Effects of a higher acute protein intake post muscle-damaging exercise on muscle recovery in older female endurance runners

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Abstract

Masters endurance athletes who train and compete in organised competitive endurance events, appear to take longer to recover after muscle-damaging exercise than younger athletes. A reduced sensitivity to increase skeletal muscle protein synthesis (MPS) in response to the anabolic stimuli of protein and exercise may be contributing to the slower repair and remodelling of muscle fibres. This decreased stimulus for MPS negatively affects recovery in this older cohort of athletes, although evidence supports the notion that the ingestion of a higher dose of protein than the current recommendation of 20 grams, post muscle-damaging exercise, demonstrates improvements in muscle function of male masters triathletes. However, the efficacy for a higher protein intake in female masters endurance athletes has not been established.

Chapter 2 investigates the effects of ingesting a higher acute dose of protein (40 g) compared to the current recommended dose (20 g) on muscle recovery following exercise-induced muscle damage in older female (≥48 y) endurance runners. The study assessed muscle recovery by measuring isometric maximal voluntary contraction of the knee extensors, peak countermovement jump height, perceived muscle soreness, and flexibility prior to, and at 3 and 24 hours following a downhill running protocol, used to elicit muscle damage. The study recruited six well trained female endurance runners, 48 years of age and older (mean ± *SD*: age; 52 ± 5.6 y, body mass; 61.4 ± 6.2 kg) to take part in three downhill running trials, each separated by one week. Participants consumed one of three energy matched (1190 kJ) recovery beverages immediately following the run. Beverage composition was either a 70 g carbohydrate placebo or one of two protein supplements; 20 g of protein and 50 g of carbohydrate, or 40 g of protein and 30 g of carbohydrate. The 40 g of protein had a likely small beneficial effect compared to the placebo (ES = 0.24 ± 0.5) and 20 g of protein (ES = 0.25 ± 0.29) for improving maximum voluntary contraction from baseline to 24 hours. The 40 g of protein

had a likely small beneficial effect for improving countermovement jump height compared to 20 g of protein from baseline to 3 hours (ES = 2.9 ± 4.9), and to 24 hours compared to the placebo (ES = 0.32 ± 0.39) and 20 g of protein (ES = 0.27 ± 0.19). The 40 g of protein had an unclear effect on muscle soreness and flexibility. The findings of this current study suggest that the acute ingestion of 40 grams of whey protein isolate following muscle-damaging exercise, had a small beneficial effect on improving muscle function within 24 hours, in masters female runners.

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Abbreviations

ACSM – American College of Sports Medicine

Ca^{2+} - Calcium ion

CHO – Carbohydrate

CHO-P - Carbohydrate-protein

CK – Creatine kinase

CL – Confidence limits

cm - Centimetre

CMJ – Counter movement jump

DHR – Downhill running

EAA – Essential amino acid

EIMD – Exercise induced muscle damage

ES – Effect size

FSR – Fractional synthesis rate

g – Gram

$g.kg^{-1}$ – Grams per kilogram

h – Hour

HR_{max} – Maximal heart rate

kg – Kilogram

kJ – Kilojoule

M- Mean

m – Metre

ml – Millilitre

mm - Millimetre

MPS – Muscle Protein Synthesis

mTORC1 – Mammalian target of rapamycin complex 1

MVC – Maximal voluntary contraction

n – number of participants

NSAID – Non-steroid anti-inflammatory drug

P – Protein

P20 – 20 grams of protein

P40 – 40 grams of protein

PIMT – Peak isometric torque

PIT – Peak isokinetic torque

PLA - placebo

RE – Resistance exercise

RBE – Repeated bout effect

RPE – Rate of perceived exertion

SD – Standard deviation

S6K1 – Ribosomal S6 Kinase 1

TW – Total work

VAS – Visual analogue scale

VO_{2max} – Maximum rate of oxygen consumption

WPI – Whey protein isolate

y – Year

4E-BP1 – Eukaryotic initiation factor 4E binding protein

°/s – Degrees per sec

Thesis Overview

The format of this thesis is presented in the style of an individual journal article, and consequently, some information may be repeated. The thesis is comprised of three chapters. Chapter One contains a review of literature and introduces the reader to aging effects on skeletal muscle slowing recovery in masters athletes as well as the concept of protein supplementation. Chapter Two focuses on the effects of acute protein supplementation improving recovery in older female endurance runners and is presented in the style of an individual journal article. The final Chapter Three summarises the overall finding from the experimental study included in this thesis and provides both practical applications and suggested areas for future research.

Chapter 1: Literature Review

Chapter 2: Effects of a higher acute protein intake post muscle-damaging exercise on muscle recovery in older female endurance runners

Chapter 3: Conclusion
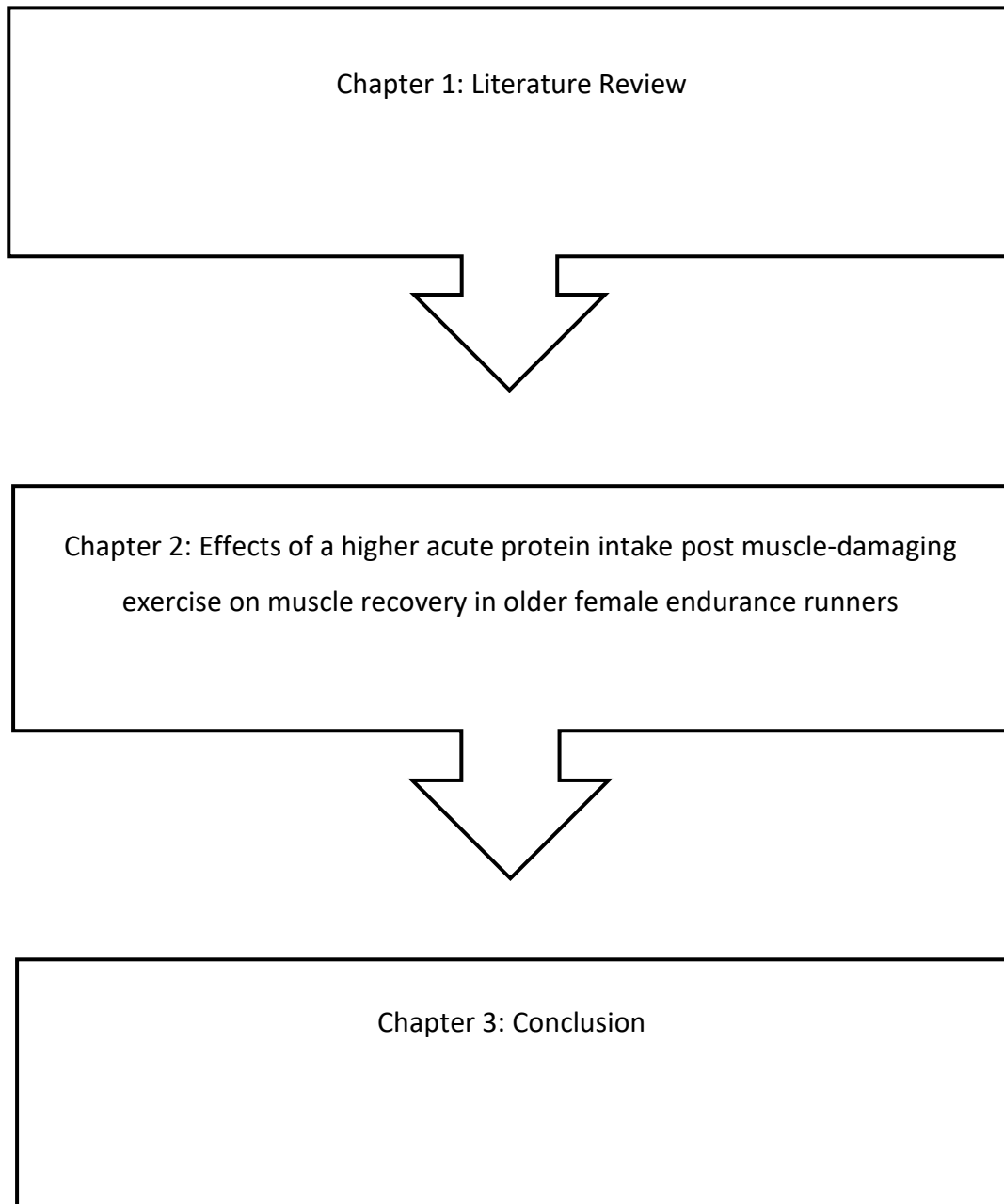
Figure 1. Schematic of thesis structure.

Chapter 1: Literature Review

Introduction

During recent decades, an increasing number of masters athletes have been participating in endurance running events, including the half marathon, marathon, and ultras (events ≥50 km) (Knechtle, Rüst, Rosemann, & Lepers, 2012; Lepers & Stapley, 2016). A masters athlete is "an individual who systematically trains and competes in organised forms of competitive sport specifically designed for older adults" (Reaburn & Dascombe, 2008, p. 31), and for the purpose of this review is defined as 45 years (y) of age or older.

Endurance running has been shown to induce muscle damage, particularly the eccentric loading of downhill running (Brisswalter & Nosaka, 2013). While the magnitude of muscle damaged has been reported to be similar between masters athletes and their younger counterparts, research suggests that masters athletes take longer to adapt to and recover from the muscle damage (Borges, Reaburn, Driller, & Argus, 2016; Brisswalter & Nosaka, 2013; Easthope et al., 2010). A slower recovery time has serious implications for the masters athlete, with the potential of forcing the athlete to progressively reduce the intensity and frequency of training sessions, limiting adaptations and ultimately performance (Barnett, 2006; Bishop, Jones, & Woods, 2008; Fell & Williams, 2008).

Masters female endurance runners are an understudied (Costello, Bieuzen, & Bleakley, 2014) yet distinct population, with slower recovery times following exercise induced muscle damage (EIMD). This is potentially due to the aging effects on muscle, namely the hormonal effects (Enns & Tiidus, 2010), and 'anabolic resistance' to muscle protein synthesis (MPS) (Breen & Phillips, 2011).

Anabolic resistance is the impaired ability of aging muscle to increase protein synthesis in response to the anabolic stimuli of protein and exercise (Cuthbertson et al., 2005; Dickinson, Volpi, & Rasmussen, 2013). To overcome this reduced sensitivity to the synthesis of new muscle proteins, research indicates that a higher dose than the recommended 20 g of protein is needed (Dickinson et al., 2013); it

has been shown that 20 g near maximally stimulates MPS in younger men (Witard et al., 2014). Information available from dose response studies of dietary protein to MPS indicate that ingesting ~40 g of protein after resistance exercise in untrained older men can near maximally stimulate MPS (Robinson et al., 2012; Yang, Breen, et al., 2012). This dose should also support the facilitation of optimal repair and remodelling of skeletal muscle and therefore enhance recovery following EIMD.

The primary aim of this review is to assess if a higher acute protein intake (40 g) post muscle-damaging exercise can improve recovery in older female endurance runners.

The first section of this review provides an understanding of EIMD, the importance of optimising recovery between training bouts for the endurance athlete, and evidence for attenuated recovery in masters athletes after EIMD. Following this is a discussion on the effects of aging on skeletal muscle in attenuating recovery in older female endurance runners and how anabolic resistance to MPS can be overcome. Lastly, given the current lack of evidence regarding protein supplementation to improve recovery after EIMD in older athletes, appropriate studies in younger populations are critically reviewed and placed in context for the older female endurance runner.

Section One - Exercise induced muscle damage and recovery

Exercise induced muscle damage has been an area of research since the 1900s, with growing interest over the last decades as to the proposed mechanisms of muscle damage and the recovery process (Hyldahl & Hubal, 2014). EIMD has a direct impact on an athlete's capacity to train, with adequate recovery fundamental for maximising training induced adaptations and performance (Fell & Williams, 2008). Research suggests that in older male endurance athletes recovery time may be delayed after EIMD compared to their younger counterparts (Borges et al., 2016).

Mechanisms, symptoms and severity of EIMD

EIMD is caused primarily by eccentrically biased exercise where the muscle lengthens while trying to contract to resist the force of gravity (Douglas, Pearson, Ross, & McGuigan, 2017). The exact underlying mechanisms responsible for EIMD remain unclear (Paulsen, Ramer Mikkelsen, Raastad, & Peake, 2012). However, mechanisms associated with muscle damage can be separated into a primary and secondary phase (Owens, Twist, Cobley, Howatson, & Close, 2018). The primary phase is a result of the mechanical damage occurring during exercise, including damage and disruption to the sarcomeres from the high tension on the muscle myofibrils (Clarkson & Hubal, 2002; Howatson & Someren, 2008), and dysfunction of excitation-contraction coupling (Howatson & Someren, 2008; Owens et al., 2018). Secondary damage involves the loss of Ca^{2+} regulation, which causes further damage to the myofibrils (Peake, Nosaka, & Suzuki, 2005), followed by an inflammatory response, required for removal of the damaged tissue and starting the repair and remodelling of skeletal muscle (Chazaud, 2016; Peake et al., 2005).

Endurance running has a large component of eccentric contractions, with damage most severe from downhill running (DHR) due to the higher mechanical stress on the muscles (Easthope et al., 2010). This damage is characterised by a set of specific symptoms following the exercise bout.

The symptoms of EIMD typically include a loss of muscle force generating capacity, soreness, swelling of the affected limb/s, a decrease in range of motion, and leakage of muscle proteins (e.g. creatine kinase, myoglobin) into the blood (Douglas et al., 2017; Howatson & Someren, 2008; Peake et al., 2005). Symptoms present immediately after the initial exercise bout, with some symptoms present for up to two weeks, depending on the extent of damage (Owens et al., 2018).

The severity of muscle damage determines the time taken to recover, with muscle damage largely dependent on the choice of exercise protocol (Owens et al., 2018). Greater damage is induced from eccentric contractions that are of faster velocity,

higher intensity, longer muscle lengths or a greater number of contractions (repetitions) (Peake et al., 2005). The damage response is also modified by the large variation between individuals in response to EIMD (Owens et al., 2018; Paulsen et al., 2012). Genetic variability among individuals (Baumert, Lake, Stewart, Drust, & Erskine, 2016), training status, age, and hormones (Owens et al., 2018) modify the damage response. The greatest modifier is the repeated bout effect (RBE), defined as the ability of skeletal muscle to adapt so that it is protected against damage in subsequent exercise bouts (Hyldahl, Chen, & Nosaka, 2017; McHugh, 2003). Irrespective of differences in the extent of muscle damage, adequate recovery is a fundamental component of an athletes training program, as muscle soreness and loss of muscle function impact directly on an athlete's ability to maintain a training program.

Optimising recovery and the proposed delayed recovery in masters athletes after EIMD

Optimising recovery between bouts of endurance training is essential to maximise training induced adaptations and improve performance in any age endurance athlete, yet there is a paucity of information and understanding on the recovery kinetics of the masters endurance athlete after EIMD (Borges et al., 2016). Women, in particular are underrepresented in this research. Much of the seminal research has been conducted using animal models, with indications that advancing age does not increase susceptibility to damage but does lead to longer recovery periods (Fell & Williams, 2008). Two studies have been undertaken in male endurance athletes that support the findings from animal models (Easthope et al., 2010; Sultana et al., 2012).

Recovery over a 72 hour (h) period was compared between 13 older (50 ± 6 y) and 10 younger (31 ± 7 y) ultra-endurance runners of a similar performance level after a 55 km trail race (Easthope et al., 2010). Fatigue levels after the race were similar, but the older athletes required an additional 24 h for maximal voluntary contractions (MVC) of knee extensors to return to baseline. A later study

measured the recovery of well-trained male triathletes after an Olympic distance triathlon (Sultana et al., 2012); in 10 masters (52 ± 10 y) and 9 younger (28 ± 6 y) athletes. While there was little difference in recovery of MVC of knee extensors at 24 h following the triathlon in either group, running speed in the masters group was significantly reduced at ventilatory threshold 2 (linked strongly with running performance) at 24 h post-exercise, compared to younger athletes. These studies suggest that after EIMD, the extent of muscle damage is comparable between younger and older athletes, but the recovery time is slower for the repair and regeneration of muscle tissue.

A slower rate of recovery in masters endurance runners after muscle-damaging exercise has the potential of forcing the athlete to progressively reduce the intensity and frequency of training sessions, thereby slowing and limiting adaptations and performance (Bishop et al., 2008; Brisswalter & Nosaka, 2013; Fell & Williams, 2008), and possibly increasing the risk of injury (Borges et al., 2016). It is therefore important, to identify the key factors for the delayed recovery and possible means by which they can be modified to enhance the recovery time.

Section Two - Factors contributing to slower recovery

A number of factors likely contribute to the attenuated recovery of masters endurance athletes after EIMD, however this review concentrates on the slower recovery in older female endurance runners. Of relevance to the older female endurance runner are the hormonal effects on muscle, and anabolic resistance to MPS in this population (Doering, Reaburn, Phillips, & Jenkins, 2016).

Hormonal effects on muscle

Aging is associated with a natural decline in sex hormones, however, the declining levels of oestrogen, specifically that of estradiol-17β (referred to for ease of communication in this review as oestrogen), may significantly impact on recovery due to its proposed protective and anabolic effect on skeletal muscle (Enns & Tiidus, 2010). Oestrogen acts as an antioxidant, limiting damage to muscle fibres,

and works to stabilise the sarcolemma which reduces disruption to the membrane in the primary damage phase (Kendall & Eston, 2002). In the secondary phase, oestrogen attenuates muscle tissue inflammation, reducing the infiltration of neutrophils to the damage site (Tiidus, Lowe, & Brown, 2013). Oestrogen also has an anabolic effect, promoting skeletal muscle repair and regeneration via enhanced satellite cell activation and proliferation, needed for the repair and regeneration of skeletal muscle (Enns & Tiidus, 2010; Velders & Diel, 2013).

Declining oestrogen levels correspond with the menopause transition beginning in the mid to late 40s (Burger, Hale, Robertson, & Dennerstein, 2007; Messier et al., 2011), with levels continuing to decline more rapidly during menopause in the late 40s to early 50s. By post-menopause oestrogen is generally at minimal concentrations in most females (Hansen, 2018). These lower concentrations of oestrogen have important implications for female endurance runners such as an increased susceptibility to muscle damage and an attenuated ability to promote skeletal muscle repair and regeneration. While low oestrogen levels likely contribute to the proposed delayed recovery in this cohort, the anabolic resistance to MPS likely exerts an effect of greater magnitude.

Anabolic resistance to muscle protein synthesis with aging

Aging is characterised by a gradual loss of skeletal muscle mass beginning in the fourth or fifth decade of life due to an imbalance between muscle protein synthesis and muscle protein breakdown, referred to as sarcopenia (Wall, Cermak, & Loon, 2014). It has been established that basal skeletal muscle net balance in older adults is not compromised (Markofski et al., 2015), and that this loss of muscle mass is primarily due to an impaired ability of skeletal muscle to increase muscle protein synthesis in response to anabolic stimuli of protein/amino acids or exercise, termed "anabolic resistance" (Breen & Phillips, 2011; Cuthbertson et al., 2005; Dickinson et al., 2013). Anabolic resistance is directly linked to sarcopenia and is also suggested to be a main factor contributing to the attenuated recovery

in masters athletes after EIMD compared to their younger counterparts (Doering, Jenkins, et al., 2016; Doering, Reaburn, et al., 2016).

Mammalian target of rapamycin complex 1 and muscle protein synthesis

A critical regulator of muscle protein synthesis is the mammalian target of rapamycin complex 1 (mTORC1) pathway (Drummond et al., 2009). The mTORC1 is activated independently in response to two main stimuli, muscle contraction and protein/amino acid feeding, through different upstream signalling pathways (Dickinson et al., 2011; Drummond et al., 2009). When protein feeding follows a bout of exercise, muscle contraction and protein feeding work synergistically to increase mTORC1 activation than either one alone (Moore et al., 2009). An increase in mTORC1 kinase activity leads to an increase in the phosphorylation of two key downstream effectors, eukaryotic initiation factor 4E binding protein (4E-BP1) and ribosomal S6 Kinase 1 (S6K1). These two effectors contribute significantly to the regulation of translation initiation and ultimately protein synthesis in skeletal muscle (Wang & Proud, 2006).

Evidence for a significant role of the mTORC1 signalling pathway in promoting MPS after endurance exercise comes from several studies in younger male athletes. These studies have found that when carbohydrate (CHO) is co-ingested with protein (P) after endurance exercise, there is an increased activation of mTORC1 and its downstream effectors (Breen et al., 2011; Cogan et al., 2018; Rowlands et al., 2014; Rowlands et al., 2011), along with MPS (Breen et al., 2011; Howarth, Moreau, Phillips, & Gibala, 2009; Rowlands et al., 2011), than CHO alone. The efficacy for carbohydrate-protein (CHO-P) over CHO alone for increasing MPS has been found with CHO-P ingested at 1.2 $g.kg^{-1}$-0.4 $g.kg^{-1}$ and 25 g-10 g and 180 g-23 g. For example, when trained male cyclists (29 ± 6 y) consumed a CHO-P beverage containing (25 g CHO-10 g P) compared to a CHO only beverage after cycling for 90 minutes, mTORC1 phosphorylation and its downstream effector S6K1 were greater at 4 hours post-exercise, and myofibrillar protein synthesis rates were ~35% higher (Breen et al., 2011).

In older (~70 y) adults however there is substantial evidence for an impaired ability to fully activate the mTORC1 pathway in response to the anabolic stimuli (Cuthbertson et al., 2005; Kumar et al., 2009). As such, it is proposed to be a key underlying mechanism for 'anabolic resistance', leading to lower rates of skeletal MPS in older populations (Dickinson et al., 2013).

Anabolic resistance to protein ingestion, exercise or both

Anabolic resistance to MPS with aging has been reported in response to protein ingestion in the rested state (Cuthbertson et al., 2005; Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2005; Wall et al., 2015), after resistance exercise in the fasted state (Fry et al., 2011; Kumar et al., 2009), and when protein is ingested in close proximity to a bout of exercise (Doering, Jenkins, et al., 2016; Durham et al., 2010; Farnfield, Breen, Carey, Garnham, & Cameron - Smith, 2012).

Anabolic resistance to protein ingestion at rest

Evidence for anabolic resistance to protein ingestion at rest comes from a study of 24 healthy elderly men (70 ± 6 y) and 24 younger (28 ± 6 y) men who consumed 10 g essential amino acids (EAA) (~20 g P). The older men had significantly lower phosphorylation of mTORC1 and its downstream effector S6K1 (Cuthbertson et al., 2005). Katsanos and colleagues (2005), investigated the differences between muscle protein accretion in older (68 ± 3 y) and younger (31 ± 2 y) men and women. With ingestion of a small bolus of ~7 g EAA (15 g P) muscle protein accretion was reduced in the older group (Katsanos et al., 2005). In a large cohort study of 35 young (22 ± 1 y) and 40 older (74 ± 1 y) men, ingesting 20 g of casein protein resulted in mixed muscle fractional synthesis rate being 16% lower over the 4 - 6 h period in the older group (Wall et al., 2015). Fractional synthesis rate (FSR) is the rate ($\%.h^{-1}$) that muscle is being synthesised. However, in contrast to these findings when a larger bolus of 15 g EAAs (~30 g P) was ingested, similar increases in mixed muscle FSR was observed in the older (~67 y) and younger (~34 y) groups of men and women (Paddon-Jones et al., 2004). These findings indicate that while the capacity to stimulate MPS with protein ingestion is kept with aging,

the skeletal muscle is less sensitive and responsive to the ingestion of lower doses of amino acids (10 g or less of EAA) (Dickinson et al., 2013). A larger dose may be needed to overcome the impaired MPS response and to synthesise muscle proteins equivalent to that in younger individuals.

Anabolic resistance to exercise in the fasted state

Anabolic resistance to MPS following exercise in the fasted state draws on findings from resistance exercise (RE) interventions due to a lack of endurance exercise-based studies. After an acute bout of resistance exercise, MPS, mTORC1 and its downstream effectors showed a reduced responsiveness in untrained elderly (67 ± 2 y) men and women (Fry et al., 2011). When an acute bout of RE was performed at intensities between 60 - 90% one repetition maximum, S6K1 and 4E-BP1 phosphorylation were reduced in older men (70 ± 5 y) compared to the younger men (24 ± 6 y), along with MPS (Kumar et al., 2009). These studies lend support to a lower MPS response to exercise in the fasted state, at least with RE and in untrained male subjects.

Anabolic resistance to endurance exercise and protein feeding

An impaired ability to increase MPS when protein was ingested in close proximity to a bout of endurance exercise was found in untrained older (67 ± 2 y) men, with MPS reduced by 40% in the older compared to younger men following 45 minutes of low intensity walking and amino acid infusion (Durham et al., 2010). Although the transferability of this study to trained masters endurance athletes is questionable, one well conducted study in male masters triathletes lends support for anabolic resistance to MPS when protein is ingested post endurance exercise (Doering, Jenkins, et al., 2016).

This study involved three consecutive days of intense endurance training. Following exercise sessions, five masters triathletes (53 ± 2 y) and six young triathletes (27 ± 2 y) consumed 20 g of whey protein isolate immediately after each exercise bout along with a daily protein intake of 1.6 g.kg^{-1}, provided as 4 doses of 0.3 g.kg^{-1} per meal, in line with sport nutrition recommendations (1.2 g.kg^{-1} per

day, 0.25 - 0.3 g.kg^{-1} per meal). Master triathletes had significantly (p=0.009) lower myofibrillar FSR compared to the younger triathletes.

Not all studies, however, have observed anabolic resistance to MPS when protein is ingested post-exercise in older adults. When seven untrained older (67 ± 2 y) and seven younger (29 ± 3 y) male and female adults ingested a protein rich meal of beef containing 90 g of protein, the synergistic response to RE and protein ingestion on MPS was the same in both groups at 3 h and 5 h post-exercise (Symons, Sheffield-Moore, Mamerow, Wolfe, & Paddon-Jones, 2011). This could be attributed to the 90 g protein dose and suggests that a higher dose of protein ingested post-exercise may be needed to counteract the anabolic resistance to MPS in the aging athlete. This highlights the importance of determining a dose to near maximally stimulate MPS post-exercise in older female endurance athletes to optimise recovery and the adaptive response.

Dose response of dietary protein to MPS at rest and after exercise

In younger resistance trained adults it is has been established that ingesting a 20 g dose of protein (~10 g EAA) near maximally stimulates the MPS anabolic response at rest (Witard et al., 2014), and after resistance exercise (Moore et al., 2008; Witard et al., 2014). In comparison to young adults, dose response studies in middle age (59 ± 2 y) and older (71 ± 5 y) male adults show that doses higher than 20 g result in greater amino acid absorption and subsequent MPS rates at rest (Moore et al., 2015; Pennings et al., 2012) and post-exercise (Robinson et al., 2012; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012).

To maximally stimulate MPS at rest and achieve equivalent rates to those of younger individuals, the findings of six previous dose-response studies were combined and a breakpoint analysis conducted (Moore et al., 2015). Protein doses were reported relative to body weight, with 0.25 g.kg^{-1} body mass maximally stimulating MPS in younger (~22 y) and 0.4 g.kg^{-1} in older (~71 y) men. Support for higher protein intakes to near maximally stimulate MPS in older adults is also

evident from dose response studies when protein is ingested post-exercise (Churchward-Venne, Holwerda, Phillips, & van Loon, 2016).

Three studies (Robinson et al., 2012; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012) have investigated the dose response of MPS to protein ingestion at rest and post-exercise. A summary is provided in Table 1. These three studies, using different sources of protein (beef, whey or soy), were comparable in their study designs. Participants were healthy but untrained men, of middle (~59 y) or older (~71 y) age. A similar unilateral knee extensor resistance exercise was used in all studies. Diet was controlled in the two days prior to the trial and until trial completion. The studies used the same amino acid tracer, and all measured myofibrillar FSR over a 240 minute time frame. When graded doses of beef containing 0, 12, 24 or 36 g of protein were ingested by 35 middle age men (59 ± 2 y) after resistance exercise, only the 36 g of protein showed a significant increase in MPS, with no significant difference found between 0, 12 or 24 g of protein in MPS (Robinson et al., 2012). The need for a greater protein dose to elevate MPS in older adults was also reported when graded intakes of whey isolate 0, 10, 20 or 40 g doses were ingested (Yang, Breen, et al., 2012). A minimum dose of 20 g was needed to increase MPS above exercise alone, with 40 g elevating MPS 32% more than 20 g post-exercise (Yang, Breen, et al., 2012). When soy protein isolate was ingested at 0, 20 or 40 g, only the 40 g dose increased MPS above exercise alone (Yang, Churchward-Venne, et al., 2012). This study also compared equivalent graded doses of soy protein to whey protein, with soy showing a reduced ability to stimulate MPS at 20 and 40 g doses (Yang, Churchward-Venne, et al., 2012). Table 1 provides a summary of dose response studies of MPS to protein ingestion.

The author of this thesis acknowledges the work done by previous authors (Churchward-Venne et al., 2016; Doering, Reaburn, et al., 2016) in producing tables to summarise dose response studies of MPS to protein ingestion. These tables were adapted for the purpose of this review to allow for ease of comparison between the different studies.

Table 1. Dose response relationship between protein ingestion and muscle protein synthesis (FSR) post resistance exercise in older adults

Study	*n*	Age (y. *Mean ± SD*)	Protein source	Protein dose (g)	Dose (g) stimulating greatest FSR	
					At rest	Post Exercise
Yang, Breen, et al. (2012)	30	70 ± 4	WI	0, 10, 20, 40	20	40
Yang, Churchward-Venne, et al. (2012)	30	71 ± 5	SI	0, 20, 40	40	40
Robinson, et al. (2013)	35	59 ± 2	Beef	0, 12, 24, 36	36	36

WI whey isolate, *SI* soy isolate, *FSR* fractional synthesis rate.

Table adapted from Churchward-Venne et al. (2016); Doering, Reaburn, et al. (2016).

Taken together these studies indicate that middle age and older men require a significantly larger amount (36-40 g) of protein post-exercise than the 20 g in younger adults to near maximally stimulate MPS, and that protein source is also an important factor in the MPS response.

Protein source

Evidence from studies in primarily male adults confirms that whey protein supports greater stimulation of MPS than soy (Phillips, Tang, & Moore, 2009; Yang, Churchward-Venne, et al., 2012) or casein protein (Burd et al., 2012; Pennings et al., 2011; Phillips et al., 2009; Yang, Churchward-Venne, et al., 2012), despite all three being considered as high quality proteins containing all the EAA needed to stimulate MPS (Phillips et al., 2009).

The greater MPS response with whey protein is attributed to its faster digestion and absorption kinetics and amino acid composition, specifically its higher leucine content (Phillips, 2016). Leucine is an EAA that directly activates the mTORC1

signalling pathway and initiates an increase in MPS (Phillips, 2016). In elderly men and women a reduced sensitivity of MPS to the anabolic effects of leucine has been demonstrated (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006; Yang, Breen, et al., 2012), with a higher leucine content (~3.0 g) required to trigger a rise in MPS to a level equivalent to that seen in younger individuals (~1.85 g) (Devries et al., 2018; Katsanos et al., 2006). The capacity to near maximally stimulate MPS with ~40 g of protein is likely linked to the increased activation of leucine with this higher dose (Churchward-Venne et al., 2016). While protein dose and source are key factors in maximising MPS in older athletes, timing of intake may also modulate the MPS response.

Protein timing

Consuming protein in the immediate post-exercise recovery period is recommended for enhancing MPS and net protein balance for repair and remodelling of muscle fibres, and to promote recovery after endurance exercise (Moore, Camera, Areta, & Hawley, 2014). However, this period for protein ingestion may be more important to masters endurance athletes, as delaying protein ingestion by as little as two hours was found to attenuate accretion of muscle protein in older men after resistance training (Esmarck et al., 2001). The MPS response to protein dose, source and timing have been investigated primarily in older males, however, there is evidence to suggest sexual dimorphism in the MPS response (Smith & Mittendorfer, 2016; Smith Gordon et al., 2012).

The effects of protein and exercise on MPS in women

The impact of protein and exercise on MPS in women has been less well studied, however, a reduced sensitivity to the anabolic stimuli of protein and its leucine content (Devries et al., 2018; Katsanos et al., 2006), and to exercise (Burd, Tang, Moore, & Phillips, 2009), has been reported in females. This reduced sensitivity compared to their male counterparts is suggested to be linked to the decline in female hormones, particularly oestrogen concentrations (Hansen, 2018; Smith &

Mittendorfer, 2016). Considering women around menopausal age (~48 y) and older are also potentially more susceptible to muscle damage, optimising protein dose, source and timing may be of even greater importance to maximise the MPS response post-exercise.

Summary

In summary, to near maximally stimulate and achieve similar rates of MPS as younger populations, the available evidence suggests that post resistance exercise, untrained middle-age and older (~59 y plus) males, need to ingest ~40 g of a rapidly digested protein with a high leucine content such as whey isolate. To date there is a lack of data available to make clear recommendations on the amount of ingested protein needed to maximally stimulate MPS after endurance exercise in older female athletes. However, considering the distinct aging effects on skeletal muscle of older females (~48 y and older) it would be reasonable to suggest this higher protein intake post-exercise would be advantageous to facilitate the optimal repair and remodelling of skeletal muscle, and enhance recovery in older female endurance runners after EIMD.

Section 3 - Acute protein supplementation post EIMD to improve recovery

The potential benefits of protein supplementation to reduce the negative effects of EIMD and improve recovery has been extensively researched in younger (<40 y) adults, with females underrepresented in these studies. To date, only one study has been conducted in male masters endurance athletes (Doering, Reaburn, Borges, Cox, & Jenkins, 2017). Evidence from studies in younger populations for positive effects of protein ingestion before, during and/or after a single or multiple exercise session is equivocal (Cockburn, 2010; Pasiakos, Lieberman, & McLellan, 2014), and likely due to the differences in study designs and methodologies.

To reduce this heterogeneity, the following section is limited to studies investigating the effects of acute ingestion (within a 2 h time frame post-exercise) of protein, or carbohydrate and protein (CHO-P) following a single bout of muscle-

damaging exercise. Excluded further from this review of studies were those including antioxidants in addition to protein or CHO-P supplement post-exercise (Ives et al., 2017), exercise protocols that were likely to result in metabolic rather than mechanical muscle damage (Lunn et al., 2012; Saunders, Kane, & Kent Todd, 2004), and the protein supplement provided in several boluses post-exercise over an extended period (Buckley et al., 2010).

Six studies are reviewed, with five demonstrating an attenuation in one or more markers of EIMD when protein or CHO-P was supplemented post-exercise (Cockburn, Hayes, French, Stevenson, & St Clair Gibson, 2008; Etheridge, Philp, & Watt, 2008; Rankin, Landy, Stevenson, & Cockburn, 2018; Rankin, Stevenson, & Cockburn, 2015; Saunders, Luden, Dewitt, Gross, & Rios, 2018). One study reported no beneficial effects (Green, Corona, Doyle, & Ingalls, 2008). A summary of these studies can be found in Table 2.

Markers used to asses muscle damage to determine the effectiveness of protein supplementation post-exercise varied between studies. All but one study (Saunders et al., 2018) measured blood creatine kinase (CK), muscle soreness, and muscle function tests of peak isometric torque (PIMT) and/or peak isokinetic torque (PIT).

Of these indirect markers, muscle function (a measure of the capacity of a muscle to generate force) is regarded as the most reliable and valid for quantifying the extent of muscle damage, as it best reflects muscle myofibrillar status (Owens et al., 2018; Paulsen et al., 2012). Five studies included one or more markers of muscle function, with four reporting a beneficial effect of CHO-P supplementation on improving muscle force generating capacity over a recovery period of 24-72 h (Cockburn et al., 2008; Etheridge et al., 2008; Rankin et al., 2018; Rankin et al., 2015).

In addition, the above-mentioned studies also included measurements for CK and muscle soreness. CK levels increased post-exercise in these studies for all

treatment groups. In two studies CK levels were reduced with the protein supplement at either 48 h (Cockburn et al., 2008) or 72 h (Rankin et al., 2015) post-exercise compared to 24 h post-exercise levels. Muscle soreness increased post-exercise but was attenuated with the protein supplement compared to the isocaloric CHO supplements (Rankin et al., 2015). It should be recognised however that CK and muscle soreness do not accurately reflect the extent of muscle damage (Warren, Lowe, & Armstrong, 1999) and weakly correlate with each other (Paulsen et al., 2012). They are not regarded as being adequately valid to be used on their own, but provide complimentary or extra information to muscle function measurements (Owens et al., 2018).

Beneficial effects of a CHO-P supplement compared to a near isocaloric CHO supplement following a marathon run were reported (Saunders et al., 2018). This study measured CK, and perceptual measures of muscle soreness, physical fatigue and mental energy. CHO-P attenuated muscle soreness and physical fatigue over 72 h recovery period. However, no measures for muscle function were included and sample size was very small (4/group), and as such results should be considered with caution.

Confounding variables were not always controlled for in these studies which makes accurate interpretation difficult for determining the effectiveness of the protein supplement. Supplement treatments used in individual studies were not all isocaloric, with one study comparing 40 g of EAA (100 g P) to a non-isocaloric placebo (Etheridge et al., 2008), while another had differing quantities of CHO in the treatment groups (Cockburn et al., 2008). Several studies did not control for diet before or during the trial/s. For example, no attempt for dietary control was reported in two studies (Green et al., 2008; Saunders et al., 2018), while Cockburn and colleagues only requested participants to keep normal dietary habits throughout the intervention (Cockburn et al., 2008). Only two studies took measures to control for diet, where participants kept food records for the 24 h prior and 72 h post-exercise. Diets were analysed for energy and macronutrient

content, with no significant difference between groups reported (Rankin et al., 2018; Rankin et al., 2015).

An absence of dietary standardisation may be one plausible reason for Green and colleagues who observed no beneficial effect from CHO-P supplementation as opposed to carbohydrate (Green et al., 2008). These reported results oppose another (Etheridge et al., 2008) given they used the same exercise protocol of a 30 minute downhill running (DHR), muscle damage markers and measurement time points. While Etheridge and colleagues used a cross over design and had participants replicate their dietary intake for the 24 h before and 72 h post-exercise for the two trials and did not allow the consumption of additional meals earlier than 3 h after supplement ingestion, Green and colleagues implemented no dietary control at all.

Two recent studies are comparable in their design and methodology (Rankin et al., 2018; Rankin et al., 2015). Both used a parallel group design, participants were team sport athletes of a similar age (~22 y), with female only participants in one study (Rankin et al., 2018), and separate groups of male and females in the other (Rankin et al., 2015). The supplement treatments were taken immediately post-exercise and were identical. The CHO placebo was isocaloric to the CH0-P supplement (milk). The protein supplement provided 17 g of protein (~0.27 g.kg^{-1} in females), which is near to the amount known to maximally stimulate MPS. Diet was controlled for in the 24 h prior to testing and during the 72 h recovery period. Results for the females in both studies showed protein supplementation improved muscle function. Peak isokinetic torque showed a likely/very likely beneficial effect from baseline to 24, 48 and 72 h (Rankin et al., 2015), and a very likely beneficial effect for PIT at 72 h and CMJ at 72 h (Rankin et al., 2018).

This review of literature suggests acute protein intake following a bout of muscle-damaging exercise has the potential to promote recovery of muscle function and enhance recovery over a 24-72 h period. There is more conclusive evidence for its potential benefit when markers of muscle function are included, the nutritional

treatments are isocaloric, diet is controlled for, and protein is provided immediately post-exercise, in amounts known to near maximally stimulate MPS in younger adults.

While these studies assessed muscle recovery over several days, the only study of masters athletes (Table 2) supports a positive effect of protein supplementation on recovery of muscle function over a short time frame of 8 h (Doering et al., 2017). The aim of this crossover study was to establish if three repeated boluses of a higher protein intakes (0.6 g.kg^{-1}) compared to lower intakes (0.3 g.kg^{-1}) after muscle-damaging exercise could improve recovery in the same day. A 30 minute DHR was used to induce muscle damage in eight well trained male masters triathletes, followed by an 8 h recovery period. The higher intakes of whey isolate protein attenuated reductions in afternoon PIMT to a greater degree, with a moderate beneficial effect compared to the lower protein. While this reduction in PIMT did not result in a beneficial effect on the time trial performance it does support the need for larger amounts of high-quality protein in older athletes, due to the changes in PIMT.

Comparison between the study in masters and those in younger athletes is problematic due to the difference in age between the two groups and study interventions i.e. several boluses of protein over the recovery period compared to protein ingestion in the immediate post-exercise period, and differences in the recovery time period under investigation. While these findings cannot be generalised to older female endurance athletes, they do lend support for protein supplementation post-exercise to potentially attenuate decrements in muscle function and improve recovery in older female endurance runners.

Section 4 - Limitations and future research

A clear gap in literature exists on the nutritional practises of older female endurance runners and it cannot be established at this stage if the practises of these athletes are contributing to the attenuated recovery. There is the need for

comprehensive and well conducted nutrition assessments to be carried out in this cohort. This information is needed to establish current practise, and to provide appropriate and practical recommendations to optimise recovery.

Research to date to determine the amount of protein ingested to maximally stimulate MPS after exercise has been primarily conducted in older untrained male subjects using resistance training protocols. There is a lack of data available to make any clear recommendations for older female endurance athletes on the amount of protein needed to maximally stimulate skeletal MPS. Research in this area is warranted given the increasing number of female athletes competing in endurance events, and the importance of dietary protein to facilitate the repair and remodelling of skeletal muscle leading to training induced adaptations and ultimately performance improvements.

The studies reviewed for the effects of acute protein ingestion following EIMD in younger athletes highlighted several limitations in study design and methodology which damaged the internal validity of the results. These included not controlling for confounding variables and not including a valid marker for quantifying muscle damage. To ensure the correct analysis of results and reporting of findings, future investigations would benefit from stricter rigour paid to study design and methodologies.

Research is in its infancy investigating the potential beneficial effects of protein supplementation to improve recovery after EIMD in older athletes. The only study in older male endurance athletes, while reporting a potential benefit in recovery of muscle function within the same day, does not allow for any conclusive recommendations to be made for this age group of males or for older females. Given the negative impact slower recovery can have on endurance training, adaptations and performance, this is an area where extensive research is warranted in both males and females to determine the efficacy of a higher protein supplementation dose on both acute and chronic muscle recovery.

Section 5 - Conclusions

Endurance running is known to induce muscle damage. While the magnitude of damage is similar between male masters and younger athletes, masters athletes appear to take longer to recover. A slower recovery impacts on the quality of training sessions, limits adaptations and ultimately performance. Identifying key factors for this delayed recovery and means by which they can be modified to improve recovery time is therefore of significance.

The older female endurance runner is an understudied yet distinct population, whose slower recovery is due to the aging effects on muscle; specifically, that of reduced oestrogen concentrations, known to augment muscle damage and attenuate the repair process, and an anabolic resistance to muscle protein synthesis.

Anabolic resistance with aging reduces the sensitivity of skeletal muscle to increase MPS in response to the stimuli of protein or exercise. This impairment can be compensated for by ingesting a higher dose of protein than what is currently recommended (~20 g). The available evidence suggests that ingesting ~40 g of a quickly digested, high leucine-content protein such as whey, in the immediate post-exercise period can near maximally stimulate MPS in older untrained men after resistance exercise.

To date there is a lack of data available to make clear recommendations on the amount of protein need to maximally stimulate MPS after endurance exercise in older female athletes. However, given that older women (~48 y plus) have been shown to have a reduced sensitivity to the anabolic stimuli of protein and its leucine content compared to their male counterparts, and an increased susceptibility to muscle damage, it would be reasonable to propose that ingesting ~40 g of whey protein in the immediate post-exercise period would be beneficial for optimising the repair and remodelling of skeletal muscle and hence recovery in older female endurance runners following EIMD.

Studies to date investigating acute protein supplementation to reduce the negative effects of EIMD and improve recovery have been limited to younger populations. Findings from these interventions suggest acute protein supplementation to be potentially beneficial in attenuating decrements in muscle function and enhancing recovery over a 24-72 h period. Evidence was more conclusive when stricter rigour was paid to study design and methodology. The one study with male masters triathletes found that higher than recommended repeated boluses of whey protein post-exercise improved recovery of muscle function over an 8 h period, lending support to the importance of protein source and the need for a larger dose in masters athletes.

The beneficial effects of protein supplementation on the recovery of muscle function reported in studies in younger populations, specifically female athletes, and the one study in male masters athletes cannot be extrapolated to the older female endurance runner. While a higher dose of whey protein proved beneficial in attenuating decrements in muscle function in male masters triathletes, there is no evidence to date for the efficacy of a higher (~40 g) acute protein dose post muscle-damaging exercise to improve recovery of muscle function in older female endurance runners.

Table 2. Summary of studies reporting effects of acute protein or protein-carbohydrate supplementation post-exercise on markers of exercise induced muscle damage following an acute bout of exercise

Reference	Participants	Study Design	Exercise Protocol	Main Muscle Damage Markers and Measurement Times	Post-Exercise Supplement Protocol	Positive Results from Protein Supplementation
Cockburn et al., (2008)	24 M (21 ± 3y) 4 groups Team sport athletes	Parallel single blind, placebo controlled	Eccentric contractions knee flexors 6 sets x 10 reps/ leg 1.05°/s	CK, Mb, muscle soreness, PIT knee extensors/quad Pre, 24, 48 h post	Immediately, 2 h, 500 ml Placebo: water CHO 64g CM: CHO-P 118 g/33 g Milk: CHO-P 49 g/34 g	CM= Milk CM and Milk > CHO and PLA for leg PIT, TW 48 h CM = Milk CM and Milk < CHO and PLA for CK, Mb 48 h
Etheridge et al., (2008)	9 M (21 ± 1y) Recreationally active	Crossover double blind 28 days between trials	Downhill running 30 min at 10% decline 75% predicted HR_{max}	CK, muscle soreness, IMST quads, PPO (cycle erg) Pre, 24,48 72 h post	Immediately 1000 ml PLA flavoured water P 100g (40 g EAA)	P > PLA for IMST quads and PPO 48 h
Green, et al., (2008)	18 F (24.6 ± 3.3y) 3 groups Recreationally active,	Parallel double blind, placebo controlled. ,	Downhill running 30 min intermittent run 12% grade at 8 mph	CK, muscle soreness, IMST quads Pre, immediately post, 24 48, 72 h post	0, 30 min, 60 min Placebo non caloric CHO Hr 1: 1.2 g.kg Hr 2: 0.6 g.kg CHO- P Hr 1: 1.2 g.kg /0.3 g.kg Hr 2: 0.6 g.kg/0.15 g.kg	No beneficial effect IMST quads returned to base line values by day 3 in all groups
Rankin et al., (2015)	16 M (23.7 ± 3.4y) 16 F (21.9 ± 2.1y) Team sport	Parallel 4 groups (1 female 1 male group for each supplement	Eccentric contractions knee flexors 6 sets x 10 reps, 60 and 180°/s	CK, muscle soreness, PIT knee flexion, CMJ, 20m sprint performance pre, 24, 47 and 72 h post	Immediately post, 500 ml Isocaloric Energy 910 kJ CHO 53.6 g Milk: CHO-P-FAT 25.5 g/17 g/5 g	Both genders: Milk < muscle soreness, CK, Females: Milk > CHO PIT 24, 48 and 72 h, 20 m sprint performance 72 h

CHO carbohydrate, **CK** creatine kinase, **CM** chocolate milk, **CMJ** counter movement jump, **CRP** C reactive protein, **F** female, **IKST** isokinetic strength, **IMST** isometric strength, **LDH** lactate dehydrogenase, **M** male, **Mb** myoglobin, **PIMT** peak isometric torque, **PIT** peak isokinetic torque, **PLA** placebo, **PPO** peak power output **P** protein, **TTP** time trial performance, **TTE** time to exhaustion, **TW** total work, **WPI** whey protein isolate, < indicates lower values, **>** indicates greater values.

Table 2. Summary of studies reporting effects of acute protein or protein-carbohydrate supplementation post-exercise on markers of exercise induced muscle damage following an acute bout of exercise (cond't)

Rankin et al., (2018)	18 F (21.6 ± 3.4y) Team sport athletes	Parallel 2 groups	Sprints 15 x 20 m max 10 plyometric jumps	CK, CRP, muscle soreness, CMJ, PIT knee flexion, sprint performance pre, 24, 47 and 72 h post	Immediately post, 500 ml Isocaloric Energy 910 kJ CHO 53.6g Milk: CHO-P-FAT 25.5g/17g/5g	Milk > CHO for muscle function: PIT, CMJ 24,48 and 72 h
Saunders et al., (2018)	4 F, 4 M (18-26y) 2 groups mix Novice runners	Parallel double blind	Marathon run (42km)	CK, muscle soreness, physical fatigue, mental energy Pre, 24, 72 h post	Food bags similar caloric value Immediately post-exercise CHO 111 g ± 31 g CHO-P 57 g ± 9 g/ 28 ± 9 g	CHO-P < CHO for muscle soreness, physical fatigue and > for mental energy 72 h
Doering et al., (2017)	8 M (52.1 ±2.1y) Trained triathletes	Crossover double blind 7 days between trials.	Downhill running 30 min at 10% decline 70% VO_{2max}	TTP Mb, muscle soreness, fatigue, motivation, PIMT, Pre, immediately post (Mb, recovery perceptions) 8 h (all),	Immediately post, 2 and 4 h High P: CHO-P 1 g.kg/0.6 g.kg Moderate P: CHO-P 1.3 g.kg/0.3 g.kg WPI for both	High P > Mod P for PIMT knee extensors at 8 h post High P > Mod P for perceptual measures of fatigue

CHO carbohydrate, **CK** creatine kinase, **CM** chocolate milk, **CMJ** counter movement jump, **CRP** C reactive protein, **F** female, **IKST** isokinetic strength, **IMST** isometric strength, **LDH** lactate dehydrogenase, **M** male, **Mb** myoglobin, **PIMT** peak isometric torque, **PIT** peak isokinetic torque, **PLA** placebo, **PPO** peak power output **P** protein, **TTP** time trial performance, **TTE** time to exhaustion, **TW** total work, **WPI** whey protein isolate, < indicates lower values, > indicates greater values.

Chapter 2:

Effects of a higher acute protein intake post muscle-damaging exercise on muscle recovery in older female endurance runners

Abstract

Masters athletes appear to take longer to recover after muscle-damaging exercise than their younger counterparts. The purpose of this study was to compare the effect of a higher (40 g) acute protein intake versus the recommended intake (20 g) immediately following muscle-damaging exercise in older female endurance runners on the recovery of muscle function, perceived muscle soreness, and flexibility within a 24 hour (h) period. Six well trained female endurance runners, 48 years of age and older (mean ± SD: age; 52 ± 5.6 y, body mass; 61.4 ± 6.2 kg) completed three trials separated by seven days, in a randomised Latin square, cross-over placebo-controlled study, with participants blinded to treatments. Prior to the first trial baseline measures for isometric maximal voluntary contraction of the knee extensors, peak countermovement jump height (CMJ), muscle soreness and hamstring flexibility were taken. These measures were retested at 3 h and 24 h post run. Trials consisted of a morning (between 7 am and 10.30 am) 30 minute downhill run (-10% grade, 80% age predicted HR_{max}) to induce eccentric muscle damage, followed by the consumption of a higher protein intake (P40) (protein-carbohydrate 40 g-30 g, 1190 kJ), a recommended protein intake (P20) (protein-carbohydrate 20 g-50 g, 1190 kJ) or a placebo (PLA) (carbohydrate 70 g, 1190 kJ). The P40 had a likely small beneficial effect compared to the PLA (ES = 0.24 ± 0.5) and P20 (ES = 0.25 ± 0.29) for improving maximum voluntary contraction from baseline to 24 h. The P40 had a likely small beneficial effect for improving countermovement jump height compared to P20 from baseline to 3 h (ES = 2.9 ± 4.9) and to 24 h compared to the PLA (ES = 0.32 ± 0.39) and P20 (ES = 0.27 ± 0.19). P40 had an unclear effect on muscle soreness and flexibility. In conclusion the acute ingestion of 40 g of whey protein isolate, double the recommended, post-exercise intake, improved recovery of muscle function within 24 h. This intake may be beneficial in enabling these athletes to better maintain the quality of training sessions, promoting training induced adaptations and improving performance, and as such warrants a larger study.

Introduction

Endurance running has been shown to induce muscle damage, particularly the eccentric loading of downhill running (DHR) (Brisswalter & Nosaka, 2013). Masters (≥ 45 years) male endurance athletes display a similar magnitude of muscle damage as their younger counterparts after muscle-damaging exercise but appear to take longer to adapt to and recover from the muscle damage (Brisswalter & Nosaka, 2013; Easthope et al., 2010). A slower rate of recovery can negatively impact on the quality of subsequent bouts of training, limiting adaptations and ultimately performance (Barnett, 2006; Bishop et al., 2008; Fell & Williams, 2008). Identifying factors for this delayed recovery and means by which they can be modified to improve recovery time is therefore of utmost importance.

One reason for attenuated recovery may be the age-associated impairment of protein synthesis in response to the anabolic stimuli of dietary protein/amino acids and exercise, termed 'anabolic resistance' (Cuthbertson et al., 2005; Dickinson et al., 2013). While it is well known that adequate dietary protein intake in the post-exercise period is vital for improving protein balance, and hence the repair and remodelling of skeletal muscle, research indicates that untrained older adults (Yang, Churchward-Venne, et al., 2012) and masters endurance athletes (Doering, Jenkins, et al., 2016) have a reduced sensitivity to the protein dose (20 g) associated with maximal stimulation of post-exercise muscle protein synthesis (MPS) signalling in younger adults (~22 y) (Moore et al., 2008; Witard et al., 2014).

This anabolic resistance to protein ingestion in older adults can be offset by ingesting a higher dose of protein; 36-40 g protein, post resistance exercise (RE), inducing a near maximal stimulation of MPS signalling in middle age and older untrained males (Robinson et al., 2012; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012). Post-exercise muscle anabolism may also be modulated by the source and timing of ingestion, with a quickly digested source of protein, with a high leucine content such as whey (Phillips, 2016), and ingestion

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in the immediate (0-1 h) post-exercise period (Esmarck et al., 2001; Moore et al., 2014) maximising the MPS signalling response. These findings suggest that the acute ingestion of ~40 g of whey protein immediately following muscle-damaging exercise should facilitate the optimal repair and remodelling of skeletal muscle and enhance recovery time in male masters athletes.

Studies to date investigating acute protein supplementation to reduce the negative effects of exercise induced muscle damage (EIMD) and improve recovery are limited to younger populations (Cockburn et al., 2008; Etheridge et al., 2008; Green et al., 2008; Rankin et al., 2018; Rankin et al., 2015; Saunders et al., 2018). Although these findings cannot be generalised to older populations, the results from five of the six studies provide evidence for a potential benefit for attenuating decrements in muscle function and enhancing recovery over 24-72 h. The one study in masters endurance athletes (male masters triathletes) reported that higher than recommended repeated boluses (3 feedings of 0.6 $g.kg^{-1}.bolus^{-1}$ at 2 h intervals, commencing immediately post-exercise) of whey protein isolate (WPI) post-exercise improved recovery of muscle function over an 8 h period (Doering et al., 2017). While this was not an acute supplementation protocol, and in male master triathletes, it lends support to the importance of protein source (whey) and the need for a larger dose in masters athletes than their younger counterparts.

Currently, there is limited data to identify the effects of acute protein supplementation on recovery from daily bouts of training in older (≥48 y) female endurance runners, despite increasing participation in endurance running events in this population (Knechtle et al., 2012; Lepers & Stapley, 2016). Older female endurance runners are an understudied yet distinct population whose reduced recovery may be further influenced by the decline in oestrogen levels associated with the onset of menopause (mid to late 40s), specifically that of estradiol-17β (Hansen, 2018; Smith & Mittendorfer, 2016). Lower oestrogen levels have been linked to a reduced sensitivity to the anabolic stimuli of protein (Devries et al.,

2018; Katsanos et al., 2006), an augmentation in muscle damage and attenuation in the repair process (Enns & Tiidus, 2010).

The primary aim of the current study was to compare the effect of a higher (40 g) acute protein intake versus a recommended intake for endurance athletes (20 g) immediately following muscle-damaging exercise on recovery of muscle function, perceived muscle soreness and flexibility within a 24 h recovery period in older female endurance runners. It was hypothesised that the higher acute protein intake post muscle-damaging exercise would improve recovery and reduce decrements in muscle function, flexibility and soreness within a 24 h recovery period in older ($\geq$ 48 y) female endurance runners.

Inappropriate recovery practises in this cohort may also contribute to the compromised recovery, however, to date no nutritional assessments have been conducted to support this. Therefore, the secondary aim of this study, was to assess the typical dietary practises of this cohort post training, specifically protein intake during the immediate post-exercise period, as compared to current sport nutrition guidelines and the proposed higher dose of 40 g.

Methods

Participants

Six well-trained, female endurance runners 48 y of age and older (52 $\pm$ 5.6 y) participated in the study. Runners were recruited through running groups, running events and by word of mouth from the Bay of Plenty Region, New Zealand. Participant characteristics can be viewed in Table 3. Exclusion criteria were age less than 48 y, training less than 5 h a week or for a race distance less than 10 kilometres in competition, on hormone replacement therapy, cardiovascular or kidney diseases, diabetes or joint injuries of the lower body, and unable to consume whey protein. The study was approved by The University of Waikato

Human Research Ethics Committee. All subjects provided voluntary written informed consent prior to participation in the study. The study took place from November 2018 to February 2019.

Table 3. Participant characteristics

Characteristics	Mean ± SD
Age (y)	51.5 ± 5.6
Height (cm)	167 ± 4.2
Body mass (kg)	61.4 ± 6.2
Body mass index	22 ± 1.9
Years in endurance running	11.3 ± 9.5
Training volume (h.week^{-1})	7.3 ± 4.3

Study design

Participants completed a familiarisation session, followed 6-8 days later by the first of three trials that were conducted exactly one week apart at the same time of day. The study was a Latin square, cross-over placebo-controlled design, with participants blinded to supplement treatments. Randomisation to supplement order was computer generated using publicly available software (http://www.randomization.com). Preliminary testing and trials took place in the laboratory at the University of Waikato Adams Centre for High Performance, Mount Maunganui, Tauranga, New Zealand.

Preliminary testing

Participants completed a physical activity readiness questionnaire (PAR-Q & You) and a training questionnaire. Anthropometric measurements for body mass (kg), measured to the nearest 100 g (portable digital scales: Seca, Germany), and height

(cm), measured to the nearest 0.1 cm (portable stadiometer: Seca, Germany) were taken. Following this, instructions for filling in food records and training logs were given. A familiarisation session was then completed for tests to measure muscle recovery: an isometric squat for perceived muscle soreness, sit and reach test for hamstring flexibility, a countermovement squat jump (CMJ), and an isometric maximal voluntary contraction (MVC) of the knee extensors. A treadmill (Steelflex PT 10 Commercial Treadmill, UK) test to establish downhill running speed at 80% maximum heart rate (HR_{max}) was then conducted.

Treadmill testing commenced with a 5-minute warm up at a self-selected pace on a level gradient. Following the warm up, the treadmill gradient was set at a 10% decline and subjects ran for 30 minutes at a target average heart rate (heart rate monitor: H10 Polar Electro, Finland) of 80% predicted HR_{max}. Heart rate max was calculated as: HRmax = 208 – (0.7 x age) (Tanaka, Monahan, & Seals, 2001). This intensity corresponds to ~70% VO_{2max} and is consistent with previous research that uses DHR to induce muscle damage (Braun & Dutto, 2003; Etheridge et al., 2008). Treadmill speed, heart rate and rate of perceived exertion (RPE) (Borg, 1982) were monitored continuously and recorded every 5 minutes with treadmill speed altered accordingly at these time points to maintain heart rate as close as possible to 80% HR_{max} (heart rate was kept within ± 5 beats/minute of participants 80% HR_{max}) and a RPE between 12-14.

Participants were provided with detailed verbal and written instructions along with templates for food recording. Participants were asked to record the date, time the meal was eaten, meal type (e.g. breakfast), detailed description of the food, including brand if applicable, cooking method, and portion size. Portions were estimated from either standard household measures, food packaging labels (volume or weight), and diagrams provided to participants. Recipes or amounts for combination foods or mixed dishes were also recorded. Participants were asked to include any protein supplements (Phillips, 2016) they took in addition to

their normal dietary intakes. Participants were asked to maintain their normal eating habits throughout the recording periods.

Instructions for filling in the training log along with a template was provided to participants. Participants recorded the date, start and finish time of training, description of training and intensity as either light (little effort, can carry on full conversation), moderate (moderate effort, noticeably accelerates the heart rate), vigorous (large amount of effort, rapid breathing, substantial increase in heart rate).

The familiarisation session served to acclimate subjects to exercise protocols with the goal of minimising any possible order effects in the subsequent trials and systematic errors, specifically from the repeated bout effect (RBE) (Hyldahl et al., 2017) on muscle damage, and the learning effect (Green, Parro, & Gabriel, 2014; Pekünlü & Özsu, 2014) from performing MVC of knee extensors and CMJ.

Trial overview

A schematic overview of the experimental trials can be found in Figure 2. Participants reported to the lab between 7 a.m. and 10.30 a.m. for each trial. Baseline measures for the dependent variables were taken prior to the DHR in the following order: perceived muscle soreness of the lower body, hamstring flexibility, peak isometric strength of the knee extensors and maximum countermovement jump height. Following baseline testing participants completed a 5-minute warm up at a self-selected pace on a level treadmill before completing a 30 minute DHR protocol to induce muscle damage. Immediately post run (0 h) the same measures for dependant variables were taken, followed by participants consuming one of three experimental beverages. Only water could be consumed in the 3 h period between supplement ingestion and the 3 h post-test for dependent variables. Measures for dependant variables were taken again at 3 h and 24 h post run. A 10-minute standardised warm up was conducted before

baseline testing and at 3 h and 24 h post run testing for dependant variables to avoid injuries and prepare the muscles for testing.

Participants refrained from exercise training, caffeine, non-steroid anti-inflammatory drugs (NSAID) and alcohol for 24 h before trials. For the 24 h period post DHR, participants were asked to avoid training, NSAID, supplements (protein, vitamins or minerals) and the use of other recovery modes (e.g. compression garments, massage).

DHR

Pre   O h   3 h   24 h

Supplement

Measurement of
dependent variables

Dietary   48h pre   24hpost
standardisation
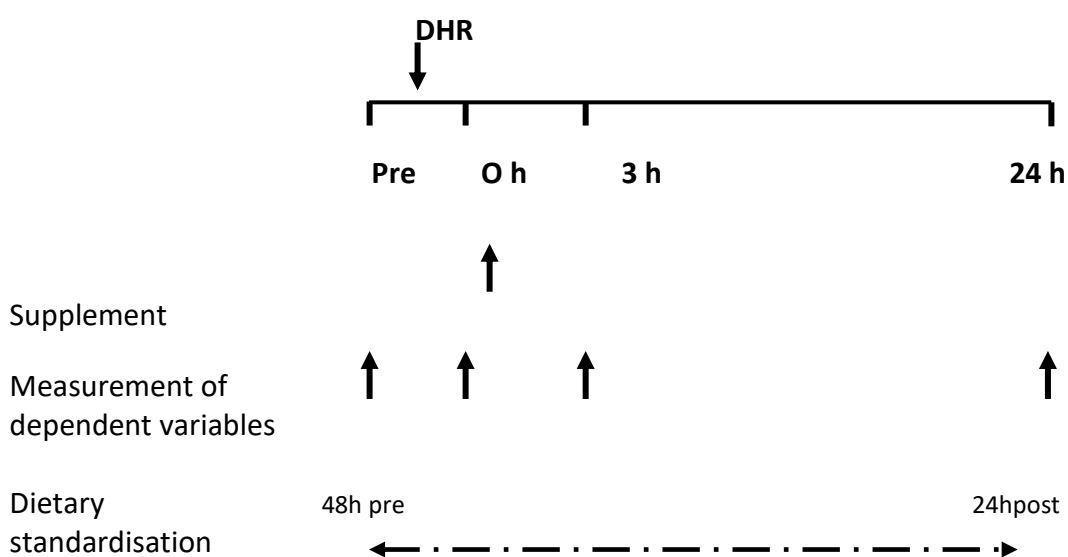
Figure 2. Schematic of testing protocol illustrating the time point when the supplement was consumed and when the measures for dependent variables were taken.

Downhill run protocol

Participants completed a 5-minute warm up at self-selected pace, on a level gradient followed by a 30 minute DHR at 10% decline, at an intensity corresponding to as close as possible to 80% HR_{max} to induce muscle damage. Downhill running speeds at 80% HR_{max} established in preliminary testing were used in trial one, however if required adjustments to running speed were made accordingly during trial one (at 5-minute intervals). To ensure standardisation

across trials, the running speeds and corresponding times in trial one was replicated for trial two and three. Heart rate, RPE, and running speed were monitored and recorded every 5 minutes in all trials.

Dietary and exercise standardisation

Participants completed a dietary food record for 48 h prior to each trial and throughout the trial period until the 24 h post-test. For dietary standardisation (Jeacocke & Burke, 2010) across trials, participants were asked to replicate these diets for the subsequent two trials. Participants noted any changes on their food record they made during these periods. All records were checked by the researcher before Trial 2 and 3 for compliance and to ensure any dietary changes that had been made were of an energy and macronutrient content similar to the original food record. Food records were analysed for energy and macronutrient content using dietary analysis software FoodWorks 9 (Xyris Software, Brisbane, Australia).

For exercise standardisation a training log was kept for a week before the first trial and participants were asked to keep to a similar training plan (i.e. no new blocks of training started) during the entire study period.

Supplementation protocol

Immediately following measurements for dependent variables post run (0 h), participants consumed one of three recovery beverages. Energy and macronutrient composition of the recovery beverages are shown in Table 4. All beverages were isovolumetric (250 ml), isocaloric (1190 kJ) and were matched for taste, colour and consistency. Beverages were consumed within 3 minutes of being provided**.** All beverages consisted of commercially available ingredients that were easy to prepare as a smoothie-based drink.

Carbohydrate (CHO) placebo CHO 70 g (PLA). Supplement composition: 25 g glucose (King), 10 g Nesquik banana flavour (Nestle) and 175 g banana. Xanthan

gum (0.25 g) was used to thicken the beverage and 1.2 ml vanilla essence used for flavouring.

Protein-Carbohydrate 20 g-50 g (P20). Supplement composition: 22.8 g of WPI vanilla flavour protein powder containing 2.4 g leucine (Balance), 10 g of glucose powder (King), 10 g Nesquik banana flavour (Nestle) and 150 g of banana. Twenty grams of protein is in line with current recommendations to support post-exercise recovery (Beelen, Burke, Gibala, & Van Loon, 2010).

Protein-Carbohydrate 40 g-30 g (P40). Supplement composition was 45.6 g of WPI vanilla flavour protein powder containing 4.8 g leucine (Balance), 10 g Nesquik, banana flavour (Nestle), 92 g banana. Forty grams of protein is the suggested amount to near maximally stimulate MPS in older adults after resistance training (Yang, Breen, et al., 2012) and 30 grams of CHO is sufficient to maximise hyperinsulinemia and to maximise the inhibition of protein breakdown (Glynn et al., 2010).

Table 4. Nutritional content of supplements

Supplement	Energy (kJ)	CHO (g)	Protein (g)	Leucine (g)	Fat (g)
Placebo	1190	70	0	0	0
Protein 20 g	1190	50	20	2.4	Trace
Protein 40 g	1190	30	40	4.8	Trace

To determine blinding of participants to the supplements, they were given a questionnaire after the final trial to determine which supplement condition they perceived to have been assigned to for each trial and to assess how well-matched supplements were for flavour, texture, colour and palatability.

Muscle recovery testing procedures

Knee Extensor Isometric Strength

Peak isometric strength of the knee extensors was assessed using a digital force gauge (MARK-10 Series 3 Force Gauge, USA). Participants sat on a custom-made bench to which the strain gauge was attached. The participants' dominant leg was firmly strapped to the bench across the mid-thigh. Immediately above the malleoli on the dominant leg an ankle cuff was attached, with a chain connecting the cuff to the strain gauge. Maximal voluntary contraction of the knee extensors was performed at a knee joint angle of 90°. The angle was assessed before each repetition with a goniometer, at the lateral condyle of the femur. Two submaximal contractions were performed prior to the first of the three MVC tests. MVC were performed for 3 seconds, with 60 seconds rest between tests. A 3 second countdown preceded tests and strong verbal encouragement was given to the participants during each attempt. MVC were recorded (Newtons) with the maximum MVC value used for data analysis.

Countermovement Jump Height

Counter movement jump height (measured by flight time) was determined using MyJump 2 app on an iPhone 6s (Apple Inc, USA), a reliable and valid tool for evaluating vertical jump performance (Driller, Tavares, McMaster, & O'donnell, 2017; Stanton, Wintour, & Kean, 2017). To record the jump, the researcher lay prone on the ground facing the participant (frontal plane) at 1.5 m from the participant and zoomed in on the participant's feet. Take off phase was the first frame in which both feet were off the ground and landing phase was the first frame in which one foot was touching the ground (Driller et al., 2017). Participants stood on the floor with their feet shoulder width apart, hands on hips, and descended rapidly to approximately 90° knee angle and then in one continuous movement jumped vertically as high as possible with legs fully extended and hands remaining on hips throughout the jump. Two submaximal jumps were performed

before the first of 3 maximal test jumps. Test jumps were separated by 90 seconds with the highest jump used for analysis. Verbal encouragement was given before jumps.

Muscle Soreness

Muscle soreness of the lower limbs was evaluated using a visual analogue scale (VAS) (Hawker, Mian, Kendzerska, & French, 2011). The VAS was a 100 mm long horizontal line with anchor points on either end stating no muscle soreness on the far left, to muscle soreness as bad as it could be on the far right. Subjects performed a 5 second isometric squat with their ankles, knees and hips at 90°, or as close to 90° as possible. Participants then placed a vertical line at the point on the scale corresponding to their perceived level of soreness. The distance (mm) between the no muscle soreness anchor and the participant's mark was measured and recorded for analysis.

Hamstring flexibility

Hamstring flexibility was measured with a YMCA sit and reach test using a standardised testing procedure (Pescatello, 2014, p. 106). In brief, participants slowly reached forward as far as possible with the fingertips of both hands overlapped and in contact with the measuring tape and held this position for 2 seconds. Each participant performed the test three times and the highest value achieved was used for analysis.

Dietary intake: Assessment of actual vs guidelines

In addition to conducting the main study, the study also assessed post-exercise protein intake in the immediate (0-1 h) recovery period for two typical training days. Food records and training logs were kept in the same manner as previously described. Dietary software FoodWorks 9 (Xyris Software, Brisbane Australia) was used for all analysis. To improve the quality of the analysis and reduce coding

errors, the same researcher who was familiar with the dietary analysis software entered all records.

Statistical analysis

Simple descriptive statistics are expressed as means ± between subject standard deviation (*SD)*. Baseline values were established as the first MVC, CMJ, and hamstring flexibility score achieved at the first trial, and used in subsequent data analysis. Mean effects and 90% confidence limits (CL) of protein dose (P20 vs P40) and placebo were estimated using a published spreadsheet (Hopkins, 2006). Data for CMJ, MVC and sit and reach measurements were log-transformed to reduce bias from non-uniformity of error, and back-transformed to express changes in means as percent. Soreness values were not log-transformed because of interval scaling (Nevill & Lane, 2007). Supplement effects were analysed by comparing changes in MVC, CMJ, muscle soreness and hamstring flexibility across trials (PLA vs. P20 vs. P40).

Effects were expressed as raw units, percentage changes and standardised effects. The magnitude of effect size (ES) is expressed using Cohen's thresholds of <0.2 (trivial), 0.2-0.6 (small), 0.6-1.2 (moderate), 1.2-2.0 (large), 2.0-4.0 (very large) >4.0 (extremely large) (Hopkins, Marshall, Batterham, & Hanin, 2009). The smallest worthwhile effect was assumed to be 1% (Hopkins, 2017).

Post-exercise protein intakes were assessed for the proportion (%) and number of total training sessions undertaken by participants in which protein intake either met suggested intakes for older athletes (Doering, Reaburn, et al., 2016), met current sport nutrition guidelines (Moore, 2015; Thomas, Erdman, Burke, & Mackillop, 2016), did not meet current sport nutrition guidelines, or there was no intake of protein during the first hour of recovery.

Results

Subjects

Of the 19 participants assessed for eligibility in the study, 13 were excluded, two for not meeting the inclusion criteria and 11 declined to participate for other reasons. Of the 6 participants recruited, all 6 completed all three trials, and all data from the 6 participants was analysed.

Maximal Voluntary Contraction

Mean baseline MVC was 334.7 ± 55.4 N. MVC at 3 h post DHR was 296.3 ± 57.5 N (PLA), 297.7 ± 49.2 N (P20), and 303.0 ± 45.3 N (P40). The difference between P20 and PLA was 0.9 ± 10.6%, P40 and PLA 2.9 ± 13.4% and P40 and P20 2.0 ± 13.4%. There are unclear outcomes for any beneficial effect for P20 and PLA, P40 and PLA, and P40 and P20.

Mean MVC at 24 h post was 305.0 ± 65.0 N (PLA), 301.7 ± 49.9 N (P20) and 318.2 ± 41.4 N (P40). The difference between P20 and PLA was 0.2 ± 8.4%, P40 and PLA was 5.8 ± 12.5%, and P40 and P20 was 5.9 ± 7.1%. There is a likely small beneficial effect for P40 relative to PLA and P20 (ES = 0.24 ± 0.5 and 0.25 ± 0.29) respectively.
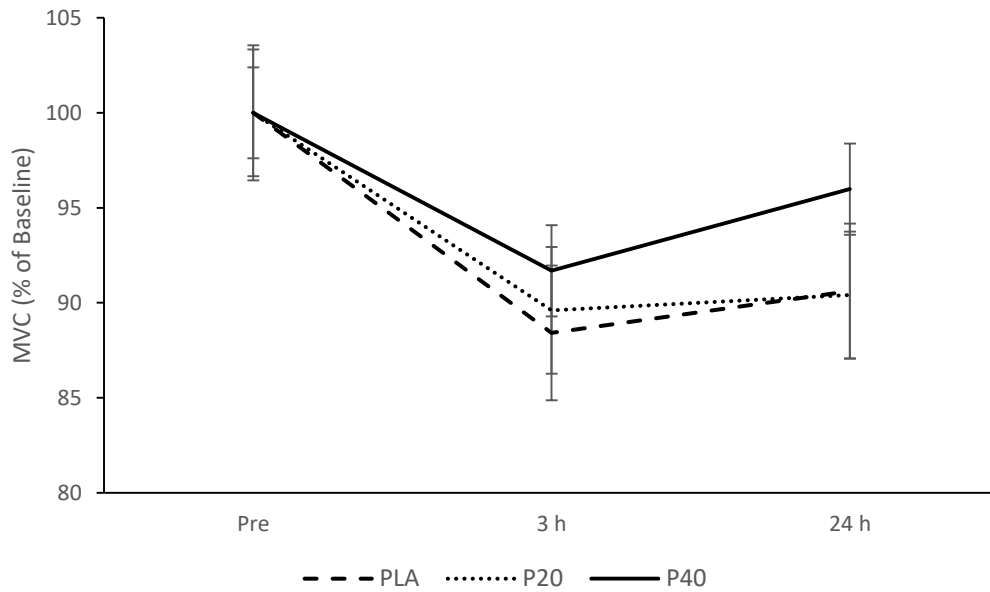
Figure 3. Maximal voluntary contraction performance (% change from baseline) post downhill run in the placebo (*n* = 6), protein 20 g (P20) *(n* = 6), and protein 40 g (P40) *(n* = 6) groups.

Countermovement Jump

Mean baseline CMJ was 17.8 ± 1.4 cm. Mean values for CMJ at 3 h post was 18.4 ± 2.3 cm placebo (PLA), 17.9 ± 2.2 cm protein 20 g (P20) and 18.4 ± 1.5 protein 40 g (P40). The difference between P20 and PLA was -2.9 ± 4.5%, P40 and PLA, 0.0 ± 5.2%, and P40 and P20**,** 2.8 ± 4.2%. There was a likely small beneficial effect for PLA and P40 relative to P20 (ES = PLA: 0.24 ± 0.36, P40: 0.23 ± 0.34).

Mean values for CMJ at 24 h post was 17.5 ± 1.7 cm, 17.5 ± 1.1 cm, 18 ± 1.3 cm observed for PLA, P20 and P40 respectively. The difference between P20 and PLA was 0.1 ± 3.3%, P40 and PLA was 3 ± 3.7% and P40 and P20 2.9 ± 1.9%. There was a likely small beneficial effect for P40 relative to PLA and P20, (ES = 0.32 ± 0.39 and 0.27 ± 0.19) respectively.
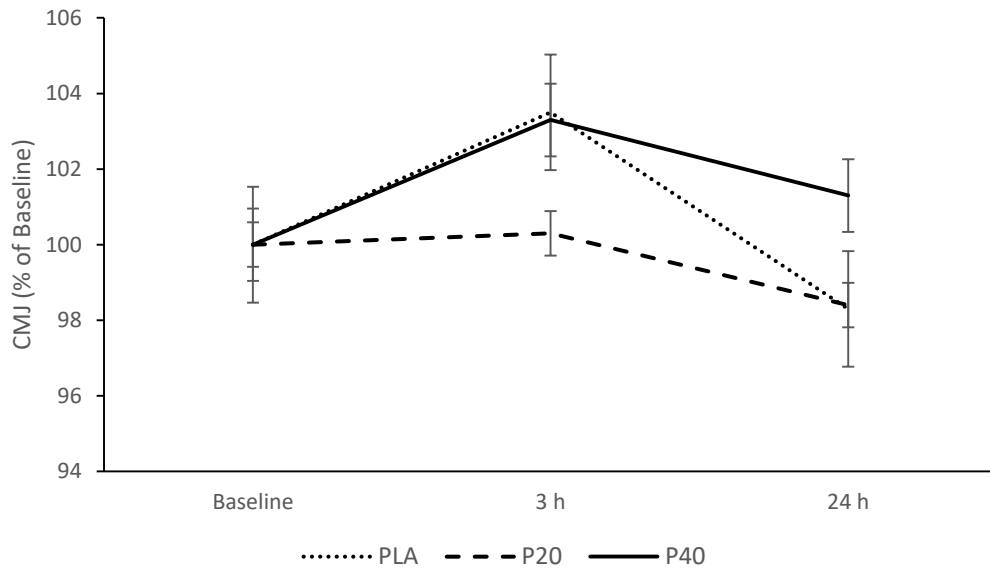
Figure 4. Countermovement jump performance (% change from baseline) post downhill running in the placebo (*n* = 6), protein 20 g (P20) (*n* = 6) and protein 40 g (P40) (*n* = 6) groups.

Hamstring Flexibility: (500 mm at heels for sit and reach test)

Mean baseline hamstring flexibility was 360.5 ± 77.3 mm. Hamstring flexibility at 3 h post was 407.7 ± 40 mm (PLA), 408.5 ± 63 mm (P20), and 419.7 ± 58.1 mm (P40). The difference between P20 and PLA was -0.4 ± 8.4%, P40 and PLA 2.5 ± 6.4% and P40 and P20 2.9 ± 3.8%. There was a likely trivial beneficial effect for P40 relative to P20 (ES = 0.14 ± 0.18).

Hamstring flexibility at 24 h post was 389.0 ± 51.6 mm (PLA), 388.7 ± 46.7 mm (P20), and 406.7 ± 63.7 mm (P40). The difference between P20 and PLA was 0.1 ± 8.1%, P40 and PLA 4.3 ± 15.3% and P40 and P20 4.2 ± 13.7%. There were unclear outcomes for any beneficial effect for P20 and PLA, P40 and PLA, and P40 and P20.

Muscle Soreness

Mean baseline muscle soreness was 10.3 mm (VAS 0-100 mm). Muscle soreness at 3 h post was 17.8 ± 24.8 mm (PLA), 19.2 ± 19.4 mm (P20), and 23.0 ± 19.1 mm (P40). The difference between P20 and PLA was 1.3 ± 26.6%, P40 and PLA 5.2 ±

15.2% and P40 and P20 3.8 ± 14.4%. There were unclear outcomes for any beneficial effect between P20 and PLA, P40 and PLA, and P40 and P20.

Muscle soreness at 24 h post was 18.3 ± 22.1 mm (PLA), 21.1 ± 22.5 mm (P20) and 14.7 ± 19.6 mm (P40). The difference between P20 and PLA was 2.8 ± 22.6%, P40 and PLA -3.7 ± 8.2% and P40 and P20 -6.4 ± 20.0%. There were unclear outcomes for any beneficial effect for P20 and PLA, P40 and PLA, and P40 and P20.

For analysis it was decided to not include the immediate 0 time point measurements for CMJ, MVC, hamstring flexibility and muscle soreness. Metabolic fatigue immediately post-exercise is likely to contribute to reductions in MVC and muscle soreness (Warren et al., 1999). Post activation potentiation and increased intramuscular temperature may influence CMJ height (Hughes, Massiah, & Clarke, 2016), and an increase in muscle temperature and eccentric muscle actions influence hamstring flexibility (O'Sullivan, McAuliffe, & DeBurca, 2012). These factors would have made a positive change and not negative change to CMJ and hamstring flexibility, hence their omission.

Table 5. Summary of the effect of the supplements on maximal voluntary contraction, countermovement jump height, and hamstring flexibility 3 hours and 24 hours following the downhill run

| | | Time frame | | | |
| | | 3 hours | | 24 hours | |
Exercise	Comparison	Mean	Qualitative	Mean	Qualitative
MVC	P20-PLA	0.9 ± 10.6	unclear	-0.2 ± 8.4	unclear
	P40-PLA	2.9 ± 13.4	unclear	5.8 ± 12.5	likely[b]
	P40-P20	2.0 ± 13.4	unclear	5.9 ± 7.1	likely[b]
CMJ	P20-PLA	-2.9 ± 4.5	unlikely	0.1 ± 3.3	unclear
	P40-PLA	0 ± 5.2	unclear	3.0 ± 3.7	likely[b]
	P40-P20	2.8 ± 4.2	likely[b]	2.9 ± 4.9	likely[b]
HSF	P20-PLA	-0.4 ± 8.4	unclear	0.1 ± 8.1	unclear
	P40-PLA	2.5 ± 3.8	unclear	4.3 ± 15.3	unclear
	P40-P20	2.9 ± 3.8	likely[b]	4.2 ± 13.7	unclear

Note. Results for log-transformed data only CI = confidence interval; CMJ = countermovement jump; HSF= hamstring flexibility.

[a] With reference to a smallest worthwhile change of 1.0%.

[b] Likely beneficial.

Dietary Standardisation

According to the food logs kept by participants compliance was high for the replication of diets for the 48 h pre and 24 h post downhill run in all three trials. Energy and macronutrient intakes over the 72 h (48 h prior to trials and 24 h post DHR) were energy intake: 9796 ± 1904 kJ, 160 ± 32 $kJ.kg^{-1}$, CHO: 225 ± 98 g, 3.7 ± 1.8 $g.kg^{-1}$, PRO: 105 ± 20 g, 1.7 ± 0.3 $g.kg^{-1}$, Fat: 100 ± 44 g, 1.6 ± 0.7 $g.kg^{-1}$.

Protein intakes for all participants were over 1.2 $g.kg^{-1}$ body mass for the 48 h pre DHR (1.9 ± 0.4 $g.kg^{-1}$) and in the 24 h post DHR (1.5 ± 0.3 $g.kg^{-1}$).

Blinding of Subjects to Treatments

Testing for blinding of participants to the supplement treatments can be viewed in Table 6. For the 18 supplement trials participants either incorrectly identified the supplement or did not know what it was 67% of the time, and correctly identified the supplement 33% of the time. Mean scores for how well the different supplements were matched for flavour, texture, colour and palatability were 3.9, 4.2, 4.8, 4.7 out of five with five being no difference between treatments.

Table 6. Participant blinding to the supplement treatments (n = 18)

Supplement	Identified correctly (n)	Identified incorrectly (n)	Did not know (n)
Placebo	2	2	2
Protein 20 g	2	2	2
Protein 40 g	2	1	3
Total	**6**	**5**	**7**

Secondary Study Aim: Protein Intake Immediately After Training

Dietary practises of this cohort post training, specifically protein intake during the immediate (0-1 h) post-exercise period are shown in Figure 5. Of the 13 training sessions analysed 3 sessions had no protein intake in the immediate post-exercise period, 6 sessions had an intake below the recommended 20 g, and 4 sessions had an intake of 20 g or more, with 1 of these 4 sessions having an intake over 40 g. No protein supplements were not taken in the immediate post-exercise period.
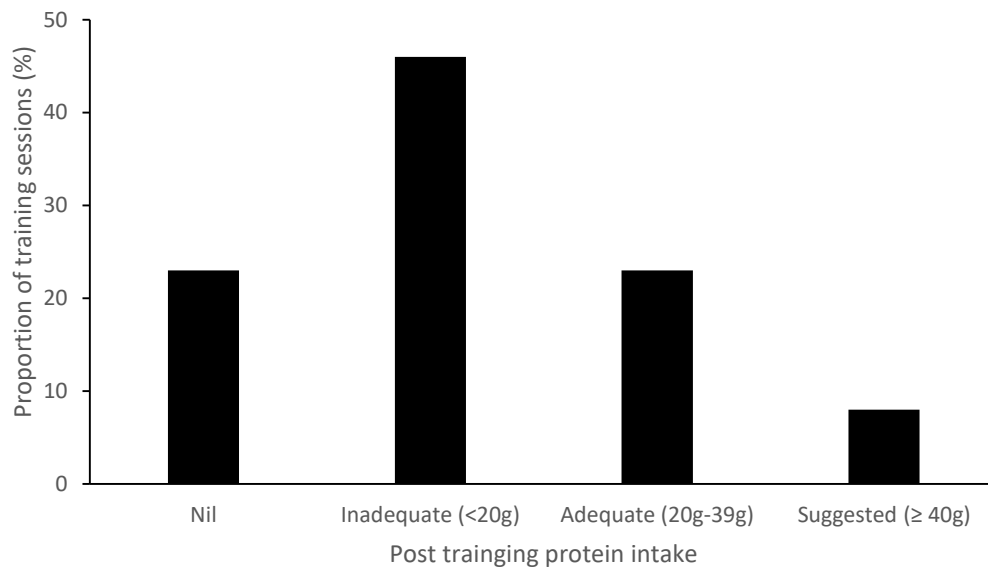
Figure 5. Proportion of total training sessions (*n* = 13) where protein intake during the first hour of recovery was reported as no intake, inadequate amounts to meet sport nutrition guidelines, adequate amount to meet sport nutrition guidelines, or a protein amount suggested for older athletes.

Discussion

This study is the first to investigate the effects of double the recommended protein intake, taken acutely post muscle-damaging exercise, on 24 h recovery in older female endurance runners.

The primary findings of this study indicate that an acute dose of 40 g of WPI following a downhill run can improve/accelerate recovery of muscle function in older female endurance runners. The 40 g dose showed a likely small beneficial effect for attenuating decreases in MVC and improving CMJ height performance compared to 20 g of WPI or an isocaloric placebo at 24 h post-exercise, and a likely small beneficial effect for improving CMJ height at 3 h compared to 20 g of WPI. The observed benefits of 40 g of WPI are proposed to be from the larger dose of protein facilitating greater repair and remodelling of the myofibrillar fibres, and hence a more rapid improvement in the recovery of muscle function.

The change in muscle protein metabolism with aging is suggested to be the underlying reason for the larger dose of protein improving muscle function in this study. There is strong evidence that in both older men and women, there is a reduced sensitivity with aging to the anabolic stimuli of protein ingestion (Cuthbertson et al., 2005; Dickinson et al., 2013). Research has shown that male master endurance athletes (Doering, Jenkins, et al., 2016) have significantly lower (p = 0.009) myofibrillar fractional synthesis rates post-exercise compared to their younger counterparts, to the 20 g dose known to maximally stimulate MPS in younger athletes (Moore et al., 2008; Witard et al., 2014). Furthermore, in middle age and older men a dose of ~36-40 g of protein is needed to near maximally stimulate MPS following RE (Robinson et al., 2012; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012). To date however, there is a lack of research to make clear recommendations on the amount of protein needed to maximally stimulate MPS in older female endurance runners. Muscle protein synthesis rates may be further influenced in older women due to a reduced sensitivity to protein

and its leucine content, associated with the decline in oestrogen levels at the onset of menopause (mid to late 40s), in addition lower oestrogen levels are known to augment muscle damage and attenuate the repair process (Devries et al., 2018; Hansen, 2018; Katsanos et al., 2006). Although not directly measured, it would therefore be reasonable to suggest that the results of this study are from 40 g of protein being able to enhance the MPS response compared to 20 g, and hence facilitate a greater repair and remodelling of the myofibrillar fibres, and a more rapid improvement in the recovery of muscle function in this cohort.

While studies of acute protein supplementation post-exercise in younger populations cannot be directly compared to the results of this study due to differences in age, protein metabolism, and methodologies, most lend support for acute protein supplementation to be beneficial in improving recovery of muscle function (Cockburn et al., 2008; Etheridge et al., 2008; Rankin et al., 2018; Rankin et al., 2015; Saunders et al., 2018). Furthermore, two of these studies demonstrate that when age specific protein doses known to maximally stimulate MPS are ingested, recovery of muscle function is improved (Rankin et al., 2018; Rankin et al., 2015). In both of these studies, peak isokinetic torque showed a very likely beneficial effect from baseline at 72 h when 17 g (~0.27 $g.kg^{-1}$) was ingested by female team sport athletes. The only study in male masters athletes showed that repeated boluses of WPI (0.6 $g.kg^{-1}$), improved recovery of muscle function in comparison to a lower dose (0.3 $g.kg^{-1}$) (Doering et al., 2017), supporting the findings of the current study for a higher dose of whey protein isolate in improving muscle function.

The unclear benefits of P20 compared to the placebo in any of the tests for measuring recovery in the current study may potentially be due to the leucine content (2.4 g) in this protein dose not being enough to activate the mammalian target of rapamycin complex 1 (mTORC1) pathway. Research suggests that ~3 g of leucine is required to activate the mTORC1 pathway and trigger a rise in MPS to a

level equivalent to that seen in younger individuals (Devries et al., 2018; Katsanos et al., 2006).

Isometric MVC was reduced with all treatments at 3 h and 24 h from baseline measurements, indicating that the DHR protocol was effective in inducing a muscle damage response. Though not a direct marker of muscle damage, MVC is a functional measure and considered to be the most reliable and valid for quantifying the extent of muscle damage (Warren et al., 1999). The loss in muscle function at 24 h (9.6%) was of a smaller magnitude than in several studies in younger female population at this time point (Green et al., 2008; Rankin et al., 2018; Rankin et al., 2015). This is likely due to differences in the training status of participants, exercise protocol used to induce muscle damage, and participants in this study unlikely to have benefited from the repeated bout effect. All were well trained endurance runners who regularly included DHR in their weekly training program.

Although it is known that multi-joint performance is better maintained after EIMD than single joint (Douglas et al., 2017), the increases in CMJ height above baseline measures at 3 h for all supplements and at 24 h for P40 was an unexpected outcome of this study. One possible mechanism for this improved performance, is that the optimal length of tension development in the muscle was shifted to a longer muscle length following the DHR (Brughelli & Cronin, 2007). This adaptation potentially changed the mechanical properties of the muscle, increasing leg stiffness and enhancing the stretch shorten cycle of the CMJ, and hence improving performance (Brughelli & Cronin, 2007; Elmer, Hahn, McAllister, Leong, & Martin, 2012). It is proposed that this enhancement in the stretch shorten cycle outweighed muscle damage at 3 h while at 24 h this acute adaptation was diminished, with P40 showing a beneficial effect over P20 and the placebo due to the improved repair and remodelling of muscle fibres from this higher dose. This adaptive response to eccentric exercise has important implications for these athletes, as endurance running involves stretch shortening cycle actions and in

principal chronic eccentric loading would lead to sustained increases in leg stiffness, and as such improved performance. Future research in this area is warranted given that with aging endurance running performance is known to decrease (Lepers & Stapley, 2016).

Studies of acute protein supplementation in younger populations, where CMJ was measured in females (Rankin et al., 2018; Rankin et al., 2015), do not support these findings for improved CMJ over baseline measures. These conflicting results may be from a difference in the muscular response to eccentric exercise with aging (O'Sullivan et al., 2012), different training programs of team sport athletes compared to older female endurance runners influencing CMJ performance after muscle-damaging exercise, and the time points for measuring CMJ performance (acute time frame vs. days).

Reasons for the likely beneficial effect of placebo and P40 increasing CMJ above P20 at 3 h post recovery are not clear. It is possible the higher protein and carbohydrate facilitated a more rapid re-uptake of carbohydrate into the muscle, while the P20 resulted in a low glycogen content in the muscle, impairing the ability to release Ca^{2+} and reducing muscle power generation (Gejl et al., 2014), hence the lower jump height. However, this remains speculative and warrants further investigation with a larger sample size.

The sit and reach test showed no clear benefits for a higher protein intake nor did it show a reduction in flexibility of the hamstrings from baseline measures. These findings are likely from this single test not relating to the flexibility of the knee extensor muscles that absorb the most force when running downhill, which the most damage is inflicted upon (Douglas et al., 2017), and/or the proposed increased lower limb flexibility from eccentric training (O'Sullivan et al., 2012). This stresses the importance of considering the battery of tests that will most accurately reflect the recovery of those muscles damaged in the eccentric exercise protocol. Testing for isokinetic MVC of the knee extensors would be a more valid

test (Warren et al., 1999) to include for measuring recovery from exercise induced muscle damage.

Muscle soreness increased with all supplements following the downhill run which agrees with acute protein supplementations in younger adults (Cockburn et al., 2008; Rankin et al., 2018; Rankin et al., 2015; Saunders et al., 2018). At 24 h post-exercise muscle soreness was still increasing with P20 and the PLA, while P40 showed an attenuation, although there was no clear beneficial effect. This disparity between the benefit of P40 at 24 h improving muscle function and no clear benefit for muscle soreness is likely related to muscle soreness being a subjective measurement, and/or any clear benefit for protein supplementation for attenuating muscle soreness is seen at a later time point in the recovery. In younger athletes (team sport and endurance runners), a very likely beneficial effect for protein supplementation for attenuating muscle soreness was seen at 72 h post-exercise, with no clear benefit seen at 24 h post EIMD (Rankin et al., 2015; Saunders et al., 2018). Future research should determine if a similar effect is seen after supplementing with 40 g of protein in older female endurance runners at later time points post-exercise.

A limitation of this study was it did not measure oestrogen levels or strictly control for them. The decline in oestrogen associated with the onset of menopause (mid to late 40s), specifically that of estradiol-17β is linked to a reduced sensitivity to protein (Devries et al., 2018), an augmentation in muscle damage and attenuation in the repair process (Enns & Tiidus, 2010). While all participants were of an age where oestrogen levels generally decline, levels would have differed due to differences in participants menopausal status; 3 participants being post-menopausal, 2 in the menopausal transition and 1 pre-menopausal. With the small sample size of this study and varying estrogen levels, it cannot be determined how muscle damage, recovery, or the results of this study were influenced.

While diet was not strictly controlled, there was high compliance by participants in replicating their diets for the 48 h prior to the downhill run and for the 24 h post. However, as dietary intake during the trial period may influence the trial intervention and performance measures, future studies could look to standardise diets not just within subjects but also between subjects by providing food in amounts scaled to body mass.

Strengths of this study which allowed for a more accurate interpretation for determining the effectiveness of the supplements include ensuring that all supplements were isocaloric, and participants being effectively blinded to the supplement treatments, which is important given the performance testing involved in this study and the potential placebo effect.

The secondary aim of this study was to assess the typical immediate post-exercise protein intake in these athletes after two training days.

Post-exercise protein intake did not meet current sport nutrition guidelines for athletes after nearly 70% of the training sessions. It is recommended that endurance athletes ingest 20 g (approximately $0.25-0.3$ $g.kg^{-1}$ body mass) of a rapidly digested high leucine content protein, such as whey, in the immediate post-exercise recovery period to maximise MPS for the repair and remodelling of skeletal muscle and support a faster recovery following endurance exercise (Moore, 2015; Thomas et al., 2016).

These guidelines do not differentiate between younger and masters athletes and this amount of protein does not represent the growing evidence for an increased requirement with aging (Robinson et al., 2012; Yang, Breen, et al., 2012). Doering and colleagues suggest that endurance athletes should consume a dose of 35-40 g (~0.4 $g.kg^{-1}$) of protein after muscle-damaging exercise to facilitate muscle repair and remodelling (Doering, Reaburn, et al., 2016). The findings of the current study support these higher protein recommendations, however after only one of the 13 training sessions was this suggested protein dose consumed.

These results suggest that poor dietary protein intake in the immediate post-exercise recovery period may be compromising post-exercise MPS rates for the repair and remodelling of muscle fibres, and potentially contributing to a slower recovery in this cohort. Further research into the nutritional recovery practises in this immediate post-exercise period and across the day is warranted to establish current practises, so that appropriate and practical recommendations can be provided to these athletes to optimise recovery.

Summary

The primary findings of this study support the hypothesis that an acute dose of 40 g of whey protein isolate following a DHR can improve recovery of muscle function in older female endurance runners. The 40 g dose showed a likely small beneficial effect for attenuating decreases in MVC and improving CMJ height performance compared to 20 g of WPI or an isocaloric placebo at 24 h post-exercise. The observed benefits of 40 g of WPI, though not directly measured, are proposed to be from this larger dose of protein facilitating a greater repair and remodelling of the myofibrillar fibres and hence a more rapid improvement in the recovery of muscle function. Improving the recovery of muscle function is relevant to older female endurance runners as it will allow these athletes to better maintain the quality of subsequent daily bouts of training, enhancing adaptations and ultimately performance.

Chapter 3: Conclusion

Summary

The current study was designed to compare the effect of a higher (40 g) acute protein intake versus the recommended intake (20 g) immediately following muscle-damaging exercise in older female endurance runners on the recovery of muscle function, perceived muscle soreness, and flexibility within a 24 h period. The main findings of this research indicate that older female endurance runners benefit from the 40 g protein intake as compared to the recommended 20 g or placebo, for improving muscle function following exercise induced muscle damage. Relative to baseline measures 40 g of protein had a likely small beneficial effect compared to the placebo (ES = 0.24 ± 0.5) and 20 g of protein (ES = 0.25 ± 0.29) for improving MVC at 24 h. Relative to baseline measures 40 g of protein had a likely small beneficial effect for improving CMJ compared to 20 g of protein at 3 h (ES = 2.9 ± 4.9) and at 24 h compared to the placebo (ES = 0.32 ± 0.39) and 20 g of protein (ES = 0.27 ± 0.19). While this study had a small sample size, the trend for improving muscle function warrants further investigation with a larger number of older female endurance runners, given that an enhanced recovery would allow these athletes to better maintain the quality of work during subsequent daily bouts of training.

Practical Applications

The evidence of this research supports the use of a higher protein intake of 40 g to improve recovery of muscle function in older female endurance runners. These findings provide insight for recovery recommendations in this population after daily bouts of training, specifically intense training bouts where eccentric muscle-damage is induced. In practical terms, consuming 40 g of WPI post endurance running will help athletes better maintain the quality of work during subsequent training sessions, supporting training induced adaptations and ultimately performance. The ingestion of 40 g of protein post-exercise may be particularly

relevant during intensified blocks of training when the older female endurance athlete needs to recover adequately before the next acute training bout.

Future Research

- A larger study to expand on the findings of the current study given the trend of improved muscle function seen in this pilot study.
- Investigate if the modest beneficial effects seen with the higher acute protein intake on muscle function can be further enhanced with additional doses of protein evenly distributed over the day to accelerate the recovery process.
- Determine the effects of chronic protein intake post-exercise during blocks of intense endurance training, on the ability to maintain a prescribed training program.
- Chronic eccentric loading interventions to increase leg stiffness in older female endurance runners in the aim of improving performance and potentially reducing the risk of injury.
- More comprehensive and well conducted nutrition assessments in this cohort. Assessment of the nutritional recovery practises in the immediate post-exercise period and across the day to establish current practise, and to provide appropriate and practical recommendations to optimise recovery.

Limitations

The small sample size of this study reduced statistical power, decreasing the ability to detect meaningful effects and reducing the external validity. The small sample size was due to difficulty in recruiting participants, with only six of the 19 participants assessed for eligibility participating in the study. The main reasons for withdrawing from the study were having to abstain from coffee for the 24 h before trials, unable to commit to the time, and the study interfering with their training. These factors should be taken into consideration for future studies. This study

does serve as a pilot study, with none of the six participants recruited withdrawing during the three weeks of trials and all 18 data sets analysed.

Diet was not strictly controlled for in the study. Although there was a high compliance by individual participants in replicating their diets for the 48 h prior to the downhill runs and the 24 h post.

Oestrogen levels were not measured or controlled for in the study. While all participants were of an age when oestrogen levels generally decline, levels would have been different due to the variation in participants menopausal status. With the small sample size of this study and differing oestrogen concentrations, it cannot be determined how muscle damage, recovery or the results of this study were influenced. Measuring oestrogen levels and recruiting a larger sample size of only postmenopausal female endurance runners, with stabilised hormone levels would provide more insight into the mechanisms responsible for the results determined in the current study.

The inclusion of another functional test, such as measuring isokinetic MVC of the knee extensors, would provide another more valid assessment of muscle recovery from downhill running.

Conclusions

The current study has demonstrated that the acute ingestion of 40 g of whey protein isolate improves recovery of muscle function within 24 hours following muscle-damaging exercise in older female endurance runners. The data from the six participants indicates a trend for improving muscle function and warrants a larger study. This higher protein intake post-exercise may be advantageous to these athletes for promoting training induced adaptations and improving performance.

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Appendices

Appendix 1 – Ethics approval letter

The University of Waikato
Private Bag 3105
Gate 1, Knighton Road
Hamilton, New Zealand

Human Research Ethics Committee
Julie Barbour
Telephone: +64 7 837 9336
Email:humanethics@waikato.ac.nz

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

22 May 2018

Zoe Neureuter
By email: zoes1@xtra.co.nz

Dear Zoe

UoW HREC(Health)#2018-34 : Acute protein supplementation post exercise to enhance recovery in post-menopausal female endurance runners

Thank you for submitting your amended application HREC(Health)#2018-34 for ethical approval.

We are now pleased to provide formal approval for your study of the effect of protein supplements on recovery in post-menopausal women who are endurance runners. This will involve the following research activities:

1. DEXA bone density test at Toi-Ohomai bone health lab
2. Preliminary assessments (screening for readiness to exercise PAR-Q, training questionnaire), height and weight, food diary instructions and templates
3. Familiarisation run and training trial (maximal voluntary contraction MVC), plus information around diet and training schedule around trials
4. Trials x3, 3 weeks apart, including saliva samples, MVC measurements, consumption of supplement. Samples and MVC repeated at 3 hours and 24 hours after run.

:

Please contact the committee by email (humanethics@waikato.ac.nz) if you wish to make changes to your project as it unfolds, quoting your application number with your future correspondence. Any minor changes or additions to the approved research activities can be handled outside the monthly application cycle.

We wish you all the best with your research.

Regards,

_____

Julie Barbour PhD
Chairperson
University of Waikato Human Research Ethics Committee

Appendix 2 – Introduction letter for potential participants

Dear Club Member

I am currently enrolled in a Master of Health, Sport and Human Performance at the University of Waikato. As part of my thesis and a partial requirement of my Masters, I am required to carry out a research project on a chosen topic, which is:

"Acute protein supplementation post-exercise to enhance recovery in post-menopausal female endurance runners"

The primary objective of this study is to determine if higher than recommended acute protein supplementation after muscle-damaging exercise can enhance recovery in post-menopausal female endurance runners. Secondary, to the main aim is to gather nutrition data on the recovery practices of these athletes.

This study is relevant for several reasons. The nutritional recovery practices of endurance athletes impact on the quality of subsequent training sessions, adaptations, and ultimately performance. Secondly, after muscle-damaging exercise, as with downhill running recovery is delayed in Masters athletes due to impairments in the synthesis of protein for the repair and remodelling of muscle. Higher than currently recommended doses of protein after resistance training in untrained older males, has been found to increase muscle protein synthesis. However, to date there is limited research on age specific protein feedings post-exercise to enhance muscle recovery in Masters endurance athletes and none have included older female endurance runners.

Your voluntary participation in this project would mean you would be involved in novel research. This research will provide valuable information where none currently exists on acute protein supplementation to enhance recovery in post-

menopausal female endurance runners, and secondly on the nutrient intakes and recovery practices of this cohort. Additionally, you will be provided with individual feedback on your nutrient intakes and recovery practices.

If you are potentially interested in participating in this project, please contact me via email or phone and I will provide you with a Participant Information Sheet. Please take the time to read the sheet and feel free to contact me if you require further information.

A meeting will then be held for all those interested in participating in the study at the University of Waikato Adams Centre for High Performance, Mount Maunganui, on ……… date, to go over the information sheet with you and answer any queries. Should you choose to participate, you will be required to read and voluntarily sign an informed consent form.

Thank you

Zoe Neureuter   **Phone: 027 3109580**   **Email: zoes1@xtra.co.nz**

Participant Information Sheet

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Title of Project

Acute protein intake post muscle-damaging exercise to improve recovery in older female endurance runners

Project Purpose

The primary purpose of this project is to determine if higher than recommended acute protein supplementation following muscle-damaging exercise can improve recovery in older female endurance runners.

Secondary to the main purpose, the project will collect nutrition data on the recovery practises of older female endurance runners.

Exclusion criteria from the study

- Women who are less than 48 years of age
- Endurance runners training for less than 5 hours per week or for a distance less than 10km in competition
- On hormone therapy
- Have cardiovascular disease or kidney disease, diabetes, arthritis or other joint injury of the lower body
- Not able to consume whey protein

Participant Involvement

Preliminary assessments and familiarisation

If you voluntarily sign the informed consent form, you will be asked to complete two questionnaires, a health screening questionnaire and a short training questionnaire. Measurements for height and weight will be taken. Instructions will be given for keeping a food record diary (2 training days and 1 rest day) and a training log (approx. 15 mins a day to fill in diaries). The information from these records will be used for dietary and exercise training standardisation for trials and for collecting data on your nutrition intake and recovery practises. A familiarisation session for what is expected of you in the trials will then take place. This will include instruction on the correct form for executing a knee extension (using a strain gauge), a countermovement jump and a sit and reach test for

flexibility and range of motion. These exercises are a measure of muscle function and recovery. To determine running speed for subsequent trials you will then perform a 30 minute treadmill run at a -10% decline at 80% of your maximal heart rate. Heart rate and rate of perceived exertion will be monitored during this run. Time commitment approximately 90 minutes.

Trial overview

Three trials will be held a week apart the first commencing a week after the familiarisation session. Trials will be held in the morning and begin with baseline measurements of muscle function and muscle soreness. A 30 minute treadmill run, (-10% decline ~80% maximal heart rate) will follow. On completion of the run, muscle soreness and muscle function measurements will be taken. You will then be given either a placebo solution or whey protein isolate solution to drink. Muscle soreness and muscle function measurements will be retaken at 3 hours and 24 hours after the run.

Dietary, Exercise and Additional Standardisation

You will be asked to replicate your habitual diet for the 48 hours before each trial and during the 24 hours post run. Exercise training for the week prior to all three trials is to be kept the same/similar. In the 24 hours prior to each trial you will be asked to refrain from all exercise, alcohol, caffeine and non-steroid anti-inflammatory drugs (NSAID). In the 24 hours post run you will be asked to refrain from taking NSAID, other supplements, or the use of other recovery modes (e.g. massage, compression garments).

Location

The University of Waikato Adams Centre for High Performance, Mt Maunganui, or the Avantidrome, Cambridge will be used for all preliminary assessments, familiarisation sessions and trials.

Risk of Harm

Measurements for height and weight will be taken in a private setting, with the correct terminology and language used. Weight or such will not be discussed with anyone.

The most recent research supports the notion that consuming higher levels protein (2.5g/kg/day) than currently recommended (1-1.2g/kg/day) is unlikely to be harmful to your health.

Precautionary measures have been put in place to minimise the risk of physical injury associated with the execution of a countermovement jump, knee extension and in the running component of this project. Should an injury or an unforeseen medical condition occur, the researcher and research assistants (supervisor) are first aid certified, there is medical support at the Centre and a doctor will be on call.

Use of Information Collected

This research is a partial requirement for Master of Health, Sport and Human Performance. The information collected will be used in the researcher's thesis. A printed and digital copy of the thesis will be publicly available through the University of Waikato. It is possible that journal articles and presentations may be the outcome of the research.

Benefits from Participating

Individual feedback will be given on dietary recovery practises, and once the study is concluded a summary of the findings will be available.

Participation

Your participation in this project is completely voluntary. Please take the opportunity to talk through your potential involvement with family and friends. You have the right to have answered any questions about the project prior to signing the consent form and you are free to request further information at any stage during your participation. You are free to withdraw from the study up to two weeks after participating in the research activities, by informing a member of the research team, without disadvantage.

Participant Privacy:

Your data will not be recognised by name in text or analysis. Your information will be kept private and secure and only the researcher and supervisors will have access to this information. On completion of the project all personal identifying information will be removed from paper records and electronic files which represent the data from the project, and these will be placed in secure storage and kept for at least five years. There is still a small risk of identification from this project, even with the de-personalisation of all identifying information.

Further Inquiries

If you have any questions or concerns regarding this project, either now or in the future, please feel free to contact either:

Researcher: Zoe Neureuter   e: zoes1@xtra.co.nz   Ph: 027 310 9580

Supervisor: Dr. Stacy T Sims   e: stacy.sims@waikato.ac.nz

Co-supervisor: Lillian Morton   e: Lillian.Morton@hpsnz.org.nz

This research project has been approved by the Human Research Ethics Committee (Health) of the University of Waikato under HREC(Health)#2018-34. Any questions about the ethical conduct of this research may be addressed to the Secretary of the Committee,

email humanethics@waikato.ac.nz, postal address, University of Waikato, Te Whare Wananga o Waikato, Private Bag 3105, Hamilton 3240.

Appendix 4 – Consent form for participants

Consent form for participants

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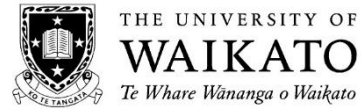
Title of Project

Acute protein supplementation post-exercise to enhance recovery in post-menopausal female endurance runners

Name of participant: ....

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that I meet the criteria for participation which are explained in the Information Sheet.
4. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
6. I have been informed and understand the nature of my participation
7. I have been informed of any potential harm to me by taking part in this project.
8. I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at five years.
9. I understand that the results of the project may be published and be available in the University of Waikato Library, and it is possible that journal articles and presentations may be the outcome of the research.
10. I understand but that any personal identifying information will remain confidential between myself and the researcher and supervisors during the study and will not appear in any spoken or written report of the study.

11. I know that with the de-personalization of all identifying information, there still is a small risk of identification from publications.
12. I provide consent for the publication of research papers, presentations, student thesis using average and/or range of data to be published.

This research project has been approved by the Human Research Ethics Committee (Health) of the University of Waikato under HREC(Health)#2018-34. Any questions about the ethical conduct of this research may be addressed to the Secretary of the Committee, email humanethics@waikato.ac.nz, postal address, University of Waikato, Te Whare Wananga o Waikato, Private Bag 3105, Hamilton 3240.

Signature of participant: Date:

Signature of researcher: Date:

Appendix 5 – Participant training questionnaire

Participant Training Questionnaire

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Name: …………………………………………...

Age: ……….

Menopausal Status: (tick which applies to you)

- **Premenopausal:** no decreased regularity in menstrual bleeding in last year

- **Early perimenopause:** decreased menses regularity in previous three months

- **Late perimenopausal**: no menses for last 3-11 months

- **Postmenopausal:** no menses for 12 or more months

Training:

   Endurance Running Training

- Are you an endurance (tick all that apply)

     o   Road runner

     o   Trail runner

     o   Both

- What running distance do you train for (tick all that apply)

     o   10km

     o   Half marathon

     o   Marathon

     o   Ultra

- How many years have you been endurance running …………

- In the last 3 months:

     o   What is the average number of **hours** per week you run   ………

     o   What is the average number of **times** per week you run   ………

- Have you regularly (once or more a week) included hill work in your training **yes/no**

Resistance Training

- Do you regularly (once or more a week) include resistance training (body weight, and/or with weights, not yoga) in your weekly training **yes/no**
- **If yes,** in the last 3 months

    - What is the average number of **hours** per week you train   ………
    - What is the average number of **times** per week you train   ………

Multiple training sessions in a day

- Do you regularly (once or more a week) include two or more training sessions in a day in your weekly training **yes/no**
- **If yes** in the last 3 months what is the average number of **times** per week you do two or more training sessions for

    - Running only   ……….
    - Running and resistance training   ……….
    - Running and other (e.g. cycling, swimming, boot camp, yoga/Pilates) ……….

Appendix 6- Food record diary package for participants: instructions, sample day and template

Instructions for Completing Food Record Diary

Please complete the food record diary for the **three days before the first running trial**, the **day of the trial and the morning before retesting at 24 hours post run**. If these three days do not include two training days (rest day is the day before the run trial) please include an additional day which is a training day. It is a good idea to carry the food record with you and record entries as soon after eating as possible. Don't wait until the end of the day or you will forget.

It is important for you to record **ALL** food and beverages you consume for meals or snacks at home or away from home. **Please do not change your normal eating habits for the days of recording.** Your **honesty** is crucial for collecting accurate data on the nutritional recovery practises of post-menopausal female endurance runners and for providing you with valuable feedback on your current nutrient intakes and recovery practises.

Please include the following information for **Day 1, Day 2 and Day 3** on your Food Record.

- **Date:** fill in the section at the top of each page to indicate the date
- **Time:** time the meal or snack was eaten
- **Meal:** indicate if it was breakfast, lunch, dinner, a snack
- **Food Description:** enter all foods and beverages consumed at a meal or snack. Please include if applicable:
    - **Type of food:** e.g**.** soy and linseed bread, timtams biscuits, canned tuna in water
    - **Brand of food:** e.g. Vogels, Arnotts
    - **Cooking method:** e.g. fried, poached, raw,
    - **Homemade or Purchased:** e.g. packaged supermarket, fast food, restaurant
    - **Other relevant information:** e.g**.** fat content: low-fat milk, lean beef, skinless and boneless chicken, flavour: chocolate milk, blueberry yoghurt
- **Amount:** for every food or beverage you consume enter the amount using either
    - **Common household measures**: teaspoon, tablespoon, cup (metric cup = 250ml)
    - **Refer to volume or weight on food package**
    - **Number of items of predetermined size**

- o **Estimate portion size:** use either the grid (dimension and shape) or portion size guide provided

  NB: combination foods and mixed dishes please provide details and amounts of each food or include the recipe.

  If applicable, please state if the amount recorded is a raw or cooked amount.

- **Supplements:** include any protein supplements you take: type (powder, bar, drink, brand, flavour and amount. Use the food label on the protein supplement to help provide details.

Daily Check: please go over your entries at the end of the day to check you have included as much detail as possible. Can a stranger reproduce what you ate for the day accurately

Food record - Sample Day

Participant Name:

Contact Details:

Date:

Time	Meal	Description: include food/beverage amount, type, brand, cooking method, flavour, etc
6am	Pre-run	1 cup instant black coffee
	Snack	1 home baked Anzac biscuit – 3cm diameter
8 am	Breakfast	1 cup plunger black coffee
		2 slices Vogels soy and linseed toast
		3 tsp Adams smooth peanut butter
		1 tsp honey
		½ cup low fat unsweetened natural Greek yoghurt
		¼ cup Sanitarium natural muesli
		¼ cup frozen blueberries
11 am	Snack	1 cup light reduced fat trim (blue top) milk, 1 med banana
1.30 pm	Lunch	1 large cup black tea, 1 med homemade Anzac bici
		1 Freya's wholemeal wrap
		3 slices tasty cheese – business card size each
		1.5 tsp Best Foods mayonnaise
		1.5 tsp of homemade tomato relish
		1 small tomato, 1 Tbsp alfalfa sprouts, 1 handful/1 cup salad greens
5 pm	Snack	1 medium orange, sm handful unroasted almonds, 1 Timtam bici
		1 cup (250 ml) homemade kombucha tea
7.45 pm	Dinner	Frittata: 12 med eggs, 4 handfuls spinach, 2 medium tomatoes,
		1 med sweet red pepper, 1 small onion, 2 med kumura,
		1 cup grated tasty cheese, 4 slices Grandpas lean middle bacon
		¼ small pumpkin, 2 Tbsp olive oil I ate ¼ of this frittata
		Salad: 2cups salad greens, 1 sm carrot, 1/4 cup alfalfa sprouts

		½ med tomato 1Tbsp of Paul Newman Balsamic dressing
		2 standard glassed of white wine (pinot gris)

Food record template

Participant Name:
Contact Details:
Date:

Time	Meal	**Description**: include food/beverage amount, type, brand, cooking method, flavour, etc
NB: Please check that you have provided enough detail that if you gave this record to a stranger could they ACCURATELY reproduce what you ate today??		

Appendix 7 – Training log template

TRAINING LOG: please fill in for the week before the first running trial

Participant Name:

Contact Details:

Date	Start	Description of Training	Intensity	Finish

Intensity of Training

- **Light:** Requires little effort, can carry on a full conversation
- **Moderate:** Requires a moderate amount of effort and noticeably accelerates the heart rate
- **Vigorous:** Requires a large amount of effort and causes rapid breathing and a substantial increase in heart rate

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RESEARCH

STUDY

"Acute protein supplementation post-exercise to enhance recovery in post-menopausal female endurance runners"

Volunteers needed now to participate in this novel research study.

Your participation will provide valuable information where none currently exists!

This study is a partial requirement for a Master of Health, Sport and Human Performance at the University of Waikato

For more information contact :

Zoe Neureuter   email: zoes1@xtra.co.nz   Ph: 027 3109580

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Appendix 9 – Protein supplement composition

Protein Supplements

Brand: BALANCE: WPI vanilla flavour (B 14187)

Nutrient analysis: quantity/100g

- Protein: 87.8g (leucine 10.5)
- Carbohydrate (CHO): 3.6
- Fat: 0.7

SUPPLEMENTS:

- Volume: 250ml
- Energy: ~ 1190 kJ
- Matched for taste/colour/consistency

40 g Protein Supplement: 40g P and 30g CHO

- 40g P/45.6g protein powder (leucine 4.8g)
- 1.6g CHO/ 45.6g p powder
- 10g CHO/ 10g Banana Nesquik
- 18.4g CHO/92g Banana

20g Protein Supplement: 20g P and 50g CHO

- 20g P/22.8g protein powder (leucine 2.4g)
- 0.8g CHO/ 22.8g p powder
- 10g CHO/10g Banana Nesquik
- 10g CHO/10g Glucose
- 29.2g CHO/ 150g Banana

CHO Placebo: 70g CHO

- 10g CHO/10g Banana Nesquik
- 25g CHO/25g glucose
- 35g CHO/ 175g banana
- ⅛tsp Xanthan Gum (thickening agent)
- ¼ tsp vanilla essence

Appendix 10 – Supplement questionnaire

<u>SUPPLEMENT QUESTIONNAIRE</u>

Please tick which supplement you think you had for each trial.

	Carbohydrate	Protein 20 g	Protein 40 g	I Don't Know
Trial One				
Trial Two				
Trial Three				

Flavour: on a scale of 1-5 how well matched were the supplements for flavour?

　　　1 = totally different flavour   5 = no difference in flavour

Texture: on a scale of 1-5 how well matched were the supplements in texture?

　　　1 = totally different texture   5 = no difference in texture

Colour: on a scale of 1-5 how well matched were the supplements for appearance/colour?

　　　1 = totally different colour/appearance   5 = no difference in appearance/colour

Palatability: on a scale of 1-5 how easy and enjoyable were the supplements to drink?

　　　1 = disgusting hard to get down   5 = tasty and easy to drink

Visual Analogue Scale for Muscle Soreness

Participant ID: ……………………………………....

Trial Number: …………….

Measurement Period

☐ Baseline   ☐ 0 hrs post exercise ☐ 3 hrs post exercise   ☐ 24 hrs
post exercise

            No muscle soreness   Muscle soreness as bad as it
could possibly be

Visual Analogue Scale for Muscle Soreness

Participant ID: …………………………………….

Trial Number: ………….

Measurement Period

☐ Baseline   ☐ 0 hrs post exercise ☐ 3 hrs post exercise   ☐ 24 hrs
post exercise

            No muscle soreness   Muscle soreness

as bad as it could possibly be

Appendix 12 – Physical activity readiness questionnaire

Par Q Form

Name:_____Date: _____

Telephone: _____

Date of Birth:_____Age: _____   Height: _____   Weight:_____

In Case of Emergency Contact:_____Relationship:_____

Address: _____   Phone: _____

Physician: _____   Specialty: _____

Address: _____   Phone: _____

Are you currently under a doctor's care: Yes ☐   No ☐

If yes, explain: _____

When was the last time you had a physical examination? _____

Have you ever had an exercise stress test: Yes ☐ No ☐ Don't Know If ☐

yes, were the results: Normal ☐ Abnormal Do ☐

you take any medications on a regular basis?   Yes No   ☐

If yes, please list medications and reasons for taking: _____

Have you been recently hospitalized?   Yes ☐   No ☐

If yes, explain: _____

Do you smoke?   Yes ☐   No ☐

Are you pregnant?   Yes ☐   No ☐

Do you drink alcohol more than three times/week?   Yes ☐   No ☐

Is your stress level high?   Yes ☐   No ☐

Are you moderately active on most days of the week?   Yes ☐   No ☐

Do you have:

High blood pressure?   Yes ☐   No ☐

High cholesterol?   Yes ☐   No ☐

Diabetes?   Yes ☐   No ☐

Have parents or siblings who, prior to age 55 had: Yes ☐   No ☐

A heart attack?   Yes ☐   No ☐

A stroke?   Yes ☐   No ☐

High blood pressure?   Yes ☐   No ☐

To the best of my knowledge the above information is true

Signature _____

Date _____   Witness _____