The physiological and molecular response to repeated-sprints in male and female team-sport athletes

A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science

In

Sport and Exercise Science

At Massey University, Palmerston North,

New Zealand

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2009

Abstract

Background: Due to the unique demands of the sport, athletes playing football perform a variety of differing training methods to improve physiological performance. These include strength, endurance and sprint training. While the effects of strength and endurance training have been well researched, the effects of repeated-sprint training on blood and muscle variables in well trained males and females are not well known. An understanding of changes to the blood and muscle during and following an exercise bout are important, so to gain an understanding of the type of stress and resulting adaptations that may occur. Also, while a large volume of research in training adaptations has been performed on males; little has been done on females. To date, some research indicates metabolism during moderate-intensity exercise may differ between males and females; however, no study has compared repeated-sprint exercise. Therefore, it is unclear as to whether males and females would have a differing physiological response to repeated-sprint training.

Purpose: The purpose of this study was to determine the effects of a repeated-sprint bout on molecular signalling in muscle and blood measures and heart rate in well-trained footballers. Additionally, we compared running times and sprint decrement (%).

Research Design: Eight female senior University football players (Mean ± SD, age, 19 ± 1 y, $\dot{V}O_{2peak}$ 53.0 ± 5.1 ml·kg^{-1}min^{-1}) and seven male senior University football players (Mean ± SD, age, 19 ± 3 y, $\dot{V}O_{2peak}$ 59.0 ± 6.6 ml·kg^{-1}min^{-1}) volunteered to participate in this study. Participants performed four bouts of 6 x 30 m maximal sprints spread equally over a 40 min period. Sprint time was measured (at 30 m) for each sprint and sprint decrement was also calculated for all bouts. Muscle biopsies were taken from the vastus lateralis muscle at rest, 15 min following exercise and 2 h into recovery. Venous blood

samples were taken at the same time points as the biopsies while capillary blood lactate was measured at rest and 3 min following each sprint bout. Repeated measures ANOVA and *Post hoc* t-tests were performed to determine significant differences between the two groups (male vs. female) and time points.

Findings: Both groups had a significant (P<0.05) increase in blood lactate (mM) after the first bout of repeated sprints, with no differences between females (pre 0.9 ± 0.4 mM – post 10.0 ± 1.6 mM) and males (pre 0.8 ± 0.3 mM – post 10.0 ± 3.5 mM). Blood lactate remained elevated compared to rest (P<0.05) following bouts 2, 3 and 4 for both females (12.0 ± 3.6, 12.0 ± 3.3, 12.2 ± 3.8 mM respectively) and males (11.9 ± 2.9, 11.6 ± 2.3, 11.5 ± 4.0 mM respectively), with no differences between groups or time points (P>0.05). There were no differences (P>0.05) between the female and male athletes in mean heart rate attained at the end of each bout of repeated sprints (187 ± 2 v 190 ± 2 bpm respectively) or during recovery between sprints (140 ± 2 v 130 ± 2 bpm respectively). There were no differences between groups or time points in blood insulin (P>0.05). Fastest 30 m sprint time and mean 30 m sprint time during the repeated-sprint bout was faster for the males than females (4.58 ± 0.12 v 5.26 ± 0.27 s respectively; (P>0.05)). However, there were no differences in running velocity during the sprints between the males and females (165 ± 0.4 % vs. 155 ± 0.05 %; P>0.05) when expressed relative to velocity at $\dot{V}O_{2peak}$ ($v\dot{V}O_{2peak}$). Also, mean % decrement during the repeated-sprint bout was lower in the males then females (4.9 ± 1.3 v 7.1 ± 1.9 % respectively; P<0.05). No changes were observed in total or phosphorylated Akt at any time-point or between genders. However, while total 4E-BP1 was lower, the ratio of total to phosphoryalated 4E-BP1 at rest was greater in males than females (P<0.05). Finally, there was also a significant decrease in 4E-BP1 phosphorylation post-exercise in males (P<0.05), but not females.

Conclusions: There were no sex differences in blood lactate or heart rate throughout the repeated-sprint bout. These findings suggest that there were no cardio respiratory or lactate production/clearance differences in the response to a repeated-sprint-training bout between sexes. However, while males were faster than their female counterparts, the average relative speed was similar between sexes, suggesting a similar relative volume of work was performed during the sprint bouts. However, the females did have a greater decrement in sprint performance indicating a greater ability to recover sprint performance in the males. Sex differences in resting total and phosphorylated 4E-BP1 may indicate greater potential for muscle growth in the male athletes during basal conditions. However, differences could be due to factors other than sex, including previous training history. There was a lack of change in plasma insulin or Akt, but, similar to resistance exercise, a significant decrease in post-exercise 4E-BP1 phosphorylation for the males, but not females. The sex differences in the 4E-BP1 phosphorylation response post-exercise could be due to differences in the metabolic disturbance in the muscle during and following maximal sprints.

Keywords: blood lactate, heart rate, muscle

Author's Publications

Dent J, Edge J, Mündel T, Hawke E, Short M, McMahon C, Coffey V (2008). The effects of sex on the physiological response to repeated sprints. Post Graduate Sport Research Conference, Massey University, Palmerston North (Oral Communication).

Hawke E., Edge J., **Dent J**., Mundel T., Short M.J., Mcmahon C., Coffey V., The effects of sex and repeated-sprints on muscle protein signalling. Coaching & Sport Science Journal (2008) 3, 2:37.

Acknowledgements

Firstly, I would like to thank my supervisor Dr. Johann Edge, thank you for inspiring me to enter the world of research. The amount of time you invested into this research project is appreciated and my fountain of knowledge flows faster after my 3 years learning from you.

To Emma Hawke, Dr. David Simcock, Chris McMahon and Matt Barnes, thank you for sharing your time and experience in the lab with me. Your expertise and patience have not gone unappreciated.

To my research assistants Chelsea Aim and Warrick Raymond who ensured the smooth running of all exercise trials and made participants feel at ease. Good Attitude!

Next, I would like to thank my co-supervisor Dr. Toby Mundel. Thanks for always being there to push things along. Also to Associate Professor Hugh Morton, thank you for the time you spent reading over my literature review.

To those who without, this research could not have taken place, the participants and my friends. I wish you luck in soccer and life.

To Mum and Dad, thank you for always believing in me and inspiring me with your own work ethic.

Lastly, to the love of my life, Andy. Thank you for your unconditional love and support.

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List of Abbreviations

RSA	Repeated sprint ability
mRNA	Messenger Ribonucleic Acid
ATP	Adenosine Triphosphate
PDK1	Pyruvate dehydrogenase kinase-1
IGF	Insulin Like Growth Factor
P13K	Phosphatidylinositol- 3 Kinase
Akt	Serine/Threonine-specific protein-kinase 1
mTOR	Mammalian Target of Rapamycin
4E-BP1	Eukaryotic translation-initiation factor 4E binding protein 1
p70S6k	p70S6 Kinase
eIF4F	Eukaryotic initiation factor 4F complex
eIF4G	Eukaryotic initiation factor 4G
eIF4E	Eukaryotic initiation factor 4E
5'TOP	5'Terminal polypryrimidine tract
MEF2	Myocyte enhancer factor 2
AMPK	5'AMP-activated protein kinase
AMP	Adenosine monophosphate
TSC2	Tuberons sclerosis protein
FFM	Fat free mass
GH	Growth Hormone
FFA	Free fatty acids
IMCL	Intramyocellular lipid
Grb10	Growth factor receptor bound-bound 10
CHF	Chronic heart failure
CHO	Carbohydrate

1. Introduction

Team sports such as the various football codes are intermittent in nature with periods of high-intensity exercise interspersed with low-intensity exercise periods. In addition to performing sprints that are interspersed with recovery periods long enough to allow recovery during match-play, athletes are also required to perform a number of sprints with limited active and passive recovery periods between them that result in temporary fatigue [1]. For this reason, an essential fitness component of many team based sports is repeated-sprint ability (RSA). While a number of different training strategies have been shown to benefit RSA [2, 3], it is likely that training that mimics this fitness component will result in optimal improvements in the ability to perform repeated sprint efforts.

Previously, both endurance and resistance training have been shown to improve RSA due to improvements in either aerobic capacity or muscle cross-sectional area and strength [2, 3]. However, endurance and resistance training result in diverse acute and chronic physiological adaptations [4]. The similar improvements in RSA following aerobic and resistance training [2, 3], despite diverse adaptations are likely due to RSA requiring both high force production for performance and high O_2 delivery and utilisation to aid maintenance of performance and recovery between efforts. However, despite well published data on the acute effects of resistance and endurance type training on skeletal muscle, there is little information on the acute effects of repeated-sprint type training.

The long term adaptation of skeletal muscle is due to a number of acute training bouts that overload various components within muscle. Therefore, identifying the acute response in skeletal muscle to a particular training session is important to understanding possible mechanisms underlying long term adaptation. Recent advances in technology have meant that there is a significantly greater understanding as to why repetitive high-force muscle

contraction results in muscle hypertrophy and low-force, long-duration muscle contraction results in improved aerobic capacity of the muscle [5]. However, the physiological response of a task that requires both a high force and aerobic capacity is not clear.

Cell-signalling pathways, including the Protein Kinase B/Akt and Mammalian Target of Rapamysin (mTOR) pathways have been identified as important regulators of skeletal muscle mass. The phosphorylation of Akt, mTOR and their downstream targets such as Eukaryotic translation-initiation factor 4E binding protein 1 (4E-BP1) are important regulators of mRNA initiation and elongation and hence affect protein synthesis and cell development. Some reports identify an increase in phosphorylation of Akt and its downstream targets following a resistance training session [4, 6-9], while others show no change [10-14] (see Table 2.1). These discrepancies are likely to be due to differences in nutritional status, metabolic load, prior training and timing of the muscle sample, as these have all been shown to affect the phosphorylation of Akt and its downstream targets [7, 12, 14-16] (see Table 2.1). However, while a number of reports have identified changes to the phosphorylation of Akt and other proteins involved in cell growth after resistance or endurance training, one study has determined the response following intermittent sprint exercise (4 x 30 s sprints) [17]. Furthermore, no studies to date has determined the effects of short repeated-sprints similar to that performed during training and match-play of team-sport athletes on the activation of these proteins. Therefore, the cell-signalling response following repeated-sprint type activity is largely unknown.

Additionally, sex differences in the activation of proteins regulating cell growth are not well understood. The number of women participating in team-based sports such as football is rapidly increasing and therefore, identifying sex differences in response to this type of exercise warrants investigation also. Previously, little sex difference has been

identified in the phosphorylation status of proteins involved in muscle metabolism or growth. However, differences in the activation of AMPK (5'AMP-activated protein kinase) [18] and MEF2 (myocyte enhancer factor 2) [19] have been identified following moderate-intensity aerobic exercise in untrained participants, suggesting that there may be some sex differences. Furthermore, sex differences in the phosphorylation status of 4E-BP1 and the eIF4F complex have been reported in rats [20], indicating there could be sex differences in the metabolic machinery regulating muscle mass in addition to circulating hormonal differences [21, 22]. However, any sex differences in phosphorylation of proteins of the Akt pathway in a human athletic population have yet to be determined.

Therefore, the purposes of the present study were to determine (1) the physiological, and specifically, the skeletal muscle response during and following repeated-sprint exercise by team-sport athletes and (2) any sex differences at rest or following repeated-sprint exercise.

2. Literature Review

This literature review is written with two main purposes in mind; (1) to provide an introduction to skeletal muscle protein signalling pathways and their activation during different exercise modalities and (2) to highlight specific sex differences in exercise performance, metabolism, and protein signalling. As these areas are complex on their own, they will be discussed in their own right in two different sections before being brought together to introduce the aims of the present study.

2.1. Regulation of Skeletal Muscle Mass

Skeletal muscle comprises approximately 40-50% of the total body weight in healthy humans (typically slightly less in females). Skeletal muscle fibre numbers differ between individuals and as numbers seem to remain unaltered following training it is suggested that this variation is due to genetic inheritance [23, 24]. However, muscle fibre size has been shown to be greatly affected by exercise training [25, 26], and appropriate nutrition [27, 28]. Skeletal muscle is a malleable tissue and its regulation involves complex interactions between structural, neural and enzymatic adaptations of the muscle. The purpose of this chapter is to discuss in detail the physiological and biochemical processes regulating skeletal muscle adaptation.

Human skeletal muscle is composed of a number of individual muscle fibres, connective tissue surrounding the fibres, and intra-and extra-cellular water. Muscle mass is determined by the intricate balance between protein synthesis and degradation. The increases in fibre dimensions are a result of increases in the cross-sectional area of the individual fibres [29]. The greatest muscle hypertrophy gains are reported following high-tension muscle contraction that overloads the tissue. Therefore, strength or resistance training is commonly used to induce muscle hypertrophy [30-32]. Following training, the

body adapts to the demands of the muscle to work against increasing weight by increasing the cross-sectional area of the muscle via increases in the size of the actin and myosin filaments which can lead to the addition of sarcomeres in series (whole contractile unit) [29, 33]. While the strength of the muscle is directly proportional to the cross sectional area of the muscle, a portion of the strength gains are due to neural adaptations in the muscle [34].

In contrast, regular endurance training increases the ability of a muscle to sustain low- to high-intensity exercise for prolonged periods by improving skeletal muscle oxidative capacity. Changes in mitochondrial density and capillarisation of the muscle are evident with regular prolonged endurance training, resulting in improved O_2 delivery and utilisation [35]. In contrast to strength trained athletes, endurance trained athletes have a greater ratio of type I to type II fibres and have a greater skeletal muscle mitochondrial density. Furthermore, and in contrast to strength training, endurance training generally does not result in marked muscle hypertrophy, although small muscle hypertrophy has been reported in type I muscle Fibres following endurance training [36].

The effects of sprint-training on muscle fibre hypertrophy are less clear. Some studies have reported an increase in cross-sectional area following sprint-training [37, 38], although others have not [39, 40]. These inconsistencies could be due to the type of sprint training employed, as training factors such as the length of the sprint and the rest period in between sprints could markedly affect such adaptations. Indeed, when longer sprint intervals are performed (30 s) with short - moderate length rest periods, a number of aerobic type adaptations similar to endurance training occur, without marked changes in anaerobic enzymes or fibre size [41]. However, when short sprints are performed (4 – 6 s), similar to that encountered by team-sport athletes, in some [37, 42] but not all cases [40] muscle hypertrophy has been reported. As this type of training is regularly performed by team-sport

athletes, it would seem pertinent to understand the effects that this type of training has on skeletal muscle adaptation.

2.2. Skeletal Muscle and Protein Synthesis

Following an acute resistance training bout, muscle protein synthesis and protein degradation both increase. When there is a positive net balance i.e. protein synthesis is greater than degradation, there is an increase in protein accretion contributing to muscle hypertrophy. During intense muscle contraction (i.e. a training session) protein synthesis decreases below basal rates to conserve energy [8]. However, following exercise, protein synthesis is elevated above basal rates and can continue to be elevated some 24 -72 h post exercise [43-46], with the greatest increase in the initial 24 h [45, 46]. Protein synthesis occurs in large part by increases in *translation* (messenger ribonucleic acid (mRNA) is decoded and used to assemble polypeptides), *initiation* (assembly of active ribosome and the reading of the first mRNA codon), *elongation* (covalent linkage of new amino acid to growing polypeptide chain) and *protein assembly* within the sarcoplasm of the Fibres. During the time period post-exercise, protein degradation is also elevated [47], however, protein synthesis is elevated above degradation resulting in a positive protein balance, especially when amino acids and carbohydrates are ingested [27, 28]. While a single bout of exercise causes an acute increase in protein synthesis, several bouts of resistance exercise are required to obtain significant hypertrophy of the fibres (i.e. weeks to months depending on training status and exercise overload).

2.3. Signalling Pathways Involved in Translation Initiation

A number of important signalling pathways have been identified that are responsible for protein synthesis and degradation within skeletal muscle. These signalling pathways involve the phosphorylation and dephosphorylation of a number of proteins and kinases that

eventually result in altering rates of protein synthesis and degradation. For instance the insulin-like growth factor (IGF) pathway has been shown to be important to increases in muscle mass, as it activates key proteins involved in muscle development and elevated (mRNA) messenger ribonucleic acid (mRNA) initiation, elongation and termination rates required for the synthesis of new proteins.

Mechanical stimuli (i.e. muscle stretch), altered energy status (i.e. changed ATP, glycogen levels) [48], and endocrine changes (i.e. elevated insulin/IGF) have been identified as key regulators of cell signalling and muscle mass [49]. Indeed it has been shown that increasing the tension on skeletal muscle can enhance the efficiency of translation [50] stimulating protein synthesis. The initiation of translation is synchronized by co-ordinated cell signalling events that alter the phosphorylation status of a series of proteins and translation initiation factors. This results in faster and more efficient rates of amino acid sequencing and the resultant increase in new proteins.

2.3.1. P13K/Akt/mTOR Pathway

While still under investigation, a series of pathways have been identified as important for muscle hypertrophy. The IGF pathway has been shown to be stimulated following resistance exercise and lead to muscle hypertrophy [6] (see Figure 2.1). The binding of IGF-1 to its receptor on the cell membrane stimulates the activation of phosphatidylinositol-3 kinase (P13K) which subsequently modulates the creation of a lipid binding site on the membrane for serine/threonine-specific protein kinase 1 (also known as Akt) [51]. Akt is the mediator of most P1K3 downstream effects, mediating both the normal and pathological effects of its pathway. IGF stimulation of P13K stimulates and phosphorylates Akt by recruiting it from the cytosol to the membrane, whereas inhibition of P13K leads to the inhibition of Akt (see Figure 2.1).

Once activated, Akt can phosphorylate and activate many downstream targets both in the cytoplasm and the nucleus of the muscle cell. Akt phosphorylates a number of substrates with regulatory functions in cell growth, glucose metabolism and the initiation of translation of proteins. Notably, Akt shares a complex reciprocal relationship with mammalian target of rapamycin (mTOR) to initiate multiple downstream cell processes including protein synthesis. Accordingly, the response of Akt to physical exercise, especially resistance exercise has been well documented in recent years (Table 2.1).

Within the first 30 min of recovery, Akt has shown to be reduced or unchanged after a session of resistance training in untrained participants ([10-12] [8, 52]). While similar results have been recorded with endurance trained subjects [4, 13, 14]. However, Creer et al, 2005 [16] reported conflicting results with an increase in phosphorylation of Akt within 10 min of a low-intensity resistance training session. Wilson et al. 2006 [53] and Thorell et al. 1999 [9] also recorded an increase in phosphorylation of Akt up to 50% immediately post cycling endurance exercise. With feeding, most studies have reported an increase in Akt phosphorylation [7, 10, 14-16]. Reasons for these differences in Akt phosphorylation post exercise are unclear. The intensity (high v low-intensity), mode (resistance vs. endurance) and duration of the exercise are likely factors, while training status of the participants is also a probable cause. An increase in AMPK due to reductions in cellular energy in the stages immediately proceeding exercise could possibly explain the reduced phosphorylation of Akt.

mTOR has been recognised as an essential constituent of the signalling pathways that control the translational machinery involved in cell growth and protein synthesis. Signalling through mTOR has been observed in exercise-induced growth of skeletal muscle [6, 54]. Although the exact mechanisms involved in the mechanical/contraction induced activation

of mTOR in skeletal muscle are still to be established, it is generally accepted that the stimulation of P13K and successive activation of Akt is one method by which mTOR is activated. However, it has also been shown that amino acids alone, without activation of Akt, can activate mTOR signalling [55]. The downstream targets of mTOR, Eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) and p70S6 Kinase (p70S6k) are important to protein synthesis as they regulate mRNA translation, initiation and elongation (see Figure 2.1). Therefore, greater activation of mTOR and its downstream targets such as 4E-BP1 could potentially result in greater protein synthesis and in the long term, muscle mass.

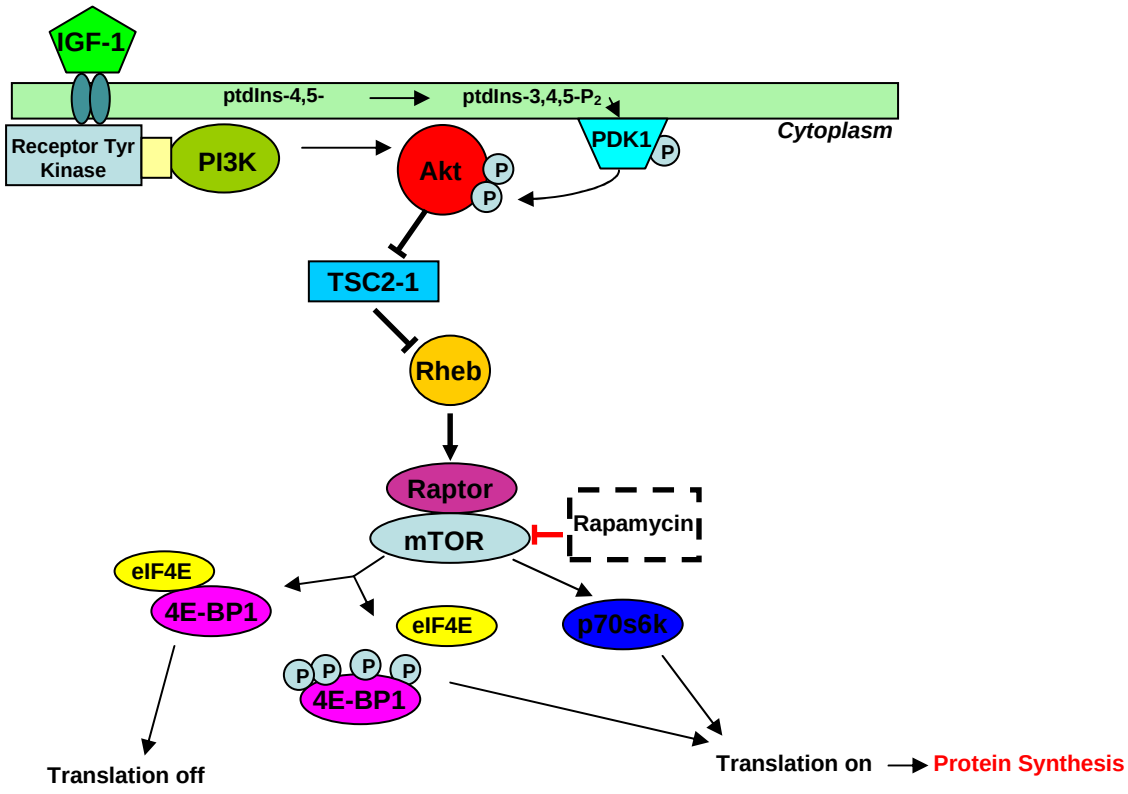

Figure 2.1. Simplified version of the Akt/mTOR signalling pathway. Growth factor (IGF-1) activation of P13K and subsequent activation of Akt controls protein synthesis at the level of translation initiation. Akt can directly phosphorylate and activate mammalian target of rapamycin (mTOR). Downstream targets of mTOR include 4E-BP1 and p70S6k. Once phosphorylated by mTOR, 4E-BP1 is free to dissociate from eIF4E which leads to initiation of translation.

2.3.2. 4E-BP1 via The eIF4F complex

The eukaryotic initiation factor 4F complex (eIF4F) collectively serves to recognize, unfold, and guide the mRNA to the 43s pre-initiation complex [56]. Phosphorylation of eIF4E is suggested to stimulate translation rates through an increase in association with eIF4G [56], an association controlled by the eukaryotic binding protein 4E-BP1 (see figure 2.2; [57]). The binding site of 4E-BP1 on eIF4E shares an overlap with the binding site for eIF4G, thus if 4E-BP1 is bound to eIF4E, eIF4G cannot bind and the eIF4 formation complex cannot occur [58]. 4E-BP1 must be dephosphorylated to bind with eIF4E to prevent translation occuring. Thus when 4E-BP1 is phosphorylated by its upstream effector mTOR, its attraction for eIF4E is depressed. This allows for the binding of eIF4G with eIF4E and the formation of the eIF4 complex [58]. The active eIF4 complex will then associate with the 5'-cap structure of the mRNA before binding to the 43s pre-initiation complex of mRNA translation (see figure 2.2). Therefore, identifying the phosphorylation of a given protein allows a measure of the activation/deactivation of that protein. When 4E-BP1 is dephosphorylated it then becomes more active, which can reduce translation initiation and protein synthesis. The measurement of 4E-BP1 phosphorylation has previously been used to determine if various interventions affect the initiation of protein synthesis. However, there are a number of events occurring during protein synthesis and 4E-BP1 cannot be solely used to inference differences in protein synthesis per se. In spite of this it is still a very useful marker and may provide mechanisms as to why protein synthesis may be altered.

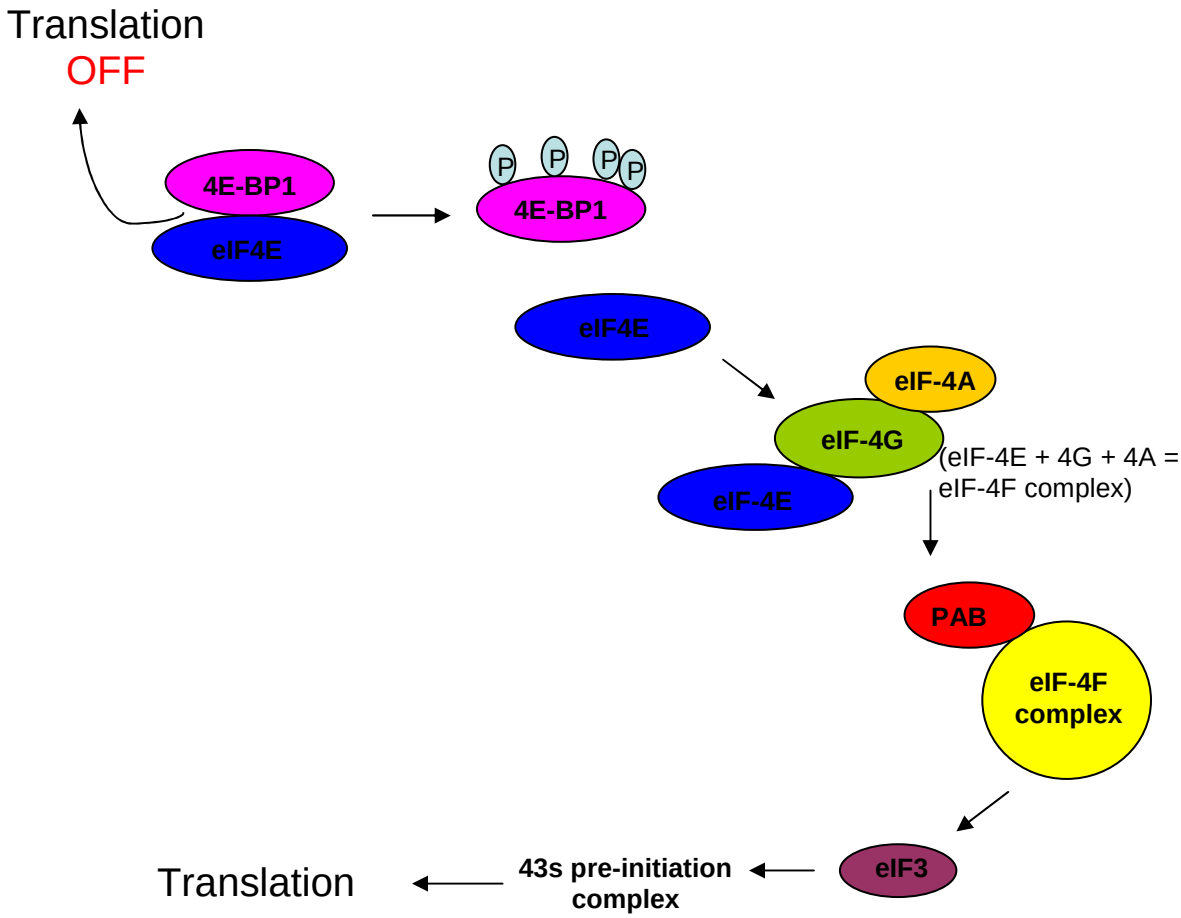

Figure 2.2. Simplified diagram of the formation of the eIF4 complex. The initiation-factor complex, eIF4F is composed of (eIF4A-eIF4E-eIF4G). Mitogens or IGF-1 induce the release of eIF4E by stimulating the phosphorylation of 4E-BP1 by the activation of the PI3-K/Akt/mTOR pathway. Release of eIF4E from 4E-BP1 allows it to join and form the eIF4F complex leading to the initiation of translation.

2.3.3. p70S6k

Parallel to the mTOR activated protein 4E-BP1, is the enzyme p70S6k. This signalling protein is also involved in regulating protein synthesis and is implicated as a regulator in cell size ([59]; Figure 2.3). When stimulated by insulin or mitogens, p70S6k phosphorylates the 40s ribosomal protein S6K. p70S6k undergoes multisite phosphorylation, becoming activated through phosphorylation of Serine and Threonine residues [60]. Pyruvate dehydrogenase kinase-1 (PDK1) has been shown to activate p70S6k through phosphorylation on the Threonine 252 site, however recent evidence suggests that p70S6k activation is also dependent on the phosphorylation of two other sites; Threonine 412 and

Serine 394 [61]. Activation of p70S6k enhances phosphorylation of the S6 ribosomal protein increasing the translation rate of 5'TOP (5' Terminal polypyrimidine tract) mRNAs. In the absence of phosphorylation of these sites, there is no detectable activation of any substrate downstream of p70S6k. Acute exercise studies have shown that p70S6k is phosphorylated after exercise and that this phosphorylation correlates with increases in skeletal muscle protein synthesis and muscle mass [62, 63].

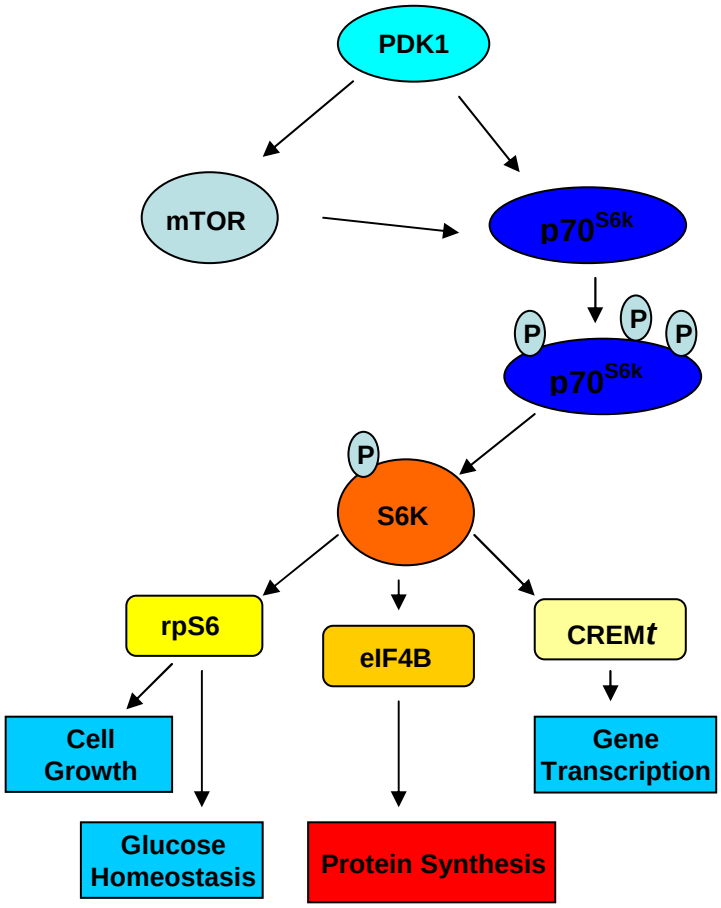

Figure 2.3. p70S6k pathway involved in protein synthesis. The sequential activation of the P13K/Akt/mTOR leads to the phosphorylation of the ribosomal protein S6K by p70S6k which stimulates the translation of mRNAs leading to initiation of protein synthesis.

2.3.4. AMPK

5'AMP-activated protein kinase (AMPK) is a heterotrimeric (a macromolecule composed of three subunits of at least one differs from the other two) complex, consisting of a catalytic alpha sub-unit and the regulatory beta and gamma sub-units. For activation of AMPK, the

gamma sub-unit must undergo a conformational change to expose the active Thr-172 site on the catalytic alpha sub-unit. The conformational change on the gamma sub-unit takes place under states of increased AMP (adenosine monophosphate) content within the cell. AMPK has been termed a metabolic switch [64, 65], becoming activated throughout states of energy stress such as hypoxia, low glucose and physical exercise, acting to reverse the energy depleted state by switching off anabolic consuming processes and stimulating catabolic processes [66]. Contrary to the other pathways discussed previously in this chapter, the AMPK pathway is a negative regulator of mTOR signalling via the phosphorylation and subsequent activation of TSC2 (Tuberous sclerosis protein 2), and/or the phosphorylation and subsequent inactivation of mTOR [67] (see Figure 2.4). Skeletal muscle contraction causes the ratio of AMP to ATP (Adenosine triphosphate) to rapidly increase; this change causes AMPK phosphorylation to subsequently increase. It is suggested that differences in type and intensity of exercise will activate the two AMPK isoforms (α1 and α2) differently. AMPK is also thought to have a glycogen-binding domain thus its activity increases during periods of low glucose concentrations.

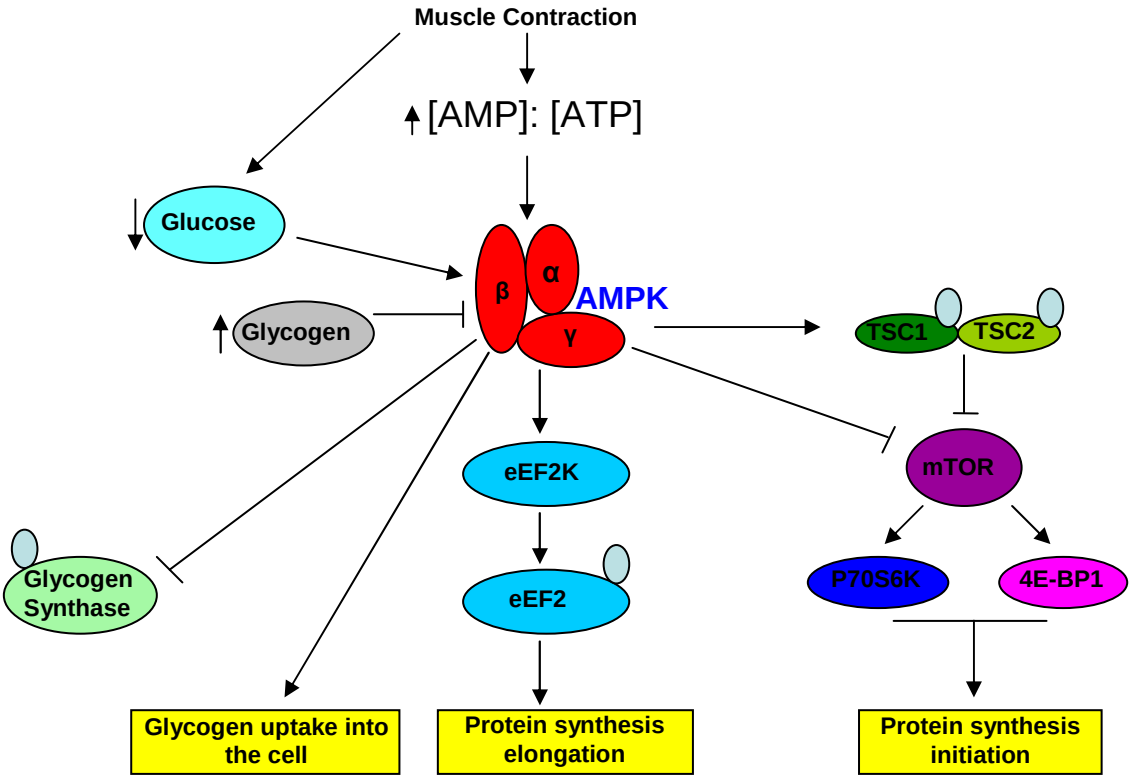

Figure 2.4. The influence of AMPK on protein synthesis and glucose homeostasis during and post exercise. Skeletal muscle contraction causes the ratio of AMP to ATP to increase which stimulates the phosphorylation of AMPK leading to the inhibition of mTOR and subsequent inhibition of protein synthesis.

2.4. Effect of Exercise on Skeletal Muscle Protein Signalling

2.4.1. Resistance Exercise and protein signalling

It has been well documented that resistance exercise is a potent stimulus of muscle protein synthesis and muscle cell growth, with an increase in protein synthesis being detected within 2-3 hours post exercise and remaining elevated for up to 72 h [44]. Resistance exercise can either suppress protein synthesis [8, 68, 69], or elevate protein synthesis [43] depending on the intensity of the exercise, and the amount of time passed since the exercise bout. Akt has been implicated as a key signalling protein that is able to provoke protein synthesis pathways. Once activated (i.e. in response to an exercise stimulus), Akt can stimulate (by changing the phosphorylation status) many downstream targets in the muscle cell that ultimately result in hypertrophy of the muscle. However, during and immediately post resistance exercise, protein synthesis is suppressed or decreases in human skeletal

muscle. Measurements of cell signalling events support these findings and provide some mechanisms and insight as to why protein synthesis is suppressed or decreased during and in the short term following exercise [4].

A likely reason that protein synthesis is suppressed is the large energy deficit that is often caused by repeated muscle contraction. A number of studies employing various acute resistance training protocols have reported reduced 4E-BP1 phosphorylation (~20 - 36%) [12, 70] [7, 8, 71] and protein synthesis immediately post exercise [7, 8]. In the absence of food intake, 4E-BP1 phosphorylation usually returns to baseline levels within 30 min – 2 h after exercise [7, 8, 71]. With the ingestion of amino acids and carbohydrate, both protein synthesis and 4E-BP1 phosphorylation are elevated above baseline levels [7, 72] [10, 12]. These results suggest 4E-BP1 phosphorylation may play an important part in the regulation of skeletal muscle protein synthesis.

Accompanying the reduction in 4E-BP1 phosphorylation after exercise, a number of studies also report an increase in AMPK phosphorylation following exercise ([7, 8, 71]. It has recently been shown that AMPK is a regulator of skeletal muscle energy status and may indeed inhibit the activation of the Akt/mTOR pathways until muscle energy stores begin to be replenished (see Figure 2.4). Indeed, AMPKα2 activity is increased immediately following exercise, and can remain elevated for 1 – 2 h [46]. Therefore, AMPK through its effect on TSC2 and ultimately downstream effectors of the Akt/mTOR pathways is a likely mechanism by which protein synthesis is reduced during and in the short term after exercise. However, not all reports support the notion of an AMPK/Akt switch and therefore further research in this area is required. However, to date it does seem that 4E-BP1 and protein synthesis are reduced in the short term after exercise and this is likely due to changes in cell energy status.

Another downstream target of mTOR that is activated in a similar but parallel manner to 4E-BP1 following muscle contraction is p70S6k (see Figure 2.1) Resistance training consisting of either 70 maximal eccentric contractions or 70 maximal concentric contractions were completed by rats. Eccentric contractions resulted in a 4-fold increase of phosphorylation on the Ser424/Thr421 site, as well as a 2-fold increase on the Thr 389 site directly after exercise with these levels remaining high 1 h and 2 h post exercise. In contrast, maximal concentric contractions did not cause any significant changes in phosphorylation of p70S6k [52], highlighting tha differences existed in the response between concentric and eccentric exercise. Additionally, both an acute resistance and endurance training session have been shown to cause an increase in the phosphorylation of p70S6k. However, after ten weeks of training, only resistance training exhibited significant chronic increases in p70S6k [73]. Significant increases in p70S6k phosphorylation have been regularly reported 30 min and up to 6 h after exercise [7, 15, 52, 63, 71]. Furthermore, an increase in p70S6k phosphorylation has been reported up to 36 hours post exercise [62].

2.4.2. Endurance Exercise

While it has long been thought that endurance training does not result in a positive protein balance i.e. net protein synthesis, recent reports have shown that endurance exercise also results in elevated protein synthesis and activation of the IGF-1 signalling pathway. However, there may be a difference in the type of proteins developed following endurance as opposed to resistance exercise, with greater mitochondrial protein synthesis present following endurance exercise and myofribrillar protein synthesis following resistance exercise [73]. Therefore, despite similar increases in protein synthesis, the divergent contraction modes with endurance versus resistance exercise are likely to induce different functional protein accretion and resultant exercise-induced adaptation.

Although recent findings [73] have provided some insight into the effects of endurance exercise on muscle protein synthesis, this area has received much less attention than resistance type exercise. Evidence suggests that protein synthesis is elevated following endurance exercise [74]. A further study investigating the effect of a 4 month endurance-training program (3 sessions per week at 70% heart rate max for 20 min) on protein synthesis found that despite no change in whole body protein turnover, muscle protein synthesis was increased following endurance exercise [75]. In a similar response to that observed following resistance exercise, endurance exercise also reduces protein synthesis in the immediate recovery stages [46]. The formation of the 4E-BP1· eIF4E complex (inactive complex = reduced translation initiation) has been shown to be elevated following endurance exercise [56], therefore, reducing the binding of eIF4E to the active eIF4F complex (active complex = increase translation initiation; see Figure 2.2). Consequently, a reduction in protein synthesis is likely. This suggests that the same mechanistic pathway may be responsible for the reduction in protein synthesis immediately post exercise following both resistance and endurance exercise.

The effect of endurance exercise on the activity of p70S6k is less well-known than the effects of resistance exercise. A study involving three intensities of contraction stimulus; high frequency electrical stimulus (HFES), low frequency electrical stimulus (LFES) and treadmill running (RN) investigated the response of signaling proteins including p70S6k of mice skeletal muscle to different types of exercise. Treadmill running exercise did not result in either p70S6k or PKB (associated with the regulation of p70S6k) phosphorylation at any of the studied time points (0, 3 and 6 h post exercise) [76]. These findings are supported by a previous study in which rats completed 2 h of treadmill running at 26m/min at ~75% $\dot{V}O_{2max}$. No significant activation of p70S6k occurred either at 1 h or 2 h after exercise, even when accompanied by refeeding, There was also a 26% decrease in protein synthesis

[56]. In contrast, 2 recent human studies have shown that acute endurance exercise (45 min – 1 h at 75% $\dot{V}O_{2max}$) increased phosphorylation of p70S6k in both the untrained and trained state [13, 73]. Conflicting findings indicate there may be differences in the p70S6k response between mice and humans following endurance exercise.

Although full regulation of AMPK following endurance exercise is still unclear, phosphorylation of AMPK by its kinase (AMPKK) and a reduction in creatine phosphate may increase skeletal muscle AMPK activity in response to light endurance type contractile activity. Some studies now suggest that the two isotopes of AMPK are activated at different intensities of exercise, the α1 isotope activated during endurance exercise at ~60% $\dot{V}O_{2max}$, with the α2 isotope thought to be activated only during high intensity exercise (> 60% $\dot{V}O_{2max}$) [77, 78]. Phosphorylation of the α2 isotope has been shown to increase up to 8 fold during high-intensity exercise, thus depending on the length of sprints and the length of rest periods, it could be expected that repeated-sprint activity will cause an increase in skeletal muscle AMPK [78, 79].

2.4.3. High-Intensity Repeated-Sprint Exercise

Heavy resistance exercise and low-intensity endurance exercise sit at the opposite ends of the exercise/training continuum and yet a number of athletes compete and train for sports that fall somewhere within this continuum. Furthermore, some sports require their athletes to be both strong/powerful and have high levels of endurance ability, including any team-sports. Indeed, football players are required to perform intense sprint efforts interspersed with periods of moderate to low-intensity activities [1, 80]. Often team-sport athletes will perform repeated-sprint type training, that has both a high power component and due to short rest periods employed, a high aerobic component. To date, there is little research as to the protein signalling response in human participants following this type of activity.

Depending on the type of sprint training employed, either aerobic or anaerobic type adaptations have been reported following sprint training. To date, there is only one published article that has determined the effects of sprint training on the muscle signalling response to exercise, with no studies yet to determine the effects of this type of training on protein synthesis. While not specific to team-sport athletes, a recent study did measure protein signalling in active males immediately following 1 x and 4 x 30-s maximal sprints and 3 h into recovery [17]. Phosphorylation of Akt, p70S6k and 4E-BP1 remained unchanged during and after 1 and 4 x 30 s maximal sprints, while AMPK and the α1 and α2 subunits were higher after sprint 4 than any other time point. This suggests that these high-intensity efforts had no effect on skeletal muscle growth signalling, however with only 6 participants this research may be underpowered [17]. Yet, with limited research into the muscle signalling response following this type of exercise, the answer to whether high-intensity sprint exercise resembles either endurance or resistance type training in terms of the activation of signalling proteins remains equivocal.

Table 2.1. Summary of research investigating Akt phosphorylation after acute exercise in skeletal muscle

Study	Participants	Exercise	Muscle Biopsies	Results
Humans				
Eliasson et al, 2006	n = 16m	4 x 6 sets concentric contrac. 4 x 6 sets eccentric contrac.	VL : rest, immed post ex., 1 h post ex, 2 h post ex.	No change
Coffey et al, 2006	n = 13 m	Cycle 60 min at 70% $\dot{V}O_{2max}$ 8 x 5 sets leg extension	VL : rest, immed post ex, 3 h post ex.	Unaltered after cycling, 50% ↑ after resistance post ex.
Dreyer et al, 2006	n = 7m + 4f	10 x 10 sets leg extension at 70% 1RM	VL : rest, during ex, 60 min post ex and 120 min post ex	100% ↑ post ex.
Wilson et al, 2006	n = 9m	Cycle 60 min at 70% $\dot{V}O_{2max}$	VL : rest, immed. Post ex., 3 h post ex.	50% ↑ immed. Post ex.
Thorell et al, 1999	n = 7m + 2f	Cycle 60 min at 70% $\dot{V}O_{2max}$	VL: rest, 1 h and 2 h post ex.	↑ 2.8 fold post ex.
Ivy, et al, 2008	n = 8m	Cyle 45 min at 75% $\dot{V}O_{2max}$ 5 x 1 min sprints at 90% $\dot{V}O_{2max}$	VL : rest, 45 min post ex.	No change
Drummond et al, 2008	n = 7m	8 x 10 sets of leg extension at 70% 1RM	VL: rest, 1, 3 and 6 h post ex.	No change 1 hr post ex.
Dreyer et al, 2008	n = 8m	10 x 10 sets of leg extension at 70% 1RM	VL: rest, immed, 1 and 2 h post ex.	Significantly ↑ 1 hr post ex. Returned to baseline by 2 h post
Glover et al, 2008	n = 9m	4 x 10 sets leg press at 10RM (fasted)	VL: rest, 360 min post ex	Significantly ↑ 360 min post ex.

Fujita et al, 2008	n = 7m + 4f	10 x 10 sets of leg extension at 70% 1RM	VL : rest, immed post ex., 1 and 2 h post ex.	No change.
Mascher et al, 2007	n = 6m	Cycle 1 h at 75% $\dot{V}O_{2max}$	VL : rest, immed post ex., 30, 60, 120, 180 min post ex.	No change immed post ex., 2 to 4 Fold 1 and 2 h post ex., returned to Baseline 3 h post ex.
Rats				
Bolster et al, 2003	Male Sprague-dawley	50 contractions of hindlimb	Gastroc : rest, 5, 10, 15	↑ 200% post ex.
Williamson et al, 2005	Male mice	Treadmill running at 10% incline At 26m/min for 10, 20 or 30 min	Gastroc excised post ex.	No change
With feeding				
Creer et al, 2005	n = 8m	3 x 10 sets of knee extension HCOH, LCOH meals	VL : rest, immed. Post ex., 10 min post ex	Akt level at all time points in LCOH, ↑ 1.5 fold at 10 min post in HCOH
Ivy, et al, 2008	n = 8m	Cycle 45 min at 75% $\dot{V}O_{2max}$ 5 x 1 min sprints at 90% $\dot{V}O_{2max}$ CHO-PRO drinks	VL : rest, 45 min post ex.	↑ 65% 45 min post ex
Drummond et al, 2008	n = 7m	8 x 10 sets of leg extension at 70% 1RM 20g EAA 1 hr post ex.	VL: rest, 1, 3 and 6 h post ex.	Significantly ↑ 3 h post ex.
Dreyer et al, 2008	n = 8m	10 x 10 sets of leg extension at 70% 1RM EAA + CHO solution following 1 h recovery	VL: rest, immed, 1 and 2 h post ex.	Significantly ↑ 1 hr post ex. Remained elevated 2 hr post ex
Glover et al, 2008	n = 9m	4 x 10 sets leg press at 10RM	VL: rest, 360 min post ex	Significantly ↑ at rest in the fed

		Mixed meal drink at 90, 180, 270 Post ex		state, did not increase significantly post ex
Fujita et al, 2008	n = 6m + 5f	10 x 10 sets of leg extension at 70% 1RM EAA + CHO solution 1 hr pre ex.	VL : rest, immed post ex., 1and 2 h post ex.	Significantly ↑ pre ex., remained Elevated at 1 h post, returned to Baseline 2 h post.

m = male; **f** = female; **Ex** = exercise; **RM** = repetition maximum; **CHO** = Carbohydrate diet/meal; **Pro** = Protein diet/meal $\dot{V}O_{\mathbf{2max}}$ = maximal oxygen upatake; **VL** = Vastus lateralis muscle

Table 2.2. Summary of research investigating 4E-BP1 phosphorylation after acute exercise in skeletal muscle

Study	Participants	Exercise	Muscle Biopsies	Results
Human				
Dreyer, et al 2006	n = 7m + 4f	10 sets of 10 repetitions leg extension at 70% 1RM	VL: rest, during ex, 60 min and 120 post ex.	4E-BP1p ↓ immediately following exercise but returned to baseline 2 h post ex.
Koopman et al, 2006	n = 8m	8 sets of 10 repetitions leg press and leg extension at 75% 1RM	VL: Rest, 30 min and 2 h post	4E-BP1 phosphorylation ↓ post ex, returned to baseline at 30 min post Ex.
Deldicque et al, 2005	n = 6m	10 x 10 sets of leg press at 70% 1RM	VL : rest, 3 h and 24 h post ex.	↑ 200% 3 h post ex, ↓ towards Basal levels 24 h post
Drummond et al, 2008	n = 7m	8 x 10 sets of leg extension at 70% 1RM	VL: rest, 1, 3 and 6 h post ex.	No change 1 h post ex.
Dreyer et al, 2008	n = 8m	10 x 10 sets of leg extension at 70% 1RM	VL: rest, immed, 1 and 2 h post ex.	Significantly ↑ 1 hr post ex. Returned to baseline 2 h post ex.
Fujita et al, 2008	n = 7m + 4f	10 x 10 sets of leg extension at 70% 1RM	VL : rest, immed post ex., 1and 2 h post ex.	Significantly ↓ post ex, returned to Baseline during recovery.
Rats				
Bolster et al, 2003	Male Sprague-dawley rats	50 contractions of hindlimb musculature	Gastroc : rest, 5, 10, 15 30, 60 min post ex	↑ 4E-BP1 phosporylation 10 min post ex.
Williamson et al, 2005	Male mice	Treadmill running at 10% incline	Gastroc excised post ex.	↓ 4E-BP1 phosphorylation post ex.

		At 26m/min for 10, 20 or 30 min		
Morrison et al, 2007	n = 60 Male Sprague-Dawley rats	3 h swimming with 3% weight attached to tails	Quadriceps excised immed post, 60 and 90 min post ex	↓ 55% 4E-BP1 phosphorylation post ex
With Feeding				
Morrison et al, 2008	n = 60 Male Sprague-Dawley rats	3 h swimming with 3% weight attached to tails. CHO, CHO + Pro, Pro diets.	Quadriceps excised immed post, 60 min and 90 min post Ex.	CHO+Pro more phosphorylated at 30 min post exercise. Significantly Higher 90 min post than other diets But same as exercise only group.
Koopman et al, 2007	n = 7m	8 x 10 sets of leg press and leg Extension at 75% 1 RM CHO, CHO + Pro Diets	VL : rest, immed post ex, 60 min and 240 min post ex.	↓ 4E-BP1 phosphorylation post ex (48% in CHO group, 15% in CHO + Pro group) ↑ during recovery but higher in CHO+Pro
Drummond et al, 2008	n = 7m	8 x 10 sets of leg extension at 70% 1RM 20g EAA 1 hr post ex.	VL: rest, 1, 3 and 6 h post ex.	Significantly ↑ 3 h post ex.
Dreyer et al, 2008	n = 8m	10 x 10 sets of leg extension at 70% 1RM EAA + CHO solution following 1 h recovery	VL: rest, immed, 1 and 2 h post ex.	Significantly ↓ 1 h post ex. Significantly ↑ 2 h post ex
Fujita et al, 2008	n = 6m + 5f	10 x 10 sets of leg extension at 70% 1RM EAA + CHO solution 1 hr pre ex.	VL : rest, immed post ex., 1and 2 h post ex.	Significantly ↑ immed pre ex. Returned to 1 h pre ex levels after Ex., was higher immed post and 1 h Post ex compared to unfed

m = male; **f** = female; **Ex** = exercise; **RM** = repetition maximum; **CHO** = Carbohydrate diet/meal; **Pro** = Protein diet/meal; **VL** = Vastus lateralis muscle

2.5. Sex Differences in the human body that may affect exercise performance and subsequent muscle response

As the participation and acceptance of females in athletic activities increases, so does the interest of sex differences in performance. Only 40 years ago were woman first allowed to compete in an Olympic marathon event, decades after men first started competing. Ten years earlier woman were only just being granted permission to compete in running events longer than 800m. These restrictions on women were set in place due to the unguided perception that women were physiologically not suited to such distance events. In fact, now science suggests that women may have a greater resistance to aerobic fatigue than men.

2.5.1. Body Size and Composition

It is accepted that on average, women are shorter and lighter than their male counterparts and have less lean mass and greater fat mass [81, 82]. However, until puberty at ~12 y in females and ~14 y in males, sex differences in body composition are non-existent. By the ages of 12 -13 y, fat free mass (FFM) in females begins to show sign of a plateau, peaking at around 15 y, whereas males reach their peak FFM at around the age of 20 y [83].

Body composition differences between sexes tend to occur at the onset of puberty due to endocrine changes. The secretion of the sex hormones, testosterone and oestrogen influence growth in males and females differently, so that by adulthood, men have a greater muscle mass [82] and a significantly different distribution of their muscle mass, in that men carry a greater percentage of their muscle mass in the upper body compared with women [81, 84]. Men will typically be ~13 cm taller, weigh ~18kg more (~20kg heavier in FFM) and have ~10% less body fat than women [85]. Anthropometrically men have broader shoulders, narrower hips and carry more fat in the abdomen, where women will carry more fat on the hips and lower body [86, 87].

Sexual dimorphism also exists in skeletal muscle in the size of individual muscle fibres, metabolic enzyme activity, gene expression, substrate oxidation, fatigability and overall mass of the muscle. Muscle fibres are larger in men [88] [89], but the sex difference is particularly prominent in type 2 fibres, in which men have a greater type II to type I fibre ratio when compared to women [88, 90].

2.5.2. Sex Hormones

Women have less muscle than men and research suggests gains in muscle mass are smaller and slower with strength training [91, 92] which is a potent stimulator of muscle growth [93-97]. Why it is that women are at a disadvantage in terms of mass and growth of skeletal muscle is still not clear, however, the lower levels of circulating testosterone may be an important factor.

Testosterone is known to increase both protein synthesis and net muscle protein balance [98] which results in increases in muscle mass [99]. Until puberty, no differences exist in levels of circulating hormones in boys and girls. At the onset of puberty however, there is a dramatic increase in testosterone levels in males which is accompanied by transient increases in muscle mass, significantly surpassing muscle gains in females. Testosterone is also understood to help maintain muscle throughout the male lifespan. There is no evidential data that female sex hormones (particularly oestrogen) prevent or inhibit muscle protein synthesis or maintenance of muscle mass. Hypogonadal men tend to have lower fat-free mass and higher fat mass than eugonadal men. Testosterone replacement therapy (10+ weeks at ~100mg testosterone weekly, intramuscularly or sublingualy) results in significant increases in fat-free mass ranging from ~2 - 15% with some studies showing concomitant increases in strength of up 22% [100], where others have recorded no change or decreases in strength [101].

Oestrogens are present in both men and women, but levels are significantly higher in females than males post puberty. Oestrogens have been shown to influence patterns of metabolism at rest and during exercise [102-104]. Oestrogens promote deposition of subcutaneous fat [105] and possibly alter appetite by modifying levels of circulating fat. Oestrogens are also known to increase insulin sensitivity [106], possibly stimulating increased levels of lipolysis in fat cells. Progesterone also stimulates fat accumulation by exciting adipocyte ligases. It is probably these female hormones that are responsible for the observations of less oxidative stress during endurance exercise, and less exercise-induced muscle soreness following eccentric contraction in females [107].

During detraining or deweighting, the muscle mass lost is less in women than in men [91]. Again the reasons for this are not clear, including whether the female sex hormones have a direct effect on this response. However, rat studies have shown that ovariectomy (essentially removal of the female sex hormones) prevents the recovery of atrophied muscle following hind limb suspension and is accompanied by reduced activation of Akt and p70S6K (and its downstream substrates) [108]. This suggests that removal of the ovaries (and ovarian hormones) alters the physiological environment required for female muscle to grow following atrophy. Extending these results to humans is difficult due to the large protein turnover in rats compared to humans. However, these findings do indicate sex differences in protein synthesis/degradation may be due to hormonal differences.

Although research into this area is far from conclusive, it seems evident that differences in levels of sex hormones, particularly the levels of testosterone are in some way partly responsible for the significant differences in muscle mass between sexes.

Menstrual Cycle

As the percentage of female athletes in both recreational and competitive sport increases it is important to gain an understanding of how and when the menstrual cycle has an effect on exercise performance and adaptation, both as a comparison within females and between female and male athletes. The ovaries (female sex organs) produce a number of hormones, the production and release of which will oscillate both throughout the lifespan and during each individual cycle. The ovaries also produce the male hormone testosterone (in much lower doses than in men), production of which continues beyond menopause. To date the effect of the menstrual cycle on female exercise performance is inconclusive. Some studies have reported improved performance during particular phases of the cycle where others conclude muscular strength and fatigability, and exercise capacity, as defined by both $\dot{V}O_{2\,peak}$ and the lactate threshold are not affected by menstrual cycle phase in young women [109-111].

Glycogen storage and ultilisation have been shown to be affected by metabolic hormones. Rodent studies have shown that the female hormone oestrogen can alter glycogen storage and reduce the utilisation of glycogen during long duration sub-maximal exercise [112, 113], most probably by way of 'glycogen sparing' due to increased oxidation of fatty acids as the prominent energy sources [114]. In human female endurance athletes under sex hormone manipulation (within levels found naturally in healthy menstruating women), it was found that high circulating concentrations of the female sex steroid hormones resulted in an enhanced reliance upon the oxidation of lipid as an energy substrate, accompanied by a significant reduction in carbohydrate oxidation [115] .This situation was reversed during levels of low sex hormone circulation. In physical exercise of long duration that is heavily reliant on glycogen, the 'metabolic shift' in energy substrate caused by circulating female hormones could result in small performance improvements.

While research into the effect of the menstrual cyle phases on myofibrillar protein synthesis has been almost non-existent, a recent study found no effect of menstrual cycle phase (follicular phase, or luteal phase) at rest or in response to an acute bout of exercise (60 min of one-legged kicking at 67% W_{max}) on myofibrillar protein synthesis in women [116]. Based on the research to date investigating the effect of the menstrual cycle and its phases on exercise performance, and exercise response in the muscle, it appears that the response to resistance type exercise or anaerobic exercise that does not rely heavily on the oxidation of substrates for fuel, will be uneffected by the phase of the menstrual cycle.

2.5.3. Sex differences in Growth Hormone release during exercise

An exercise bout has shown to be a powerful stimulus for acute growth hormone release (GH) in both men and women which could affect substrate use and adaptation to exercise [117]. The intensity of the exercise is suggested to regulate the dose response of GH release, and a threshold which must be exceeded to elicit release. It is known that young females secrete a one and a half to two-fold greater mass of GH per pulse at rest than similar aged men, and some studies have found that females will reach the peak of their absolute GH concentration at a particular set intensity sooner than their male counterparts [21]. A study with male and female participants exercising for 30 min at ~70% $\dot{V}O_{2\,max}$ found that this duration and intensity of exercise was stressful enough to elicit the growth hormone response to exercise. Despite the resting differences in pulsatile GH secretion (higher in women than men) [118], the magnitude of the incremental increase in GH release induced by the aerobic exercise protocol was similar between the sexes [21]. However, as with rest, females secreted greater levels of GH per pulsatile burst during constant load exercise, resulting in greater mean production. Females also reached their peak GH concentration sooner than men, but the fold increase in GH release was greater in males than in females [21]. Despite the differences in resting growth hormone release and the time to reach peak

growth hormone release during exercise, the absolute magnitude of release during sub maximal exercise is similar between the sexes.

2.5.4. The effect of Sex on Substrate Metabolism during Exercise

Woman have a lower resting metabolic rate than males, a consequence of differences in overall muscle mass and body composition [119]. Females also display the advantage of having a greater ability to metabolise lipids [120]. Apart from very high-intensity exercise (>80% $\dot{V}O_{2\,max}$), fuel substrate utilisation in skeletal muscle during exercise comes from both carbohydrate and fat stores. While it is well known that relative contributions of carbohydrate to fat utilization depends on both the duration and intensity of the exercise, sex has also been shown to affect substrate utilisation during exercise [121].

During sub-maximal prolonged exercise, endogenous triglycerides are an important source of energy for the exercising skeletal muscles. Lipolysis of these triglycerides increases during exercise, freeing fatty acids into circulation for their oxidation by the working muscles. Lipolysis of intramuscular triglycerides is also stimulated by endurance-type exercise, releasing fatty acids for direct oxidation by the mitochondria. However, the rate of fat to carbohydrate usage during endurance exercise has been shown to differ in some, but not all studies comparing males and females. Earlier studies have reported no significant differences in the substrate reliance during sub-maximal exercise between sexes [122], whereas later studies have shown that women have a lower respiratory exchange ratio during sub maximal exercise when compared with men [120, 123], indicating greater fat utilization. Indeed, both sedentary and recreationally active women compared to their male counterparts have recorded less glycogen utilization, and higher concentrations of plasma free fatty acids (FFA), and oxidation of intramyocellular lipid (IMCL) [124, 125] during endurance exercise, reflected in lower respiratory gas exchange ratio at any given relative

sub-maximal intensity. At higher intensities (>80% $\dot{V}O_{2\,max}$) of exercise the sex difference in substrate choice for fuel disappears.

While many studies have reported sex differences in metabolism [119, 121, 124, 125], others have not [122, 126]. Differing results between studies could be due to differences in body composition and relative fitness levels between male and female participants, as both can independently alter rate of substrate use during exercise. In a study where male and females were matched on both aerobic fitness and adiposity, a stable isotope infusion protocol was conducted to perform lipid kinetics at rest and during exercise and participants performed cycle exercise at 50% of their $\dot{V}O_{2\,max}$. Whole body lipolytic rate and plasma and FFA availability and uptake was higher in women than in similarly matched men, while whole body total fatty acid oxidation was similar between sexes. Women also oxidized greater plasma FFA and less nonplasma fatty acids [125]. Despite a growing body of literature in this area, the effect of sex on both the mobilization and utilisation of triglycerides and carbohydrates during endurance exercise is still not completely clear.

2.5.5. Sex differences in factors regulating protein synthesis

The clear sex differences in adult human muscle mass and the increasing evidence for substrate differences during exercise between males and females suggest that there may be sex differences in protein metabolism. However, research to date has failed to exhibit any significant sex differences in signalling protein pathways directly related to protein synthesis in skeletal muscle at rest or following exercise. On the other hand, recent studies in rats have shown sexual dimorphism in skeletal muscle protein synthesis following chronic alcohol consumption. Following up to 26 wk of an alcohol diet, the inactive 4E-BP1. eIF4E complex was increased more than two-fold in male rats, with no alterations in complex levels shown in females. Similarly the amount of active eIF4G.eIF4F complex was

reduced up to 60% in males with again no change in levels in females. In support of these alterations in muscle signalling, male rats exhibited a decline in protein synthesis (20%) following chronic alcohol administration [20]. Interestingly, this study also reported lower basal (prior to alcohol treatment) protein synthesis and 4E-BP1 phosphorylation in females compared to males.

Although most of the data to date shows no sex differences in basal muscle protein balance, it is possible that current methods of measurement are not sensitive enough (either in sample size, or method) to detect small but possibly meaningful differences. One of the very few studies that directly compared males with females, investigated MEF2 regulatory pathway stimulation at rest and during sub maximal (60% $\dot{V}O_{2\,max}$) cycle exercise. While no differences were present at rest, in response to exercise, MEF2A mRNA expression increased exclusively in women, whereas MEF2D mRNA expression tended to increase only in men [19]. However, failure to find any sex differences in proteins associated with translation and initiation of protein synthesis may lend further support to the idea that sex hormones are the main influence on differences on musculature between males and females.

The Grb10 adapter protein

The Grb10 (growth factor receptor-bound 10) adapter protein is capable of interacting with a variety of receptor tyrosine kinases, including the binding to and signal regulation of the insulin receptor, a signalling molecule involved in the stimulation of protein synthesis in skeletal muscle. Grb10 has been implicated in a number of cellular processes, including regulating muscle cell growth, muscle metabolism, and apoptosis [127]. Grb10 has also been shown to produce a protein that prevents IGF signalling [128]. Although it has previously been shown that plasma IGF-1 levels or IGF1 muscle gene expression does not demonstrate a sex difference, studies have shown that levels of Grb10 (which is an inhibitor

of IGF signalling) are elevated in women [129]. However levels of IGF receptor gene expression were shown to be 3 –5 fold higher in females than males which may act to minimize effects on insulin sensitivity bought on by the higher levels of Grb10.

2.5.6. The Effect of Sex on Exercise Performance and Adaptation

Endurance Exercise

Regular endurance training results in physiological adaptations of the cardio-respiratory and neuromuscular systems that improve the delivery of oxygen to the mitochondria and facilitate a tighter regulation of muscle metabolism [130]. Improvements in any of these physiological parameters will result in improved aerobic endurance performance including an increased ability to sustain sub-maximal exercise at a given workload for a longer period of time, or achieve a higher average work output over a constant distance or time [131]. However, it has been demonstrated that women have a greater capacity for sub-maximal endurance activity than men. Women have shown that at the same relative intensity of sub-maximal exercise they continue exercise for longer than men [132-134]. Although the mechanisms responsible for this sex difference are still being researched, it is proposed that men rely more on glycolytic pathways during exercise while women rely more on fat oxidation, suggesting that women can last longer during sub-maximal exercise due to their utilisation of fat as a fuel source and glycogen sparing.

Unique sex differences have been shown in baseline capillary density and capillary denisty change in response to aerobic exercise. In sedentary overweight participants, women have been shown to have significantly lower baseline capillary density than similarly matched men [135]. After a long term running program (20 miles per wk x 24 wk) exercise capacity increased by the same relative amount (20%) in both males and females. However, only females showed an increase in capillary density, suggesting that male and female muscle undergo different adapatations with exercise and it is likely that improved

skeletal muscle capillary density plays a greater role in women than men for improving aerobic exercise capacity [136].

Anaerobic and High-Intensity Repeated- Sprint Exercise

Anaerobic power determines the ability of a person to undertake short sprints and jumping movements and is a reflection of phosphagen stores in active muscles as well as the potential to mobilise the stores of energy quickly when required. Due to the smaller overall muscle mass, women have the disadvantage of having only about 70% the total fibre area of that in men, a significant shortcoming when developing maximum power. Males out-perform females in maximal power production, mean power production and total work performed during a 30 s cycle ergometer sprint [137]. A study by Hill et al, [138] reported a 35% greater total work completed by males then females during a 30 s Wingate test. Additionally, the males' anaerobic energy production was twice that of the females in the final 5 s, suggesting that males have higher anaerobic energy provisions during maximal prolonged exercise. Reports suggest that females achieve only ~70% the anaerobic power achieved in this type of test relative to males. However, when Wingate test performance is calculated relative to body mass the sex gap is reduced [137]. Furthermore, when performance is calculated relative to lean body mass, the gender gap in peak power was shown to be reduced to 10% of male maximal values.

A further study [139] comparing Wingate sprint performance between males and females found a similar linear association between absolute lean mass of the lower extremities and peak power output. This relationship was so similar between males and females that when expressed relative to lean mass in the lower extremities, the mean peak power output achieved during the Wingate was ~50 $W \cdot kg^{-1}$ in both sexes. These studies indicate that when normalized to lean body mass, power output does not differ markedly

between males and females. This suggests that the absolute sex differences in anaerobic performance (≤ 30 s in duration) are predominantly due to differences in muscle mass.

Top male sprinters are about 7.3% faster than their female counterparts [140]. Sprint performance depends on the ability to generate power in the lower limbs as well as achieving a high ratio of power production to body mass. Correlations between leg muscle mass and maximal running velocity have been reported as quite high [139]. As muscle mass is a large determinant of force production, (and the resulting power [(force x the velocity at which the force was produced)] one could assume that when scaled for lean muscle mass men and women could produce the same sprint performance. Also, when discussing and comparing sprint studies it is important to note the differences between running and cycle sprinting. During sprint running, the athlete must transport their own body mass and perform both concentric and eccentric contraction whereas sprint cycling is weight independent and contains no eccentric contraction. For ease of laboratory control and work parameters most studies have used cycle sprinting as their mode of sprint exercise [2, 141-146] .

The ability to repeatedly sprint at maximal effort is important in many team sports. This area has been regularly studied in the past few years; though most studies used only male participants and very few directly compared males and females. However, one comparative study found recovery time required between sprints to reproduce the same relative mean power is similar between males and females [141]. This said, women showed greater decrement in power output from peak values, and had a greater loss in power towards the end of the sprints. This finding suggests that women may be more prone to fatigue (loss of power or slower sprint times) with repeated maximal efforts.

2.5.7. Blood Insulin

Although the blood insulin response following exercise can differ amongst individuals and with different exercise modes and intensities, clear sex differences have been identified. Following acute cycle exercise at 85% lactate threshold, men were found to have a significantly lower whole body insulin action than women when measured 3-4 h post exercise. This result is likely due to the relative decrease in the stimulation of peripheral glucose uptake in men while no sex differences existed in endogenous glucose production [147]. Similar results were recorded following treadmill running at ~65% $\dot{V}O_{2\,max}$ for 15.5 km, with men found to have significantly lower blood insulin levels than females [120]. Sex differences in insulin sensitivity have also been recorded at rest, with females having higher levels than males [148]. Also, after feeding of a high-starch meal, females exhibit a higher glucose flux and greater whole-body insulin sensitivity than males [149].

Although, research on sex specific response in insulin sensitivity following exercise is not widespread most studies on the topic have observed greater sensitivity in females. Collectively, the findings suggest that sex differences do exist in post exercise insulin action in skeletal muscle.

2.5.8. Blood Glucose

Whole body carbohydrate metabolism has generally been observed to be proportionally less in females compared with men, while oxidizing more lipids as already discussed earlier. During mild to moderate exercise, circulation of blood glucose and intramuscular glycogen stores contribute to total carbohydrate oxidation. The lower carbohydrate oxidation observed in females has been suggested to be due to lower utilization of circulating blood glucose and muscle glycogen. Like many areas in exercise metabolism research, the sex differences in glucose and glycogen levels has been under-studied. Due to this lack of

research, discrepancies exist in the small volume of available findings. Some studies report similar glycogen levels [150, 151] while others report lower rates of glucose turnover and muscle glycogen utilisation in females versus males [120]. Differences in results are likely to be due to do the differences in direct methods of measuring glucose turnover [124].

Table 2.3. Summary of research investigating Sex-differences at rest and with exercise

Study	Participants	Exercise/Intervention	Measurement	Results
Janssen et al, 2000	n = 268 m + 200 f	Rest	MRI: Transverse images 10 mm Thick taken every 40 mm from Hand to foot	Males had significantly more SM in absolute and relative terms Males had significantly more SM in Upper body compared to lower body in comparison to females
Wideman et al, 1999	n =9m + 9f	Cycle exercise for 30 min at pre-Determined power output	Growth Hormone measure in blood samples	Rest: 3.69 fold > mass of GH secreted per burst Ex: Resulted in > mean mass of GH secreted per burst in females Women obtained max serum GH Conc. Earlier than males Similar max serum GH conc in Males and females
Arciero et al, 1993	n = 328 m + 194 f	Rest	Resting metabolic rate	RMR 23% higher in males than females in absolute terms After controlling for FM and FFM Sex difference (3%) remained
Horton et al, 1998	n = 14m + 13f	2 h cycling at 40% $\dot{V}O_{2max}$ 2 h post exercise recovery	Substrate metabolism Indirect calorimetry	More total energy expended from fat oxidation in women. Males Derived proportionally more Energy from carbohydrate Oxidation
Mittendorfer et al, 2002	n = 5m + 5f	Cycling at 50% $\dot{V}O_{2max}$	Substrate metabolism Isotope infusion	Whole body lipolytic rate and plasma FFA availability and uptake During exercise greater in females Than males

Duscha et al, 2001	n = 25 m = 13 f with Chronic heart failure (CHF) n = 10 m + 10 f	Graded upright cycle exercise beginning at 150 kpm/min + 3 min stages	Capillary density MB: Vastus lateralis rest & post ex	Normal males have greater baseline capillary density then females ↓ $\dot{V}O$ 4.5 mets in men = ↑ capillary density compared to CHF men with $\dot{V}O$ > 4.5 mets Females with CHF have greater Capillary density then normal females
Duscha et al, 2003	n = m + f	24 weeks at 20 miles running Per week	Capillary density MB: Vastus lateralis Pre & post training	Males and females ↑ exercise capacity by same relative amount (20%). Only females significantly ↑ Capillary density
Hill et al, 1993	n = 16 m + 22 f	Modified Wingate tests Against resistances of 0.086 kg^{-1} body mass		Total work was 30% lower in females then males Aerobic contribution was 7% lower In females compared to males
Perez-Gomez et al, 2008	n = 123 m + 32 f	30 s Wingate tests		Peak power output expressed Per kg lean mass was similar in Males and females. Mean power Was 22% higher in males
Perreault et al, 2004	n = 10 m + 10 f	Cycle exercise for 90 min at 85% Lactate threshold	3-h hypoinsulinemic euglycemic clamp (30 $mU.m^{-2}.min^{-1}$)	Males exhibited relatively lower whole body insulin action in 3-4 h post exercise Lower glucose rate of disap-pearance in males

Robertson et al, 2002	n = m + f	Feeding of a high-starch meal	Insulin-sensitivity Derived using Cumulative Gd/time & integrated insulin concentration	Females had significantly higher insulin-dependent GD per pmol of insulin. Greater whole body insulin sensitivity in males
Horton et al, 2006	n = 12 m + 10 f	Cycle exercise for 90 min at 85% lactate threshold	Isotope infusion [6,6-$^{2}H_2$]	Circulating blood glucose flux was significantly lower during 90 min Exercise and immediately post ex.
Lang et al, 2006	n = m + f Sprague-Dawley rats	26 wk on an ethanol-containing diet	Gastrocnemius portions post ex.	Protein synthesis ↓ 20% in males Accompanied by a 60% ↓ in active eIF4E.eIF4G complex Protein synthesis unaltered in Females. Translation initiation also Unaltered in females

m = male; **f** = female; **Ex** = exercise; **GD** = glucose disappearance; **RMR** = resting metabolic rate; **FM** = fat mass; **FFM** = fat free mass; **MB** = muscle biopsy

2.5.9. Summary of reviewed literature

1. Team sport athletes are required to perform repeated high-intensity/maximal efforts interspersed with varying active recovery periods as part of regular match play
2. Resistance exercise and endurance exercise cause increases in myofibrillar and mitochondrial protein synthesis respectively
3. The effect of high-intensity/sprint type exercise on signalling proteins has not been extensively investigated
4. Sex differences exist in musculature and skeletal muscle metabolism at rest and during exercise
5. No previous research has exclusively compared relative male and female sprint performance and the subsequent muscle signalling response

2.5.10. Aims

1. To investigate repeated sprint performance in both male and female team sport athletes;
2. To investigate blood and muscle response to high-intensity repeated sprint exercise

3. Methods

3.1. Participants

Eight female and seven male competitive football players from the University top level senior football teams (see Table 3.1) volunteered to participate in this study. Participants were informed of the study requirements, benefits and risks before providing written consent. Approval for the study's procedures was granted by the Massey University Human Ethics Committee and conformed to the Declaration of Helsinki.

Table 3.1. Subject Characteristics, Mean ± SE

	Male	Female
Age (y)	20.1 ± 2.1	19.4 ± 2.2
Weight (Kg)	79.0 ± 11.1	62.6 ± 6.8 *
$\dot{V}O_{2\,peak}$ (mL·Kg·min)	58.5 ± 5.8	51.2 ± 4.4 *

* Significantly different from male values (P<0.05).

3.2. Experimental overview.

All participants attended the laboratory for baseline measures, where they completed a graded exercise test to determine peak oxygen uptake ($\dot{V}O_{2\,peak}$) and running velocity at $\dot{V}O_{2\,peak}$ ($v\dot{V}O_{2\,peak}$). All subjects performed a familiarization trial of the sprint protocol at least 48 h before experimental testing began. The sprint protocol required participants to complete 4 sets of 6 x 30 m sprints (see Figure 3.1). Prior to, 15 min post and again 2 h post the sprint protocol, muscle biopsies and venous blood samples were collected. All participants performed the sprint protocol at the same time of day (± 2 h).

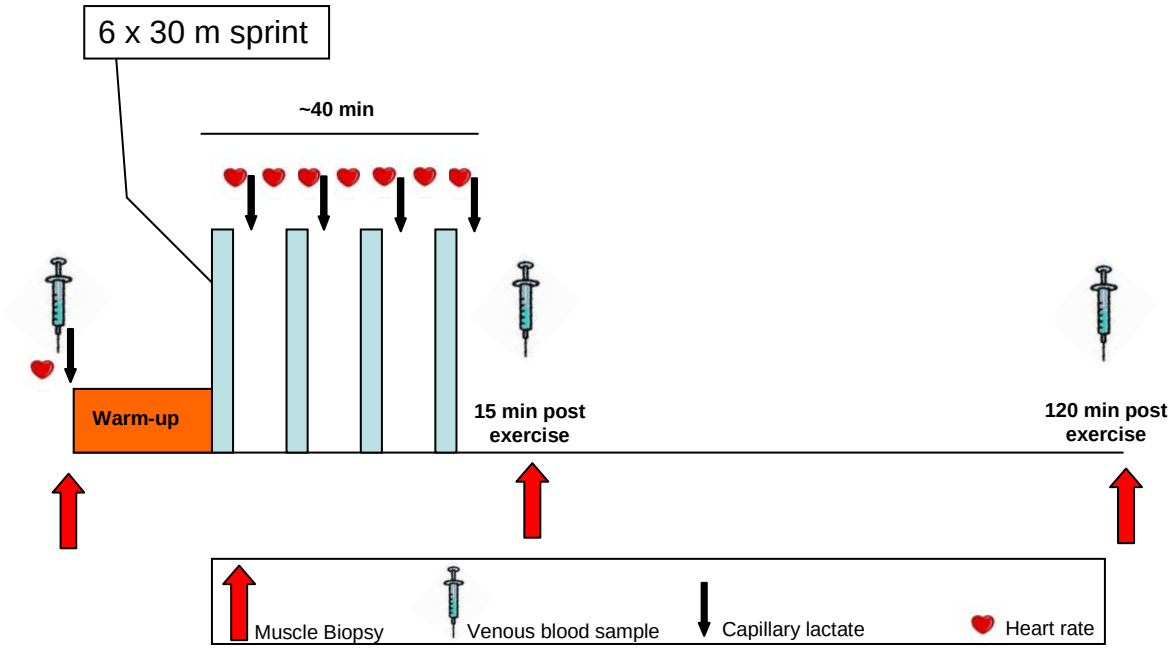

Figure 3.1. Overview of Experimental protocol.

3.3. Graded exercise test

The graded exercise test (GXT) to determine $\dot{V}O_{2peak}$ and $v\dot{V}O_{2\,peak}$ was conducted on an electronically-controlled treadmill (True 825, Fitness Technology Inc, Missouri, USA) and consisted of graded exercise steps (2.5-min stages), using a continuous protocol [152]. The test commenced at 10 km·h^{-1} with the speed increased to 12 km h^{-1} and thereafter, by 1 km h^{-1} each stage, until volitional exhaustion. The test was completed when the subject could no longer continue running at the speed required and grabbed onto the hand rails due to volitional exhaustion. Verbal encouragement was provided to each subject throughout the test to ensure maximal effort. Expired gas was collected using Douglas Bags and measured with an Ametek analyser (Applied Electrochemistry, Pittsburgh, PA) and a dry gas meter (Harvard, UK) to determine $\dot{V}O_2$. The value used for $\dot{V}O_{2peak}$ corresponded to the highest value achieved over a 1-min collection period. The velocity at $v\dot{V}O_{2peak}$ was determined from the speed at the last completed stage of the graded exercise test.

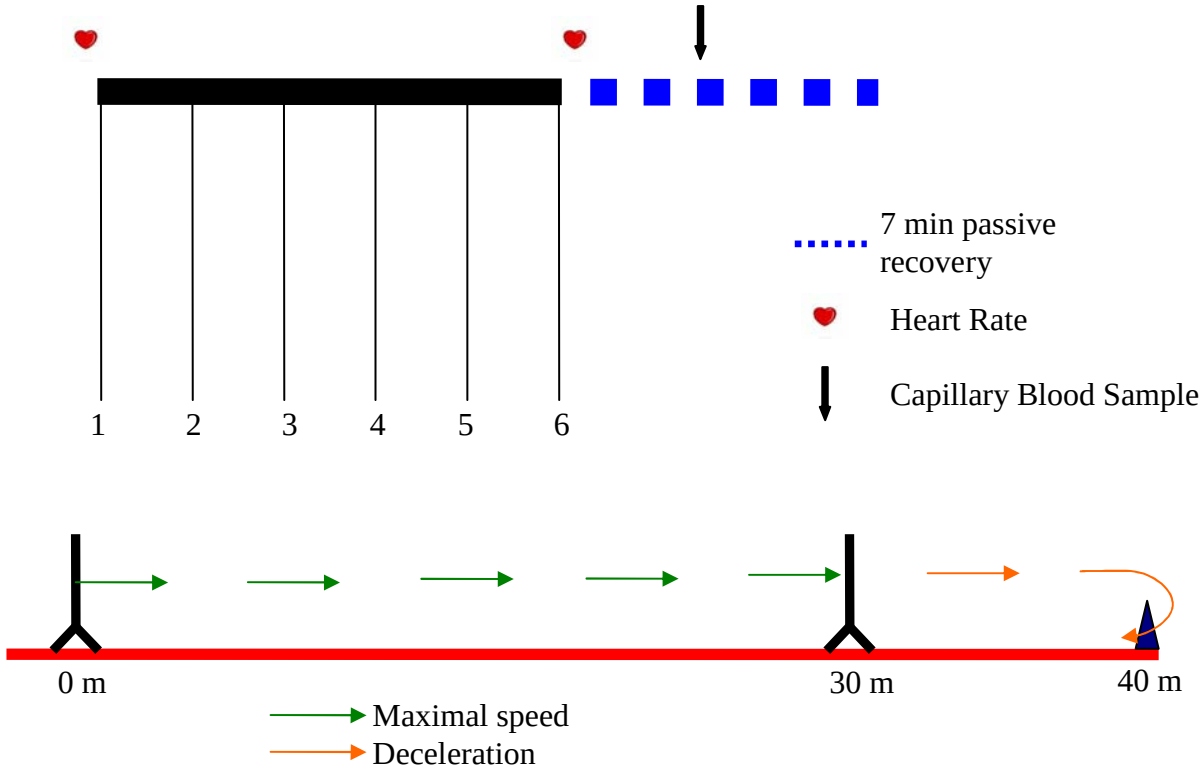

Figure 3.2. Diagram of the repeated-sprint protocol. *Upper diagram:* Representation of one full sprint bout. *Lower diagram:* Representation of specific sprint requirements; participants ran at maximal speed from point 0 m (timing gate 1) to point 30 m (timing gate 2), decelerated between points 30 m and 40 m and then jogged around the cone back to the starting position within 30 seconds.

3.4. Repeated-sprint protocol

The repeated-sprint protocol was preceded by a 15 min warm-up consisting of jogging (~400m), stretches and 6 x 30 m progressive runs as performed during the repeated-sprint protocol. The repeated-sprint protocol consisted of four sprint bouts (Figure 3.1) with each sprint bout consisting of 6 x 30 m maximal running sprints (see Figure 3.2). Electronic timing gates (Speed Light Sports timing system, SWIFT, NSW) were placed at the start line and at 30 m to measure time (s) for each sprint. A cone was placed at 40 m, allowing 10 m for participants to decelerate before turning around the cone and jogging back to the start line; hence recovery between individual sprints was active. Participants were required to sprint every 30 s during each sprint bout, so that there was ~20 s for the participants to decelerate, jog back to the start line and assume a stationary ready position and a 5 s

countdown to the next sprint. A capillary blood sample was taken from the earlobe at rest and 3 min post each sprint bout. Participants had 7 min passive rest between bouts (Sprint bouts started every 10 min) and were allowed to consume water only.

Calculation of Sprint Data

Average speed over 30 m was calculated by dividing the distance by recorded time. This average speed was expressed as a percentage of the maximum velocity reached during the $\dot{V}O_{2peak}$ test:

$$\text{percentage speed} = \frac{\text{average speed}}{\text{max } \dot{V}O_{2\,peak} \text{ speed}} \times 100\%$$

A relative (percentage decrement over the repeated efforts) RSA score was calculated for each of the 6 x 30m sprint bouts, and total decrement for all 24 x 30m sprints as explained below [142]:

Ideal sprint time = Fastest 30 m sprint time x 6

e.g.= 4.47s x 6

= 26.28 s

Decrement (%) = 100 – (Total sprint time / ideal sprint time x100)

e.g = 100 – (28.82 / 22.35 x 100)

= 6.5%

3.5. Muscle sampling and analysis

On the day of the trial, two incisions were made under local anesthesia (5 mL 2% xylocaine) into the *vastus lateralis* muscle of the right leg. The first incision was used for the resting biopsy and then closed off with a Steri-strip. The second incision was cut proximally to the first incision and closed off with a Steri-strip until after the sprint protocol session was completed when it was subsequently reopened and used for the 15 min post exercise biopsy. Just prior to the 2 h time point another incision was made under local anesthesia into the vastus lateralis muscle of the contralateral (left) leg. This incision was used for the 2 h post biopsy and then closed with a Steri-strip. All biopsies were taken in the supine position. Samples were immediately removed from the biopsy needle, blotted (to remove blood) and placed in liquid nitrogen and stored at -80°C until analysis.

Western Immunoblot Analyses

Muscle samples (40 – 60 mg) were homogenized in a low salt buffer containing Hepes, MgCl, KCl, Vanadate, IGEPAL and millipore water (ratio 1:1, so 50mg muscle = 50mL buffer). Samples were homogenised with a grinder probe (Untraturex) for 20 s. Samples were then place on ice for 10 min. and then centrifuged at 15,000 c for 5 min and a portion (10µl) of the supernatant was used for protein determination. Protein concentration of the samples was determined using a BSA kit. After determination of protein concentration, 200µl lysate was re-suspended in Laemmli sample buffer and boiled at 90°C for 5 min. Samples were separated by SDS-PAGE and transferred to 4–20% gels (Bio-Rad Laboratories, Richmond, CA). Polyvinylidine fluoride membranes were blocked with 5% non-fat milk, washed with TBST (10mM Tris HCl, 100mM NaCl, 0.02% Tween 20) and incubated with primary antibody (1:1000) overnight at 4°C. Membranes were incubated with secondary antibody (1:2000) and proteins were detected via chemiluminescence (Amersham Biosciences, Buckinghamshire, UK) and quantified by densitometric scanning

using a Gel Doc 2000 in combination with Quantity One version 4.4.0 (Bio-Rad Laboratories). All sample time-points for each subject were run on the same gel. Monoclonal anti-phospho- Akt ^{ser473} and polyclonal 4E-BP1 were from cell signaling Technology (Danvers, MA).

3.6. Heart Rate

Heart rate monitors were used to record heart rate (F1, Polar Electro Dy, Finland) immediately pre and immediately post each sprint bout. Heart rates were converted to percentage of maximal heart rate calculated with the following equations:

Estimated maximal heart rate (EMHR) = 220 – Age

Percentage of maximal heart rate = HR/ (EMHR)*100

3.7. Capillary blood sampling and analysis

A hyperaemic ointment (Finalgon, Boehringer Ingelheim) was applied to the earlobe ~6 min before pre blood sampling. Capillary blood samples (50 µl) were taken at rest and 3 min post each sprint bout of the sprint training protocol (5 samples in total). Capillary blood lactate concentration ($[La^-]_b$) was measured using an YSI Sport lactate analyzer (YSI Inc., Ohio, USA.) which was calibrated prior to each testing session.

3.8. Venous Blood Sampling

The anterior forearm was wiped down with an alcohol swab and left to dry. Venous blood samples (5 mL) were taken from the medial cubital vein on the anterior side of the elbow into 4-mil lithium heparin-contain vacutainer tubes (Becton-Dickinson, Plymouth, UK) then placed on ice for ~10 min. Samples were then centrifuged at 805 G for 10 min after which plasma was removed and pipetted into 300 µl aliquots and frozen at -80^{o}C. Samples were

taken at rest, 10 min post exercise and 1 h and 55 min post exercise. Venous blood sampling immediately preceded muscle sampling.

3.9. Insulin Analysis

The samples were assayed using a human insulin specific RIA kit (Cat # HI-14k, Linco Research Inc, MO, USA). All samples were assayed in duplicate.

3.10. Dietary and Exercise Controls

To reduce the potential for dietary-induced variability in resting muscle metabolic profile and exercise metabolism, breakfast (51 kJ energy per kg of body mass; ~78 %CHO, ~8 % Fat, ~14 % Protein) was provided on the morning of the trial. Participants then fasted (water ingestion only) for 4 h prior to the commencement of the sprint protocol. No post-exercise nutrition was permitted within 2 h of completion of the exercise and until the third and final muscle sample was collected. Participants were also required to refrain from caffeine on the day of the trial and alcohol in the 24 h preceding the trial. Subjects were asked to maintain their normal diet and prescribed training program throughout the study. As the study was undertaken in-season and it was not practical to ask the athletes to not train in the 24 h prior to the sprint protocol, all participants were required to perform 60 – 90 min of intermittent exercise (soccer training) in the evening (~18-24 h) before the sprint protocol commenced.

3.11. Menstrual Cycle

Before commencing the study all female participants were required to map their menstrual cycle. Female participants only completed the trial when within days 8-12 (follicular phase) of their cycle.

3.12. Statistical Analysis

Data was analyzed by using a two-factor repeated-measures ANOVA. To determine any differences between the two groups (male vs. female) 2-tailed *t*-tests were performed post hoc to locate where differences were between groups and time points. The level of significance for the analyses was set at ($P<0.05$). All data are presented as means ± SE.

4. Results

4.1. Sprint Performance

All participants successfully completed 24 x 30 m maximal sprints. As expected, the fastest 30 m sprint time was significantly faster ($P<0.05$; Figure 4.1) in males (4.38 ± 0.27 s) than females (4.76 ± 0.15 s). Maximal sprint time in males or females was not recovered during the sprint protocol ($P<0.05$). In addition, the mean sprint time over all sprints (24 x 30 m) was significantly faster ($p<0.05$; Figure 4.2) in males (4.58 ± 0.12 s) than females (5.26 ± 0.27 s).

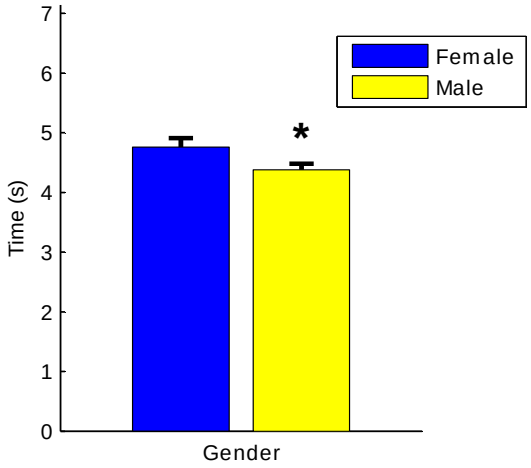

Figure 4.1. Overall fastest 30 m sprint time during the repeated sprint protocol for males and females. Values are mean ± SE. * significantly different from females ($P<0.05$).

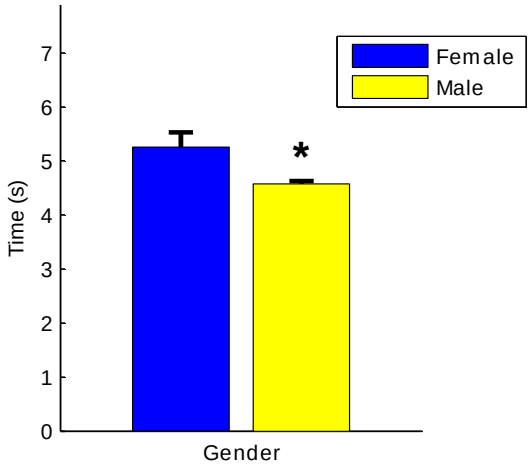

Figure 4.2. Overall average 30 m sprint time during the repeated sprint protocol for males and females. Values are mean ± SE. * significantly different from females ($P<0.05$).

However, despite these absolute differences in sprint times there were no sex differences in the relative intensity of the mean sprint efforts (in velocity) when expressed as a percentage of $v\dot{V}O_{2\,peak}$ in females (165 ± 0.4%) and males (155 ± 0.05 %; P>0.05) (see Figure 4.3).

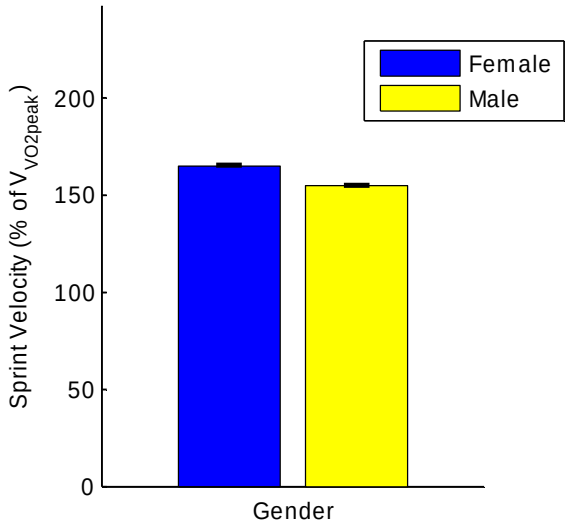

Figure 4.3. Relative intensity of the mean sprint efforts expressed as a percentage of $v\dot{V}O_{2\,peak.}$ Values are means ± SE.

Mean percent sprint decrement over 30 m for each sprint bout (6 x 30 m) was significantly lower (P<0.05; Figure 4.4) in males (4.7 ± 0.6, 5.0 ± 0.6, 5.3 ± 0.9, 4.4 ± 0.5%) than females (7.1 ± 0.8, 6.7 ± 1.0, 7.5 ± 0.6, 7.0 ± 1.0%). It followed that mean percentage decrement across all sprints (24 x 30 m) was significantly lower (P<0.05; Figure 4.5) in males (4.9 ± 1.6 %) then females (7.1 ± 1.9 %).

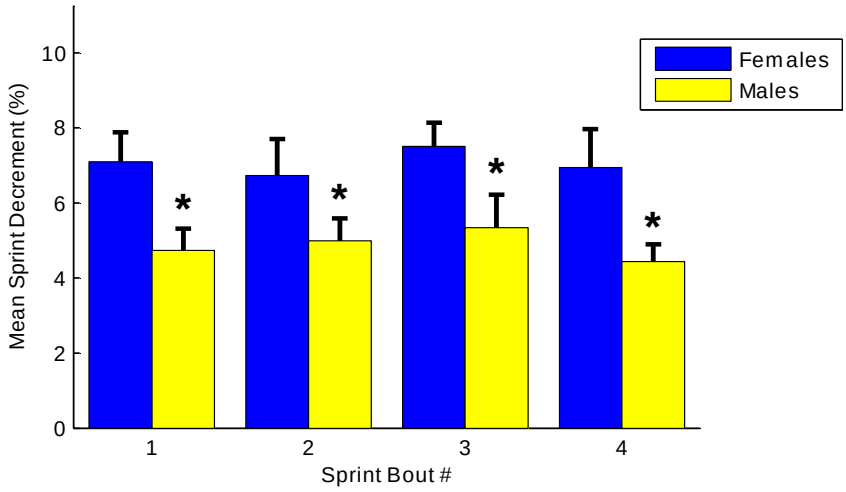

Figure 4.4. Average sprint decrement for each bout for males and females. Values are mean ± SE. * significantly different from females (P<0.05).

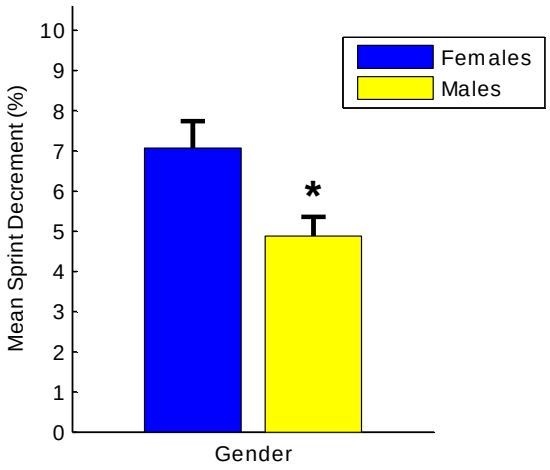

Figure 4.5. Mean sprint decrement measured across all four sprint bouts for males and females. Values are mean ± SE. * significantly different from females (P<0.05).

4.2. Physiological Markers

There was a significant (P<0.05) increase in heart rate from rest to post the first bout of sprints (see Figure 4.6). Heart rate continued to be elevated above resting levels at pre and post each sprint bout, however, there were no differences between males and females (P>0.05) at any time point.

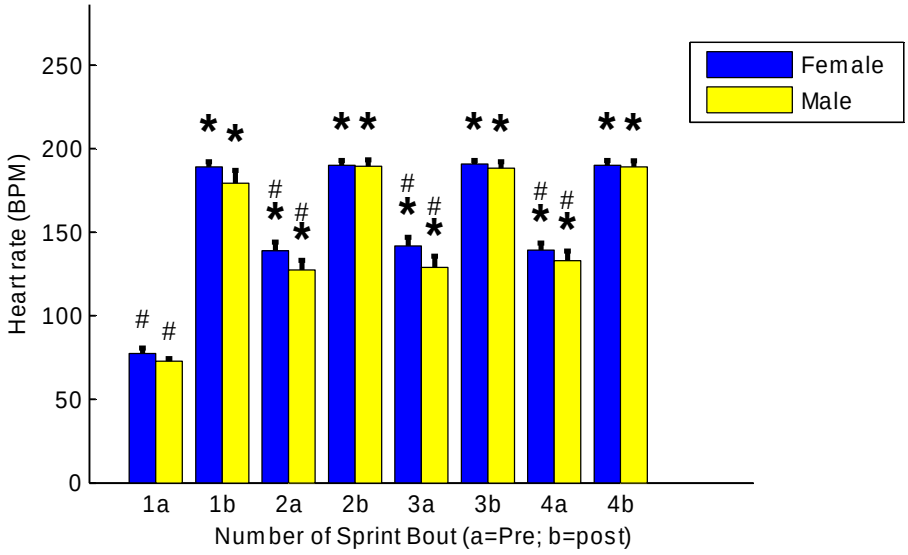

Figure 4.6. Heart rate measured at rest, immediately post each sprint bout, and immediately prior to each sprint bout in males and females. Values are mean ± SE. * significantly different from resting levels, # significantly different from post bout levels.

Similar responses were recorded in the percentage of estimated maximal heart rates reached during the protocol. There was a significant (P<0.05) increase in percentage maximal heart rate from rest after the first sprint bout (see Figure 4.7) and this continued to be significantly higher than rest at pre and post each sprint bout. No differences in response were recorded between males and females (P>0.05) at any time point.

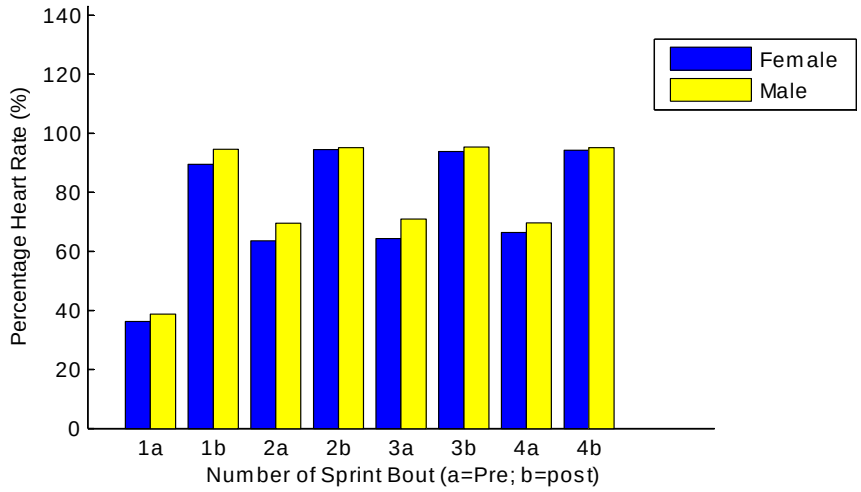

Figure 4.7. Percentages of estimated maximal heart rate reached in males and females pre and post each sprint bout. Values are means.

There was a significant (P<0.05; Figure 4.8) increase in blood lactate from rest to post the first bout of sprints, in both males and females. Similarly blood lactate concentration was significantly higher than rest after sprint bouts 2, 3, and 4, with no differences between males and females. Furthermore, while there was a small elevation in blood lactate from bout 1 to bout 2 of the sprints, there was no significant differences between bouts 1, 2, 3, or 4 (P>0.05).

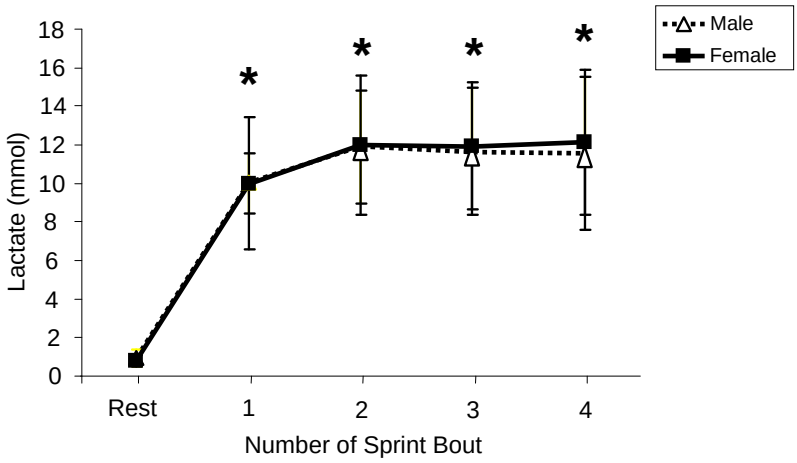

Figure 4.8. Plasma lactate measured at rest and 3 min post each sprint bout for male and female subjects. Values are mean ± SE. * significantly different from resting levels (P<0.05).

There was no significant difference in plasma insulin concentrations at rest ($P<0.05$; Figure 4.9) between sexes. Post exercise plasma insulin was not significantly changed from resting levels and did not exhibit any sex differences at any time points post exercise.

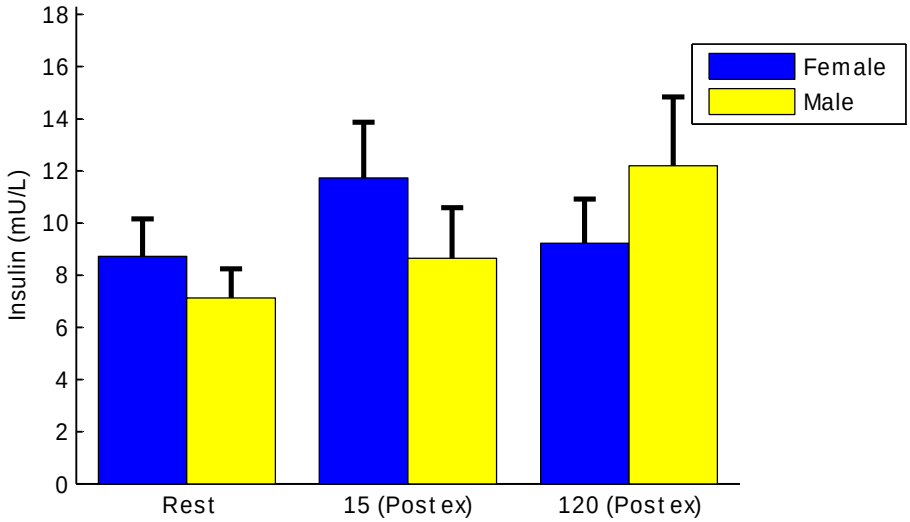

Figure 4.9. Plasma insulin measured at rest, 15 min post exercise and 120 min post exercise for male and female subjects. Values are mean ± SE.

4.3. Markers of Protein Signalling

The total protein expression and the ratio of phosphorylated to total protein expression of Akt were measured to record any changes in protein signalling upstream of the mammalian target of rapamycin (mTOR). The ratio of phosphorylated to total protein is an indication of the activation/deactivation levels of the measured protein. No sex or time effects were recorded for Total Akt (*P*>0.05; Figure 4.10).

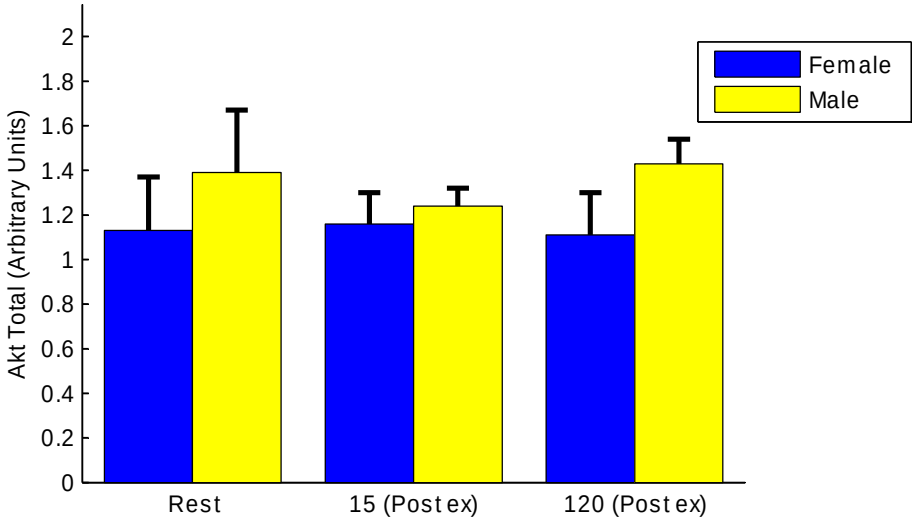

Figure 4.10. Total Akt measured at rest, 15 min post exercise and 120 min post exercise for male and female subjects. Values are mean ± SE.

Levels of Akt phosphorylation were similar between males and females at rest, with this relationship not differing at any time point between sexes (see Figure 4.11). No significant time effects were recorded for either sex.

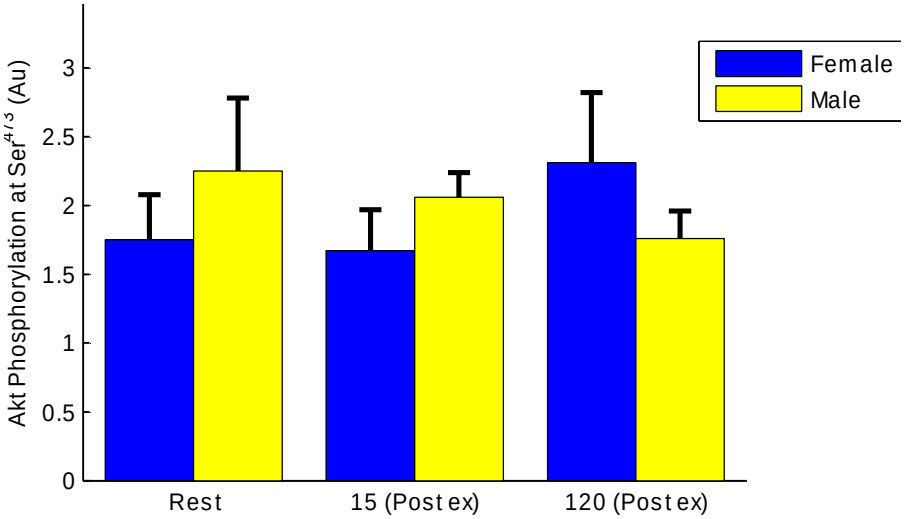

Figure 4.11. Ratio of phosphorylated to total Akt measured at rest, 15 min post exercise and 120 min post exercise in males and females. Values are means ± SE.

To further determine skeletal muscle regulation post acute sprint exercise, the total protein expression and the ratio of phosphorylated to total protein expression of 4E-BP1 was determined. Total 4E-BP1 at rest was significantly higher (P<0.05; Figure 4.12) in females (3.59 ± 0.30) when compared with males (1.97 ± 0.31). There was no significant change in Total 4E-BP1 at any time point post exercise (P>0.05).

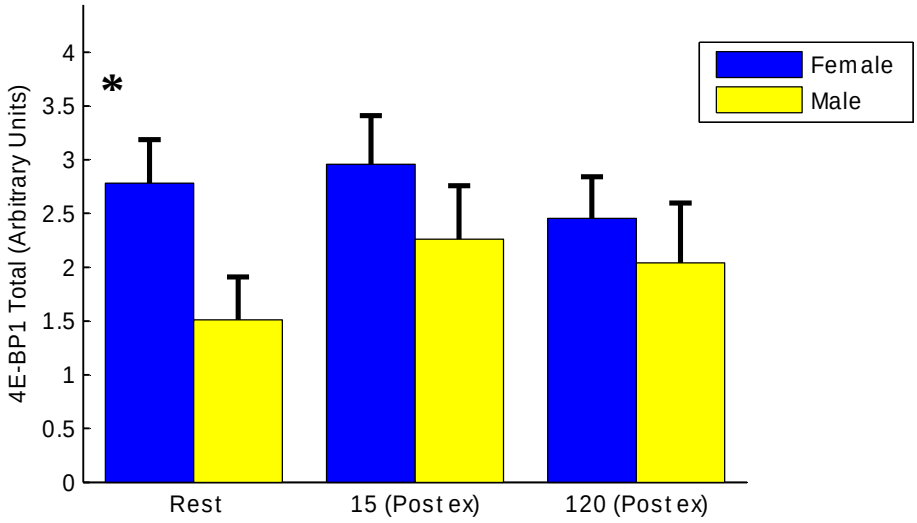

Figure 4.12. Total 4E-BP1 measured at rest, 15 min post exercise and 120 min post exercise for male and female subjects. Values are mean ± SE. * Significantly different from males (P<0.05).

In contrast to Total 4E-BP1, levels of phosphorylated 4E-BP1 at rest were higher (P<0.05; Figure 4.13) in males (0.29 ± 0.06) than females (0.11 ± 0.20). At 15 min post exercise phosphorylated 4E-BP1 exhibited a significant decrease (P<0.05; Figure 4.13) below resting levels in males only, whereas no significant differences in phosphorylated levels were recorded at any time point in females.

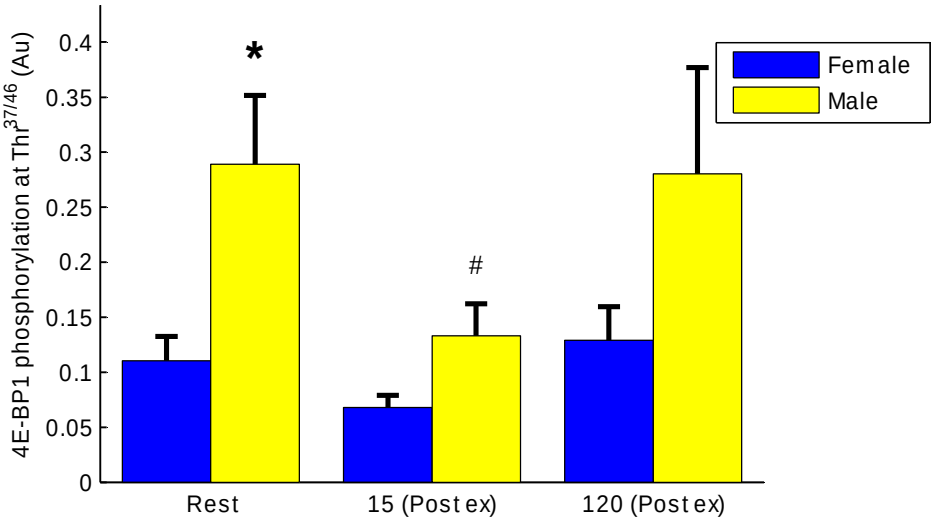

Figure 4.13. 4E-BP1 phosphorylation measured at rest, 15 min post exercise and 120 min post exercise in males and females. Values are means ± SE. *significantly different from females (p<0.05). # Significantly different from resting levels (P<0.05).

5. Discussion

The purpose of this study was to determine and compare the effects of a repeated-sprint bout on the physiological response and molecular signalling in muscle of male and female team-sport athletes. The results show that in the absence of post-exercise nutrition, an acute bout of repeated-sprint exercise results in an early reduction in the post-exercise skeletal muscle phosphorylation of 4E-BP1 (sample taken 15 min post-exercise) before returning to baseline levels by 2 h post-exercise. However, these changes in 4E-BP1 phosphorylation were more marked and reached significance in males but not females. Furthermore, at rest, the phosphorylation of 4E-BP1 was greater in males than females. There were no changes in skeletal muscle Akt phosphorylation or blood insulin levels at any of the time points sampled in the present study for either males or females and there were no sex differences at any time point. Additionally, other physiological responses such as heart rate and blood lactate were significantly elevated during and following the exercise, but responded similarly between sexes.

5.1. Sprint performance

The fastest sprint time was recorded during the first sprint bout for most participants. Maximal sprint speed over 30 m (fastest sprint time recorded during the first sprint) was not recovered during the exercise protocol ($P<0.05$). This suggests that a passive recovery of 7 min is not long enough to recover maximal 30 m sprint speed after a series of maximal efforts. During team sports where the recovery time between sprints is likely to be considerably less than 7 min it is likely that maximal sprint speed will be more greatly affected. This supports previous findings that players' ability to perform high-intensity exercise in terms of both sprint distance and sprint performance is reduced towards the end

of the match as well as immediately following a period of game-play that requires the athlete to undertake a large amount of high-intensity exercise [1, 153-155].

5.2. Heart rate and blood lactate

Heart rate increased to ~90-95% of estimated maximal heart rate after each sprint bout and decreased to ~65% of maximal heart rate by the start of the next bout with no differences between males and females. Heart rates recorded in the present study were similar to the levels recorded following intense exercise in and during recovery of game-play of soccer players [1, 153, 156]. The first sprint bout resulted in a large increase in blood lactate (~11 fold) from resting levels in both males and females. Further, blood lactate did not change significantly after sprint bouts 2, 3 or 4. The changes in blood lactate in the present study are similar to those previously reported following repeated-sprint exercise [144, 145]. However, during actual team-sport game-play, lactate levels are not observed as high as that recorded during the present study [154, 157]. This is likely to be due to football players performing sprints of various distances (from 1 - 50m) and multiple other game-based activities of lower exercise-intensity during match-play and the difficulty in obtaining accurate blood samples/measures following high-intensity exercise periods in game-play [158]. The high blood lactate levels reported following each of the repeated-sprint bouts indicate a high level of anaerobic metabolism throughout the protocol. This differs to some studies that have performed prolonged (30 – 90 min) repeated-sprint protocols where lactate declines or is lower [159] throughout exercise when compared to the present study. The greater blood lactate recorded following the sprints in the present study is likely to be due to the prolonged rest periods (~7 min) between each sprint bout allowing repeated maximal efforts, similar to that performed at rest and therefore, allowing high ATP resynthesis from glycolysis.

In contrast to the similarities in physiological changes mentioned above, and as would be expected, sex differences were observed in sprint performance. Males were significantly faster than females in terms of absolute maximal-sprint performance and maintained a significantly faster sprint performance across all 24 sprints. However, there were no differences in the relative speed that the male and female athletes ran at, when running speed is expressed relative to $v\dot{V}O_{2peak}$ (i.e. males at 155% and the females at 165% $v\dot{V}O_{2peak)}$. Therefore, despite the differences in absolute running speed, the male and female athletes ran at a similar relative intensity (% of $v\dot{V}O_{2peak)}$. As such, this may be an explanation as to why physiological responses such as HR and blood lactate were similar between sexes.

The use of % of $v\dot{V}O_{2peak}$ is often used when expressing running speed or power output during sub-maximal exercise to match metabolic demand across various groups but rarely during sprint activities, where bouts are maximal. A recent report used the differences obtained between maximal power output during a GXT ($\dot{V}O_{2peak}$ test) and a repeated-sprint test to determine possible contributions of aerobic and anaerobic energy sources to sprint exercise [160]. The peak power recorded during the GXT was termed the aerobic power range (indicative of the aerobic contribution), while the difference between peak power during the measured sprints and the pre-determined GXT was termed the anaerobic power reserve (indicative of the anaerobic contribution). In the current study the difference recorded during the pre-detemined $v\dot{V}O_{2peak}$ and running speed during the sprints were comparable between the male and females. This suggests there may have been a similar reliance on anaerobic metabolism between the two groups. This is supported by the almost identical post-exericse blood lactate values recorded by the males and females. Therefore, it is likely that the sex differences that were exhibited in sprint performance are due at least in

part to physical characteristics such as differences in muscle mass and force output [81] as opposed to a true sex difference.

However, the female athletes did experience a greater sprint decrement than the males, which may suggest that the males were better adapted to cope with repeated maximal efforts. Additionally, sprint decrement has been shown to be related to aerobic fitness and therefore [142], the greater aerobic capacity of the male athletes in the present study could partially explain this difference in sprint decrement [142]. Furthermore, males have been reported to have greater levels of the muscle acid buffer – carnosine than females [161], which could help offset the large changes in hydrogen ion accumulation that occur during repeated-sprint exercise [142]. While the current findings do not offer a clear explanation for the differences in sprint decrement they do indicate that there may be a greater drop-off in sprint performance during training and possibly match-play in female compared to male team-sport athletes.

5.3. Muscle

In the present study, there were no changes in Akt phosphorylation at 15 min or 2 h post-exercise compared to pre exercise for either the male or female athletes. In previous studies, immediate post-exercise Akt phosphorylation has been shown to be either increased [7, 8] or in the majority of studies, not changed [10-12, 14] in the absence of nutrient supplementation. However, these previous reports employed resistance exercise (65-70 % 1RM, 5- 8 sets x 10 – 12 reps). Similarly, a recent report [17] shows that Akt phosphorylation is not altered immediately following 1 or 4 x 30-s sprints (120 s total sprint duration) or 3 h into recovery in recreationally active males. The current results support these findings in that there were no changes in Akt phosphorylation following 24 x ~4-5 s sprints (~120 s total sprint duration) for either male or female athletes. Therefore, it seems

that following single or repeated maximal exercise of either long (30 s) or short (<10 s) duration there is little change in Akt phosphorylation. Due to its positive role in muscle growth, it might be expected that Akt phosphorylation would be elevated following high-intensity exercise such as resistance exercise and/or repeated-sprint exercise. However, in the absence of nutrients such as amino acids, there seems to be little change or a slight decrease in Akt phosphorylation following high-intensity intermittent exercise.

The reason for the differences that are reported in post-exercise Akt phosphorylation (i.e. no change v decrease v increase; Table 2.1) may to due to differences in exercise intensity, duration, mode and training background of the participants which can affect the signalling of other proteins [4]. The small decrease in or little change in Akt phosphorylation following many exercise interventions may be due to post-exercise increases in AMPK activity, which has previously been shown to remain elevated up to one hour post exercise [8, 71]. Furthermore, repeated-sprint exercise has been shown to increase AMPK phosphorylation [17]. A decrease in ATP levels as well as glycogen levels stimulates the activation of AMPK which has been suggested to inhibit the activation of growth-related proteins [8, 71]. Previous reports have shown that a repeated-sprint bout of 5 x 6 s sprints results in ~40% decrease in ATP levels in males [144] and females [2]. A low energy status signified by low ATP concentration after repeated maximal-sprint exercise, such as that performed in the current study is likely to stimulate an increase in AMPK activity which may act to inhibit the post-exercise Akt response and hence delay the muscle hypertrophy response, especially in the absence of nutrient supply.

In addition to exercise and nutrient availability related factors, the timing of the muscle sampling may have also influenced the finding of no change in Akt phosphorylation. A muscle biopsy is only a snapshot of what is happening in the muscle at a particular time

point. To date there is little data regarding the time course of post-exercise Akt phosphorylation in humans, although some work has been done in rat models [6], showing the optimal time point to be ~10 min post-exercise. However, protein synthesis rates are quite different in rats compared to humans, indicating there is likely to be differences in the signalling response also. Therefore, it should be acknowledged that if the timing of the muscle biopsy is not cued with the physiological response of a specific protein any significant change could be missed. In one study, Akt phosphorylation was shown not to be changed immediately post exercise, then elevated at 1 h post exercise and then returned to baseline levels by 2 h post-exercise [7], which could have also occurred in the present study, indicating we may have missed significant changes in Akt. However, for ethical reasons and due to the athletic level and time of season for the participants in the present study, muscle biopsy sampling was kept to a minimum. Time course research is required to be done in human studies to determine the optimal time point for muscle sampling for proteins such as Akt.

While there were no changes in Akt phosphorylation, there were significant changes in 4E-BP1 phosphorylation following the repeated-sprint protocol for the males, but not the females. Similar to a number of previous reports employing a resistance training mode [8, 12, 71, 72], 4E-BP1 phosphorylation was reduced below resting levels by 15 min post exercise in the males and was then returned to basal levels 2 h post exercise. Although statistical significance was not reached, the female muscle response tended to follow the same pattern. The lower resting levels of 4E-BP1 phosphorylation and therefore, smaller absolute change may have contributed to the changes in 4E-BP1 not reaching statistical significance in the females. Alternatively, a reduced metabolic disturbance i.e. reduced change in ATP, may explain these differences.

The similar reduction in 4E-BP1 phosphorylation in males and return to basal levels reported previously following resistance exercise [8, 12, 71] and in the present study using repeated sprints may be due to the activation of AMPK. AMPK activation may then have reduced mTOR phosphorylation, reducing activation of specific downstream targets such as 4E-BP1 (refer to Figure 2.1). However, while the current study reports a decrease in 4E-BP1, a recent study did not report any changes in 4E-BP1 phosphorylation following 4 x 30 s sprints by males [17]. The reasons for the differences between that study and the present study are not clear, but could be due to the mode and/or intensity of exercise. The present study employed sprint running and therefore, had an eccentric component similar to resistance exercise, whereas the protocol employed by Gibala et al, 2008 [17] employed cycling exercise which only has a concentric phase. There may be a greater muscle disturbance when concentric exercise is accompanied by eccentric exercise resulting in differences in protein signalling. Furthermore, the present study required the participants to perform repeated, short, (4 - 6 s) sprints, compared to the long 30 s sprints employed by Gibala et al., [7] which result in a marked decline in exercise intensity over a bout and during subsequent bouts. In contrast despite a small drop off in maximal sprint performance after each bout, sprint performance was well maintained in the present study. These differences in exercise intensity and/or mode may explain the differences in 4E-BP1 phosphorylation between studies.

The greater post-exercise decrease in 4E-BP1 in males compared to females could also be due to differences in activation of AMPK. Previously, Roepstorff, et al, [18], reported that AMPK phosphorylation and AMP:ATP ratio increased in male, but not significantly in female muscle following 90 min of sub maximal exercise (60% $\dot{V}O_{2peak}$). Further, a study involving repeated bouts of high-intensity sprint exercise (3 x 30 s cycle sprints separated by 20 min) produced a smaller reduction in cellular ATP and a smaller

accumulation of IMP in women than men. It could be that in the present study, (following 24 maximal sprints) female muscle was better able to maintain the cellular energy balance of the muscle, resulting in a lower ratio of AMP:ATP and lower activation of AMPK, reducing the inactivation of anabolic processes (i.e. dephosphorylation of 4E-BP1 and subsequent reduced protein synthesis). While further investigation is required, this study is the first to report differences in the 4E-BP1 response between males and females as well as changes to 4E-BP1 phosphorylation following short repeated-sprint exercise similar to that performed by team-sport athletes during training and match play.

Additionally, there were significant differences in the resting levels of total and phosphorylated 4E-BP1 between males and females in the present study. As phosphorylation of 4E-BP1 is an important step in translation initiation and is related to protein synthesis, the higher resting levels of phosphorylated 4E-BP1 and lower total 4E-BP1 could contribute to higher levels of muscle protein synthesis in the males, although without further muscle measures (i.e. fractional protein synthesis) this is speculative.

The male and female athletes in the current study were equally matched in terms of playing level (Premier Division) and team training volume per week (2 x soccer specific training sessions + 1 x 90 min game) and were recruited at the same time of the season. Furthermore, each of the athletes was required to perform a similar soccer specific training session ~18 h prior to the exercise trial, after which they were required to abstain from any more training before their muscle biopsies and exercise trial. All athletes were tested at the same time of the day (1-2pm). The athletes were also given a food parcel that contained the same nutrients per kg of body mass for breakfast on the day of the trial and were required to refrain from nutrient intake in the 4 h prior to the trial. Therefore, it does not seem that the sex differences in resting levels of phosphorylated or total 4E-BP1 are due to differences in

the time of day, recently completed exercise or food intake prior to the muscle biopsy sample. This is supported by similar resting levels in other physiological measures between the male and female athletes, such as blood insulin and lactate levels and Akt phosphorylation. Furthermore, unlike phosphorylated 4E-BP1, it is unlikely that total 4E-BP1 would differ due to acute differences in exercise or food intake. Therefore, it would seem that these differences are due to chronic differences between the two groups of athletes.

The differences in resting levels of 4E-BP1 could be due to a number of sex related characteristics such as circulating hormones or non-sex related differences such as training history and training activities outside of specific soccer training. Testosterone is associated with significant increases in fat-free mass and muscle size, most noticeably in males [100]. Though basal levels of testosterone in males are significantly higher than females, an acute bout of resistance exercise has been shown to increase levels of serum testosterone in females [162] and affect the pattern of response similarly in both sexes. In contrast, others have shown that serum testosterone concentration does not change in females following resistance exercise [163] or the absolute change in serum testosterone is significantly greater in males [164]. It is still uncertain exactly how testosterone increases muscle mass, but it is likely the differing levels between the sexes may partly explain chronic differences in muscle growth and could explain the differences in the resting levels of 4E-BP1 activation between groups. Unfortunately, the training outside of soccer specific work was not recorded. Therefore, it is possible that the males were actively involved in a greater volume of resistance training or had a longer history of high-intensity type training that could also alter 4E-BP1 levels.

While there is limited sex comparative data, sex differences in protein synthesis and protein phosphorylation have been reported in the heart muscle of rats. Male Sprague-Dawley rats had significantly larger hearts, greater basal protein synthesis and 4E-BP1 phosphorylation than female rat hearts [165]. Furthermore, while a much different intervention was used in a rat study (26 weeks of alcohol feeding) compared to the exercise intervention in the present study, similarly there were no changes in 4E-BP1 phosphorylation in the females. Conversely, there was a significant decrease in 4E-BP1 phosphorylation in the males. These findings indicate that there may be sex differences in the present levels of particular proteins involved in muscle growth in mammals.

To date, most human studies, predominantly involving resistance exercise, are yet to elucidate any sex differences involved in translation or changes in protein synthesis at rest or after an acute bout of exercise [8, 12]. The difference between these studies and the current study are the training history and physical activity of the participants. Accordingly, it could be that in the untrained state, sex differences in muscle machinery are negligible, but improvements made with training are greater in males than females, elucidating a more noticeable sex gap in the muscle hypertrophy response. Though, future studies are needed to outline the relationship between training status and sex response to acute high-intensity exercise in skeletal muscle.

Summary

The results of present study show that in similarly competitive male and female team-sport athletes, males have a smaller sprint decrement, suggesting they have a greater ability to maintain performance during repeated sprint exercise compared with females. However, the results of this study also show that males and females run at approximately the same relative intensity, when sprint performance is expressed relative to $v\dot{V}O_{2peak.}$ We provide

data that suggest sex differences may exist in basal proteins associated with protein synthesis and that sprint exercise may elicit sex differences in the protein response. Specifically our findings show that at rest, Total 4E-BP1 is higher in females, and phosphorylation of 4E-BP1 following 40 min of repeated sprint exercise is reduced in males but not females. No differences were recorded in Akt, and no changes in Akt phosphorylation occurred during the exercise protocol and into recovery at the time points measured. However, the measurement of mTOR, p70S6k, and AMPK and protein synthesis would give a greater understanding of how the pathway responds to the particular exercise stimulus, and may provide greater insight into whether the Akt/mTOR pathway is the primary pathway activated following repeated sprint efforts. The sex differences in 4E-BP1 presented in the current study should be investigated further to determine whether the sex differences in basal and post-exercise levels in protein phosphorylation are due strictly to differences in sex, or whether training status is also implicated. In addition, as the results in the present study appear to mimic those observed after an acute resistance session it would be important to see whether long term repeated-sprint-interval training would result in similar chronic adaptations observed in the muscle following regular resistance training. In summary, this is the first study to show that females have a reduced phosphorylated 4E-BP1 at baseline compared to males, and that ~120 s of high-intensity repeated-sprint exercise significantly reduces the level of phosphorylated 4E-BP1 in males but not females. Therefore these results indicate that there may be sex differences in the translational machinery of skeletal muscle that may affect muscle growth.

6. References

1. Mohr, M., P. Krustrup, and J. Bangsbo, *Match performance of high-standard soccer players with special reference to development of fatigue.* J Sports Sci, 2003. **21**(7): p. 519-28.

2. Edge, J., et al., *Effects of high- and moderate-intensity training on metabolism and repeated sprints.* Med Sci Sports Exerc, 2005. **37**(11): p. 1975-82.

3. Hill-Haas, S., et al., *Effects of rest interval during high-repetition resistance training on strength, aerobic fitness, and repeated-sprint ability.* J Sports Sci, 2007. **25**(6): p. 619-28.

4. Coffey, V.G., et al., *Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans.* FASEB J, 2006. **20**(1): p. 190-2.

5. Atherton, P.J., et al., *Selective activation of AMPK-PGC-1alpha or PKB-TSC2-mTOR signaling can explain specific adaptive responses to endurance or resistance training-like electrical muscle stimulation.* FASEB J, 2005. **19**(7): p. 786-8.

6. Bolster, D.R., et al., *Immediate response of mammalian target of rapamycin (mTOR)-mediated signalling following acute resistance exercise in rat skeletal muscle.* J Physiol, 2003. **553**(Pt 1): p. 213-20.

7. Dreyer, H.C., et al., *Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle.* Am J Physiol Endocrinol Metab, 2008. **294**(2): p. E392-400.

8. Dreyer, H.C., et al., *Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle.* J Physiol, 2006. **576**(Pt 2): p. 613-24.

9. Thorell, A., et al., *Exercise and insulin cause GLUT-4 translocation in human skeletal muscle.* Am J Physiol, 1999. **277**(4 Pt 1): p. E733-41.

10. Drummond, M.J., et al., *Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging.* J Appl Physiol, 2008. **104**(5): p. 1452-61.

11. Eliasson, J., et al., *Maximal lengthening contractions increase p70 S6 kinase phosphorylation in human skeletal muscle in the absence of nutritional supply.* Am J Physiol Endocrinol Metab, 2006. **291**(6): p. E1197-205.

12. Fujita, S., et al., *Essential amino acid and carbohydrate ingestion prior to resistance exercise does not enhance post-exercise muscle protein synthesis.* J Appl Physiol, 2008.

13. Mascher, H., et al., *Changes in signalling pathways regulating protein synthesis in human muscle in the recovery period after endurance exercise.* Acta Physiol (Oxf), 2007. **191**(1): p. 67-75.

14. Ivy, J.L., et al., *Post exercise carbohydrate-protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation.* Amino Acids, 2008. **35**(1): p. 89-97.

15. Glover, E.I., et al., *Resistance exercise decreases eIF2Bepsilon phosphorylation and potentiates the feeding-induced stimulation of p70S6K1 and rpS6 in young men.* Am J Physiol Regul Integr Comp Physiol, 2008. **295**(2): p. R604-10.

16. Creer, A., et al., *Influence of muscle glycogen availability on ERK1/2 and Akt signaling after resistance exercise in human skeletal muscle.* J Appl Physiol, 2005. **99**(3): p. 950-6.

17. Gibala, M.J., et al., *Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1{alpha} in human skeletal muscle.* J Appl Physiol, 2008.

18. Roepstorff, C., et al., *Higher skeletal muscle alpha2AMPK activation and lower energy charge and fat oxidation in men than in women during submaximal exercise.* J Physiol, 2006. **574**(Pt 1): p. 125-38.

19. Vissing, K., et al., *Effect of sex differences on human MEF2 regulation during endurance exercise.* Am J Physiol Endocrinol Metab, 2008. **294**(2): p. E408-15.

20. Lang, C.H., R.A. Frost, and T.C. Vary, *Skeletal muscle protein synthesis and degradation exhibit sexual dimorphism after chronic alcohol consumption but not acute intoxication.* Am J Physiol Endocrinol Metab, 2007. **292**(6): p. E1497-506.

21. Wideman, L., et al., *Effects of gender on exercise-induced growth hormone release.* J Appl Physiol, 1999. **87**(3): p. 1154-62.

22. Aizawa, K., et al., *Sex differences in steroidogenesis in skeletal muscle following a single bout of exercise in rats.* J Appl Physiol, 2008. **104**(1): p. 67-74.

23. Simoneau, J.A. and C. Bouchard, *Genetic determinism of fiber type proportion in human skeletal muscle.* FASEB J, 1995. **9**(11): p. 1091-5.

24. Nakamura, T., et al., *Heredity of muscle fibre composition estimated from a selection experiment in rats.* Eur J Appl Physiol Occup Physiol, 1993. **66**(1): p. 85-9.

25. Green, H., et al., *Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise.* Am J Physiol, 1999. **276**(2 Pt 2): p. R591-6.

26. Luthi, J.M., et al., *Structural changes in skeletal muscle tissue with heavy-resistance exercise.* Int J Sports Med, 1986. **7**(3): p. 123-7.

27. Bolster, D.R., L.S. Jefferson, and S.R. Kimball, *Regulation of protein synthesis associated with skeletal muscle hypertrophy by insulin-, amino acid- and exercise-induced signalling.* Proc Nutr Soc, 2004. **63**(2): p. 351-6.

28. Cribb, P.J. and A. Hayes, *Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy.* Med Sci Sports Exerc, 2006. **38**(11): p. 1918-25.

29. Russell, B., D. Motlagh, and W.W. Ashley, *Form follows function: how muscle shape is regulated by work.* J Appl Physiol, 2000. **88**(3): p. 1127-32.

30. Frontera, W.R., et al., *Strength conditioning in older men: skeletal muscle hypertrophy and improved function.* J Appl Physiol, 1988. **64**(3): p. 1038-44.

31. Starkey, D.B., et al., *Effect of resistance training volume on strength and muscle thickness.* Med Sci Sports Exerc, 1996. **28**(10): p. 1311-20.

32. Staron, R.S., et al., *Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women.* Eur J Appl Physiol Occup Physiol, 1990. **60**(1): p. 71-9.

33. Butterfield, T.A., T.R. Leonard, and W. Herzog, *Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent.* J Appl Physiol, 2005. **99**(4): p. 1352-8.

34. Carroll, T.J., S. Riek, and R.G. Carson, *Neural adaptations to resistance training: implications for movement control.* Sports Med, 2001. **31**(12): p. 829-40.

35. Ingjer, F., *Effects of endurance training on muscle fibre ATP-ase activity, capillary supply and mitochondrial content in man.* J Physiol, 1979. **294**: p. 419-32.

36. Coggan, A.R., et al., *Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women.* J Appl Physiol, 1992. **72**(5): p. 1780-6.

37. Cadefau, J., et al., *Biochemical and histochemical adaptation to sprint training in young athletes.* Acta Physiol Scand, 1990. **140**(3): p. 341-51.

38. Harridge, S.D., et al., *Sprint training, in vitro and in vivo muscle function, and myosin heavy chain expression.* J Appl Physiol, 1998. **84**(2): p. 442-9.

39. Allemeier, C.A., et al., *Effects of sprint cycle training on human skeletal muscle.* J Appl Physiol, 1994. **77**(5): p. 2385-90.

40. Linossier, M.T., et al., *Ergometric and metabolic adaptation to a 5-s sprint training programme.* Eur J Appl Physiol Occup Physiol, 1993. **67**(5): p. 408-14.

41. Burgomaster, K.A., et al., *Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans.* J Appl Physiol, 2005. **98**(6): p. 1985-90.

42. Sleivert, G.G., R.D. Backus, and H.A. Wenger, *Neuromuscular differences between volleyball players, middle distance runners and untrained controls.* Int J Sports Med, 1995. **16**(6): p. 390-8.

43. Chesley, A., et al., *Changes in human muscle protein synthesis after resistance exercise.* J Appl Physiol, 1992. **73**(4): p. 1383-8.

44. MacDougall, J.D., et al., *The time course for elevated muscle protein synthesis following heavy resistance exercise.* Can J Appl Physiol, 1995. **20**(4): p. 480-6.

45. Miller, B.F., et al., *Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise.* J Physiol, 2005. **567**(Pt 3): p. 1021-33.

46. Rose, A.J., et al., *Skeletal muscle eEF2 and 4EBP1 phosphorylation during endurance exercise is dependent on intensity and muscle fiber type.* Am J Physiol Regul Integr Comp Physiol, 2009. **296**(2): p. R326-33.

47. Biolo, G., et al., *Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans.* Am J Physiol, 1995. **268**(3 Pt 1): p. E514-20.

48. Kimball, S.R., *Interaction between the AMP-activated protein kinase and mTOR signaling pathways.* Med Sci Sports Exerc, 2006. **38**(11): p. 1958-64.

49. DeVol, D.L., et al., *Activation of insulin-like growth factor gene expression during work-induced skeletal muscle growth.* Am J Physiol, 1990. **259**(1 Pt 1): p. E89-95.

50. Hornberger, T.A., et al., *Mechanical stimuli regulate rapamycin-sensitive signalling by a phosphoinositide 3-kinase-, protein kinase B- and growth factor-independent mechanism.* Biochem J, 2004. **380**(Pt 3): p. 795-804.

51. Latres, E., et al., *Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway.* J Biol Chem, 2005. **280**(4): p. 2737-44.

52. Blomstrand, E., et al., *Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise.* J Nutr, 2006. **136**(1 Suppl): p. 269S-73S.

53. Wilson, C., M. Hargreaves, and K.F. Howlett, *Exercise does not alter subcellular localization, but increases phosphorylation of insulin-signaling proteins in human skeletal muscle.* Am J Physiol Endocrinol Metab, 2006. **290**(2): p. E341-6.

54. Zanchi, N.E. and A.H. Lancha, Jr., *Mechanical stimuli of skeletal muscle: implications on mTOR/p70s6k and protein synthesis.* Eur J Appl Physiol, 2008. **102**(3): p. 253-63.

55. Proud, C.G., *Amino acids and mTOR signalling in anabolic function.* Biochem Soc Trans, 2007. **35**(Pt 5): p. 1187-90.

56. Gautsch, T.A., et al., *Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise.* Am J Physiol, 1998. **274**(2 Pt 1): p. C406-14.

57. Vary, T.C., L.S. Jefferson, and S.R. Kimball, *Role of eIF4E in stimulation of protein synthesis by IGF-I in perfused rat skeletal muscle.* Am J Physiol Endocrinol Metab, 2000. **278**(1): p. E58-64.

58. Kimball, S.R. and L.S. Jefferson, *Control of protein synthesis by amino acid availability.* Curr Opin Clin Nutr Metab Care, 2002. **5**(1): p. 63-7.

59. Fingar, D.C., et al., *Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E.* Genes Dev, 2002. **16**(12): p. 1472-87.

60. Hannan, K.M., G. Thomas, and R.B. Pearson, *Activation of S6K1 (p70 ribosomal protein S6 kinase 1) requires an initial calcium-dependent priming event involving formation of a high-molecular-mass signalling complex.* Biochem J, 2003. **370**(Pt 2): p. 469-77.

61. Isotani, S., et al., *Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase alpha in vitro.* J Biol Chem, 1999. **274**(48): p. 34493-8.

62. Baar, K. and K. Esser, *Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise.* Am J Physiol, 1999. **276**(1 Pt 1): p. C120-7.

63. Terzis, G., et al., *Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects.* Eur J Appl Physiol, 2008. **102**(2): p. 145-52.

64. Hardie, D.G., *The AMP-activated protein kinase pathway--new players upstream and downstream.* J Cell Sci, 2004. **117**(Pt 23): p. 5479-87.

65. Ruderman, N.B., et al., *AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise.* Acta Physiol Scand, 2003. **178**(4): p. 435-42.

66. Hue, L. and M.H. Rider, *The AMP-activated protein kinase: more than an energy sensor.* Essays Biochem, 2007. **43**: p. 121-37.

67. Deshmukh, A.S., et al., *Role of adenosine 5'-monophosphate-activated protein kinase subunits in skeletal muscle mammalian target of rapamycin signaling.* Mol Endocrinol, 2008. **22**(5): p. 1105-12.

68. Durham, W.J., et al., *Leg glucose and protein metabolism during an acute bout of resistance exercise in humans.* J Appl Physiol, 2004. **97**(4): p. 1379-86.

69. Rasmussen, B.B., et al., *An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise.* J Appl Physiol, 2000. **88**(2): p. 386-92.

70. Morrison, P.J., et al., *Adding protein to a carbohydrate supplement provided after endurance exercise enhances 4E-BP1 and RPS6 signaling in skeletal muscle.* J Appl Physiol, 2008. **104**(4): p. 1029-36.

71. Koopman, R., et al., *Increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers.* Am J Physiol Endocrinol Metab, 2006. **290**(6): p. E1245-52.

72. Koopman, R., et al., *Nutritional interventions to promote post-exercise muscle protein synthesis.* Sports Med, 2007. **37**(10): p. 895-906.

73. Wilkinson, S.B., et al., *Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle.* J Physiol, 2008. **586**(Pt 15): p. 3701-17.

74. Sheffield-Moore, M., et al., *Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise.* Am J Physiol Endocrinol Metab, 2004. **287**(3): p. E513-22.

75. Short, K.R., et al., *Age and aerobic exercise training effects on whole body and muscle protein metabolism.* Am J Physiol Endocrinol Metab, 2004. **286**(1): p. E92-101.

76. Nader, G.A. and K.A. Esser, *Intracellular signaling specificity in skeletal muscle in response to different modes of exercise.* J Appl Physiol, 2001. **90**(5): p. 1936-42.

77. Fujii, N., et al., *Exercise induces isoform-specific increase in 5'AMP-activated protein kinase activity in human skeletal muscle.* Biochem Biophys Res Commun, 2000. **273**(3): p. 1150-5.

78. Wojtaszewski, J.F., et al., *Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle.* J Physiol, 2000. **528 Pt 1**: p. 221-6.

79. Chen, Z.P., et al., *Effect of exercise intensity on skeletal muscle AMPK signaling in humans.* Diabetes, 2003. **52**(9): p. 2205-12.

80. Bangsbo, J., L. Norregaard, and F. Thorso, *Activity profile of competition soccer.* Can J Sport Sci, 1991. **16**(2): p. 110-6.

81. Janssen, I., et al., *Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr.* J Appl Physiol, 2000. **89**(1): p. 81-8.

82. Lynch, N.A., et al., *Muscle quality. I. Age-associated differences between arm and leg muscle groups.* J Appl Physiol, 1999. **86**(1): p. 188-94.

83. Rogol, A.D., *Leptin and puberty.* J Clin Endocrinol Metab, 1998. **83**(4): p. 1089-90.

84. Janssen, I., et al., *Estimation of skeletal muscle mass by bioelectrical impedance analysis.* J Appl Physiol, 2000. **89**(2): p. 465-71.

85. Najjar, M.F. and M. Rowland, *Anthropometric reference data and prevalence of overweight, United States, 1976-80.* Vital Health Stat 11, 1987(238): p. 1-73.

86. Lemieux, S., et al., *Sex differences in the relation of visceral adipose tissue accumulation to total body fatness.* Am J Clin Nutr, 1993. **58**(4): p. 463-7.

87. Vague, J., *The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease.* Am J Clin Nutr, 1956. **4**(1): p. 20-34.

88. Miller, A.E., et al., *Gender differences in strength and muscle fiber characteristics.* Eur J Appl Physiol Occup Physiol, 1993. **66**(3): p. 254-62.

89. Staron, R.S., et al., *Fiber type composition of the vastus lateralis muscle of young men and women.* J Histochem Cytochem, 2000. **48**(5): p. 623-9.

90. Simoneau, J.A. and C. Bouchard, *Human variation in skeletal muscle fiber-type proportion and enzyme activities.* Am J Physiol, 1989. **257**(4 Pt 1): p. E567-72.

91. Ivey, F.M., et al., *Effects of strength training and detraining on muscle quality: age and gender comparisons.* J Gerontol A Biol Sci Med Sci, 2000. **55**(3): p. B152-7; discussion B158-9.

92. Roth, S.M., et al., *Muscle size responses to strength training in young and older men and women.* J Am Geriatr Soc, 2001. **49**(11): p. 1428-33.

93. Charette, S.L., et al., *Muscle hypertrophy response to resistance training in older women.* J Appl Physiol, 1991. **70**(5): p. 1912-6.

94. Fiatarone, M.A., et al., *Exercise training and nutritional supplementation for physical frailty in very elderly people.* N Engl J Med, 1994. **330**(25): p. 1769-75.

95. Higbie, E.J., et al., *Effects of concentric and eccentric training on muscle strength, cross-sectional area, and neural activation.* J Appl Physiol, 1996. **81**(5): p. 2173-81.

96. McCall, G.E., et al., *Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training.* J Appl Physiol, 1996. **81**(5): p. 2004-12.

97. Young, A., et al., *The effect of high-resistance training on the strength and cross-sectional area of the human quadriceps.* Eur J Clin Invest, 1983. **13**(5): p. 411-7.

98. Sheffield-Moore, M., *Androgens and the control of skeletal muscle protein synthesis.* Ann Med, 2000. **32**(3): p. 181-6.

99. Wittert, G.A., et al., *Oral testosterone supplementation increases muscle and decreases fat mass in healthy elderly males with low-normal gonadal status.* J Gerontol A Biol Sci Med Sci, 2003. **58**(7): p. 618-25.

100. Bhasin, S., et al., *Testosterone replacement increases fat-free mass and muscle size in hypogonadal men.* J Clin Endocrinol Metab, 1997. **82**(2): p. 407-13.

101. Wang, C., et al., *Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men--a clinical research center study.* J Clin Endocrinol Metab, 1996. **81**(10): p. 3654-62.

102. D'Eon, T.M., et al., *Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women.* Am J Physiol Endocrinol Metab, 2002. **283**(5): p. E1046-55.

103. Braun, B. and T. Horton, *Endocrine regulation of exercise substrate utilization in women compared to men.* Exerc Sport Sci Rev, 2001. **29**(4): p. 149-54.

104. Campbell, S.E. and M.A. Febbraio, *Effects of ovarian hormones on exercise metabolism.* Curr Opin Clin Nutr Metab Care, 2001. **4**(6): p. 515-20.

105. Cooke, P.S. and A. Naaz, *Role of estrogens in adipocyte development and function.* Exp Biol Med (Maywood), 2004. **229**(11): p. 1127-35.

106. Lindheim, S.R., et al., *A possible bimodal effect of estrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin.* Fertil Steril, 1993. **60**(4): p. 664-7.

107. Stupka, N., et al., *Gender differences in muscle inflammation after eccentric exercise.* J Appl Physiol, 2000. **89**(6): p. 2325-32.

108. Sitnick, M., et al., *Ovariectomy prevents the recovery of atrophied gastrocnemius skeletal muscle mass.* J Appl Physiol, 2006. **100**(1): p. 286-93.

109. Lebrun, C.M., et al., *Effects of menstrual cycle phase on athletic performance.* Med Sci Sports Exerc, 1995. **27**(3): p. 437-44.

110. Janse de Jonge, X.A., et al., *The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans.* J Physiol, 2001. **530**(Pt 1): p. 161-6.

111. Redman, L.M., G.C. Scroop, and R.J. Norman, *Impact of menstrual cycle phase on the exercise status of young, sedentary women.* Eur J Appl Physiol, 2003. **90**(5-6): p. 505-13.

112. Kendrick, Z.V., et al., *Effect of estradiol on tissue glycogen metabolism in exercised oophorectomized rats.* J Appl Physiol, 1987. **63**(2): p. 492-6.

113. Rooney, T.P., et al., *Effect of estradiol on the temporal pattern of exercise-induced tissue glycogen depletion in male rats.* J Appl Physiol, 1993. **75**(4): p. 1502-6.

114. Hatta, H., et al., *The effects of ovarian hormones on glucose and fatty acid oxidation during exercise in female ovariectomized rats.* Horm Metab Res, 1988. **20**(10): p. 609-11.

115. Hackney, A.C., D. Muoio, and W.R. Meyer, *The Effect of sex steroid hormones on substrate oxidation during prolonged submaximal exercise in women.* Jpn J Physiol, 2000. **50**(5): p. 489-94.

116. Miller, B.F., et al., *No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle.* Am J Physiol Endocrinol Metab, 2006. **290**(1): p. E163-E168.

117. Wideman, L., et al., *Growth hormone release during acute and chronic aerobic and resistance exercise: recent findings.* Sports Med, 2002. **32**(15): p. 987-1004.

118. van den Berg, G., et al., *Combined amplification of the pulsatile and basal modes of adrenocorticotropin and cortisol secretion in patients with Cushing's disease:*

evidence for decreased responsiveness of the adrenal glands. J Clin Endocrinol Metab, 1995. **80**(12): p. 3750-7.

119. Arciero, P.J., M.I. Goran, and E.T. Poehlman, *Resting metabolic rate is lower in women than in men.* J Appl Physiol, 1993. **75**(6): p. 2514-20.

120. Tarnopolsky, L.J., et al., *Gender differences in substrate for endurance exercise.* J Appl Physiol, 1990. **68**(1): p. 302-8.

121. Horton, T.J., et al., *Fuel metabolism in men and women during and after long-duration exercise.* J Appl Physiol, 1998. **85**(5): p. 1823-32.

122. Costill, D.L., et al., *Lipid metabolism in skeletal muscle of endurance-trained males and females.* J Appl Physiol, 1979. **47**(4): p. 787-91.

123. Devries, M.C., et al., *IMCL area density, but not IMCL utilization, is higher in women during moderate-intensity endurance exercise, compared with men.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(6): p. R2336-42.

124. Horton, T.J., et al., *Glucose kinetics differ between women and men, during and after exercise.* J Appl Physiol, 2006. **100**(6): p. 1883-94.

125. Mittendorfer, B., J.F. Horowitz, and S. Klein, *Effect of gender on lipid kinetics during endurance exercise of moderate intensity in untrained subjects.* Am J Physiol Endocrinol Metab, 2002. **283**(1): p. E58-65.

126. Powers, S.K., W. Riley, and E.T. Howley, *Comparison of fat metabolism between trained men and women during prolonged aerobic work.* Res Q Exerc Sport, 1980. **51**(2): p. 427-31.

127. Smith, F.M., A.S. Garfield, and A. Ward, *Regulation of growth and metabolism by imprinted genes.* Cytogenet Genome Res, 2006. **113**(1-4): p. 279-91.

128. Dufresne, A.M. and R.J. Smith, *The adapter protein GRB10 is an endogenous negative regulator of insulin-like growth factor signaling.* Endocrinology, 2005. **146**(10): p. 4399-409.

129. Welle, S., R. Tawil, and C.A. Thornton, *Sex-related differences in gene expression in human skeletal muscle.* PLoS ONE, 2008. **3**(1): p. e1385.

130. Gibala, M.J., et al., *Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance.* J Physiol, 2006. **575**(Pt 3): p. 901-11.

131. Jones, A.M. and H. Carter, *The effect of endurance training on parameters of aerobic fitness.* Sports Med, 2000. **29**(6): p. 373-86.

132. Clark, B.C., et al., *Gender differences in skeletal muscle fatigability are related to contraction type and EMG spectral compression.* J Appl Physiol, 2003. **94**(6): p. 2263-72.

133. Hunter, S.K., et al., *Men are more fatigable than strength-matched women when performing intermittent submaximal contractions.* J Appl Physiol, 2004. **96**(6): p. 2125-32.

134. Hunter, S.K. and R.M. Enoka, *Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions.* J Appl Physiol, 2001. **91**(6): p. 2686-94.

135. Robbins, J.L., et al., *A Gender Specific Relationship between Capillary Density and Anaerobic Threshold.* J Appl Physiol, 2009.

136. Duscha, B.D., et al., *Baseline and exercise response sex diffrences in skeletal muscle capillary density.* Medicine & Science in Sports & Exercise, 2003. **35(5) Supplement 1**: p. S388.

137. Gratas-Delamarche, A., et al., *Lactate and catecholamine responses in male and female sprinters during a Wingate test.* Eur J Appl Physiol Occup Physiol, 1994. **68**(4): p. 362-6.

138. Hill, D.W. and J.C. Smith, *Gender difference in anaerobic capacity: role of aerobic contribution.* Br J Sports Med, 1993. **27**(1): p. 45-8.

139. Perez-Gomez, J., et al., *Role of muscle mass on sprint performance: gender differences?* Eur J Appl Physiol, 2008. **102**(6): p. 685-94.

140. Cheuvront, S.N., et al., *Running performance differences between men and women:an update.* Sports Med, 2005. **35**(12): p. 1017-24.

141. Billaut, F., M. Giacomoni, and G. Falgairette, *Maximal intermittent cycling exercise: effects of recovery duration and gender.* J Appl Physiol, 2003. **95**(4): p. 1632-7.

142. Bishop, D., J. Edge, and C. Goodman, *Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women.* Eur J Appl Physiol, 2004. **92**(4-5): p. 540-7.

143. Bishop, D., et al., *The validity of a repeated sprint ability test.* J Sci Med Sport, 2001. **4**(1): p. 19-29.

144. Dawson, B., et al., *Muscle phosphocreatine repletion following single and repeated short sprint efforts.* Scand J Med Sci Sports, 1997. **7**(4): p. 206-13.

145. Gaitanos, G.C., et al., *Human muscle metabolism during intermittent maximal exercise.* J Appl Physiol, 1993. **75**(2): p. 712-9.

146. Jones, N.L., et al., *Muscle performance and metabolism in maximal isokinetic cycling at slow and fast speeds.* J Appl Physiol, 1985. **59**(1): p. 132-6.

147. Perreault, L., et al., *Gender differences in insulin action after a single bout of exercise.* J Appl Physiol, 2004. **97**(3): p. 1013-21.

148. Yki-Jarvinen, H., *Sex and insulin sensitivity.* Metabolism, 1984. **33**(11): p. 1011-5.

149. Robertson, M.D., G. Livesey, and J.C. Mathers, *Quantitative kinetics of glucose appearance and disposal following a 13C-labelled starch-rich meal: comparison of male and female subjects.* Br J Nutr, 2002. **87**(6): p. 569-77.

150. Tarnopolsky, M.A., et al., *Carbohydrate loading and metabolism during exercise in men and women.* J Appl Physiol, 1995. **78**(4): p. 1360-8.

151. Tarnopolsky, M.A., et al., *Gender differences in carbohydrate loading are related to energy intake.* J Appl Physiol, 2001. **91**(1): p. 225-30.

152. Hawley, J.A. and T.D. Noakes, *Peak power output predicts maximal oxygen uptake and performance time in trained cyclists.* Eur J Appl Physiol Occup Physiol, 1992. **65**(1): p. 79-83.

153. Bangsbo, J., *The physiology of soccer--with special reference to intense intermittent exercise.* Acta Physiol Scand Suppl, 1994. **619**: p. 1-155.

154. Reilly, T., *Energetics of high-intensity exercise (soccer) with particular reference to fatigue.* J Sports Sci, 1997. **15**(3): p. 257-63.

155. Reilly, T. and V. Thomas, *Estimated daily energy expenditures of professional association footballers.* Ergonomics, 1979. **22**(5): p. 541-8.

156. Mohr, M., et al., *Muscle temperature and sprint performance during soccer matches--beneficial effect of re-warm-up at half-time.* Scand J Med Sci Sports, 2004. **14**(3): p. 156-62.

157. Deutsch, M.U., et al., *Heart rate, blood lactate and kinematic data of elite colts (under-19) rugby union players during competition.* J Sports Sci, 1998. **16**(6): p. 561-70.

158. Krustrup, P., et al., *Muscle and blood metabolites during a soccer game: implications for sprint performance.* Med Sci Sports Exerc, 2006. **38**(6): p. 1165-74.

159. Bishop, D. and B. Claudius, *Effects of induced metabolic alkalosis on prolonged intermittent-sprint performance.* Med Sci Sports Exerc, 2005. **37**(5): p. 759-67.

160. Mendez-Villanueva, A., P. Hamer, and D. Bishop, *Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity.* Eur J Appl Physiol, 2008. **103**(4): p. 411-9.

161. Mannion, A.F., et al., *Carnosine and anserine concentrations in the quadriceps femoris muscle of healthy humans.* Eur J Appl Physiol Occup Physiol, 1992. **64**(1): p. 47-50.

162. Copeland, J.L., L.A. Consitt, and M.S. Tremblay, *Hormonal responses to endurance and resistance exercise in females aged 19-69 years.* J Gerontol A Biol Sci Med Sci, 2002. **57**(4): p. B158-65.

163. Hakkinen, K. and A. Pakarinen, *Acute hormonal responses to heavy resistance exercise in men and women at different ages.* Int J Sports Med, 1995. **16**(8): p. 507-13.

164. Weiss, L.W., K.J. Cureton, and F.N. Thompson, *Comparison of serum testosterone and androstenedione responses to weight lifting in men and women.* Eur J Appl Physiol Occup Physiol, 1983. **50**(3): p. 413-9.

165. Vary, T.C., S.R. Kimball, and A. Sumner, *Sex-dependent differences in the regulation of myocardial protein synthesis following long-term ethanol consumption.* Am J Physiol Regul Integr Comp Physiol, 2007. **292**(2): p. R778-87.

Appendix A

Massey University

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Participant Information Sheet

Project Title: **The effect of a soccer-specific exercise protocol on muscle and blood hormonal response in athletes**

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You have been invited to participate in a study investigating muscle and blood response to a soccer-specific exercise protocol in competitive male and female soccer players. Participation in this study is on a voluntary basis and all participants have the right to pull out, or ask questions at any time.

Aim

The aim of this study is to investigate the muscle and blood hormones after soccer training in male and female soccer players.

Test Procedure

Approximately 5 days before the testing date you will be asked to come into the lab and perform a maximal endurance test ($\dot{V}O_{2peak}$ test) and a familiarisation session in which you will perform half of the soccer specific training session. On the day of actual testing you will be asked to complete a sprint training session consisting of two 24 x 30 metre sprints. You will perform 6 sprints in a row (approximately 25 s between each sprint) x 4 sets. Each set will be separated by 7 minutes of passive rest.

We ask that you come to the training session having fasted for the 3 hours prior to the session. We will provide a standardised meal for you to take 4-5 hours prior to the exercise session. Before the session commences a resting capillary and venous blood sample will be taken. A resting muscle biopsy will also be performed 30 minutes prior to the

commencement of the session. Further capillary samples will be taken at the completion of each repeated-sprint test (x 4). Ten minutes after the training session is completed a second venous blood sample and muscle biopsy will also be taken. Two hours after the completion of the training session, a final muscle biopsy, capillary, and venous blood sample will be taken and thus conclude your participation in the study.

This experiment will involve a total of 3 venous blood samples, 6 capillary blood samples and 3 muscle biopsies over a period of 4 hours.

Potential risks and discomforts

Muscle Biopsy

This procedure will be performed by an experienced medical Doctor (Dr. Michael Short). This is a common procedure used to determine muscle adaptations to exercise.

- Upon arrival to the laboratory the sites will be prepared for muscle samples. There will be a total of 3 muscle biopsies during the session.
- The muscle biopsy will involve the administering of a local anaesthetic to the outside part of the upper thigh. This will be followed by a small incision made through the skin. A 5 millimetre biopsy needle will then be inserted through the incision and into the muscle, in order to remove a small piece of muscle tissue (~80 – 100 mg, size of a match head).
- Finally the incision will be closed with steri-strips (like a band aid). After the last muscle biopsy appropriate recovery processes will be performed including, rest, ice, compression and elevation of the leg.
- The muscle biopsy process may result in slight discomfort (a mild "cork"), which may last for the following 1-3 days. This may be accompanied by local temporary bruising (although normally rare), along with the very small risk of superficial nerve damage in the skin (caused by the incision), which if present, may cause a temporary (approximately 1 - 10 days) loss of sensation to the area. However, this is also extremely rare. You should not perform vigorous exercise for the next 24-48 hours. However, you may resume light exercise approximately 24 hours after the procedure. If you get a haematoma due to the biopsy procedure the Doctor will apply pressure to the sample area to reduce the bleeding and follow the RICE procedures of rest, ice, compression and elevation. There is the extremely remote risk of a small decrease in muscle size at the site of the muscle biopsy. In the event that the intramuscular bleeding is not reduced using RICE procedures, we will book an immediate appointment to see Dr. Michael Short, who will recommend appropriate procedures to deal with this issue."

- In the circumstance you feel unwell at any stage during the recovery period you should immediately call Johann Edge (Chief Investigator, see phone numbers at the end of this information sheet)

Venous blood sampling

Three x 10 mL blood samples will be taken from your forearm (totalling 30 mL). There is a small needle prick and therefore, discomfort associated with placement

of a needle. The researcher (Toby Mundel) is trained in venous blood sampling. This method of blood sampling is routinely practiced in clinical research studies. The risk of infection is extremely low, however there is chance of minor bruising. You can request to have any/all portions of your samples returned to you.

Capillary blood sampling

Six earlobe capillary blood samples (100 micro litres each sample) will be taken (totalling 600 micro litres). A small pin prick will be performed to pierce the skin while capillary tubes are used to sample the blood. This may result in mild discomfort.

As in any physical activity, there is a very small possibility of injury that includes, but not restricted to, muscle, ligament or tendon damage, breathing irregularities and dizziness. All protocols are commonly performed in sport science research and potential risks to participants have been minimized by allowing thorough warm-up prior to the testing session.

Benefits and compensation

For the time you invest in this study, you will receive a $50 movie theatre voucher. You will also have your peak aerobic fitness ($\dot{V}O_{2peak}$) score and sprint performance given to you at the end of the study. Additionally, a meal will be provided both before exercise for study control purposes and after exercise to aid in your recovery. From the muscle and blood we will measure proteins (Mammalian target of rapamysin; mTOR) and hormones (growth hormone, insulin-like growth factor) important to the growth of your muscle, your personal results will be provided to you.

Participants' rights

- **You can ask questions on any aspect of the project at any time, and we will do our best to answer them to your satisfaction.**
- **As a participant in the study you will provide information on the understanding that your name will not be used unless you give permission to the researcher.**
- **You have the right to view your own data at any stage and have it explained to you.**
- **You have the right to have any blood samples returned to you after they have been analyzed.**
- **You will also be given access to a summary of the project findings when it is concluded.**
- **You can withdraw from the project at any time, without giving any reason and without penalty.**

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and

entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury. If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Eligibility

To participate in this study we require you are a competitive male or female in-season soccer player with a moderate to high fitness level and void of injury. It is also important for the female participants that you have a regular menstrual cycle. You must also be of good health. You will **not** be eligible to participate if any of the following apply:

- ***You have any known heart or cardiovascular condition or if a member of your family died below the age of fifty (50) as a result of a heart condition.***
- ***In the last six months you have suffered from any painful injury or condition that lasted more than one week.***
- ***You have ever had an injury or any medical condition that you think may affect your ability to sense pain or discomfort.***
- ***You have ever had persistent or regular lower back pain.***
- ***You are taking prescribed medication.***
- ***You have cultural or religious sensitivities about human body measurements.***
- ***You have any other reason to consider that you are not in good health and of average, or better than average, fitness.***
- ***You are diabetic***
- ***You are pregnant***
- ***You or a family member has a bleeding disorder***

Further Information

You will be required to wear suitable sporting clothing and footwear that you feel comfortable exercising in. Water will be provided throughout the testing procedure and showers are also available should you need them. We also require you to fill in a food diary and training diary. For female participants we also ask that you map your menstrual cycle prior to testing.

All data obtained from this study will be kept strictly confidential. Data will identified as a code only. Results will be made available to you at the completion of the study.

If you are Interested in Taking Part

Contact:

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This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 07/46. If you have any concerns about the conduct of this research, please contact Professor John O'Neill, Chair, Massey University Human Ethics Committee: Southern A, telephone 06 350 5799 x 8771, email humanethicsoutha@massey.ac.nz.

Appendix B

Study Instructions: Food and exercise diary

Bring this food/exercise diary to your trial. We will supply you with breakfast and morning tea. You cannot eat for the 3 hours prior to your exercise trial (you may have water). Record your food for the day prior to the trial and on the day of the trial (i.e. your supplied snacks), put approximate portion sizes (i.e. 1 Cup of vegetables, 1 cheese sandwich).

1 day before trial

Breakfast	
Snack	
Lunch	
Snack	
Dinner	
Snack	

Day of trial

Breakfast	
Snack	
Lunch	
Snack	
Dinner	
Snack	

Exercise for 2 days and 1 day before trial

Record your exercise for 2 days before trial	
Record your exercise for 1 day before trial	

Appendix C Raw Data

Lactate

Subject #	Sex	Rest Lac	post bout 1 Lac	post bout 2 Lac	post bout 3 Lac	post bout 4 Lac
(2)	f	0.53	7.87	9.27	9	8.55
(6)	f	0.83	6.69	10.23	11.37	12.44
(3)	f	0.88	17.39	18.54	15.29	16.88
(4)	f	1.2	8.19	9.55	9.76	8.44
(8)	f	0.41	7.84	7.86	7.8	8.04
(10)	f	1.09	9.89	11.8	12.33	12.85
(9)	f	0.71	12.39	15.73	17.68	17.85
(12)	f	0.62	9.83	12.95	12.44	12.33
Mean		0.8	10.0	12.0	12.0	12.2
SD		0.3	3.5	3.6	3.3	3.8
SE						
(1)	m	1.51	7.84	7.27	6.85	4.36
(5)	m	0.37	9.21	9.22	7.95	8.06
(7)	m	0.56	9.89	12.49	12.34	12.45
(11)	m	1.25	9.67	11.73	11.77	12.12
(13)	m	1.25	9.79	12.22	12.33	13.94
(14)	m	0.73	10.81	15.53	16.35	15.82
(15)	m	0.82	12.91	14.83	13.92	14.09
	m					
Mean		0.9	10.0	11.9	11.6	11.5
SD		0.4	1.6	2.9	3.3	4.0