Intermittent-sprint exercise: Performance and muscle adaptations in health and chronic disease

Submitted by

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ABSTRACT

The aim of this thesis was to investigate the acute and chronic effects of high-intensity intermittent exercise, in the form of repeated-sprint exercise (RSE) and indoor football (futsal), on performance responses and skeletal muscle molecular adaptations in young, healthy and middle-aged, sedentary individuals. This was accomplished through four studies.

Study I part I. Ten young, healthy adults (Mean ± SD; age 22.3 ± 4.1 years; height 174.40 ± 9.0 cm; mass 70.2 ± 11.6 kg; 7 males, 3 females) performed an incremental test on a treadmill, a Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1), and one RSE trial. RSE comprised three sets of 5 x 4-s maximal sprints on a non-motorised treadmill, with 20 s of passive recovery between repetitions and 4.5 min of passive recovery between sets. After ten repeated-sprint training sessions, participants performed all tests again. During RSE, performance was determined by measuring acceleration, mean and peak power/velocity. Recovery heart rate (HR), HR variability, and finger-tip capillary lactate concentration ($[Lac^-]$) were measured. Performance progressively decreased across the three sets of RSE, with the indices of repeated-sprint ability being impaired to a different extent before and after training. Training induced a significant increase ($p < 0.05$) in all indices of performance, particularly acceleration by 21.9, 14.7 and 15.2 % during sets 1, 2 and 3, respectively. Training significantly increased Yo-Yo IR1 performance by 8 % and decreased $\Delta[Lac^-]$/work ratio by 15.2, 15.5, and 9.4 % during sets 1, 2 and 3, respectively, and recovery HR during RSE. There were strong correlations between Yo-Yo IR1 performance and indices of RSE performance, especially acceleration post-training ($r = 0.88$, $p = 0.004$). Repeated-sprint

training, comprising only 10 min of exercise overall, effectively improved performance during multiple-set RSE. This exercise model better reflects team-sport activities than single-set RSE. The rapid training-induced improvement in acceleration, quantified here for the first time, has wide applications for team-sport activities.

Study I part II. Ten young, healthy adults (age 22.3 ± 4.1 years; height 174.40 ± 90 cm; mass 70.2 ± 11.6 kg; 7 males, 3 females) performed an RSE trial. After 4 weeks of repeated-sprint training (3 x wk.) participants repeated the RSE. Pre- and post-training muscle biopsies were obtained at rest, immediately after, 1h and 4h after RSE. Real time RT-PCR and western blotting were used to measure mRNA expression and protein abundance, respectively. Acute RSE increased the phosphorylation of Acetyl-CoA Carboxylase (ACC; 86 %; effect size (ES) ± confidence interval: 1.4 ± 0.8; $p < 0.001$) and Ca^{2+} calmodulin-dependent protein kinase II (CaMK II; 69 %; ES 0.7 ± 0.6). Peroxisome proliferator-activated receptor γ, co-activator 1α (PGC-1α; 208 %; ES 1.5 ± 0.7; $p < 0.001$), and nuclear respiratory factor 1 (NRF1; 92 %, ES 0.7 ± 0.8) mRNA expression were increased following RSE. Four weeks of training increased PGC-1α protein abundance at rest (33 %, ES 0.9 ± 0.7). Both acute and chronic RSE, despite only 60 s and 12 min of exercise respectively, altered the molecular signalling associated with mitochondrial adaptations and PGC-1α mRNA expression in skeletal muscle. However, the small-to-moderate changes in resting PGC-1α protein abundance after training, together with the absence of changes in aerobic fitness, indicate that further research is required to understand the functional significance of PGC-1α in response to repeated-sprint exercise.

Study II. Skeletal muscle protein signalling associated with mitochondrial biogenesis was assessed in response to a futsal game vs. work-matched, moderate-intensity

continuous running exercise. Sixteen young, healthy men (age 21.4 ± 1.7 years; height 179.20 ± 6.0 cm; mass 74.8 ± 5.9 kg) performed an incremental exercise test on a treadmill. After 48 h, a resting muscle biopsy was performed. Then, after one week, participants were randomly assigned to either a futsal group (FUT, n = 8) or a moderate-intensity running exercise group (MOD, n = 8). The FUT group performed a 2 x 20-min halves futsal game on a parquet indoor court. The MOD group performed moderate-intensity exercise on a treadmill with triaxial accelerometers used to match the work performed by the two groups. Immediately after exercise, all participants underwent another muscle biopsy. Protein signalling was assessed via western blotting. An acute FUT game was associated with increased ATF2 phosphorylation (50 %, ES 0.59 ± 0.13, p = 0.001), while no change was detected in response to MOD (interaction time x training, p = 0.02). Both FUT and MOD increased ACC phosphorylation, by 119 and 75 %, respectively (main effect for time, p = 0.01). Similarly, both training interventions increased p38 MAPK phosphorylation by 85 and 36 % for FUT and MOD, respectively (main effect for time, p = 0.003). In conclusion, both FUT and MOD induced similar protein signalling responses in human skeletal muscle, which signifies that FUT may be used as an appropriate alternative to traditional aerobic exercise to promote mitochondrial biogenesis.

Study III. The effects of FUT and MOD training on skeletal muscle and systemic adaptations associated with the reduction of diabetes risk factors were then assessed. Twenty middle-aged, sedentary men (age 44.2 ± 6.3 years; height 177.00 ± 0.1 cm; mass 91.6 ± 14.2 kg) undertook either FUT (n = 12) or MOD (n = 8) for 8 weeks, 3 times per week. Before and after the training interventions, participants underwent an oral glucose tolerance test (OGTT), a resting muscle biopsy, and an incremental

exercise test on a treadmill. Plasma glucose and insulin concentration, and peripheral insulin sensitivity were measured in response to the OGTT. The abundance of selected proteins associated with mitochondrial biogenesis and glucose transport was measured at rest and analysed via western blotting. Blood lipids, HbA1c, blood pressure, anthropometry and self-reported dietary intake were also assessed. Peripheral insulin sensitivity was improved only in FUT (+31 %, ES 0.67 ± 0.53). Mitochondrial complex IV, subunit II (COXII) resting protein abundance was increased in FUT only (+16 %, ES 0.79 ± 0.24), while glucose transporter 4 (GLUT4) was equally increased in FUT and MOD (+45 %, ES 0.78 ± 0.57; and +44 %, ES 0.67 ± 0.57, respectively). Only FUT training resulted in a lowered plasma total cholesterol and triglycerides concentration (-8 %, ES 0.44 ± 0.24; and -32 %, ES 0.52 ± 0.31, respectively). No changes were detected for BMI, waist circumference, and self-reported dietary intake for either training intervention. These data suggest that FUT training may be employed as an effective exercise model to assist in the prevention of diabetes in middle-aged sedentary males.

Conclusions. Acute RSE increased protein signalling associated with mitochondrial biogenesis and glucose metabolism. These increases were replicated in response to an acute game of futsal and were comparable to a work-matched, continuous running exercise. Chronic participation in RSE improved sprint performance in young adults, however it did not induce significant changes in the abundance of proteins associated with mitochondrial biogenesis. On the contrary, futsal training was effective in promoting molecular changes associated with mitochondrial biogenesis and glucose metabolism, and assisted in the reduction of diabetes risk factors in middle-aged men.

STUDENT DECLARATION

I, Fabio R. Serpiello, declare that the Ph.D. thesis entitled “Intermittent-sprint exercise: performance and muscle adaptations in health and chronic disease” is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Fabio R. Serpiello Thursday, 17th May 2012

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Dedicated to my parents and my brother

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I would also like to dedicate this thesis to Jess

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LIST of PUBLICATIONS

The following publications are presented in support of this thesis:

1. **Serpiello FR**, McKenna MJ, Stepto NK, Bishop DJ, Aughey RJ. Performance and physiological responses to repeated-sprint exercise: a novel multiple-set approach. *Eur J Appl Physiol* 2011; **111(4):**669-78.

2. **Serpiello FR,** McKenna MJ, Bishop DJ, Aughey RJ, Caldow MK, Cameron-Smith D, Stepto NK. Repeated sprints alter signalling related to mitochondrial biogenesis in humans. *Med Sci Sports Exercise* 2012; **44(5):**827-834.

3. **Serpiello FR,** McKenna MJ, Wyckelsma VL, Banting LK, Steward C, Stepto NK, Aughey RJ, Anderson MJ, Bishop DJ. Futsal training lowers risk factors associated with diabetes in middle-aged men: the "FIT-SAL" project. Submitted to *PLOS ONE* (October 2012).

LIST of CONFERENCE PRESENTATIONS

The following oral presentations and poster communications are associated with this thesis:

1. **Serpiello FR**, Bishop DJ, Wyckelsma VL, Banting LK, Steward C, Stepto NK, Aughey RJ, Anderson MJ, McKenna MJ. Futsal training for diabetes prevention in middle-aged males: the "FIT-SAL" project. POSTER. 17th Annual Congress of the European College of Sport Sciences, Bruges, Belgium (2012).

2. **Serpiello FR**. The effects of high-intensity intermittent exercise on muscle gene and protein expression in humans. ORAL E-PRESENTATION. Victoria University & University of Texas at El Paso Health Research Symposium, Melbourne, Australia (2011).

3. **Serpiello FR**, Stepto NK, Bishop DJ, Aughey RJ, McKenna MJ. Repeated-sprint exercise and training alter skeletal muscle protein signalling and expression in humans. POSTER. 2nd National Congress of the Italian Society of Sport and Exercise Sciences, Turin, Italy (2010).

4. **Serpiello FR**, McKenna MJ, Stepto NK, Bishop DJ, Aughey RJ. Repeated-sprint training is an effective method to improve specific fitness in intermittent sports. ORAL PRESENTATION. 16th International Tennis Federation Worldwide Coaches Conference, Valencia, Spain (2009).

LIST of AWARDS

The following scholarships and awards are associated with this thesis:

1. **2011 João Havelange Research Scholarship**

   Awarded by: Fédération Internationale de Football Association (FIFA) and the International Centre for Sports Studies (CIES)

   Amount: CHF 30,000

   Purpose: Research Grant to fund research-related expenses (Study III)

2. **2011 CooperInt Travel Grant**

   Awarded by: University of Verona, Italy

   Amount: Euro 5,000

   Purpose: Two-month Visiting Scholarship at the Department of Physiology, The University of Melbourne, Australia

3. **2009 International Postgraduate Research Scholarship**

   Awarded by: Victoria University

   Amount: Au$ 22,854 per annum

   Purpose: Living expenses

4. **2008 Endeavour Europe Award**

   Awarded by: Department of Education, Australian Government

   Amount: Au$ 50,000

   Purpose: Twelve-month research scholarship (living expenses and fees)

ABBREVIATIONS

ACC	Acetyl-CoA Carboxylase
ADP	Adenosine diphosphate
Akt	V-akt murine thymoma viral oncogene homolog
AMP	Adenosine monophosphate
AMPK	5'AMP-activated protein kinase
ANOVA	Analysis of variance
AS160	Akt substrate of 160 kDa
ATF2	Activating transcription factor 2
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
$[Ca^{2+}]$	Calcium ion concentration
$[Ca^{2+}]_i$	Intracellular calcium ion concentration
CaM	Calmodulin
CaMKII	Ca^{2+}/CaM-dependent protein kinase II
CaMKIV	Ca^{2+}/CaM-dependent protein kinase IV
CaMKKβ	Ca^{2+}/calmodulin-dependent protein kinase kinase β
cDNA	Complementary DNA
CI	Confidence interval
COX II	Cytochrome c oxidase subunit II
COX IV	Cytochrome c oxidase subunit IV
CRE	cAMP response element
CS	Citrate synthase
CV	Coefficient of variation

DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycoltetraacetic acid
ERK	Extracellular signal-regulated kinase
ES	Effect size
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
[Glu]	Glucose concentration
GLUT4	Glucose transporter 4
h	Hour
H^+	Hydrogen ion
$[H^+]$	Hydrogen ion concentration
Hb	Haemoglobin
Hct	Haematocrit
HAD	3-hydroxyacyl CoA dehydrogenase
HDAC 5	Histone deacetylase 5
HR	Heart rate
Hz	Hertz
IL-6	Interleukin-6
JNK	C-Jun N-terminal kinase
IRS-1	Insulin receptor substrate 1
KCl	Potassium chloride
kg	Kilogram
kJ	KiloJoule
km	Kilometre
L	Litre

Lac^-	Lactate ion
$[Lac^-]$	Lactate ion concentration
LT	Lactate threshold
MAPK	Mitogen-activated protein kinase
MCT1	Monocarboxylate transporter 1
MCT4	Monocarboxylate transporter 4
MEF2A	Myocyte enhancer factor 2A
$MgCl_2$	Magnesium chloride
MKK	MAPK kinase
m	Metre
μL	Microlitre
min	Minute
mL	Millilitre
μM	Micromolar
mM	Millimolar
mRNA	Messenger RNA
mtTFA	Mitochondrial transcription factor A
NaN_3	Sodium azide
NaCl	Sodium chloride
NaF	Sodium fluoride
Na_3VO_4	Sodium orthovanadate
$Na_4O_7P_2$	Sodium pyrophosphate
ng	Nanogram
nM	Nanomolar
NP-40	Tergitol-type NP-40 (nonyl phenoxypolyethoxylethanol)
NRF1	Nuclear respiratory factor 1

N_2	Nitrogen
OBLA	Onset of blood lactate accumulation
PCr	Phosphocreatine
P_i	Inorganic phosphate
PGC-1α	peroxisome proliferator-activated receptor γ, co-activator 1 α
PMSF	Phenylmethanesulfonylfluoride
PVDF	Polyvinylidene fluoride
RIP140	Receptor-interacting protein 140
RMSSD	Root-mean-square difference of successive normal R-R intervals
RNA	Ribonucleic acid
RSE	Repeated-sprint exercise
RT-PCR	Reverse transcription – polymerase chain reaction
s	Second
SDNN	Standard deviation of normal R-R intervals
SDS-PAGE	Sodium dodecyl sulphate – polyacrylamide gel electrophoresis
Ser	Serine
STK11	Serine/threonine kinase 11
Thr	Threonine
TNF-α	Tumoral necrosis factor α
Tris	2-Amino-2-hydroxymethyl-propane-1,3-diol
Tyr	Tyrosine
$\dot{V}O_{2peak}$	Peak pulmonary oxygen uptake
$\dot{V}O_{2max}$	Maximal pulmonary oxygen uptake
W	Watt
Yo-Yo IR1	Yo-Yo Intermittent Recovery Test level 1

TABLE of CONTENTS

LIST of FIGURES

LIST of TABLES

CHAPTER 1 – INTRODUCTION

The prevention of chronic disease through exercise participation has attracted extensive scientific research (Shaw *et al.*, 2006; Thomas *et al.*, 2006b). The projections of the increased prevalence of type 2 diabetes and obesity for the next two decades not only highlight the risks to public health, but also point to the economic and psychosocial burden that many countries will face if insufficient action is taken (Kelly *et al.*, 2008; Whiting *et al.*, 2011). There is general consensus on the importance of prevention strategies and lifestyle modifications to reduce the risk factors associated with chronic diseases (Mayor, 2007), and one of the main common risk factors is a sedentary lifestyle.

The majority of pioneer research on the use of exercise as lifestyle modification has employed continuous aerobic exercise, usually performed at a low or moderate intensity. Large randomised controlled trials have reported beneficial effects of aerobic exercise on the health of middle-aged individuals (Eriksson and Lindgarde, 1991; Lindstrom *et al.*, 2003). Aerobic exercise is thus an effective exercise model that can easily be employed in research, primarily due to the advantage of being reproducible in a laboratory setting and applicable in the field. However, aerobic exercise also has the disadvantage of not being motivating for many participants, with a consequent risk of high drop-out rates. This originates from the social interaction components presented by this type of activity to participants (Deci and Ryan, 1985; Hagger and Chatzisarantis, 2007), and the large time commitment required to obtain physical benefits, which is one

of the main constraints to physical activity participation (Australian Bureau of Statistics (2007)).

Therefore, alternative exercise models have been investigated, especially high-intensity interval training (Earnest, 2008). The rationale behind the proposed efficacy of interval training was based on both physiological and psychosocial aspects. Firstly, it has been demonstrated that 6 weeks of interval training, in the form of repeated, 30-s "all-out" bouts, were as effective as aerobic exercise in inducing molecular adaptations associated with mitochondrial biogenesis within skeletal muscle, despite the training volume being approximately 10 times lower (Burgomaster *et al.*, 2008). Secondly, interval training was perceived as more enjoyable than aerobic training by participants (Bartlett *et al.*, 2011; Tjønna *et al.*, 2008).

Despite the increasing evidence supporting the efficacy of interval training in inducing muscle molecular adaptations, it remains difficult to apply this type of exercise to a real-life context, as it is not always practical and it is very demanding from a metabolic perspective (Little *et al.*, 2010). An appropriate alternative is intermittent exercise that comprises shorter periods of activity at maximal intensity, interspersed with periods of recovery or low-intensity activity. Intermittent-sprint exercise models such as repeated sprints and indoor football (futsal) belong to this category. Repeated-sprint exercise is based on the repetition of short sprints (usually less than 10 s in duration) and recovery periods. It is usually well tolerated, and is more reflective of the activity patterns of many team sports (Spencer *et al.*, 2005). Futsal is an intermittent team-sport game which comprises repeated accelerations and decelerations interspersed with periods of

low-intensity activity or recovery (Castagna *et al.*, 2009). This intermittent nature, together with its high participation rate worldwide, makes futsal a promising exercise model for applied research.

The aim of this doctoral thesis was to investigate the performance and muscle adaptations to repeated-sprint exercise and futsal in health and chronic disease.

CHAPTER 2 – REVIEW of LITERATURE

This chapter reviews the effects of acute and chronic high-intensity intermittent exercise (RSE and futsal) on performance and muscle adaptations. The definitions of the types of physical activity which have been used in the thesis will be provided, followed by a review of the effects of RSE and futsal on muscle adaptations, with a specific focus on those adaptations that have been associated with the reduction of the severity of diabetes risk factors.

2.1 Intermittent- and repeated-sprint exercise

2.1.1 Definition of sprint, intermittent-sprint exercise and repeated-sprint exercise

Establishing a clear definition of the different types of exercise is a complex task, and largely depends on the background of the researchers and on the criteria chosen to create the comparison (Winter and Fowler, 2009). It is fundamental to define three types of activities that recur throughout the chapters of this thesis: these are "sprint", "intermittent-sprint exercise" and "repeated-sprint exercise".

2.1.1.1 Definition of sprint

While the term "sprint" is ubiquitous in exercise-related research, its definition has been associated with very different types of activity, ranging from 5-s maximal efforts (Linossier *et al.*, 1993), to "all-out", 30-s (McKenna *et al.*, 1993) or 1-min exercise bouts (Wittekind and Beneke, 2011). It is intuitive that the term "sprint" cannot identify both a 5-s and a 1-min exercise period. A recent review has defined a sprint as "brief exercise, in general $\leq$ 10 seconds, where maximal workout (i.e., power/speed) can be

nearly maintained until the end of the exercise" (Girard *et al.*, 2011). The rationale of such definition was based on the difference in the velocity profile during the 100-m and 200-m world-record sprint performances. During the 100-m sprint the peak velocity reached during the acceleration phase was maintained almost constant until the last 10-m split. In contrast, during a 200-m effort the velocity at the end of exercise was reduced to approximately 88 % of the peak velocity (Girard *et al.*, 2011). This observation supports the choice of 10 s as cut-off duration for the definition of sprint. However, this definition was based on elite athletes, therefore its applicability may be limited in exercise-related research utilising sedentary or moderately-trained individuals. To confirm the rationale behind the definition of "sprint", the velocity profile of a 4-s, 6-s, 10-s, and 30-s maximal running effort on a non-motorised treadmill was assessed in a recreationally-active individual (unpublished data, Serpiello, 2009). While during the shorter exercises there was almost no decrease in the velocity, during the 30 s exercise the velocity at the end of exercise corresponded to approximately 70 % of the peak velocity (Figure 1).

Therefore, for this thesis the term "sprint" will be defined as an effort performed at the maximal possible intensity, with a duration of less than, or equal to 10 s. All types of maximal exercise longer than 10 s will be defined as "all out".

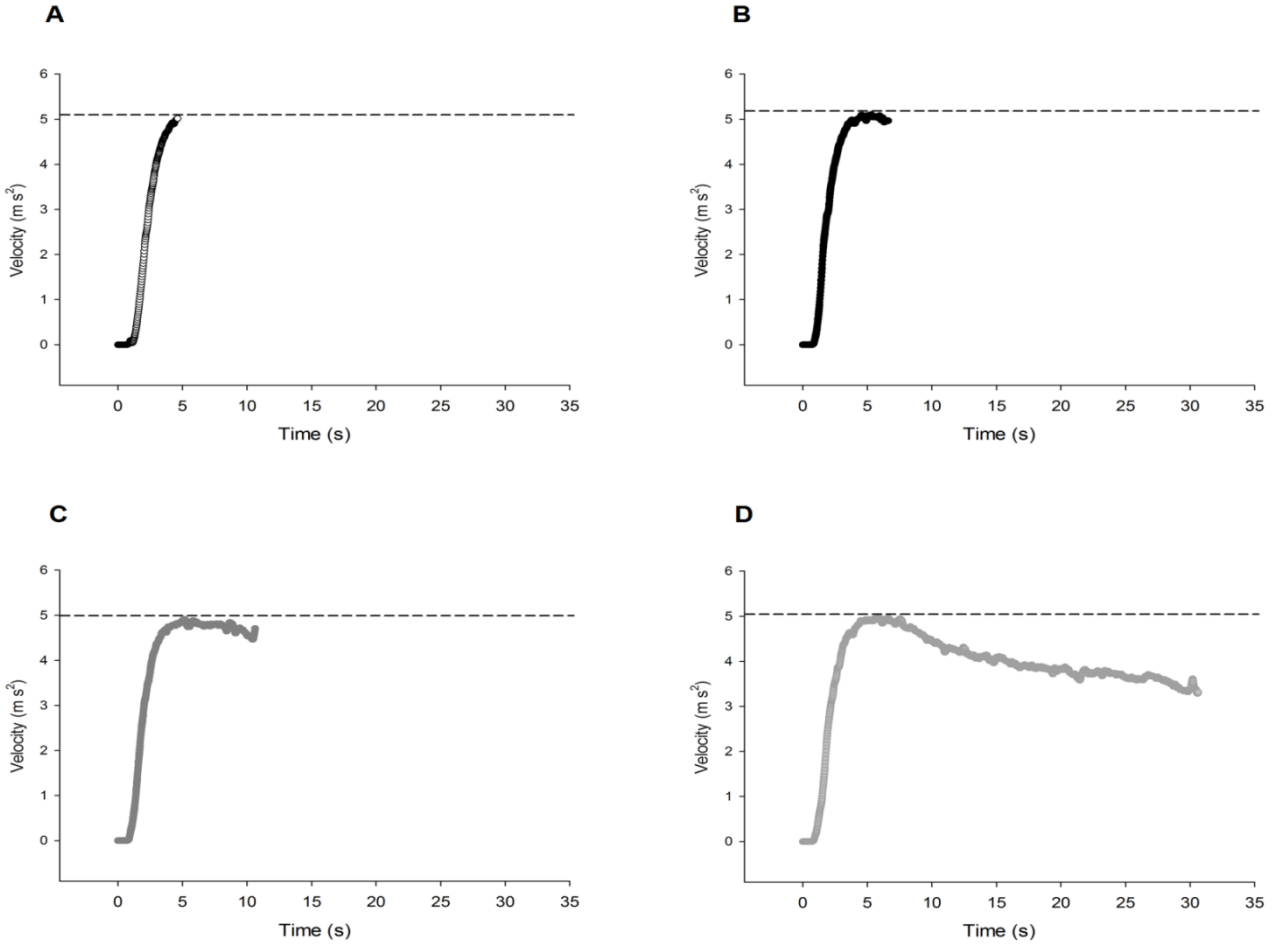

Figure 1; Velocity profile of 4-s (A), 6-s (B), 10-s (C), and 30-s (D) maximal running efforts on a non-motorised treadmill.

Sampling frequency: 50 Hz; n=1

2.1.1.2 Definition of intermittent-sprint and repeated-sprint exercise

When sprints are repeated over time, with a period of passive rest or low-intensity recovery between the subsequent efforts, the resulting exercise is generally referred to as "intermittent-sprint exercise". However, there is a more specific definition of "intermittent-sprint exercise", which differentiates it from "repeated-sprint exercise" (RSE). Intermittent-sprint exercise is usually defined as characterised by recovery periods with a duration longer than 60 s (Bishop and Claudius, 2005; Nevill *et al.*, 1993), while RSE has traditionally been defined as employing shorter recovery periods of less than 30 s (Dawson *et al.*, 1997). Such distinction might appear unnecessary, but it is based on early research demonstrating a significant difference in the decline of sprint performance when 6-s sprints are repeated with 30, 60, or 120 s of recovery (Balsom *et al.*, 1992). In the present thesis RSE will be defined as a repetition of sprints interspersed with a recovery period of less than, or equal to 30 s.

2.1.2 RSE reflects high-intensity activity during a football game

RSE is often used in research as a reflection of the high-intensity activity component of team sports, in particular football. This section will discuss the rationale for this choice.

The activity profile of football has been thoroughly investigated in the last three decades, to improve understanding of the physiological responses to the game, thus allowing better training interventions (Ekblom, 1986). In particular, attention has been directed to the assessment of high-intensity activity during the game, possibly initiated by the conviction that an improvement in high-intensity activity would be correlated with a better performance during the game. While some basic parameters, such as the total distance covered by players during a game, were assessed rather early (Bangsbo *et*

al., 1991), the quantification of high-intensity activity and sprints presented itself as a more complex task. In particular, the analysis of sprint frequency during a football game has been proven difficult (Table 1), mainly due to differences in the choice of the cut-off running velocities, the methods employed (e.g., manual recording, video etc.) and the player positions (Spencer *et al.*, 2005). However, when a more systematic approach is taken, for example considering only data referring to all players together (i.e., with no role distinction) and measured with video analysis, the frequency of high-intensity efforts can be narrowed to a range of approximately 20 to 40 sprints per game, with a between-sprint recovery from 135 to 270 s (Bangsbo *et al.*, 1991; Carling *et al.*, 2012; Di Salvo *et al.*, 2009; Mohr *et al.*, 2003). This data would suggest that the ability to repeat sprints during a football game is important for a player's physical performance, especially in the final stages of a game. When short sprints (30-40 m) are performed with a recovery of 60 or 120 s, the decline in sprint performance is significant after 7 and 11 sprints, respectively (Balsom *et al.*, 1992).

Study	Sport	Subjects	Positional role	Method	Sprint duration (sec)	Sprint distance (m)	Sprint frequency	Recovery time between sprints (sec)	Change in motion[a] (sec)
Bangsbo et al.[1]	Soccer	14 E M	All players	Video	2.0		19	284	~7
		4 E M	Defenders	Video	2.0		16	338	
		7 E M	Mid-fielders	Video	2.1		17	318	
		3 E M	Forwards	Video	1.7		24	225	
Barros et al.[17]	Soccer	25 E M	All players	Video		13	55	98	
Drust et al.[18]	Soccer	23 E M	All players	Video					4.0
Mayhew and Wenger[3]	Soccer	3 E M	All players	Video	4.4[b]		519[b]	40[b]	6.1
Mohr et al.[2]	Soccer	18 E M	All players	Video	2.0		39	138	
		24 E M	All players	Video	1.9		26	208	
Reilly and Thomas[4]	Soccer	40 E M	All players	Manual-audio		15.7	62	90	6.4
		11 E M	Mid-fielders	Manual-audio		15.6	68	79	6.0
		8 E M	Full-backs	Manual-audio		15.1	52	104	6.3
		14 E M	Forwards	Manual-audio		16.4	65	83	6.7
		7 E M	Centre-backs	Manual-audio		14.1	59	92	6.7
Withers et al.[5]	Soccer	20 E M	All players	Video	3.7[b]	22.4[b]	~30 (97[b])	~180 (56[b])	
		5 E M	Full-backs	Video	3.7[b]	24.3[b]	~38 (110[b])	49[b]	
		5 E M	Centre-backs	Video	3.6[b]	20.8[b]	~19 (80[b])	68[b]	
		5 E M	Mid-fielders	Video	3.8[b]	22.6[b]	~29 (110[b])	49[b]	
		5 E M	Forwards	Video	3.5[b]	21.2[b]	~32 (88[b])	61[b]	
Yamanaka et al.[6]	Soccer	10 E M	All players	Audio	3.0		35	154	7.3
		39 T M	All players	Audio	4.5		44	123	5.9

a Using standard categories of motion (stand, walk, jog, stride and sprint).

b Denotes studies that have combined the motions of sprinting and striding into one category.

audio = analysis via audio play-back, no visual coding; **computer** = analysis via computer tracking; **E** = elite; **F** = females; **M** = males; **manual** = analysis via real-time recording/charting; **manual-audio** = analysis via combination of audio and manual charting; **T** = trained; **video** = analysis via video play-back and usually computer software.

Table 1; Sprint frequency during a football game measured with different match analysis methods.

Modified from original Table 1 in Spencer *et al.*, 2005

High-intensity actions (e.g., sprints) are not evenly distributed during team-sport games, but rather are grouped in periods with higher frequency, alternated with periods of relative recovery. This was confirmed in field-hockey games (Spencer *et al.*, 2004b), where an average of 4 repeated-sprint bouts were found, defined as a minimum of 3 sprints within each bout and a recovery of less than 21 s. These results have not encountered a conclusive corroboration in football (Buchheit *et al.*, 2010; Carling *et al.*, 2012), therefore generating uncertainty about the actual relationship between RSE and high-intensity activity during a game. However, evidence to support the rationale that RSE reflects high-intensity activity during a football game can be found in the analysis of construct and logical validity of RSE. The first point of evidence originates from the assessment of the construct validity of a repeated-sprint test. The ability to repeat sprints with a limited decrement in performance has a strong correlation with very-high intensity running and sprinting distance during a football game (Rampinini *et al.*, 2007). Similarly, players with a lower performance decrement during a repeated-sprint test (six 6-s sprints, 20-s recovery) were able to perform more high-intensity exercise bouts with short recovery during a match (recovery of less than 20 and 30 s), compared to those players with poorer repeated-sprint test scores (Carling *et al.*, 2012).

Further support is to be obtained by looking at an underestimated component of many team sports: acceleration. Traditional match analyses have attempted to categorise movement demands based on running velocity bands. However, it has been overlooked that an important component of team sports is the ability to repeatedly accelerate (Little and Williams, 2005). Since an acceleration is an action requiring a large energy expenditure (di Prampero *et al.*, 2005), it is safe to assume that, when an assessment of

accelerations is included into traditional match analysis, the resulting movement demand is increased. This has been demonstrated in professional football matches (Osgnach *et al.*, 2010). Also, when maximal accelerations (> 2.78 m s^{-2}) were included in the total calculation of high-intensity activity (high-velocity running + sprinting + accelerations), the frequency of high-intensity efforts was increased by almost 20 % compared to running efforts alone (Varley *et al.*, 2011). However, little information is available on the importance of acceleration during RSE, and this will be investigated in this thesis. These findings together support the use of RSE as an exercise model to mimic the high-intensity activity which occurs during football practice.

In the next chapter, the molecular adaptations induced by RSE and football in skeletal muscle will be reviewed, with a focus on mitochondrial biogenesis and exercise-induced glucose metabolism.

2.2 Skeletal muscle molecular responses induced by exercise

To date, there is a limited knowledge regarding the use of intermittent-sprint exercise to induce molecular responses in skeletal muscle. This chapter reviews the effects of intermittent-sprint exercise on the main molecular adaptations associated with two fundamental areas of cellular physiology, mitochondrial biogenesis and the relationship with metabolism and oxidative phosphorylation; and exercise-induced glucose metabolism.

2.2.1 The molecular bases of exercise-induced adaptations: from contraction to protein activity

Any physiological adaptation to exercise within skeletal muscle is a measurable phenomenon that is the consequence of a change in the abundance, activation, activity, or localisation of a protein within the muscle cell. The process linking muscle contraction to a measurable adaptation within the cell requires several steps to occur. These are presented in Figure 2, followed by a general overview of the processes.

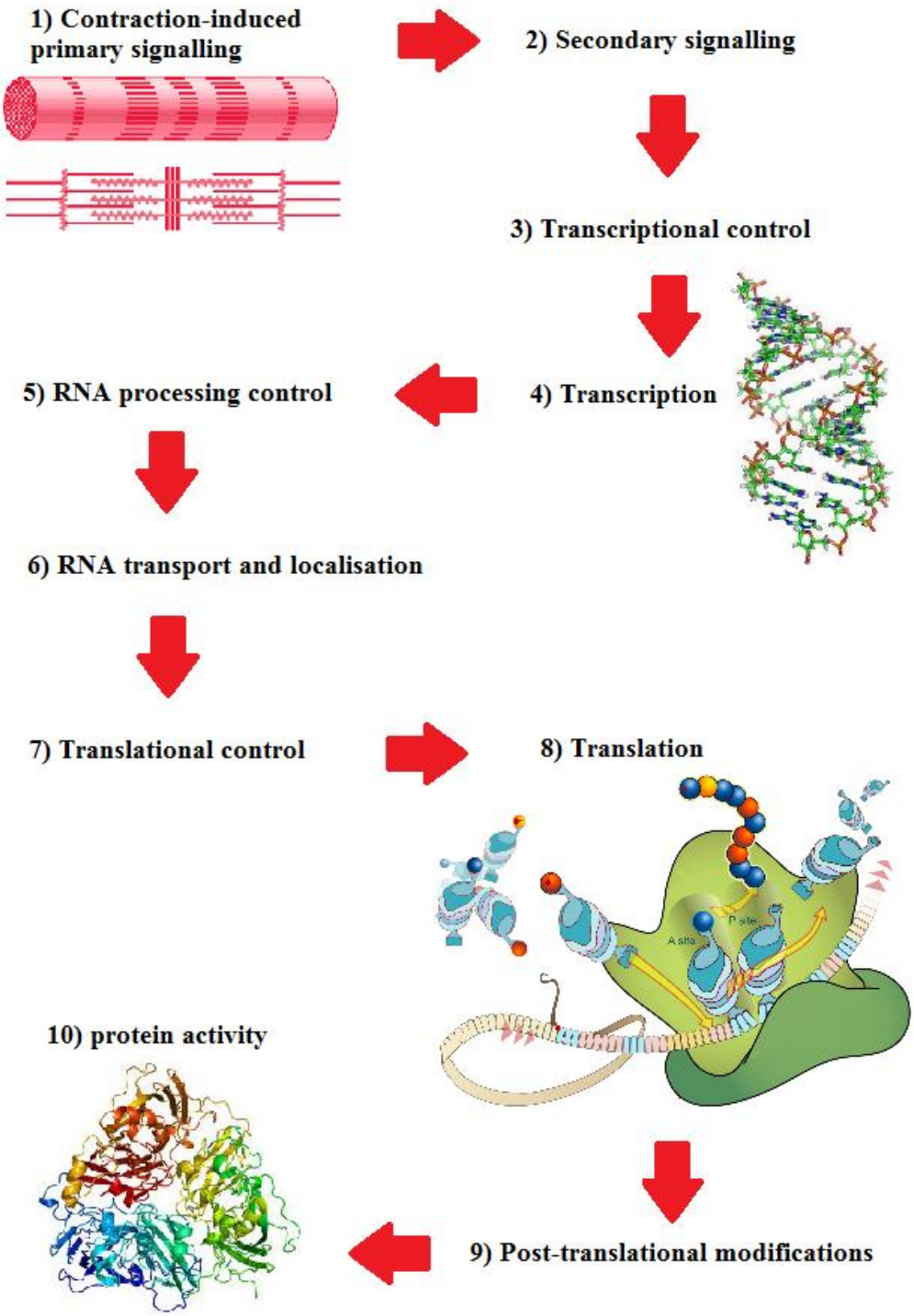

Figure 2; Schematic representation of the main steps involved in contraction-induced molecular adaptations

When a skeletal muscle contracts during exercise, many acute physiological responses occur within its cellular structure. Amongst these responses, there is a coordinated change in the muscular content of different molecules, such as adenosine triphosphate (ATP), the lactate ion (Lac^-), calcium ion (Ca^{2+}), hydrogen ion (H^+), and glycogen. In traditional exercise physiology the exercise-induced increase or decrease in these molecules, especially metabolites, has been considered as the endpoint of a specific physiological process. An example may be found in the role of ATP in muscle. Almost two decades of research were necessary to move from the simple concept of ATP as energetic molecule required for excitation-contraction coupling (Mommaerts and Wallner, 1967; Takahashi *et al.*, 1965), to its role as a primary messenger of signalling processes in the muscle (Benham and Tsien, 1987).

The term "primary messenger" refers to the mechanism of translating the muscle contraction into a biological message whose role is to initiate a cascade of molecular events. From a biological perspective, this initial signalling step occurs consequent to a primary messenger reacting with or modifying a target protein using a specific binding site (Coffey and Hawley, 2007). These target proteins are defined as "secondary messengers" and are usually kinases with a multi-subunit structure, with some subunits being responsible for catalysing a specific enzymatic reaction (i.e., catalytic subunits) and others having a regulatory role. The function of a kinase is to activate/deactivate a target protein usually by adding a phosphate group to a specific binding site, a process called phosphorylation. Phosphorylation is not the only modification for a target protein, as it will discussed later; however, it is a very common modification in the contraction-induced signalling pathways. Specifically related to skeletal muscle, these kinases are responsible for the phosphorylation of two main categories of downstream

targets: transcriptional co-activators and transcriptional co-repressors. A transcriptional co-activator is a protein which acts as a regulator of multiple transcription factors, which are responsible for initiating the transcription of DNA into messenger RNA (mRNA), usually by allowing the enzyme RNA polymerase II to access the binding site within a specific region of the gene (Tamura *et al.*, 1980). The action of transcriptional co-activators on the transcription factors is not always a simple direct activation, but is usually a multi-level process requiring the deactivation of one or more co-repressors (Reece and Platt, 1997). Transcriptional co-repressors are proteins which, in a resting state, are associated with a specific DNA binding site and do not allow transcription factors to initiate transcription. Once transcriptional co-repressors become inactive, they separate from the binding site, allowing transcription to commence.

Transcription commences when RNA polymerase recognises a precise sequence of nucleotides, called a promoter, which corresponds to the initial portion of the gene to be copied. Similarly, transcription is concluded when RNA polymerase detects a specific nucleotide sequence called a terminator (Eron and Block, 1971). The resulting mRNA obtained from this process is not fully functional. In bacteria, for example, there is generally a perfect correspondence between the gene, the mRNA and the final protein synthetised. In eukaryotes, however, the pre-mRNA contains redundant nucleotide sequences (introns) which are interspersed to the essential sequences (exons). Introns are removed and exons joined together, producing a mature mRNA; this process is defined as alternative splicing (Nadal-Ginard *et al.*, 1991). Once mRNA is completed, it enters the cytoplasm and joins the ribosomal complex to undergo translation, which is the process of converting mRNA into an inactive polypeptide chain. This needs to undergo post-translational modifications to become active and reach its biological

target. An example of post-translational modification is the addition of a phosphate group via an enzymatic reaction. Other common modifications are acetylation (i.e., addition of an acetyl group) and glycosylation (i.e., addition of a glycosyl group) (Walsh, 2006). Once these modifications occur, the protein is ready to undertake its specific biological activity.

The following section outlines the three main molecular pathways involved in the regulation of mitochondrial biogenesis and exercise-induced glucose metabolism: the AMPK pathway, the CaMKII pathway, and the p38 MAPK pathway.

2.2.2 AMPK pathway

Skeletal muscle requires a great increase in energy consumption when contracting. This energy is provided through the breakdown of different substrates, such as fatty acids, glycogen, phosphocreatine, or the available glucose to produce ATP, which is directly utilised in the actin-myosin interaction occurring in the sarcomere (Takahashi *et al.*, 1965), by the sarcoplasmic reticulum Ca^{2+}-ATPase and by the Na^{+},K^{+}-ATPase. Substrate utilisation and ATP synthesis are largely dependent on the characteristics of the contraction, such as intensity and duration. During contraction, ATP hydrolysis results in the production of adenosine diphosphate (ADP) + inorganic phosphate (P_i). Therefore, during contraction there is a reduction of ATP and a concurrent increase in ADP, leading in turn to an increase in the content of adenosine monophosphate (AMP) (Mommaerts and Wallner, 1967). In addition to their physiological role, the decrease in ATP and the parallel increase in ADP and AMP content have also been proposed as the main triggers for the activation of one of the secondary messengers that has received

considerable attention in molecular biology: the 5'AMP-activated protein kinase (AMPK) (Hardie and Carling, 1997). AMPK is a heterotrimer comprised of a catalytic α subunit, and two regulatory subunits, β and γ (Hardie *et al.*, 2003). Two isoforms exist for the α subunit, two for the β, and three for γ, allowing twelve isoform combinations which have specific signalling functions (Oakhill *et al.*, 2009). The activation of AMPK occurs via a double mechanism (Sanders *et al.*, 2007). Firstly, it can be directly phosphorylated at the Thr^{172} site on the α subunit, by its upstream kinases Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) and serine/threonine kinase 11 (STK11), also named LKB1. Secondly, AMPK can be activated allosterically (i.e., the binding occurs in a site that is different from the active sites) on the γ subunit by an increase in the *free* AMP-to-ATP ratio. Recently, a role has also been suggested for ADP as a direct activator of AMPK (Hardie *et al.*, 2011). Once activated, AMPK phosphorylates a multitude of target proteins involved in diverse areas of cellular physiology (Figure 3). The numerous roles of AMPK in the different tissues are beyond the scope of this review, which focuses on AMPK's role as the main sensor of the metabolic changes within skeletal muscle (Hardie and Sakamoto, 2006). In particular, the links between AMPK and the signalling processes associated with mitochondrial biogenesis and glucose metabolism will be reviewed.

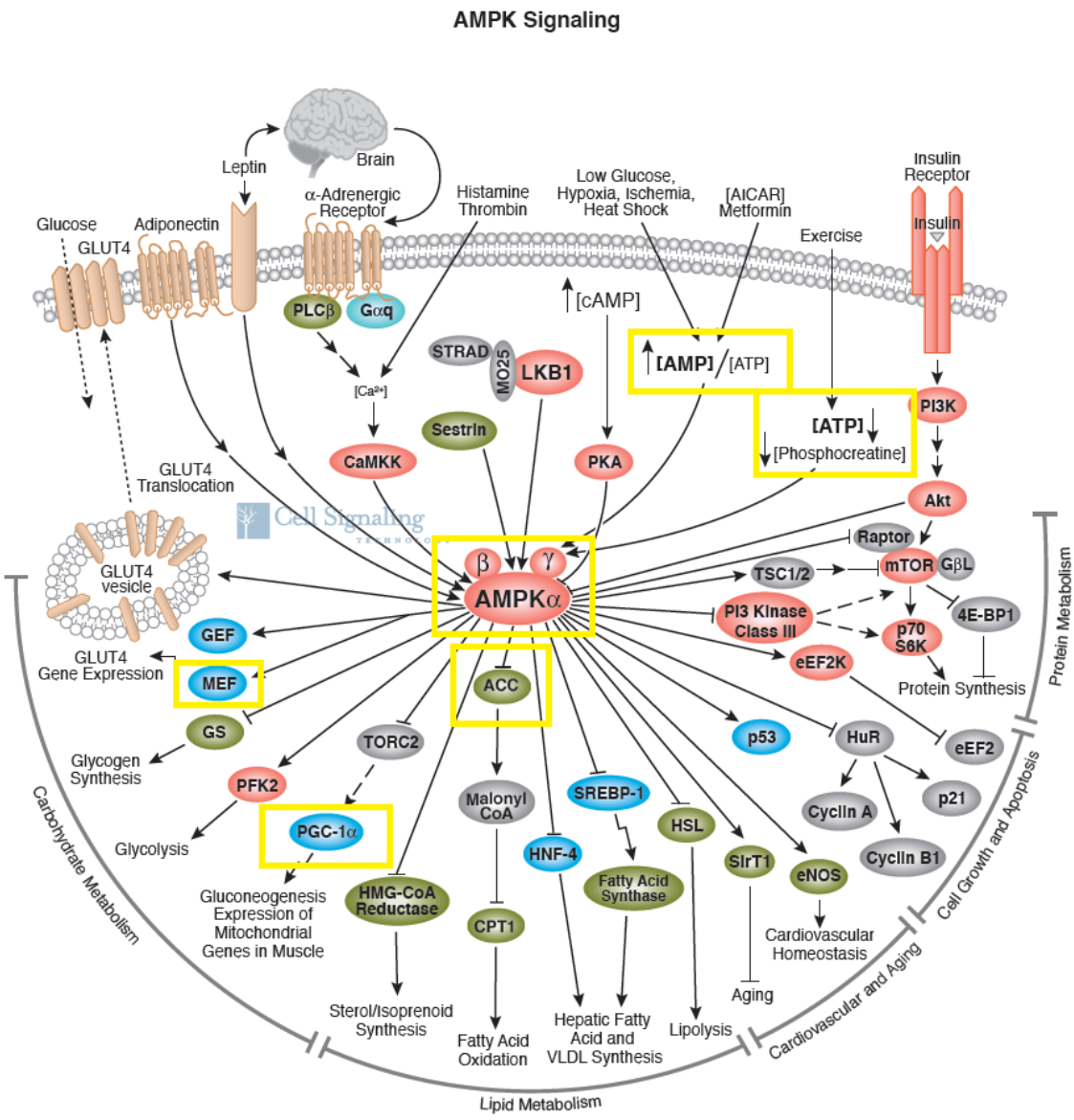

Figure 3; Overview of the AMPK signalling pathway.

Those molecules that are the object of this review of literature are highlighted in yellow.

Modified from www.cellsignal.com.

One of the main actions of AMPK in the signalling processes associated with mitochondrial biogenesis occurs through direct phosphorylation of one of the key transcriptional co-activators in skeletal muscle, the peroxisome proliferator-activated receptor γ, co-activator 1 α (PGC-1α) (Jager *et al.*, 2007). Following phosphorylation by AMPK at the Thr^{177} and Ser^{538} sites (Jager *et al.*, 2007), PGC-1α regulates several transcription factors, in particular the mitochondrial transcription factor A (mtTFA) and the nuclear respiratory factor 1 and 2 (NRF1/2) (Wu *et al.*, 1999). These transcription factors control the transcription of the nuclear and the mitochondrial genome, which are both required for the transcription of mitochondrial genes. In addition to AMPK's role in the signalling processes associated with mitochondrial biogenesis, AMPK also participates in the insulin-mediated and exercise-mediated glucose metabolism. One of the main actions of AMPK is exerted by controlling the gene expression of the main transporter of glucose across the cellular membrane, the glucose transporter 4 (GLUT4). The mechanism is presented in Figure 4. In the resting condition, GLUT4 mRNA expression is inhibited by a transcriptional co-repressor, the histone deacetylase 5 (HDAC5). HDAC5 is associated with the myocyte enhancer factor 2 (MEF2), the transcription factor that is in charge of controlling GLUT4 mRNA transcription, and it blocks the access of MEF2 to the DNA binding site (Lu *et al.*, 2000). With contraction, AMPK phosphorylates HDAC5, causing its extrusion from the nucleus and allowing GLUT4 mRNA transcription to take place (McGee and Hargreaves, 2011). Parallel to the regulation of GLUT4 mRNA transcription, AMPK also participates in the insulin signalling process. For example, AMPK can phosphorylate the Ser^{789} site within the insulin receptor substrate 1 (IRS-1), which is required for the insulin-mediated glucose metabolism (Jakobsen *et al.*, 2001).

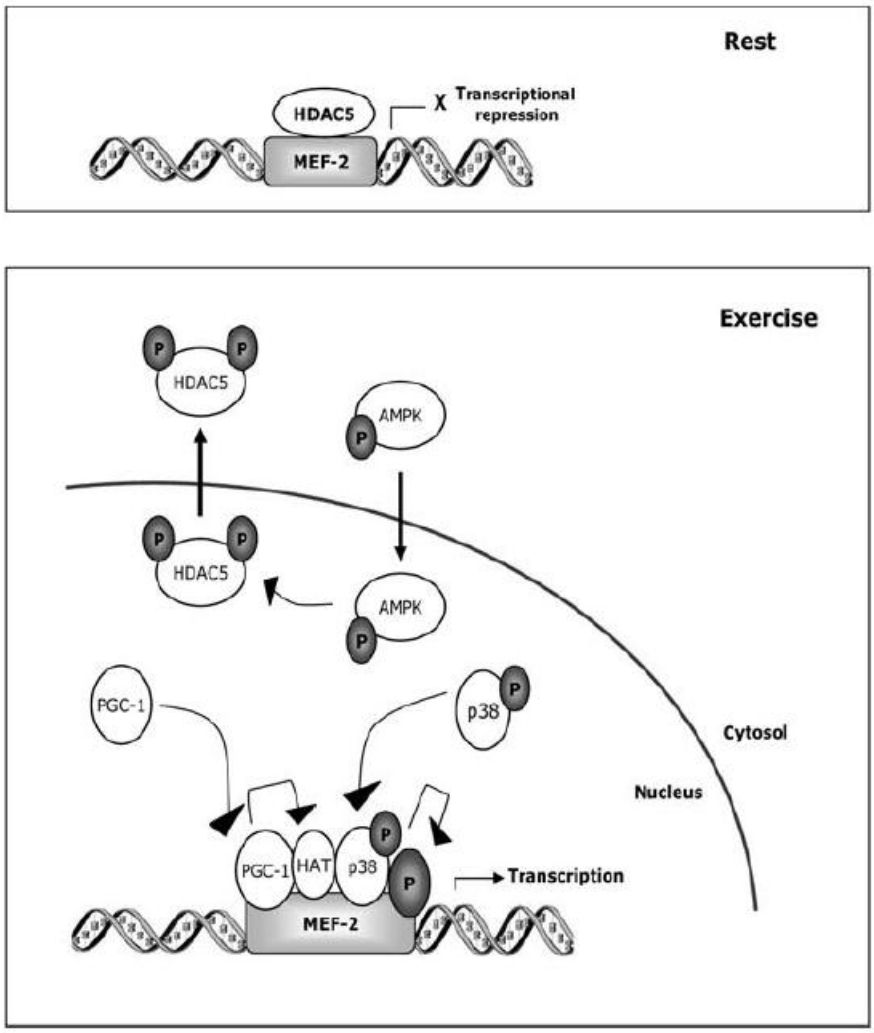

Figure 4; Schematic representation of the AMPK-HDAC5-MEF2 interaction

(McGee and Hargreaves, 2006)

2.2.2.1 The effects of exercise on AMPK

Extensive research has investigated the effects of exercise on the AMPK pathway, particularly AMPKα phosphorylation and activity. The majority of this research has utilised prolonged aerobic exercise as the experimental method. Continuous exercise of 20 to 30 min, performed at an intensity between 30 % and 80 % $\dot{V}O_{2\text{peak}}$ or $\dot{V}O_{2\text{max}}$ (depending on the method used), resulted in an increase in AMPK phosphorylation of between 30 and 400 % (Birk and Wojtaszewski, 2006; Kristensen *et al.*, 2007; Nielsen *et al.*, 2003a; Wadley *et al.*, 2006). At similar intensities, but with longer duration (60-120 min), AMPK phosphorylation was increased by 200 to 1500 % (Akerstrom *et al.*, 2006; Benziane *et al.*, 2008; Lee-Young *et al.*, 2006; Roepstorff *et al.*, 2005; Roepstorff *et al.*, 2006). Similarly, AMPK α2 activity was increased between 30 and 800 % depending on the duration and intensity of exercise (Chen *et al.*, 2003; McConell *et al.*, 2005; Wojtaszewski *et al.*, 2003). A large inter-study variability is noticeable in these results, possibly due to differences in the population used (e.g. trained versus untrained), the type of exercise (e.g., cycling, running, or one legged exercise) and the timing of the muscle biopsy sampling. However, it is evident that continuous aerobic exercise increased AMPK phosphorylation and activity.

Since AMPK activation/activity may be dependent on the exercise intensity (Chen *et al.*, 2003), the efficacy of high-intensity interval exercise on the activation of AMPK has been investigated. Different protocols have been used, such as repeated 5-min bouts at 85 % $\dot{V}O_{2\text{peak}}$ (Yu *et al.*, 2003), repeated 10-min bouts at 70-90 % $\dot{V}O_{2\text{peak}}$ (De Filippis *et al.*, 2008) and repeated 30-s, “all-out” bouts (Gibala *et al.*, 2009). These protocols increased AMPK phosphorylation by 40 to 100 %. However, whilst the increase in AMPK phosphorylation was much lower than in response to aerobic

exercise of longer duration, the increase in AMPK activity was similar between the two types of exercise (Yu *et al.*, 2003). This suggests that high-intensity interval exercise might be able to transduce contraction-induced signalling, despite the reduced exercise volume, by increasing AMPK activity. One of the aims of this thesis will be to investigate whether maximal-intensity exercise with an even smaller volume is capable of activating AMPK.

2.2.3 CaMKII pathway

Contraction is initiated within the cell by an increase in the intracellular calcium ion concentration ($[Ca^{2+}]_i$) in skeletal muscle, as a result of Ca^{2+} release from the sarcoplasmic reticulum. The main physiological role of an increased $[Ca^{2+}]_i$ is the excitation-contraction coupling process, enabling the actin-myosin interaction in muscle fibres (Berchtold *et al.*, 2000). An important additional function has been assigned to $[Ca^{2+}]_i$ as a main signal initiator in what has been named the "excitation-transcription coupling" process (Chin 2005). Depending on the characteristics of the contractions, the increase in $[Ca^{2+}]_i$ occurs in a transient fashion with different amplitude and frequency. Calcium transients are detected by a signal transducer protein named calmodulin (CaM). Each CaM protein has the capacity to bind four Ca^{2+} ions and to decode the amplitude and frequency of the transients by undergoing conformational changes and by modifying its affinity to Ca^{2+} (De Koninck and Schulman, 1998). Once activated, the Ca^{2+}/CaM complex binds to different downstream proteins. Amongst these, the family of Ca^{2+}/CaM-dependent protein kinases, and in particular CaMKII, are believed to play a pivotal role in the signalling cascades within skeletal muscle. CaMKII is composed of 12 subunits arranged in two sets of 6 subunits. When a contraction-induced rise in

$[Ca^{2+}]_i$ occurs, the Ca^{2+}/CaM complex binds to a site of CaMKII which is specifically designed to bind CaM. This results in the activation of CaMKII and commences a process of auto-phosphorylation which leads to a Ca^{2+}-independent activity (i.e., CaMKII autonomous activity) (Chin 2005). Similarly to AMPK, the family of CaM kinases has been implicated in the signalling processes leading to mitochondrial biogenesis, although this has not yet been well established in human skeletal muscle (Kusuhara *et al.*, 2007). Studies using transgenic mice have shown that CaMKIV, a similar kinase of the same family, upregulates enzymes involved in fatty acid oxidation and induces PGC-1α mRNA expression (Wu *et al.*, 2002). CaMKII has also been suggested to play a pivotal role in the signalling processes associated with glucose metabolism and in particular with GLUT4 mRNA expression (Ojuka, 2004). The mechanisms via which CaMKII regulates GLUT4 are similar to as described for AMPK. An acute caffeine-induced increase in cytosolic $[Ca^{2+}]$ in C_2C_{12} myotubes resulted in CaMKII activation, leading to a reduced nuclear abundance of HDAC5 (thus suggesting its dissociation from MEF2), increased binding of MEF2A to the promoter region of GLUT4, and hyper-acetylation of H3 histones (Mukwevho *et al.*, 2008). The exact mechanisms via which CaMKII regulates glucose metabolism are still debated. Recently, it has been proposed that the specific function of CaMKII in regulating glucose metabolism is identified in the ability of CaMKII to regulate contraction-dependent, but not insulin-dependent, glucose uptake (Witczak *et al.*, 2010). On the other hand, research conducted on L6 myotubes has demonstrated that CaMKII plays a fundamental role in the insulin signalling pathways by interfering with the insulin-induced phosphorylation of the Akt substrate of 160 kDa (AS160) (Mohankumar *et al.*, 2012).

2.2.3.1 The effects of exercise on CaMKII

Much less is known about the effects of exercise on the CaMK signalling than for AMPK. The majority of the early research regarding exercise and CaMKII employed continuous, long-duration aerobic exercise as the experimental model. The phosphorylation of CaMKII at Thr^{287} was increased by approximately 60 to 200 % in response to 60-90 min of exercise performed at 67 to 76 % $\dot{V}O_{2\text{peak}}$ (Benziane *et al.*, 2008; McGee *et al.*, 2009; Rose *et al.*, 2006). These results taken in isolation would not suggest a strong involvement for CaMKII in the exercise-induced signalling within human skeletal muscle. However, an interesting finding highlighted the possibility that high-intensity exercise may induce larger CaMKII activation than low-intensity activity. Skeletal muscle signalling responses to two isocaloric exercises at either 40 or 80 % $\dot{V}O_{2\text{peak}}$ were compared in young, sedentary individuals (Egan *et al.*, 2010). Immediately following exercise, CaMKII phosphorylation was increased by 84 % following the high-intensity protocol, whereas no change was detected following low-intensity exercise (Figure 5). However, high-intensity interval exercise comprising repeated, 30-s "all-out" bouts found very limited changes in CaMKII phosphorylation (Gibala *et al.*, 2009). Therefore, there is the need to expand on research regarding the effects of different types of exercise on CaMKII activation.

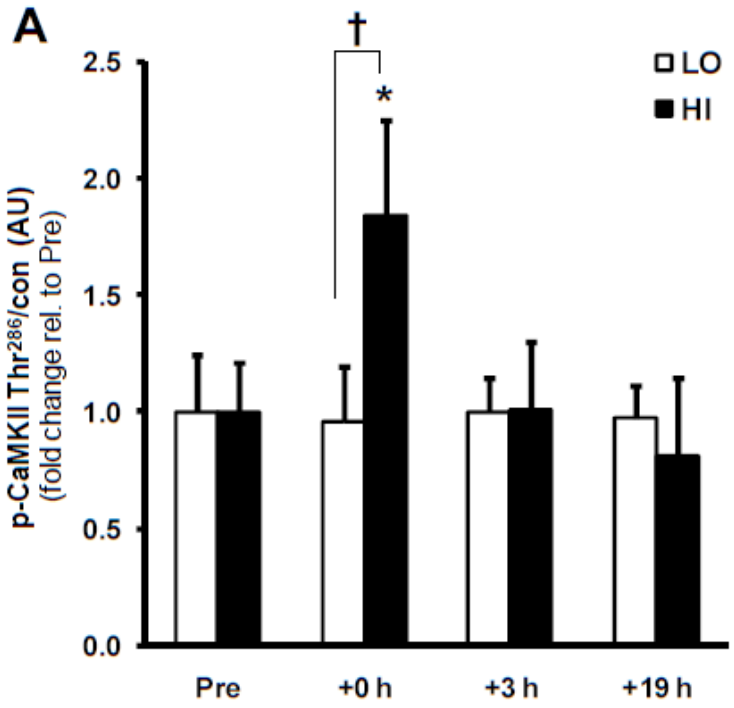

Figure 5; CaMKII phosphorylation in response to continuous exercise performed at 40 % (LO) and 80 % (HI) $\dot{V}O_{2\text{peak}}$.

From original Figure 5A in Egan *et al.*, 2010

2.2.4 p38 MAPK pathway

Unlike AMPK and CaMKII, the p38 mitogen-activated kinase (p38 MAPK) has received interest mainly due to the complexity of its activation by different upstream proteins, rather than its functions on downstream targets (Figure 6). The p38 MAPK family comprises four kinases (α, β, γ, δ) which are differently expressed in human tissues. These subunits are activated by three upstream kinases named MAPK kinases (MKK), specifically MKK3, MKK4, and MKK6 (Cuadrado and Nebreda, 2010). These three MKKs are indirectly activated by different physiological stimuli, the main being an increase in inflammatory cytokines (Herlaar and Brown, 1999), reactive oxygen species (Kulisz *et al.*, 2002), and tumor necrosis factor α (McLeish *et al.*, 1998). Upon phosphorylation by its upstream kinases, p38 MAPK is able to activate multiple target proteins involved in gene transcription, cytokine production and apoptosis. In regards to the molecular signalling leading to mitochondrial biogenesis and oxidative metabolism, p38 MAPK can phosphorylate PGC-1α protein and, in parallel, inactivate a repressor of PGC-1α, the p160 myb binding protein, therefore increasing PGC-1α activity as a transcriptional co-activator (Fan *et al.*, 2004). Also, similar to AMPK and CaMKII, p38 MAPK is important for its involvement in the exercise-induced glucose metabolism, as it promotes GLUT4 mRNA expression via phosphorylation of MEF2 (Montessuit *et al.*, 2004). Also, p38 MAPK has a fundamental role in stimulating liver gluconeogenesis via inducing gene transcription, partly through the involvement of PGC-1α (Liu and Cao, 2009).

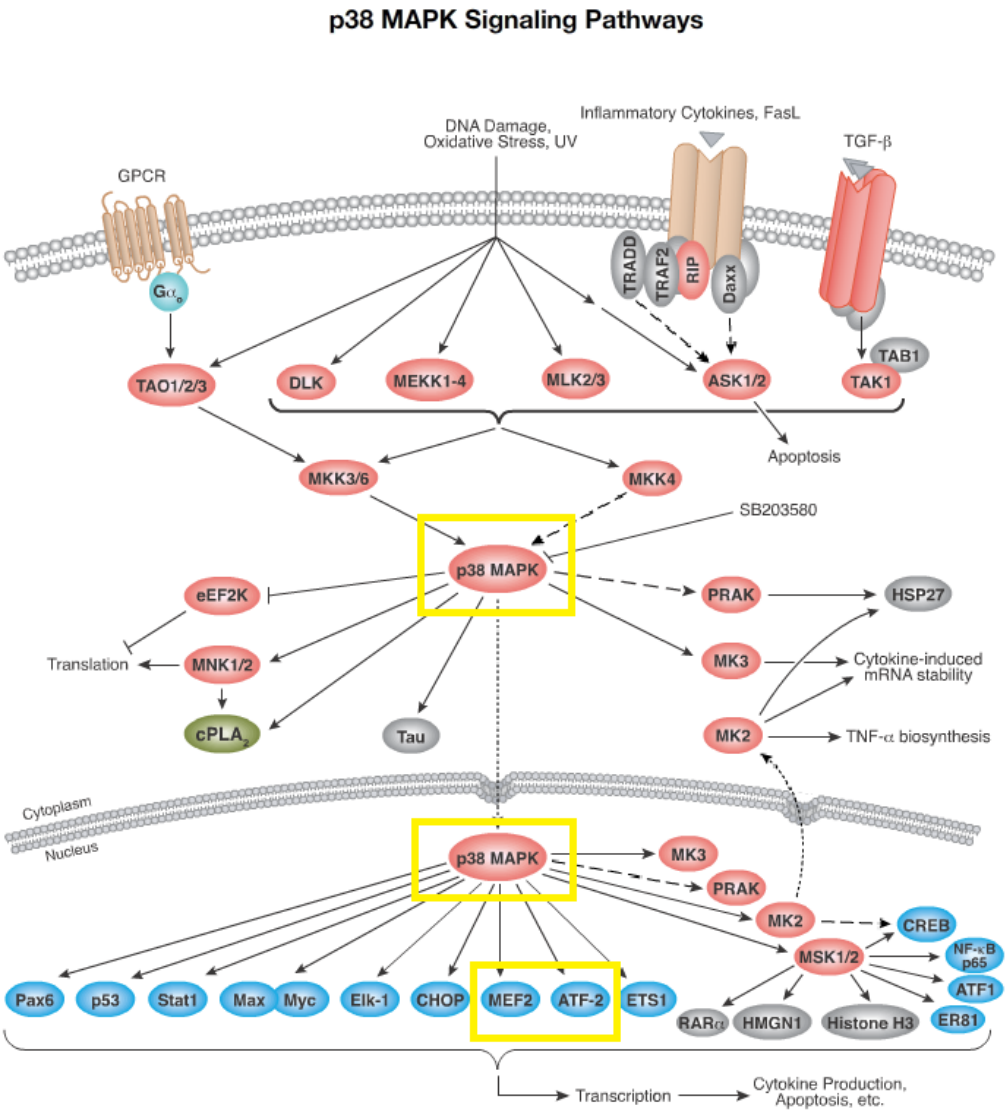

Figure 6; Overview of the p38 MAPK signalling pathway

Those molecules that are the object of this review of literature are highlighted in yellow.

Modified from www.cellsignal.com.

2.2.4.1 The effects of exercise on p38 MAPK

Research has investigated the effects of both continuous aerobic exercise and interval exercise on the activation of p38 MAPK. The results of these investigations are very heterogeneous. Some of the early studies showed that, in response to 60 min of exercise at approximately 70 % $\dot{V}O_{2\text{peak}}$, p38 MAPK phosphorylation was increased by 130 to 480 % (Coffey *et al.*, 2006; McGee and Hargreaves, 2004). Similar large increases were measured in response to 3 h of exercise at 60 % $\dot{V}O_{2\text{peak}}$ (Watt *et al.*, 2004) and to a marathon lasting approximately 4 h (Yu *et al.*, 2001). However, a similar type of continuous aerobic exercise (60 min at 71 % $\dot{V}O_{2\text{peak}}$) induced a 1000 % increase in p38 MAPK phosphorylation (Benziane *et al.*, 2008), and even larger changes were reported following only 20 min of exercise at 80 % $\dot{V}O_{2\text{peak}}$ (Nielsen *et al.*, 2003b). Since the main upstream signalling messengers of p38 MAPK, and in particular inflammatory cytokines and reactive oxygen species, are possibly more sensitive to changes in response to high-intensity exercise, research was conducted on more intense protocols. However, repeated 5-min bouts at 85 % $\dot{V}O_{2\text{peak}}$, as well as repeated 30-s, "all-out" bouts, resulted in only moderate increases in p38 MAPK phosphorylation (Gibala *et al.*, 2009; Yu *et al.*, 2003), leaving this topic still debated.

2.3 The effects of intermittent-sprint exercise on molecular signalling processes

Earlier sections have reviewed the mechanisms involved in exercise-induced muscle cellular adaptations in response to aerobic interval exercise. The following section investigates how intermittent-sprint exercise interventions, in the form of RSE and football, lead to those adaptations. This first focuses on research conducted on the specific effects of RSE and football on muscle metabolites and key signalling proteins associated with mitochondrial biogenesis and glucose metabolism. There is a relatively large amount of research on the effects of intermittent-sprint exercise on the AMPK signalling pathway but very limited information on the other pathways. Therefore, additional information will be gathered from other experimental models in order to provide a clear rationale for the use of intermittent-sprint exercise for the experiments of this thesis.

2.3.1 RSE and the AMPK pathway

The rationale supporting the concept that intermittent-sprint activities are an efficient method to induce molecular adaptations in skeletal muscle is mainly based on the fact that AMPK functions as the main sensor of metabolic alterations within the cell. Both a single sprint and RSE induce large metabolic perturbations in the cell and these perturbations involve, to different extents, all energy systems. A single short sprint requires the energy to be provided through PCr utilisation (55 %), anaerobic glycolysis (32 %), pre-existing ATP (10 %), but also from aerobic metabolism (Spencer *et al.*, 2005). Therefore, the repetition of sprints with short recovery should induce even larger metabolic perturbations, in turn activating AMPK. In order to understand the extent of

the metabolic alterations caused by RSE, a systematic approach will be taken to review the changes in metabolites following single and repeated-sprints (Table 2). After an accurate screening of the relevant literature, those research articles which investigated changes in the muscle content of ATP, PCr, lactate and glycogen were selected. Original articles were considered for the review using the following systematic inclusion criteria: i) studies performed with humans; ii) sprints with a duration of less or equal to 10 s; iii) recovery duration between sprints of less or equal to 30 s (for RSE); iv) metabolite content measured in whole muscle, no single fibre analysis; and v) metabolite content measured by enzymatic assay analysis.

Table 2. Whole-muscle metabolite content following a single sprint on a cycle ergometer

ATP	Sprint duration (s)	Subjects (n)	Rest	Post-exercise	Change (%)
(Howlett *et al.*, 1999)*	10	10	22.4 ± 3.8	17.0 ± 3.2	-24.1
(Bogdanis *et al.*, 1998)*	10	8	25.6 ± 2.0	20.2 ± 3.7	-21.1
(Gaitanos *et al.*, 1993)	6	8	24.0 ± 2.7	20.9 ± 2.5	-12.9
(Dawson *et al.*, 1997)	6	7	24.3 ± 2.4	20.5 ± 2.3	-15.6
(Gray *et al.*, 2006)	6	8	23.3 ± 1.7	19.6 ± 1.4	-15.9
(Boobis *et al.*, 1982)	6	4	24.4 ± 0.9	22.2 ± 1.1	-9.0
PCr					
(Howlett *et al.*, 1999)*	10	10	93.7 ± 7.6	40.2 ± 10.1	-57.1
(Bogdanis *et al.*, 1998)*	10	8	80.7 ± 10.1	36.1 ± 9.5	-55.3
(Gaitanos *et al.*, 1993)	6	8	76.5 ± 7.2	32.9 ± 2.6	-57.0
(Dawson *et al.*, 1997)	6	7	81.0 ± 7.4	44.9 ± 6.4	-44.6
(Gray *et al.*, 2006)	6	8	74.1 ± 5.7	41.7 ± 6.1	-43.7
(Boobis *et al.*, 1982)	6	4	84.3 ± 2.3	54.8 ± 11.3	-35.0
LACTATE					
(Howlett *et al.*, 1999)*	10	10	5.5 ± 4.7	58.8 ± 11.7	+969
(Bogdanis *et al.*, 1998)*	10	8	4.5 ± 1.1	51.0 ± 13.0	+1033
(Gaitanos *et al.*, 1993)	6	8	3.8 ± 1.1	28.6 ± 5.7	+652
(Dawson *et al.*, 1997)	6	7	6.8 ± 1.9	42.5 ± 5.0	+525
(Gray *et al.*, 2006)	6	8	5.3 ± 0.8	22.0 ± 4.1	+315
(Boobis *et al.*, 1982)	6	4	9.3 ± 1.8	28.4 ± 7.7	+205
GLYCOGEN					
(Bogdanis *et al.*, 1998)*	10	8	403.8 ± 56.8	357.4 ± 52.6	-11.6
(Gaitanos *et al.*, 1993)	6	8	316.8 ± 74.8	273.3 ± 79.9	-13.7
(Gray *et al.*, 2006)	6	8	483.0 ± 154.0	447.0 ± 161.0	-7.5
(Boobis *et al.*, 1982)	6	4	266.9 ± 28.1	229.0 ± 42.5	-14.2

Data are presented as mean ± SD (unit, mmol kg^{-1} dry muscle mass). *, SD calculated from the original data expressed as mean ± SE.

Single-sprint exercise induces marked metabolic changes in skeletal muscle, including an approximate decrease by 10 to 25 % in ATP content, 35 to 57 % in PCr content, and 8 to 15 % in glycogen following a single maximal sprint, with some differences depending on the duration of the sprint. Also, single-sprint exercise induces up to a 1000 % increase in muscle lactate content. These results suggest that the repetition of multiple single sprints with limited recovery might be efficient to trigger AMPK activation in skeletal muscle cells, possibly leading to mitochondrial biogenesis and improved glucose metabolism. Table 3 presents the muscle metabolite content in response to acute RSE protocols.

Table 3. Whole-muscle metabolite content following RSE on a cycle ergometer

ATP	Protocol	Subjects (n)	Rest	Post-exercise	Change (%)
(Edge *et al.*, 2005)	5 x 6-s	10	20.6 ± 0.7	16.5 ± 1.7	-19.9
(Edge *et al.*, 2005)	5 x 6-s	10	21.5 ± 1.0	15.2 ± 2.1	-29.3
(Spencer *et al.*, 2008) §	6 x 4-s	9	21.3 ± 2.4	18.0 ± 2.7	-15.5
(Gaitanos *et al.*, 1993)	10 x 6-s	7	24.0 ± 2.7	16.4 ± 3.9	-31.7
(Dawson *et al.*, 1997)	5 x 6-s	8	22.8 ± 2.7	15.1 ± 2.3	-33.8
PCr					
(Edge *et al.*, 2005)	5 x 6-s	10	69.5 ± 10.8	33.9 ± 7.7	-51.2
(Edge *et al.*, 2005)	5 x 6-s	10	75.3 ± 9.5	34.1 ± 14.4	-54.7
(Spencer *et al.*, 2008) §	6 x 4-s	9	75.8 ± 9.2	38.0 ± 12.3	-49.9
(Spencer *et al.*, 2006)	6 x 4-s	6	72.7 ± 6.6	33.2 ± 15.3	-54.3
(Gaitanos *et al.*, 1993)	10 x 6-s	7	76.5 ± 7.2	12.2 ± 3.7	-84.1
(Dawson *et al.*, 1997)	5 x 6-s	8	77.1 ± 4.9	21.1 ± 5.6	-72.6
LACTATE					
(Edge *et al.*, 2005)	5 x 6-s	10	8.3 ± 4.2	70.8 ± 23.4	+753.0
(Edge *et al.*, 2005)	5 x 6-s	10	10.0 ± 2.5	56.9 ± 16.1	+469.0
(Spencer *et al.*, 2008) §	6 x 4-s	9	4.7 ± 3.1	50.9 ± 20.1	+983.0
(Spencer *et al.*, 2006)	6 x 4-s	7	11.5 ± 2.1	55.2 ± 15.7	+380.0
(Gaitanos *et al.*, 1993)	10 x 6-s	7	3.8 ± 1.1	112.3 ± 30.6	+2855.3
(Dawson *et al.*, 1997)	5 x 6-s	8	7.7 ± 3.2	103.6 ± 17.5	+1245.5
(Bishop *et al.*, 2004)	5 x 6-s	23	13.1 ± 10.6	61.4 ± 20.7	+368.7
(Bishop and Edge, 2006)	5 x 6-s	16	7.6 ± 2.2	62.7 ± 13.6	+725.0
(Bishop and Edge, 2006)	5 x 6-s	16	10.0 ± 2.1	60.6 ± 7.5	+506.0
GLYCOGEN					
(Gaitanos *et al.*, 1993)	10 x 6-s	7	316.8 ± 74.8	201.4 ± 90.1	-63.6

Data are presented as mean ± SD (unit, mmol kg^{-1} dry muscle mass)
§, only RSE protocol with low-intensity recovery was considered.

Acute RSE induces large metabolic changes in skeletal muscle. The different RSE protocols resulted in a 20 to 34 % decrease in ATP, 50 to 72 % decrease in PCr, 64 % decrease in glycogen, and 370 to 2900 % increase in muscle lactate content. These changes in metabolites indicate that large metabolic perturbations following RSE should lead to the activation of signalling proteins within the AMPK pathway.

To the best of knowledge, there is no research investigating RSE-induced adaptations within the AMPK pathway. However, two studies have assessed AMPK activation in response to an intermittent-sprint protocol of 10 x 6-s sprints on a cycle ergometer, with a 1-min recovery between sprints (Coffey *et al.*, 2009; Coffey *et al.*, 2011). Both studies combined RSE with resistance exercise and measured AMPK phosphorylation following each of the two individual components. In the first study, AMPKα phosphorylation at Thr^{172} was increased by approximately 100 % at 15 min after the last sprint (estimated from their original Figure 3F and reproduced in Figure 7 (Coffey *et al.*, 2009)). In the same article, the authors also reported an approximate 300 % increase in PGC-1α mRNA expression measured 3 h following RSE and resistance exercise. However, a conclusive effect of intermittent-sprint exercise on PGC-1α cannot be established due the potential cumulative effects of the two types of exercise on the final mRNA expression.

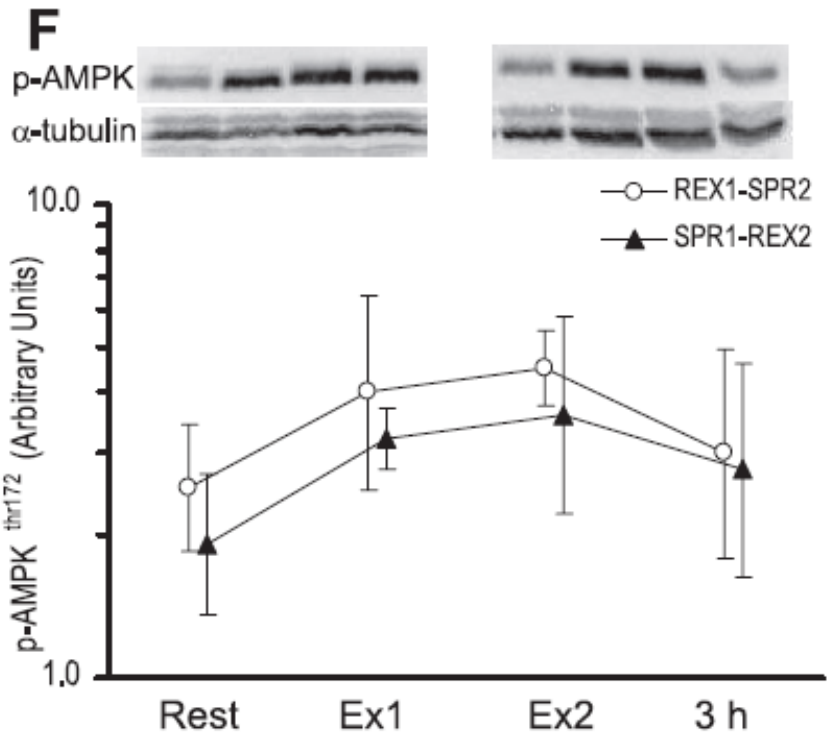

Figure 7; AMPK phosphorylation measured in response to 10 x 6-s sprints on a cycle ergometer.

(Coffey *et al.*, 2009)

In the second study, which replicated the first but with inclusion of a nutrient or placebo infusion, no changes in AMPK phosphorylation were detected (Coffey *et al.*, 2011). The reasons behind the difference in the two studies are not clear, but the absence or limited magnitude of AMPK phosphorylation in response to RSE suggests that a revision of the long-proposed fundamental role of AMPK is needed. Increased attention has been progressively directed to one of the downstream targets of AMPK, the Acetyl-CoA Carboxylase (ACC), a 280 kDa protein that is directly activated at Ser^{221} by AMPK (Winder and Hardie, 1996). Despite always being considered a direct downstream target of AMPK, ACC phosphorylation might be AMPK-independent in

transgenic mice (Dzamko *et al.*, 2008). Interestingly ACC phosphorylation at Ser^{221} appears to anticipate AMPK phosphorylation at Thr^{172} following a single "all-out" exercise of 30 s in duration (Guerra *et al.*, 2010). To date, there is no information regarding the effects of RSE on both AMPK and ACC activation, and this has been investigated in this thesis.

2.3.2 RSE and the CaMKII pathway

This section reviews evidence on whether repeated high-intensity exercise bouts can provide an optimal stimulus for Ca^{2+}-related signalling. There is limited information on the regulation of cytosolic Ca^{2+} concentration in response to muscle contraction with different characteristics (e.g., intensity) in humans, because of methodological difficulties associated with measuring $[Ca^{2+}]_i$ and Ca^{2+} transients in human skeletal muscle fibres both at rest and during contraction. Therefore, to assess the potential of RSE to promote signalling within the Ca^{2+}-related pathway, the contraction-induced rise in $[Ca^{2+}]_i$ from studies performed on mouse single fibres was assessed. Then, to understand whether high-intensity exercise can produce an optimal stimulus for CaMKII activation, a systematic review of the data available in regards to $[Ca^{2+}]_i$ in mouse skeletal-muscle single fibres in response to high- (> 50 Hz) or low-frequency (< 50 Hz) electrical stimulation was performed (Table 4). It is acknowledged that the application of a concept originating from rodent isolated muscle experiments to exercising humans is speculative. However, this approach allows a precise quantification of the physiological process, thus strengthening the rationale to the hypotheses that RSE can activate CaMKII.

At rest, the $[Ca^{2+}]_i$ measured in mouse skeletal muscle single-fibre cytosol is approximately 50-60 nM, with a range of approximately 25-100 nM depending on the preparation of the fibre and on the experimental condition (Bruton *et al.*, 2010; Weigl *et al.*, 2000; Westerblad and Allen, 1993). There is no information regarding $[Ca^{2+}]_i$ in human skeletal muscle single fibres, however similar values to those presented above have been reported for cultured human skeletal muscle cells, with resting $[Ca^{2+}]_i$ ranging from approximately 70 nM (Vandebrouck *et al.*, 1999) to 120-130 nM (Benders *et al.*, 1996; May *et al.*, 2006). During electrical stimulation, $[Ca^{2+}]_i$ rapidly rises up to 1600 % in the single fibre preparation. Original articles were considered for the review only when adhering to the following systematic inclusion criteria: i) measurement of $[Ca^{2+}]_i$ performed with injection of calcium indicators *indo-1* or *fura-2*; ii) mouse skeletal muscle single fibres only, no other animal models; iii) in the presence of multiple experimental protocols, only data associated with a control condition (stimulation only) were considered. No data were collected from experiments combining electrical stimulation and injection of substances (e.g., caffeine); and iv) where possible, only data from short stimulation periods were considered (e.g., 10 tetani). If long fatigue protocols were adopted, only data from the initial measurements of the protocol were considered, when available.

Table 4. Mouse single-fibre $[Ca^{2+}]_i$ following high- (> 50 Hz) and low-frequency (< 50 Hz) electrical stimulation

HIGH FREQUENCY	**Frequency (Hz)**	**Duration (s)**	**Rest (nM)**	**Stimulation (μM)**	**Change (%)**
(Moopanar and Allen, 2006)	100	0.4	~100	0.96	+860
(Westerblad *et al.*, 1993)	100	0.35	~50	0.88	+1660
(Chin *et al.*, 1997)	100	0.35	47	0.98	+1620
(Chin and Allen, 1996)	100	0.35	76	0.77	+910
(Westerblad and Allen, 1996)	100	0.35	43	0.62	+1340
(Bruton *et al.*, 2010)	100	0.5	61	2.19	+3490
(Westerblad and Allen, 1993)	70	0.35	26	0.47	+1690
(Place *et al.*, 2008)	70	0.35	~100	1.47	+1360
(Aydin *et al.*, 2007)	70	0.35	~100	1.33	+1230
(Tavi *et al.*, 2003)	70	0.35	~100	1.26	+1160
(Bruton *et al.*, 2010)	70	0.5	61	1.34	+2096
LOW FREQUENCY					
(Aydin *et al.*, 2007)	20	0.35	~100	0.44	+340
(Bruton *et al.*, 2010)	20	0.5	61	0.54	+785
(Westerblad *et al.*, 1993)	30	0.35	~50	0.31	+520
(Bruton *et al.*, 2010)	30	0.5	61	0.72	+1080
(Bruton *et al.*, 2010)	40	0.5	61	0.91	+1391
(Westerblad *et al.*, 1993)	50	0.35	~50	0.49	+880
(Bruton *et al.*, 2010)	50	0.5	61	1.09	+1686

The rise in $[Ca^{2+}]_i$ in mouse single fibres following high-frequency stimulation is up to double compared to low-frequency stimulation. This confirms that high-intensity muscle contraction results in higher $[Ca^{2+}]_i$ spikes. The following explores whether higher and more frequent $[Ca^{2+}]_i$ spikes lead to an increased CaMKII activation. Single-fibre experiments in mice demonstrated that CaMKII activation is dependent on the frequency of Ca^{2+} release from the sarcoplasmic reticulum (Aydin *et al.*, 2007). Similarly, during continuous tetani stimulation at 70 Hz, CaMKII phosphorylation continued to increase with each single tetanus, and the increase in phosphorylation closely reflected the increase in $[Ca^{2+}]_i$ spikes until the stimulation was ended (i.e., n=10 tetani) (Tavi *et al.*, 2003). However, CaMKII undergoes autonomous activity following initial activation (Rose and Hargreaves, 2003), and this could potentially signify a plateau of its capability to become active, independent of the type of stimulation. The peak phosphorylation and autonomous activity of CaMKII both occur within the first minute of exercise in humans (Rose *et al.*, 2006), then stabilise to a lower level for the remaining duration. However, this has only been demonstrated for continuous exercise at approximately 67 % $\dot{V}O_{2\text{peak}}$. To date, it is not known whether CaMKII activation can be increased following RSE or other intermittent-sprint exercises. Nonetheless, there is evidence that the CaMKII pathway is sensitive to exercise intensity in humans. The phosphorylation of phospholamban, a substrate of CaMKII in skeletal muscle, was measured following 3 continuous bouts of 10-min exercise where the intensity was increased to 35 %, 60 %, and 85 % $\dot{V}O_{2\text{peak}}$, respectively. After the exercise bout at 60 % $\dot{V}O_{2\text{peak}}$ phospholamban phosphorylation was unchanged compared to the previous bout performed at 35 %, but it almost doubled following the 10-min period at the higher-intensity (Rose *et al.*, 2006). These results taken together suggest that RSE

potentially represents a strong biological stimulus to trigger signalling within the CaMK pathway. This was investigated in this thesis.

2.3.3 RSE and the p38 MAPK pathway

In skeletal muscle, p38 MAPK is activated by direct upstream kinases MKK3, MKK4, and MKK6 which, in turn, are triggered by an increased oxidative stress and inflammatory cytokines (section 2.2.4). Alternative activation stimuli that have been identified include increased tumor necrosis factor α (TNFα) activation and increased mechanical stress. High-intensity exercise results in a larger oxidative stress, inflammation, and mechanical stress in skeletal muscle, compared to a matched amount of lower-intensity exercise. As a consequence, p38 MAPK should be activated following RSE. To date, there is no research directly demonstrating the effects of acute RSE on skeletal muscle oxidative stress or markers of inflammation. Nonetheless, information can be collected from the analysis of the end-products of such processes in the blood. A common inflammatory measurement is Interleukin-6 (IL-6), a mixed pro-inflammatory and anti-inflammatory cytokine secreted by skeletal muscle (Pedersen and Febbraio, 2008). A single bout of intermittent-sprint exercise, comprising repeated 30-m sprints commencing every 35 s, induced a 70 to 80 % increase in the serum IL-6 concentration (Abbey and Rankin, 2011; Opheim and Rankin, 2012). However, repeated 10-s sprints performed with 3-min recovery between efforts, did not increase blood biomarkers of oxidative stress, protein carbonyls and malondialdehyde (Bloomer *et al.*, 2006), possibly because of the extremely large work-to-rest ratio. These data together do not provide conclusive evidence that acute RSE may represent a strong activation stimulus for p38 MAPK. This uncertainty is supported by observations of the

effects of different exercise protocols on p38 MAPK activation. A very demanding protocol of four, 30-s "all-out" exercise bouts, which should induce large oxidative stress and inflammation (Cuevas *et al.*, 2005; Groussard *et al.*, 2003), increased p38 MAPK phosphorylation at Thr^{180}/Tyr^{182} by only approximately 30 % (Gibala *et al.*, 2009). Also, 36 min of exercise performed at approximately 80 % $\dot{V}O_{2\text{peak}}$ induced p38 MAPK phosphorylation identical to a volume-matched exercise performed at 40 % $\dot{V}O_{2\text{peak}}$ (Egan *et al.*, 2010). This might suggest that p38 MAPK activation is not intensity-dependent and might not be strongly upregulated by RSE. However, the phosphorylation of the activating transcription factor 2 (ATF2), a downstream target of p38 MAPK, was significantly greater after the high-intensity exercise bout compared to the low-intensity protocol (Egan *et al.*, 2010). This suggests that a similar mechanism to that described above for AMPK and ACC might also occur within the p38 MAPK pathway; however, research is needed to corroborate this scenario.

2.4 The potential football-induced molecular adaptations in skeletal muscle

This section reviews the literature investigating whether an acute game of football is capable of inducing molecular adaptations in skeletal muscle, adaptations that have been identified as important in the reduction of risk factors associated with type 2 diabetes. Firstly, there is no research to date on molecular adaptations associated with either skeletal muscle mitochondrial biogenesis or glucose metabolism following a game of football. This provides the present thesis with a unique research perspective.

2.4.1 Could football activate the AMPK pathway?

Information supporting the idea that a football game might trigger the AMPK signalling cascades in skeletal muscle can be found in the measurement of muscle metabolite content following a 90-min football game (Krustrup *et al.*, 2006). A decrease of 13 % in ATP content, 11 % and 43 % in PCr and glycogen content respectively, and a 210 % increase in muscle lactate content was found at the end of the game compared to rest. Figure 8 shows the individual data with respect to the changes in muscle lactate and glycogen content after each half of the game compared to rest.

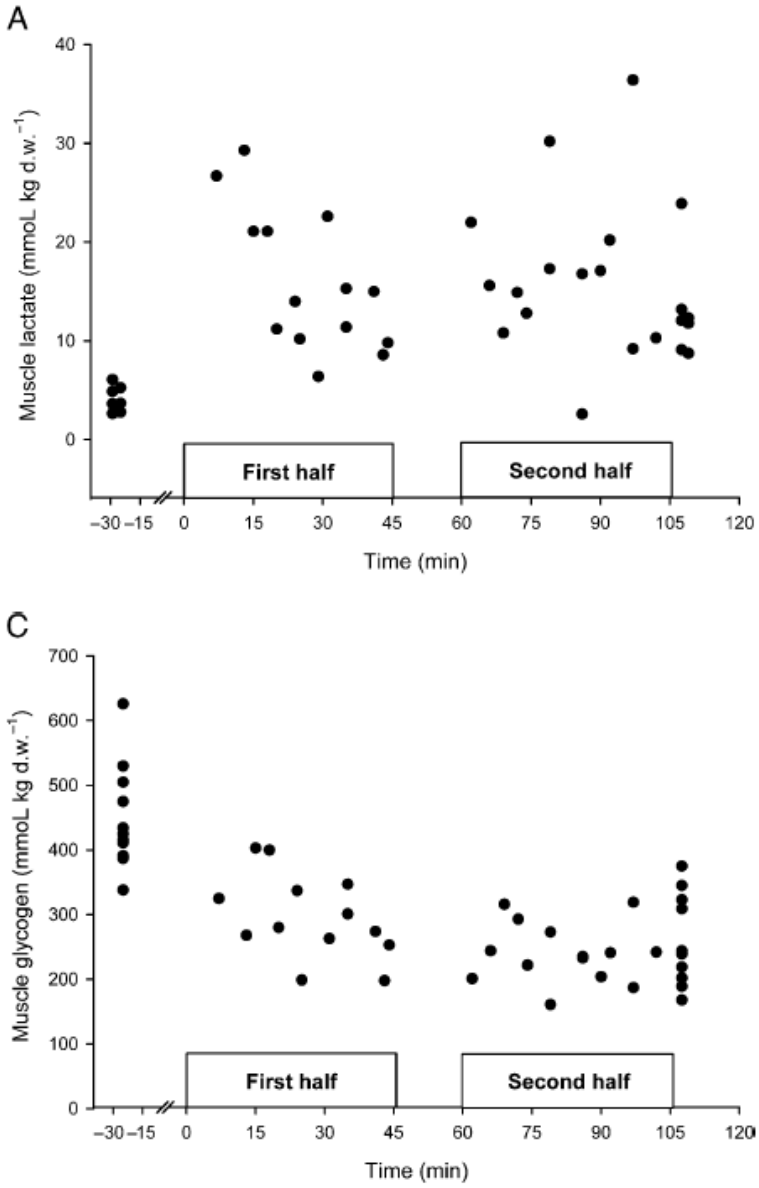

Figure 8; Individual changes in muscle lactate and glycogen content in response to a football match.

Modified from the original Figure 1 in Krustrup *et al.*, 2006

These metabolic perturbations are lower than those in response to acute RSE (Table 3), and hence would not support the idea of football being effective in triggering the AMPK pathway. However, such measurements performed at the end of a game do not necessarily reflect the metabolic demands of the entire exercise, as they are usually associated with the intensity of the period immediately preceding the sampling time, with the exception of glycogen. Also, the time delay between the end of exercise and the muscle collection is crucial, as the ATP and PCr recovery kinetics are particularly fast (Bendahan *et al.*, 2003). To account for this, muscle metabolites were also measured immediately after an intense period of play in both the first and second half of the game (Krustrup *et al.*, 2006). These were defined as 5-min periods where fast running (> 18 km h^{-1}) and sprinting (> 25 km h^{-1}) occurred. Immediately after these bouts, muscle ATP, PCr, and glycogen content were decreased by 14 %, 24 %, and 47 %, respectively. Also, muscle lactate content was increased by 300 %. In support of this, a more intense activity consisting of a 1-h small-sided football game (7 vs. 7), more similar to futsal, resulted in approximately 40 % decrease in PCr content, 30 % decrease in glycogen and an approximate 200 % increase in muscle lactate (Randers *et al.*, 2010b). Despite the magnitude of these responses being lower than that reported in response to other types of exercise, it is possible that the repetition of several intense periods of exercise, each containing high-intensity running efforts, would lead to an up-regulation of the kinases involved in the AMPK signalling processes.

2.4.2 Could football activate the p38 MAPK pathway?

No research to date has investigated the adaptations associated with p38 MAPK in response to a football game. There is, however, some information regarding the blood markers of inflammatory processes in response to a football game and to football simulation models.

Immediately following a 90-min football game, the serum IL-6 concentration was increased by 400 %, being approximately 0.85 and 4.25 pg mL^{-1} at rest and immediately post-game, respectively (Ispirlidis *et al.*, 2008). Similarly, a football game increased plasma IL-6 concentration by 80 to 430 %; the plasma concentration of tumor necrosis factor α was increased by approximately 300 % (Andersson *et al.*, 2010). These results are supported by investigations employing high-intensity intermittent simulation protocols designed to replicate a football game. A protocol consisting of five, 15-min high-intensity bouts (running + ball shooting test) followed by a final shuttle run, resulted in a 560 % increase in plasma IL-6 concentration (Abbey and Rankin, 2009). Another protocol of six, 15-min periods of shuttle sprinting, running and walking, separated by 3-min recovery periods, induced a 300 % increase in plasma IL-6 and a 68 % increase in plasma TNF-α (Bishop *et al.*, 2002). The exercise-induced inflammatory and oxidative stress responses have also been investigated in response to an acute futsal match. A regular game of futsal, consisting of two halves of 20 min, resulted in a 400 % increase in serum IL-6 but no increase in TNF-α concentration (de Moura *et al.*, 2012). However, they found an increased neutrophil TNF-α concentration by more than 500 % and an approximate 15 % increase in the neutrophil content of reactive oxygen species.

2.5 Football in the reduction of risk factors of diabetes: the state of the art.

One of the main purposes of this thesis is to investigate the hypothesis that high-intensity intermittent exercise, and in particular futsal participation, induces muscle adaptations that may assist in the reduction of risk factors of type 2 diabetes. To date, no research has directly assessed the effects of futsal training on muscle adaptations associated with mitochondrial biogenesis and glucose metabolism. However, the concept of high-intensity intermittent exercise/training for the prevention of diabetes has catalysed research in this area (Hawley and Gibala, 2012). These authors supported the efficacy of high-intensity intermittent training by presenting two main arguments. Firstly, they proposed that one of the main advantages of high-intensity intermittent training was the time efficiency. Secondly, they focused on the molecular mechanisms of muscle adaptations induced by this type of exercise. These skeletal muscle molecular adaptations identified some of the protein signalling pathways that have been presented in this review of literature (Figure 9).

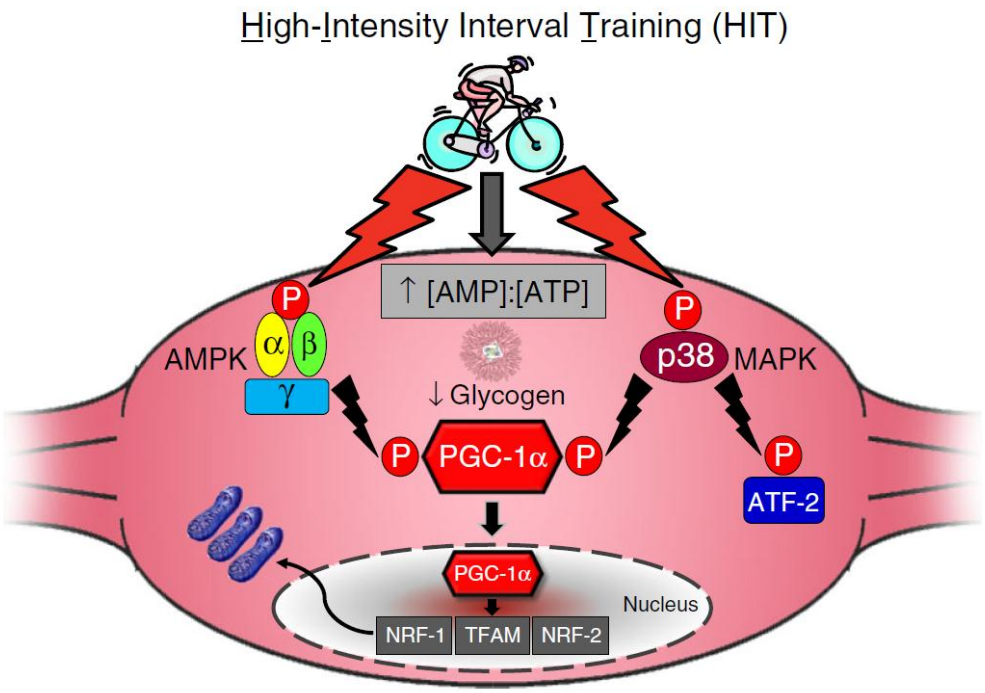

Figure 9; The key signalling pathways associated with mitochondrial biogenesis in response to high-intensity intermittent training.

(Hawley and Gibala, 2012)

After almost a decade of investigation, there is now strong evidence that low-volume, high-intensity intermittent exercise is as effective, if not superior to, continuous moderate-intensity exercise to promote adaptations associated with mitochondrial biogenesis and glucose metabolism in skeletal muscle. Training protocols employing intermittent, 30-s, "all-out" bouts have been demonstrated to produce similar muscle oxidative adaptations to a continuous programme with 10 times larger volume (Gibala *et al.*, 2006). Also, PGC-1α protein abundance and maximal activity of the enzymes citrate synthase (CS) and 3-hydroxyacyl CoA dehydrogenase (HAD) increased to the same extent following the above-mentioned two protocols (Burgomaster *et al.*, 2008). Similarly, a training intervention consisting of six sessions of up to 12, 60-s intervals

performed at 100 % of peak power output, significantly increased the abundance of COX II, COX IV, mtTFA, and PGC-1α (nuclear), and increased citrate synthase activity (Little *et al.*, 2010). These results show that high-intensity intermittent training induces positive molecular adaptations in skeletal muscle. However, little is known about the specific effects of football participation. To date, the only data about muscle molecular/biochemical adaptations following football are on the activity of CS and HAD enzymes. Sixteen weeks of small-sided football games (5 vs. 5, 7 vs. 7, and 9 vs. 9) resulted in an 11 % increase in muscle CS activity and a 9 % increase in HAD activity in women (Bangsbo *et al.*, 2010). These increments were of similar magnitude to those following 16 weeks of continuous running training performed at 82 % of maximal heart rate. In another study, 12 weeks of small-sided football games (4 vs. 4 and 5 vs. 5) produced an 18 % and 20 % increase in muscle CS and HAD activity in men, respectively (Randers *et al.*, 2010a). It is clear that, to date, it is not possible to determine whether or not football participation is effective to induce skeletal muscle adaptations associated with mitochondrial biogenesis or glucose homeostasis. However, complementary information is to be found on the effects of football training upon the common haematological risk factors associated with diabetes. The effects of football training on such risk factors are presented in Table 5. The following risk factors, which have been recently identified in the updated worldwide definition of metabolic syndrome by the International Diabetes Federation (Alberti *et al.*, 2005), were considered:

i. Systolic and diastolic blood pressure
ii. Fasting plasma glucose concentration
iii. Plasma high-density lipoprotein (HDL) cholesterol concentration

iv. Plasma triglycerides concentration

Table 5. The effects of a small-sided football training intervention on the haematological risk factors associated with type 2 diabetes

SYSTOLIC BLOOD PRESSURE (mmHg)	Subjects (n)	Age (y)	Duration (weeks)	Pre	Post	Change (%)
(Andersen *et al.*, 2010) [1]	13	46.7	12	150 ± 11	138 ± 7	-8.0 %
(Randers *et al.*, 2010a) [2]	10	20-43	12	125 ± 6	116 ± 6	-7.2 %
(Knoepfli-Lenzin *et al.*, 2010) §[3]	15	37.0	12	~135	~125	-7.5 %
(Krustrup *et al.*, 2010) [4]	21	37.2	16	113 ± 9	106 ± 9	-6.2 %
DIASTOLIC BLOOD PRESSURE (mmHg)						
(Andersen *et al.*, 2010)	13	46.7	12	91 ± 7	84 ± 7	-7.7 %
(Randers *et al.*, 2010a)	10	20-43	12	78 ± 6	75 ± 6	-3.9 %
(Knoepfli-Lenzin *et al.*, 2010) §	15	37.0	12	~88	~79	-10.3 %
(Krustrup *et al.*, 2010)	21	37.2	16	74 ± 9	70 ± 9	-6.5 %
FASTING PLASMA GLUCOSE (mM)						
(Randers *et al.*, 2010a)	10	20-43	12	5.2 ± 0.6	5.2 ± 0.3	n/a
(Krustrup *et al.*, 2010)	21	37.2	16	5.6 ± 0.9	5.6 ± 0.5	n/a
HDL CHOLESTEROL (mM)						
(Knoepfli-Lenzin *et al.*, 2010)	15	37.0	12	1.3 ± 1.5	1.4 ± 1.2	+7.7 %
(Krustrup *et al.*, 2010)	21	37.2	16	1.6 ± 0.5	1.7 ± 0.5	+6.3 %
(Randers *et al.*, 2010a)	10	20-43	12	1.2 ± 0.3	1.3 ± 0.3	+8.3 %
TRIGLYCERIDES (mM)						
(Andersen *et al.*, 2010)	13	46.7	12	1.80 ± 0.7	1.60 ± 0.7	-11.1 %
(Krustrup *et al.*, 2010)	21	37.2	16	1.02 ± 0.5	0.96 ± 0.5	-5.9 %
(Randers *et al.*, 2010a)	10	20-43	12	1.03 ± 0.8	0.97 ± 0.6	-5.8 %

Data are presented as Mean ± SD calculated from the original data expressed as mean ± SE
§, data calculated from the graphical representation. [1], training details: 5vs5, 6vs6, 7vs7. [2], training details: 4vs4, 5vs5. [3], training details: 3vs3, 4vs4, 5vs5. [4], training details: 5vs5, 7vs7, 9vs9

Thus, regular participation in football games, particularly in the form of small-sided activities, is beneficial in reducing the severity of the main risk factors of diabetes. In the majority of cases, these changes were comparable to those in response to traditional aerobic training.

2.6 Aims and hypotheses

2.6.1 Aims

This review of literature has presented the rationale behind the concept that RSE and futsal effectively induce performance and skeletal muscle adaptations in young adults, and that those adaptations can lead to a reduction of diabetes risk factors in middle-aged sedentary adults. This concept was developed through four original investigations. The first two studies aimed to:

i) Investigate the physiological and performance responses to a multiple-set model of RSE

ii) Assess skeletal muscle molecular changes associated with mitochondrial biogenesis following acute and chronic RSE

Then, the concept of short, high-intensity intermittent exercise was applied into a "real-life" physical activity context. Futsal was selected to closely reflect the high-intensity patterns of the RSE used in the previous studies, with the aim to:

iii) Understand the molecular responses to one session of futsal compared to one session of work-matched, continuous moderate-intensity running exercise

iv) Investigate the efficacy of futsal training, compared to aerobic training, for the reduction of risk factors of diabetes in middle-aged, sedentary males.

2.6.2 Hypotheses

Based on the rationale presented in this review of literature, and according to the aims of the investigations, it was hypothesised that:

i) Four weeks of multiple-set repeated-sprint training, despite the limited training volume, would improve RSE performance in young adults.

ii) Acute RSE would increase the mRNA expression of transcription factors MEF2A, NRF1 and mtTFA, primarily via a large activation of the AMPK and CaMKII signalling pathways; and this would result in a training-induced increase in PGC-1α protein abundance.

iii) An acute game of futsal, due to its intermittent nature, would induce protein signalling associated with mitochondrial biogenesis equal to, or greater than a work-matched continuous running exercise in young healthy men.

iv) Eight weeks of futsal training would induce equal or greater skeletal muscle protein abundance and insulin sensitivity compared to moderate-intensity, continuous running training in middle-aged, sedentary men.

CHAPTER 3 – PERFORMANCE AND PHYSIOLOGICAL RESPONSES TO REPEATED-SPRINT EXERCISE: A NOVEL MULTIPLE-SET APPROACH

3.1 Introduction

In many team sports, the number of sprints during a match is between 20 and 40, with an average duration of 2 to 3 s, and recovery periods ranging from approximately 50 to 300 s (Bangsbo *et al.*, 1991; Dawson *et al.*, 2004; Mohr *et al.*, 2003; Spencer *et al.*, 2004b). This combination of sprints and recovery is unlikely to be linked to a performance decrement during a game. When 6-s sprints are performed with 60 s of recovery, a decline in performance is only evident after the tenth consecutive repetition (Balsom *et al.*, 1992). However, during a game sprints are often clustered, with short recovery between repetitions (i.e., < 60 s) and longer recovery between bouts (Spencer *et al.*, 2004b). These repeated-sprint bouts are typically associated with important phases of the game, such as gaining advantage over an opponent or creating scoring opportunities. Therefore, the ability to repeat sprints (i.e., repeated-sprint ability (Wadley and Le Rossignol, 1998)), is fundamental for team-sport participants.

A single set of 5 to 15 sprints, with between-sprint recovery of less than 30 s, has traditionally been used to assess performance during repeated-sprint exercise (RSE) (Spencer *et al.*, 2005). This model poorly reflects the demands of team-sport games, where players are often required to perform multiple bouts of sprints (Spencer *et al.*, 2004b). Therefore, the use of multiple sets of RSE allows a more accurate investigation of team-sport performance. To the best of knowledge, only three studies have

investigated multiple-set RSE by assessing performance in response to an acute intervention (Beckett *et al.*, 2009; Carr *et al.*, 2008; Sim *et al.*, 2009). However, all three studies measured only sprint time as their performance variable. Given the short duration of sprints during a match (Spencer *et al.*, 2005), a key factor in team-sport performance is the ability of players to accelerate, rather than the ability to maintain a mean velocity. Further research is therefore required to assess changes in acceleration during multiple-set RSE, and to investigate the effects of training on performance and physiological responses. This was investigated in the present thesis.

Given the strong correlation between repeated-sprint ability and very-high intensity running/sprinting distance during a game (Rampinini *et al.*, 2007), it is surprising that little attention has been directed towards the efficacy of sprint training to improve RSE performance. Sport-specific training and small-sided games (Buchheit *et al.*, 2009; Hill-Haas *et al.*, 2009; Spencer *et al.*, 2004a), interval and continuous training (Bishop and Edge, 2005; Buchheit *et al.*, 2009; Buchheit *et al.*, 2008; Edge *et al.*, 2005; Ferrari Bravo *et al.*, 2008; Glaister *et al.*, 2007), and resistance training (Edge *et al.*, 2006; Hill-Haas *et al.*, 2007) have been investigated. However, only three studies to date have employed repeated-sprint training (Buchheit *et al.*, 2008; Dawson *et al.*, 1998; Ferrari Bravo *et al.*, 2008). These studies indicated an improvement in repeated-sprint ability ranging from 1.0 to 2.2 %, but only used mean/total sprint time to assess performance. Repeated-sprint training differs from protocols utilising longer between-sprint recovery (e.g., 55 s; (Linossier *et al.*, 1993)), or longer efforts (e.g., repeated “all-out” 30 s bouts; (Gibala *et al.*, 2006; McKenna *et al.*, 1993)). Further, these types of training do not reflect the specificity of team-sport performance. Also, laboratory cycling and outdoor

running have been traditionally employed as RSE modalities. While cycle ergometry testing has limited applications to team sports, field-based sprinting can be difficult to control, and performance difficult to measure. Instead, non-motorised treadmills offer a suitable methodology to expand on previous research and to reliably asses performance in a standardised laboratory setting (Ross *et al.*, 2009).

Therefore, the aims of the present study were a) to comprehensively assess the performance and physiological characteristics of acute, multiple-set RSE on a non-motorised treadmill; b) to investigate the effects of repeated-sprint training on RSE performance; and c) to quantify relationships between $\dot{V}O_{2\text{peak}}$, intermittent running capacity, and indices of RSE performance.

3.2 Methods

3.2.1 Participants

Ten young, healthy adults (7 males, 3 females) gave written informed consent and participated in this study, which was approved by the Victoria University Human Research Ethics Committee and designed to conform to the Declaration of Helsinki. The baseline physical characteristics of the subjects were (Mean ± SD): age 22.3 ± 4.1 years; height 174.40 ± 9.0 cm; mass 70.2 ± 11.6 kg; $\dot{V}O_{2\text{peak}}$ 53.7 ± 6.9 mL kg^{-1} min^{-1}. All participants were physically active and seven of them were involved in team sports at a recreational level.

3.2.2 Experimental overview

Prior to training, participants visited the laboratory on three separate occasions and performed an incremental exercise test on a treadmill, a Yo-Yo Intermittent Recovery Test level 1 (Yo-Yo IR1), and a familiarisation trial of the RSE; each test was separated by at least 48 h. After two weeks, all participants performed the pre-training RSE. Then, participants commenced their 10-session training program (Monday, Wednesday, Friday), with each session consisting of a standardised warm up followed by a repeated-sprint protocol which replicated the pre-training RSE. Forty-eight hours after the last training session the participants performed the post-training RSE. The incremental test and the Yo-Yo IR1 were then performed in this order and with each test separated by 48 h. Participants were asked to refrain from exercise, alcohol and caffeine consumption for 24 h before all tests.

3.2.3 Incremental test

This test was performed on a motorised treadmill (Quinton Q65, Seattle, WA, USA) and comprised 4-min exercise stages interspersed by 1 min of passive rest. The test commenced at a speed of 8 km h^{-1} for females and 9.1 km h^{-1} for males. The intensity was thereafter increased by 1.5 km h^{-1} every 4 min, with no gradient, until volitional exhaustion, defined as the subject's inability to maintain the running speed. Expired gases were analysed using a custom-made metabolic cart. Briefly, subjects breathed through a Hans-Rudolph 3-way non-rebreathing valve, with expired air passed through flexible tubing into a mixing chamber; expired volume was measured using a ventilometer (KL Engineering, Sunnyvale, CA, USA); mixed expired O_2 and CO_2 contents were analysed by rapidly-responding gas analysers (S-3lA/II and CD-3A

analysers, Ametek, PA, USA). The gas analysers were calibrated immediately prior to each test using commercially-prepared gas mixtures (BOC, Australia). The ventilometer was calibrated prior to each test using a standard 3-L syringe. $\dot{V}O_{2\text{peak}}$ was calculated as the average of the two highest values in two consecutive 15-s periods. At rest, and immediately after the completion of each stage, a finger-tip capillary blood sample was collected from the participants while in a standing position, and analysed for lactate concentration ([Lac^-]) (Lactate Pro Analyser, Arkray Inc., Kyoto, Japan). The lactate threshold was calculated according to two different methods, i.e. the intensity that precedes an increment of [Lac^-] of more than, or equal to 1 mM (defined as "lactate threshold", LT), and the intensity corresponding to a fixed lactate concentration of 4 mM (defined as "onset of blood lactate accumulation", OBLA). Heart rate (HR) was recorded every second using a heart-rate monitor (RS800sd, Polar Electro Oy, Kempele, Finland). Ten minutes after the completion of the incremental test, subjects undertook an initial familiarisation on a non-motorised treadmill (Woodway Force, Waukesha, WI, USA), which comprised a 1-min walk, two 10-s runs (8 and 10 km h^{-1}) and one 4-s sprint. The purpose of this initial familiarisation was to habituate the participants to the non-motorised treadmill under a range of different exercise intensities.

3.2.4 Yo-Yo IR1

Forty-eight hours after the completion of the incremental test, the participants performed the Yo-Yo IR1, which is a field-based test that is broadly used in team sports to measure intermittent running capacity (Bangsbo *et al.*, 2008). This test comprised 2 x 20-m shuttle runs at increasing speeds, separated by 10 s of active recovery. The participants were required to run on a parquet-floor indoor court, guided by a beep

signal, until they were unable to maintain the desired speed. The test was terminated when the subjects were no longer able to reach the finish line on the beep signal on two consecutive occasions. The stage reached, along with the total distance covered (m), was recorded as the final result of the test. The reliability of the Yo-Yo IR1 in participants with a similar aerobic power to the subjects of this study has been previously reported, with the coefficient of variation (CV) ranging from 4.9 to 8.7 % (Krustrup *et al.*, 2003; Thomas *et al.*, 2006a).

3.2.5 Familiarisation trial

At least 48 h after completing the Yo-Yo IR1, the participants reported to the laboratory to perform the main familiarisation session. The test began with a standardised warm up which consisted of 4 min of running on the motorised treadmill at a velocity corresponding to 60 % of the velocity associated with $\dot{V}O_{2\text{peak}}$, followed by three runs on the non-motorised treadmill. In detail, the female participants performed two 4-s runs at 13 km h^{-1}, interspersed with 20 s of passive recovery, followed by 1 min of rest, before performing a final 4-s run at 15 km h^{-1}; males performed the same three 4-s runs at 15 and 17 km h^{-1}, respectively. After 1 min of passive recovery the subjects were asked to complete two sets of 5 x 4-s maximal sprints separated by 20 s of passive recovery, with 4.5 min of active recovery between the sets. Participants completed only two sets of sprints in order to minimise the possibility of vasovagal episodes. For the same reason, participants were asked to slowly walk in the first 2 min of recovery. However, during the experimental RSE subjects were instructed to stand and limit movements, to closely replicate the pre- and post-training recovery conditions during which physiological responses (e.g., HR and blood [Lac^-]) were measured.

3.2.6 RSE

Two weeks following the familiarisation trial the subjects performed the main RSE test. Participants initially undertook the above warm up protocol, with the exception that the three 4-s runs on the non-motorised treadmill were performed at 70 and 90 % of the actual peak velocity reached during the familiarization trial. Participants were required, from a standing start, to reach as rapidly as possible the required velocity indicated on the treadmill, and to maintain that velocity for 4 s. The same warm up was subsequently used for all training sessions. The RSE commenced immediately (1 min) after the completion of the warm up, and consisted of three sets of 5 x 4-s sprints with 20 s of passive recovery between sprints and 4.5 min of passive rest between sets. During the RSE, mean and peak power, mean and peak velocity, and acceleration were measured. All measurements from the non-motorised treadmill were acquired with a sampling rate of 50 Hz, and the force transducers were calibrated before each trial according to the manufacturer's guidelines. The height of the horizontal force transducer was adjusted for each subject by measuring and reproducing the distance between the subject's anterior superior iliac spine and the belt of the treadmill, and maintained constant throughout the study. However, the imperfect horizontal position of the force transducer must be taken into consideration when interpreting results from the calculation of power (Morin *et al.*, 2010). The mean HR during the 4.5-min recovery period was defined as the recovery HR and was measured as described above. In addition, two time domain measures of HR variability were calculated from the R-R intervals measured during the last 3 min of recovery with a HR monitor (RS800sd, Polar Electro Oy, Kempele, Finland). The calculation of the root-mean-square difference of successive normal R-R intervals (RMSSD), and the standard deviation of normal R-R intervals (SDNN) were

applied to the last 3 min of data in order to evaluate the part of the recovery period that provided a more stationary HR. Finger-tip blood samples were collected at rest, immediately after sets 1 and 2, and 1 min after the last set, and were analysed for [Lac^-] (Lactate Pro Analyser, Arkray Inc., Kyoto, Japan). A Δ[Lac^-]-to-work ratio (Δ[Lac^-]/work) was calculated as the difference of [Lac^-] after each set and the baseline, and corrected for the total work performed (calculated from mean power and expressed as kiloJoules (kJ)). This ratio allowed comparisons under conditions where the total work completed during RSE pre- and post-training was different. No correction for change in plasma volume was applied, as no statistically significant difference in plasma volume was found between RSE before and after training, for any of the three sets. Compared to rest, the change in plasma volume for sets 1, 2 and 3 was respectively -17.7 ± 6.2 %, - 17.7 ± 3.9 %, and -17.4 ± 5.1 % before training, and -16.3 ± 2.8 %, -17.5 ± 3.8 %, and -19.4 ± 3.3 % after training. A rating of perceived exertion was recorded immediately after the completion of the exercise using a 6-20 point scale (6 = very very light; 20 = very very hard (Borg, 1982)). Laboratory temperature and relative humidity during the trials were 21.8 ± 0.4°C and 37.0 ± 5.8 %, respectively.

3.2.7 Calculation of measurements from the non-motorised treadmill

During the RSE, both visual and audio instructions ("3-2-1-Go") were given to the participants by the same operator, in order to reduce the variability caused by differences in the sprint start. However, data analysis revealed an appreciable level of anticipation in each subject. To minimise the impact of this, the start point for the calculation of the sprints was defined as when a velocity of 1 m s^{-1} was attained; from then, a 4-s period was calculated.

Mean power and mean velocity were calculated as the average of all data sampled in the 4-s period. Peak power and peak velocity were determined as the highest single value recorded during a sprint. Preliminary analyses revealed no differences in peak power or velocity when the values were obtained from a single peak value or from an average of multiple peaks (2 to 5 highest values) recorded during the sprint. Acceleration was calculated as the rate of change in velocity in the 0.5 s immediately after attaining the 1 m s^{-1} velocity. This 0.5-s period was preferred to a 1-s period, as initial analysis demonstrated 0.5 s to better represent the maximal acceleration. That is, if velocity is measured during a 1-s period, a commencement of plateau in acceleration often occurs, therefore failing to reflect maximal acceleration (Figure 10).

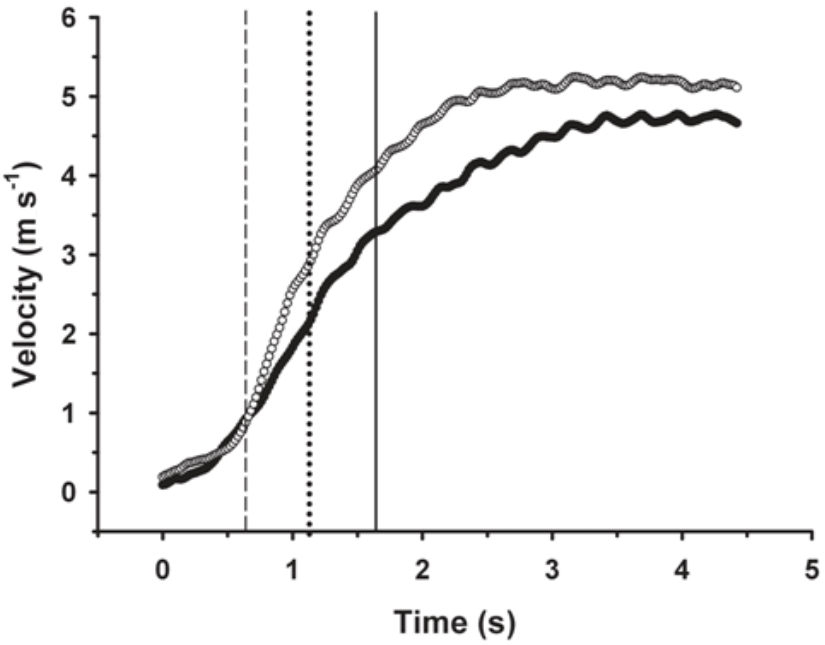

Figure 10; Typical trend of the increase in velocity during a 4-s sprint, for the same participant, before (*filled circle*), and after (*open circle*) training.
The dashed line represents the starting point for the 4-s calculations, which corresponded to the attainment of the 1 m s^{-1} velocity. The dotted and solid lines represent the conclusion of a 0.5-s and 1-s period, respectively.

3.2.8 Test-retest reliability and smallest worthwhile change

The pre-training RSE and the first training session (48 h apart) were used to determine the reliability of all performance measurements from the non-motorised treadmill, of the recovery HR, and of the HR variability (i.e., RMSSD and SDNN). Calculations of reliability allow a more correct interpretation of the results in a single-group, pre-post research design, with similar information to those provided by the use of a control group. Reliability was calculated as typical error expressed as a coefficient of variation (CV) (Hopkins, 2000). The CV was respectively 4.7 % and 10.8 % for mean and peak power; 2.6 % and 3.5 % for mean and peak velocity; 7.6 % for acceleration, 7.3 % for

recovery HR, and 19.3 % and 23.9 % for SDNN and RMSSD. The same data sets were used to calculate the smallest worthwhile change, which was estimated as 0.2 x between-subject standard deviation expressed as a CV (%) (Batterham and Hopkins, 2006). The smallest worthwhile change was 5.7 % and 7.5 % for mean and peak power, 2.8 % and 2.7 % for mean and peak velocity, 5.7 % for acceleration, 3.8 % for recovery HR, and 10.9 % and 12.8 % for SDNN and RMSSD.

3.2.9 Statistical analysis

Data are expressed as Mean ± SD. Results were tested for normal distribution using a Shapiro-Wilk W test. All data met the assumption of normal distribution ($p > 0.05$) with the exception of SDNN and RMSSD. A log transformation was applied to these two measures in order to reduce bias due to non-uniformity of error. Then, performance and physiological responses to RSE were analysed using a Two-Way ANOVA with Repeated Measures, with Bonferroni *post hoc* tests. Results from the incremental test and the Yo-Yo IR1 were analysed using a paired t-test. Statistical significance was set at $p < 0.05$. Pearson product moment correlation coefficient was used to examine the relationships between indices of RSE performance, $\dot{V}O_{2\text{peak}}$, Yo-Yo IR1 performance and physiological responses. The magnitude of the changes was assessed using effect size (ES) statistic with 90 % confidence intervals (CI) and percentage change (Batterham and Hopkins, 2006; Hopkins, 2007). ES were defined as a follows: smaller than 0.2 = trivial, 0.2 to 0.6 = small, 0.6 to 1.2 = moderate, 1.2 to 2.0 = large, larger than 2.0 = very large (Hopkins, 2003 (Access Date 24-12-2011)).

3.3 Results

3.3.1 RSE performance

Before training, performance progressively decreased across the three sets of acute RSE, with a reduction in the majority of the indices in set 3 vs. set 1 (Table 6). Mean power decreased by 4.8 % in set 3 vs. set 1 ($p = 0.006$, ES = -0.21 ± 0.07), peak power was reduced by 9.2 % ($p = 0.027$, ES = -0.28 ± 0.11), and mean velocity declined by 2.2 % ($p = 0.019$, ES = -0.18 ± 0.33). Ten sessions of repeated-sprint training produced improvements in all measurements except for peak power, with changes between 5 and 10 %, and small to moderate effects (Table 6). During the post-training RSE, mean power in set 3 was 4.1 % lower than set 1 ($p = 0.006$, ES = -0.19 ± 0.06), and mean velocity was reduced by 2.1 % ($p = 0.014$, ES = -0.17 ± 0.06), hence similar to the pre-training decrements. The reduction in peak power after training in set 3 was unclear ($p = 0.051$, ES = -0.19 ± 0.10).

Table 6. Summary of changes in RSE performance before and after ten sessions of repeated-sprint training (n = 10).

	set #	pre-training	post-training	% change	ES ± 90 % CI
Mean power (W)	1	734 ± 172	800 ± 188 §	8.8	0.38 ± 0.08
	2	720 ± 161	787 ± 177 §	9.2	0.41 ± 0.07
	3	698 ± 165 *	764 ± 167 * §	9.6	0.39 ± 0.08
Peak power (W)	1	2096 ± 622	2276 ± 864	6.2	0.29 ± 0.17
	2	2036 ± 650	2180 ± 724	6.7	0.22 ± 0.14
	3	1916 ± 624 *	2113 ± 628	10.7	0.31 ± 0.13
Mean velocity ($m\ s^{-1}$)	1	4.10 ± 0.53	4.42 ± 0.57 §	7.7	0.59 ± 0.10
	2	4.08 ± 0.50	4.37 ± 0.55 §	7.2	0.59 ± 0.09
	3	4.01 ± 0.51 *	4.32 ± 0.52 * §	7.8	0.60 ± 0.11
Peak velocity ($m\ s^{-1}$)	1	4.98 ± 0.62	5.25 ± 0.63 §	5.5	0.44 ± 0.10
	2	4.92 ± 0.60	5.23 ± 0.66 §	6.2	0.50 ± 0.11
	3	4.90 ± 0.57	5.18 ± 0.62 §	5.6	0.48 ± 0.14

Data are presented as Mean ± SD; RSE consisted of three sets of 5 x 4-s maximal sprints on a non-motorised treadmill
* significantly less than set 1 ($p < 0.05$);
§ significantly greater than pre-training ($p < 0.05$)

Before training, there was no difference in the acceleration between sets. After training, acceleration was substantially improved, being 21.9 %, 14.7 % and 15.2 % greater than pre-training values for set 1, 2, and 3, respectively; ES were 0.81 ± 0.16, 0.60 ± 0.18 and 0.63 ± 0.22, respectively. During the post-training RSE, acceleration in set 3 was 6.9 % less than that recorded in set 1 ($p = 0.010$; ES = -0.32 ± 0.33; Figure 11).

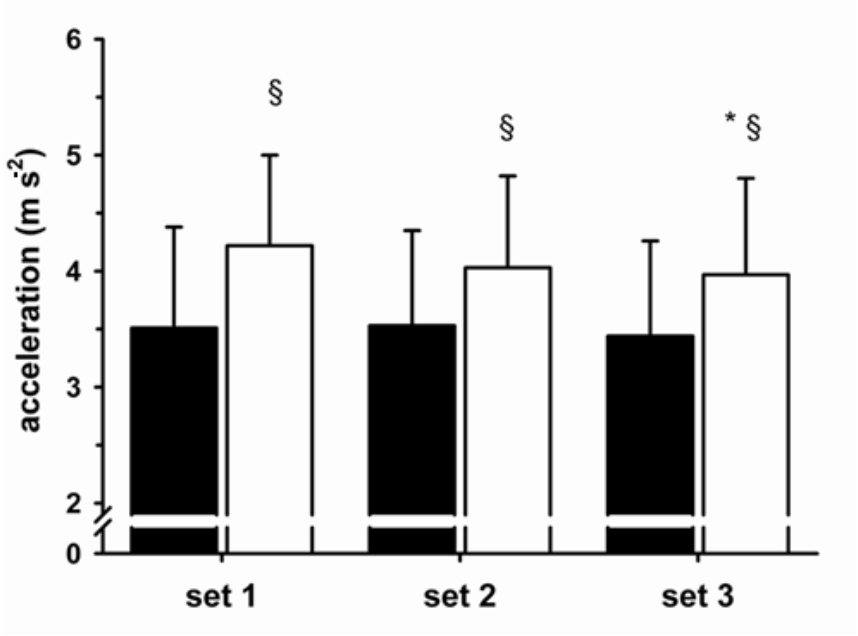

Figure 11; Effects of training on acceleration during acute RSE (n = 10). *Significantly less than set 1 ($p<0.05$); §significantly greater than pre-training ($p<0.05$); filled bars pre-training; open bars post-training. Mean ± SD.

3.3.2 Physiological responses to acute and chronic RSE

Capillary blood [Lac^-] did not change between pre- and post-training RSE, being 1.2 ± 0.3 vs. 1.4 ± 0.4 mM at rest, 9.1 ± 2.1 vs. 8.8 ± 2.6 mM after set 1, 11.5 ± 2.6 vs. 11.5 ± 3.0 mM after set 2, and 12.3 ± 2.8 vs. 12.6 ± 3.2 mM after set 3. However, due to the increase in the total work performed, the $\Delta[Lac^-]$/work after training was lower compared to pre-training at all time-points (Figure 12). Percentage change and ES post-training vs. pre-training were respectively, -15.2 % and -0.60 ± 0.44 for set 1; -12.7 % and -0.50 ± 0.32 for set 2; -9.4 % and -0.74 ± 0.52 for set 3.

Before training, average HR during the 4.5 min recovery period following sets 1, 2, and 3 was, respectively, 136 ± 9, 138 ± 9 and 138 ± 7 beats min^{-1} and did not differ between sets (n = 8, due to technical problems with HR monitoring). After the training

intervention, recovery HR decreased in all three sets, being 125 ± 11 (-8 %, $p < 0.001$, ES = -1.03 ± 0.37), 132 ± 8 (-4.2 %, $p = 0.001$, ES = -0.55 ± 0.23) and 135 ± 8 beats min^{-1} (-2.7 %, $p = 0.024$, ES = -0.49 ± 0.26) for sets 1, 2 and 3 respectively. During the post-training RSE, the average recovery HR increased after set 2 and 3, compared to set 1. Before training, SDNN was 36.4 ± 14.5 ms, 30.9 ± 12.4 ms, and 26.7 ± 8.0 ms ($p = 0.050$ set 3 vs. set 1), and RMSSD was 6.1 ± 2.4 ms, 5.7 ± 3.4 ms, and 5.3 ± 3.7 ms, after set 1, 2 and 3 respectively (n = 7). Post-training, SDNN increased by 22.3 % after set 1 ($p = 0.085$, ES = 0.50 ± 0.56), 16.7 % after set 2 ($p = 0.179$, ES = 0.37 ± 0.53), and 46.3 % after set 3 ($p = 0.003$, ES = 1.06 ± 0.54). RMSDD increased by 53.3 % ($p = 0.005$, ES = 0.90 ± 0.71) and 12 % ($p = 0.399$, ES = 0.20 ± 0.30) after set 1 and 2 respectively, but decreased by -3.9 % after set 3 ($p = 0.762$, ES = -0.07 ± 0.40). Rating of perceived exertion measured immediately after exercise did not show any change, being 16.8 ± 2.4 post-training vs. 16.7 ± 2.3 pre-training (n = 10).

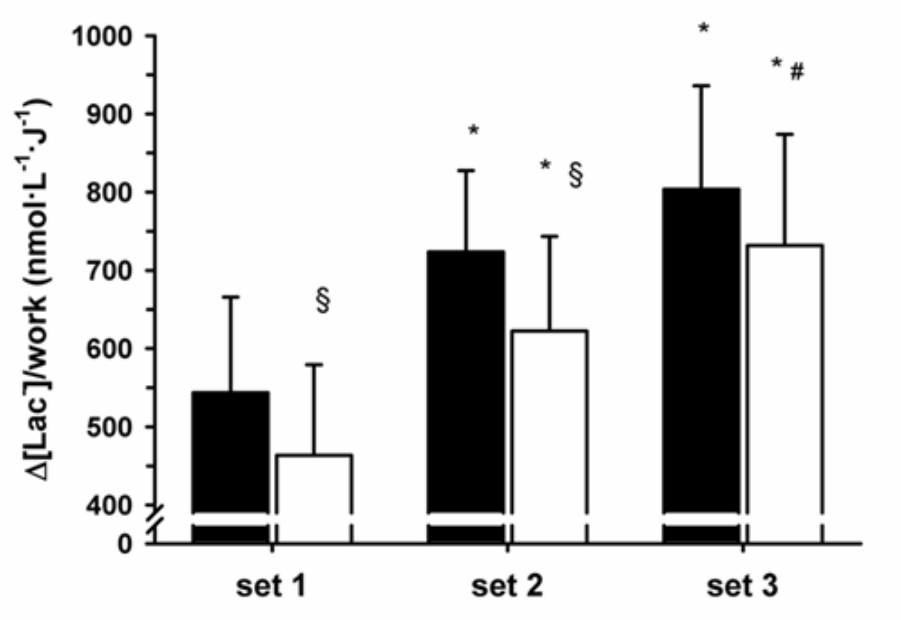

Figure 12; Δ[Lac$^-$]/work ratio during acute RSE before and after training (n = 10); *significantly greater than set 1 ($p<0.05$); #significantly greater than set 2 ($p<0.05$); §significantly less than pre-training ($p<0.05$); filled bars pre-training; open bars post-training; Mean ± SD.

3.3.3 Yo-Yo IR1, $\dot{V}O_{2peak}$ and lactate threshold

Pre-training Yo-Yo IR1 performance was 1305 ± 709 m and increased after training by 8 % to 1400 ± 715 m (p = 0.021, ES = 0.12 ± 0.08) (n = 8). Absolute $\dot{V}O_{2\text{peak}}$ before-training was 3.79 ± 0.63 L min^{-1} and did not differ after training, being 3.86 ± 0.70 L min^{-1} (p = 0.223, ES = 0.16 ± 0.11). Relative $\dot{V}O_{2\text{peak}}$ was 53.7 ± 6.9 and 54.8 ± 6.6 mL kg^{-1} min^{-1} pre- and post-training respectively (p = 0.074, ES = 0.18 ± 0.13). There were no changes in the LT and OBLA. LT was 11.4 ± 2 km h^{-1} before training, corresponding to 70.5 ± 9.4 % of the velocity associated with $\dot{V}O_{2\text{peak}}$, and 11.0 ± 1.7 km h^{-1} after training (-3.3 %, p = 0.34, ES = -0.18 ± 0.34). OBLA before training was 12.4 ± 1.8 km

h^{-1} and corresponded to 76.5 ± 6 % of the velocity associated with $\dot{V}O_{2peak}$; after training, OBLA was 12.4 ± 1.8 km h^{-1} with a 0.7 % change (p = 0.57, ES = 0.04 ± 0.13).

3.3.4 Relationships between Yo-Yo IR1, $\dot{V}O_{2peak}$ and RSE performance

A summary of the relationships between the indices of RSE performance, the $\dot{V}O_{2peak}$ and Yo-Yo IR1 is provided in Table 7. There were large significant correlations between Yo-Yo IR1 and RSE performance, with a very large correlation between Yo-Yo IR1 and acceleration after training. There were weak non-significant relationships between $\dot{V}O_{2peak}$ and indicators of RSE, except for acceleration and mean velocity after training. There were very large correlations between $\dot{V}O_{2peak}$ and Yo-Yo IR1 performance before (r = 0.87, p = 0.012) and after training (r = 0.90, p = 0.006), but a non-significant correlation between the percent changes in the two measures after training (r = 0.56, p = 0.191). There were no significant correlations between RSE performance and LT or OBLA.

Table 7. Summary of the relationships between indices of RSE performance and measures of aerobic power before and after training

	acceleration ($m\ s^{-2}$)		**mean power (W)**		**peak power (W)**		**mean velocity ($m\ s^{-1}$)**		**peak velocity ($m\ s^{-1}$)**	
	pre	**post**	**pre**	**post**	**pre**	**post**	**pre**	**post**	**pre**	**post**
Yo-Yo IR1 (m)	0.61	0.88 *	0.75 *	0.82 *	0.73 *	0.73 *	0.74 *	0.86 *	0.77 *	0.83 *
$\dot{V}O_{2peak}$ ($mL\ kg^{-1}\ min^{-1}$)	0.35	0.71 *	0.45	0.62	0.48	0.61	0.45	0.67 *	0.49	0.63

n = 8 for Yo-Yo IR1; n = 9 for $\dot{V}O_{2peak}$
Relationships are expressed as a Pearson product moment correlation coefficient (r);
* statistically significant ($p < 0.05$)

3.4 Discussion

There were three main findings in the present study. Firstly, ten sessions of repeated-sprint training, comprising a total of only 10 min of exercise, induced a) a moderate, practically-important improvement in acceleration, b) an improvement in indices of RSE performance, $\Delta[Lac^-]$/work and recovery HR, and c) an increase in the intermittent running capacity, despite no changes in the $\dot{V}O_{2peak}$, the LT or OBLA. Secondly, there were strong correlations between Yo-Yo IR1 performance and indices of RSE performance. Finally, performance during multiple sets of RSE progressively decreased from set 1 to set 3, with peak power reporting the biggest decrement pre-training, and acceleration being most affected after training.

3.4.1 Training improves acceleration during multiple-set RSE

To the best of knowledge, this is the first study to quantify the training-induced improvements in the capacity to accelerate. A conventional method to assess acceleration during a sprint is to record the time or the average velocity over the initial 5 or 10 m (Harrison and Bourke, 2009; Spinks *et al.*, 2007). However, despite acceleration being the main component in the initial phase of a sprint, these indirect measurements do not provide information on the rate of change of the instantaneous velocity during the sprint. Instead, by using a non-motorised treadmill, the actual change in acceleration during RSE was detected. Such a change was 2 to 3 times greater than the CV, and 3 to 4 times greater than the smallest worthwhile change calculated

before training; therefore, this enhancement in acceleration was genuine and can be attributed to the training intervention.

The mechanisms underlying the improvement in acceleration after training might include an increase in power output and reactive strength at the beginning of the sprints, possibly via improved neuromuscular adaptations (e.g., change in fibre recruitment pattern). In support of this, 8 weeks of repeated-sprint training has previously been reported to produce an increase in both the velocity over 15-m sprints and countermovement jump height, without any significant changes in lower-body kinematic variables and stride length/frequency in the first two steps (Spinks *et al.*, 2007). Amongst all indices of RSE performance, acceleration returned the largest improvement, which was up to four times greater than the change in peak velocity. This highlights the importance of including a measure of acceleration in match analysis, RSE testing, and training studies that investigate sport-specific sprint performance. For example, traditional analysis of high-intensity activity, taking only high-velocity running into account, ignores the physically-demanding nature of accelerations (Osgnach *et al.*, 2010), and therefore underestimates the real amount of high-intensity activity (Little and Williams, 2007). By directly measuring acceleration, one can not only gain a deeper understanding of the components of repeated-sprint performance in a controlled laboratory environment, but also a more complete interpretation of the real demands of the task. This ultimately allows a better targeting of subsequent training interventions.

3.4.2 Indices of repeated-sprint ability

Multiple sets of repeated sprints induced a significant reduction in performance during set 3 compared to set 1. This is in line with previous research showing a significant reduction of mean/total sprint time after the third set (approximately -1.2 %) when between-set passive recovery was performed (Beckett *et al.*, 2009). The results of the present study also suggest that peak power is affected to a greater extent than mean power or velocity during multiple-set RSE before training. However, after the training intervention it was the capacity to accelerate that reported the largest decrement (-6.9 %, ES = -0.32 ± 0.33) in set 3 vs. set 1. The training-induced enhancement in acceleration was accompanied by small-moderate improvements in mean power/velocity and peak velocity, and a small-trivial change in peak power. Interestingly, the improvements in these indices were greater than those measured in the three previous studies which employed repeated-sprint training (Buchheit *et al.*, 2008; Dawson *et al.*, 1998; Ferrari Bravo *et al.*, 2008). Firstly the lower values of $\dot{V}O_{2\text{peak}}$ of the participants in this study, which may reflect a lower training status, could partly explain the greater changes in RSE performance. In addition, the use of a non-motorised treadmill might have produced, concomitant with the training adaptations, a learning effect which is not present in field-based sprinting. As the participants of this study had to run on a non-motorised treadmill wearing a belt that was connected to a force transducer, this may also represent a small resistance that requires additional work to perform, similar to what occurs during resisted sprinting (Harrison and Bourke, 2009; Spinks *et al.*, 2007). Ultimately, it is interesting to note that in the present study the training intervention consisted of only ten sessions, while previous studies employed from 14 to 18 sessions distributed over 6 to 9 weeks (Buchheit *et al.*, 2008; Dawson *et al.*, 1998; Ferrari Bravo

et al., 2008). However, in one study it has been observed that repeated-sprint ability improves during the first weeks of interval training, reaching a peak at the fourth week, followed by a plateau in the following weeks despite an increase in the training load (Bishop and Edge, 2005). Thus, it is possible that the ten sessions of training employed in this study were optimal to elicit peak changes in RSE performance. Nonetheless, further research is required to understand if a plateau in RSE performance also occurs with repeated-sprint training. Verification of the observation that repeated-sprint training interventions produce positive adaptations in a limited amount of time, would provide essential information on how to optimally train this important fitness component for team sports.

3.4.3 Training reduces Δ[Lac⁻]/work and recovery HR

Ten sessions of repeated-sprint training produced a reduction in Δ[Lac^-]/work measured during acute RSE. This could be explained through a greater participation of the aerobic system during RSE, an increased production and/or clearance of lactate (Stallknecht *et al.*, 1998), or possibly an increase in blood volume. A greater aerobic ATP contribution during RSE might have supported the increase in work despite an unchanged [Lac^-]. Six sessions of repeated, "all-out" exercise bouts improved oxygen uptake and muscle deoxygenation kinetics during exercise at two constant intensities, with a concomitant reduction of the blood [Lac^-]; this was not detected after continuous training performed at a lower intensity (Bailey *et al.*, 2009). The lower Δ[Lac^-]/work after training might also reflect a greater lactate clearance during exercise. One of the mechanisms responsible for this is the action of the monocarboxylate transporters (MCT), which transport lactate across the muscle cell and mitochondrial membrane. Skeletal muscle

MCT1 and MCT4 protein abundance increased after a training intervention involving multiple 6-s sprints interspersed with 1 min of recovery (Mohr *et al.*, 2007). It is likely that both an improved oxidative metabolism and enhanced lactate clearance contributed to the reduction in the $\Delta[Lac^-]$/work after training in the present study.

Ten sessions of training also reduced recovery HR during acute RSE. This might be explained through an improvement in autonomic control, either via increased parasympathetic function or sympathetic withdrawal, during the recovery following the three sets of RSE. The improvements in markers of HR variability support the idea of an enhanced autonomic control, in particular after the first set of sprints. Similar to the results of this thesis, nine weeks of repeated-sprint training improved indices of HR recovery from approximately 5 to 13 %, with a 52 % increase in RMSSD and a 13 % increase in SDNN, following a 6-min exercise at a constant intensity (Buchheit *et al.*, 2008). In addition, the training-induced changes in HR recovery were positively correlated ($r = 0.62$) with the improvements in repeated-sprint ability (Buchheit *et al.*, 2008), linking a practical measure of recovery to performance during short, "all-out" exercise. Likewise, in this thesis a large positive correlation was found between the training-induced decrement in recovery HR after set 1, and the increase in mean power during set 2 ($r = 0.81$, $p = 0.016$). Further research is required to elucidate the mechanisms underlying an improved HR recovery and variability after repeated-sprint training, and to investigate whether a causal relationship exists between an enhanced recovery after one set of sprints and the performance during the subsequent set.

3.4.4 RSE performance and Yo-Yo IR1 performance are strongly correlated

One of the focal points of research investigating RSE has been an attempt to understand the extent to which different physical capacities determine performance, with a particular interest in aerobic power. It is well accepted that maximal oxygen uptake might not be the most important determinant of repeated-sprint ability, and this is supported by the moderate correlations that have been found between these two parameters (Aziz *et al.*, 2000; Bishop *et al.*, 2004). However, it was of interest to see whether aerobic power might be correlated with RSE performance when participants were asked to complete multiple sets of sprints. The results from the present study indicate no significant correlations between $\dot{V}O_{2\text{peak}}$ and any of the indices of RSE performance ($r = 0.45$ to $r = 0.61$, $p > 0.05$). After training, all correlations became stronger, but only those with mean velocity and acceleration reached statistical significance. No correlation was present between the training-induced changes in $\dot{V}O_{2\text{peak}}$ and the improvement in acceleration or velocity. Conversely, large correlations between Yo-Yo IR1 performance and RSE performance were found before training, and even larger correlations after training (Table 7). Importantly, acceleration during pre-training RSE was the physical capacity that had the poorest correlation with Yo-Yo IR1 performance ($r = 0.61$, $p = 0.109$), but the strongest correlation after training ($r = 0.88$, $p = 0.004$). This may be explained by the fact that both types of exercise require repeated accelerations with short recovery between repetitions, and repeated-sprint training might have created specific adaptations which are important for intermittent running performance.

3.5 Conclusions

Ten sessions of repeated-sprint training were effective in improving acceleration, repeated-sprint ability, and intermittent running capacity in young, healthy adults. This repeated-sprint intervention had a remarkably-low training volume, with a total of only 10 min of exercise (140 min including recovery periods). This is therefore a time-efficient intervention that can be integrated into the training programmes of many intermittent sports. Further, low-volume, maximal-intensity training can improve specific components important for team-sport performance, in particular acceleration, without inducing a concomitant decrement in the maximal aerobic power. This is of great practical application in the modern sport environment, where the competition calendar limits the time that can be dedicated to training. In addition, this study adds important information on the extent to which the different indices of repeated-sprint ability are involved in the decline of performance across multiple sets of sprints. This type of exercise more closely resembles the activity profile of team sports and might be more appropriate than a traditional single-set test to evaluate repeated-sprint ability in intermittent sport activities. Furthermore, this study is in line with the increasing interest towards short-term, "all-out" exercise programs to improve sport performance and health-related indices (Benziane *et al.*, 2008; Burgomaster *et al.*, 2008; Thomassen *et al.*, 2010).

CHAPTER 4 – REPEATED SPRINTS ALTER SIGNALLING RELATED TO MITOCHONDRIAL BIOGENESIS IN HUMANS

4.1 Introduction

Physical exercise is used, both in an acute and chronic form, as a method to investigate the mechanisms of mitochondrial adaptations in human skeletal muscle. The mitochondria play an important role in the etiopathogenesis of chronic diseases (Patti and Corvera, 2010); therefore it is important to understand the molecular events that contribute to exercise-induced mitochondrial adaptations. To date, continuous exercise, performed at a low- or moderate-intensity, has predominantly been employed as the experimental model, and has been shown to activate the molecular pathways associated with mitochondrial biogenesis (Egan *et al.*, 2010; Mahoney *et al.*, 2005; Pilegaard *et al.*, 2005). However, prolonged continuous exercise might not represent an attractive option for many individuals to engage in regular physical activity (Krustrup *et al.*, 2009). Consequently, different experimental models have been explored, for example acute and chronic high-intensity intermittent exercise. In particular, sub-maximal intervals of 4-5 min in duration (Benziane *et al.*, 2008; Gurd *et al.*, 2010) or repeated 30-s "all-out" bouts (Gibala *et al.*, 2009) have been employed. However, the practical applications of such models appear to be limited, as these exercises do not reflect typical patterns of physical activities, and are often perceived as very demanding (Doherty *et al.*, 2004). Repeated-sprint exercise (RSE) comprises short maximal sprints (less than 6 s in duration) interspersed with recovery of less than 30 s. This type of exercise is usually well tolerated, and is a typical component of many sports, in particular team sports

(Spencer *et al.*, 2005). However, despite this, there is almost no research that has investigated the effects of RSE on molecular pathways associated with mitochondrial biogenesis.

A coordinated interaction of signalling molecules, transcription co-activators, transcription factors, and repressors, is responsible for exercise-induced mitochondrial adaptations in skeletal muscle. The mitochondrial transcription factor A (mtTFA) (Fisher *et al.*, 1987), nuclear respiratory factor 1 (NRF 1) (Wu *et al.*, 1999), and myocyte enhancer factor 2 (MEF 2) (McGee and Hargreaves, 2011) have been identified as key transcription factors. The peroxisome proliferator-activated receptor γ, co-activator 1α (PGC-1α) is considered the master regulator of these transcription factors, and is targeted, amongst other pathways, by the 5'AMP-activated protein kinase (AMPK), Ca^{2+} calmodulin-dependent protein kinase II (CaMK II) (Wu *et al.*, 2002), and p38 mitogen-activated protein kinase (p38 MAPK) (Akimoto *et al.*, 2005) signalling pathways. Also, repressors interact either with co-activators or with transcription factors, for example the histone deacetylases (HDAC) (McGee and Hargreaves, 2011), to help regulate mitochondrial biogenesis.

Despite the remarkably-low exercise volume, RSE might represent an effective physiological stimulus to up-regulate the signalling processes associated with mitochondrial adaptations. For example, repeated 4-s sprints performed with 25 s of recovery, produce a 15 % decrease in ATP content in the human vastus lateralis (Spencer *et al.*, 2008). As the change in the AMP-to-ATP ratio during muscle contraction is the primary trigger of AMPK activation (Hardie and Carling, 1997), this

suggests that RSE may provide an ideal exercise stimulus to activate AMPK and its downstream Acetyl-CoA Carboxylase (ACC). Also, the intracellular concentration of Ca^{2+} is the main initiator of the CaMKII signalling cascade, and it has been demonstrated that CaMKII is able to decode high-frequency Ca^{2+} spikes better than low-frequency ones (De Koninck and Schulman, 1998). Therefore, the repetition of maximal sprints may phosphorylate CaMKII protein, ultimately leading to mitochondrial biogenesis.

It has been shown that a single set of intermittent sprints (10 x 6-s sprints; 1-min recovery) increases AMPK protein phosphorylation and PGC-1α mRNA expression (Coffey *et al.*, 2009; Coffey *et al.*, 2011). However, there is paucity of information about the effects of multiple-set RSE on a more comprehensive sequence of molecular events. Also, the effects of chronic RSE, i.e., repeated-sprint training, have not been assessed. Therefore, the aim of this study was to investigate the effects of acute and chronic RSE on skeletal muscle mRNA expression and protein abundance/phosphorylation associated with mitochondrial adaptations. It was hypothesised that acute RSE would increase the expression of transcription factor genes via a large activation of the signalling pathways, resulting in a training-induced increase in PGC-1α protein abundance.

4.2 Methods

4.2.1 Participants

Ten healthy young adults (7 males, 3 females) gave written informed consent and

participated in this study, which was approved by the Victoria University Human Research Ethics Committee and conforms to the Declaration of Helsinki. Their physical characteristics were (Mean ± SD): age 22.3 ± 4.1 years; height 174.40 ± 9.00 cm; mass 70.2 ± 11.6 kg; $\dot{V}O_{2\text{peak}}$ 53.7 ± 6.9 mL kg^{-1} min^{-1}. All participants were recreationally active and seven were involved in team sports at a recreational level.

4.2.2 Experimental overview

Prior to training, participants performed an incremental exercise test on a motorised treadmill, followed by a first habituation of the non-motorised treadmill used for the RSE, as previously described in detail in chapter 3.2.3. At least 48 h later, participants returned to the laboratory for the main RSE familiarisation trial. After one week, participants performed the pre-training RSE trial. Then, participants commenced a 4-week training program, three times per week, with each session comprised of a standardised warm up followed by a repeated-sprint protocol which replicated the pre-training RSE. The post-training RSE trial was conducted 48 h after the last training session and the incremental test was then performed a further 48 h later. Muscle biopsies and venous blood samples were obtained during pre- and post-training RSE trials. Participants refrained from exercise, and from alcohol and caffeine consumption for 24 h before all tests. Participants were asked to complete a dietary report, with a detailed description of the last three meals consumed before the pre-training RSE trial. The same diet was then replicated before the post-training RSE trial.

4.2.3 RSE trial

The participants arrived at the laboratory between 08:00 and 10:00 a.m. After remaining

supine for ten minutes, a resting venous blood sample and a muscle biopsy were taken. Immediately after the biopsy, participants completed a standardised warm-up consisting of two parts. First, they ran on a treadmill for 4 min at a velocity corresponding to 60 % of the velocity associated with their $\dot{V}O_{2\text{peak}}$. Then, participants quickly moved to the non-motorised treadmill and performed two 4-s runs at the 70 % of their peak sprinting velocity (measured from the sprints during the familiarisation trial) with 20 s of passive recovery; after 1 min of rest, one 4-s run at the 90 % of the peak sprinting velocity was performed. Participants were required, from a standing start, to reach the required velocity indicated on the treadmill as rapidly as possible, and to maintain that velocity for 4 s. The same warm up was subsequently used for all training sessions and the post-training RSE. The RSE commenced 1 min after the completion of the warm up, and consisted of three sets of 5 x 4-s sprints with 20 s of passive recovery between sprints and 4.5 min of passive rest between sets. Laboratory temperature and relative humidity during the trials were 22.3 ± 0.2 °C and 43.0 ± 4.1 % respectively, and did not differ between pre- and post-training RSE trials.

4.2.4 Venous blood sampling and analyses

A 20-gauge catheter (Optiva, Medex Medical Ltd., UK) was inserted in the antecubital vein, and a blood sample of approximately 3 mL was drawn at rest, after each set of sprints, and at 1, 2, 5, 10, 20 and 30 min after RSE, and immediately placed in a tube containing lithium heparin. Glucose ([Glu]) and lactate ([Lac^-]) concentrations were measured in duplicate using an automated analyser (2300 STAT plus, YSI Inc., Yellow Springs, OH, USA). Total haemoglobin concentration ([Hb]) and the haematocrit (Hct) were measured in duplicate using an automated haematology analyser (Sysmex K-800,

TOA Medical Electronics, Kobe, Japan), in order to assess the change in plasma volume during exercise and recovery. The typical error, expressed as coefficient of variation (%) of duplicates for [Glu], [Lac^-], [Hb], and Hct, was 3.8 %, 3.9 %, 0.6 %, and 1.0 % respectively.

4.2.5 Muscle biopsies

Skeletal muscle samples were taken from the vastus lateralis muscle using a biopsy needle with suction. After injection of a local anaesthetic into the skin and fascia (Xylocaine 1 %, AstraZeneca, North Ryde, NSW, Australia) four small incisions were made 1 cm apart. Biopsies were taken before, immediately after, and 1 h and 4 h after RSE, both before and after training. Opposite legs were chosen for the pre- and post-training biopsies. After sampling, muscle samples were rapidly blotted on a filter paper to remove excess blood, and immediately frozen in liquid N_2. All samples were then stored at -80 °C before being analysed.

4.2.6 RNA extraction and RT-PCR

Total cellular RNA was extracted as previously described (Trenerry *et al.*, 2007). RT-PCR was performed using a Real Time PCR System (7500, Applied Biosystems, Beverly, MA, USA). PCR was performed in duplicate with reaction volumes of 20 µL, containing POWER SYBR Green 1 (Applied Biosystems), forward and reverse primers, and cDNA template (1.25 ng μL^{-1}). Data were analysed using a comparative critical threshold (Ct) method, where the amount of target gene normalised to the amount of endogenous control relative to control value is given by $2^{-\Delta\Delta Ct}$. The efficacy of 18s as an endogenous control was examined using the equation $2^{-\Delta Ct}$. 18s was considered an

appropriate control for this study when no changes in the expression of the mRNA were observed (data not shown). Primers were designed using Primer Express software package version 3.0 (Applied Biosystems) from gene sequences obtained from GenBank (Table 8). Primers were designed spanning intron-exon boundaries to prevent amplification of the target region for any contaminating DNA. Primer sequence specificity was also confirmed using the Basic Local Alignment Search Tool (BLAST). A melting point dissociation curve was generated by the PCR instrument for all PCR products to confirm the presence of a single amplified product.

Table 8. Primer sequences and accession numbers for RT-PCR of skeletal muscle genes

Gene	Accession Number	Forward Sequence (5' – 3')	Reverse Sequence (5' – 3')
COX IV	NM_001861	CATGTGGCAGAAGCACTATGTGT	GCCACCCACTCTTTGTCAAAG
MEF2a	NM_005587	TGGCATGACTCTTTCCATTC	TTGCTATCATCCATTATTTGTCTTCCT
NRF 1	NM_005011	TGATTTTCTGGGATTGGTGAAGA	AAGAAGTCCTGCCACAATTTGG
PGC1α	NM_013261	CCGCACGCACCGAAAT	TCGTGCTGATATTCCTCGTAGCT
mtTFA	NM_003201	AAGACCTCGTTCAGCTTATAACGTTT	TTTCCAGTTTTCCTTTACAGTCTTCA
18s	NR_003286	TTCGGACGTCTGCCCTATCAA	ATGGTAGGCACGGCGACTA

COX IV, cytochrome oxidase subunit IV; MEF2a, myocyte enhancer factor 2a; NRF 1, nuclear respiratory factor 1; PGC-1α, peroxisome proliferator-activated receptor γ, co-activator 1 α; mtTFA, mitochondrial transcription factor A.

4.2.7 Immunoblotting

Approximately 40 mg of frozen muscle samples were homogenised in ice-cold buffer containing 20 mM Tris pH 7.8 (Bio-Rad Laboratories, Hercules, CA, USA), 1mM EDTA, 137 mM NaCl, 2.7 mM KCl (Merck, Darmstadt, Germany), 1 mM $MgCl_2$, 5 mM $Na_4O_7P_2$, 10 mM NaF, 1 % Triton X-100, 10 % Glycerol, 0.5 mM Na_3VO_4, 1 μg mL^{-1} Leupeptin, 1 μg mL^{-1} Aprotinin, 200 mM PMSF, 1 mM DTT, 1 mM Benzamidine.

All reagents were analytical grade (Sigma-Aldrich, St. Louis, MO, USA), unless otherwise specified. Samples were homogenised (1:37.5 dilution (wt/vol)) for 2 x 20 s, using a tissue homogeniser (TH220, Omni International, Kennesaw, GA, USA). Homogenates were then rotated for 60 min at 4 °C, centrifuged at 15000 g for 10 min at 4 °C, and protein concentration of the resulting supernatant was determined using a DC Protein Assay kit (Bio-Rad). Aliquots of the muscle lysate were mixed with Laemmli sample buffer, and 60 μg of total protein per sample were separated by 6 to 20 % gradient SDS-PAGE, for 2 h at 80 mA and overnight at 16 mA, in a standard vertical electrophoresis unit (SE 600 Chroma®, Hoefer Inc., Holliston, MA, USA). Following electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (Bio-Rad) for 3 h at 320 mA using a semi-dry blotter (TE70X, Hoefer). Membranes were blocked in TBST buffer (10 mM Tris, 100 mM NaCl, 0.02 % Tween-20) containing 7.5 % non-fat milk, for 1 h at room temperature. After being washed 3 x 10 min in TBST, membranes were incubated with the appropriate primary antibody overnight at 4 °C. Primary antibodies were diluted in TBS buffer containing 0.1 % NaN_3 and 0.1 % albumin bovine serum. All membranes were incubated with the same volume of buffer.

To determine protein abundance and phosphorylation, membranes were incubated with antibodies for ACC (Cell Signaling Technology, Danvers, MA, USA, # 3662), phospho-ACC Ser^{79} (Cell Signaling, # 3661), AMPKα (Cell Signaling, # 2532), phospho-AMPKα Thr^{172} (Cell Signaling, # 2535), phospho-CaMKII Thr^{286} (Cell Signaling, # 3361), COX IV (Cell Signaling, # 4844), p38 MAPK (Cell Signaling, # 9212), phospho-p38 MAPK Thr^{180}/Tyr^{182} (Cell Signaling, # 9211), PGC-1α (Calbiochem, Merck, # KP9803), phospho-HDAC5 Ser^{259} (kindly donated by Dr. Sean McGee and raised as previously described (McGee *et al.*, 2008)), and RIP 140 (Abcam, # ab3425). All

membranes were normalised for loading with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA, # sc-25778). A dilution of 1:1000 was used for most antibodies, with the exception of ACC/phospho-ACC (1:750), RIP 140 (1:500), COX IV (1:6000) and GAPDH (1:2000). Following incubation with the primary antibodies, membranes were washed 3 x 10 min in TBST buffer and incubated with the appropriate anti-rabbit (PerkinElmer, Waltham, MS, USA # NEF812001EA) or anti-mouse (PerkinElmer # NEF822001EA) horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Secondary antibodies were diluted 1:5000 in TBST buffer containing 5 % non-fat milk. After washing the membranes in TBST, immunoreactive proteins were detected using chemiluminescence reagents (Immobilon™ HRP Substrate, Millipore, Billerica, MS, USA), and quantified by densitometric scanning (VersaDoc™ Imaging System, Bio-Rad). Only linear adjustments to the whole images were performed with a dedicated software (Quantity One v.4.6.6, Bio-Rad), with no modifications to the gamma settings and no external image-processing software used. Where possible, images are presented with a height of approximately five bands above and below the band of interest (Wada *et al.*, 2004).

4.2.8 Statistical analysis

Data are presented as mean ± SD. Results were tested for normal distribution using a Shapiro-Wilk W test, and when this assumption was not met ($p < 0.05$) data were log transformed to reduce bias due to the non-uniformity of error. For balanced data sets (i.e., no missing values), a two-way ANOVA with Repeated Measures and Bonferroni post hoc test were applied. For the data sets with missing values, a linear mixed model

analysis was used, with training and time as fixed effects and with Restricted Maximum Likelihood as the estimation method for missing values. For each variable, three covariance structures were tested, i.e., compound symmetry, first-order autoregressive, and unstructured. The appropriate structure was then chosen by comparing the Akaike's Information Criterion of each covariance type, and accounting for the number of parameters included in the estimation. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS 15.0 for Windows (IBM Corporation, USA). The magnitude of the changes was assessed using effect size (ES) statistic with 90 % confidence intervals (CI) and percentage changes. ES were defined as a follows: smaller than 0.2 = trivial, 0.2 to 0.6 = small, 0.6 to 1.2 = moderate, 1.2 to 2.0 = large, larger than 2.0 = very large (Hopkins, 2003 (Access Date 24-12-2011)).

4.3 Results

4.3.1 Performance and physiological responses

During the pre-training RSE, mean power was 710 ± 180 W, 711 ± 163 W, and 688 ± 152 W for set 1, 2, and 3 respectively. Peak power was 2106 ± 724 W, 2056 ± 608 W, and 1974 ± 535 W for set 1, 2, and 3 respectively. Training increased mean power ($p < 0.001$ main effect), being 802 ± 196 W (+13.0 %), 780 ± 182 W (+9.6 %), and 750 ± 164 W (+9.5 %) for set 1, 2, and 3 respectively. Peak power did not change after training (+2.4 %, 6.1 %, and 4.4 % for sets 1, 2, and 3 respectively). The [Lac^-] and [Glu] in response to acute RSE before and after training are presented in Figure 13. There were no differences in plasma volume changes between the pre- and post-training

RSE. Training did not change $\dot{V}O_{2peak}$ (53.7 ± 6.9 and 54.8 ± 6.6 mL kg^{-1} min^{-1}, pre- and post-training respectively), the onset of blood lactate accumulation (OBLA; 12.4 ± 1.8 km h^{-1} and 12.4 ± 1.8 km h^{-1}, pre- and post-training, respectively), or the lactate threshold (LT; 11.4 ± 2.0 km h^{-1} before training, 11.0 ± 1.7 km h^{-1} after training).

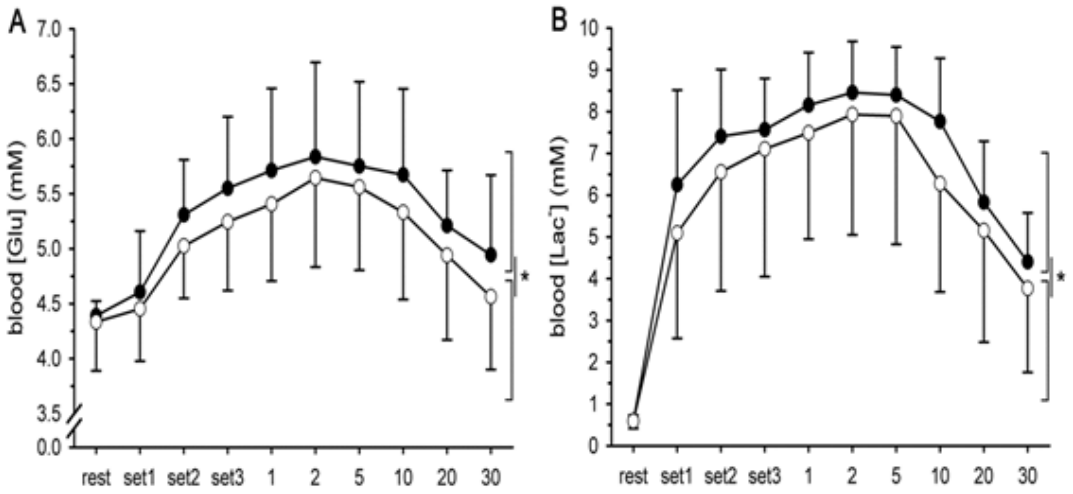

Figure 13; Blood glucose (A) and blood lactate (B) concentrations during acute repeated-sprint exercise and recovery.

Filled circles, pre-training; *open circles,* post-training. *, significantly less than pre-training (main effect, $p < 0.05$). Data are presented as mean ± SD; n = 10.

4.3.2 Skeletal muscle protein signalling

Representative Western blot images are presented in Figure 14. Acute RSE was associated with a small change in AMPKα phosphorylation (32 %; ES 0.7 ± 0.4), when 1 h after exercise was compared to rest (Table 9). ACC abundance (51 %; ES 0.7 ± 0.3), as well as ACC phosphorylation (86 %; ES 1.4 ± 0.8), were increased immediately after

exercise compared to rest ($p < 0.001$; Figure 15A). Acute RSE increased CaMKII phosphorylation 1 h after exercise compared to rest (69 %; ES 0.7 ± 0.6; Figure 15B), but there were only small changes in p38 MAPK abundance (17 %; ES 0.3 ± 0.3) and phosphorylation (19 %; ES 0.3 ± 0.3) immediately after exercise compared to rest. Training resulted in a small change in resting AMPKα protein abundance (18 %; ES 0.6 ± 0.8; $p = 0.027$ main effect), and moderate changes in AMPKα phosphorylation (38 %; ES 0.7 ± 0.4; $p<0.05$), and ACC abundance by (46 %; ES 0.8 ± 0.8). After training, acute RSE increased ACC phosphorylation by 83 % (ES 1.4 ± 0.8; $p < 0.001$).

Table 9. Skeletal muscle protein abundance and phosphorylation in response to acute repeated-sprint exercise before and after four weeks of repeated-sprint training (3 times per week).

	Pre-training			**Post training**		
Protein	**Rest**	**End**	**1 h**	**Rest**	**End**	**1 h**
AMPKα	3.4±0.9	3.6±1.3	4.0±1.8	4.1±1.2 [b]	4.4±1.6	4.5±1.8
p-AMPKα	7.1±3.9	8.4±2.9	9.4±3.2 [a]	9.8±4.0 [b]	9.6±5.4	8.4±4.2
p-AMPKα/ AMPKα	2.5±1.8	2.7±1.0	2.9±1.0	3.0±1.7	2.6±1.2	2.2±1.0
ACC	15.6±8.3	23.6±11.1 [a]	22.7±9.4 [a]	22.8±9.1[b]	24.5±11.2	20.1±9.9
p-ACC/ACC	8.7±7.5	8.5±5.4	5.7±3.6	5.1±2.7	9.3±6.2	6.0±3.7
p38 MAPK	5.1±3.1	5.9±3.3	5.6±2.7	5.2±3.2	5.3±3.1	4.6±2.3
p-p38 MAPK	6.9±6.0	8.2±5.0	7.5±4.1	6.4±2.8	8.4±7.3	7.3±4.5
p-p38/p38	1.5±0.7	1.5±0.7	1.6±0.7	1.6±0.8	2.0±1.2	1.9±1.1
Pgc-1α	3.5±1.8	3.8±2.1	3.4±1.7	4.6±1.7	4.2±1.5	3.2±2.3
p-HDAC5	5.3±4.2	10.2±10.9 [a]	9.0±9.2	4.5±2.9	5.5±5.9	4.8±4.3
RIP140	11.0±4.2	11.2±5.0	9.9±4.4	11.4±6.4	10.4±4.3	8.9±4.4
COX IV	9.1±3.5	9.0±2.9	9.5±3.8	10.4±4.3	9.2±3.7	7.1±2.8
GAPDH	9.6±2.3	9.7±2.6	9.6±2.1	9.5±2.2	9.1±2.3	9.5±2.3

Data are presented as mean ± SD and expressed as arbitrary units normalised for GAPDH; n = 10 except for ACC (n = 9, unable to detect band for one individual). [a], moderate effect size for acute RSE-induced changes in protein abundance and phosphorylation compared to rest. [b], moderate effect size for training-induced changes in resting protein abundance and phosphorylation.

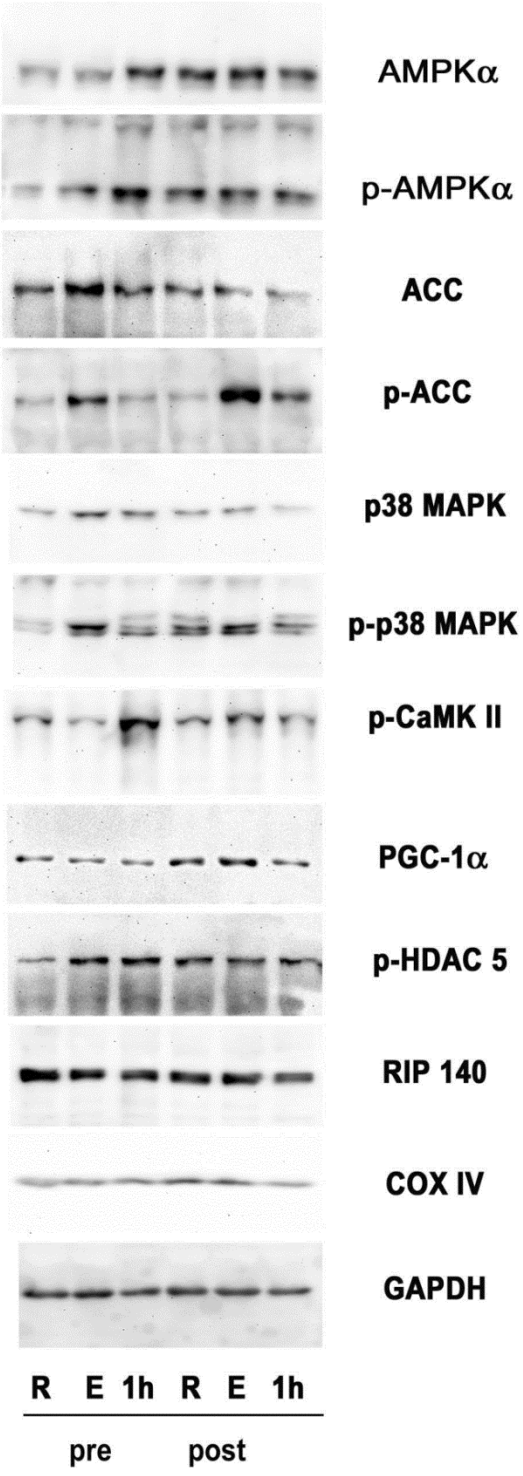

Figure 14; Representative western blot images for one individual.

R, rest; E, end of exercise; 1h, 1 hour post-exercise. Pre, pre-training; post, post-training.

4.3.3 PGC-1α, phospho-HDAC5, RIP 140

Before training, acute RSE increased PGC-1α mRNA expression by 208 % at 4 h after exercise (ES 1.5 ± 0.7; p = 0.002; Figure 16A). There was a small change in the phosphorylation of HDAC5 immediately after exercise compared to rest (92 %; ES 0.6 ± 0.8), but no change in RIP 140 abundance (-10 %; ES -0.2 ± 0.6). Four weeks of repeated-sprint training induced a 33 % increase in PGC-1α protein abundance at rest (ES 0.9 ± 0.7; Figure 16B). After training, acute RSE resulted in an increase of PGC-1α mRNA expression at 4 h after exercise (ES 1.4 ± 1.3) and a small change in phospho-HDAC5 immediately after exercise, both compared to rest.

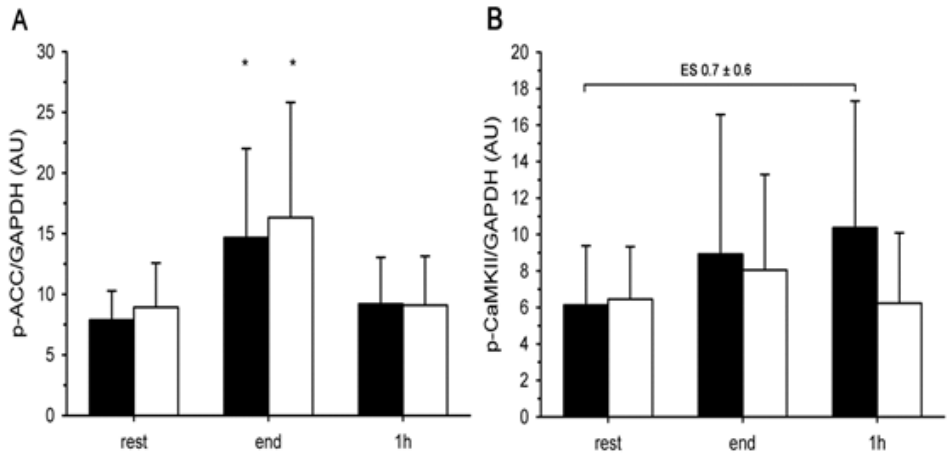

Figure 15; Skeletal muscle ACC (A) and CaMKII (B) protein phosphorylation in response to acute repeated-sprint exercise before (*filled bars*) and after (*open bars*) four weeks of repeated-sprint training.

*, significantly greater compared to rest ($p < 0.05$); ES, effect size statistic ± 90 % confidence interval. Data are presented as mean ± SD; n = 9 for p-ACC, n = 10 for p-CaMKII.

4.3.4 Transcription factors

As a result of acute RSE, the mRNA expression at 4 h after exercise and compared to rest was moderately changed for NRF1 (92 %; ES 0.7 ± 0.8) and MEF2a (202 %; ES 1.0 ± 1.3). Training produced a trivial change in the resting mRNA expression of NRF1 (33 %; ES 0.1 ± 0.6), mtTFA (18 %; ES 0.1 ± 1.0), and MEF2a (16 %; ES 0.1 ± 0.9). After training, acute RSE resulted in a moderate change of mRNA expression at 4 h after exercise compared to rest for NRF1 (105 %; ES 0.9 ± 1.5) and mtTFA (34 %; ES 0.7 ± 1.0).

4.3.5 COX IV

Acute RSE induced a trivial change in COX IV mRNA expression at 4 h after exercise compared to rest (25 %, ES 0.1 ± 0.8), and no change in the protein abundance. Training resulted in a small change in protein abundance at rest (14 %, ES 0.3 ± 0.7). Following the acute RSE after training, COX IV mRNA expression was moderately changed at 4 h after exercise compared to rest (74 %, ES 0.8 ± 1.5).

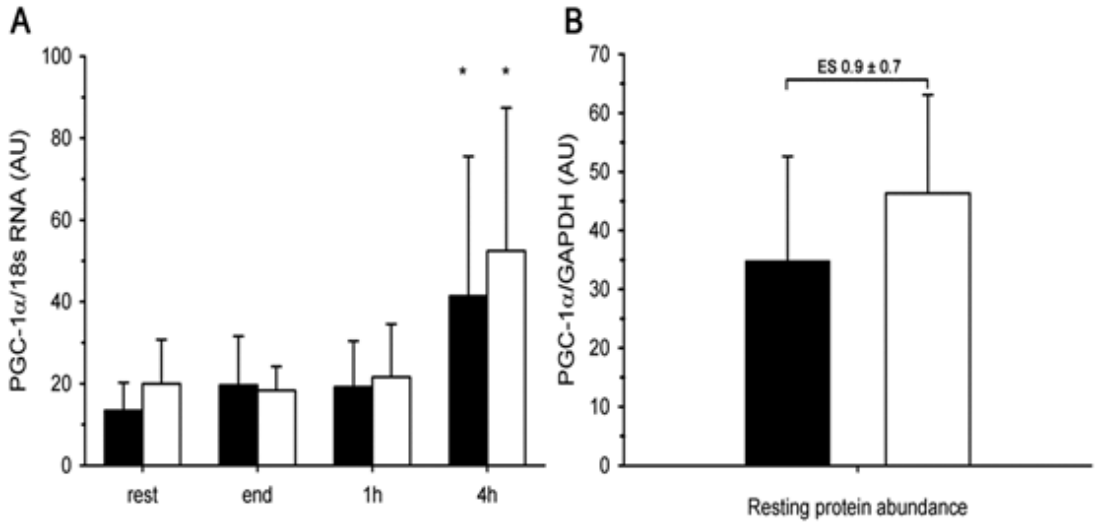

Figure 16; Skeletal muscle PGC-1α gene expression in response to acute repeated-sprint exercise (A) and resting protein abundance (B), before (*filled bars*) and after training (*open bars*).

*, significantly greater compared to rest ($p < 0.05$); ES, effect size statistic ± 90 % confidence interval. Data are presented as mean ± SD, n = 10.

4.4 Discussion

There were three main findings in this study: a) acute RSE produced a large increase in ACC protein phosphorylation, and a moderate increase of CaMKII protein phosphorylation; b) acute RSE induced a large increase in PGC-1α mRNA expression; c) four weeks of training induced a moderate change in resting PGC-1α protein abundance.

4.4.1 Acute RSE activates AMPKα and CaMKII signalling pathways

The effects of RSE on the main molecular pathways associated with mitochondrial biogenesis are presented here for the first time. As hypothesised, despite the extremely-low exercise volume (only 60 s), the repetition of maximal-intensity efforts and short recovery periods achieved during RSE activated AMPKα and CaMKII protein signalling. This suggests that intensity plays an important role in the exercise-induced adaptations associated with mitochondrial biogenesis. In support of this, 10 min of exercise at 50 % $\dot{V}O_{2\text{peak}}$ followed by 3.5 min at 100 of $\dot{V}O_{2\text{peak}}$ increased CaMKII autonomous activity to a similar extent to that measured in response to 40 min of exercise at 76 % $\dot{V}O_{2\text{peak}}$, despite the volume of the latter being more than three times greater (Rose and Hargreaves, 2003). However, maximal-intensity exercise does not seem to influence all signalling proteins in the same manner. In a recent study, the effect of 4 x 30-s, "all-out" exercise bouts on protein signalling was investigated (Gibala *et al.*, 2009). The authors detected a similar increase in the phosphorylation of AMPK compared to this study, but approximately 6-fold greater phosphorylation of ACC. As AMPK is mainly triggered by a change in the metabolic status in skeletal muscle cell, it

is of interest to consider the differences in ATP depletion following a 6 x 4-s repeated-sprint exercise (Spencer *et al.*, 2008), and one bout of "all-out", 30-s exercise (Gibala *et al.*, 2009). Both exercises had similar duration and peak power, and both were performed on air-braked cycle ergometers. The two protocols show a similar reduction in muscle ATP content (-16 and -20 %, respectively) compared to rest. This might explain the similar increase in AMPKα protein phosphorylation in this thesis and the one by Gibala and colleagues (Gibala *et al.*, 2009). However, the marked difference in ACC protein phosphorylation remains undetermined. It has been shown that contraction-induced ACC phosphorylation is AMPK independent in a model of transgenic mice expressing a kinase-dead AMPK (Dzamko *et al.*, 2008). The results of this study suggest that the exercise characteristics, such as intensity, duration, and volume, might be differently involved in the regulation of signalling proteins within this pathway.

4.4.2 Acute RSE increases PGC-1α mRNA expression

Together with the activation of the AMPKα and CaMKII protein signalling, acute RSE was associated with a 200 % increase in PGC-1α mRNA expression 4 h after exercise compared to rest. A similar increase in PGC-1α mRNA expression has been shown in response to other low-volume exercise methods, such as combined intermittent-sprint and resistance exercise (Coffey *et al.*, 2009), and repeated 30-s, "all-out" efforts (Gibala *et al.*, 2009). The study by Gibala and colleagues also reported, similarly to this study, an unchanged PGC-1α protein abundance 3 h after exercise. These results might depend on the time course of PGC-1α protein synthesis. In fact, PGC-1α protein abundance was elevated 24 h, but not 4 h after an exercise comprising of 10 x 4-min cycling at 90

% $\dot{V}O_{2peak}$ (Perry *et al.*, 2010). The absence of an increase in PGC-1α protein abundance in the present study does not signify an interruption in the sequence of molecular events. In fact, 4 x 30-s "all-out" efforts increased the nuclear abundance, but not the whole-muscle abundance of PGC-1α protein at 3 h after exercise (Little *et al.*, 2011), suggesting an important role for the protein localisation. Also, other types of post-translational modification of PGC-1α protein, such as phosphorylation (Jager *et al.*, 2007) and deacetylation (Cantó *et al.*, 2010), might be of more importance in the short-term adaptation to exercise rather than the simple increase in abundance. It is acknowledged that the specificity of the PGC-1α antibody represents an area of current discussion, and the use of different commercially-available antibodies might partly explain the differences in the results obtained from different research groups. In the present study, a band of a molecular weight of approximately 100 kDa was detected.

This study also showed for the first time an increased phosphorylation of HDAC 5 protein in response to RSE. At rest, transcription is inhibited by the association of HDAC 5 with MEF2 transcription factor (Lu *et al.*, 2000). During exercise, AMPK and CaMKII phosphorylate HDAC 5 causing its export from the nucleus, ultimately allowing gene transcription to occur (McGee and Hargreaves, 2011). Sixty minutes of stimulation with 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside induced an approximate 65 % increase of HDAC 5 phosphorylation at Ser^{259} in human primary myotubes (McGee *et al.*, 2008). The fact that the magnitude of the change reported in that study is similar to the change observed in this thesis suggests that the intensity of exercise might be more important than its duration or volume. This is supported by the observation that 36 min of cycling at 80 % $\dot{V}O_{2peak}$ induced a phosphorylation of HDAC 4/5/7 that was almost double that following a 70-min isocaloric exercise

performed at 40 % $\dot{V}O_{2peak}$ (Egan *et al.*, 2010). Conversely, no increase in the abundance of RIP 140 protein was found after acute or chronic RSE. This is of interest considering that research showed a 7-fold increase in the abundance of RIP 140 in response to 1 h of exercise at 70 % $\dot{V}O_{2peak}$ (unpublished data). Further research is required for a comprehensive understanding of the role of repressors in the exercise-induced molecular adaptations, with particular focus on exercise intensity and volume.

4.4.3 The effects of repeated-sprint training

One of the findings of this study is that short-term repeated-sprint training resulted in a moderate increase in PGC-1α resting protein abundance. The training protocol employed in this research comprised only 12 min of repeated-sprint exercise, probably representing an unprecedented low training volume in an experimental study. It is of interest to compare the results of this study with those from similar exercise protocols. Other intermittent training interventions, all with exercise volume higher than the protocol employed here, returned discordant results. On one hand, two weeks (six sessions) of training comprising of 8-12 x 60-s cycling at 100 % of the peak power output did not change PGC-1α protein abundance at rest (Little *et al.*, 2010), while eighteen sessions of 10 x 4-min exercise at 90 % $\dot{V}O_{2peak}$ produced only a 16 % increase (Gurd *et al.*, 2010). On the other hand, six weeks (18 sessions) of 4-6 x 30-s, "all-out" efforts resulted in approximately 100 % increase in PGC-1α abundance, an adaptation comparable to that induced by endurance training with 10 times higher volume (Burgomaster *et al.*, 2008). This suggests that the mechanisms of molecular adaptations to repeated-sprint training, in particular those regarding the role of PGC-1α, might be more complex than a simple dose-response concept. Supportive to this is

the analysis of the molecular adaptations induced by acute RSE post-training compared to pre-training. Despite the change in the abundance/phosphorylation of almost all proteins considered was blunted in response to acute exercise after training, the magnitude of PGC-1α mRNA expression was fully preserved. This might signify that only small changes in signalling are required to maintain PGC-1α mRNA gene expression in a trained muscle.

4.5 Conclusions

This study shows for the first time the effects of acute and chronic repeated-sprint exercise, with short maximal sprints and brief recovery, on the molecular events associated with mitochondrial biogenesis. As hypothesised, acute RSE was capable of altering the signalling pathways dependent on the metabolic state and the Ca^{2+} signalling in skeletal muscle, in turn increasing PGC-1α mRNA expression. The fact that such a low-volume intervention is capable of altering the signalling pathways and the PGC-1α mRNA expression in skeletal muscle is, without a doubt, of interest. Possibly as a result of the repeated acute effect of exercise, a moderate increase in PGC-1α protein abundance was detected after training. However, the changes in PGC-1α mRNA expression and protein abundance do not appear to be sufficient to promote beneficial adaptations in the systemic aerobic capacity, as highlighted by the absence of changes in $\dot{V}O_{2\text{peak}}$ and LT/OBLA.

Despite these changes described above, the trivial-to-small changes of many genes and proteins observed in this study, either before or after the training intervention, are surprising. On one hand, the small changes in the expression of the transcription factor

genes following acute RSE and in the abundance of COX IV protein after training are in contrast with the hypothesis that RSE would produce an ideal stimulus for mitochondrial biogenesis. However, the observation of a greater COX IV mRNA expression following acute RSE post-training compared to pre-training, together with a preserved increase in PGC-1α mRNA expression, is encouraging to extend on research regarding the effects of low-volume, high-intensity physical activity on muscle adaptations associated with mitochondrial adaptations. In particular, further investigation is required to understand whether a RSE intervention alone, perhaps with a greater training volume, might be sufficient to induce mitochondrial biogenesis in skeletal muscle, or whether RSE can be more effective in combination with other physical activity regimes, to even better mimic the activity patterns of team sports.

CHAPTER 5 – A GAME OF FUTSAL AND WORK-MATCHED, MODERATE INTENSITY CONTINUOUS EXERCISE INDUCE SIMILAR CHANGES IN SKELETAL MUSCLE SIGNALLING

5.1 Introduction

Exercise training is used as one of the primary lifestyle modifications adopted in an attempt to reduce of the risk factors associated with type 2 diabetes (Shaw *et al.*, 2006; Thomas *et al.*, 2006b). Skeletal muscle is one of the largest tissues contributing to metabolism and glycaemic control. As a consequence, research has focused on the effects of exercise on skeletal muscle adaptations which may contribute to the reduction of risk factors for diabetes (Eriksson and Lindgarde, 1991; Lindstrom *et al.*, 2003). Particular attention has been directed towards understanding those molecular adaptations that are believed to be fundamental in the prevention of this chronic disease. The molecular mechanisms involved in mitochondrial biogenesis and in exercise- or insulin-mediated glucose transport have received considerable interest. Diabetic individuals present impaired mitochondrial function and a downregulation of key markers of mitochondrial biogenesis such as PGC-1α in skeletal muscle (Mensink *et al.*, 2007). Similarly, offspring of diabetic individuals showed altered mitochondrial function and elevated IRS-1 phosphorylation (Befroy *et al.*, 2007; Morino *et al.*, 2005). These observations led researchers to investigate whether impaired mitochondrial function/biogenesis could be implicated in the etiopathogenesis of diabetes (Patti and Corvera, 2010). To date, results about a cause/effect relationship between these physiological adaptations are inconclusive (Garcia-Roves, 2011).

Despite this, it is interesting that both mitochondrial biogenesis and glucose metabolism share several key signalling proteins in their molecular pathways. For example, the activation of AMPK induces at the same time an up-regulation of mitochondrial transcription factors via the activation of PGC-1α, an increased mRNA transcription of GLUT4, and IRS-1 phosphorylation (Jager *et al.*, 2007; Jakobsen *et al.*, 2001; McGee and Hargreaves, 2011). Similarly, the activation of CaMKII induces an increase in PGC-1α mRNA expression and a parallel increase in GLUT4 mRNA expression via the HDAC5-MEF2A mechanism described in section 2.2.2 (Mukwevho *et al.*, 2008; Wu *et al.*, 2002).

All the above signalling proteins are highly sensitive to acute and chronic exercise (Coffey and Hawley, 2007). Traditionally, research investigating skeletal muscle molecular adaptations in response to exercise has employed continuous aerobic exercise as the experimental model. Latterly, increased focus was directed toward low-volume, high-intensity protocols, in an attempt to establish whether exercise intensity plays an important role in such adaptations (Gibala *et al.*, 2009). However, all of these laboratory-based exercise models are not applicable to "real-life" conditions, as they are likely to be poorly motivating or too exhausting for the general population.

Indoor football (futsal) may be an appropriate alternative to study the effect of real-life physical activity on the molecular adaptations involved in mitochondrial biogenesis and glucose metabolism. The rationale behind this idea is based on two different considerations. Firstly, football is the sport with the highest participation rate worldwide, therefore research involving football has a broad practical application.

Secondly, it has been demonstrated that the repetition of high-intensity exercise efforts is a more efficient physiological stimulus to continuous exercise to induce adaptations in skeletal muscle (Burgomaster *et al.*, 2008).

Therefore, this study tested the hypothesis that an acute session of futsal would induce signalling responses equal to, or greater than a work-matched continuous running exercise in young, healthy men.

5.2 Methods

5.2.1 Participants

Sixteen young, healthy men took part in this study, which was conducted as a randomised parallel-group, pre-post design. The study was approved by the Ethics Committee of the Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona. Participants' physical characteristics were (Mean ± SD): age 21.4 ± 1.7 years, height 179.2 ± 6.0 cm, body mass 74.8 ± 5.9 kg, and $\dot{V}O_{2\text{peak}}$ 52.0 ± 6.3 mL kg^{-1} min^{-1} (FUT: range 37.5 – 59.5; MOD: range 44.9 – 60.3 mL kg^{-1} min^{-1}). All participants had previous futsal experience.

5.2.2 Experimental overview

For the baseline testing, participants visited the laboratory on two different occasions. During the first visit, participants performed an incremental test to exhaustion on a treadmill. At least 48 h after, participants returned to the laboratory to undergo a resting muscle biopsy. Before each test, participants were asked to refrain from alcohol and

caffeine intake for the 48 h preceding the visit, and to avoid physical exercise. One week after the completion of baseline testing, participants were randomly allocated to either a futsal group (FUT) or a moderate-intensity running group (MOD). The FUT group performed a single futsal game, while MOD performed a work-matched running exercise on a treadmill. Immediately after the acute exercise, another muscle biopsy was taken. Participants were asked to accurately report the three meals preceding the resting biopsy and to exactly replicate the same diet before the main exercise trial.

5.2.3 Incremental exercise test

The test was performed on a motorised treadmill (RUNRACE^{TM}, Technogym, Gambettola, Italy) and consisted of an initial 3-min stage at 8 km h^{-1}, with the intensity thereafter increased by 1 km h^{-1} every minute until exhaustion, defined as the subject's inability to maintain the required intensity. During the test, expired gases were analysed breath-by-breath using a commercially-available metabolic cart (Quark PFT, COSMED srl, Rome, Italy). Peak oxygen uptake was calculated as the average of the two highest $\dot{V}O_2$ values in two consecutive 10-s periods. Ventilatory threshold 1 (VT1) was calculated as an increase in the ventilatory equivalent for oxygen ($\dot{V}E/\dot{V}O_2$) without a concurrent increase in the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$) (Caiozzo *et al.*, 1982). During the test, participants wore a triaxial accelerometer (X6-2; sampling rate 160 Hz, ± 6 g, 16-bit; Gulf Coast Data Concepts, USA) placed in a dedicated vest between the scapulae. The changes in acceleration on the three anatomical axes were used to calculate the work corresponding to each speed of the incremental test, using the following vector magnitude equation:

$$work\ rate\ (AU) = \frac{\sqrt{((x1 - x0)^2 + (y1 - y0)^2 + (z1 - z0)^2)}}{100}$$

Where x represents vertical accelerations, y represents lateral accelerations, z represents frontal accelerations, and 100 represent a scaling factor.

5.2.4 Exercise trials

One week after the completion of baseline testing, participants performed the main exercise trial according to their group allocation. The FUT group performed a regular futsal game consisting of a standardised 5-min warm-up, immediately followed by two 20-min halves with 5 min of rest in between. Due to the inability to perform multiple post-exercise biopsies at the same time, participants commenced and finished the game with a 10-min time delay between them, with the missing players being temporarily substituted by a corresponding number of individuals who did not participate in the study. On a different occasion, the MOD group performed a continuous exercise on a treadmill performed at an intensity corresponding to their individual VT1. The duration of each individual's exercise was calculated based on the total work performed during FUT and matched according individual VT1 work. Work matching between groups was achieved by the use of triaxial accelerometers. This method is widely used in research to quantify physical activity, and an acceptable validity has been found on the comparison of work measured during treadmill- and ground-based physical activity (Hendrick *et al.*, 2010). During the FUT game participants wore an accelerometer, and the work for each game (including warm-up) was recorded. The duration of exercise required for MOD to match the work performed during the FUT games was then calculated by dividing the average FUT work by the work corresponding to 1 min of exercise at VT1. For example, a work of 600 units during the FUT game corresponded to 30 min of treadmill exercise for a MOD participant with a work at VT1 of 20 units per minute. The average

duration of the MOD trial was 23.1 ± 1.9 min. For a more detailed explanation of the procedures followed to achieve work matching see Appendix 10.

5.2.5 Muscle biopsy

A muscle biopsy was performed at rest and immediately after exercise on the vastus lateralis muscle of the participants' dominant leg. Briefly, after injection of a local anaesthetic (lidocaine hydrochloride 2 %, Monico SpA, Venezia, Italy) a muscle sample was collected using a semi-automatic biopsy needle (Vantage 13G, ZAMAR srl, Suzzara, Italy). After collection, the samples (~ 60 mg) were immediately blotted on a filter paper to remove excess blood and quickly frozen in liquid N_2 before being stored at -80 °C for subsequent analysis.

5.2.6 Immunoblotting

Representative western blotting images are presented in Figure 17. Approximately 50 mg of frozen muscle samples were homogenised in ice-cold buffer containing 20 mM Tris-HCl pH 7.4, 10mM EDTA, 1mM EGTA, 137 mM NaCl, 1 mM $Na_4O_7P_2$, 20 mM NaF, 1 % NP-40, 10 % Glycerol, 1 mM Na_3VO_4, 4 μg mL^{-1} Leupeptin, 4 μg mL^{-1} Aprotinin, 4 μg mL^{-1} Pepstatin, 1 mM PMSF. Samples were homogenised (1:15 dilution (wt/vol)) for 2 x 10 s with a 5-min break on ice, using a tissue homogeniser (Bead Ruptor 24, Omni International, Kennesaw, GA, USA) and single-use homogenising mix tubes (Omni International, # 19-628). Homogenates were then rotated for 60 min at 4 °C, centrifuged at 13000 rpm for 15 min at 5 °C, and protein concentration of the resulting supernatant was determined using a BCA Protein Assay kit (Pierce, ThermoFisher Scientific, Albany, New Zealand). Samples were then diluted

to a standard concentration of 5 μg mL^{-1} for analysis. Lysates aliquots were mixed with Laemmli buffer and proteins separated via 8 % SDS polyacrylamide gel electrophoresis at 120 V in a standard electrophoresis unit (Mini Trans-Blot® Cell, Bio-Rad Laboratories, Hercules, CA, USA). Following electrophoresis, proteins were transferred to PVDF membranes (Millipore, Billerica, MS, USA) for 10 min at 25 V (2.5 A) using a semi-dry blotter (Trans-Blot® Turbo transfer cell, Bio-Rad). Membranes were blocked in TBST buffer containing 5 % BSA with 0.1 % Tween 20, for 1 h at room temperature. After being washed, membranes were incubated overnight at 4 °C with primary antibodies for: ATF2 (Cell Signaling Technology, Danvers, MA, USA, # 9226), phospho-ATF2 Thr^{71} (Cell Signaling, # 5112), phospho-ACC Ser^{79} (Cell Signaling, # 3662), AMPKα (Cell Signaling, # 2603), phospho-AMPKα Thr^{172} (Cell Signaling, # 2535), CaMKII (Santa Cruz Biotechnology, Santa Cruz, CA, USA, # SC-13082), phospho-CaMK II Thr^{286} (Cell Signaling, # 3361), p38 MAPK (Cell Signaling, # 9212), phospho-p38 MAPK Thr^{180}/Tyr^{182} (Cell Signaling, # 9211), phospho-HDAC4/5/7 (Cell Signaling # 3443). All membranes were normalised for loading with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Abcam, Sapphire Biosciences, Waterloo, NSW, Australia, ab8245). A dilution of 1:1000 was used for most antibodies, with the exception of CaMKII (1:200) and GAPDH (1:10000). Primary antibodies were diluted 1:1000 in TBST containing 5 % BSA. Following incubation with the primary antibodies, membranes were incubated with polyclonal goat anti-rabbit or anti-mouse horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, Glostrup, Denmark, # PO448), for 1 h at room temperature (1:10,000 in TBST with 5 % non-fat milk). Immunoreactivity was detected using a chemiluminescence reagent (ECL Advance western blotting detection kit, GE Healthcare, Pittsburgh, PA, USA, #

RPN2135), and quantified by densitometric scanning (LAS-4000 Luminescent image analyser, Fujifilm, Tokyo, Japan).

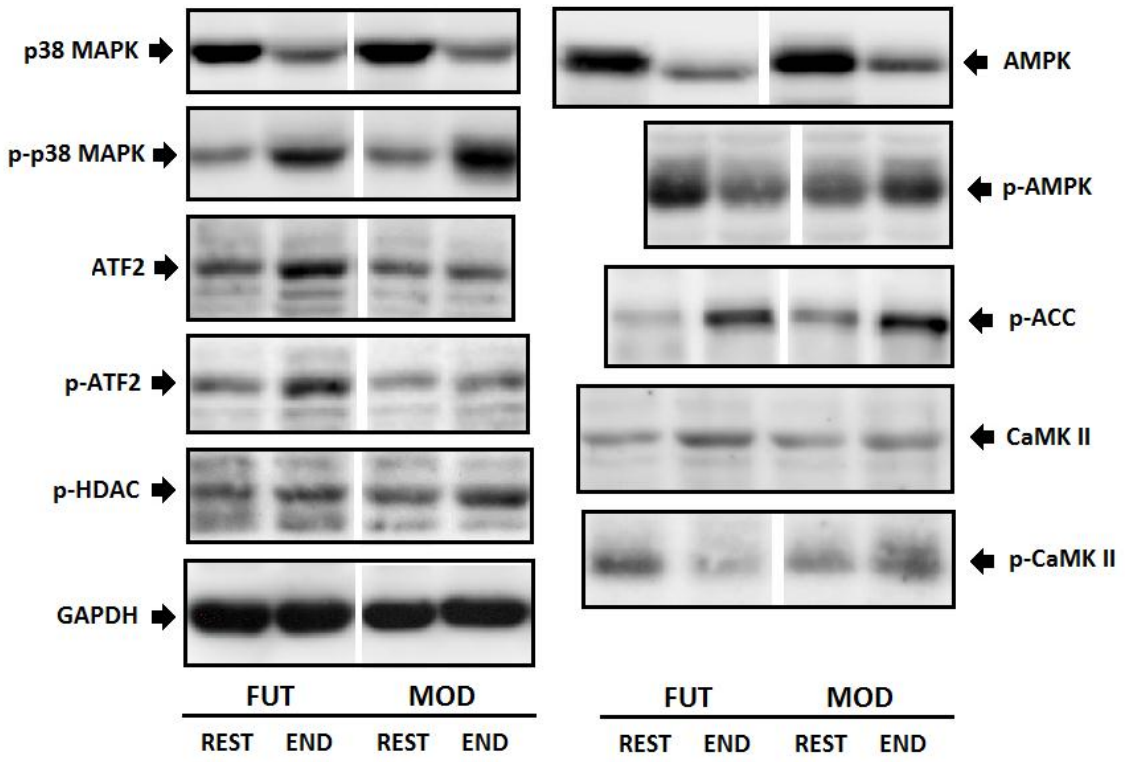

Figure 17; Representative western blot images for one individual.

FUT, futsal game; MOD, moderate-intensity continuous exercise

5.2.7 Statistical analysis

Data are presented as mean ± SD. Results were tested for normal distribution using a Shapiro-Wilk W test and, when the assumption of normality was not met, data were log transformed to reduce bias due to the non-uniformity of error. The magnitude of the changes was assessed using effect size (ES) statistic with 90 % confidence intervals, and percentage changes. ES were defined as a follows: smaller than 0.2 = trivial, 0.2 to 0.6 =

small, 0.6 to 1.2 = moderate, 1.2 to 2.0 = large, larger than 2.0 = very large (Hopkins, 2003 (Access Date 24-12-2011)). The effects were defined as substantially meaningful when there was ≥ 75 % of chance of the true value of the statistic being practically positive (Batterham and Hopkins, 2006). Student's independent t test and two-way ANOVA with Repeated Measures were used to assess baseline and group x training differences, respectively.

5.3 Results

5.3.1 Skeletal muscle protein signalling

An acute FUT game was associated with a 50 % increase in ATF2 phosphorylation ($p = 0.001$, Figure 18), while no change was detected in response to MOD (time x training interaction, $p = 0.02$). Both FUT and MOD increased ACC phosphorylation by 119 and 75 %, respectively (main effect for time, $p = 0.01$, Figure 19). Similarly, both training interventions increased p38 MAPK phosphorylation by 85 and 36 % for FUT and MOD, respectively (main effect for time, $p = 0.003$). A summary of the changes in protein abundance and phosphorylation in response to FUT and MOD is presented in Table 10.

Table 10. Skeletal muscle protein abundance and phosphorylation following an acute futsal game and work-matched continuous exercise

	FUT			MOD		
Protein	**Pre**	**End**	**ES ± CI**	**Pre**	**End**	**ES ± CI**
AMPKα	1.2 ± 0.5	1.0 ± 0.4	-0.33 ± 0.66	1.0 ± 0.4	0.8 ± 0.3	-0.39 ± 0.57
p-AMPKα	1.0 ± 0.5	0.9 ± 0.3	-0.17 ± 0.58	1.3 ± 0.5	1.5 ± 0.6	0.24 ± 0.61
p-AMPKα/AMPKα	1.1 ± 0.7	1.1 ± 0.5	0.14 ± 0.60	1.1 ± 0.2	2.2 ± 1.4	2.01 ± 2.23
p-ACC	6.1 ± 6.2	13.4 ± 6.9	1.07 ± 0.56 [a]	9.8 ± 9.0	17.2 ± 18.6	0.46 ± 0.48 [a]
CaMKII	1.7 ± 1.2	1.6 ± 0.9	-0.09 ± 0.44	1.1 ± 0.4	1.3 ± 0.8	0.10 ± 0.27
p-CaMKII	2.5 ± 2.7	1.9 ± 1.3	-0.16 ± 0.37	2.5 ± 1.7	2.9 ± 3.0	0.03 ± 0.45
p-CaMKII/CaMKII	1.7 ± 2.0	1.3 ± 0.7	-0.10 ± 0.41	2.8 ± 2.0	3.6 ± 3.8	0.06 ± 0.63
p38 MAPK	1.8 ± 0.5	1.4 ± 0.6	-0.91 ± 0.70 [a]	1.9 ± 0.7	1.4 ± 0.6	-0.69 ± 0.57 [a]
p-p38 MAPK	8.6 ± 4.0	15.9 ± 8.8	0.77 ± 0.51 [a]	10.1 ± 2.1	13.7 ± 2.7	1.16 ± 1.14 [a]
p-p38/p38 MAPK	4.5 ± 2.1	14.4 ± 13.0	1.40 ± 0.78 [a]	6.4 ± 3.4	12.2 ± 6.2	1.10 ± 0.79 [a]
ATF2	6.0 ± 2.6	11.1 ± 4.4 *	1.22 ± 0.59 [a,b]	8.0 ± 2.5	8.2 ± 2.9	0.04 ± 0.52
p-ATF2	1.0 ± 0.6	1.4 ± 0.8	0.59 ± 0.13 [a,b]	1.1 ± 0.7	1.1 ± 1.0	-0.04 ± 0.36
p-ATF2/ATF2	1.7 ± 0.8	1.5 ± 0.9	-0.25 ± 0.34	1.5 ± 0.9	1.4 ± 0.8	-0.06 ± 0.27
p-HDAC4/5/7	1.0 ± 0.5	1.2 ± 0.5	0.18 ± 0.46	1.3 ± 0.7	1.3 ± 0.3	0.14 ± 0.48
GAPDH	1.1 ± 0.1	1.1 ± 0.1	0.18 ± 0.51	1.0 ± 0.2	1.0 ± 0.1	0.20 ± 0.64

Data are presented as mean ± SD and expressed as arbitrary units normalised for GAPDH; n = 8. AMPKα, 5'AMP-activated protein kinase α; ACC, Acetyl-CoA carboxylase; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; p38 MAPK, p38 mitogen-activated protein kinase; ATF2, Activating transcription factor 2, HDAC, Histone deacetylase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase. FUT, futsal training group; MOD, moderate-intensity continuous training group; ES, effect size; CI, confidence interval; [a], substantially meaningful change compared to pre-training; [b], substantially meaningful change compared to MOD; *, significantly different between groups ($p < 0.05$).

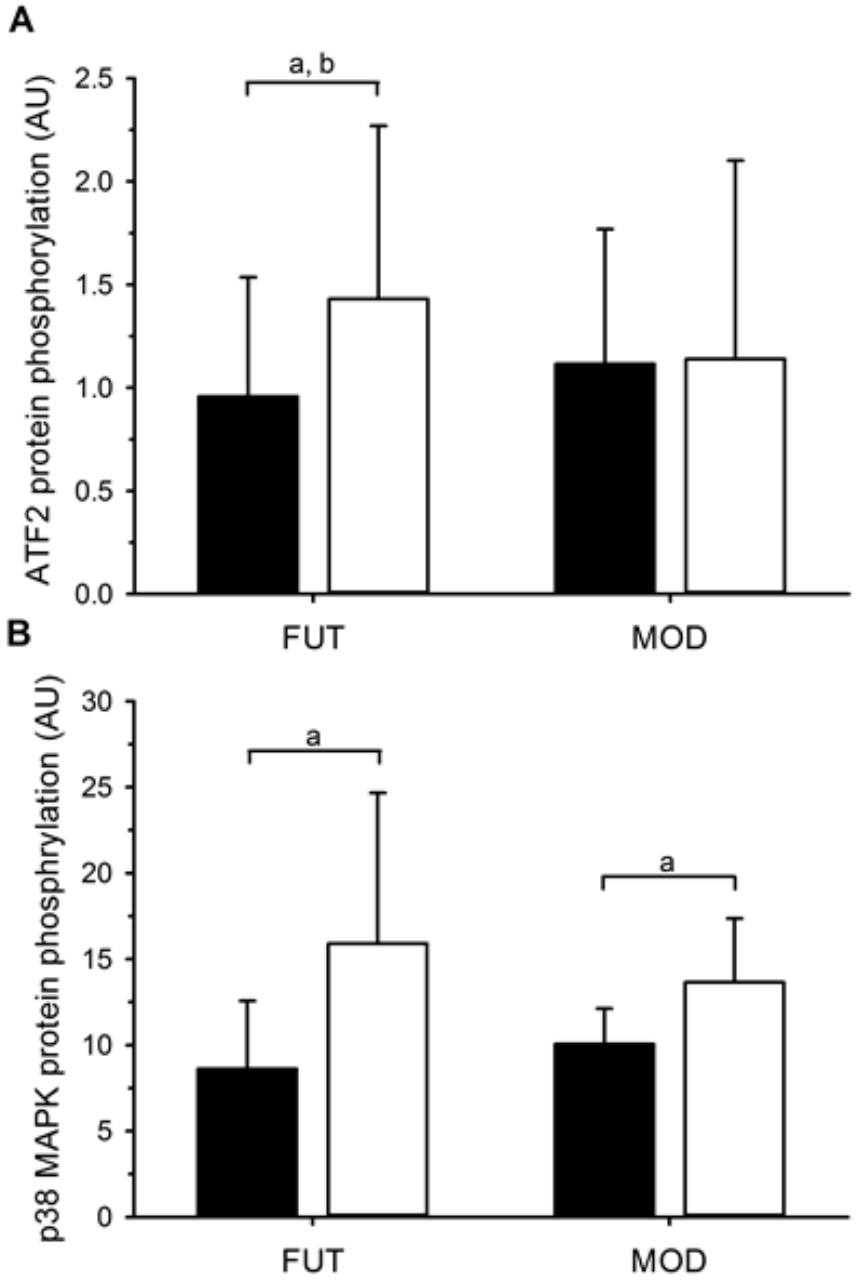

Figure 18; Skeletal muscle ATF2 (A) and p38 MAPK (B) protein phosphorylation at rest (*filled bars*) and immediately after (*open bars*) an acute game of futsal (FUT) and a work-matched running exercise performed at a moderate intensity (MOD).

a, meaningful change compared to rest; b, meaningful change compared to MOD. Data are presented as Mean ± SD; n = 8 for both groups

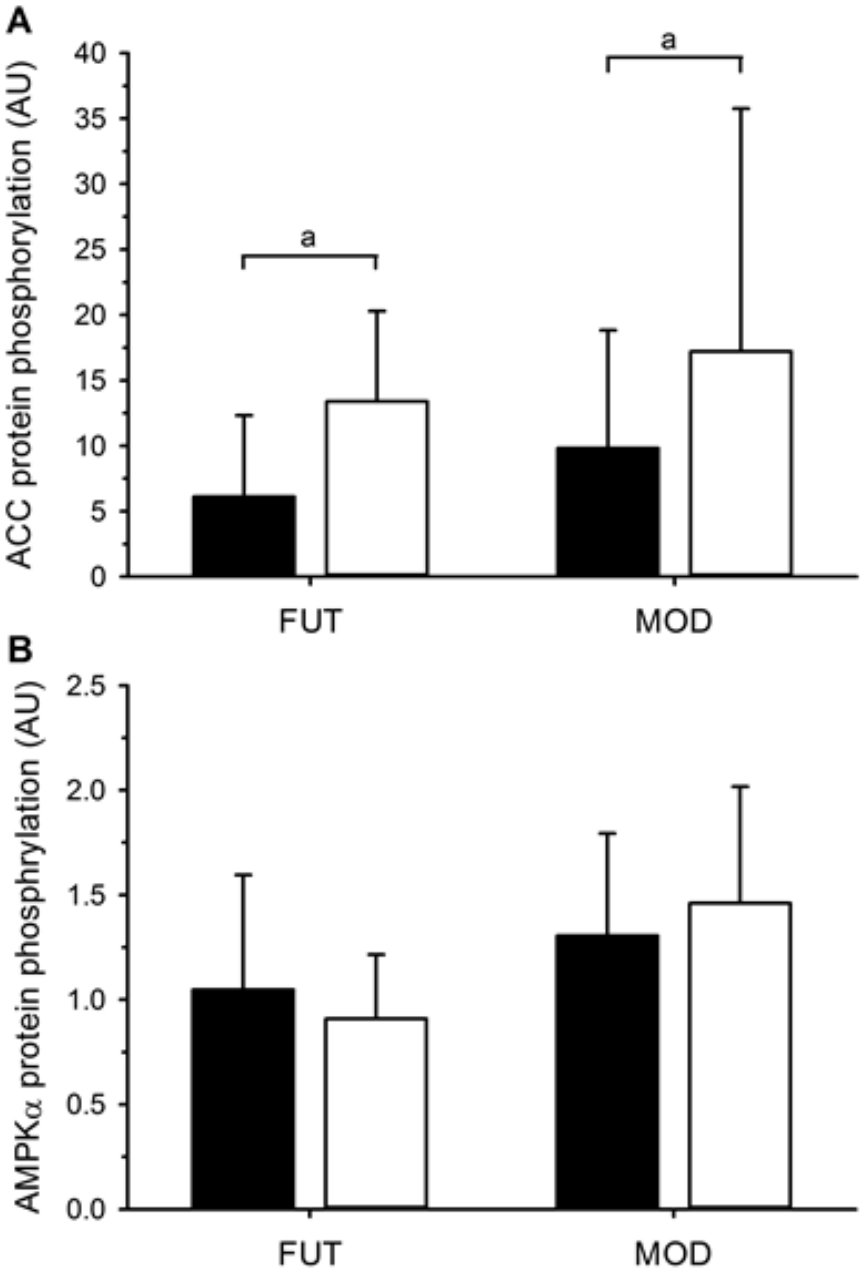

Figure 19; Skeletal muscle ACC (A) and AMPKα (B) protein phosphorylation at rest (*filled bars*) and immediately after (*open bars*) an acute game of futsal (FUT) and a work-matched running exercise performed at a moderate intensity (MOD).

a, meaningful change compared to rest; Data are presented as Mean ± SD; n = 8 for both groups.

5.4 Discussion

5.4.1 ATF2 phosphorylation is dependent on exercise intensity

One of the main results of this study was that ATF2 phosphorylation was increased in response to FUT, while no change was detected with MOD. This is interesting, considering that p38 MAPK, which is the direct upstream kinase of ATF2, was increased to the same extent in both FUT and MOD.

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The role of ATF2 in the regulation of gene expression in response to exercise has received little attention. It has been demonstrated that exercise-induced PGC-1α mRNA expression was increased via a mechanism involving p38 MAPK, ATF2, and the cAMP response element (CRE) (Akimoto *et al.*, 2005), which is the main transcription factor implicated in PGC-1α gene expression. In this proposed mechanism, upon contraction-induced phosphorylation of ATF2 by p38 MAPK, ATF2 binds to CRE which in turn increases PGC-1α mRNA expression. However, the results of this thesis suggest a differential response to work-matched intermittent or continuous exercise for ATF2 and its direct upstream regulator p38 MAPK. These results are consistent with previous research, showing that ATF2 phosphorylation was increased only in response to exercise performed at 80 % $\dot{V}O_{2\text{peak}}$ but not to an isocaloric exercise at 40 % $\dot{V}O_{2\text{peak}}$, despite an almost identical increase in p38 MAPK phosphorylation (Egan *et al.*, 2010). Therefore, there is emerging evidence pointing to the differential response of ATF2 and its upstream regulator p38 MAPK in skeletal muscle. This suggests that ATF2 might be regulated by upstream kinases other than p38 MAPK. Despite mechanistic research on this topic being lacking in human skeletal muscle experiments, it has been shown that

ATF2 can be activated by signalling pathways involving the extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in human cancer cells (Lee *et al.*, 2010). Therefore, ATF2 should be the preferred target within the MAPK pathway to be measured when comparing different exercise characteristics on molecular responses in skeletal muscle, as ATF2 appears to be more sensitive to exercise intensity than p38 MAPK. Also, measurements of p38 MAPK and ATF2 should be integrated with ERK and JNK to better understand the importance of MAPKs in exercise-induced signalling.

5.4.2 Differential responses of ACC and AMPK to acute exercise

There was a significant increase in ACC phosphorylation in response to both FUT (119 %) and MOD (75 %). The magnitude of these increases was consistent with those measured in response to a single bout of repeated-sprint exercise (section 4.3.2). On one hand, this is supportive of the idea that futsal and RSE are similar types of exercise, as they both comprise repeated, short bouts of high-intensity exercise. On the other hand, however, it must be noted that the increase in ACC phosphorylation in response to FUT is only marginally larger than the increase with MOD. This is in contrast with the hypothesis that the AMPK pathway is sensitive to exercise intensity (Chen *et al.*, 2003). This discrepancy is more evident when ACC phosphorylation is compared to the activation of its direct upstream kinase, AMPK (Figure 19). Neither FUT nor MOD increased AMPK phosphorylation immediately after exercise. However, when considering the ratio between the abundance of the phosphorylated and total AMPK protein (i.e., phospho/total AMPK ratio) there was a detectable difference between the two groups, with the ratio being increased by approximately 100 % in MOD and no

change in FUT (Table 10). This signifies that continuous, moderate-intensity running exercise is more effective than futsal in inducing AMPK phosphorylation, but the two protocols are similar in promoting ACC phosphorylation. It has been previously discussed that the activation of ACC might be independent of AMPK (Dzamko *et al.*, 2008), this also being supported by the lack of chronological sequence in the phosphorylation of the two proteins, as highlighted by the results of this thesis (section 4.4.1). It is evident that further research is required to define the role of AMPK and ACC in skeletal muscle signalling. It is also evident that these two proteins are not optimal markers to differentiate high- and moderate-intensity exercise when matched by total volume.

5.4.3 Neither FUT nor MOD increase CaMKII phosphorylation

An interesting finding was that CaMKII total protein abundance and phosphorylation were not changed in response to either FUT or MOD. One of the concepts presented in the review of literature of this thesis was that the activation of CaMKII would be a main factor explaining the molecular adaptations induced by high-intensity intermittent exercise. This was supported by the highest $[Ca^{2+}]_i$ recorded in response to muscle fibre stimulation at higher frequencies (Table 4). It was demonstrated that the phosphorylation of both CaMKII and its downstream target phospholamban were increased after exercise performed at a higher, but not after a lower intensity (Egan *et al.*, 2010; Rose *et al.*, 2006). Also, the results of the study presented in Chapter 4 demonstrated that an acute exercise comprising only 60 s of sprinting was capable of increasing CaMKII phosphorylation by approximately 70 % (Figure 15B). Therefore, the results of the present study are surprising. A possible explanation might be found in

the observation that both CaMKII autonomous activity and phosphorylation at Thr^{287} were initially increased after 1 min of continuous exercise at 67 % $\dot{V}O_{2\text{peak}}$, then reduced by 100 to 200 % during the following 90 min (Rose *et al.*, 2006). However, this is in contrast with the results presented in Figure 15B showing a detectable increase in CaMKII phosphorylation 1 h after the conclusion of RSE. Therefore, it is not possible to conclude that the activation of CaMKII is dependent on exercise intensity when two different exercise protocols are matched by total work performed.

5.5 Conclusions

This study examined for the first time the effects of an acute game of futsal on skeletal muscle protein signalling in young healthy adults. The effects of futsal were compared to a work-matched exercise consisting of continuous running performed at an intensity corresponding to VT1. The results indicate that FUT was comparable to MOD with respect to inducing protein signalling in skeletal muscle. This has important practical applications, as futsal might be employed as an alternative to traditional aerobic training to promote muscle adaptations. This study also suggests that, amongst the different proteins analysed, the activating transcription factor 2 (ATF2) might represent a valid target to investigate the effects of acute exercise of different intensities on protein signalling in skeletal muscle.

CHAPTER 6 – FUTSAL TRAINING LOWERS RISK FACTORS ASSOCIATED WITH DIABETES IN MIDDLE-AGED MEN: THE "FIT-SAL" PROJECT

6.1 Introduction

The prevalence of diabetes mellitus worldwide is expected to rise by almost 50 % by 2030 compared to 2011 (Whiting *et al.*, 2011). Diabetes is associated with an increased economic burden directly on those individuals affected by the pathology (Barcelo *et al.*, 2003), and with negative psychological and social outcomes (Speight *et al.*, 2012), stemming from the direct physical symptoms. There is general consensus about the importance of undertaking preventive actions directed towards those population groups considered to be at high-risk, such as individuals with pre-diabetes, metabolic syndrome, or from a low socioeconomic background (Alberti *et al.*, 2007). Therefore, the scientific community has investigated the effects of lifestyle modifications specifically targeted to individuals with a high risk of developing diabetes. Amongst the different interventions investigated, exercise training has been thoroughly researched.

The majority of research into the benefits of exercise training to counter the occurrence of diabetes has employed continuous, aerobic exercise usually performed at a low or moderate intensity (Hays *et al.*, 2006; Hughes *et al.*, 1993; Hutchison *et al.*, 2012). However, motivation or long-term adherence issues may arise when these exercise models are applied to real-life situations, especially if aerobic training is performed individually (Tjønna *et al.*, 2008). For an activity to be inherently motivating it should be enjoyable, challenging, and include positive social interaction (Deci and Ryan,

1985). Research has identified intermittent exercise as a promising alternative for those otherwise not inclined towards conventional aerobic exercise training (Earnest, 2008). There is evidence that intermittent exercise training induces adaptations to reduce the risk of chronic disease (Little *et al.*, 2010; Tjønna *et al.*, 2008), and that it is more motivating than continuous exercise for participants (Tjønna *et al.*, 2008).

Indoor football (futsal) may be an excellent alternative to aerobic training. Futsal is a high-intensity intermittent activity, with approximately 70 % of a game being performed between 85 and 100 % of an individual's maximal heart rate (Castagna *et al.*, 2009). Therefore, futsal training may provide an optimal physiological stimulus to elicit health benefits. In support of this hypothesis, small-sided football training similar to futsal produced health benefits in a positive psychosocial environment in different groups of population (Bangsbo *et al.*, 2010; Knoepfli-Lenzin *et al.*, 2010; Randers *et al.*, 2010a). In addition, small-sided football games are perceived as more enjoyable and motivating than continuous aerobic exercise (Krustrup *et al.*, 2009).

The benefits of exercise for the prevention of diabetes have been traditionally associated with systemic adaptations, such as a reduction in fasting glycaemia and body fat, an improvement in the blood lipid profile and/or enhanced insulin sensitivity. However, a key role in the attainment of systemic benefits has been established for those adaptations occurring at a cellular level, in particular in skeletal muscle. Despite the underlying mechanisms still being debated, insulin-dependent glucose transport (Wang *et al.*, 2009) and mitochondrial function (Lowell and Shulman, 2005) have gained particular interest within exercise-related research. However, no study has investigated

the effects of futsal participation on these skeletal muscle adaptations associated with the reduction of diabetes risk factors in a high-risk population. Therefore, we compared 8 weeks of futsal versus moderate-intensity, continuous treadmill training on skeletal muscle markers of glucose metabolism and mitochondrial biogenesis in sedentary, middle-aged male individuals. It was hypothesized that futsal training would induce equal or greater skeletal muscle adaptations and insulin sensitivity compared to moderate-intensity, continuous exercise, matched by total work performed.

6.2 Methods

6.2.1 Participants

Twenty participants completed the study, which was conducted as a two-group, pre-post design. The study was approved by the Victoria University Human Research Ethics Committee and conformed to the Declaration of Helsinki. Participants' baseline physical characteristics are shown in Table 11.

Table 11. Baseline anthropometric, physiological, and medication-related characteristics of participants

Variable	**FUT (n=12)**	**MOD (n=8)**
Age (y)	41.2 ± 5.8	48.6 ± 3.9 *
Body mass (kg)	95.1 ± 16.3	86.3 ± 8.8
BMI (kg m^{-2})	29.9 ± 3.1	27.7 ± 2.3
Waist circumference (cm)	104.3 ± 9.7	98.3 ± 3.0
Resting plasma [Glu] (mmol L^{-1})	5.4 ± 0.4	5.7 ± 0.3
2 h plasma [Glu] (mmol L^{-1})	5.2 ± 1.3	5.4 ± 1.1
Resting plasma insulin (mU L^{-1})	7.5 ± 3.8	9.0 ± 5.0
2 h plasma insulin (mU L^{-1})	44.8 ± 22.2	56.6 ± 23.3
Systolic blood pressure (mmHg)	126.3 ± 9.4	124.0 ± 5.2
Diastolic blood pressure (mmHg)	83.7 ± 6.7	83.0 ± 7.0
Running velocity at VT1 (km h^{-1})	6.7 ± 0.7	7.1 ± 0.6
$\dot{V}O_2$ at VT1 (mL kg^{-1} min^{-1})	20.7 ± 4.3	23.8 ± 5.0
Medications		
Angiotensin II receptor antagonist (n=)	1	1
Statins (n=)	-	2
Calcium channel blocker (n=)	-	1
Other (n=)	1	1
Risk factor classification		
IFG (n=)	3	3
Metabolic syndrome ^ (n=)	10	5

Data are presented as mean ± SD.
FUT, futsal training group; MOD, moderate-intensity continuous training group; BMI, body mass index; [Glu], glucose concentration; VT1, ventilatory threshold; 2 h, 2 h post glucose ingestion during OGTT. *, significantly different at baseline ($p < 0.05$). ^, definition according to (Alberti *et al.*, 2006)

6.2.2 Experimental overview

Individuals who responded to recruitment were contacted to assess their eligibility. The inclusion criteria required individuals to *a)* be 35-55 years of age, *b)* have a BMI between 25 and 35 kg m^{-2}, and *c)* be physically inactive, defined as less than 2 h of formal physical activity per week. Individuals with diagnosed diabetes or major cardiac pathologies were excluded. Individuals with previously-diagnosed prediabetes (n=1) were only included in the study if they also matched the general inclusion criteria. Eligible participants (n = 22, two drop-outs) completed a cardiovascular risk questionnaire and obtained written clearance from their physician. Participants then undertook an OGTT, a resting muscle biopsy (with blood sampling), and an incremental exercise test (with anthropometric measurements), in this order and with one week between tests. Individuals were allocated, according to personal preference, to either a futsal training group (FUT) or a moderate-intensity continuous training group (MOD), and two weeks after the completion of the incremental test commenced their training intervention, 3 times per week for 8 weeks. Sixty-two hours (range 60 to 64 h) after the last training session, participants underwent a post-training resting muscle biopsy, followed by an OGTT and incremental exercise test, in this order and with 48 h between tests. In the 24 h before each OGTT and biopsy trial, participants were instructed to fast overnight, to refrain from exercise, and from caffeine and alcohol consumption.

6.2.3 OGTT

Participants arrived at the laboratory between 07:00 and 09:00 a.m. After remaining seated for 5 min, resting blood pressure was measured using a standard sphygmomanometer. A blood sample was drawn from the antecubital vein, immediately

placed in a tube containing lithium heparin, and centrifuged at room temperature for 2 min at 12000 rpm to obtain plasma for analysis. Then, participants drank a solution of 75 g of glucose in 300 mL of water (Point of Care Diagnostics, Artarmon, NSW, Australia), in less than five minutes. Two hours after ingestion, another antecubital venous blood sample was taken. Plasma glucose concentration ([Glu]) was measured using an automated analyser (2300 STAT plus, YSI Inc., Yellow Springs, OH, USA). Plasma insulin concentration was measured using a solid-phase, two-site chemiluminescent immunometric assay (Immulite® 2000, Siemens AG, Munich, Germany). From these two measurements, peripheral insulin sensitivity was calculated using the Cederholm index (Cederholm and Wibell, 1990).

6.2.4 Muscle biopsy and blood collection

Forty-eight hours after the OGTT, participants returned to the laboratory between 07:00 and 09:00 a.m. for blood and muscle sampling. After remaining supine for 5 min, a blood sample was collected at rest from an antecubital vein. Plasma glycated haemoglobin (HbA_{1c}) was measured via ultra-affinity HPLC. Plasma cholesterol, triglycerides, and HDL-C were measured via enzymatic colorimetric assay (Olympus, Center Valley, PA, USA). Low-density lipoprotein cholesterol was calculated using the Friedewald equation (Friedewald *et al.*, 1972). Immediately after the blood collection, a muscle sample was taken from the vastus lateralis at rest using a biopsy needle with suction, as previously described in section 4.2.4. The post-training resting muscle biopsy was performed on the same leg, approximately 62 h (range 60-64 h) after the last training session.

6.2.5 Incremental exercise test

Before the commencement of the test, body mass, height, BMI, and waist circumference were measured. The incremental test was performed on a treadmill (Quinton Q65, Seattle, WA, USA) and comprised an initial 3-min stage at 3 km h^{-1}, with the intensity thereafter increased by 1 km h^{-1} every minute, with no gradient. Calibration of the equipment was conducted prior to testing and expired gases and volume were analysed as previously described in section 3.2.3, with data displayed in real-time. The test was terminated one stage after the detection of the ventilatory threshold 1 (VT1), defined as an increase in the ventilatory equivalent for oxygen ($\dot{V}E/\dot{V}O_2$) without a concurrent increase in the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$) (Caiozzo *et al.*, 1982). During the test, participants wore a triaxial accelerometer (X6-2; sampling rate 160 Hz, ± 6 g, 16-bit; Gulf Coast Data Concepts, Waveland, MS, USA) placed in a dedicated vest between the scapulae. The changes in acceleration on the three anatomical axes were used to calculate the work rate corresponding to each speed of the test, using the following vector magnitude equation,

$$work\ rate\ (AU) = \frac{\sqrt{((x1 - x0)^2 + (y1 - y0)^2 + (z1 - z0)^2)}}{100}$$

Where x represents vertical accelerations, y represents lateral accelerations, and z represents frontal accelerations.

6.2.6 Group allocation and training intervention

Participants were allocated, according to their preference, to either FUT (n = 12) or MOD (n = 10), and both groups trained three times per week for 8 weeks. The FUT

group performed 5 vs. 5 futsal games on a parquet-floor indoor court. The game duration was progressively increased from 2 x 12 min to 2 x 26 min, with 5 min of rest between halves and 2-min rotations for each player. Each training session was preceded by a standard 5-min warm-up comprising of running and mobility exercises. The MOD group performed treadmill exercise training, matched to FUT for total work, with the intensity of exercise progressively increased from 85 to 105 % of the velocity corresponding to VT1.

6.2.7 Work matching

Work matching between groups was achieved by the use of triaxial accelerometers. This method is widely used in research to quantify physical activity, and an acceptable validity has been found on the comparison of work rate measured during treadmill- and ground-based physical activity (Hendrick *et al.*, 2010). During all FUT games participants wore an accelerometer, and the total work for each game (including warm-up) was recorded. The duration of exercise required for MOD to match the work performed during each FUT game was then calculated by dividing the average FUT work by the work corresponding to 1 min of exercise at VT1. For example, a total work of 600 units during a FUT game corresponded to 30 min of treadmill exercise for a MOD participant with a work at VT1 corresponding to 20 units per minute. For a more detailed explanation of the procedures followed to achieve work matching see Appendix 10.

6.2.8 Immunoblotting

Approximately 60 mg of muscle was homogenised as described in section 4.2.7. Lysate aliquots were mixed with Laemmli buffer, and protein separated by pre-cast gel electrophoresis (Criterion TGX, Bio-Rad Laboratories, Hercules, CA, USA), for 1 h at 200 V. Different gel gradients were used in accordance with the molecular weight of the proteins to be separated. The amount of sample to be loaded for each protein of interest was selected on the basis of preliminary optimisation blots, which tested the linearity of the relationship between the amount of protein loaded and the density outcome. Following electrophoresis, proteins were transferred to PVDF membranes (Bio-Rad) for 100 min at 25 V using a semi-dry blotter (Trans-Blot transfer cell, Bio-Rad). Membranes were blocked in TBST buffer containing 7.5 % non-fat milk, for 1 h at room temperature. After being washed, membranes were incubated overnight at 4 °C with primary antibodies for complexes I-V of the mitochondrial electron transport system (MitoProfile® total OXPHOS human antibody cocktail, Abcam, Sapphire Biosciences, Waterloo, NSW, Australia, ab110411), peroxisome proliferator-activated receptor γ, co-activator 1α (PGC-1α, Calbiochem, EMD Millipore, Billerica, MS, USA, ST1202), glucose transporter 4 (GLUT4, Millipore, CBL243), myocite enhancer factor 2a (MEF2a, Abcam, ab87975), and mitochondrial transcription factor A (mtTFA , Abcam, ab47517). Primary antibodies were diluted 1:1,000 (except GLUT4, 1:300) in TBS containing 0.1 % NaN_3 and 0.1 % albumin bovine serum. Following incubation with the primary antibodies, membranes were incubated with anti-rabbit (PerkinElmer, Waltham, MS, USA, NEF812001EA) or anti-mouse (PerkinElmer, NEF822001EA) horseradish peroxidase (HRP)-conjugated secondary antibodies, for 1 h at room temperature (1:10,000 in TBST with 5 % non-fat milk). Immunoreactivity was detected

using a chemiluminescence reagent (Luminata™ Classico Western HRP Substrate, Millipore), and quantified by densitometric scanning (VersaDoc™ MP4000, Bio-Rad). Only linear adjustments to the whole images were performed (Quantity One, Bio-Rad), with no modifications to gamma settings. Representative images are presented with a height of at least five bands above and below the band of interest (Wada *et al.*, 2004). Loading control was performed using a modified version of the Coomassie stain protocol (Welinder and Ekblad, 2011). Briefly, after removing the HRP substrate, membranes were incubated with a Coomassie stain solution (0.1 % Brilliant Blue R-250 in 1:1 methanol/distilled water) for 2 min, followed by 5 min of de-staining (1:5:4 acetic acid/ethanol/distilled water). After washing, the membranes were analysed as described above.

6.2.9 Diet reports

During the first and last week of training, participants were asked to complete a 3-day diet report to assess changes in energy and macronutrient intake. Participants were encouraged to accurately record food and drink intake over three consecutive days, comprising of two weekdays and one weekend day. They were instructed to report exact quantities in grams and fat content whenever possible or, alternatively, to use standard references (e.g., 1 cup).

6.2.10 Statistical analysis

Data are presented as mean ± SD. Results were tested for normal distribution using a Shapiro-Wilk W test and, when the assumption of normality was not met, data were log transformed to reduce bias due to the non-uniformity of error. The magnitude of the

changes was assessed using effect size (ES) statistic with 90 % confidence intervals, and percentage changes. ES were defined as a follows: smaller than 0.2 = trivial, 0.2 to 0.6 = small, 0.6 to 1.2 = moderate, 1.2 to 2.0 = large, larger than 2.0 = very large (Hopkins, 2003 (Access Date 24-12-2011)). The effects were defined as substantially meaningful when there was equal to, or larger than 75 % of chance of the true value of the statistic being practically positive (Batterham and Hopkins, 2006). Independent Student's t test and two-way ANOVA with Repeated Measures were used to assess baseline and group x training differences, respectively.

6.3 Results

6.3.1 Insulin sensitivity and glucose metabolism

There was an increase in peripheral insulin sensitivity in response to training in FUT only, being 84 ± 37 and 110 ± 42 mg L^{-2} $mmol^{-1}$ mU^{-1} min^{-1}, pre- and post-training, respectively (+31 %, ES 0.67 ± 0.53) (Figure 20). No change was detected for MOD (69 ± 14 and 74 ± 11 mg L^{-2} $mmol^{-1}$ mU^{-1} min^{-1}, pre- and post-training, respectively). There was no change after training in plasma glucose concentration at rest, while there was a meaningful decrease 2 h after ingestion for FUT only (-9.7%, ES 0.41 ± 0.38) (Table 12). Plasma insulin concentration at rest did not change, while there was a meaningful decrease 2 h after ingestion for both FUT (-28.1 %, ES 0.88 ± 0.67) and MOD (-20.6 %, ES 0.53 ± 0.49).

6.3.2 Triglycerides, Cholesterol, HDLC, LDLC, and HbA_{1c}

Resting plasma lipids and glycated haemoglobin measured before and after training are shown in Table 12. FUT training induced a meaningful decrease in total cholesterol (-8 %, ES 0.44 ± 0.24) and triglycerides (-32 %, ES 0.52 ± 0.31), whilst no changes were detected for MOD.

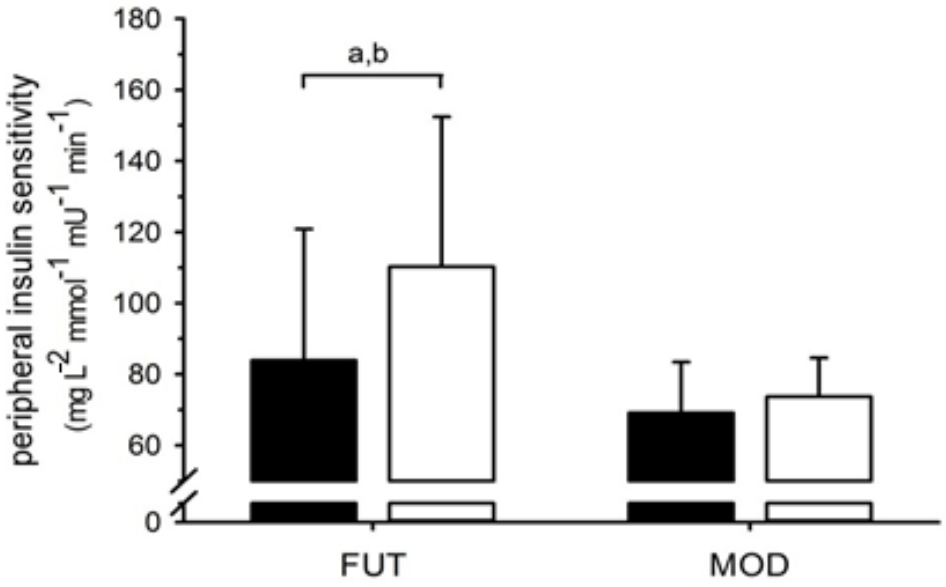

Figure 20; Peripheral insulin sensitivity (Cederholm index) measured before and after 8 weeks of training.

FUT, futsal training group; MOD, moderate-intensity continuous training group; Filled bars, pre-training; open bars, post-training; a, substantially meaningful change compared to pre-training; b, substantially meaningful change compared to MOD.

Table 12. Haematological changes in response to 8 weeks of futsal versus moderate-intensity continuous training.

Variable	FUT (n=12)			MOD (n=8)		
	Pre-training	**Post-training**	**ES ± CI**	**Pre-training**	**Post-training**	**ES ± CI**
Resting glucose (mmol L^{-1}) §	5.4 ± 0.4	5.4 ± 0.5	0.14 ± 0.45	5.7 ± 0.3	5.7 ± 0.3	0.10 ± 0.28
2 h OGTT glucose (mmol L^{-1}) §	5.2 ± 1.3	4.6 ± 1.2	0.41 ± 0.38 [a, b]	5.4 ± 1.1	5.4 ± 0.8	0.03 ± 0.51
Resting insulin (mU L^{-1}) §	7.5 ± 3.8	7.2 ± 5.0	0.21 ± 0.57	9.0 ± 5.0	7.4 ± 5.0	0.23 ± 0.16
2 h OGTT insulin (mU L^{-1}) §	45 ± 22	26 ± 17	0.88 ± 0.67 [a]	57 ± 23	41 ± 13	0.53 ± 0.49 [a]
HbA_{1c} (mmol $molHb^{-1}$)	38.0 ± 2.0	35.0 ± 2.0 *	1.36 ± 0.51 [a]	39.0 ± 2.0	37.0 ± 3.0	0.93 ± 0.50 [a]
Cholesterol (mmol L^{-1})	5.3 ± 1.0	4.9 ± 0.9	0.44 ± 0.24 [a]	5.2 ± 1.0	5.2 ± 1.0	0.04 ± 0.80
Triglycerides (mmol L^{-1})	2.1 ± 1.1	1.6 ± 0.7	0.52 ± 0.31 [a, b]	1.5 ± 0.7	1.8 ± 1.1	0.26 ± 0.61
HDL-C (mmol L^{-1})	1.1 ± 0.2	1.1 ± 0.1	0.10 ± 0.28	1.3 ± 0.4	1.2 ± 0.4	0.16 ± 0.29
LDL-C (mmol L^{-1})	3.2 ± 0.8	3.0 ± 0.8	0.27 ± 0.30	3.2 ± 0.7	3.1 ± 0.8	0.06 ± 0.97

Data are presented as mean ± SD. §, n=11 for FUT and n=7 for MOD

FUT, futsal training group; MOD, moderate-intensity continuous training group; ES, effect size; CI, confidence interval; [a], substantially meaningful change compared to pre-training; [b], substantially meaningful change compared to MOD; 2 h OGTT, 2 h post glucose ingestion during OGTT; *, significantly different from MOD ($p < 0.05$ main effect training)

HbA_{1c}, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

6.3.3 Skeletal muscle protein abundance

Representative western blotting images are presented in Figure 21. A summary of the training-induced abundance of proteins associated with glucose transport and mitochondrial biogenesis is presented in Table 13. There was a meaningful increase in COXII protein abundance in FUT only (16 %, ES 0.79 ± 0.24, Figure 22), while both FUT and MOD training resulted in a similar meaningful increase in GLUT4; the increase being 45 % (ES 0.78 ± 0.47) and 44 % (ES 0.67 ± 0.57) respectively, as shown in Figure 22.

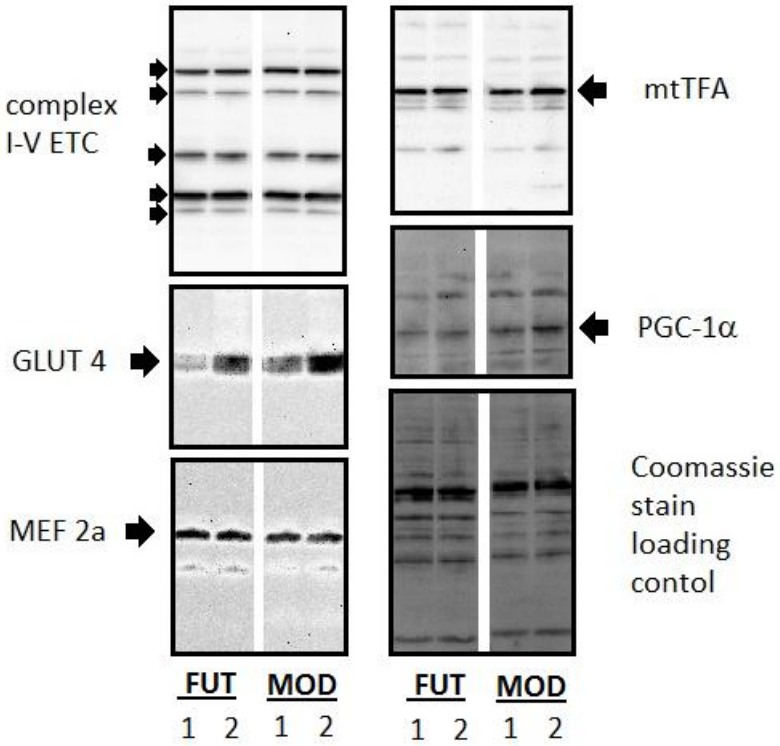

Figure 21; Representative western blot images for one individual of each group. FUT, futsal training group; MOD, moderate-intensity continuous training group; 1, pre-training; 2, post-training; ETC, electron transport chain; GLUT 4, glucose transporter 4; MEF2a, myocite enhancer factor 2a; mtTFA, mitochondrial transcription factor A; PGC-1α, peroxisome proliferator-activated receptor γ, co-activator 1 α.

Table 13. Summary of the changes in the skeletal muscle abundance of proteins associated with mitochondrial biogenesis in response to 8 weeks of futsal versus moderate-intensity continuous training.

Variable	FUT (n=12)			MOD (n=8)		
	Pre-training	**Post-training**	**ES ± CI**	**Pre-training**	**Post-training**	**ES ± CI**
CI-20	11 ± 3	14 ± 4	0.55 ± 0.29 [a]	12 ± 3	13 ± 2	0.39 ± 0.75
CII-30	24 ± 9	25 ± 9	0.15 ± 0.14	23 ± 9	25 ± 9	0.14 ± 0.29
CIII-core2	10 ± 4	12 ± 4	0.36 ± 0.24 [a]	11 ± 5	13 ± 5	0.32 ± 0.21 [a]
COX II	39 ± 7	45 ± 8	0.79 ± 0.24 [a, b]	48 ± 8 *	50 ± 5	0.42 ± 0.45 [a]
CV-alpha	27 ± 5	29 ± 4	0.30 ± 0.30	31 ± 7	34 ± 7	0.49 ± 0.19 [a]
GLUT 4	11 ± 9	16 ± 8	0.78 ± 0.47 [a]	14 ± 7	20 ± 9	0.67 ± 0.57 [a]
MEF2a	9 ± 7	9 ± 8	0.06 ± 0.17	8 ± 5	10 ± 7	0.23 ± 0.34
mtTFA	51 ± 14	54 ± 17	0.13 ± 0.41	58 ± 21	64 ± 20	0.30 ± 0.40
PGC-1α	23 ± 12	24 ± 10	0.19 ± 0.40	33 ± 12 *	40 ± 18	0.38 ± 0.29 [a]

Data are presented as mean ± SD (arbitrary units, normalised with Coomassie stain loading control density).

FUT, futsal training group; MOD, moderate-intensity continuous training group; CI-20, complex I subunit NDUFB8; CII-30, complex II-FeS subunit 30kDa; CIII-core 2, complex III subunit core 2; COX II, cytochrome c oxidase subunit II, CV-alpha, ATP synthase subunit alpha; GLUT 4, glucose transporter 4; MEF2a, myocite enhancer factor 2a; mtTFA, mitochondrial transcription factor A; PGC-1α, peroxisome proliferator-activated receptor γ, co-activator 1 α.

ES, effect size; CI, confidence interval; [a], substantially meaningful change compared to pre-training; [b], substantially meaningful change compared to MOD; *, significantly different between groups at baseline ($p < 0.05$).

6.3.4 Anthropometric measurements

There were no training-induced changes in body mass, BMI, waist circumference or the running velocity associated with VT1, with no difference between groups Table 11.

6.3.5 Dietary report

There was no difference after training or between groups in the dietary nutrient and energy intake. Average daily energy intake for FUT was 8798 ± 1968 kJ pre-training and 9017 ± 2263 kJ post-training (n = 10), whilst for MOD was 9101 ± 1880 kJ pre-training and 9419 ± 1733 kJ post-training for (n = 6). Macronutrient intake (protein, fat, carbohydrate) was 20 ± 3, 31 ± 7, and 45 ± 7 % pre-training and 20 ± 4, 32 ± 4, and 43 ± 7 % post-training for FUT; and 17 ± 6, 33 ± 4, and 46 ± 6 % pre-training and 20 ± 6, 29 ± 5, and 47 ± 7 % post-training for MOD.

6.3.6 Adherence and injuries

Attendance rate to the training sessions was 92 ± 5 % for FUT and 93 ± 7 % for MOD. Two drop-outs occurred, both in MOD group (from 10 to 8 participants). During the 8 weeks of training, 3 minor musculoskeletal injuries occurred in FUT (n = 1 quadriceps, n = 1 biceps femoris, n = 1 adductor muscles) and 1 in MOD (tibialis anterior). All injuries resulted in absence from training for up to three sessions. The three participants affected by injury in FUT returned to training without further complications. The MOD participant returned to training and suffered from the same problem, but to a lower degree, for the rest of the study.

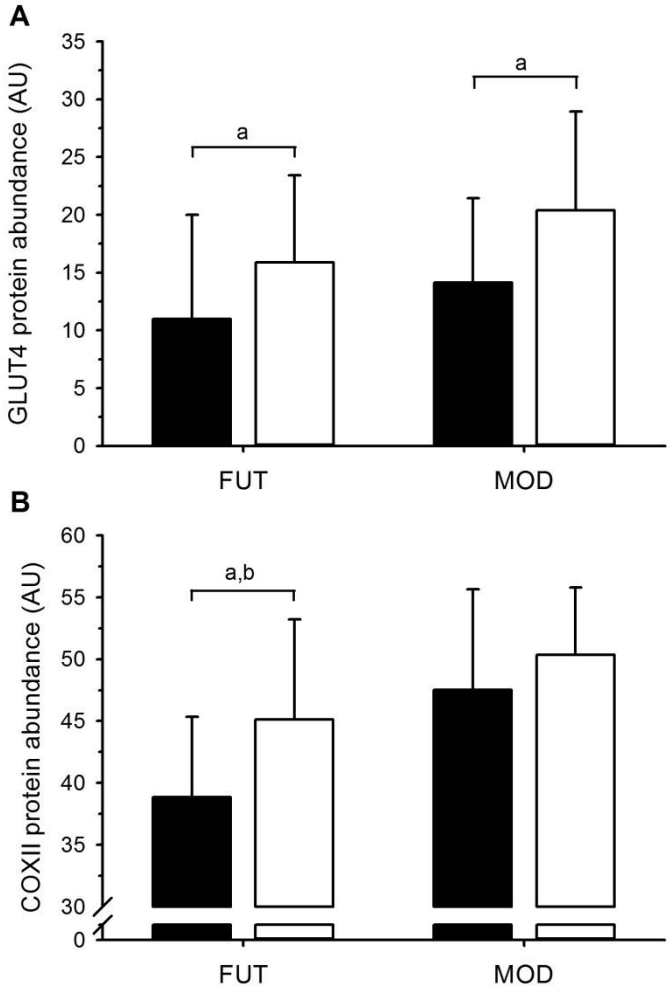

Figure 22; Resting glucose transporter 4 (GLUT4) (A) and mitochondrial complex IV, subunit II (COXII) (B) skeletal muscle protein abundance, before and after 8 weeks of training.

Filled bars, pre-training; *open bars*, post-training; [a], substantially meaningful change compared to pre-training; [b], substantially meaningful change compared to MOD.

6.4 Discussion

There were three main findings in this study. Firstly, FUT improved peripheral insulin sensitivity in middle-aged males, whereas a work-matched continuous running training did not. Secondly, FUT was associated with a greater increase in the abundance of COXII protein, despite the fact that PGC-1α, a key co-activator of mitochondrial biogenesis, was increased in MOD only. Lastly, only FUT lowered resting plasma cholesterol and triglycerides.

6.4.1 Futsal training increases peripheral insulin sensitivity in middle-aged males

One of the main results was that 8 weeks of FUT resulted in a meaningful improvement in peripheral insulin sensitivity. To the best of knowledge, this is the first study to quantify the changes in insulin sensitivity in response to football/futsal training. The increase with FUT was larger than with MOD; this is in accordance with evidence of larger improvements in insulin sensitivity following training at 80 % $\dot{V}O_{2\text{peak}}$ compared to a volume-matched intervention at 65 % $\dot{V}O_{2\text{peak}}$ in older women (DiPietro *et al.*, 2006). Similarly, insulin sensitivity was improved in response to 7 days of training at 70 % $\dot{V}O_{2\text{peak}}$, but not following training at 50 % $\dot{V}O_{2\text{peak}}$ in obese men (Kang *et al.*, 1996). Taken together, these results suggest that exercise intensity, rather than volume, plays an important role in the training-induced improvement in insulin sensitivity. This has important implications for the choice of the modality of exercise to adopt as a prevention method, as one of the main advantages of intermittent training is the ability of an individual to exercise for longer time at higher intensities.

As there were no differences between FUT and MOD for the training-induced reduction in plasma insulin concentration, adaptations in the insulin-mediated glucose uptake may have occurred. A larger clearance of glucose from the blood in response to insulin secretion is primarily due to an increased abundance, translocation, and/or activity of the GLUT4 protein (Maarbjerg *et al.*, 2011). As these results show an almost identical increase in resting GLUT4 protein abundance in both FUT and MOD after training (45 % and 44 %, respectively), this does not appear to explain the difference in insulin sensitivity between the groups. Similarly, in a study investigating the effects of training on insulin sensitivity in individuals from different age groups, heterogeneous adaptations in insulin sensitivity were found across groups, despite a similar increase in GLUT4 protein abundance (Short *et al.*, 2003). In support of this, an enhanced insulin sensitivity detected after acute exercise in rats was found to be the consequence of an increased GLUT4 translocation to the cell surface, rather than of a simple increase in total abundance (Hansen *et al.*, 1998). Hence, FUT may be superior to MOD in improving peripheral insulin sensitivity, via the positive effect of high-intensity intermittent exercise on GLUT4 protein translocation, although this remains to be demonstrated.

6.4.2 COXII is increased in FUT despite no changes in PGC-1α, mtTFA, and MEF2A

The relationship between mitochondrial function and the development of insulin resistance has been the focus of recent research (Handschin, 2011; Szendroedi and Roden, 2008). It is unclear whether an improvement in insulin sensitivity is at least partially the consequence of an enhanced mitochondrial function, or if both are merely co-adaptations induced by exercise. This study assessed for the first time the changes induced by FUT and MOD in the protein abundance of a) representative subunits of

complex I to V of the mitochondrial electron transport system; b) PGC-1α, a co-activator involved in mitochondrial biogenesis; c) mtTFA, a key factor for mitochondrial genome transcription; and d) MEF2A, a transcription factors involved in both mitochondrial biogenesis and GLUT4 expression. The main finding was that FUT induced a meaningful increase in COXII protein abundance, which was larger than the change produced by MOD. This is consistent with what appears to be a larger increase in COXII protein abundance following interval training compared to endurance training (Gibala *et al.*, 2006). It must be also acknowledged that part of this difference might be explained by the different COXII abundance between groups at baseline.

It was hypothesised that FUT would result in larger adaptations at the mitochondrial level compared to MOD due to the effects of the higher exercise intensity on the chronic up-regulation of transcription factors and co-activators. Changes in mRNA expression and protein abundance of PGC-1α and mtTFA are believed to be dependent on the intensity of exercise (Egan *et al.*, 2010; Psilander *et al.*, 2010; Tjønna *et al.*, 2008). However, meaningful changes in the protein abundance of mtTFA or MEF2A were not detected in either FUT or MOD and, paradoxically, PGC-1α protein abundance was increased in MOD only. It has been proposed that PGC-1α might not be necessary to promote training adaptations associated with mitochondrial biogenesis and oxidative phosphorylation (Leick *et al.*, 2008). Alternative mechanisms, such as PGC-1α protein translocation or post-translational modifications, might be involved in the mitochondrial responses to exercise training and additional investigation is required to understand the importance of exercise intensity on such responses.

6.4.3 Futsal training-induced reduction in plasma lipids

FUT lowered total cholesterol and triglyceride concentration at rest, while no changes were detected after MOD. These results have important practical implications, as an increased plasma lipid concentration is one of the main variables to be counteracted in the prevention of risk factors associated with diabetes and its co-morbidities. Similar reductions in total cholesterol have been found in response to 12 weeks of small-sided football games in 20-45 year-old men (Knoepfli-Lenzin *et al.*, 2010; Randers *et al.*, 2010a). Together, these results suggest that intermittent activities, such as futsal, are a promising alternative type of exercise for improving the blood lipid profile. Although some evidence exists suggesting that high-intensity training might be more effective than low-intensity interventions, the mechanisms behind the exercise-induced reduction in blood lipids are still debated (Trejo-Gutierrez and Fletcher, 2007). Traditionally, reductions in body mass and adipose tissue are considered as the main factors responsible for a reduction in lipaemia. However, there were no detectable differences between FUT and MOD in regards to the changes in body mass (-1.1 and -1.4 %, respectively), waist circumference (-1.0 and 0.6 %, respectively), daily energy intake (2.5 and 3.5 %, respectively), and macronutrient intake. A simple explanation for the differences between groups might be found in the fact that the FUT group commenced training with a higher body mass and waist circumference; this was associated with higher total cholesterol and triglycerides at baseline, hence the larger reductions after training. Alternatively, high-intensity exercise might have exerted a positive effect at a more specific level, such as cholesterol morphology, transport mechanisms and cholesterol absorption by cellular targets (e.g., the liver) (Trejo-Gutierrez and Fletcher, 2007). Further research is required to confirm the superior efficacy of high-intensity

intermittent exercise on plasma lipid reduction, and to investigate the underlying mechanisms.

6.4.4 Limitations

The main limitation associated with the present study was the self-selection of the training programme by participants. This study was designed to compare the efficacy of two training interventions to reduce risk factors associated with diabetes in a very specific population. It is acknowledged that a randomised controlled trial would represent a stronger design. However, when comparing the effects of two different interventions, a desirable outcome is to obtain a high compliance from participants, especially when the sample size is limited. In the present study a high adherence of more than 90 % occurred in both groups, with only two drop-outs occurring, both in MOD. By choosing their training intervention, participants might have commenced the program with a positive attitude and self-efficacy in relation to participation to exercise, both factors contributing to high adherence to physical activity programmes (Stiggelbout *et al.*, 2006).

6.5 Conclusions

This study combined for the first time the use of indoor soccer training with blood and skeletal muscle measurements associated with the reduction of risk factors of diabetes, specifically targeting middle-aged males. The results indicate that 8 weeks of high-intensity intermittent training were more efficacious than moderate-intensity continuous training in reducing several risk factors associated with diabetes. The findings of this

study expand on previous knowledge on the efficacy of team sports, in particular futsal, as an effective method of increasing muscle adaptations and reducing risk factors of a chronic disease.

CHAPTER 7 – GENERAL DISCUSSION

7.1 Intermittent-sprint exercise induces skeletal muscle molecular adaptations in young, healthy adults

One of the main findings of this thesis was that intermittent-sprint exercise induced protein signalling associated with mitochondrial biogenesis and glucose metabolism in skeletal muscle. Chapters 4 and 5 tested the hypotheses that a) an acute bout of RSE would increase mRNA expression of transcription factors via protein signalling, especially within the AMPK and CaMKII pathways; and b) an acute game of futsal would induce similar or superior protein signalling compared to a work-matched continuous running exercise (section 2.6.2).

The experiments presented in Chapter 4 attempted to clarify whether maximal exercise intensity (sprinting) would be sufficient to trigger molecular cascades associated with mitochondrial biogenesis and glucose metabolism, despite an extremely-low exercise volume comprising a total of only 60 s of sprinting. The rationale underpinning this concept was based on the observation that high-intensity exercise produced measurable physiological perturbations in skeletal muscle cell (Section 2.3). Particularly, it was expected that the decrease in metabolite content after RSE (Dawson *et al.*, 1997; Gaitanos *et al.*, 1993) and the large increase in $[Ca^{2+}]_i$ measured after high-frequency contractions (Bruton *et al.*, 2010; Westerblad and Allen, 1993) would provide an optimal stimulus to trigger AMPK and CaMKII signalling. The results of Chapter 4 confirmed the hypothesis. An acute bout of RSE activated the signalling cascade within the AMPK and CaMKII pathways, which was associated with an approximate 200 %

increase in PGC-1α mRNA expression (Figure 16). However, the magnitude of the training-induced changes in most of the selected markers of mitochondrial biogenesis was smaller than expected. Also, an acute bout of RSE did not induce significant activation of p38 MAPK. This was partially contrasting with the mechanisms behind the activation of the p38 MAPK pathway, but it was consistent with the results of other high-intensity exercise interventions of similar duration (Gibala *et al.*, 2009; Yu *et al.*, 2003). The conclusion to the findings of Chapter 4 was that perhaps RSE might be more effective when combined with other types of activity, due to an increase in the total exercise volume (section 4.5). This hypothesis was tested through the experiments presented in Chapter 5. Skeletal muscle protein signalling within the AMPK, CaMKII, and p38 MAPK pathways was assessed following an acute game of futsal, and compared to a work-matched bout of moderate-intensity running exercise on a treadmill. The hypothesis was that futsal, due to the combination of high-intensity intermittent bouts and a higher exercise volume compared to RSE, would induce similar or greater signalling responses compared to traditional aerobic exercise. The results of Chapter 5 indicated that a game of futsal induced similar protein signalling responses compared to traditional aerobic exercise (Table 10). This was in line with research showing similar molecular responses following "all-out" interval training and endurance training (Burgomaster *et al.*, 2008). It was also interesting to verify the concept that futsal would be superior to RSE in regards to promoting signalling responses in skeletal muscle. This concept was only partially confirmed. In fact, the three signalling pathways selected for Study I and II responded differently to the two exercise stimuli. Both RSE and futsal had limited effects on AMPK activation, and increased ACC phosphorylation to a similar extent (86 and 119 % for RSE and FUT, respectively)

despite the substantial differences in the exercise volume. This provided a mechanistic confirmation to the observation that ACC activation might be independent of AMPK (Dzamko *et al.*, 2008). Acute RSE also increased CaMKII phosphorylation (69 %), whilst no change was detected in response to a futsal game. This might be explained to the peculiarities of CaMKII activation, which has been shown to be maximal at 1 min after the commencement of exercise, then reducing with the progression of the activity (Rose *et al.*, 2006). Finally, futsal induced a larger increase in p38 MAPK phosphorylation compared to RSE (85 and 19 % for FUT and RSE, respectively), pointing to the possibility that this signalling pathway might be responding to the volume of exercise, rather than its intensity. However, an interesting finding was that the ATF2, which is the direct downstream target of p38 MAPK, was activated in FUT only. This aligned with previous research showing ATF2 activation after high-intensity but not after low-intensity exercise (Egan *et al.*, 2010), and it was speculated that ATF2 might be more sensitive to exercise intensity than p38 MAPK. To summarise, intermittent-sprint exercise was capable of promoting skeletal muscle protein signalling in young adults. However, the importance of exercise intensity, as opposed to exercise volume or duration, is yet to be established.

7.2 High-intensity intermittent training improves performance and lowers risk factors associated with type 2 diabetes

This thesis provided novel findings regarding the effects of high-intensity intermittent training on high-intensity running performance, and its application as a method to lower risk factors of chronic disease in a high risk population. The experiments presented in Chapters 3, 4, and 6 tested the hypotheses that a) four weeks of RSE training would improve specific sprinting performance and induce molecular adaptations associated with mitochondrial biogenesis in skeletal muscle of young individuals; and b) eight weeks of futsal training would increase skeletal muscle protein abundance and insulin sensitivity in middle-aged, sedentary men.

The results of Chapter 3 confirmed the first hypothesis. Ten sessions of repeated-sprint exercise, comprising a total of only 10 min of exercise (approximately 140 min including recovery periods), improved multiple-set, repeated-sprint performance, as well as intermittent running capacity (section 3.3). An important finding of this experiment was that the capacity to accelerate was improved by approximately 20 % (Figure 11), and this was the performance index that returned the larger training adaptation. This has practical implications for team sport-based research, as recent investigations have demonstrated the importance of accelerations during team-sport activities (Osgnach *et al.*, 2010; Varley *et al.*, 2011). Considering the meaningful performance adaptations obtained with this intervention in limited training time, it was expected that repeated-sprint training would also induce selected muscle adaptations. This was supported by the concept that RSE generated remarkable physiological perturbations in skeletal muscle. Therefore, the chronic repetition of RSE should lead to

adaptations of those genes and proteins that are sensitive to physiological perturbations. In support to this hypothesis, intermittent-sprint exercise had been previously shown to induce AMPK phosphorylation and PGC-1α mRNA expression (Coffey *et al.*, 2009). However, the results presented in Chapter 4 demonstrated that only small-to-moderate molecular responses were detected after four weeks of training (section 4.3). Similar to what discussed in section 7.1, it was speculated that a training intervention with a larger volume might have been more effective in inducing muscle adaptations. This concept was developed in Chapter 6. To provide a meaningful output which could apply to a "real-life" situation, the effects of futsal training were compared to work-matched traditional aerobic training in middle-aged, sedentary men who were at high risk of developing type 2 diabetes. The results indicated that, despite most of the molecular adaptations being similar between groups (section 6.3), futsal training was more effective in improving insulin sensitivity and lowering plasma cholesterol and triglyceride concentrations in middle-aged, sedentary individuals. These results added important body of knowledge to the concept of football participation as an appropriate tool to assist in the prevention of chronic disease (Andersen *et al.*, 2010; Krustrup *et al.*, 2010).

7.3 Conclusions

The specific conclusions of this thesis are:

1. Ten sessions of repeated-sprint training were effective in improving acceleration, repeated-sprint ability, and intermittent running capacity in young, healthy adults.
2. Acute RSE altered the signalling pathways dependent on the metabolic state and the Ca^{2+} signalling in skeletal muscle, in turn increasing PGC-1α mRNA expression.
3. Possibly as a result of the repeated acute effect of RSE, a moderate increase in PGC-1α protein abundance was detected after four weeks of repeated-sprint training.
4. The changes in PGC-1α mRNA expression and protein abundance of markers of mitochondrial biogenesis do not appear to be sufficient to promote beneficial adaptations in the aerobic power.
5. An acute FUT game was comparable to MOD with respect to inducing protein signalling within the AMPK, CaMKII and p38 MAPK pathways in skeletal muscle.
6. Skeletal muscle abundance of proteins associated with mitochondrial biogenesis and glucose transport was similar in response to FUT and MOD training.
7. Eight weeks of high-intensity intermittent training were more efficacious than moderate-intensity continuous running training in reducing several risk factors associated with type 2 diabetes.

CHAPTER 8 – RECOMMENDATIONS FOR FURTHER RESEARCH

This thesis demonstrated for the first time that both acute RSE and futsal exercise induced skeletal muscle molecular adaptations in young, healthy adults. Possibly as a result of the accumulation of the acute responses, high-intensity intermittent training improved RSE performance and lowered some risk factors associated with type 2 diabetes, in particular an increase in insulin sensitivity and an improvement in the lipid profile. These findings have important implications for physical activity participation, as high-intensity intermittent exercise might become a preferred alternative to traditional aerobic exercise to improve performance and induce muscle adaptations that might assist in health maintenance at different stages of an individual's life. However, in a "real world" context it is likely that individuals would engage in multiple types of physical activity during their lifespan, rather than in one type only. It is possible that by combining different types of exercise, skeletal muscle adaptations might be potentiated. Further research should investigate which combinations of physical activities, for example a combination of RSE and futsal exercise, or futsal and other intermittent exercises, might be optimal to promote those adaptations assessed in this thesis. In particular, focus should be directed to identify the best combinations of exercise to improve mitochondrial biogenesis in the skeletal muscle, in health and chronic disease. Stemming from these research questions, it would be of interest to investigate the optimal training frequency required to maximise health benefits in a sedentary population, as well as to compare the effects of training amongst different population groups, in particular children and adolescents. Regarding the training frequency, two main questions can be investigated. Firstly, it would be of interest to investigate the

optimal training frequency (e.g. two versus three days per week) to obtain the best performance improvements in RSE in a team-sport setting. Secondly, the minimum futsal training frequency necessary to obtain health benefits in middle-aged men should be investigated. The protocol employed in Chapter 6 utilised a three-day-per-week intervention, however this is uncommon in a "real-world" setting, where individuals would more likely play futsal once or twice a week.

Also, to date it is unknown what the long-term health benefits of futsal participation are, and this should be investigated by comparing a medium-term intervention (e.g. six months) to a long-term program of several years, similarly to what has been already investigate in regards to outdoor football. Finally, this thesis provided several points of discussion about the mechanisms behind exercise-induced molecular adaptations in skeletal muscle. Additional research will be required to clarify what the relationships are between muscle adaptations and the characteristics of exercise, such as intensity, duration, and volume. Also, differences in muscle adaptations in relation to fibre type and sex might be useful to investigate. This will add invaluable information to the knowledge generated from this novel thesis, in regards to the adoption of exercise interventions as a preventive tool to counteract the onset of secondary health conditions. In performing these additional research studies, a strong emphasis should be placed on the research design, and in particular randomisation, to minimise risk of differences in the baseline measures in the experimental intervention.

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LIST of APPENDICES

APPENDIX 1

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH (STUDY I)

You are invited to participate

You are invited to participate in a research project entitled **"THE EFFECTS OF REPEATED-SPRINT EXERCISE AND TRAINING ON HUMAN SKELETAL MUSCLE GENE EXPRESSION"**.

This project is being conducted by a student researcher Fabio Rubens Serpiello as part of a PhD at Victoria University under the supervision of Prof. Michael J McKenna [School of Human Movement, Recreation and Performance].

Project explanation

The project aims to investigate the effects of both a single session of repeated sprint exercise and 4 weeks of repeated sprint-training on the expression of genes and proteins from the thigh muscle. Such genes are fundamental to obtain adaptations important for health and performance.

What will I be asked to do?

We will ask you to fill in several short questionnaires about your family medical history and your exercise habits.You will be asked to do three testing session before training, a 4-week period of training and two additional testing sessions after training. You will be asked for your permission to a medical doctor to take a small sample of your muscle (the equivalent of 3-4 rice grains) using a needle biopsy before and after one of the testing sessions, on two occasions - before and after training. A venous blood sample will be also taken during four sessions.

What will I gain from participating?

From participating to this study you can expect to gain strong benefits for your aerobic fitness, increase your understanding of fitness, fitness tests. You will also gain the experience of having participated in an exercise science experiment designed to increase knowledge about muscles and genes.

How will the information I give be used?

Your samples will be stored under alphanumeric codes (i.e. without your name or personal details) and only the researchers will be able to connect the samples to you. All of the muscle sample collected will be used to analyse some proteins, genes and energy sources involved with the function of your thigh muscle. In the event of any tissue remaining it will be disposed of in a de-identified container (i.e. no coding present) via incineration using Victoria University's waste disposal contractor. The data that will be collected during the study will be used/published in peer-reviewed journals and conference presentations. No personal details will be revealed without your written consent.

What are the potential risks of participating in this project?

The maximal treadmill incremental exercise test involves risks of sudden death due to myocardial infarct, vasovagal episodes, muscle soreness and stiffness. Risks associated with venous catheterisation include discomfort, bruising and infection (for example puss, tenderness and/or redness). Risks associated with muscle biopsy include discomfort, pain, bruising, bleeding, soreness, localised altered sensation of skin reduced/absent/tingle/hypersensitive) and infection. Risk associated with blood sample includes slightly uncomfortable, with possibility of bruising and infection.

How will this project be conducted?

Participants will initially be screened for cardiovascular risk factors and any health issues of relevance to the study. There will be two main phases in the study: Phase 1 (acute exercise) and Phase 2 (training).

Phase 1: Acute Exercise Responses

In the first phase, all subjects will visit the laboratory four (4) times. During their first visit, participants will undertake measures of aerobic fitness (VO_{2max} and lactate threshold) during an incremental exercise test on a treadmill. A venous blood sample will be taken at rest, during exercise and in recovery. In the second visit, subjects will perform a Yo-Yo Intermittent Recovery Test 2, which is an aerobic performance test specific for field-based team sports. The third visit will be a familiarisation trial of the repeated 4-sec sprint exercise and will comprise the participant performing 3 sets of 5 repetitions of sprints lasting 4 seconds each. These sprints will be performed on a non-motorised treadmill. After at least 4 days the fourth visit will be the main experimental trial consisting of 3 sets of five repeated 4-sec sprints. During this trial, a muscle biopsy will be taken from the thigh muscle prior to, immediately after, and at 1 and 4 hours after exercise, totalling four samples. A venous blood sample from the antecubital vein will also be collected at rest, during exercise and in recovery.

Phase 2: Exercise Training and Post-Training Tests

The main experimental session of the Phase 1 will be considered as the first training session of the program. After one week of recovery, subjects will be then asked to

train for 4 weeks, three times a week for a total of 12 training sessions, with each session fully supervised by the investigators (usually PhD student Mr F. Serpiello). On the day following the tenth training session, participants will repeat the Yo-Yo IR2 test. On the day following the eleventh training session the participants will repeat the incremental exercise with antecubital venous blood sampling, as indicated above. The final training session will also be used as the main post-training test, with repeat of the pre-training invasive test. This will involve muscle biopsies and venous blood samples as stated above.

Who is conducting the study?

The study is conducted by the School of Human Movement, Recreation and Performance, Victoria University

Main Investigators:

Prof. Michael J. McKenna, Telephone number 9919 4499, Mobile 0488 475 735, email michael.mckenna@vu.edu.au

Dr. Nigel K Stepto, Telephone number 99195416, Mobile 0409 338 696, email nigel.stepto@vu.edu.au

Dr. Robert Aughey, Telephone number 9919 5551, Mobile 0448 153 597, email robert.aughey@vu.edu.au

Mr. Fabio Rubens Serpiello, Mobile 0458 656 280, email fabiorubens.serpiello@live.vu.edu.au

Any queries about your participation in this project may be directed to the Principal Researcher listed above.

If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4781.

APPENDIX 2

UNIVERSITÀ DEGLI STUDI DI VERONA

DIPARTIMENTO DI SCIENZE NEUROLOGICHE, NEUROPSICOLOGICHE, MORFOLOGICHE E MOTORIE

SEZIONE DI SCIENZE MOTORIE

FOGLIO INFORMATIVO

TITOLO DELLO STUDIO:

"FIT-sal. I benefici del calcio a 5 per la prevenzione del diabete"

Lo studio è stato approvato dal Comitato Etico del Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie dell'Università degli Studi di Verona. Prima di decidere se prendere parte allo studio, per favore legga attentamente le seguenti informazioni. Se ha delle domande o necessità di ulteriori chiarimenti non esiti a farlo. Se deciderà di partecipare, Le verrà chiesto di firmare un "Modulo di consenso informato". Lei sarà ad ogni modo libero di ritirarsi dallo studio in qualsiasi momento senza dover dare spiegazioni.

OBIETTIVI DELLO STUDIO: L'obiettivo dello studio è quello di paragonare gli effetti di una partita di calcio a 5 (futsal) e di una sessione di corsa moderata, sulle risposte molecolari nel muscolo scheletrico.

METODO DI SVOLGIMENTO DELLA SPERIMENTAZIONE: Ad ogni partecipante verra' chiesto di svolgere i seguenti test:

Esercizio massimale incrementale su treadmill. Questo test consiste nell' effettuare un esercizio di corsa ad intensita' crescente su treadmill. Durante il test verra' misurato il consumo di ossigeno tramite l'impiego di un metabolimetro.

Biopsia muscolare a riposo. Questa procedura consente di prelevare un campione di tessuto muscolare della grandezza di uno-due chicchi di riso dalla porzione distale del muscolo vasto laterale (coscia). Dopo aver disinfettato la parte appropriata, verra' effettuata un iniezione di anestetico locale (lidocaina), al fine di anestetizzare i recettori del dolore situati sotto la cute. Una volta confermato l'effetto dell'anestetico, si procedera' al prelievo del campione di tessuto muscolare tramite l'utilizzo di un ago semi-automatico totalmente sterile.

Uno dei seguenti due tipi di esercizio fisico:

Una partita di calcio a 5 seguita da due biopsie muscolari (immediatamente dopo e 3 ore dopo la fine dell'esercizio. Ai partecipanti che verranno assegnati a questo gruppo

verra' chiesto di prendere parte ad una partita di calcio a 5. Subito dopo la fine della partita, e tre ore dopo, verra' ripetuta la procedura per il prelievo di un campione di tessuto muscolare.

Una sessione di corsa ad intensita' moderata, seguita da due biopsie muscolari (immediatamente dopo e 3 ore dopo la fine dell'esercizio. Ai partecipanti che verranno assegnati a questo gruppo verra' chiesto di svolgere una sessione di corsa su treadmill a intensita' moderata. Subito dopo la fine dell'esercizio, e tre ore dopo, verra' ripetuta la procedura per il prelievo di un campione di tessuto muscolare.

COSA ACCADRA' AI RISULTATI DELLO STUDIO?

I dati ottenuti nello studio verranno analizzati dai ricercatori coinvolti ed utilizzati per la pubblicazione di articoli scientifici. In nessun modo verrà fatto riferimento a dati personali dei pazienti. Solo dati medi del gruppo verranno riportati.

E' PREVISTO UN RIMBORSO?

Si, in questo studio e' previsto un rimborso che verra' consegnato al completamento dei test.

DOVE E QUANDO?

Lo studio verrà eseguito presso il Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie, presso i laboratori della Facoltà di Scienze Motorie, Via Felice Casorati 43, Verona. Lo studio avrà luogo nel periodo Ottobre 2011-Novembre 2011

PRIVACY, COME VERRANNO TRATTATI I MIEI DATI?

Nel caso in cui Lei decidesse di prendere parte al presente studio Le verrà chiesto di firmare il "Modulo di consenso informato" attraverso il quale Lei dichiara di essere consapevole delle attività previste e delle modalità di una Sua adesione, ed autorizzerà il responsabile della ricerca e i suoi collaboratori, ai sensi e per gli effetti del D.lgs. del 30 giugno 2003, n. 196:

- ad esaminare i Suoi dati personali, inclusi quelli relativi al Suo stato di salute;

- ad utilizzare i dati dello studio, in forma anonima, per elaborarli al fine di ottenere le informazioni cui è finalizzata la ricerca. Unicamente le persone autorizzate e direttamente coinvolte nello studio sperimentale avranno accesso ai dati che registreremo. Tutte le informazioni saranno mantenute confidenziali.

La partecipazione allo studio non comporta per Lei alcun aggravio di spesa.

Lo studio è stato approvato dal Comitato Etico del Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie ed il suo svolgimento ed i suoi risultati sono monitorati dallo stesso Comitato.

Si precisa che i risultati dello studio verranno portati a conoscenza della comunità scientifica ed i dati raccolti durante la sperimentazione non potranno rimanere di proprietà di singoli o gruppi che li possano utilizzare secondo il loro esclusivo interesse.

La procedura dello studio garantisce, peraltro, la riservatezza dei Suoi dati personali con riferimento al relativo Codice (D.lgs. del 30 giugno 2003, n. 196). Ai sensi dell'art. 13 del D.lgs. del 30 giugno 2003, n. 196 si sottopone la seguente informativa.

In conformità alle disposizioni del Codice in materia di protezione dei dati personali (di seguito "Codice"), il Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie La informa che intende svolgere attività di trattamento di dati personali (di seguito "Dati"), anche sensibili[1], che La riguardano.

FINALITA' E MODALITA' DEL TRATTAMENTO DEI DATI:

I Dati forniti vengono acquisiti e trattati nel rispetto della normativa sopra richiamata, con il supporto di mezzi cartacei, informatici o telematici atti a memorizzare, gestire e trasmettere i dati stessi e comunque mediante strumenti idonei a garantire la loro sicurezza e riservatezza, nel rispetto delle regole fissate dal Codice, per le finalità della ricerca in precedenza descritta riguardo agli obiettivi, alle procedure, ai benefici ed rischi della partecipazione, all'impegno operativo e temporale richiesto.

NATURA DEL CONFERIMENTO E CONSEGUENZE DI UN EVENTUALE RIFIUTO:

L'eventuale rifiuto di fornire i Dati funzionali all'esecuzione della ricerca su menzionata, non comporta alcuna conseguenza relativamente ad eventuali trattamenti terapeutici in corso, salva l'eventuale impossibilità di dare seguito alle operazioni connesse alla ricerca.

Lei è libero/a di non partecipare alla ricerca o di ritirarsi dallo stessa anche senza preavviso o motivazione. Qualora, durante la ricerca, divengano disponibili dati che possano influenzare la Sua volontà di continuare Lei sarà tempestivamente ed opportunamente informato e, se necessario, Le sarà richiesto nuovamente il Consenso Informato a proseguire il trattamento in corso.

COMUNICAZIONE DEI DATI:

I Dati potranno essere accessibili solo ai responsabili della sperimentazione.

[1] L'art. 4 del Codice definisce "*sensibili*" i dati personali idonei a rivelare l'origine razziale ed etnica, le convinzioni religiose, filosofiche o di altro genere, le opinioni politiche, l'adesione a partiti, sindacati, associazioni od organizzazioni a carattere religioso, filosofico, politico o sindacale, nonché i dati personali idonei a rivelare lo stato di salute o la vita sessuale.

I Dati relativi ai risultati della ricerca sono strettamente confidenziali e soggetti ad anonimato. I risultati potranno essere portati a conoscenza di terzi o pubblicati, ma escludendo ogni possibile riferimento personale.

DURATA DEL TRATTAMENTO DEI DATI:

Ai sensi del Decreto Legge n° 196/03 (Art.7 e 13) relativo alla tutela delle persone per il trattamento dei dati personali, La informiamo che i Suoi dati personali verranno raccolti ed archiviati in modo adeguato e saranno utilizzati esclusivamente a scopi di ricerca scientifica o didattica. I Dati verranno trattati dalla Sezione di Scienze Motorie solamente per la durata della ricerca. La durata della ricerca sarà di circa 6 mesi. L'art. 7 del Codice riconosce all'interessato numerosi diritti che La invitiamo a considerare attentamente. Tra questi, Le ricordiamo sinteticamente i diritti di: ottenere la conferma dell'esistenza o meno dei Dati che La riguardano, anche se non ancora registrati, e la loro comunicazione in forma intelligibile; ottenere l'indicazione dell'origine dei Dati, delle finalità e modalità del trattamento, degli estremi identificativi del titolare, dei responsabili, dei soggetti o delle categorie di soggetti ai quali i Dati possono essere comunicati o che possono venirne a conoscenza in qualità di responsabili o incaricati; ottenere l'aggiornamento, la rettificazione o l'integrazione dei Dati (qualora vi sia un interesse in tal senso) ovvero la cancellazione, la trasformazione in forma anonima o il blocco dei dati trattati in violazione di legge, nonché l'attestazione che tali operazioni sono state portate a conoscenza di coloro ai quali i Dati sono stati comunicati o diffusi; opporsi, in tutto o in parte, al trattamento dei Dati che La riguardano per motivi legittimi ovvero per fini di invio di materiale pubblicitario o di vendita diretta o per il compimento di ricerche di mercato o di comunicazione commerciale.

Lei, per l'intera durata del trattamento, potrà chiedere informazioni o porre domande agli sperimentatori circa i dati acquisiti nel corso della sperimentazione e circa l'andamento della stessa relativamente al suo caso; allo stesso modo, al termine della ricerca, se richiesto, i risultati che La riguardano saranno comunicati a Lei.

DANNI CONSEGUENTI A PROCEDURE SPERIMENTALI.

Con il presente modulo Le viene proposto di prendere parte al programma di studio, ricercatore referente è il Dott. Fabio Serpiello. La avvertirà prontamente di qualunque informazione diventi disponibile durante lo studio e provvederà a sospendere la Sua partecipazione allo studio qualora ciò risulti nel Suo interesse, comunicandoLe i motivi di tale sospensione.

Né il Sistema Sanitario Nazionale, né l'ospedale fornirà assistenza medica a lungo termine o risarcimenti economici per tali danni, eccetto per quanto già normalmente previsto dalla legge.

TITOLARE DEL TRATTAMENTO.

Titolare del trattamento è il Dipartimento di Scienze Neurologiche, Neuropsicologiche, Morfologiche e Motorie

COME POSSO AVERE MAGGIORI INFORMAZIONI?

Se ha qualche domanda riguardo al progetto di ricerca La preghiamo di contattare:

Fabio Serpiello; tel. 045 842 5139; mail fabiorubens.serpiello@univr.it

UNIVERSITY OF VERONA

DEPARTMENT OF NEUROLOGICAL, NEUROPSYCHOLOGICAL, MORFOLOGICAL E MOVEMENT SCIENCES

SCHOOL OF EXERCISE SCIENCE

TRANSLATION

INFORMATION TO PARTICIPANTS

TITLE OF THE STUDY

"FIT-sal; the benefits of futsal training for the prevention of diabetes"

This study has been approved by the Ethics Committee of the Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona. Before you decide whether you wish to take part in this study, please read carefully the following information. Should you have any question or should you need clarifications regarding the project, do not hesitate to ask. If you decide to participate, you will be asked to complete a Consent Form. You will have the right to withdraw from the study at any time and without having to give any explanation to the researchers.

AIMS OF THE STUDY

The aim of this study is to compare the effects of a single futsal (5-a side soccer) game and a moderate intensity running exercise on the molecular responses in the skeletal muscle.

METHODS

You will be asked to take part in the following tests:

1. Incremental test to exhaustion; this test requires to run at a progressively-increased velocity on a treadmill. During the test, oxygen consumption will be measured via the use of a metabolic cart
2. Resting muscle biopsy; this procedure allows taking a muscle sample, approximately the size of two-three rice grains, from the distal third of your thigh. After disinfecting the part, an injection of a local anaesthetic (lidocaine) will be performed, in order to anaesthetise the pain receptors situated under the skin. Then, a muscle samples will be taken using a sterile, semi-automatic needle.
3. One of the two following exercises:

a. A futsal game, followed by two muscle biopsies (immediately after and 3 h after the game). All participants of this group will be asked to play a game of 5-a side football. Immediately after the game, and 3 h after, a muscle biopsy will be taken.
b. An acute running session, performed at moderate intensity, followed by two muscle biopsies (Immediately after and 3 h after). All participants of this group will be asked to perform a treadmill running exercise at moderate intensity. Immediately after exercise, and 3 h after, a muscle biopsy will be taken.

WHAT WILL HAPPEN TO THE RESULTS OF THE STUDY

The data obtained in this study will be analysed by the researchers involved in the project and they will be used for the publication of scientific articles. In no way there will be a reference to the personal details of the patients. Only average data will be used.

IS THERE ANY REIMBURSEMENT?

Yes, there will be a reimbursement for this study. The reimbursement will be given upon completion of all tests.

WHEN AND WHERE?

The stud will take place in the facilities of the Department of Neurological, Neuropsychological, Morphological and Movement Sciences, at the School of Exercise Science, via Felice Casorati 43, Verona. The study will take place in the period October-November 2011

PRIVACY: HOW WILL MY DETAILS BE TREATED?

If you decide to take part in this study, you would be asked to complete a Consent Form, via which you will declare to be fully aware of the procedures involved and of the modalities of participation, and you will authorise the principal investigator and collaborators, according to the D.lgs n. 196, 30 June 2003, to:

- Take view of your personal details, including those relative to your health
- Use the results of the study, in anonymous form, to obtain the information necessary for research. Only the authorised persons which are directly involved in the study will have access to the data. All information will be kept confidential

The participation to this study does not require you to pay any fee

This study has been approved by the Ethics Committee of the Department of Neurological, Neuropsychological, Morphological and Movement Sciences, and the procedures and results will be monitored by the Committee.

Please note: the results of the study will be presented to the scientific community and the data collected during the experiments will not be owned by individuals or groups which may use them at their own interest.

The study guarantees the privacy of your personal details, with reference to the relative Code of Rules (D.lgs. 196, 30 June 2003). Following the D.lgs. 196, 30 June 2003 we present the following information.

In accordance to the Code about privacy and personal details ("the Code"), the Department of Neurological, Neuropsychological, Morphological and Movement Sciences informs you that it is expected to treat your personal details, even those defined as "sensible"[1].

1. Art. N. 4 of the Code defines as "*sensible*" all personal data that can reveal the ethnic origins, religion, philosophical belief, political opinions, membership to political parties, unions, associations and religious, philosophical or political organisations, and the personal details that can reveal your health status or sexual preferences.

AIM OF TREATMENT OF PERSONAL DETAILS

The personal details are acquired and processed in respect of the above rules in paper or electronic form, provided that these can allow to store and process the data in a secure and private way, in accordance to the Code, with the described scientific scopes regarding the aims, benefits, risks and commitment related to the participation.

WITHDRAWAL

You can refuse to give us your personal details and this will not incur in any change regarding the procedures involved, unless this makes impossible to undertake the research.

You are free to refuse to participate to the research, even without notice or explanations. If during the research, we will have information that may preclude your participation, you will be immediately informed and, if necessary, we will again ask your consent to continue the procedures.

DISSEMINATION OF RESULTS

The data coming from this study will be accessed only by the researchers involved.

The data are strictly confidential and are anonymous. The results may be presented to third parties or published, always excluding any reference to personal information

DURATION

In accordance to D.L. 196/03 (Art 7 and 13), we inform you that your personal details will be processed adequately and will only be used for research or didactical purposes by the School. The total duration of this project is 6 months. We would like to remind you of your rights concerning personal details:

- You can ask to be provided with evidence of the existence of your details in our database
- You can obtain indications about the origin of your details and the details of the individuals responsible
- You can request the modification or integration of your details, the cancellation, transformation or block of any detail dissemination

- You can oppose to the treatment of your details for legitimate reasons, for examples commercial use or market research

For the entire duration of the study you will be able to ask for information or questions to the researchers about the data collected during the experiments, and about the status of the research for what concerns your person.

DAMAGE CONSEQUENT TO EXPERIMENTAL PROCEDURES

With this form we propose you to take part in this study, the person responsible is Mr. Fabio Serpiello. He will contact you to inform of any change during the study and will suspend your participation should he have any concern about your person.

Nor the National Healthcare System, neither the Hospital will provide long-term medical assistance nor economic reimbursement for damage connected to this study, except for what already regulated by law.

ORGANISATION RESPONSIBLE OF THE RESEARCH

The Department of Neurological, Neuropsychological, Morphological and Movement Sciences

HOW CAN I OBTAIN ADDITIONAL INFORMATION?

Should you require any further information, please contact:

Fabio Serpiello

Ph., 045 842 5139

Email, fabiorubens.serpiello@univr.it

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

APPENDIX 3

INFORMATION TO PARTICIPANTS INVOLVED IN RESEARCH (STUDY III)

You are invited to participate

You are invited to participate in a research project entitled **"The effect of exercise training to improve the health of middle-aged pre-diabetic individuals".**

This project is being conducted by a student researcher Mr Fabio Rubens Serpiello as part of a PhD study at Victoria University under the supervision of Prof. Michael J. McKenna from the Institute of Sport, Exercise and Active Living (ISEAL)

Project explanation

The aim of this study is to investigate the effects of different types of exercise training on the prevention of risk factors for type 2 diabetes. In particular, we are interested in understanding how high-intensity intermittent exercise differs from low-intensity exercise in producing adaptations in the skeletal muscle genes and proteins. Such genes are fundamental to obtain adaptations important for health.

What will I be asked to do?

We will ask you to fill in several short questionnaires about your family medical history and your exercise habits. You will be then asked to perform three testing sessions before the commencement of the training, a 12-week training program involving one of different types of physical activities, and again three testing sessions after training. You will be asked your permission to a medical doctor to take a small sample of your thigh muscle (the equivalent of 3-4 rice grains) using a needle biopsy before and after training. Venous bloods samples will also be taken, before and after training, as part of a 2-hours oral glucose tolerance test.

What will I gain from participating?

From participating to this study you can expect to gain strong benefits for your health and fitness status. A complete and successful adherence to the training sessions will increase your possibilities to prevent the occurrence of type II diabetes and correlated pathologies via rapid adaptations of your skeletal muscles.

How will the information I give be used?

Your samples will be stored under alphanumeric codes (i.e. without your name or personal details) and only the researchers will be able to connect the samples to you. All of the muscle sample collected will be used to analyse some proteins, genes and energy sources involved with the function of your thigh muscle. In the event of any tissue remaining it will be disposed of in a de-identified container (i.e. no coding present) via incineration using Victoria University's waste disposal contractor. The data that will be collected during the study will be used/published in peer-reviewed journals and conference presentations. No personal details will be revealed without your written consent.

What are the potential risks of participating in this project?

The maximal treadmill incremental exercise test involves risks of sudden death due to myocardial infarct, vasovagal episodes, muscle soreness and stiffness. Risks associated with venous catheterisation include discomfort, bruising and infection (for example puss, tenderness and/or redness). Risks associated with muscle biopsy include discomfort, pain, bruising, bleeding, soreness, localised altered sensation of skin reduced/absent/tingle/hypersensitive) and infection. Risk associated with blood sample includes slightly uncomfortable, with possibility of bruising and infection.

How will this project be conducted?

You will be initially screened for cardiovascular risk factors and any health issues of relevance to the study. There will be three main phases:

Phase 1: Pre-training testing: In this phase you will visit the laboratory three (3) times. During the first visit, you will undertake an incremental exercise test on a treadmill to obtain measurements of your aerobic fitness (maximal oxygen uptake and lactate threshold). A fingertip capillary blood sample will be taken at rest and after each stage of the incremental exercise. In the second visit you will be asked to have a 2-hours oral glucose tolerance test (OGTT) that will allow us understanding how the glucose metabolism is regulated in your body. The test will require you to ingest a glucose solution and to have venous blood sampled at regular intervals for 2 hours. During the third visit, you'll undergo a resting muscle biopsy.

Phase 2: Training. After one week of recovery from the muscle biopsy, you will be starting your 12-wk training program. You will be randomly allocated to one of three groups, two performing physical activity and one acting as a control group. For scientific purposes, you will not be told what kind of physical training the other group is performing.

Phase 3: Post-training testing. After training, you will be repeating all the testing sessions described in Phase 1.

Who is conducting the study?

This study is conducted by the Institute of Sport, Exercise and Active Living and the School of Sport and Exercise Science, Victoria University

Main Investigators:

Prof. Michael J. Mckenna, Telephone number 9919 4499, email michael.mckenna@vu.edu.au

Prof. David J. Bishop, Telephone number 9919 9471, email david.bishop@vu.edu.au

Dr. Nigel K Stepto, Telephone number 99195416, email nigel.stepto@vu.edu.au

Dr. Robert J. Aughey, Telephone number 9919 5551, email robert.aughey@vu.edu.au

Mr. **Fabio Rubens Serpiello**, email fabiorubens.serpiello@live.vu.edu.au

Any queries about your participation in this project may be directed to the Principal Researcher listed above.

If you have any queries or complaints about the way you have been treated, you may contact the Ethics and Biosafety Coordinator, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4148.

APPENDIX 4

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

CARDIOVASCULAR AND OTHER RISK FACTORS QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating **"The effect of exercise training to improve the health of middle-aged pre-diabetic individuals"** you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.

Name: _____ **Date:** _____

Age: _____ **years** **Weight:** _____ **kg** **Height:** _____ **cm**

Gender: **M** **F**

Give a brief description of your average activity pattern in the past 2 months:

_____
_____
_____

Circle the appropriate response to the following questions.

1.	Are you overweight?	Yes	No	Don't know
2.	Do you smoke?	Yes	No	Social
3.	Are you an asthmatic?	Yes	No	Don't Know
4.	Are you a diabetic?	Yes	No	Don't Know
5.	Does your family have a history of diabetes?	Yes	No	Don't Know
6.	Do you have a thyroid disorder?	Yes	No	Don't Know

7. Does your family have a history of thyroid disorders?

Yes No Don't Know

8. Do you have a pituitary disorder? Yes No Don't Know

9. Does your family have a history of pituitary disorders?

Yes No Don't Know

10. Do you have a heart rhythm disturbance? Yes No Don't Know

11. Do you have a high blood cholesterol level? Yes No Don't Know

12. Do you have elevated blood pressure? Yes No Don't Know

13. Are you being treated with diuretics? Yes No

14. Are you on any other medications? Yes No

List all medications? (Including oral contraceptives)

_____

_____

_____

15. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise?

Yes No

If yes, please elaborate

_____

16. Have you had any musculoskeletal problems that have required medical treatment (e.g., broken bones, joint reconstruction etc.)? Yes No

If Yes, please provide details (including dates)

_____

17. Are you currently pregnant or expect to become pregnant during the time in which this experiment is conducted? Yes No

18. Does your family have a history of premature cardiovascular problems (E.g., heart attack, stroke)? Yes No Don't Know

I, _____, believe that the answers to these questions are true and correct.

Signature: _____ Date: _____

APPENDIX 5

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

MUSCLE BIOPSY, ARTERIAL & VENOUS CATHETERISATION QUESTIONNAIRE:

"The effect of exercise training to improve the health of middle-aged pre-diabetic individuals"

NAME: _____

ADDRESS: _____

_____

DATE: _____ **AGE:** _____ **years**

1. Have you or your family suffered from any tendency to bleed excessively? (E.g. Haemophilia) or bruise very easily? Yes No Don't Know

   If yes, please elaborate

_____

2. Are you allergic to local anaesthetic? Yes No Don't Know

   If yes, please elaborate

_____

3. Do you have any skin allergies? Yes No Don't Know

   If yes, please elaborate

_____

4. Have you any other allergies? Yes No Don't Know

If yes, please elaborate

_____

5. Are you currently on any medication? Yes No

If yes, what is the medication?

_____

6. Do you have any other medical problems? Yes No

If yes, please elaborate

_____

7. Have you ever fainted when you had an injection or blood sample taken?

Yes No Don't know

If yes, please elaborate

_____

8. Have you previously had heparin infused or injected?

Yes No Don't know

If yes, please elaborate

_____

9. Do you or other members of your family have Raynaud's disease, or suffer from very poor circulation in the fingers, leading to painful fingers that turn white/blue?

Yes No Don't know

If yes, please elaborate

_____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____ Date: _____

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APPENDIX 6

RAW DATA FROM CHAPTER 3

Table 14. Mean velocity data (m s^{-1}) for each sprint (S) during the pre-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	3.99	4.01	3.85	3.79	3.76	4.22	4.09	4.09	3.82	3.94	4.20	3.92	3.91	4.04	3.91
Subject 2	3.46	3.59	3.62	3.54	3.57	3.60	3.56	3.53	3.52	3.55	3.62	3.58	3.57	3.55	3.51
Subject 3	4.71	4.55	4.55	4.37	4.47	4.48	4.52	4.53	4.39	4.29	4.63	4.48	4.47	4.25	4.44
Subject 4	3.31	3.27	3.27	3.31	3.29	3.38	3.25	3.31	3.32	3.32	3.43	3.35	3.20	3.26	3.35
Subject 5	4.31	4.10	4.00	3.87	3.88	4.16	4.14	3.93	3.89	3.79	4.08	3.66	3.84	3.73	3.71
Subject 6	5.11	5.04	4.95	4.90	4.87	4.96	4.98	4.84	4.83	4.77	4.92	4.88	4.84	4.75	4.73
Subject 7	4.15	4.00	4.11	4.16	3.95	4.25	4.22	4.15	3.94	3.98	4.04	4.03	3.84	3.77	3.68
Subject 8	3.73	3.60	3.63	3.52	3.57	3.74	3.70	3.57	3.51	3.39	3.65	3.45	3.53	3.42	3.45
Subject 9	4.28	4.19	4.28	4.41	4.33	4.32	4.17	4.01	4.16	4.10	4.01	4.15	3.85	4.11	3.68
Subject 10	4.91	4.78	4.72	4.65	4.67	4.88	4.76	4.78	4.66	4.58	5.01	4.78	4.61	4.82	4.61
Mean	**4.20**	**4.11**	**4.10**	**4.05**	**4.03**	**4.20**	**4.14**	**4.07**	**4.00**	**3.97**	**4.16**	**4.03**	**3.97**	**3.97**	**3.91**
SD	**0.60**	**0.55**	**0.53**	**0.53**	**0.52**	**0.51**	**0.53**	**0.52**	**0.50**	**0.48**	**0.54**	**0.54**	**0.52**	**0.53**	**0.50**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 15. Mean velocity data (m s^{-1}) for each sprint (S) during the post-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	4.66	4.49	4.44	4.51	4.35	4.70	4.54	4.44	4.42	4.35	4.62	4.47	4.46	4.21	4.35
Subject 2	3.95	3.83	3.86	3.78	3.78	3.83	3.83	3.75	3.74	3.83	3.86	3.82	3.74	3.87	3.75
Subject 3	5.41	5.27	5.13	5.04	4.90	5.23	5.09	4.92	4.78	4.67	5.07	4.84	4.71	4.66	4.63
Subject 4	3.43	3.27	3.28	3.31	3.33	3.36	3.34	3.36	3.34	3.34	3.20	3.18	3.26	3.26	3.50
Subject 5	4.78	4.66	4.42	4.43	4.34	4.79	4.70	4.52	4.43	4.28	4.61	4.65	4.49	4.20	4.10
Subject 6	5.14	5.07	5.07	4.98	5.01	5.08	5.05	4.96	4.88	4.89	5.02	5.05	4.82	4.95	4.94
Subject 7	4.71	4.42	4.44	4.46	4.35	4.75	4.55	4.41	4.41	4.30	4.62	4.42	3.97	4.25	4.29
Subject 8	4.17	4.07	3.93	3.88	3.91	3.96	4.08	4.00	3.94	3.77	4.06	4.13	3.99	3.98	3.86
Subject 9	4.40	4.17	4.18	4.29	4.31	4.49	4.11	4.02	4.18	4.16	4.36	4.08	4.38	4.25	4.35
Subject 10	5.21	5.00	5.03	4.95	4.95	5.20	5.05	5.07	4.88	4.89	5.07	4.98	4.93	4.89	4.79
Mean	**4.59**	**4.42**	**4.38**	**4.36**	**4.32**	**4.54**	**4.44**	**4.35**	**4.30**	**4.25**	**4.45**	**4.36**	**4.27**	**4.25**	**4.26**
SD	**0.61**	**0.61**	**0.59**	**0.57**	**0.54**	**0.63**	**0.58**	**0.56**	**0.51**	**0.50**	**0.60**	**0.58**	**0.53**	**0.50**	**0.46**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 16. Peak velocity data (m s^{-1}) for each sprint (S) during the pre-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	5.12	5.02	4.57	4.84	4.70	5.36	4.85	4.69	4.43	4.78	5.09	5.17	4.60	4.84	4.93
Subject 2	4.22	4.25	4.19	4.21	4.20	4.33	4.29	4.23	4.20	4.18	4.41	4.51	4.44	4.12	4.16
Subject 3	5.62	5.48	5.37	5.31	5.39	5.50	6.03	5.43	5.25	5.46	5.54	5.42	5.31	5.24	5.82
Subject 4	4.04	3.92	3.91	3.97	3.94	4.12	3.98	4.18	4.10	4.01	4.39	4.34	3.92	4.24	4.47
Subject 5	5.35	4.99	4.94	5.08	5.12	5.12	5.17	4.80	4.77	4.78	5.11	4.69	4.77	4.90	4.89
Subject 6	5.88	5.84	5.70	5.65	5.58	5.84	5.75	5.62	5.95	5.76	5.75	5.85	6.01	5.49	5.49
Subject 7	5.15	4.85	4.82	4.84	4.94	5.40	4.98	4.71	4.53	4.62	5.14	4.67	4.46	4.43	4.50
Subject 8	4.92	4.41	4.54	4.43	4.37	4.76	4.69	4.42	4.17	3.99	4.46	4.25	4.27	4.17	4.12
Subject 9	5.28	4.99	5.08	5.54	5.06	5.26	5.00	4.69	4.85	4.93	4.85	5.05	4.66	5.14	4.52
Subject 10	6.38	5.79	5.64	5.98	5.49	5.82	5.69	5.71	5.59	5.37	5.90	5.66	5.51	5.83	5.43
Mean	**5.20**	**4.95**	**4.87**	**4.98**	**4.88**	**5.15**	**5.04**	**4.85**	**4.78**	**4.79**	**5.06**	**4.96**	**4.80**	**4.84**	**4.83**
SD	**0.70**	**0.64**	**0.59**	**0.65**	**0.56**	**0.58**	**0.65**	**0.55**	**0.63**	**0.61**	**0.55**	**0.56**	**0.63**	**0.59**	**0.59**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 17. Peak velocity data (m s^{-1}) for each sprint (S) during the post-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	5.47	5.41	5.18	5.62	5.17	5.47	5.32	5.21	5.57	5.00	5.77	5.24	5.13	5.22	5.08
Subject 2	4.71	4.49	4.76	4.49	4.43	4.56	4.57	4.49	4.43	4.68	4.54	4.81	4.38	4.77	4.37
Subject 3	6.34	6.40	5.91	5.77	5.71	6.22	6.42	5.74	5.58	5.79	6.05	5.76	5.40	5.53	5.39
Subject 4	4.17	3.92	4.00	4.23	4.30	4.07	4.02	4.05	3.97	4.12	4.03	3.91	4.10	3.92	4.14
Subject 5	5.77	5.54	5.31	5.22	5.15	5.54	5.88	5.38	5.18	5.11	5.45	5.73	5.25	5.17	4.90
Subject 6	6.04	5.96	5.90	5.86	5.84	6.40	5.93	6.25	5.78	5.68	5.96	5.90	5.77	5.73	6.18
Subject 7	5.69	5.29	5.51	5.23	5.20	5.75	5.39	5.30	5.53	4.99	5.46	5.61	5.12	5.05	5.33
Subject 8	4.80	4.96	4.50	4.49	4.48	4.60	4.72	4.96	4.68	4.42	4.85	4.71	4.58	4.57	4.51
Subject 9	5.23	4.95	5.19	5.07	5.49	5.62	4.90	4.84	5.03	5.06	5.37	5.00	5.09	5.09	5.16
Subject 10	6.12	5.95	5.92	5.73	5.72	6.18	5.95	5.86	5.67	5.72	6.36	5.82	5.94	5.69	6.04
Mean	**5.43**	**5.29**	**5.22**	**5.17**	**5.15**	**5.44**	**5.31**	**5.21**	**5.14**	**5.06**	**5.38**	**5.25**	**5.08**	**5.07**	**5.11**
SD	**0.70**	**0.74**	**0.65**	**0.60**	**0.57**	**0.79**	**0.75**	**0.66**	**0.61**	**0.56**	**0.72**	**0.64**	**0.58**	**0.55**	**0.67**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 18. Mean power data (W) for each sprint (S) during the pre-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	710	700	671	635	632	785	730	716	647	672	781	690	675	704	664
Subject 2	543	539	566	557	552	579	549	546	538	531	580	564	552	538	543
Subject 3	969	910	895	831	866	906	896	902	841	792	946	877	872	776	863
Subject 4	501	478	482	484	478	513	480	481	484	485	505	493	459	461	495
Subject 5	820	741	708	634	630	777	718	662	660	609	734	622	638	558	576
Subject 6	1065	1050	1009	991	963	1043	1035	972	972	927	1007	984	948	932	924
Subject 7	752	719	735	746	691	790	767	736	673	687	718	713	658	626	595
Subject 8	604	557	567	533	545	578	593	543	524	488	584	484	527	485	530
Subject 9	815	767	783	824	814	790	738	709	734	720	724	728	679	717	615
Subject 10	988	953	919	881	890	965	886	897	873	841	1011	933	860	901	856
Mean	**777**	**741**	**733**	**711**	**706**	**773**	**739**	**716**	**695**	**675**	**759**	**709**	**687**	**670**	**666**
SD	**193**	**187**	**171**	**168**	**167**	**174**	**170**	**167**	**161**	**150**	**180**	**177**	**160**	**166**	**156**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 19. Mean power data (W) for each sprint (S) during the post-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	872	813	809	820	776	895	836	819	783	778	874	828	817	732	775
Subject 2	627	599	595	582	578	617	596	580	581	597	607	595	565	591	569
Subject 3	1122	1097	1052	1019	981	1094	1049	964	925	875	1046	938	868	844	828
Subject 4	511	474	489	480	480	501	486	488	486	483	475	451	477	467	523
Subject 5	901	860	774	767	741	903	853	813	750	712	830	799	771	705	663
Subject 6	1042	1030	1002	1004	981	1024	1013	987	974	938	1023	997	960	966	952
Subject 7	895	813	794	796	768	901	832	799	770	737	848	786	677	735	723
Subject 8	672	622	579	581	575	609	634	621	608	548	646	638	605	599	570
Subject 9	854	779	769	803	774	881	754	743	780	757	846	754	798	770	790
Subject 10	1084	1024	993	980	954	1097	1015	1001	943	939	1088	995	953	930	910
Mean	**858**	**811**	**786**	**783**	**761**	**852**	**807**	**782**	**760**	**736**	**828**	**778**	**749**	**734**	**730**
SD	**202**	**204**	**191**	**188**	**177**	**209**	**191**	**176**	**163**	**158**	**201**	**178**	**164**	**155**	**147**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 20. Peak power data (W) for each sprint (S) during the pre-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	1,994	2,074	1,661	1,710	1,791	1,822	2,131	1,997	1,642	1,854	2,256	1,791	1,855	1,688	1,648
Subject 2	1,543	1,923	1,549	1,441	1,342	1,460	1,453	1,307	1,509	1,588	1,326	1,296	1,335	1,301	1,301
Subject 3	3,515	3,424	3,078	2,978	2,729	2,630	2,949	3,829	2,937	3,639	3,000	2,815	2,928	2,954	3,216
Subject 4	1,362	1,455	1,104	1,203	1,171	1,193	1,144	1,077	1,179	1,251	1,198	1,304	1,028	1,094	1,124
Subject 5	2,029	1,658	1,861	1,495	1,704	1,757	1,810	1,798	1,505	1,327	1,821	1,507	1,425	1,654	1,384
Subject 6	3,043	2,648	2,758	3,094	3,075	3,150	2,513	2,931	2,555	2,795	3,046	2,531	2,086	2,607	2,064
Subject 7	1,651	1,514	1,499	1,599	1,556	1,592	1,524	1,470	1,408	1,394	1,593	1,479	1,217	1,429	1,253
Subject 8	1,902	2,064	2,149	1,847	2,257	2,072	2,158	1,906	2,154	1,909	1,641	1,991	2,085	2,101	2,004
Subject 9	2,430	2,224	2,368	2,227	2,423	2,223	2,095	2,248	2,167	2,084	1,895	1,867	1,642	1,842	1,629
Subject 10	2,800	2,419	2,403	2,699	2,333	2,935	2,784	2,871	2,531	2,737	3,079	2,744	2,490	2,875	2,356
Mean	**2,227**	**2,140**	**2,043**	**2,029**	**2,038**	**2,083**	**2,056**	**2,143**	**1,959**	**2,058**	**2,086**	**1,932**	**1,809**	**1,954**	**1,798**
SD	**702**	**591**	**618**	**679**	**621**	**648**	**586**	**849**	**591**	**776**	**722**	**578**	**599**	**659**	**637**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 21. Peak power data (W) for each sprint (S) during the post-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	2,299	2,026	1,943	2,131	2,285	2,591	2,160	2,435	2,208	2,219	2,552	2,069	2,374	2,233	2,101
Subject 2	1,543	1,724	1,601	1,487	1,647	1,412	1,506	1,917	1,608	1,810	1,512	1,651	1,442	1,665	1,571
Subject 3	4,386	4,823	4,419	4,198	3,860	4,330	3,982	3,605	3,633	3,633	3,379	3,501	3,185	3,180	3,140
Subject 4	1,183	1,083	1,049	952	1,012	1,128	1,255	1,019	1,035	940	1,107	1,093	1,091	1,013	1,030
Subject 5	2,000	2,157	1,987	1,940	1,796	1,998	2,185	2,000	1,785	1,679	1,887	2,349	1,746	1,518	1,416
Subject 6	3,037	2,895	3,022	3,104	2,521	2,730	2,680	2,860	2,297	2,812	2,828	2,799	2,603	2,837	2,952
Subject 7	2,116	2,095	1,998	1,773	1,954	2,107	1,953	1,950	1,968	1,708	2,322	2,014	1,710	1,895	1,900
Subject 8	2,135	2,085	1,698	2,126	1,745	2,022	1,907	1,943	2,043	1,643	2,091	1,982	1,778	1,729	1,858
Subject 9	2,605	2,158	1,984	2,074	2,230	2,501	2,045	1,814	2,071	1,747	2,118	1,747	2,274	2,008	2,420
Subject 10	2,909	2,435	2,683	2,534	2,332	2,585	2,450	2,436	2,424	2,215	2,885	2,366	2,203	2,407	2,105
Mean	**2,421**	**2,348**	**2,238**	**2,232**	**2,138**	**2,341**	**2,212**	**2,198**	**2,107**	**2,041**	**2,268**	**2,157**	**2,041**	**2,048**	**2,049**
SD	**894**	**985**	**940**	**898**	**745**	**873**	**746**	**692**	**667**	**741**	**678**	**659**	**609**	**640**	**656**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 22. Acceleration data (m s^{-2}) for each sprint (S) during the pre-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	3.22	3.50	3.30	3.19	3.38	3.77	3.69	4.09	3.07	3.37	3.43	2.84	3.36	3.83	3.34
Subject 2	2.30	3.07	3.32	2.84	3.14	2.73	2.68	2.99	3.05	3.02	3.09	3.03	3.43	3.23	3.02
Subject 3	4.01	4.05	3.80	3.95	4.06	3.36	3.53	3.58	3.38	3.63	4.08	3.52	4.23	3.40	3.47
Subject 4	1.81	2.22	2.31	2.35	2.34	2.57	2.19	2.36	2.56	2.73	2.99	2.80	2.15	2.29	2.21
Subject 5	3.19	3.15	2.56	2.96	2.66	2.52	3.10	2.93	2.41	2.78	2.33	2.36	2.79	3.27	2.72
Subject 6	5.51	5.18	5.48	5.10	5.21	4.94	5.38	5.18	5.45	4.95	4.94	4.78	5.14	4.98	4.76
Subject 7	3.47	3.42	3.57	4.04	3.34	3.73	3.76	4.01	4.08	3.94	3.29	3.67	3.29	2.91	3.73
Subject 8	2.79	2.74	2.85	2.65	2.82	2.75	3.02	3.02	2.86	2.76	2.74	2.54	2.77	2.70	2.62
Subject 9	3.58	3.58	4.38	4.11	4.28	3.67	3.80	3.58	3.84	3.98	3.45	4.02	3.26	3.78	2.98
Subject 10	3.91	3.95	4.17	4.30	4.33	4.52	4.22	4.36	4.13	4.65	4.48	4.29	4.19	5.43	4.25
Mean	**3.38**	**3.49**	**3.57**	**3.55**	**3.55**	**3.45**	**3.54**	**3.61**	**3.48**	**3.58**	**3.48**	**3.38**	**3.46**	**3.58**	**3.31**
SD	**1.02**	**0.81**	**0.94**	**0.88**	**0.89**	**0.83**	**0.88**	**0.83**	**0.92**	**0.80**	**0.80**	**0.80**	**0.86**	**0.98**	**0.78**
n	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 23. Acceleration data (m s^{-2}) for each sprint (S) during the post-training RSE trial

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Subject 1	4.38	4.13	4.14	4.41	4.00	4.76	4.57	3.86	3.88	4.20	4.24	4.56	4.45	4.00	4.10
Subject 2	3.52	3.81	3.65	3.31	3.50	3.40	3.11	3.12	3.25	3.30	3.55	3.29	3.40	3.75	3.50
Subject 3	5.63	5.45	5.39	5.11	4.64	5.21	4.96	4.90	4.69	4.34	4.66	3.97	4.73	4.06	4.78
Subject 4	2.59	2.70	2.69	2.56	2.87	2.57	2.67	2.85	2.77	2.37	1.71	2.13	2.33	2.55	2.92
Subject 5	4.51	4.37	4.01	4.12	3.95	4.38	4.52	3.90	4.25	3.59	4.18	4.77	4.12	3.42	3.52
Subject 6	5.14	4.84	5.36	4.66	5.18	4.95	5.07	4.67	4.81	5.00	4.85	5.06	4.40	5.06	4.95
Subject 7	4.06	3.57	3.89	4.29	3.81	4.16	4.08	3.21	4.22	4.09	4.51	4.00	1.95	3.77	3.99
Subject 8	3.91	4.17	4.66	3.77	4.19	3.70	3.80	4.00	3.38	3.44	3.66	4.09	3.52	3.85	3.57
Subject 9	4.38	4.11	3.89	4.43	3.85	3.74	3.77	3.20	3.77	3.61	3.78	3.44	4.54	3.71	4.83
Subject 10	5.54	4.84	5.10	4.88	5.24	5.24	4.97	5.26	4.72	5.13	4.65	4.88	5.06	4.85	4.63
Mean	**4.36**	**4.20**	**4.28**	**4.15**	**4.12**	**4.21**	**4.15**	**3.90**	**3.97**	**3.91**	**3.98**	**4.02**	**3.85**	**3.90**	**4.08**
SD	**0.93**	**0.76**	**0.85**	**0.76**	**0.73**	**0.87**	**0.82**	**0.83**	**0.69**	**0.82**	**0.92**	**0.89**	**1.04**	**0.70**	**0.70**
N	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 24. Capillary blood lactate concentration (mM) at rest and following each set of sprints during the pre- and post-training RSE

	Pre-training				Post-training			
	Rest	**Set1**	**Set2**	**Set3**	**Rest**	**Set1**	**Set2**	**Set3**
Subject 1	0.8	7.3	8.8	9.6	1.3	6.8	9.8	10.2
Subject 2	1.2	6.6	9.9	10.0	1.4	6.1	7.9	8.7
Subject 3	1.2	13.2	15.7	16.8	0.8	15.0	16.9	16.6
Subject 4	1.0	6.2	7.8	7.8	1.9	6.9	6.8	7.7
Subject 5	1.4	10.8	13.2	13.0	1.3	9.7	13.4	15.4
Subject 6	1.4	10.0	13.9	14.9	1.1	9.3	11.7	12.3
Subject 7	1.9	9.4	13.0	15.1	2.2	8.1	11.8	15.8
Subject 8	1.4	10.0	10.2	11.3	1.4	7.4	10.0	10.7
Subject 9	1.0	9.4		11.9	1.1	10.7	14.4	13.8
Subject 10	1.0	7.7	11.1	12.9		8.1	12.2	14.9
Mean	**1.2**	**9.1**	**11.5**	**12.3**	**1.4**	**8.8**	**11.5**	**12.6**
SD	**0.3**	**2.1**	**2.6**	**2.8**	**0.4**	**2.6**	**3.0**	**3.2**
n	**10**	**10**	**9**	**10**	**9**	**10**	**10**	**10**

Table 25. The $\Delta[Lac^-]$-to-work ratio (nmol L^{-1} J^{-1}) following each set of sprints during the pre- and post-training RSE

	Pre-training			Post-training		
	Set1/rest	**Set2/rest**	**Set3/rest**	**Set1/rest**	**Set2/rest**	**Set3/rest**
Subject 1	485	563	626	336	517	553
Subject 2	490	793	792	394	547	624
Subject 3	671	836	900	673	820	873
Subject 4	536	696	705	514	501	606
Subject 5	665	861	927	519	751	936
Subject 6	423	631	704	405	537	572
Subject 7	515	759	997	363	594	902
Subject 8	766	807	948	495	712	760
Subject 9	525		787	603	849	802
Subject 10	362	566	652	333	541	693
Mean	**544**	**724**	**804**	**464**	**637**	**732**
SD	**123**	**104**	**132**	**116**	**133**	**143**
n	**10**	**9**	**10**	**10**	**10**	**10**

Table 26. Mean heart rate (beats min^{-1}) during the 4.5 min recovery following each set of sprints during the pre- and post-training RSE

	Pre-training			**Post-training**		
	Set1	**Set2**	**Set3**	**Set1**	**Set2**	**Set3**
Subject 1	125	130	132	116	123	126
Subject 2	123	124	131	117	127	127
Subject 3	145	145	142	131	137	140
Subject 4	140	143	146	137	139	143
Subject 5	129	127	128	107	120	124
Subject 6						
Subject 7	147	149	141	138	142	143
Subject 8	140	143	143	128	135	136
Subject 9						
Subject 10	139	141	143	128	133	136
Mean	**136**	**138**	**138**	**125**	**132**	**135**
SD	**9**	**9**	**7**	**11**	**8**	**8**
n	**8**	**8**	**8**	**8**	**8**	**8**

Table 27. Standard deviation of the normal R-R heart rate intervals (ms) during the last 3 min of recovery following each set of sprints during the pre- and post-training RSE

	Pre-training			**Post-training**		
	Set1	**Set2**	**Set3**	**Set1**	**Set2**	**Set3**
Subject 1						
Subject 2	35.6	23.5	22.5	32.3	20.1	31.6
Subject 3	23.1	26.3	25.3	49.0	31.7	27.1
Subject 4	29.1	23.6	18.2	27.8	26.5	36.6
Subject 5	65.9	47.5	37.0	90.2	52.7	48.3
Subject 6						
Subject 7	26.2	21.4	34.5	38.6	41.9	37.2
Subject 8	32.7	24.0	16.9	30.9	34.1	30.0
Subject 9						
Subject 10	42.4	50.3	32.5	49.6	41.4	61.8
Mean	**36.4**	**30.9**	**26.7**	**45.5**	**35.5**	**38.9**
SD	**14.5**	**12.4**	**8.0**	**21.5**	**10.8**	**12.2**
n	**7**	**7**	**7**	**7**	**7**	**7**

Table 28. Root-mean-square difference of successive normal R-R heart rate intervals (ms) during the last 3 min of recovery following each set of sprints during the pre- and post-training RSE

	Pre-training			**Post-training**		
	Set1	**Set2**	**Set3**	**Set1**	**Set2**	**Set3**
Subject 1						
Subject 2	7.1	5.6	4.3	12.0	6.0	5.1
Subject 3	3.4	3.1	3.4	6.6	4.2	2.9
Subject 4	7.5	5.4	3.8	8.3	5.4	3.8
Subject 5	9.7	13.1	13.5	32.8	12.3	7.2
Subject 6						
Subject 7	3.4	2.9	4.3	5.3	4.3	3.7
Subject 8	4.6	4.9	3.1	3.7	3.9	3.8
Subject 9						
Subject 10	6.8	5.1	4.6	8.8	7.0	6.1
Mean	**6.1**	**5.7**	**5.3**	**11.1**	**6.2**	**4.7**
SD	**2.4**	**3.4**	**3.7**	**9.9**	**2.9**	**1.5**
n	**7**	**7**	**7**	**7**	**7**	**7**

Table 29. Peak oxygen uptake ($\dot{V}O_{2peak}$), lactate threshold (LT), onset of blood lactate accumulation (OBLA) and Yo-Yo Intermittent Recovery Test Level 1 (Yo-Yo IR1), before and after training

	$\dot{V}O_{2peak}$ (mL kg^{-1} min^{-1})		**$\dot{V}O_{2peak}$ (L min^{-1})**		**LT (km h^{-1})**		**OBLA (km h^{-1})**		**Yo-Yo IR2 (m)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Subject 1	63.3	64.5	4.18	4.25	13.4	13.4	14.8	15.1	1960	2000
Subject 2									440	640
Subject 3	58.2	59.8	3.90	4.07	12.0	10.5	12.0	12.5	2080	2120
Subject 4	48.0	46.8	3.08	2.81						
Subject 5	54.1	55.4	3.73	3.77	9.1	10.5	10.2	10.8	960	1040
Subject 6	58.9	61.2	4.65	4.77	12.0	12.0	13.4	13.3	1880	2120
Subject 7	48.5	49.6	3.30	3.47	12.0	10.5	12.0	11.7		
Subject 8	52.5	53.4	2.78	2.83	8.0	8.0	10.2	10.0	720	840
Subject 9	41.3	45.2	4.01	4.34					520	480
Subject 10	58.7	57.5	4.47	4.43	13.4	12.0	13.9	13.7	1880	1960
Mean	**53.7**	**54.8**	**3.79**	**3.86**	**11.4**	**11.0**	**12.4**	**12.4**	**1305**	**1400**
SD	**6.9**	**6.6**	**0.63**	**0.70**	**2.1**	**1.7**	**1.8**	**1.8**	**709**	**715**
n	**9**	**9**	**9**	**9**	**7**	**7**	**7**	**7**	**8**	**8**

APPENDIX 7

RAW DATA FROM CHAPTER 4

Table 30. Nuclear respiratory factor 1 mRNA expression (AU) at rest, immediately after, and 1 h and 4 h after RSE, pre- and post-training

	Pre-training				Post-training			
	Rest	**End**	**1 h**	**4 h**	**Rest**	**End**	**1 h**	**4 h**
Subject 1	11.2	5.7	4.0	9.0	9.2	4.4	14.7	66.2
Subject 2	7.6	23.0	8.6	5.3	4.9	3.8	1.8	4.7
Subject 3	2.7	4.8	5.9	3.3	1.3	9.1	3.4	23.3
Subject 4	3.0	2.1	0.6			4.0	4.5	8.4
Subject 5	3.0	7.0	0.8	17.0		6.2	4.4	0.8
Subject 6	3.4	1.6	1.3	1.9	3.6	6.6	5.0	3.5
Subject 7	7.0	9.3	10.8	12.6			5.2	21.7
Subject 8	7.8	10.4	13.4	19.4	10.9		9.2	9.0
Subject 9	6.9	21.4	4.1	10.7	15.6	12.3	7.2	12.8
Subject 10	5.2	10.8	6.3	20.9	7.6	7.4	6.0	5.0
Mean	**5.8**	**9.6**	**5.6**	**10.0**	**5.3**	**5.4**	**6.1**	**15.5**
SD	**2.8**	**7.4**	**4.3**	**7.4**	**5.4**	**3.8**	**3.6**	**19.3**
n	**10**	**10**	**10**	**9**	**7**	**8**	**10**	**10**

Table 31. Myocyte enhancer factor 2A mRNA expression (AU) at rest, immediately after, and 1 h and 4 h after RSE, pre- and post-training

	Pre-training				**Post-training**			
	Rest	**End**	**1 h**	**4 h**	**Rest**	**End**	**1 h**	**4 h**
Subject 1	4.8	2.6	2.2	3.3	4.4	2.2	8.9	10.7
Subject 2	1.8	5.8	3.4	2.7	2.0	2.5	0.8	1.8
Subject 3	1.5	2.3	2.5	1.9	0.3	2.6	1.0	3.6
Subject 4	3.4	1.0	0.8			1.9	2.3	4.0
Subject 5	0.3	1.3	11.7	40.3		7.1	1.9	1.0
Subject 6	4.7	2.3	7.4	1.8	2.6	3.9	2.7	2.2
Subject 7	2.2	3.0	3.0	2.9			2.6	3.6
Subject 8	2.5	2.3	3.7	3.8	2.4		2.5	1.8
Subject 9	2.6	7.2	3.5	3.9	6.3	2.5	3.3	2.8
Subject 10	1.7	2.0	4.1	9.0	2.9	3.0	3.7	4.9
Mean	**2.6**	**3.0**	**4.2**	**7.7**	**3.0**	**3.2**	**3.0**	**3.6**
SD	**1.4**	**2.0**	**3.1**	**12.4**	**1.9**	**1.7**	**2.3**	**2.7**
n	**10**	**10**	**10**	**9**	**7**	**8**	**10**	**10**

Table 32. Mitochondrial transcription factor A mRNA expression (AU) at rest, immediately after, and 1 h and 4 h after RSE, pre- and post-training

	Pre-training				Post-training			
	Rest	**End**	**1 h**	**4 h**	**Rest**	**End**	**1 h**	**4 h**
Subject 1	10.4	8.5	10.8	12.9	19.7	9.5	36.6	52.0
Subject 2	10.3	24.2	13.3	7.4	10.5	19.1	6.9	14.7
Subject 3	10.1	5.5	20.2	13.6	2.2	20.3	8.9	13.3
Subject 4	24.7	11.8	5.1			20.7	26.5	35.2
Subject 5	15.4	21.3	19.5	11.8		7.8	18.2	9.6
Subject 6	10.1	5.5	2.7	5.9	13.4	16.9	12.7	13.3
Subject 7	13.5	17.8	17.4	19.8			13.3	19.4
Subject 8	15.1	14.4	28.1	30.1	9.1		17.6	15.6
Subject 9	18.5	42.4	21.0	23.1	34.3	16.3	19.0	15.2
Subject 10	9.9	10.8	23.2	62.5	24.6	20.4	19.9	29.2
Mean	**13.8**	**16.2**	**16.1**	**20.8**	**16.3**	**16.4**	**18.0**	**21.7**
SD	**4.8**	**11.2**	**8.1**	**17.4**	**10.8**	**5.0**	**8.7**	**13.2**
n	**10**	**10**	**10**	**9**	**7**	**8**	**10**	**10**

Table 33. Peroxisome proliferator-activated receptor γ, coactivator 1 α (AU) mRNA expression at rest, immediately after, and 1 h and 4 h after RSE, pre- and post-training

	Pre-training				Post-training			
	Rest	**End**	**1 h**	**4 h**	**Rest**	**End**	**1 h**	**4 h**
Subject 1	1.9	12.9	20.8	2.7	31.8	15.5	45.2	102.4
Subject 2	21.1	42.4	23.8	37.6	14.3	11.9	7.7	8.5
Subject 3	10.2	9.4	43.7	27.9	3.6	18.0	10.6	98.3
Subject 4	24.0	24.3	8.7			29.2	30.5	47.9
Subject 5	12.7	25.9	23.4	67.2		11.3	23.7	44.4
Subject 6	15.4	7.2	3.6	19.9	17.2	17.8	14.6	56.7
Subject 7	5.8	10.6	10.9	12.2			8.3	15.6
Subject 8	18.2	18.3	23.8	32.0	13.8		14.6	20.4
Subject 9	15.6	34.8	20.3	59.1	33.0	20.1	22.2	36.1
Subject 10	9.4	10.4	12.8	113.9	26.1	22.8	38.4	93.9
Mean	**13.4**	**19.6**	**19.2**	**41.4**	**20.0**	**18.3**	**21.6**	**52.4**
SD	**6.8**	**11.9**	**11.2**	**34.2**	**10.8**	**5.9**	**13.0**	**35.0**
n	**10**	**10**	**10**	**9**	**7**	**8**	**10**	**10**

Table 34. Cytochrome c oxidase subunit IV mRNA expression (AU) at rest, immediately after, and 1 h and 4 h after RSE, pre- and post-training

	Pre-training				**Post-training**			
	Rest	**End**	**1 h**	**4 h**	**Rest**	**End**	**1 h**	**4 h**
Subject 1	45.9	22.9	36.7	54.4	37.2	33.4	145.1	121.7
Subject 2	57.2	81.2	45.2	27.0	9.7	17.9	8.3	13.4
Subject 3	21.9	25.9	22.0	11.3	2.8	29.3	16.4	95.8
Subject 4	40.7	20.1	4.7			25.6	30.0	96.1
Subject 5	24.0	20.2	36.5	15.3		24.5	20.4	9.2
Subject 6	32.3	11.7	5.6	11.8	41.0	46.4	38.2	32.1
Subject 7	18.0	27.9	28.2	26.9			26.7	23.8
Subject 8	34.6	29.0	39.1	39.7	20.6		14.2	13.4
Subject 9	27.2	57.3	24.4	52.6	43.9	28.6	29.3	36.5
Subject 10	13.5	26.2	45.2	116.2	35.6	37.8	58.1	30.1
Mean	**31.5**	**32.2**	**28.8**	**39.5**	**27.3**	**30.4**	**38.7**	**47.2**
SD	**13.5**	**20.9**	**14.7**	**33.1**	**16.2**	**8.7**	**39.9**	**41.1**
n	**10**	**10**	**10**	**9**	**7**	**8**	**10**	**10**

Table 35.Total AMPKα/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			Post-training		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	2.8	2.6	3.2	3.8	4.0	4.2
Subject 2	2.8	2.6	2.2	3.3	3.0	2.3
Subject 3	2.5	2.5	6.5	5.3	7.9	5.1
Subject 4	4.0	2.5	3.4	3.2	5.2	6.8
Subject 5	3.1	5.3	3.8	4.9	3.1	2.3
Subject 6	2.8	2.8	2.8	2.3	3.3	6.1
Subject 7	3.7	4.1	4.7	4.9	3.6	3.3
Subject 8	3.2	3.9	3.8	3.5	3.8	3.3
Subject 9	4.9	6.2	7.7	6.2	6.4	7.6
Subject 10	4.8	3.2	1.9	3.3	4.2	4.1
Mean	**3.4**	**3.6**	**4.0**	**4.1**	**4.4**	**4.5**
SD	**0.9**	**1.3**	**1.8**	**1.2**	**1.6**	**1.8**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 36.Phosphorylated AMPKα/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	2.7	4.5	4.8	4.8	4.7	4.8
Subject 2	4.5	7.7	6.8	7.5	7.7	5.0
Subject 3	4.6	10.7	12.9	13.9	20.9	13.3
Subject 4	12.1	13.3	13.1	15.6	16.0	17.1
Subject 5	7.6	5.4	10.2	6.3	5.2	4.5
Subject 6	4.2	5.0	6.3	8.3	7.1	9.2
Subject 7	14.9	10.3	11.8	15.8	10.3	8.5
Subject 8	8.9	10.9	9.8	9.8	10.3	8.6
Subject 9	4.6	8.1	12.5	9.7	10.4	8.6
Subject 10	6.8	8.5	5.5	6.3	3.7	4.4
Mean	**7.1**	**8.4**	**9.4**	**9.8**	**9.6**	**8.4**
SD	**3.9**	**2.9**	**3.2**	**4.0**	**5.4**	**4.2**
	10	**10**	**10**	**10**	**10**	**10**

Table 37. Total ACC/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			Post-training		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1						
Subject 2	14.4	15.1	14.3	18.9	12.2	8.7
Subject 3	4.0	8.5	22.9	18.4	24.6	15.5
Subject 4	10.9	16.8	12.3	17.0	22.0	16.4
Subject 5	13.1	20.3	16.1	29.9	39.3	25.2
Subject 6	30.7	46.2	39.1	29.9	24.2	15.1
Subject 7	9.0	20.0	23.6	23.5	21.9	27.8
Subject 8	25.4	23.6	15.0	13.9	14.7	18.2
Subject 9	13.7	31.2	34.7	40.9	45.7	41.3
Subject 10	19.7	30.8	25.9	13.2	15.7	13.1
Mean	**15.6**	**23.6**	**22.7**	**22.8**	**24.5**	**20.1**
SD	**8.3**	**11.1**	**9.4**	**9.1**	**11.2**	**9.9**
n	**9**	**9**	**9**	**9**	**9**	**9**

Table 38. Phosphorylated ACC/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1						
Subject 2	9.6	10.4	5.7	6.1	5.9	5.7
Subject 3	9.3	13.5	10.1	8.7	17.6	6.0
Subject 4	7.0	12.8	8.0	12.0	15.6	13.8
Subject 5	9.2	21.4	11.8	11.4	31.8	14.7
Subject 6	7.1	24.5	10.9	7.9	30.2	12.8
Subject 7	11.9	18.7	16.7	16.0	16.6	9.3
Subject 8	7.3	21.5	9.5	7.5	15.5	7.7
Subject 9	4.0	3.8	6.1	7.0	7.8	9.2
Subject 10	5.5	5.4	3.9	3.9	6.0	2.8
Mean	**7.9**	**14.7**	**9.2**	**8.9**	**16.3**	**9.1**
SD	**2.4**	**7.3**	**3.8**	**3.6**	**9.5**	**4.0**
n	**9**	**9**	**9**	**9**	**9**	**9**

Table 39. Total p38 MAPK/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	6.0	5.8	6.2	4.9	4.4	6.2
Subject 2	3.2	4.1	2.4	2.0	1.8	1.9
Subject 3	2.4	5.0	6.5	3.3	4.6	2.0
Subject 4	2.7	4.2	5.7	4.8	5.1	7.9
Subject 5	12.4	14.8	11.3	12.2	9.5	5.5
Subject 6	5.2	6.5	7.8	7.5	10.4	8.2
Subject 7	6.7	6.2	6.1	7.0	8.7	3.6
Subject 8	3.7	3.5	1.9	1.7	2.3	2.4
Subject 9	2.2	3.1	3.7	2.7	3.8	3.9
Subject 10	6.1	6.0	4.6	5.6	3.0	4.3
Mean	**5.1**	**5.9**	**5.6**	**5.2**	**5.3**	**4.6**
SD	**3.1**	**3.3**	**2.7**	**3.2**	**3.1**	**2.3**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 40. Phosphorylated p38 MAPK/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	8.4	9.0	9.8	4.7	3.6	12.9
Subject 2	4.7	7.2	4.1	2.9	3.9	2.1
Subject 3	3.0	8.4	9.0	8.9	11.9	7.2
Subject 4	7.6	15.4	6.7	8.9	6.5	13.5
Subject 5	22.4	17.9	13.8	10.0	8.6	7.4
Subject 6	5.3	6.0	10.6	5.0	9.3	9.7
Subject 7	9.1	7.9	12.1	9.9	27.6	11.1
Subject 8	1.8	3.2	3.3	5.2	6.2	4.7
Subject 9	2.5	2.2	2.9	2.9	3.1	2.1
Subject 10	4.1	5.2	2.7	5.0	3.6	2.0
Mean	**6.9**	**8.2**	**7.5**	**6.4**	**8.4**	**7.3**
SD	**6.0**	**5.0**	**4.1**	**2.8**	**7.3**	**4.5**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 41. Phosphorylated CaMKII/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	8.1	27.4	11.5	9.1	17.9	14.2
Subject 2	11.0	17.0	12.8	6.6	6.7	3.5
Subject 3	11.0	2.5	6.5	4.0	3.7	1.8
Subject 4	2.3	9.4	4.1	5.3	11.4	7.3
Subject 5	5.7	5.1	22.0	4.4	4.5	3.8
Subject 6	3.7	6.2	9.1	3.9	2.8	10.3
Subject 7	6.1	5.6	7.7	9.5	14.0	6.0
Subject 8	3.5	4.9	2.1	3.4	3.5	3.0
Subject 9	7.6	7.6	22.2	6.3	11.3	8.2
Subject 10	2.4	3.6	5.7	12.0	4.6	4.2
Mean	**6.1**	**8.9**	**10.4**	**6.5**	**8.0**	**6.2**
SD	**3.2**	**7.6**	**7.0**	**2.9**	**5.2**	**3.9**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 42. Total PGC-1α/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			Post-training		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	4.8	3.3	2.7	3.6	2.3	1.5
Subject 2	2.2	2.0	2.7	3.0	4.1	2.4
Subject 3	2.1	8.0	5.6	6.3	3.9	3.0
Subject 4	4.9	4.5	3.0	7.4	3.6	2.5
Subject 5	2.0	2.7	2.1	4.0	5.6	1.9
Subject 6	2.6	2.4	1.2	3.4	3.9	1.5
Subject 7	2.2	3.3	4.0	4.9	6.2	2.7
Subject 8	4.4	2.7	3.9	3.6	3.9	4.0
Subject 9	2.3	1.7	2.4	3.1	2.0	2.8
Subject 10	7.3	6.9	6.7	7.0	6.2	9.5
Mean	**3.5**	**3.8**	**3.4**	**4.6**	**4.2**	**3.2**
SD	**1.8**	**2.1**	**1.7**	**1.7**	**1.4**	**2.3**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 43. Phosphorylated HDAC5/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			Post-training		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	7.1	33.9	11.3	8.4	21.0	10.3
Subject 2	5.1	8.1	3.7	1.1	1.0	1.0
Subject 3	1.5	26.4	1.0	1.9	1.5	1.0
Subject 4	2.4	6.2	1.8	1.5	4.8	2.9
Subject 5	14.0	8.8	30.4	7.8	4.6	6.5
Subject 6	10.8	7.9	16.4	5.2	3.5	14.0
Subject 7	5.4	4.0	8.1	8.8	7.7	3.5
Subject 8	2.3	3.3	2.0	2.9	2.4	4.2
Subject 9	2.6	2.1	12.8	3.8	7.0	3.6
Subject 10	2.0	1.6	2.6	3.9	1.5	1.1
Mean	**5.3**	**10.2**	**9.0**	**4.5**	**5.5**	**4.8**
SD	**4.2**	**10.9**	**9.2**	**2.9**	**5.9**	**4.3**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 44. Total RIP 140/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			**Post-training**		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	8.1	11.7	13.7	12.5	10.7	12.6
Subject 2	12.8	13.0	12.0	16.9	16.8	16.9
Subject 3	3.4	7.2	6.1	7.0	8.7	8.2
Subject 4	18.5	17.2	13.3	3.8	13.9	11.4
Subject 5	13.7	7.2	4.8	3.1	3.0	2.3
Subject 6	8.1	7.2	4.0	20.1	13.2	5.2
Subject 7	12.0	4.7	9.7	10.5	5.3	4.4
Subject 8	10.7	10.6	6.8	6.9	7.2	8.1
Subject 9	8.4	12.3	11.0	12.6	11.7	12.3
Subject 10	14.1	21.1	17.5	20.9	13.2	7.2
Mean	**11.0**	**11.2**	**9.9**	**11.4**	**10.4**	**8.9**
SD	**4.2**	**5.0**	**4.4**	**6.4**	**4.3**	**4.4**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 45. Total COX IV/GAPDH protein abundance (AU) at rest, immediately after, and 1 h after RSE, pre- and post-training

	Pre-training			Post-training		
	Rest	**End**	**1 h**	**Rest**	**End**	**1 h**
Subject 1	14.4	12.9	13.2	12.4	12.4	10.7
Subject 2	8.1	9.9	13.7	15.1	15.5	11.4
Subject 3	9.2	8.8	6.7	6.8	7.2	5.8
Subject 4	14.0	12.1	13.2	13.5	12.5	9.2
Subject 5	12.8	7.5	8.3	6.9	5.8	4.0
Subject 6	8.6	10.2	14.3	17.8	13.1	8.7
Subject 7	6.7	4.9	5.9	7.9	5.6	3.4
Subject 8	7.4	9.3	6.8	7.9	7.1	6.3
Subject 9	3.7	3.9	3.9	4.2	5.0	5.7
Subject 10	6.5	10.8	9.1	11.7	8.4	5.8
Mean	**9.1**	**9.0**	**9.5**	**10.4**	**9.2**	**7.1**
SD	**3.5**	**2.9**	**3.8**	**4.3**	**3.7**	**2.8**
n	**10**	**10**	**10**	**10**	**10**	**10**

Table 46.Venous blood lactate concentration (mM) at rest, following each set of sprints, and after 1, 2, 5, 10, 20, and 30 min of recovery after the pre-training RSE

	Rest	**Set1**	**Set2**	**Set3**	**+1**	**+2**	**+5**	**+10**	**+20**	**+30**
Subject 1	0.47	6.59	7.31	7.60	8.75	8.57	7.93	6.76	4.77	3.36
Subject 2	0.69	6.89	7.57	7.45	7.63	7.83	7.51	6.88	5.32	4.37
Subject 3	0.56	11.05	11.10	10.35	10.30	10.65	10.20	10.30	7.62	5.37
Subject 4	0.64	6.70	7.12	6.86	6.50	7.33	6.86	6.20	3.89	3.52
Subject 5										
Subject 6	0.60	3.67	5.91	6.64	9.39	9.60	9.55	8.86	6.56	4.83
Subject 7	0.77	5.70	7.32	8.04	7.92	8.96	9.25	9.29	8.12	6.68
Subject 8										
Subject 9	0.34	5.15	6.83	6.73	7.78	7.21	7.67	6.53	5.19	3.48
Subject 10	0.71	4.26	6.12	6.88	6.99	7.52	8.22	7.32	5.22	3.64
Mean	**0.60**	**6.25**	**7.41**	**7.57**	**8.16**	**8.46**	**8.40**	**7.77**	**5.83**	**4.40**
SD	**0.14**	**2.27**	**1.60**	**1.23**	**1.26**	**1.22**	**1.15**	**1.51**	**1.46**	**1.17**
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Table 47.Venous blood lactate concentration (mM) at rest, following each set of sprints, and after 1, 2, 5, 10, 20, and 30 min of recovery after the post-training RSE

	Rest	**Set1**	**Set2**	**Set3**	**+1**	**+2**	**+5**	**+10**	**+20**	**+30**
Subject 1	0.83	4.95	5.87	5.59	6.12	6.42	5.62	4.27	2.24	1.74
Subject 2	0.45	5.01	5.35	5.46	4.71	4.72	5.04	4.38	3.20	2.09
Subject 3	0.54	10.70	12.55	13.35	11.80	12.60	12.95		9.08	6.79
Subject 4	0.54	2.22	2.67	3.27	4.08	4.14	3.64	2.88	2.16	1.70
Subject 5										
Subject 6	0.61	3.14	5.17	5.88	7.29	7.34	8.00	6.87	4.78	3.26
Subject 7	0.89	5.34	7.93	9.35	9.52	10.65	10.70	10.45	8.71	6.42
Subject 8										
Subject 9	0.39	5.22	6.46	6.74	8.44	8.80	8.38	7.30	5.20	3.62
Subject 10	0.49	4.19	6.47	7.24	7.99	8.77	8.86	7.80	5.88	4.55
Mean	**0.59**	**5.09**	**6.56**	**7.11**	**7.49**	**7.93**	**7.90**	**6.28**	**5.15**	**3.77**
SD	**0.18**	**2.52**	**2.85**	**3.06**	**2.54**	**2.88**	**3.07**	**2.59**	**2.67**	**2.01**
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Table 48.Venous blood glucose concentration (mM) at rest, following each set of sprints, and after 1, 2, 5, 10, 20, and 30 min of recovery after the pre-training RSE

	Rest	**Set1**	**Set2**	**Set3**	**+1**	**+2**	**+5**	**+10**	**+20**	**+30**
Subject 1	4.50	4.87	5.95	6.41	6.87	6.92	6.50	6.30	5.74	5.10
Subject 2	4.33	4.03	4.515	4.49	4.55	4.59	4.5	4.54	4.28	4.21
Subject 3	4.33	3.89	4.82	4.88	4.90	5.00	4.93	4.84	4.68	4.23
Subject 4	4.22	4.82	5.65	6.24	6.24	7.08	6.88	7.05	5.75	6.46
Subject 5										
Subject 6	4.59	4.86	5.61	5.81	6.09	6.12	5.96	5.76	5.26	4.67
Subject 7	4.54	5.62	5.71	5.79	5.72	5.78	5.74	5.68	5.30	5.20
Subject 8										
Subject 9	4.28	4.47	5.24	5.45	5.91	5.71	5.75	5.62	5.33	4.57
Subject 10	4.34	4.30	4.98	5.34	5.44	5.52	5.77	5.61	5.38	5.14
Mean	**4.39**	**4.61**	**5.31**	**5.55**	**5.71**	**5.84**	**5.75**	**5.67**	**5.21**	**4.94**
SD	**0.13**	**0.56**	**0.50**	**0.65**	**0.75**	**0.86**	**0.77**	**0.78**	**0.50**	**0.73**
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**

Table 49. Venous blood glucose concentration (mM) at rest, following each set of sprints, and after 1, 2, 5, 10, 20, and 30 min of recovery after the post-training RSE

	Rest	**Set1**	**Set2**	**Set3**	**+1**	**+2**	**+5**	**+10**	**+20**	**+30**
Subject 1	4.32	4.24	4.81	4.81	5.08	5.40	5.16	4.90	3.95	4.25
Subject 2	4.13	4.38	4.56	4.33	4.21	4.15	4.17	4.14	4.09	4.00
Subject 3	4.36	4.37	5.35	5.76	5.77	6.26	6.02		4.85	3.97
Subject 4	4.88	4.67	5.15	5.65	5.94	6.19	6.14	6.26	6.14	5.94
Subject 5										
Subject 6	4.03	4.34	5.11	5.13	5.37	5.43	5.39	5.15	4.69	4.07
Subject 7	5.02	5.43	5.80	6.15	6.34	6.67	6.48	6.22	5.74	4.89
Subject 8										
Subject 9	4.30	4.48	5.16	5.51	5.81	6.05	6.07	5.83	5.40	4.62
Subject 10	3.65	3.74	4.27	4.63	4.75	5.02	5.07	4.84	4.69	4.79
Mean	**4.33**	**4.45**	**5.03**	**5.25**	**5.41**	**5.65**	**5.56**	**5.33**	**4.94**	**4.56**
SD	**0.44**	**0.48**	**0.47**	**0.63**	**0.70**	**0.81**	**0.76**	**0.79**	**0.77**	**0.66**
n	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**7**	**8**	**8**

APPENDIX 8

RAW DATA FROM CHAPTER 5

Table 50. Pulmonary oxygen consumption at the ventilatory threshold 1 and at exhaustion (mL kg^{-1} min^{-1})

	FUT			MOD	
	$\dot{V}O_{2peak}$	VT1		$\dot{V}O_{2peak}$	VT1
Subject 1	49.33	35.55	Subject 9	60.31	50.33
Subject 2	47.68	40.12	Subject 10	58.75	49.82
Subject 3	51.80	43.06	Subject 11	54.36	45.13
Subject 4	49.06	39.22	Subject 12	57.47	39.50
Subject 5	59.46	37.04	Subject 13	44.91	36.79
Subject 6	37.46	26.36	Subject 14	52.18	37.15
Subject 7	46.18	38.68	Subject 15	52.23	38.68
Subject 8	51.56	40.18	Subject 16	59.59	46.18
Mean	**49.07**	**37.53**	**Mean**	**54.98**	**42.95**
SD	**6.17**	**5.03**	**SD**	**5.17**	**5.59**
n	**8**	**8**	**n**	**8**	**8**

Table 51. Phosphorylated ACC/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	20.9	19.4	Subject 9	2.2	11.1
Subject 2	1.9	11.0	Subject 10	6.1	3.6
Subject 3	5.2	4.7	Subject 11	1.8	2.8
Subject 4	2.2	13.4	Subject 12	6.9	5.4
Subject 5	2.2	3.9	Subject 13	1.7	12.0
Subject 6	5.4	18.6	Subject 14	18.6	15.9
Subject 7	4.8	12.7	Subject 15	15.9	28.6
Subject 8	6.2	23.3	Subject 16	25.4	58.2
Mean	**6.1**	**13.4**	**Mean**	**9.8**	**17.2**
SD	**6.2**	**6.9**	**SD**	**9.0**	**18.6**
n	**8**	**8**	**n**	**8**	**8**

Table 52. Total AMPK/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			MOD	
	Pre	**End**		**Pre**	**End**
Subject 1	0.6	0.8	Subject 9	0.5	0.5
Subject 2	1.6	0.9	Subject 10	1.5	0.9
Subject 3	1.5	1.9	Subject 11	1.4	1.4
Subject 4	1.5	0.6	Subject 12	1.0	0.5
Subject 5	0.4	0.8	Subject 13	1.1	0.6
Subject 6	1.6	0.7	Subject 14	1.1	1.0
Subject 7	0.9	0.7	Subject 15	0.6	0.7
Subject 8	1.5	1.4	Subject 16	0.6	0.9
Mean	**1.2**	**1.0**	**Mean**	**1.0**	**0.8**
SD	**0.5**	**0.4**	**SD**	**0.4**	**0.3**
n	**8**	**8**	**n**	**8**	**8**

Table 53. Phosphorylated AMPKα/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	0.9	1.4	Subject 9	2.2	1.2
Subject 2	2.1	1.3	Subject 10	1.5	1.9
Subject 3	0.6	0.8	Subject 11	1.5	1.2
Subject 4	1.6	0.7	Subject 12	1.3	2.4
Subject 5	1.1	0.7	Subject 13	1.6	1.9
Subject 6	0.6	0.5	Subject 14	1.0	1.4
Subject 7	0.7	1.0	Subject 15	0.8	0.6
Subject 8	0.8	0.8	Subject 16	0.6	1.1
Mean	**1.0**	**0.9**	**Mean**	**1.3**	**1.5**
SD	**0.5**	**0.3**	**SD**	**0.5**	**0.6**
n	**8**	**8**	**n**	**8**	**8**

Table 54. Total ATF2/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	3.8	18.3	Subject 9	8.8	10.8
Subject 2	10.7	14.7	Subject 10	8.8	8.8
Subject 3	5.7	13.4	Subject 11	11.0	7.1
Subject 4	5.2	6.7	Subject 12	6.3	4.3
Subject 5	2.5	5.4	Subject 13	3.9	3.9
Subject 6	8.7	11.2	Subject 14	7.0	8.4
Subject 7	6.3	11.6	Subject 15	11.2	11.5
Subject 8	5.5	7.9	Subject 16	6.7	11.0
Mean	**6.0**	**11.1**	**Mean**	**8.0**	**8.2**
SD	**2.6**	**4.4**	**SD**	**2.5**	**2.9**
n	**8**	**8**	**n**	**8**	**8**

Table 55. Phosphorylated ATF2/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	0.5	0.7	Subject 9	0.2	0.4
Subject 2	0.4	0.6	Subject 10	0.5	0.4
Subject 3	0.4	0.7	Subject 11	0.9	0.8
Subject 4	1.1	1.5	Subject 12	1.6	0.7
Subject 5	0.6	1.0	Subject 13	0.7	0.7
Subject 6	1.9	2.7	Subject 14	1.1	0.7
Subject 7	1.6	2.6	Subject 15	2.0	2.7
Subject 8	1.2	1.6	Subject 16	1.9	2.6
Mean	**1.0**	**1.4**	**Mean**	**1.1**	**1.1**
SD	**0.6**	**0.8**	**SD**	**0.7**	**1.0**
n	**8**	**8**	**n**	**8**	**8**

Table 56. Total CaMKII/GAPDH protein abundance (AU) at rest and, immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	4.4	3.2	Subject 9		2.9
Subject 2	1.4	2.5	Subject 10	1.4	1.0
Subject 3	1.2	1.4	Subject 11	1.2	1.1
Subject 4	2.0	1.0	Subject 12	0.7	0.4
Subject 5	0.7	0.6	Subject 13	0.3	0.4
Subject 6	1.4	1.5	Subject 14	1.2	1.0
Subject 7	1.5	1.4	Subject 15	1.4	1.7
Subject 8	1.2	1.3	Subject 16	1.5	1.7
Mean	**1.7**	**1.6**	**Mean**	**1.1**	**1.3**
SD	**1.2**	**0.9**	**SD**	**0.4**	**0.8**
n	**8**	**8**	**n**	**7**	**8**

Table 57. Phosphorylated CaMKII/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1	2.5	2.3	Subject 9	3.9	1.6
Subject 2	9.0	4.8	Subject 10	5.6	10.2
Subject 3	2.5	2.2	Subject 11	3.8	2.6
Subject 4	1.5	0.6	Subject 12	1.6	2.3
Subject 5	1.2	1.4	Subject 13	1.8	3.0
Subject 6	0.9	0.7	Subject 14	1.2	1.6
Subject 7	1.2	2.0	Subject 15	1.3	0.9
Subject 8	0.9	1.2	Subject 16	0.8	0.8
Mean	**2.5**	**1.9**	**Mean**	**2.5**	**2.9**
SD	**2.7**	**1.3**	**SD**	**1.7**	**3.0**
n	**8**	**8**	**n**	**8**	**8**

Table 58. Phosphorylated HDAC 4-5-7/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

FUT			**MOD**		
	Pre	**End**		**Pre**	**End**
Subject 1	1.0	1.7	Subject 9		1.3
Subject 2	2.1	1.7	Subject 10	1.9	1.9
Subject 3	1.1	1.5	Subject 11	2.3	1.4
Subject 4	0.5	0.3	Subject 12	0.6	1.2
Subject 5	0.8	0.8	Subject 13	0.9	0.8
Subject 6	0.6	0.8	Subject 14	0.9	0.9
Subject 7	1.2	1.6	Subject 15	1.6	1.6
Subject 8	1.0	1.3	Subject 16	0.7	1.1
Mean	**1.0**	**1.2**	**Mean**	**1.3**	**1.3**
SD	**0.5**	**0.5**	**SD**	**0.7**	**0.3**
n	**8**	**8**	**n**	**7**	**8**

Table 59. Total p38 MAPK/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			MOD	
	Pre	**End**		**Pre**	**End**
Subject 1	0.8	0.7	Subject 9	2.8	0.9
Subject 2	2.5	1.6	Subject 10	2.3	2.0
Subject 3	2.0	2.2	Subject 11	1.8	1.5
Subject 4	1.8	0.7	Subject 12	1.9	2.1
Subject 5	1.5	1.5	Subject 13	1.9	1.8
Subject 6	1.7	0.8	Subject 14	0.9	0.5
Subject 7	1.8	1.3	Subject 15	2.5	1.2
Subject 8	2.3	2.2	Subject 16	0.9	0.8
Mean	**1.8**	**1.4**	**Mean**	**1.9**	**1.4**
SD	**0.5**	**0.6**	**SD**	**0.7**	**0.6**
n	**8**	**8**	**n**	**8**	**8**

Table 60. Phosphorylated p38 MAPK/GAPDH protein abundance (AU) at rest and immediately after acute futsal game and moderate-intensity continuous exercise

	FUT			**MOD**	
	Pre	**End**		**Pre**	**End**
Subject 1		33.9	Subject 9	7.9	16.1
Subject 2	8.3	17.0	Subject 10	11.1	14.2
Subject 3	8.4	16.6	Subject 11	10.1	10.6
Subject 4	5.0	10.6	Subject 12	10.8	7.6
Subject 5	11.6	9.0	Subject 13	6.5	17.4
Subject 6	9.2	10.0	Subject 14	11.2	10.6
Subject 7	2.9	8.0	Subject 15	13.2	18.2
Subject 8	14.9	22.1	Subject 16	9.7	14.4
Mean	**8.6**	**15.9**	**Mean**	**10.1**	**13.7**
SD	**4.0**	**8.8**	**SD**	**2.1**	**3.7**
n	**7**	**8**	**n**	**8**	**8**

APPENDIX 9

RAW DATA FROM CHAPTER 6

Table 61. Peripheral insulin sensitivity (mg L^{-2} $mmol^{-1}$ mU^{-1} min^{-1}), measured via the Cederholm index during the Oral Glucose Tolerance Test, pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	53.5	66.0	Subject 13	93.0	79.7
Subject 2	134.0	132.6	Subject 14	62.4	82.6
Subject 3	165.9	124.0	Subject 15	67.3	89.2
Subject 4	72.0	86.9	Subject 16	61.5	64.5
Subject 5			Subject 17	64.5	74.5
Subject 6	69.3	94.4	Subject 18		
Subject 7	76.4	165.6	Subject 19	83.8	66.7
Subject 8	61.8	54.7	Subject 20	51.6	58.7
Subject 9	60.3	72.0			
Subject 10	111.7	129.6			
Subject 11	64.4	189.1			
Subject 12	53.9	97.9			
Mean	**83.9**	**110.2**	**Mean**	**69.2**	**73.7**
SD	**37.0**	**42.2**	**SD**	**14.3**	**10.9**
n	**11**	**11**	**n**	**7**	**7**

Table 62. Plasma glucose concentration (mM) at rest, and 2 h after glucose ingestion during the oral glucose tolerance test, pre- and post-training

	FUT					MOD			
	Pre-training		Post-training			Pre-training		Post-training	
	Rest	2 h	Rest	2 h		Rest	2 h	Rest	2 h
Subject 1	6.0	6.2	6.3	5.3	Subject 13	6.1	4.8	6.1	5.5
Subject 2	5.7	3.5	5.3	3.4	Subject 14	5.4	4.9	5.5	4.2
Subject 3	5.1	3.1	5.3	3.3	Subject 15	5.7	5.2	5.8	4.8
Subject 4	5.3	4.9	4.8	5.3	Subject 16	6.0	6.3	5.9	6.1
Subject 5					Subject 17	5.5	5.6	5.6	5.1
Subject 6	6.2	5.2	5.6	4.4	Subject 18				
Subject 7	5.5	5.4	5.5	2.9	Subject 19	5.5	4.0	5.7	5.3
Subject 8	5.7	6.2	6.3	6.7	Subject 20	5.5	7.4	5.3	6.5
Subject 9	5.1	5.8	5.1	5.4					
Subject 10	5.0	3.9	4.9	3.7					
Subject 11	5.1	6.0	5.0	4.6					
Subject 12	5.3	7.2	5.2	5.8					
Mean	**5.4**	**5.2**	**5.4**	**4.6**	**Mean**	**5.7**	**5.4**	**5.7**	**5.4**
SD	**0.4**	**1.3**	**0.5**	**1.2**	**SD**	**0.3**	**1.1**	**0.3**	**0.8**
n	**11**	**11**	**11**	**11**	**n**	**7**	**7**	**7**	**7**

Table 63. Plasma insulin concentration (mU L^{-1}) at rest, and 2 h after glucose ingestion during the oral glucose tolerance test, pre- and post-training

	FUT					MOD			
	Pre-training		Post-training			Pre-training		Post-training	
	Rest	2 h	Rest	2 h		Rest	2 h	Rest	2 h
Subject 1	8.8	81.2	10.7	52.7	Subject 13	2.0	21.7	2.0	24.5
Subject 2	5.2	15.4	9.8	17.5	Subject 14	19.3	99.6	16.6	48.3
Subject 3	2.9	13.5	3.7	24.8	Subject 15	6.4	55.7	5.1	25.4
Subject 4	8.1	56.6	4.4	24.6	Subject 16	9.2	44.2	5.9	40
Subject 5					Subject 17	9.8	56.0	6.9	41.3
Subject 6	10.1	44.8	2.4	26.7	Subject 18				
Subject 7	4.9	32.9	4.6	13.2	Subject 19	9.5	56.0	9.6	55.3
Subject 8	5.6	47.3	8.7	54.8	Subject 20	6.5	62.8	5.8	54.2
Subject 9	14.8	73.8	11.9	44.5					
Subject 10	10.9	22.4	17.5	16.7					
Subject 11	9.1	50.9	2.9	5.0					
Subject 12	2.0	54.1	2.0	13.3					
Mean	**7.5**	**45.0**	**7.2**	**26.0**	**Mean**	**9.0**	**57.0**	**7.4**	**41.0**
SD	**3.8**	**22.0**	**5.0**	**17.0**	**SD**	**5.0**	**23.0**	**5.0**	**13.0**
n	**11**	**11**	**11**	**11**	**n**	**7**	**7**	**7**	**7**

Table 64. Resting plasma glycated haemoglobin (mmol molHb^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	41	38	Subject 13	39	38
Subject 2	38	36	Subject 14	38	36
Subject 3	39	35	Subject 15	35	31
Subject 4	38	35	Subject 16	40	41
Subject 5	39	35	Subject 17	38	34
Subject 6	36	34	Subject 18	43	38
Subject 7	41	37	Subject 19	41	39
Subject 8	38	34	Subject 20	38	36
Subject 9	36	29			
Subject 10	34	33			
Subject 11	37	35			
Subject 12	36	37			
Mean	**37.8**	**34.8**	**Mean**	**39.0**	**36.6**
SD	**2.1**	**2.3**	**SD**	**2.4**	**3.1**
n	**12**	**12**	**n**	**8**	**8**

Table 65. Resting plasma cholesterol (mmol L^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	5.8	5.6	Subject 13	5.7	5.7
Subject 2	4.8	4.6	Subject 14	4.1	4.4
Subject 3	6.0	5.9	Subject 15	5.2	4.6
Subject 4	6.4	4.9	Subject 16	5.3	4.3
Subject 5	4.1	4.6	Subject 17	6.7	6.3
Subject 6	6.5	5.6	Subject 18	3.6	6.5
Subject 7	6.5	6.2	Subject 19	5.5	5.7
Subject 8	6.3	5.7	Subject 20	5.2	4.1
Subject 9	4.4	4.0			
Subject 10	4.0	3.4			
Subject 11	4.4	3.9			
Subject 12	4.7	4.0			
Mean	**5.3**	**4.9**	**Mean**	**5.2**	**5.2**
SD	**1.0**	**0.9**	**SD**	**1.0**	**1.0**
n	**12**	**12**	**n**	**8**	**8**

Table 66. Resting plasma triglycerides (mmol L^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	1.9	1.5	Subject 13	0.9	0.7
Subject 2	2.3	1.0	Subject 14	1.6	1.0
Subject 3	1.8	1.3	Subject 15	0.9	1.4
Subject 4	0.9	1.3	Subject 16	1.8	2.4
Subject 5	2.3	2.2	Subject 17	2.2	4.3
Subject 6	5.2	3.1	Subject 18	1.4	1.7
Subject 7	2.3	2.0	Subject 19	0.7	1.5
Subject 8	2.6	2.1	Subject 20	2.8	1.7
Subject 9	1.8	1.6			
Subject 10	1.3	0.9			
Subject 11	2.1	1.4			
Subject 12	1.1	0.9			
Mean	**2.1**	**1.6**	**Mean**	**1.5**	**1.8**
SD	**1.1**	**0.7**	**SD**	**0.7**	**1.1**
n	**12**	**12**	**n**	**8**	**8**

Table 67. Resting plasma high-density lipoprotein cholesterol (mmol L^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	1.1	1.1	Subject 13	2.0	2.0
Subject 2	1.0	1.1	Subject 14	0.9	1.0
Subject 3	1.5	1.4	Subject 15	1.5	1.2
Subject 4	1.4	1.1	Subject 16	1.1	0.9
Subject 5	1.0	1.1	Subject 17	1.6	1.4
Subject 6	0.9	1.0	Subject 18	1.0	1.1
Subject 7	1.3	1.2	Subject 19	1.2	1.1
Subject 8	1.1	1.1	Subject 20	1.1	1.1
Subject 9	0.9	0.9			
Subject 10	1.1	1.2			
Subject 11	1.1	1.2			
Subject 12	0.9	0.9			
Mean	**1.1**	**1.1**	**Mean**	**1.3**	**1.2**
SD	**0.2**	**0.1**	**SD**	**0.4**	**0.4**
n	**12**	**12**	**n**	**8**	**8**

Table 68. Resting plasma low-density lipoprotein cholesterol (mmol L^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	3.9	3.8	Subject 13	3.3	3.4
Subject 2	2.8	3.1	Subject 14	2.4	2.9
Subject 3	3.6	3.9	Subject 15	3.3	2.7
Subject 4	4.6	3.2	Subject 16	3.4	2.3
Subject 5	2.1	2.5	Subject 17	4.1	2.9
Subject 6	3.2	3.2	Subject 18	2.0	4.7
Subject 7	4.1	4.1	Subject 19	4.0	3.9
Subject 8	4.0	3.6	Subject 20	2.8	2.2
Subject 9	2.7	2.3			
Subject 10	2.3	1.8			
Subject 11	2.4	2.1			
Subject 12	3.3	2.7			
Mean	**3.2**	**3.0**	**Mean**	**3.2**	**3.1**
SD	**0.8**	**0.8**	**SD**	**0.7**	**0.8**
n	**12**	**12**	**n**	**8**	**8**

Table 69. Complex I subunit NDUFB8 resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			**MOD**	
	Pre	**Post**		**Pre**	**Post**
Subject 1	10.3	14.5	Subject 13	14.8	9.3
Subject 2	14.2	15.1	Subject 14	15.5	17.3
Subject 3	18.8	20.2	Subject 15	14.1	14.3
Subject 4	11.8	18.8	Subject 16	9.7	12.0
Subject 5	8.7	7.7	Subject 17	12.9	12.3
Subject 6	6.4	11.0	Subject 18	10.6	12.9
Subject 7	8.7	10.4	Subject 19	8.6	12.6
Subject 8	11.6	12.9	Subject 20	8.3	12.0
Subject 9	8.1	8.7			
Subject 10	11.9	13.8			
Subject 11	15.9	18.0			
Subject 12	11.3	13.0			
Mean	**11.5**	**13.7**	**Mean**	**11.8**	**12.8**
SD	**3.5**	**3.9**	**SD**	**2.9**	**2.3**
n	**12**	**12**	**n**	**8**	**8**

Table 70. Complex II-FeS subunit 30kDa resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	36.6	33.9	Subject 13	28.7	27.4
Subject 2	31.2	34.2	Subject 14	34.5	40.2
Subject 3	33.5	38.6	Subject 15	33.6	26.3
Subject 4	28.7	28.4	Subject 16	13.0	19.5
Subject 5	14.3	15.7	Subject 17	14.6	12.7
Subject 6	14.1	18.1	Subject 18	11.5	13.2
Subject 7	10.4	12.3	Subject 19	24.0	29.0
Subject 8	14.8	16.2	Subject 20	26.5	29.1
Subject 9	20.0	18.6			
Subject 10	23.7	27.9			
Subject 11	28.8	30.2			
Subject 12	26.3	23.1			
Mean	**23.5**	**24.8**	**Mean**	**23.3**	**24.7**
SD	**8.7**	**8.6**	**SD**	**9.2**	**9.2**
n	**12**	**12**	**n**	**8**	**8**

Table 71. Complex III subunit core2 resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	17.5	17.2	Subject 13	14.2	13.5
Subject 2	14.9	14.9	Subject 14	18.3	22.7
Subject 3	14.9	19.7	Subject 15	19.2	17.4
Subject 4	14.8	15.5	Subject 16	5.6	6.9
Subject 5	9.0	9.1	Subject 17	8.5	10.3
Subject 6	7.9	9.0	Subject 18	7.5	8.9
Subject 7	4.8	8.1	Subject 19	9.2	12.4
Subject 8	7.4	9.6	Subject 20	8.8	11.5
Subject 9	7.5	6.8			
Subject 10	8.4	9.8			
Subject 11	9.9	10.3			
Subject 12	7.8	10.9			
Mean	**10.4**	**11.8**	**Mean**	**11.4**	**12.9**
SD	**4.0**	**4.1**	**SD**	**5.1**	**5.0**
n	**12**	**12**	**n**	**8**	**8**

Table 72. Cytochrome C oxidase subunit II resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	48.5	52.6	Subject 13	50.8	49.9
Subject 2	41.7	51.9	Subject 14	63.3	61.5
Subject 3	41.4	55.4	Subject 15	51.6	47.8
Subject 4	49.1	59.2	Subject 16	38.8	44.3
Subject 5	39.2	41.2	Subject 17	50.7	52.9
Subject 6	30.7	37.5	Subject 18	41.9	45.4
Subject 7	32.0	35.6	Subject 19	41.4	48.6
Subject 8	36.3	45.1	Subject 20	42.0	52.5
Subject 9	32.0	34.2			
Subject 10	40.3	44.8			
Subject 11	43.8	44.4			
Subject 12	31.2	39.5			
Mean	**38.9**	**45.1**	**Mean**	**47.6**	**50.4**
SD	**6.5**	**8.1**	**SD**	**8.1**	**5.4**
n	**12**	**12**	**n**	**8**	**8**

Table 73. ATP synthase subunit alpha resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	38.6	33.9	Subject 13	31.0	36.6
Subject 2	30.4	33.8	Subject 14	43.3	49.3
Subject 3	32.0	34.9	Subject 15	39.0	37.4
Subject 4	30.4	30.9	Subject 16	21.2	24.8
Subject 5	25.8	23.0	Subject 17	29.8	33.4
Subject 6	19.4	23.9	Subject 18	24.2	28.0
Subject 7	21.6	24.7	Subject 19	27.6	33.5
Subject 8	24.3	27.6	Subject 20	29.4	32.6
Subject 9	25.3	23.9			
Subject 10	25.6	28.0			
Subject 11	27.8	30.4			
Subject 12	26.1	28.8			
Mean	**27.3**	**28.6**	**Mean**	**30.7**	**34.5**
SD	**5.1**	**4.2**	**SD**	**7.3**	**7.3**
n	**12**	**12**	**n**	**8**	**8**

Table 74. Glucose transporter 4 resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			**MOD**	
	Pre	**Post**		**Pre**	**Post**
Subject 1	4.9	13.6	Subject 13	5.3	8.9
Subject 2	5.0	9.1	Subject 14	2.5	12.9
Subject 3	7.2	6.8	Subject 15	17.8	13.8
Subject 4	2.3	8.9	Subject 16	17.5	17.0
Subject 5	11.0	15.0	Subject 17	15.5	23.1
Subject 6	10.1	10.3	Subject 18	10.7	30.0
Subject 7	1.9	11.3	Subject 19	22.0	25.1
Subject 8	1.4	13.9	Subject 20	21.8	32.4
Subject 9	16.2	23.1			
Subject 10	21.8	24.8			
Subject 11	21.1	25.0			
Subject 12	28.6	28.9			
Mean	**11.0**	**15.9**	**Mean**	**14.1**	**20.4**
SD	**9.0**	**7.5**	**SD**	**7.3**	**8.5**
n	**12**	**12**	**n**	**8**	**8**

Table 75. Myocyte enhancer factor 2A resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			**MOD**	
	Pre	**Post**		**Pre**	**Post**
Subject 1	4.7	3.1	Subject 13	4.4	4.2
Subject 2	4.5	3.7	Subject 14	3.9	4.6
Subject 3	2.7	2.0	Subject 15	6.7	9.9
Subject 4	2.0	2.9	Subject 16	5.0	3.6
Subject 5	6.8	6.6	Subject 17	4.3	4.9
Subject 6	9.5	7.1	Subject 18	7.4	8.3
Subject 7	6.6	5.4	Subject 19	20.2	19.7
Subject 8	5.7	5.6	Subject 20	9.2	22.6
Subject 9	22.2	18.4			
Subject 10	9.1	13.3			
Subject 11	20.2	17.2			
Subject 12	17.2	25.4			
Mean	**9.2**	**9.2**	**Mean**	**7.6**	**9.7**
SD	**6.8**	**7.5**	**SD**	**5.4**	**7.4**
n	**12**	**12**	**n**	**8**	**8**

Table 76. Mitochondrial transcription factor A resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			**MOD**	
	Pre	**Post**		**Pre**	**Post**
Subject 1	44.1	77.2	Subject 13	45.4	48.2
Subject 2	60.0	67.3	Subject 14	55.7	80.3
Subject 3	50.3	50.6	Subject 15	85.9	101.4
Subject 4	60.6	48.5	Subject 16	47.9	58.9
Subject 5	62.4	51.9	Subject 17	75.1	61.4
Subject 6	45.6	72.8	Subject 18	81.6	63.3
Subject 7	72.8	58.8	Subject 19	27.1	36.1
Subject 8	67.3	75.3	Subject 20	46.7	63.7
Subject 9	38.4	29.4			
Subject 10	36.9	36.8			
Subject 11	43.0	44.9			
Subject 12	28.2	31.4			
Mean	**50.8**	**53.7**	**Mean**	**58.2**	**64.2**
SD	**13.7**	**16.7**	**SD**	**20.6**	**19.7**
n	**12**	**12**	**n**	**8**	**8**

Table 77. Peroxisome proliferator-activated receptor γ, co-activator 1α resting skeletal muscle protein abundance (AU), pre- and post-training

	FUT			**MOD**	
	Pre	**Post**		**Pre**	**Post**
Subject 1	49.1	46.7	Subject 13	17.2	26.1
Subject 2	42.1	34.0	Subject 14	27.8	25.0
Subject 3	18.3	22.5	Subject 15	34.8	43.3
Subject 4	20.6	15.7	Subject 16	27.5	26.5
Subject 5	18.9	16.5	Subject 17	28.3	30.0
Subject 6	15.3	11.8	Subject 18	33.6	44.5
Subject 7	10.9	22.3	Subject 19	39.6	44.4
Subject 8	21.1	28.4	Subject 20	57.3	79.3
Subject 9	25.3	15.3			
Subject 10	7.3	13.5			
Subject 11	17.1	28.0			
Subject 12	24.6	32.2			
Mean	**22.5**	**23.9**	**Mean**	**33.3**	**39.9**
SD	**12.0**	**10.4**	**SD**	**11.8**	**18.1**
n	**12**	**12**	**n**	**8**	**8**

Table 78. Body mass (kg), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	79.2	79.1	Subject 13	71.8	71.3
Subject 2	99.5	98.2	Subject 14	96.2	94.7
Subject 3	80.3	78.8	Subject 15	79.2	78.6
Subject 4	90.3	85.6	Subject 16	85.3	83.6
Subject 5	102.8	102.3	Subject 17	90.0	89.8
Subject 6	91.8	91.5	Subject 18	79.8	73.8
Subject 7	96.0		Subject 19	92.2	94.6
Subject 8	78.3	77.3	Subject 20	95.9	94.6
Subject 9	122.2	119.8			
Subject 10	103.4	105.6			
Subject 11	123.5	121.1			
Subject 12	73.8	74.3			
Mean	**95.1**	**94.0**	**Mean**	**86.3**	**85.1**
SD	**16.3**	**16.8**	**SD**	**8.8**	**9.7**
n	**12**	**11**	**n**	**8**	**8**

Table 79. Body mass index (kg $^{m-2}$), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	26.6	26.6	Subject 13	22.9	22.8
Subject 2	29.7	29.3	Subject 14	30.0	29.5
Subject 3	29.0	28.4	Subject 15	27.6	27.4
Subject 4	29.0	27.5	Subject 16	27.2	26.7
Subject 5	29.7	29.6	Subject 17	27.8	27.7
Subject 6	29.3	29.2	Subject 18	27.5	25.4
Subject 7	31.3		Subject 19	28.0	28.7
Subject 8	28.9	28.6	Subject 20	30.8	30.4
Subject 9	34.2	33.5			
Subject 10	31.0	31.7			
Subject 11	36.3	35.6			
Subject 12	24.8	25.0			
Mean	**30.0**	**29.5**	**Mean**	**27.7**	**27.3**
SD	**3.0**	**3.1**	**SD**	**2.3**	**2.4**
n	**12**	**11**	**n**	**8**	**8**

Table 80. Waist circumference (cm), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1	100.0	98.0	Subject 13	87.0	91.0
Subject 2	101.0	101.0	Subject 14	99.9	104.0
Subject 3	97.0	94.5	Subject 15	92.0	90.0
Subject 4	95.0	97.0	Subject 16	94.0	104.0
Subject 5	102.0	102.5	Subject 17	100.6	102.0
Subject 6	106.0	106.0	Subject 18	96.5	89.0
Subject 7	107.0		Subject 19	98.5	101.0
Subject 8	101.0	100.5	Subject 20	109.0	110.0
Subject 9	123.5	121.0			
Subject 10	109.5	109.5			
Subject 11	120.0	118.5			
Subject 12	90.0	86.5			
Mean	**104.3**	**103.2**	**Mean**	**97.2**	**98.9**
SD	**9.7**	**10.2**	**SD**	**6.6**	**7.8**
n	**12**	**11**	**n**	**8**	**8**

Table 81. Running velocity at the ventilatory threshold 1 (km h^{-1}), pre- and post-training

FUT			MOD		
	Pre	**Post**		**Pre**	**Post**
Subject 1			Subject 13	8.0	8.0
Subject 2	6.0	6.0	Subject 14	6.0	6.0
Subject 3	6.0	6.0	Subject 15	8.0	8.0
Subject 4	7.0	7.0	Subject 16	7.0	7.0
Subject 5	6.0	7.0	Subject 17	7.0	7.0
Subject 6	6.0	6.0	Subject 18	7.0	7.0
Subject 7			Subject 19		
Subject 8	7.0	7.0	Subject 20	7.0	7.0
Subject 9	7.0	6.0			
Subject 10	7.0	7.0			
Subject 11	7.0	7.0			
Subject 12	8.0	8.0			
Mean	**6.7**	**6.7**	**Mean**	**7.1**	**7.1**
SD	**0.7**	**0.7**	**SD**	**0.7**	**0.7**
n	**10**	**10**	**n**	**7**	**7**

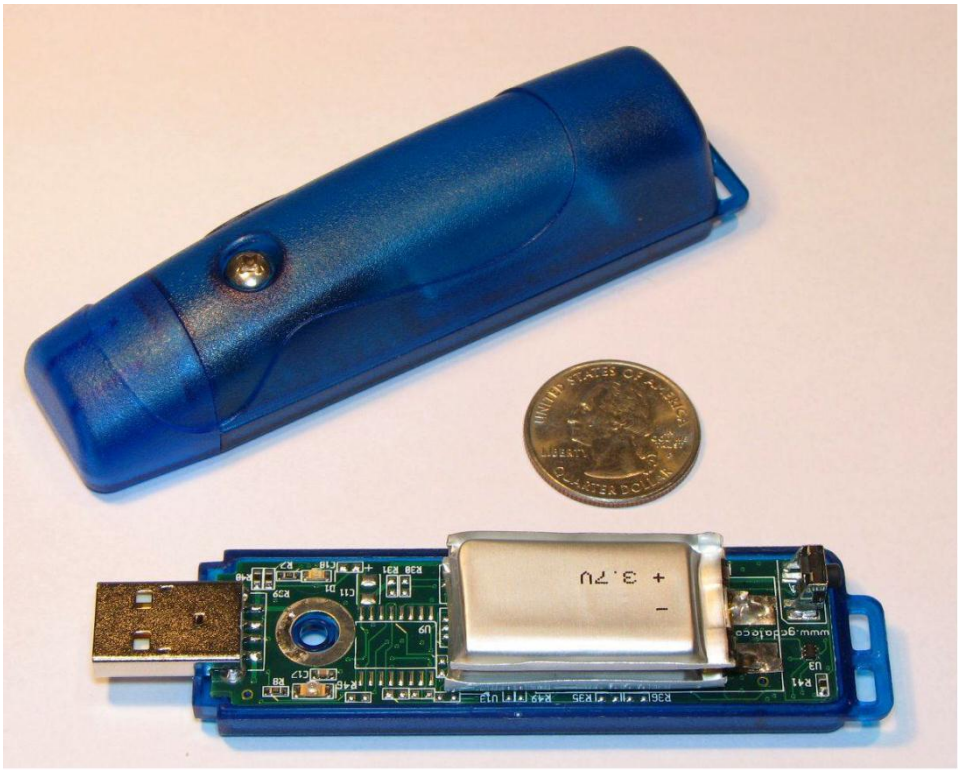
Table 82. Pulmonary oxygen consumption at the ventilatory threshold 1 (mL kg^{-1} min^{-1}), pre- and post-training

	FUT			MOD	
	Pre	**Post**		**Pre**	**Post**
Subject 1			Subject 13	31.15	30.06
Subject 2	18.80	17.87	Subject 14		
Subject 3	16.86	16.06	Subject 15	27.08	26.90
Subject 4	17.84	16.72	Subject 16	19.68	17.92
Subject 5	19.50	25.46	Subject 17	26.24	19.62
Subject 6	16.52	15.62	Subject 18	19.87	19.34
Subject 7			Subject 19		
Subject 8	25.90	21.46	Subject 20	18.82	19.31
Subject 9	17.81	14.43			
Subject 10	25.61	22.62			
Subject 11	19.41	17.21			
Subject 12	28.58	22.83			
Mean	**20.68**	**19.03**	**Mean**	**23.81**	**22.19**
SD	**4.33**	**3.74**	**SD**	**5.06**	**5.01**
n	**10**	**10**	**n**	**6**	**6**

APPENDIX 10

Explanation of work matching procedure with triaxial accelerometer units

During the incremental exercise test, participants wore a triaxial accelerometer unit placed in a dedicated vest between the scapulae. The figure below shows the accelerometer unit employed for the experiments presented in Chapters 5 and 6.



(http://www.gcdataconcepts.com)

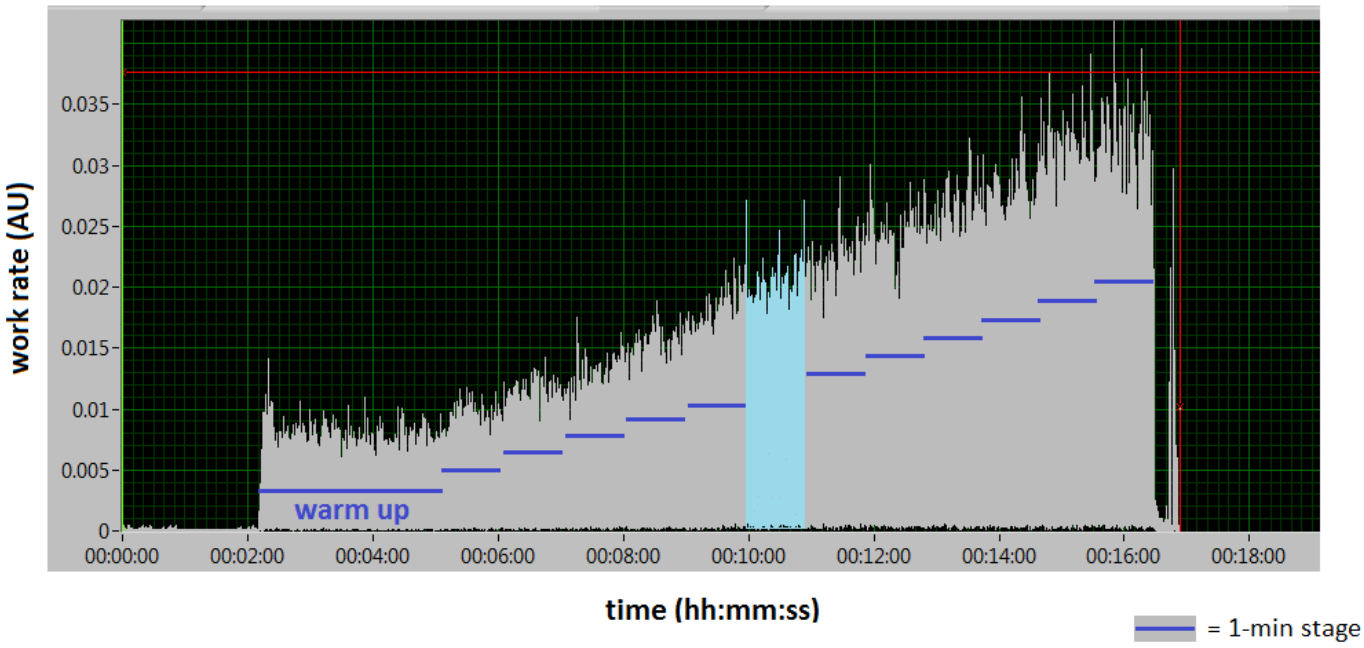
The accelerometer recorded the raw accelerations on the three anatomical axes. The sampling frequency was set at 160 Hz (low gain) and the resolution was set at 16 bit.

The work rate for every sampling point of the incremental exercise test was calculated using the following vector magnitude equation

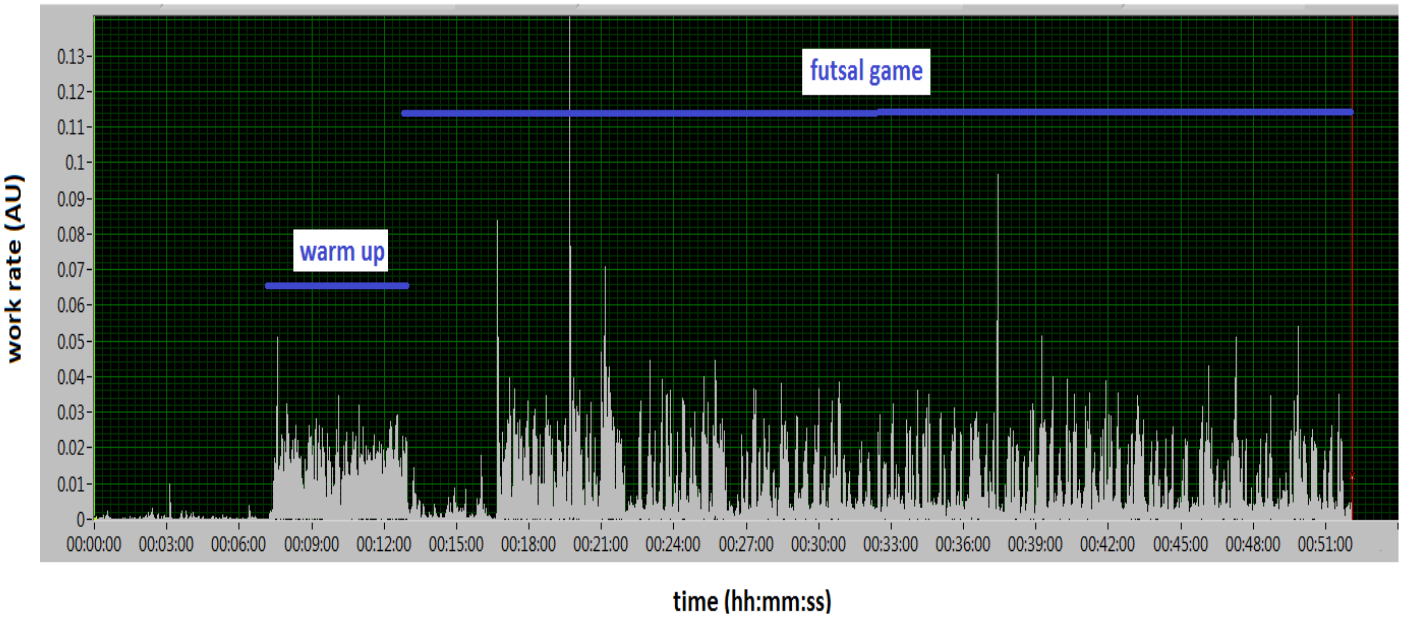
$$work\ rate\ (AU) = \frac{\sqrt{((x1 - x0)^2 + (y1 - y0)^2\ +\ (z1 - z0)^2)}}{100}$$

Where x represents vertical accelerations, y represents lateral accelerations, z represents frontal accelerations, and 100 represent a scaling factor.

The figure below shows an example of the work rate calculation originating from the recording of an incremental test for one participant. The light-blue area represents the total work corresponding to 1 min of exercise at the individual's Ventilatory Threshold 1. In this specific case, the work rate at VT1 was 31.7 arbitrary units.



During the futsal match, each player wore an accelerometer unit for the entire duration of the game. The total work performed was calculated and averaged between all players. The figure below shows an example of recording during the game for one participant. The average work during the futsal game was 621.5 ± 68.9 arbitrary units (n = 8).



To calculate the duration of the moderate-intensity running exercise (MOD) necessary to match the work performed during the futsal game, the average work was divided by the work corresponding to 1 min of exercise at VT1 measured during the incremental test. In the specific case of the subject presented above, the resulting duration of the exercise was 19.6 min, according to the following the formula:

$$MOD\ exercise\ duration = \frac{futsal\ game\ average\ work\ (AU)}{MOD\ individual's\ work\ for\ 1\ \text{min}\ at\ VT1\ (AU)}$$

$$= \frac{621.5}{31.7} = \mathbf{19.6\ min}$$