance. She has a Bsc, a Higher Diploma in Human Nutrition and a Higher Diploma in Sports Nutrition.

Mr. David Kelly and Cathal Cregg are postgraduate students and have extensive experience in laboratory and field based exercise testing.

5. CONFIDENTIALITY/ANONYMITY

5.1 WILL THE IDENTITY OF THE PARTICIPANTS BE PROTECTED?

☒ YES ☐ NO *(If NO, please explain)*

IF YOU ANSWERED YES TO 5.1, PLEASE ANSWER THE FOLLOWING QUESTIONS:

5.2 HOW WILL THE ANONYMITY OF THE PARTICIPANTS BE RESPECTED?

Confidentiality is an important issue during data collection. Participant's identity, or other personal information, will not be revealed or published. Subjects will be assigned an ID number under which all personal information will be stored in a secure file and saved in password protected file in a computer at DCU. The investigators alone will have access to the data.

5.3 LEGAL LIMITATIONS TO DATA CONFIDENTIALITY: *(Have you included appropriate information in the plain language statement and consent form? See Guidelines)*

☒ YES ☐ NO *(If NO, please advise how participants will be advised.)*

The following statement should be included in the plain language statement.
'Confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

6 DATA/SAMPLE STORAGE, SECURITY AND DISPOSAL *(see Guidelines)*

6.1 HOW WILL THE DATA/SAMPLES BE STORED? *(The REC recommends that all data be stored on campus)*

Stored at DCU ☒
Stored at another site ☐ **(***Please explain where and for what purpose)*

6.2 WHO WILL HAVE ACCESS TO DATA/SAMPLES?

Access by named researchers only ☒
Access by people other than named researcher(s) ☐ **(***Please explain who and for what purpose)*
Other *:* ☐ **(***Please explain)*

6.3 IF DATA/SAMPLES ARE TO BE DISPOSED OF, PLEASE EXPLAIN HOW, WHEN AND BY WHOM THIS WILL BE DONE?

The principal investigator will be responsible for security of the collected data. The data will be kept in locked facilities in the department through which the project is being conducted. Access to the data will only be attainable by the main researchers. Data will be kept for a minimum of five years from the date of publication of the research. Aside from the main researchers, no others will have access to the raw data. Data will be shredded after five years and Prof. Moyna will carry this out.

7. FUNDING

7.1 HOW IS THIS WORK BEING FUNDED?

The Gaelic Athletic Association

7.2 PROJECT GRANT NUMBER (If relevant and/or known)

7.3 DOES THE PROJECT REQUIRE APPROVAL BEFORE CONSIDERATION FOR FUNDING BY A GRANTING BODY?

☐ YES ☒ NO

7.4 HOW WILL PARTICIPANTS BE INFORMED OF THE SOURCE OF THE FUNDING?

In the plain language statement

7.5 **DO ANY OF THE RESEARCHERS, SUPERVISORS OR FUNDERS OF THIS PROJECT HAVE A PERSONAL, FINANCIAL OR COMMERCIAL INTEREST IN ITS OUTCOME THAT MIGHT COMPROMISE THE INDEPENDENCE AND INTEGRITY OF THE RESEARCH, OR BIAS THE CONDUCT OR RESULTS OF THE RESEARCH, OR UNDULY DELAY OR OTHERWISE AFFECT THEIR PUBLICATION?**

NO

8. PLAIN LANGUAGE STATEMENT

Plain Language Statement

Dublin City University

Project Title: Muscle adaptations in response to low volume high intensity interval training and endurance training in Gaelic games

The Research Study will take place in the School for Human Health and Human Performance DCU and the DCU Sports Grounds

The research study is being funded by the Gaelic Athletic Association.

The principal investigator is: Prof. Niall M. Moyna, (Tel: 7008802 Fax 7008888) EMAIL niall.moyna@dcu.ie

7. A good level of endurance is important for Gaelic football players. Many players spend considerable time running long distances to improve their endurance. Research studies have shown that training involving short sprints with short recovery periods (sprint interval training) can also improve endurance. The amount of time required to improve endurance is considerable less when sprint interval training is undertaken compared to distance running. No studies have compared the effect of endurance training and sprint interval training on endurance performance in Gaelic football players. The purpose of this study is to compare the effect of a 6 week endurance training and sprint interval training program on endurance performance in Gaelic football players.

You will make 3 visits to the Human Performance Laboratory in DCU before and 2 visits after taking part in the training program. The first visit before the study will last approximately 1 hour and will involve you undergoing a brief medical examination and performing an endurance exercise test. The second visit will be 2 hours in duration and will be used to your aerobic fitness. During the third visit you will have a blood and a muscle biopsy and blood sample taken. You will then be assigned, by chance, to one of two groups. One group will take part in sprint-interval training and the other group will take part in endurance training 3 times per week for 2 weeks. Subjects randomised to the endurance training group will run on a treadmill at 70-80% VO_2max. Subjects will run for 50 min during the training sessions. Subjects randomised to the sprint-interval training will sprint 100 m followed by a 50 metre recovery run. The sprint and recovery run must be completed in 40 sec. This will be repeated 3 more times followed by a 4 min recovery period (1 set). Subjects will complete 3 sets during the training session. Training sessions will be carried out on the sprint track in DCU or in the DCU gym depending on which training program you are undertaking. Each training session will be monitored by graduate students to ensure compliance. During the last training session you repeat the same endurance test that you undertook before the study. You will repeat the elements of the previous second and third visits in the human performance lab in DCU 24-48 hrs after the last training session. During the first of the post-training visits to the human performance lab in DCU you will have a blood and a muscle biopsy sample taken. For the duration of the study you will not be able to participate in any other type of exercise during this 6 week period.

8. You will receive a report summarizing the results from your tests undertaken during the study. No other benefits have been promised.

9. Your identity and other personal information will not be revealed, published or used in further studies. You will be assigned an ID number under which all personal information will be stored in a secure file and saved in a password protected file in a computer at DCU. The principal investigator, and collaborators listed on this ethics application will have access to the data. You need to be aware that confidentiality of information provided can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

10. The original documentation will be stored for a maximum of 5 years. Thereafter the documentation will be shredded.

11. Your participation in this research project is voluntary and you may withdraw your consent at any time.

If participants have concerns about this study and wish to contact an independent person, please contact:

The Secretary, Dublin City University Research Ethics Committee, c/o Office of the Vice-President for Research, Dublin City University, Dublin 9. Tel 01-7008000

9. INFORMED CONSENT FORM

INFORMED CONSENT

Title: Muscle adaptations in response to low volume high intensity interval training and endurance training in Gaelic games

Principal investigator: Prof Niall M. Moyna

Other investigators: Ms Crionna Tobin, Mr Cathal Cregg, Mr David Kelly, Dr. Noel McCaffrey, Dr. Davide Susta

Purpose: The purpose of this study is to compare the effect of a 6 week endurance training and sprint interval training program on endurance performance in Gaelic football players.

Participant Requirements

5. I will have the purpose of the study, each of the steps involved and the risks of participating in the study explained to me. I will have the opportunity to ask any questions and if I am happy with the answers I will provide written informed consent for participation in the research project. I will then complete a medical history form, which will ask questions about my general health, personal and family health history, smoking, exercise, and dietary habits. I will talk with a medical doctor about the information I have provided and I understand, based on the information provided, the medical doctor may exclude me from participating in the research project. If I agree to participate in the study I will make 3 visits to the Human Performance Laboratory in DCU before and 2 visits after taking part in the training program.
6. For the first visit, I will arrive in the morning to the Human Performance Laboratory in DCU following my normal breakfast. I will undergo a brief medical screening to evaluate my current physical condition. I will undergo an exercise test on a treadmill to measure my aerobic fitness and lactate threshold. To assess my fitness I will have a mouthpiece similar to a snorkel in my mouth to measure the amount of air I breathe in and out. To assess my lactate threshold I will have my ear pricked to collect a small blood sample.
7. On my second visit to the Human Performance Laboratory. I will take part in an endurance fitness test to measure my endurance capacity. This will involve running on a tredmill until I fatigue.
8. During the third visit I will have a blood and a muscle biopsy sample taken. The muscle biopsy sample will involve taking a small piece of muscle, about the size of a pea taken from my thigh with a special biopsy needle. A small area of the leg will be injected with a local anaesthetic, then a small incision will be made in the skin and a needle inserted briefly into the muscle. The incision will be closed with sterile strip bandaids, and my leg will be wrapped snugly with an elastic bandage to maintain pressure. Before I leave I will be given supplies to change the dressing around the biopsy sites.
9. After the third visit to the laboratory I will be assigned, by chance, to one of two groups. I will take part in sprint-interval training or endurance training 3 times per week for 2 weeks. At the end of the third weekly training session I will have my ear pricked to collect a small blood sample to measure my blood lactate levels. This test will assess how well I am responding to each training session.
10. During the last training session I will repeat the same endurance test that I undertook before the study. I will repeat the elements of the previous second and third visits in the Human Performance Laboratory in DCU 24-48 hrs after the last training session.
11. I will not participate in any other type of exercise during this 6 week period.

Sometimes there are side effects from performing exercise tests. These side effects are often called risks, and for this project, the risks are:

4. Exercise testing carries with it a very small risk of exercise induced asthma, abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. The risk of sudden death during exercise for healthy men is 1:15000-18000. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs or nausea following the maximal exercise test. It should be noted that if the experimental protocol is adhered to, the likelihood of these risks occurring is minimal.
5. I understand that the insertion and placement of a cannula (to take blood samples) should be minimally painful but a slight ache may be felt and a small bruise may appear on my arm. There is also a small risk of infection, but by using the appropriate techniques this risk is minimal.
6. I understand that there may be discomfort during the muscle biopsy with some pain and delayed soreness afterward. There is a risk of bleeding, bruising, infection and scaring of the skin. After the biopsy, my leg may feel stiff and sore. Temporary numbness of the skin near the biopsy may also occur.

There may be benefits from my participation in this study. These are:

3. I will receive a copy of my personal results, body fat and fitness measurements
4. I understand that no other benefits have been promised me.

Participant – please complete the following (Circle Yes or No for each question)

I have read the Plain Language Statement	Yes/No
I understand the information provided	Yes/No
I have had an opportunity to ask questions and discuss this study	Yes/No
I have received satisfactory answers to all my questions	Yes/No

<u>My confidentiality will be guarded:</u>

Dublin City University will protect all the information about me, and my part in this study, within the limitations of the law. My identity or personal information will not be revealed or published. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records. In addition, the study findings may be presented at scientific meetings and published in a scientific journal and/or as part of a postgraduate thesis, but my identity will not be divulged and only presented as part of a group.

If I have questions about the research project, I am free to call Prof Niall Moyna at 01-7008802.

<u>Taking part in this study is my decision.</u>

I understand that my participation in this study is voluntary and that I may withdraw my consent at any time by notifying any of the investigators. I may also request that my data and samples be removed from the database or storage and destroyed. My withdrawal from this study, or my refusal to participate, will in no way affect my relationship with Dublin City University or my entitlements as a student or staff member. I understand that my participation in this research may be terminated by the investigator without regard to my consent if I am unable or unwilling to comply with the guidelines and procedures explained to me.

<u>Signature:</u>

I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project

Participants Signature: _____

Name in Block Capitals: _____

Witness: _____

Date: _____

Email to participants:

The school of Health and Human Performance is conducting a study to compare the effect of a 6 week endurance training and sprint interval training program on speed, agility, power and endurance performance in Gaelic football players. You will make 3 visits to the Human Performance Laboratory in DCU before and 2 visits after taking part in the training program. The first visit before the study will last approximately 1 hour and will involve you undergoing a brief medical examination and lactate threshold and your aerobic fitness. The second visit will you will perform an endurance exercise test. During the third visit you will have a blood and a muscle biopsy sample taken. You will then be assigned, by chance, to one of two groups. One group will take part in sprint-interval training and the other group will take part in endurance training 3 times per week for 2 weeks. During the last training session you will take part in an endurance test. You will repeat the elements of the previous second and third visits in the Human Performance Laboratory in DCU 24-48 hrs after the last training session. We are looking for 40 healthy men, currently playing Gaelic football at any level, and between the ages of 18 – 35 years.

If you would like to hear more about this study or would consider participating, please contact one of the following;

Crionna Tobin
Email: Crionna.tobin9@mail.dcu.ie
Mobile number: 086-0705130

David Kelly
Email: david.kelly59@mail.dcu.ie
Mobile number: 085-1618207

Cathal Cregg
Email: cathal.cregg2@mail.dcu.ie
Mobile number: 087- 7633021

Thank you,

Niall M. Moyna, PhD

Standard template for ethical justification for blood sampling associated with human studies conducted within DCU.

Completion instructions:

This document is intended to prompt responses to a number of standard questions which generally need to be answered to justify the sampling of blood associated with human studies.

The document is not meant to be an exhaustive exploration of the justification for such sampling and in specific situations. Additional information may be required/ requested.

Answers are expected to be brief but should also be informative. See a sample completed form at the end.

Queries should be directed to the Secretary of the Research Ethics Committee in the OVPR office.

1) **Briefly explain why blood sampling is required**

To monitor circulating levels of glucose, lactate, free fatty acids and insulin, this will be

2) **Outline the analyses, components or general applications to be investigated in subject blood (now and any future studies)**

Blood lactate will be determined using a YSI 1500 Sport Lactate Analyzer (YSI UK limited). Glucose will be analysed using a YSI 2300 Analyzer (YSI UK limited). Free fatty acids

3) **Are any alternatives available to substitute the venous sampling of blood? yes/no.**

4) **Will sampling require cannulation or direct vein puncture?**

Direct vein puncture and ear prick with lancet

5) **Outline the minimum volume of original subject blood (i.e. not serum or plasma) required to measure the required components.**

4.0 ml for circulating levels of glucose, free fatty acids and insulin

6) **Are steps being taken in the protocol to minimise the volume of blood samples being taken? Yes**

Yes. We have taken the minimum volume of blood that will allow us to examine

7) **Are steps included to minimise the number of blood samples/vein puncture being taken? Yes**

Yes. We have taken the minimum number of blood samples that will allow us to

8) **Anticipated sampling methodology**

Volume of blood to be taken per sample	4.0 ml for glucose, insulin and free fatty acids 100µl for lactate
Maximum number of samples to be taken per "sitting"	6 for lactate 6 for glucose, insulin and free fatty acids
Maximum number of samples taken per day	Screening Visit- 0 samples Visit 1 – 6 samples Visit 2 – 6 samples During training – 2 samples Visit 3 (post training program) – 6 samples Visit 4 (post training program) – 6 samples
Maximum number of samples to be taken over the course of the full study (if long duration study indicate the amount taken in an active 1 month period)	12 blood samples and 14 lactate samples
Maximum anticipate number of vein puncture episodes	2
Total volume of blood that will be taken from subject.	50 ml

9) I certify that:-

- all persons sampling blood in this study are certified to do so through the school/unit where this work is being conducted

- that all those manipulating the resultant samples are fully trained in the safe practice of handling blood
- all persons handling this blood have received appropriate information according to current vaccination policy.

Signature of Study PI Niall Mayna Date: 06 September 2010

An original signed copy must accompany electronic submissions. Alternatively, a PDF or other scanned version with a signature may be submitted