The masters triathlete: Protein intake, muscle protein synthesis response and recovery from muscle-damaging exercise

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Abstract

Masters athletes are one of the most rapidly growing cohorts of athletes worldwide, particularly in endurance sport such as triathlon. Given the multidisciplinary nature of their sport, triathletes often train more than once a day; adequate recovery between training sessions is therefore important for maintaining training quality. Limited research evidence suggests that masters athletes recover at similar rates to younger athletes following fatiguing exercise such as cycling. However, following exercise that results in a degree of muscle damage, such as running, masters athletes appear to experience slower rates of muscle recovery compared to younger, similarly-trained athletes. This mode-dependant difference in recovery suggests an impairment to the repair and remodelling mechanisms within skeletal muscle of masters athletes.

Among other factors, an elevated rate of muscle protein synthesis (MPS) is central to the repair and remodelling of skeletal muscle. However, it is well known that older untrained adults display age-related attenuations, or "anabolic resistance", in the MPS response to two key anabolic stimuli: exercise and protein feeding. It is not clear whether this anabolic resistance persists in older/masters athletes and thus, whether it contributes to the poorer muscle recovery observed in this cohort. Despite it being widely accepted among researchers that older adults require ~40 g or ~0.40 $g \cdot kg^{-1}$ of protein post-exercise to maximise MPS, current sport nutrition recommendations do not differentiate between masters and younger athletes; the recommendations for all athletes, regardless of age, are that ~20 g of protein should be consumed post-exercise. Whether or not masters athletes consume this amount of protein post-exercise, and whether this currently recommended dose is sufficient to elevate MPS rates to levels equivalent of younger athletes, is yet to be determined.

Through a series of three related investigations, the present thesis had four primary aims: 1) to compare the post-exercise nutritional practices (protein and carbohydrate intake) of masters triathletes to both younger triathletes and current sport nutrition recommendations; 2) to compare the myofibrillar fractional synthetic rate (FSR) between masters and younger triathletes over a 72-hour period of endurance training following a muscle-damaging run, with timed protein and carbohydrate feedings consistent with current sport nutrition recommendations; 3) to compare the recovery of endurance cycling performance between masters and younger triathletes in a laboratory controlled environment following a muscle-damaging run at 10, 24 and 48 h following a downhill run; and, 4) to determine if repeated post-exercise intakes of higher doses of protein (3×0.6 g·kg^{-1}), compared to doses currently recommended (3×0.3 g·kg^{-1}), following muscle-damaging exercise led to enhanced same-day recovery of peak muscle function, perceptions of recovery, and afternoon cycling performance in well-trained masters triathletes.

Using a purpose designed and validated survey tool, study 1 demonstrated that masters triathletes typically consume post-exercise meals/snacks that contain a significantly lower amount of carbohydrate (0.7±0.4 g·kg^{-1}) than younger triathletes (1.1±0.6 g·kg^{-1}; p=0.01). Furthermore, these post-exercise meals/snacks fail to meet current recommendations for post-exercise carbohydrate intake (1.0 g·kg^{-1}; p=0.001). In addition, study 1 showed that masters triathletes typically consume post-exercise protein intakes that meet current sport nutrition recommendations (20±14 g), and are similar to doses consumed by younger athletes (26±16 g). However, on a relative (per kilogram of body mass) basis, masters triathletes typically consume significantly less protein (0.3±0.2 g·kg^{-1}) than younger triathletes (0.4±0.2 g·kg^{-1}; p=0.03).

In a parallel groups design, study 2 compared the recovery of cycling time trial performance at 10, 24 and 48 h following downhill running between masters and younger triathletes, while simultaneously comparing MPS rates between groups. Study 2 demonstrated that in response to triathlon training and protein (and carbohydrate) feedings in line with current sport nutrition recommendations (20 g protein administered post-exercise; 0.3 $g \cdot kg^{-1}$ protein per meal), masters triathletes exhibit a significantly lower myofibrillar FSR ($1.49 \pm 0.12\% \cdot d^{-1}$) compared to younger triathletes ($1.70 \pm 0.09\% \cdot d^{-1}$; $p=0.009$; $d=1.98$). Furthermore, masters triathletes tended to recover cycling performance more slowly than younger triathletes following muscle-damaging running in a laboratory-controlled environment. This difference in recovery was most evident on the same day as the muscle-damaging exercise, at 10 h post-run ($d=0.51$).

Finally, using a randomised, double-blind, crossover design, study 3 demonstrated that, compared to recommended post-exercise protein intakes (3×0.3 $g \cdot kg^{-1}$), repeated intakes of "higher" (3×0.6 $g \cdot kg^{-1}$) doses of protein following a morning downhill run provided a moderate beneficial effect ($d=0.66$) to attenuate the loss of knee extensor peak isometric torque measured that afternoon. Furthermore, higher protein intakes resulted in a large beneficial effect ($d=0.83$) to reduce perceptions of fatigue, measured eight hours post-run. Despite these differences, higher post-exercise protein intakes during the recovery period following downhill running had no beneficial effect ($d=-0.09$) on afternoon cycling time trial performance in masters triathletes.

Collectively considered, the present series of studies make a substantial and significant contribution to the field of sport nutrition and muscle protein metabolism, specific to masters endurance athletes. The knowledge gained from this thesis may inform training programs (appropriate recovery durations), enhance recovery rates via appropriate post-exercise

nutrition, and lead to improved performances and adaptations to training in masters athletes. The data show that chronic endurance-type training into later life by masters athletes does not appear to offset age-related impairments in muscle protein metabolism relative to young, similarly trained athletes, and that higher protein feedings post-exercise may be beneficial.

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Certificate of authorship and originality of thesis (declaration)

I declare that the work contained in this thesis has not been previously submitted either in whole or in part for a degree at any tertiary institution. To the best of my knowledge and belief, the material presented in this thesis is original, except where due reference is made in text.

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List of abbreviations

%·d^{-1}	percent per day
1RM	one repetition maximum
^{2}H	deuterium
4E-BP1	4E-binding protein 1
Akt/PKB	protein kinase B
AMPK	adenosine monophosphate kinase
ANOVA	analysis of variance
APE	atom percent excess
CI	confidence interval
CK	creatine kinase
CV	coefficient of variation
d	day
D_2O	deuterium oxide
eEF2	eukaryotic elongation factor 2
EIMD	exercise-induced muscle damage
FSR	fractional synthetic rate
g	grams
g·kg^{-1}	grams per kilogram of body mass
g·kg^{-1}·bolus^{-1}	grams per kilogram of body mass per bolus consumed
g·kg^{-1}·d^{-1}	grams per kilogram of body mass per day
g·kg^{-1}·h^{-1}	grams per kilogram of body mass per hour
GNKQ	general nutrition knowledge questionnaire
h / hr	hour(s)

h·wk^{-1}	hours per week
HPI	high protein intake
kg	kilogram
kJ	kilojoules
kJ·kg^{-1}	kilojoules per kilogram of body mass
km·h^{-1}·min^{-1}	kilometres per hour per minute
L·min^{-1}	litres per minute
M	mean
mL	millilitres
mL·kg^{-1}·min^{-1}	millilitres per kilogram of body mass per minute
mm	millimetres
mg	milligram
MPI	moderate protein intake
MPS	muscle protein synthesis
mRNA	messenger ribonucleic acid
miRNA	micro ribonucleic acid
mTOR	mammalian (mechanistic) target of rapamycin
mTORC1	mammalian (mechanistic) target of rapamycin complex 1
MVIC	maximal voluntary isometric contraction
NF-κB	nuclear factor-κB
ng·mL^{-1}	nanograms per millilitre
N·m	newton metres
p70S6K1	70 kilodalton S6 kinase 1
PIT	peak isometric torque

RH	relative humidity
RPE	rating of perceived exertion
rRNA	ribosomal ribonucleic acid
s	seconds
SD	standard deviation
TT	time trial
TQR	total quality of recovery
$U \cdot L^{-1}$	units per litre
$\dot{V}O_{2max}$	maximum oxygen uptake
vs	versus
VT2	ventilatory threshold two
W	watts
$W \cdot kg^{-1}$	watts per kilogram of body mass
$W \cdot min^{-1}$	watts per minute
W_{max}	peak aerobic power in watts
y	years

List of publications relevant to this thesis (in chronological order)

Chapter 2: Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Post-exercise dietary protein strategies to maximize skeletal muscle repair and remodeling in masters endurance athletes: A review. *International Journal of Sport Nutrition and Exercise Metabolism, 26*(2), 168-178. doi: http://dx.doi.org/10.1123/ijsnem.2015-0102. Impact Factor: 2.44; Quartile 2 in Sport Science (2014).

Chapter 3: Doering, T. M., Reaburn, P. R., Cox, G. R., & Jenkins, D. G. (2015). Comparison of post-exercise nutrition knowledge and post-exercise carbohydrate and protein intake between Australian masters and younger triathletes. *International Journal of Sport Nutrition and Exercise Metabolism, 26*(4), 338-346. doi: http://dx.doi.org/10.1123/ijsnem.2015-0289. Impact Factor: 2.44; Quartile 2 in Sport Science (2014).

Chapter 4: Doering, T. M., Jenkins, D. G., Reaburn, P. R., Borges, N. R., Hohmann, E., & Phillips, S. M. (2016). Lower integrated muscle protein synthesis in masters compared with younger athletes. *Medicine and Science in Sports and Exercise, 48*(8), 1613-1618. doi: http://dx.doi.org/10.1249/MSS.0000000000000935. Impact Factor: 3.98; Quartile 1 in Sport Science (2014).

Chapter 5: Doering, T. M., Reaburn, P. R., Borges, N. R., Cox, G. R., & Jenkins, D. G. (2016). The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes. *International Journal of Sport Nutrition and Exercise Metabolism* (In Press). doi: http://dx.doi.org/10.1123/ijsnem.2016-0079. Impact Factor: 2.44; Quartile 2 in Sport Science (2014).

List of conference presentations relevant to this thesis (in chronological order)

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Doering, T. M., Reaburn, P. R., Borges, N. R., & Jenkins, D. G. (2015b). Low carbohydrate intake of masters vs. young triathletes in the pre-competition phase of training. *Sports Medicine Australia Conference*. Presented on the 24th of October 2015. Gold Coast, Queensland, Australia.

Doering, T. M., Reaburn, P. R., Jenkins, D. G., Borges, N. R., Hohmann, E., & Phillips, S. M. (2016). Age-related 'anabolic resistance' in muscle protein synthesis exists in well-trained masters triathletes during a period of intensified training. *Exercise and Sports Science Australia, Research to Practice*. Presented on the 16th of April 2016. Melbourne, Victoria, Australia.

Doering, T. M., Reaburn, P. R., Borges, N. R., Cox, G. R., & Jenkins, D. G. (2016). Effect of high vs. moderate post-exercise protein feedings on recovery of masters athletes. *21st Congress of the European College of Sport Science*. Presented on the 6th of July 2016. Vienna, Austria.

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Chapter 1: Introduction

Background

A masters athlete is defined as an older adult who specifically trains for, and competes in organised sport (Reaburn & Dascombe, 2008). Participation in sport by older adults has increased considerably over the past several decades, as highlighted by an approximate 3.5 fold increase in participation in the World Masters Games from its inception in 1985 to 2009 (Cashman & Adair, 2009). Furthermore, a significant rise in the participation of masters athletes in events such as Ironman triathlon has recently been noted (Lepers, Rüst, Stapley, & Knechtle, 2013; Stiefel, Knechtle, & Lepers, 2014).

Together with the increased rate of participation, masters athletes are also significantly improving their performances in triathlon events (Lepers et al., 2013; Stiefel et al., 2014). For example, Stiefel et al. (2014) report a significant decrease in total triathlon time for masters triathletes (>40 years) in the Ironman Switzerland event between 1995 and 2010. Interestingly, within the masters cohort, the improvements in performances of the "older" age-groups (50-54 and 55-59 years) appear greater than in the "younger" age-groups (40-44 and 45-49 years) (Stiefel et al., 2014). For instance, total race times of the 55-59-year age-group appeared to decrease from approximately 12 h and 50 min to 11 h (~14%), while the total race times of 40-44-year age-groups appeared to decrease from approximately 10 h and 15 min to approximately 9 h and 20 min (~9%) over the same time-period (Stiefel et al., 2014). Taken together, the available data highlight the increased participation and performances of masters athletes in the sport of triathlon, particularly those over the age of 50 years.

The multisport nature of triathlon often demands more than one training session per day. Thus, expediting recovery from prior exercise is important to ensure subsequent training is of the highest quality, while also reducing the risk of injury and/or becoming over-trained (Meeusen et al., 2013). To expedite recovery from prior exercise, a number of post-exercise

recovery strategies, including nutritional strategies, are available to athletes (Vaile, Halson, & Graham, 2010). However, despite the growing population of masters endurance athletes, few studies have compared recovery rates between masters and younger athletes; thus, it is not clear whether age-specific recovery (nutritional) strategies are needed for masters athletes.

Age-related differences in recovery between masters and younger athletes

Few studies have examined potential age-related differences in the recovery rates of masters compared to younger endurance athletes from any sports (Bieuzen, Hausswirth, Louis, & Brisswalter, 2010; Easthope et al., 2010; Fell, Haseler, Gaffney, Reaburn, & Harrison, 2006; Fell, Reaburn, & Harrison, 2008; Louis, Hausswirth, Bieuzen, & Brisswalter, 2009; Sultana et al., 2012). The limited data suggest that masters endurance athletes recover muscle function and athletic performance at similar rates to younger athletes following fatiguing, non-damaging exercise, such as cycling (Borges, Reaburn, Driller, & Argus, 2016; Fell et al., 2006) and low-impact resistance training (Bieuzen et al., 2010; Louis et al., 2009). However, following exercise that results in exercise-induced muscle damage (EIMD), such as prolonged and/or undulating running, masters athletes appear to require longer to recover than younger athletes (Easthope et al., 2010). For example, Easthope et al. (2010) have shown well-trained masters runners (46±6 years) require an additional 24 h than younger similarly-trained runners (30±7 years) to recover peak force production of their knee extensors following a 55 km trail run. Indeed, the trail run within the study by Easthope et al. (2010) induced a significant degree of muscle damage in the masters athlete cohort, evidenced by an increased concentration of plasma creatine kinase (CK) to 1559±593 $U \cdot L^{-1}$ at 24 h post-race. Despite the limitations of utilising CK as a measure of muscle damage (Margaritis, Tessier, Verdera, Bermon, & Marconnet, 1999), others have shown considerably greater elevations in CK following an Ironman triathlon, increasing to 5834±3075 $U \cdot L^{-1}$ at a similar time-point in young triathletes

(34±5 years) (Suzuki et al., 2007). Given this, it appears that long distance triathlon, and potentially even training for such events, may induce comparable muscle damage to the trail run utilised by Easthope et al. (2010). Regardless, research conducted in less extreme, controlled conditions is required to confirm possible differences in recovery rates between masters and younger athletes following muscle-damaging endurance exercise.

In summary, the limited available research suggests that recovery following EIMD, or eccentric muscle contractions, is slower in masters compared to younger athletes. These data suggest that the rate of repair and remodelling of skeletal muscle, particularly the contractile apparatus that appears primarily susceptible to eccentric muscle damage (Tidball, 2011), is slower in masters compared to younger athletes. While multiple processes are involved in the repair of skeletal muscle (Zanou & Gailly, 2013) and thus recovery of muscle function following exercise, a protracted elevation in the rate of muscle protein synthesis (MPS) is particularly important (Hill, Wernig, & Goldspink, 2003; Zanou & Gailly, 2013), and is one component of muscle protein remodelling. Following exercise, muscle protein breakdown exceeds MPS, creating a negative net protein balance that favours the loss of muscle proteins (Kumar, Atherton, Smith, & Rennie, 2009). In order to achieve a net positive protein balance to favour muscle protein accretion for muscle repair and remodelling, MPS must be maximised, which can be achieved by inducing aminoacidemia via post-exercise dietary protein feeding (Kumar, Atherton, et al., 2009). However, research is yet to compare post-exercise protein intakes between masters and younger athletes, or consider any age-related differences in the MPS responses following training and protein consumption that may contribute to the age-related disparity in muscle repair, and thus recovery following EIMD.

Post-exercise nutrition for recovery: What do (masters) athletes consume?

The use of post-exercise recovery strategies, particularly nutritional strategies, appears poor among masters endurance athletes (Reaburn, Macgregor, & Climstein, 2013). Current sport nutrition guidelines developed by peak body organisations (Thomas, Erdman, & Burke, 2016), and promoted to athletes ("Recovery," 2012), recommend that endurance athletes regardless of age consume carbohydrate at a rate of 1.0-1.2 $g \cdot kg^{-1} \cdot h^{-1}$ post-exercise to maximise glycogen resynthesis and ~20 g of dietary protein to maximise skeletal muscle repair and remodelling (Beelen, Burke, Gibala, & van Loon, 2010; "Recovery," 2012; Thomas et al., 2016). Further, this may include 0.25-0.30 $g \cdot kg^{-1} \cdot meal^{-1}$ of protein to maximise daily rates of muscle protein synthesis (Thomas et al., 2016). In the context of this thesis, recommended carbohydrate and protein intakes refer to these guidelines. However, these guidelines are not age-specific (Tarnopolsky, 2008, 2015), despite widely accepted evidence that aging alters the MPS response to both exercise (Kumar, Selby, et al., 2009) and post-exercise protein feeding (Yang, Breen, et al., 2012). Regardless, it is unknown whether masters endurance athletes consume protein post-exercise in line with current sport nutrition recommendations.

Though several studies have examined the dietary practices of young endurance athletes, the focus has primarily been on carbohydrate intake (Burke, Cox, Culmmings, & Desbrow, 2001; Cox, Snow, & Burke, 2010; Havemann & Goedecke, 2008). Relatively few studies have examined the dietary practices of masters athletes (Beshgetoor & Nichols, 2003; Fell, Harrison, & Haseler, 2010), and none have examined the typical post-exercise intakes of protein and/or carbohydrate in either masters or younger athletes. Therefore, the primary aim of study 1, presented in Chapter 3 of the present thesis, was to compare the typical post-exercise intakes of protein and carbohydrate in masters triathletes (classified as triathletes aged 50 years

or greater), compared to younger triathletes (classified as triathletes aged 18-30 years), and to currently recommended sport nutrition guidelines.

Muscle protein synthesis for repair and remodelling: Does "anabolic resistance" persist in masters athletes?

Muscle repair following EIMD is influenced by many integrated processes including the acute inflammatory response, activation, proliferation and differentiation of satellite cells, and muscle protein synthetic responses (Zanou & Gailly, 2013). The MPS response is predominately dependant on intracellular mammalian (mechanistic) target of rapamycin complex 1 (mTORC1) signalling (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009), which is primarily responsive to muscle contraction and protein feeding (Rennie et al., 2010). While endurance-type exercise stimulates the mTORC1, post-exercise net protein balance remains negative until aminoacidemia, induced by protein feeding, maximises the MPS response and creates a positive net protein balance (Phillips & Van Loon, 2011). Therefore, historically the consumption of ~20 g (Beelen et al., 2010) and now up to 30 g of protein post-exercise (Thomas et al., 2016) is recommended to athletes to maximise the MPS response, and facilitate the repair of EIMD, and thus recovery following exercise.

It is well accepted that the mTORC1 signalling pathway and the MPS response is blunted in response to both exercise (Kumar, Selby, et al., 2009), and protein feedings (Moore et al., 2015), in older compared to younger healthy adults. This phenomenon has been termed "anabolic resistance". However, Burd, Gorissen and van Loon (2013) have suggested that habitual exercise into older age may restore skeletal muscles' anabolic sensitivity to amino acids. Their review suggests that lower habitual physical activity levels into older age, and not aging per se, may be responsible for the age-related anabolic resistance observed in healthy, but untrained older individuals. However, this hypothesis is yet to be tested in well-trained

older populations such as masters athletes. Indeed, if physical activity into older age can offset age-related anabolic resistance and thus lower rates of protein synthesis, masters athletes should display similar rates of MPS to those of similarly-trained younger athletes in response to exercise training and the recommended dietary protein feedings post-exercise and per meal. However, if masters athletes continue to display relative anabolic resistance to protein feedings and/or exercise, the resulting lower MPS rates may, at least partially, be responsible for the slowed post-exercise recovery in masters compared to younger athletes following eccentric EIMD (Baumann, Rogers, Otis, & Ingalls, 2016). Therefore, the aim of study 2, presented in Chapter 4 of the present thesis was to compare the myofibrillar fractional synthetic rate (FSR) of masters (50-60 years) and younger (18-30 years), well-trained, long distance triathletes (Half-Ironman and Ironman distance) over a 72-hour period of endurance training following a muscle-damaging downhill run, with timed protein feedings consistent with current sport nutrition recommendations. In order to determine potential age-related differences in the recovery of endurance cycling performance following EIMD, multiple cycling time trials were assessed in a controlled laboratory environment following the muscle-damaging run, at 10, 24 and 48 h post-run.

Dose-response of post-exercise protein to muscle protein synthesis: Is more protein better for masters athletes?

In younger adults, a dose-response is observed between dietary protein intake and elevations in MPS. Specifically, elevations in myofibrillar FSR, which appear exclusively responsive to protein feedings (Breen et al., 2011), are evident until protein doses reach ~20 g (Beelen et al., 2010; Witard et al., 2014) or ~0.25 $g \cdot kg^{-1}$ (Phillips, 2014). In young cohorts, protein doses higher than 20 g appear to merely increase amino acid oxidation, and the additional protein does not appear to contribute to protein synthesis (Witard et al., 2014).

However, older adults appear less responsive to these lower doses of protein, or at least the corresponding amounts (~7-10 g) of isolated essential amino acids (Cuthbertson et al., 2005; Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2005). Several studies have shown that the dose-response between protein intake and MPS is altered in older adults; specifically, myofibrillar FSR continues to increase with protein intakes higher than 20 g in older, healthy, but untrained adults (Pennings et al., 2012; Robinson et al., 2013; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012). For example, doses of protein up to 40 g when consumed post-exercise (Yang, Breen, et al., 2012), or ~0.4 $g \cdot kg^{-1}$ when rested (Moore et al., 2015), continue to elicit increases in myofibrillar FSR in older individuals. Thus, given the available literature, it is reasonable to suggest that masters athletes may also require post-exercise protein doses higher than 20 g to maximise MPS for muscle repair and remodelling.

Research in younger cohorts has shown that post-exercise consumption of approximately 20 g of milk-based protein added to a carbohydrate supplement, may enhance subsequent endurance-based performance. For example, Lunn et al. (2012) have shown that, in comparison to a carbohydrate only beverage, the consumption of an isocaloric protein (16 g) and carbohydrate beverage following a 45 min steady-state run, enhanced run time to exhaustion at VO_{2max} by 23%, 3 h later in a cohort of young (21±1 years) recreational runners. This study also showed that, in a similar cohort, consumption of this protein and carbohydrate supplement resulted in a 38% increase in mixed muscle FSR compared to carbohydrate supplement (Lunn et al., 2012). The authors did not conclude whether these findings were related. Similarly, Ferguson-Stegall et al. (2011) have found comparable results in young (32±2 years), trained cyclists. These researchers have shown that consuming a milk-based protein drink over a 4 h period following a 1.5 h moderate to high intensity cycling bout, significantly improved subsequent 40 km cycling time trial performance in comparison to an isocaloric

carbohydrate drink. Furthermore, protein supplementation increased phosphorylation of markers indicative of muscle protein synthesis (Ferguson-Stegall et al., 2011). In addition to enhancements in performance, others have suggested protein supplementation may decrease perceptions of muscle soreness following running (Millard-Stafford et al., 2005). Indeed, this effect may be more pronounced in masters athletes that have been shown to possess impaired perceptions of recovery following endurance exercise, when compared to young athletes (Fell, Reaburn, & Harrison, 2008). In summary, research supports the use of milk-based protein supplementation, even at low doses, to facilitate recovery of exercise performance in younger cohorts. Markers of MPS, or measured MPS responses have also been shown elevated in these studies where performance improvements have been noted; whether these two observations may be related is unknown. However, if a more anabolic environment is related to recovery of exercise performance, masters athletes may benefit from higher, age-specific protein doses as suggested above.

To date, research is yet to investigate whether providing greater post-exercise protein doses, which the current research suggests should maximise acute elevations in MPS, results in meaningful benefits to recovery of muscle function or exercise performance in masters athletes following EIMD. In particular, it is yet to be determined whether greater post-exercise protein intakes, delivered in a manner to maximise the MPS response (Areta et al., 2013), result in any meaningful benefit to recovery of performance over recovery periods of ~8-10 h, typically observed in triathletes who train both morning and afternoon. Therefore, the aim of study 3, presented in Chapter 5 of the present thesis was to compare the effect of repeated "high" (3×0.6 g·kg^{-1}) versus "moderate" (3×0.3 g·kg^{-1}) protein intakes following morning muscle-damaging downhill running, on recovery of afternoon peak isometric torque of the knee

extensors (PIT), perceptions of recovery, and cycling time trial performance in well-trained, standard (Olympic) and Half-Ironman distance masters triathletes aged 50 years or older.

Summary of the research problem

Masters athletes are a rapidly growing cohort within competitive endurance sport. However, little empirical and applied sport science research has been focused on this growing population. The limited available research suggests that the rate of recovery in masters and younger athletes is similar following fatiguing but non-muscle damaging exercise such as cycling. However, following muscle-damaging exercise such as running, masters athletes appear to require longer durations to recover muscle function than younger, similarly-trained endurance athletes. This suggests masters athletes may exhibit slower rates of skeletal muscle repair and remodelling than younger athletes. However, factors contributing to muscle repair and remodelling, including protein intake and skeletal muscle protein metabolism, remain to be investigated in masters athletes. This slower rate of recovery in masters athletes following muscle-damaging exercise may, at least partially, be due to: 1) poor post-exercise nutritional practices; or 2) age-related factors impairing skeletal muscle repair and remodelling processes, such as "anabolic resistance". Whether or not higher doses of post-exercise protein aimed at maximising the MPS response in masters athletes confer any benefit to the recovery of muscle function and exercise performance in masters athletes also remains to be determined.

Methodological approach to the problem

Given the available evidence, a methodological approach was developed to explore the proposal that poor post-exercise nutritional practices and/or impaired protein metabolism in masters endurance athletes slow their recovery from muscle-damaging exercise compared to younger, similarly-trained athletes. The four subsequent chapters in this present thesis address these issues.

Firstly, a comprehensive review of the literature was conducted to explore, in detail, the available research evidence regarding: 1) age-related differences in recovery rates of masters compared to younger athletes following muscle-damaging endurance exercise; 2) the intracellular signalling pathway (mTORC1) regulating elevations in MPS, and the responsiveness of the mTORC1 and MPS to endurance-type exercise; 3) the underpinning mechanisms of age-related anabolic resistance and the effect of acute and chronic endurance training on the attenuation of anabolic resistance; and, 4) the potential implications of anabolic resistance to protein feedings for masters athletes. The review of the literature comprises Chapter 2 of this thesis and has been published in the *International Journal of Sport Nutrition and Exercise Metabolism*.

Secondly, with the support of *Triathlon Australia*, an Australia-wide online survey was distributed to all currently registered and competing triathletes. Important to this thesis, the survey sought information relating to the typical post-exercise nutritional practices of masters triathletes compared to both younger triathletes, and current sport nutrition guidelines. This research comprises Chapter 3 of this thesis, and has also been accepted for publication in the *International Journal of Sport Nutrition and Exercise Metabolism*.

Thirdly, a laboratory-controlled, parallel-groups experimental trial was conducted to compare: 1) the myofibrillar FSR between well-trained masters and younger triathletes over a 72 h period of training following a muscle-damaging run, with timed protein and carbohydrate feedings consistent with current sport nutrition recommendations; and, 2) the recovery of endurance cycling performance between masters and younger triathletes in a laboratory controlled environment following a muscle-damaging run at 10, 24 and 48 h following a downhill run. This research comprises Chapter 4 of this thesis, and has been published in *Medicine and Science in Sports and Exercise*.

Finally, a double-blind, randomised, cross-over designed trial examined the effect of repeated "high" (3 × 0.6 g·kg^{-1}) verses "moderate" (3 × 0.3 g·kg^{-1}) protein feedings over an 8 h recovery period following a standardised muscle-damaging running bout, on the recovery of: 1) peak isometric torque of the knee extensors; 2) perceptions of recovery; and 3) afternoon cycling performance in well-trained masters triathletes. This research comprises Chapter 5 of this thesis, and has been accepted for publication in the *International Journal of Sport Nutrition and Exercise Metabolism*.

Research questions

Based on the currently available literature, this thesis systematically addresses the following research questions:

1. Are the typical post-exercise nutritional intakes of masters triathletes consistent with current protein and carbohydrate recommendations for athletes?
2. Does chronic endurance training, as undertaken by well-trained masters triathletes, counteract age-related anabolic resistance?
3. Is there an age-related difference in recovery of cycling performance following exercise-induced muscle damage induced by downhill running, between masters and younger well-trained triathletes, when examined in a laboratory controlled, standardised environment?
4. Does the repeated consumption of "high" (3 × 0.6 g·kg^{-1}) protein intakes following morning muscle-damaging running provide any benefit to acute (8 h) recovery of peak muscle function, perceptions of recovery and cycling performance compared to "moderate" and recommended (3 × 0.3 g·kg^{-1}) protein intakes in masters triathletes?

Experimental hypotheses

The experimental hypotheses relating to the above research questions, in order, are:

1. The typical post-exercise nutritional practices of masters (and younger) triathletes will not be consistent with current protein and carbohydrate recommendations for athletes.
2. When compared to younger athletes, masters triathletes will show age-related anabolic resistance, evidenced by a lower myofibrillar fractional synthetic rate.
3. Masters triathletes will recover cycling performance at a slower rate following exercise-induced muscle damage induced by downhill running, compared to younger similarly-trained triathletes.
4. The repeated consumption of "high" (3×0.6 g·kg^{-1}) protein intakes following morning muscle-damaging running will enhance acute (8 h) recovery of peak muscle function, perceptions of recovery and cycling performance compared to "moderate" and recommended (3×0.3 g·kg^{-1}) protein intakes in masters triathletes.

DECLARATION OF CO-AUTHORSHIP AND CONTRIBUTION

Title of Paper	Post-exercise dietary protein strategies to maximize skeletal muscle repair and remodeling in masters endurance athletes: A review
Full bibliographic reference for Journal/Book in which the Paper appears	Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Post-exercise dietary protein strategies to maximize skeletal muscle repair and remodeling in masters endurance athletes: A review. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(2), 168-178.
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Nature of Candidate's Contribution

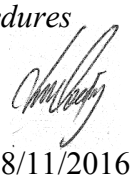
Thomas M. Doering co-conceived the idea for the review of literature, undertook the literature search, drafted the manuscript in full, edited the manuscript based on co-author feedback, submitted the manuscript, and responded to reviewer comments.

Nature of Co-Authors' Contributions

Peter R. Reaburn, David G. Jenkins and Stuart M. Phillips co-conceived the idea for the review of literature, reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscript, and reviewed responses to reviewer comments.

Candidate's Declaration

I declare that the publication above meets the requirements to be included in the thesis as outlined in the Publication of Research Higher Degree Work for Inclusion in the Thesis Procedures

Signature: 

Date: 08/11/2016

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Chapter 2 – Literature review: Post-exercise dietary protein strategies to maximise skeletal muscle repair and remodeling in masters endurance athletes: A review

This chapter is an exact copy of the manuscript that has been published in the *International Journal of Sport Nutrition and Exercise Metabolism*.

Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Post-exercise dietary protein strategies to maximize skeletal muscle repair and remodeling in masters endurance athletes: A review. *International Journal of Sport Nutrition and Exercise Metabolism, 26*(2), 168-178. doi: http://dx.doi.org/10.1123/ijsnem.2015-0102. Impact Factor: 2.44; Quartile 2 in Sport Science (2014).

Referencing format has been altered to conform to APA 6th edition. As this manuscript has been published, the references included in this manuscript are not also contained in the final references list of this thesis, but appear at the end of this chapter. Figure and Table numbers in this manuscript have been altered to also align with chapter numbers.

Abstract

Participation rates of masters athletes in endurance events such as long distance triathlon and running continue to increase. Given the physical and metabolic demands of endurance training, recovery practices influence the quality of successive training sessions, and consequently, adaptations to training. Following muscle-damaging endurance exercise, research suggests masters athletes experience slower recovery rates in comparison to younger similarly-trained athletes. Given these discrepancies in recovery rates are not observed following non-muscle-damaging exercise, it is suggested that masters athletes have impairments of the protein remodeling mechanisms within skeletal muscle. The importance of post-exercise protein feeding for endurance athletes is being increasingly acknowledged, and its role in creating a positive net muscle protein balance post-exercise well known. The potential benefits of post-exercise protein feeding include elevating muscle protein synthesis and satellite cell activity for muscle repair and remodeling, as well as facilitating muscle glycogen resynthesis. Despite extensive investigation into age-related anabolic resistance in sedentary aging populations, little is known about how anabolic resistance affects post-exercise muscle protein synthesis, and thus muscle remodeling in aging athletes. Despite evidence to suggest physical training can attenuate, but not eliminate age-related anabolic resistance, masters athletes are currently recommended to consume the same post-exercise dietary protein dose (~20 g or 0.25 $g \cdot kg^{-1} \cdot meal^{-1}$) as younger athletes. Given the slower recovery rates of masters athletes following muscle-damaging exercise, which may be due to impaired muscle remodeling mechanisms, masters athletes may benefit from higher doses of post-exercise dietary protein, with particular attention directed to the leucine content of the post-exercise bolus.

Keywords

Aging, muscle protein synthesis, exercise recovery.

Introduction

A masters athlete is defined as an older athlete who specifically trains for and competes in organized sport (Reaburn & Dascombe, 2008). Over recent decades, masters athletes have been increasingly participating in endurance-type sports such as marathon running (Lepers & Cattagni, 2012) and Ironman triathlon (Lepers, Rüst, Stapley, & Knechtle, 2013). For example, significant increases in participation rates have been noted at both Ironman Switzerland (Stiefel, Knechtle, & Lepers, 2014) and the Hawaii Ironman world championship (Lepers et al., 2013) over a 16- and 25-year period to 2010, respectively. Given a recent report that masters athletes represented 56% and 47% of male and female race finishers respectively at the 2010 Ironman world championship (Lepers et al., 2013), it is clear that increases in participation are at a global level.

Despite performances of these masters athletes improving relative to previous years (Lepers et al., 2013), recovery from exercise, both perceptually (Fell, Reaburn, & Harrison, 2008) and physiologically (Easthope et al., 2010), appears slower in masters compared to similarly-trained younger athletes. For example, impaired recovery of both muscular strength and exercise performance is evident following trail running (Easthope et al., 2010), which involves lengthening contractions known to result in structural muscle damage (Tidball, 2011). Given the degree of muscle damage experienced following a given relative intensity exercise stimulus appears similar between younger and masters athletes (Easthope et al., 2010; Fell, Haseler, Gaffney, Reaburn, & Harrison, 2006), we propose that masters athletes may have an impaired ability to repair and remodel proteins within skeletal muscle. This impaired remodeling may, at least in part, be responsible for the poorer muscle recovery observed in

older athletes. Thus, attention to more specific post-exercise dietary strategies, such as age-specific protein feedings in masters athletes may improve muscle recovery, subsequent exercise performance, and training adaptations.

Recovery of masters athletes following endurance exercise

When post-exercise recovery is monitored following exercise such as cycling that results in neuromuscular fatigue, but not necessarily muscle damage, recovery rates are similar between masters and younger athletes (Fell et al., 2006). For example, Fell and colleagues (2006) reported no difference in recovery of cycling performance between masters (45±6 years) and young (24±5 years) cyclists following three 20 km cycling time trials conducted over three consecutive days. In contrast, when post-exercise recovery is monitored following exercise that results in exercise-induced muscle damage (EIMD), such as long distance running, research suggests masters athletes take longer to recover compared to similarly-trained younger athletes (Easthope et al., 2010). Moreover, while the degree of muscle damage following a given, relative exercise stimulus may be similar between younger and masters athletes (Easthope et al., 2010; Fell et al., 2006), regeneration or repair of musculotendinous tissue appears slower in older individuals (Brisswalter & Nosaka, 2013).

Few studies to date have assessed muscle recovery following muscle-damaging endurance exercise in masters compared to younger athletes. However, of those studies that have, no studies have assessed the efficiency of the repair and remodeling mechanisms post-exercise. For example, Easthope and colleagues (2010) reported that masters runners (46±6 years) required an additional 24 h to recover maximal voluntary isometric contraction (MVIC) torque of the knee extensors following a 55 km trail running competition in comparison to younger runners (30±7 years) matched for performance. Despite suggestions that the delayed recovery in MVIC observed in masters athletes was due to neuromuscular factors, it is known

that prolonged running such as that completed by participants will result in structural muscle damage due to the associated lengthening muscle actions, and subsequently result in a loss of force generating capacity (Close, Kayani, Vasilaki, & McArdle, 2005). Similar findings have also been reported in non-athletic populations with older adults (64±1 years) requiring an additional 24 h to recover maximal strength of their elbow flexors in comparison to younger adults (25±2 years) following 30 maximal voluntary lengthening contractions (Chapman, Newton, McGuigan, & Nosaka, 2008). However, in contrast to these studies, Sultana et al. (2012) reported comparable recovery of knee extensor and flexor MVIC torque between masters (52±10 years) and younger (28±6 years) well-trained triathletes 24 h following an Olympic distance triathlon (Sultana et al., 2012). In contrast, masters triathletes in the same study exhibited significantly reduced running speed (-8.3%) at ventilatory threshold two (VT2) in comparison to younger triathletes who recovered to baseline run speed at VT2 24 h post-exercise (Sultana et al., 2012). This finding holds significant practical implications to endurance performance given the high correlation (r=-0.95) between ventilatory threshold and running performance (Loat & Rhodes, 1993). In summary, masters athletes generally experience slower rates of muscle recovery following endurance exercise that results in EIMD to contractile and connective tissue, but not following fatiguing, non-damaging exercise, when compared to similarly-trained younger athletes.

In light of the available evidence, impaired muscle protein remodeling (i.e., protein synthesis) mechanisms may be responsible for the slower post-exercise recovery of masters athletes following muscle-damaging exercise. This suggestion was recently supported within a review by Brisswalter and Nosaka (2013) who proposed that the damage to the contractile elements of muscle persist for longer in the masters athlete compared to younger, similarly-trained athletes. A review of the factors contributing to muscle repair following muscle-damaging exercise is beyond the scope of this review, although can be found elsewhere (Zanou

& Gailly, 2013). We acknowledge other factors such as lower satellite cell activity with increasing age may also contribute to the delayed recovery of skeletal muscle in older adults/athletes versus younger individuals (Fry et al., 2015). However, critical to the remodeling of skeletal muscle following muscle damage, is a protracted stimulation of muscle protein synthesis (MPS) that is observed following endurance exercise.

Mammalian target of rapamycin complex 1 and the subsequent muscle protein synthetic response to endurance exercise and feeding

The mammalian (mechanistic) target of rapamycin complex 1 (mTORC1) is a central protein in an intricate signaling pathway that integrates contractile and amino acid (feeding) signals that are imperative in mRNA translation and subsequently, MPS. The mTORC1 receives signals resulting from the systemic milieu (hormonal, aminoacidemia), and its activation results in phosphorylation of downstream targets to effect a MPS response (Figure 2.1). Importantly, both muscle contraction (mechanotransduction) and amino acid feeding can independently stimulate the mTORC1 via upstream signaling (Figure 2.1). However, when post-exercise dietary protein feeding occurs, contractile and amino acid stimuli work synergistically to confer additional stimulation of the mTORC1 signaling pathway, leading to increased rates of MPS than either stimulus alone. Independent of the upstream signal stimulating the mTORC1, downstream targets of mTORC1, namely the 70 kDa S6 kinase 1 (p70S6K1) and 4E-binding protein 1 (4E-BP1), are rate limiting to mRNA translation. As such, the phosphorylation status of these two kinases are often used as "readouts" of mTORC1 activity and have been suggested as indirect proxy markers of MPS. While a review of the primary and secondary molecular signaling events leading to increased MPS are beyond the scope of the present review, Figure 2.1 provides an abbreviated schematic overview of

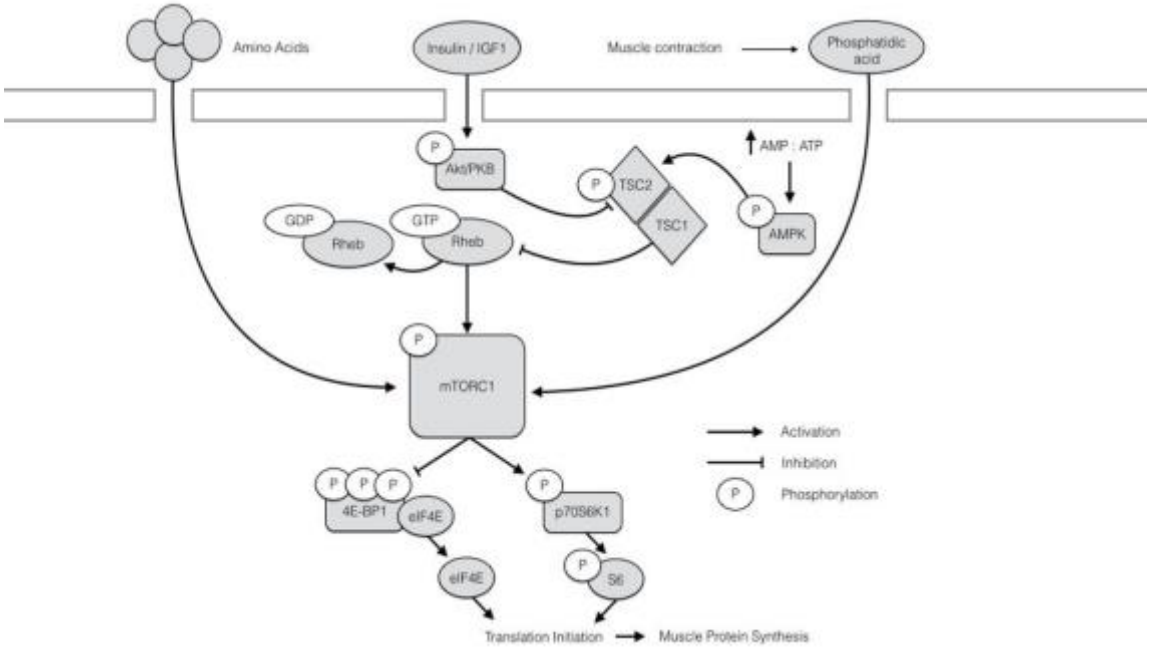

Figure 2.1: Schematic overview of relevant signal transduction pathways in skeletal muscle with exercise and protein feeding leading to protein synthesis. Akt/PKB = protein kinase B, AMPK = Adenosine monophosphate kinase, eIF4E = eukaryotic initiation factor 4E, GDP = guanosine diphosphate, GTP = guanosine triphosphate, IGF1 = Insulin like growth factor 1, mTORC1 = mammalian (mechanistic) target of rapamycin complex 1, p70S6K1 = 70 kDa S6 kinase 1, Rheb = Ras homolog enriched in brain, S6 = S6 ribosomal protein, TSC 1/2 = tuberous sclerosis 1/2, 4E-BP1 = 4E-binding protein 1.

signaling events; however, comprehensive reviews can be found elsewhere (Coffey & Hawley, 2007; Jacobs, Goodman, & Hornberger, 2014).

Substantial data shows that the mTORC1 is activated following both resistance and endurance-type stimuli. Mascher et al. (2007) reported that 1 h of cycling at 75% $\dot{V}O_{2max}$ increased phosphorylation of proteins in the mTORC1-p70 signaling axis, and significantly decreased phosphorylation of eukaryotic elongation factor 2 (eEF2) in the early post-exercise period. Furthermore, Camera et al. (2010) have shown the acute phosphorylation status of

Akt$^{Thr308/Ser473}$ and mTOR^{Ser2448} to be comparable following cycling at 70% $\dot{V}O_{2max}$, and high load (80% 1RM) resistance training. Cycling exercise similarly increased p70S6K^{Thr389} phosphorylation (62%–140%) 1 h following exercise despite a concomitant increase in AMPK^{Thr172} phosphorylation, which was seen following cycling exercise only (Camera et al., 2010). Taken together, these results suggest that endurance exercise can mediate mTORC1 signaling to a similar extent to that of resistance training, despite increases in AMPK phosphorylation, which has been suggested to inhibit mTORC1 phosphorylation (Atherton et al., 2005; Dreyer et al., 2006).

In addition to elevating mTORC1 signaling, endurance exercise has been shown to promote MPS (Table 2.1). A study by Mascher et al. (2011) found similar patterns of phosphorylation in mTOR^{Ser2448}, p70S6K^{Thr389}, and eEF2^{Thr56} in line with their previous work (Mascher et al., 2007); however, they also reported a significant concomitant increase in mixed muscle fractional synthetic rate (FSR) at 120 min following 1 h of single-leg cycling at 65-70% $\dot{V}O_{2max}$. Di Donato and colleagues (2014) also found myofibrillar FSR to be elevated following both "low" and "high" intensity endurance cycling in an intensity-dependent manner, with "high" intensity cycling (60% W_{max}) elevating myofibrillar FSR up to 28 h. In contrast, "low" intensity cycling (30% W_{max}) elevated myofibrillar FSR for only 4.5 h post-exercise. Furthermore, high intensity cycling produced a significantly greater myofibrillar and mitochondrial FSR than did low intensity cycling (Di Donato et al., 2014). As shown in Table 2.1, there are a number of other research groups reporting an elevated MPS response following low and moderate intensity endurance-type exercise with and without protein feeding.

Table 2.1: Acute effects of endurance exercise with or without nutrient interaction on post-exercise skeletal muscle protein synthesis.

Study	*n*	Age (year; *M* ±*SD*)	Exercise mode	Duration (min)	Intensity (% $\dot{V}O_{2max}$)	Feeding	Protein fraction	Timepoint assessed	Post-exercise FSR compared with rest
Carraro et al. (1990)	6	29 ± 3	Walking	240	40	No	Mixed	0–240 min	Increased
Sheffield-Moore et al. (2004)	6	29 ± 2	Walking	45	40	No	Mixed	10–180 min	Increased to 60 min
Sheffield-Moore et al. (2004)	6	69 ± 1	Walking	45	40	No	Mixed	10–180 min	Increased to 10 min
Miller et al. (2005)	6–8	25 ± 1	One-leg kicking	60	67[a]	No	Myo & sarc	6–72 hr	Myo increased to 72 hr; sarc increased to 48 hr
Durham et al. (2010)	9	30 ± 2	Walking	45	40	No	Mixed	10–180 min	Increased
Durham et al. (2010)	8	67 ± 2	Walking	45	40	No	Mixed	10–180 min	Increased
Mascher et al. (2011)	16	25 ± 2	One-leg kicking	60	65–70	No	Mixed	0–90 min & 90–180 min	Increased at 90–180 min
Di Donato et al. (2014)	8	21 ± 1	Cycling	60 & 30	30 (low) & 60 (high)[a]	No	Myo & mito	30–270 min & 24–28 hr	Myo increased at 30–270 min at both intensities and at 24–28 hr only with high intensity. Mito increased by high intensity at 24–28 hr only
Harber et al. (2010)	8	25 ± 1	Cycling	60	72	Yes	Mixed	2–6 hr	Increased with and without PRO
Coffey et al. (2011)	8	21 ± 3	Cycling	6	10 × 6[b]	Yes	Myo & mito	15–240 min	Myo increase with PRO feeding
Breen et al. (2011)	10	29 ± 6	Cycling	90	77	Yes	Myo & mito	0–240 min	Myo increase with PRO feeding
Rowlands et al. (2015)	12	30 ± 7	Cycling	100	70–85	Yes	Myo	0–240 min	Increased with PRO feeding

FSR = fractional synthetic rate, mito = mitochondrial, mixed = mixed muscle fractions, myo = myofibrillar, PRO = protein, sarc = sarcoplasmic; $\dot{V}O_2max$ = maximal oxygen uptake. [a]% Wmax. [b]s maximal.

In addition to mechanotransduction, the mTORC1 can be stimulated by hyperaminoacidemia, and thus protein feeding (Yang, Breen, et al., 2012) (Figure 2.1). Several studies have shown that pre- (Coffey et al., 2011) or post-exercise (Breen et al., 2011; Rowlands et al., 2015) protein-carbohydrate consumption further stimulates myofibrillar FSR following endurance exercise in comparison to isocaloric carbohydrate feeding (Table 2.1). Recently, Rowlands and colleagues (2015) showed that a mixed macronutrient beverage containing 23 g of protein increased myofibrillar FSR by 33% compared to an isocaloric carbohydrate control beverage following 100 min of high intensity cycling in an endurance-trained cohort (30±7 years). Taken together, the provision of protein before and/or following an endurance exercise bout appears to increase both mTORC1 activation and MPS. These observations (Breen et al., 2011; Rowlands et al., 2015) suggest that post-exercise protein consumption facilitates muscle protein remodeling and may facilitate both repair from muscle damage and exercise adaptation (Moore, Camera, Areta, & Hawley, 2014). Indeed, in some cases endurance exercise has been found to result in muscle hypertrophy (Konopka & Harber, 2014); however, increases in MPS following endurance exercise appear to reflect muscle remodeling, and often not muscle hypertrophy per se. We propose that deficits "downstream" of the mTORC1 noted in older adults, may result in lower rates of MPS in masters athletes in response to both protein feeding and exercise stimuli independently or concomitantly.

Age-related "anabolic resistance"

Muscle contraction and amino acid feeding are the two primary stimuli regulating anabolic responses in young healthy adults on an hour-to-hour basis (Rennie et al., 2010). In older adults, age-related anabolic resistance may compromise the ability of older muscle to elevate MPS to the same rate as younger muscle in response to muscle contraction (Kumar et al., 2009), and/or to amino acid feeding (Burd et al., 2013). Several studies have proposed that

the mTORC1-p70 signaling axis is impaired in healthy older adults in response to exercise (Kumar et al., 2009), and to protein feeding with both basal insulin clamps (Cuthbertson et al., 2005), and during hyperinsulinemia at rest (Guillet et al., 2004). For example, Kumar et al. (2009) reported a blunting of p70S6K1 and 4E-BP1 phosphorylation following resistance training at intensities between 60-90% 1RM in recreationally active older (70±5 years) compared to younger (24±6 years) adults. Similarly, following the provision of 10 g of essential amino acids, Cuthbertson and colleagues (2005) observed a down-regulation of mTOR and p70S6K1 phosphorylation in elderly adults (70±1 years) compared to young adults (28±1 years). Furthermore, Guillet et al. (2004) also observed blunted p70S6K1 phosphorylation and lower mixed muscle FSR in healthy older (72±2 years) compared to younger adults (25±1 years) in both the post-absorptive state, and following feeding with induced hyperinsulinemia.

Taken together, these studies suggest that age-related impairments within the mTORC1 pathway, specifically at 4E-BP1 and p70S6K1, are observed in older compared to younger individuals following exercise and/or protein feeding. Given phosphorylation status of p70S6K1^{Thr389} has been show predictive of myofibrillar FSR in endurance-trained young men (Rowlands et al., 2015), impairments at this kinase may result in lower rates of MPS and thus slower recovery from muscle-damaging exercise.

Effect of endurance exercise on age-related anabolic resistance

Previous research has suggested that the age-related decrease in physical activity levels, not aging per se, may be the underlying cause of anabolic resistance (Burd et al., 2013). This hypothesis is supported by data from Wall and colleagues (2013) who recently found 14 days of leg immobilization results in a 31±12% reduction in postprandial MPS in response to ingestion of 20 g of dietary protein in healthy young adults. Furthermore, Breen and colleagues (2013) recently confirmed that even a relative degree of inactivity in elderly males (72±1 years)

resulted in a 26% reduction in postprandial myofibrillar FSR and a 3.9% loss of lean muscle mass over a 14-day period. Given such large reductions in MPS following short-term inactivity or limb immobilization, it is clear that maintenance of physical activity into aging is important to reduce the effects of age-related anabolic resistance. However, whether or not increased levels of physical activity into older age can completely negate anabolic resistance is yet to be determined, and current evidence is lacking to suggest this is the case.

Many studies have examined muscle protein metabolism following endurance exercise (Table 2.1). However most, but not all (Breen et al., 2011; Coffey et al., 2011; Rowlands et al., 2015) of these previous studies have used "untrained" participants (De Pauw et al., 2013). Moreover, the effect of acute or chronic low volume endurance exercise on protein metabolism has rarely been compared between younger and older adults (Durham et al., 2010; Sheffield-Moore et al., 2004; Short, Vittone, Bigelow, Proctor, & Nair, 2004). The available research suggests that endurance exercise results in acute post-exercise increases in markers of MPS (Mascher et al., 2011), and increases in MPS above resting levels in young and older adults alike (Sheffield-Moore et al., 2004). However, when MPS rates of older adults are compared to younger but otherwise physiologically-matched adults, older adults display lower rates of, or a lower total capacity to elevate MPS following acute exercise bouts. For example, Sheffield-Moore and colleagues (2004) found that low intensity walking stimulated mixed muscle FSR above resting levels in both younger (29±2 years) and older adults (69±1 years). However, in contrast to older adults in which they observed a transient increase in MPS until 10 min post-exercise (a measurement that included exercise), younger adults maintained elevated rates of MPS for 60 min (Sheffield-Moore et al., 2004). Moreover, Durham and colleagues (2010) used a similar exercise protocol to Sheffield-Moore et al. (2004) and found older adults (67±2 years) displayed similar increases in $mTOR^{Ser2448}$ phosphorylation and mixed muscle FSR to younger adults (30±2 years) post-exercise. However, older adults showed

a 40% reduction in "anabolic efficiency" in comparison to younger adults (Durham et al., 2010).

Similar to the protein synthetic responses reported following acute endurance exercise, Short and colleagues (2004) reported that a 16 week aerobic exercise intervention significantly elevated resting mixed muscle FSR to a similar extent in older (60-74 years) and younger adults (19-38 years). However, given pre-intervention findings that MPS declined at a rate of 3.5% per decade, and similar post-intervention increases in MPS occurred, this suggests that despite retaining plasticity, reduced total anabolism in comparison to young adults persists despite training. Taken together, these studies suggest that age-related anabolic resistance, or reduced anabolic capacity with aging, persists following both acute endurance exercise bouts and chronic moderate-intensity endurance training interventions in previously untrained but healthy older adults.

Effect of age-related anabolic resistance on amino acid/protein feeding

In younger adults, MPS appears to be maximally stimulated with a bolus of ~20 g of protein (van Loon, 2013). In contrast, current research suggests that protein doses of ~35 g are necessary to stimulate MPS above post-absorptive levels in healthy middle-aged (Robinson et al., 2013) and elderly adults (Pennings et al., 2012). Support for these statements is found in a recent study by Moore and colleagues (2015) who suggest that older adults (71±1 years) require ~0.40 $g \cdot kg^{-1}$ of body mass of protein to maximize MPS, in contrast to younger adults (22±4 years) requiring protein boluses of ~0.24 $g \cdot kg^{-1}$ of body mass (Moore et al., 2015). These findings may explain reports of comparable MPS responses in young and older adults after protein feeding, as these studies have often provided large doses of protein (30 g) (Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009) or amino acids (15 g) (Paddon-Jones et al., 2004). Therefore, while older adults may be capable of MPS responses similar to those of

younger adults when large doses of protein are consumed, this may not be the case following exercise when lower amounts of protein (~20 g) are recommended; as such, post-exercise MPS responses may be compromised in masters athletes when this cohort consume post-exercise protein doses recommended for younger athletes.

In summary, age-related anabolic resistance to both exercise and protein/amino acid feeding is likely related to age-related deficits in the mTORC1 pathway that in turn reduces the MPS response to these stimuli. Despite suggestions that habitual physical activity into older age may ameliorate anabolic resistance, older adults/athletes undertaking endurance exercise seem unlikely to achieve "youthful" rates of anabolism regardless of training status. Thus, anabolic resistance is likely to reduce muscle protein remodeling (muscle repair) in masters athletes, which in turn may slow muscle recovery. As such, we suggest that altered post-exercise and daily feeding patterns may be necessary for masters athletes to maximize muscle remodeling and enhance muscle recovery following muscle-damaging exercise.

Potential implications to post-exercise protein feeding for the masters endurance athlete

Current sports nutrition guidelines suggest that endurance athletes should consume 1.0-1.2 $g \cdot kg^{-1}$ of carbohydrate per hour post-exercise to maximize muscle glycogen resynthesis (Rodriguez, Di Marco, & Langley, 2009). In addition, ~20 g of dietary protein should be consumed in the immediate post-exercise period to maximize muscle protein remodeling (van Loon, 2013). Furthermore, recent evidence suggests that sufficient protein intake should be repeated on multiple occasions per day, at evenly spaced intervals to maximize daily MPS (Areta et al., 2013). These guidelines are currently suggested to hold-true for all endurance athletes regardless of age (Tarnopolsky, 2008).

Despite short-term physical training attenuating anabolic resistance in comparison to age-matched sedentary counterparts (Short et al., 2004), the available evidence suggests that, despite physical training into older age, masters athletes continue to display anabolic resistance to muscle contraction and protein feeding (see previous section). Thus, ~20 g of post-exercise protein is unlikely to maximize MPS in masters athletes. Should contraction-induced MPS be down-regulated in older athletes, higher than currently recommended doses of post-exercise protein will, based on current evidence (Yang, Breen, et al., 2012), further stimulate MPS in this population to facilitate a positive net muscle protein balance, and muscle protein remodeling to support a more rapid recovery from exercise. While this is in contrast to a recent review suggesting no evidence of enhanced muscular recovery following protein supplementation in young adults (Pasiakos, Lieberman, & McLellan, 2014), we propose that this may not be the case with masters athletes. A recent review by Pasiakos and colleagues (2014) was limited to studies involving participants <50 years of age, and the small sample sizes and large variation in study designs make the findings difficult to interpret. Contrary to suggestions by Pasiakos and colleagues (2014), Moore et al. (2014) recently argued the importance of dietary protein for endurance athletes, facilitating myofibrillar and mitochondrial remodeling, and achieving a net positive protein balance. We suggest this may be of even greater importance to masters endurance athletes who may require higher protein doses to stimulate MPS for muscle remodeling following muscle-damaging exercise.

Dose-response of dietary protein to muscle protein synthesis in older adults

In contrast to young adults (van Loon, 2013), several dose-response studies conducted in middle-aged, and older populations have concluded that protein doses greater than 20 g result in further increases in MPS rates in older populations (Table 2.2). For example, a dose-response study by Pennings and colleagues (2012) showed that at rest, 35 g of whey protein

Table 2.2: Dietary protein doses inducing the highest rates of skeletal muscle protein synthesis in older adults at rest and post-exercise.

Study	*n*	Age (year; *M* ± *SD*)	Dose inducing highest FSR at rest (g)	Dose inducing highest FSR post-exercise (g)	Protein type
Pennings et al. (2012)	33	73 ± 2	35[a]	—	Whey
Yang, Breen, et al. (2012)	30	70 ± 4	20	40[a]	Whey
Yang, Churchward-Venne, et al. (2012)	30	71 ± 5	40[a]	40[a]	Soy
Robinson et al. (2013)	35	59 ± 2	36[a]	36[a]	Beef

FSR = fractional synthetic rate. [a]Dose of protein was the highest administered in the particular study.

increased MPS to a significantly greater rate than both 20 g and 10 g of whey protein in elderly (73±2 years), healthy untrained men. Furthermore, a study by Yang et al. (2012) examined the dose-response of whey protein and myofibrillar FSR at rest and post-exercise using a unilateral exercised leg model. The investigators found that in the resting leg, 20 g of whey protein was sufficient to stimulate MPS above resting levels in elderly men (71±4 years) with a 40 g dose of whey protein providing a further, but non-significant increase in MPS. However, in the exercised leg, 40 g of whey protein stimulated MPS to a significantly greater extent than 20 g, and 10 g respectively (Yang, Breen, et al., 2012). Similarly, Robinson et al. (2013) conducted a dose-response study using a similar unilateral leg model and found middle-aged adults (59±2 years) required 36 g of protein from beef to stimulate MPS above post-absorptive levels both at rest and post-exercise. Interestingly, all dose-response interventions conducted following exercise suggest that MPS rates could potentially increase further if larger quantities of dietary protein were administered (Table 2.2).

The dose-response observed between dietary protein intake and MPS may also be protein-source dependent. For example, Yang and colleagues (2012) conducted a dose-response study using soy protein to examine the MPS response with doses up to 40 g at rest,

and post-exercise using a unilateral exercised leg model in elderly men (71±5 years). The investigators reported that 40 g of soy protein was not sufficient to raise MPS above that of post-absorptive rates in the unexercised limb; however, this dose of soy protein significantly increased MPS in the exercised leg (Yang, Churchward-Venne, et al., 2012). In contrast to soy protein, 20 g of whey protein was able to stimulate MPS above post-absorptive rates in both rested and exercising limbs. Further, 40 g of whey protein produced significantly greater increases in MPS rates than did 20 g of whey protein post-exercise (Yang, Churchward-Venne, et al., 2012). Collectively, the dose-response research suggests that middle-aged and older untrained adults require quantities of protein higher than 20 g at rest, and 40 g post-exercise in order to maximally stimulate MPS. This conclusion is supported by Moore and colleagues (2015), who collated data from several dose-response studies in young and older adults. The authors concluded that older adults (71±1 years) require ~0.40 $g \cdot kg^{-1}$ of body mass of protein, in contrast to younger adults (22±4 years) requiring ~0.24 $g \cdot kg^{-1}$ of body mass of protein to maximize MPS (Moore et al., 2015). This research consolidates that older adults require greater quantities of dietary protein to maximize MPS, and suggests prescription per kilogram of body mass may be a superior method to allocate protein content per meal/feeding (Moore et al., 2015). Given the available literature, it is also suggested that the dose-response of dietary protein to MPS differs depending on the requirement of the muscle (rest vs. post-exercise), and is also protein source dependent (Table 2.2).

Effect of protein type on muscle protein synthesis in older adults

For a given quantity of protein, alternate sources have differing absorption characteristics and amino acid profiles that elicit differences in the MPS response (Pennings et al., 2011). These characteristics of differing protein sources are of particular importance to older populations and may explain the source-dependent effect of protein on MPS in this

population. A recent study by Pennings and colleagues (2011) investigated the appearance of dietary amino acids in blood following ingestion of a 20 g bolus of whey, casein or casein hydrolysate in healthy elderly men (74±1 years) at rest. The researchers found that the appearance rates of amino acids from both whey protein and casein hydrolysate were significantly higher than whole casein protein (Pennings et al., 2011). Despite comparable absorption of amino acids from whey and casein hydrolysate, whey protein was shown to elevate mixed muscle FSR to a significantly greater extent than casein hydrolysate or whole casein (Pennings et al., 2011). The researchers attributed the superior rate of MPS observed with whey protein to the amino acid composition, given the similar absorption rate of whey and casein hydrolysate. A more recent study by Burd et al. (2012) confirmed these findings, showing that elderly men (72±1 years) had significantly higher MPS responses at rest, and following resistance exercise when 20 g of whey compared to casein protein was consumed. In contrast, Dideriksen and colleagues (2011) reported no differences in MPS rates following resistance training and consumption of either whey or caseinate proteins post-exercise in untrained adults aged 61-80 years. However, the method of calculating protein dose in that study resulted in low total and largely variable protein consumptions, due to the variability of the population tested (Dideriksen et al., 2011). Taken together, the available evidence suggests that the amino acid content of differing protein sources is central to the MPS response, particularly in older adults.

Effect of leucine on muscle protein synthesis in older adults

The effectiveness of whey protein in stimulating MPS in both younger and older populations is proposed to be due, in part, to the leucine content of this protein. Leucine is an essential amino acid and a potent stimulator of anabolic activity as demonstrated in several recent studies (Churchward-Venne et al., 2013; Wall, Hamer, et al., 2013). Research by

Katsanos et al. (2006) found that 6.7 g of essential amino acids did not elevate MPS in healthy elderly adults (67±2 years) if administered as per the dose of amino acids found in whey protein (1.7 g leucine). However, 6.7 g of essential amino acids with enriched leucine (41%, 2.8 g leucine) significantly increased MPS in a similar cohort (67±2 years). Similarly, Churchward-Venne and colleagues (2013) have shown that 6.25 g of whey protein supplemented to contain 5 g of leucine was equally as effective in elevating acute MPS as was 25 g of whey protein containing 3 g of leucine in younger adults (21±1 years). Taken together, these studies firstly suggest that leucine is required to efficiently stimulate anabolic responses, and secondly provide further support for the use of whey protein in older adults given the considerably higher leucine content of whey compared to casein protein (Pennings et al., 2011). Thus, it appears that both the protein source and amino acid composition of the protein are important factors that are currently neglected when determining an adequate post-exercise recovery formula for masters athletes.

Conclusion

Increasing numbers of masters athletes are participating in endurance events. Despite a lack of controlled studies, current research suggests that masters endurance athletes recover from EIMD at a rate slower than younger, similarly-trained athletes. We suggest this may be due, at least in part, to age-related anabolic resistance underpinned by impairments in the mTORC1 pathway. These impairments seemingly lead to lower rates of post-exercise MPS, which may slow the rate of repair of EIMD and thus compromise subsequent muscle/exercise performance and adaptation. However, several recommendations regarding dietary protein intake can be made to offset this age-related decrease in MPS. Based on the limited available evidence, and in order to overcome the age-related deficits in molecular signaling, we suggest that masters endurance athletes should consume doses of leucine rich, whey protein in the range

of 35-40 g, or ~0.40 g·kg^{-1} of body mass following muscle-damaging exercise to facilitate muscle repair and remodeling. In line with findings by Areta and colleagues (2013), we suggest it may also be beneficial for masters athletes to consume at least four protein rich meals of similar doses, as evenly spaced as possible throughout the day to maximize muscle remodeling; for example, immediately following exercise and, at least, at each main meal.

Future research

Masters athletes are an exceptional example of aging, and to date, have received little scientific investigation. As such, these recommendations are based on the limited available evidence examining recovery rates of masters athletes, and MPS in older adults. Indeed, the chronological age, and training age of an individual masters athlete may influence their anabolic capacity/responses, and future research should examine this premise. Furthermore, research should aim to determine the current post-exercise nutritional practices of masters endurance athletes to determine if poor post-exercise dietary strategies may contribute to the poor recovery observed in this cohort. Finally, controlled laboratory-based studies are required to more precisely examine differences in recovery rates following muscle-damaging endurance exercise in well-trained young and masters athletes, and to determine the exact benefits of higher protein intakes on acute recovery of performance, and adaptation to training in masters athletes.

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Chapter 3 preamble

The published, peer-reviewed, narrative review of literature presented in Chapter 2 proposed evidence to suggest age-related anabolic resistance may persist in masters endurance athletes, potentially impairing muscle recovery following exercise-induced muscle damage. This suggestion is based on evidence that shows acute and chronic endurance training appears to have little effect on reinstating anabolic sensitivity in older adults, at least to rates experienced by younger adults. Chapter 2 also highlighted that little is known about the typical post-exercise dietary practices of masters athletes, which are strongly influential to post-exercise muscle recovery. Indeed, low post-exercise protein intakes, in relation to current sport nutrition recommendations (~20 g), may slow muscle recovery following exercise-induced muscle damage. However, if indeed masters athletes currently consume "adequate" intakes of protein post-exercise (~20 g), it may be that mechanisms relating to anabolic resistance then need to be examined.

Therefore, the purpose of study 1 (Chapter 3) was to present the outcomes of a survey examining the knowledge of post-exercise nutritional recommendations among Australian triathletes, and compare the typical post-exercise nutritional (protein and carbohydrate) practices of masters triathletes classified as triathletes aged 50 years or greater, to younger triathletes classified as triathletes aged 18-30 years, and current sport nutrition recommendations. Eligible triathletes had competed in the current triathlon season, or were training consistently for the upcoming season.

DECLARATION OF CO-AUTHORSHIP AND CONTRIBUTION

Title of Paper	Comparison of post-exercise nutrition knowledge and post-exercise carbohydrate and protein intake between Australian masters and younger triathletes
Full bibliographic reference for Journal/Book in which the Paper appears	Doering, T. M., Reaburn, P. R., Cox, G. R., & Jenkins, D. G. (2015). Comparison of post-exercise nutrition knowledge and post-exercise carbohydrate and protein intake between Australian masters and younger triathletes. *International Journal of Sport Nutrition and Exercise Metabolism, 26*(4), 338-346.
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Nature of Candidate's Contribution

Thomas M. Doering co-conceived the research idea, designed the survey tool, piloted the survey tool, statistically analysed the data, drafted the manuscript in full, edited the manuscript based on co-author feedback, submitted the manuscript, and responded to reviewer comments.

Nature of Co-Authors' Contributions

Peter R. Reaburn, David G. Jenkins and Gregory R. Cox co-conceived the research idea, assisted in the formulation, validation and piloting of the survey tool, reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscript, and reviewed responses to reviewer comments.

Candidate's Declaration

I declare that the publication above meets the requirements to be included in the thesis as outlined in the Publication of Research Higher Degree Work for Inclusion in the Thesis Procedures

Signature: 

Date: 08/11/2016

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Chapter 3: Comparison of post-exercise nutrition knowledge and post-exercise carbohydrate and protein intake between Australian masters and younger triathletes

This chapter is an exact copy of the manuscript that has been accepted and is In Press in the *International Journal of Sport Nutrition and Exercise Metabolism*.

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Referencing format has been altered to conform to APA 6th edition. As this manuscript has been published, the references included in this manuscript are not also contained in the final references list of this thesis, but appear at the end of this chapter. Figure and Table numbers in this manuscript have been altered to also align with chapter numbers.

Abstract

Post-exercise nutrition is a critical component of an athlete's recovery from training and competition. However, little is known about athletes' post-exercise dietary practices or knowledge of dietary recommendations, particularly among masters athletes. The purpose of this study was to compare and contrast the knowledge of post-exercise nutritional recommendations, and typical post-exercise intakes of carbohydrate and protein, between masters and younger triathletes. 182 triathletes (Male=101, Female=81) completed an online survey distributed by *Triathlon Australia*. Knowledge of post-exercise nutrition recommendations for protein and carbohydrate intake were assessed as a group, and contrasted between sub-groups of masters (≥50 years) and younger triathletes (≤30 years). Using dietary recall, post-exercise intakes of carbohydrate and protein were examined and contrasted between masters and younger triathletes. As a group, 43.1% and 43.9% of all triathletes answered, "I don't know" when asked to identify the recommended post-exercise carbohydrate and protein intakes, respectively. Dietary analysis revealed masters triathletes consumed significantly less carbohydrate (0.7±0.4 $g \cdot kg^{-1}$) post-exercise than recommended (1.0 $g \cdot kg^{-1}$; p=0.001), and in comparison to younger triathletes (1.1±0.6 $g \cdot kg^{-1}$; p=0.01). Post-exercise protein intakes were similar between masters (19.6±13.5 g) and younger (26.4±15.8 g) triathletes. However, relative to body mass, masters triathletes consumed significantly less protein (0.3±0.2 $g \cdot kg^{-1}$) than younger triathletes (0.4±0.2 $g \cdot kg^{-1}$; p=0.03), and consumed significantly less energy post-exercise (22.7±11.7 $kJ \cdot kg^{-1}$) than younger triathletes (37.8±19.2 $kJ \cdot kg^{-1}$; p=0.01). The present data suggests triathletes have poor knowledge of recommendations for post-exercise carbohydrate and protein intakes. Furthermore, low post-exercise intakes of carbohydrate and protein by masters athletes may impair acute recovery.

Keywords

Survey, triathlon, dietary recommendations, nutrition, older adults.

Introduction

Masters triathletes are one of the most rapidly expanding cohorts in the sport of triathlon. This is particularly evident in long-distance triathlon, in which participation rates of masters triathletes have increased significantly. For example, over a 16 year period from 1995 to 2010, the percentage of male race finishers over 40 years of age has increased from 23% to 48% at Ironman Switzerland (Stiefel, Knechtle, & Lepers, 2014). Furthermore, over a 25 year period from 1986 to 2010, the percentage of male masters triathletes (>40 years) completing the Hawaii Ironman world championships has increased from 31% to 56% (Lepers, Rüst, Stapley, & Knechtle, 2013).

To complete such events, high training volumes are commonly reported among masters endurance athletes (Peiffer, Abbiss, Chapman, Laursen, & Parker, 2008). However, previous research has observed a delay in recovery from muscle-damaging exercise in this cohort (Doering, Reaburn, Phillips, & Jenkins, 2016; Easthope et al., 2010). Moreover, masters athletes appear poor users of recovery practices, including nutritional recovery strategies (Reaburn, Macgregor, & Climstein, 2013). Taken together, these factors highlight the importance of post-exercise nutrition for masters athletes.

Post-exercise nutrition is a critical factor influencing post-exercise recovery following endurance exercise (Beelen, Burke, Gibala, & van Loon, 2010). Currently, sports nutrition guidelines recommend that athletes consume 1.0-1.2 $g \cdot kg^{-1} \cdot h^{-1}$ of carbohydrate during the immediate post-exercise period to replenish both muscle and liver glycogen stores (Beelen et al., 2010). Indeed, the rate of muscle glycogen resynthesis following exercise has been suggested as a primary determinant of the duration required to recover from prior exercise

(Beelen et al., 2010). Post-exercise protein intake is also vital for skeletal muscle repair and remodeling following exercise (Moore, 2015). Indeed, together with carbohydrate, protein intake is increasingly being accepted as an essential element of post-exercise recovery nutrition for endurance athletes (Moore, Camera, Areta, & Hawley, 2014). Currently, athletes are recommended to consume ~20 g of protein during the immediate post-exercise period (van Loon, 2013).

To date there has been limited research examining the dietary practices of masters athletes during the immediate post-exercise period (Beshgetoor & Nichols, 2003). Furthermore, research is yet to compare the knowledge of post-exercise nutritional recommendations, or examine the actual post-exercise nutritional practices of masters and younger endurance athletes. Therefore, the purpose of the present study was to investigate the knowledge of post-exercise nutritional recommendations among Australian triathletes, and compare and contrast the post-exercise nutritional practices of masters and younger triathletes. It was hypothesised that regardless of age, amateur triathletes would have poor knowledge of post-exercise nutrition recommendations, and would consume inadequate carbohydrate and protein post-exercise.

Methods

Participants

101 male and 81 female competitive Australian triathletes responded to an online survey. Younger triathletes were defined as triathletes 30 years of age or less ("Triathlon," 2010), while masters triathletes were defined as triathletes 50 years of age or older. Demographics of all participating triathletes, as well as sub-categories of masters and younger triathletes are shown in Table 3.1.

Table 3.1: Participant demographics.

	All triathletes	Masters (≥50 y)	Younger (≤30 y)
Number (n)	182	51	30
Male (n)	101	34	11
Female (n)	81	17	19
Age (y)	42.5±12.3	57.7±6.5*	24.3±3.8
Mass (kg)	70.9±12.2	72.8±13.0*	64.0±9.2
Swimming ($h \cdot wk^{-1}$)[#]	4.0±1.8	4.2±2.0	3.9±2.5
Cycling ($h \cdot wk^{-1}$)[#]	7.4±2.9	7.7±2.8	7.1±3.3
Running ($h \cdot wk^{-1}$)[#]	4.4±1.7	4.4±1.8	4.7±1.6

Data are mean ± SD. *Significantly different to younger triathletes. [#]n=159, 46, and 27 for All triathletes, Masters and Younger triathletes, respectively.

Survey design and implementation

The online survey (SurveyMonkey®) was designed to capture both the knowledge of post-exercise nutritional recommendations and the post-exercise nutritional practices of triathletes. The survey was tested for face and content validity by expert consensus, with a panel including two Advanced Sports Dietitians/Fellows of Sports Dietitians Australia, and two Sports Scientists. Furthermore, the survey was piloted within a similar cohort to that being tested to ensure face validity. Participants were recruited by *Triathlon Australia* via email using their 2014 membership database, and social media. The survey was released in October 2014, leading to Australia's most participated triathlon and remained open until February 2015. Triathletes participated via an opt-in approach and were provided with an information sheet to be read and acknowledged before proceeding to the survey questions. The research was approved by the Central Queensland University Human Research Ethics Committee.

Knowledge of post-exercise nutritional recommendations

Triathletes were initially asked to provide their gender via multiple-choice, their age and body mass via open text box, and hours trained per discipline via drop-down menu. Triathletes were asked to identify how many grams of carbohydrate ($g \cdot kg^{-1}$), and protein (g) an endurance athlete should consume in their post-exercise snack/meal to optimize recovery. Two separate multiple choice questions were provided in drop-down menus with answers increasing in 0.1 $g \cdot kg^{-1}$ increments for carbohydrate, and 5 g increments for protein intake. An option of "I don't know" was also provided. Correct responses were considered within the range of 1.0-1.2 $g \cdot kg^{-1}$, and 20-25 g for carbohydrate and protein intake, respectively. Finally, athletes were asked via single answer multiple choice, "What is the primary source of your information about post-exercise nutrition for recovery?".

Post-exercise nutrition practices

Post-exercise nutrition practices of only masters and younger triathletes were analysed (Table 3.1). Triathletes were asked to describe a scheduled meal, a prepared snack, a sports supplement/s, or a combination of each that they "typically" consume post-exercise with as much detail as possible. Descriptions of each meal/snack were provided by triathletes in an open text box, with examples provided as to the level of detail required. The typical duration between the completion of exercise and the intake of each post-exercise food option was provided by single answer multiple choice. On a Likert scale, triathletes were asked the likelihood which they would consume each food option reported (never, rarely, sometimes, often, always). The most likely consumed post-exercise food option was then analysed for macronutrient contribution using dietary analysis software (Foodworks 7.0®, Xyris, Australia) and expressed relative to body mass ($g \cdot kg^{-1}$) for subsequent data analysis. When a lack of detail

or absence of description inhibited analysis, the next most frequently used option was analysed. If limited or vague detail was provided in all descriptions, the response was not included.

Statistical analysis

Data were analysed using SPSS (SPSS, Version 22.0. Armonk, NY). Chi-square analysis was used to determine any between-group differences in the frequency of responses to multiple-choice questions. Answers were grouped where appropriate. Independent and one sample t-tests was used to compare carbohydrate, protein and energy intake between groups and to recommendations, respectively. Data are mean ± SD unless otherwise stated. Outliers were considered values two standard deviations from the mean, and were excluded. Normality was assessed by Shapiro-Wilk's test. Data that was not normally distributed was square root transformed to assume normality; however, transformation had no effect on statistical outcomes. Thus, parametric statistics on original data are presented. Alpha was set at 0.05.

Results

Knowledge of post-exercise nutritional recommendations

Collectively (n=123), Australian triathletes exhibited poor, or no knowledge of post-exercise nutritional recommendation for both carbohydrate (Figure 3.1) and protein intakes (Figure 3.2). As a group, 43.1% and 43.9% of all respondents stated, “I don’t know” when asked about the recommended post-exercise intake of carbohydrate and protein, respectively. There was no significant difference between the percentage of masters (44.4%, n=36) and younger triathletes (50.0%, n=18) that answered, “I don’t know” when asked to identify the recommended quantity of post-exercise carbohydrate required to optimize recovery (p=0.70) (Figure 3.1). Similarly, there was no significant difference between the percentage of masters

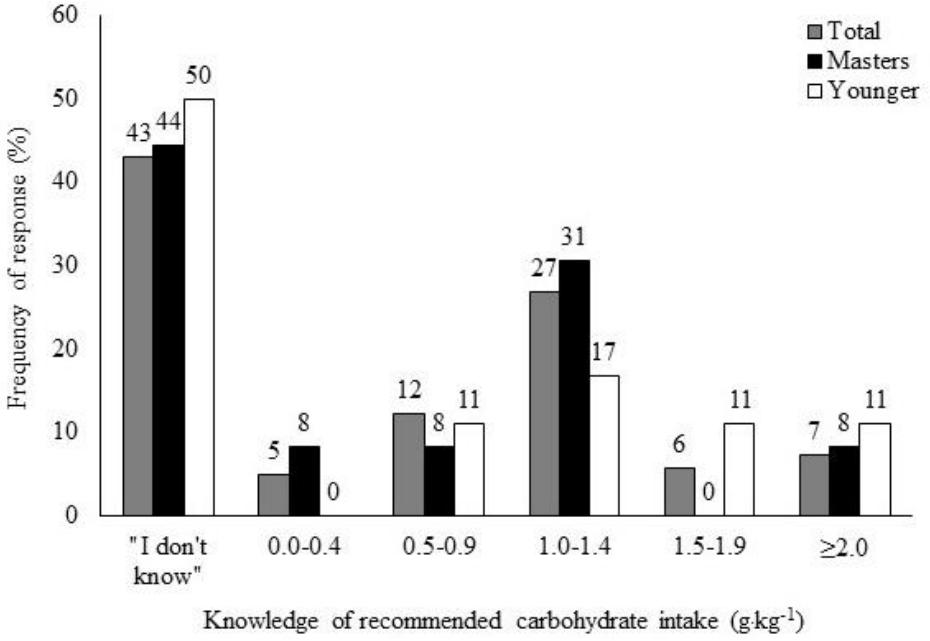

Figure 3.1: Frequency of response (%) by combined triathletes (n=123), masters (n=36) and younger (n=18) triathletes, when asked to identify the recommended post-exercise carbohydrate intake ($g \cdot kg^{-1}$) for recovery from endurance exercise.

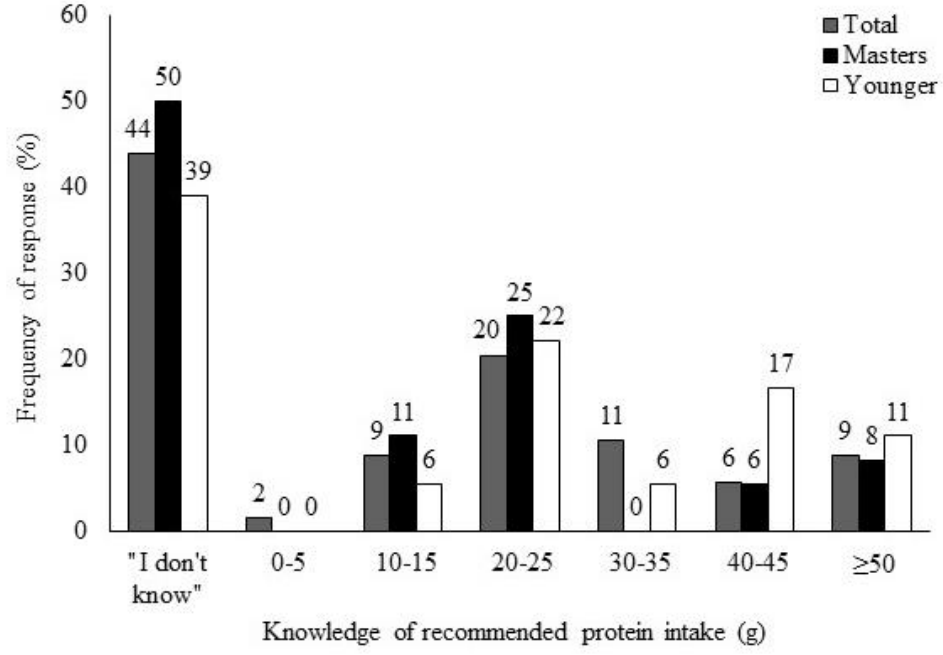

Figure 3.2: Frequency of response (%) by combined triathletes (n=123), masters (n=36) and younger (n=18) triathletes, when asked to identify the recommended post-exercise protein intake (g) for recovery from endurance exercise.

(50.0%, n=36) and younger triathletes (38.8%, n=18) that answered, "I don't know" when asked to identify the recommended quantity of post-exercise protein required to optimize recovery (p=0.44) (Figure 3.2).

25.2% of total respondents were able to correctly identify the recommended quantity of post-exercise carbohydrate required (1.0-1.2 $g \cdot kg^{-1}$) in a post-exercise meal. There was no significant difference in the frequency in which masters (30.5%, n=36) and younger triathletes (16.7%, n=18) correctly identified the recommended quantity of post-exercise carbohydrate (p=0.27).

20.3% of total respondents were able to correctly identify the recommended quantity of post-exercise protein required (20-25 g) in a post-exercise meal. There was no significant difference in the frequency in which masters (25.0%, n=36) and younger triathletes (22.2%, n=18) correctly identified the recommended quantity of post-exercise protein (p=0.82).

When asked, "What is the primary source of your information about post-exercise nutrition for recovery?" the most common response (n=110) was "own previous knowledge" (17.3%). Figure 3.3 shows the response rates to each option provided. Options with <5% response rates were omitted and included scientific journals (3.6%), Accredited Dietitians (3.6%), Nutritionist (2.7%), other magazines (1.8%) and Accredited Sport Scientists (0.9%).

Post-exercise nutrition practices

Table 3.2 displays response rates (never, rarely, sometimes, often, always) for different food options used post-exercise by masters and younger triathletes. There was no significant difference (p>0.05) in the frequencies which masters or younger triathletes utilized each food option during the post-exercise period (Table 3.2). Eating a scheduled meal (breakfast, lunch or dinner) or having a sports supplement was reported as being most often consumed post-exercise.

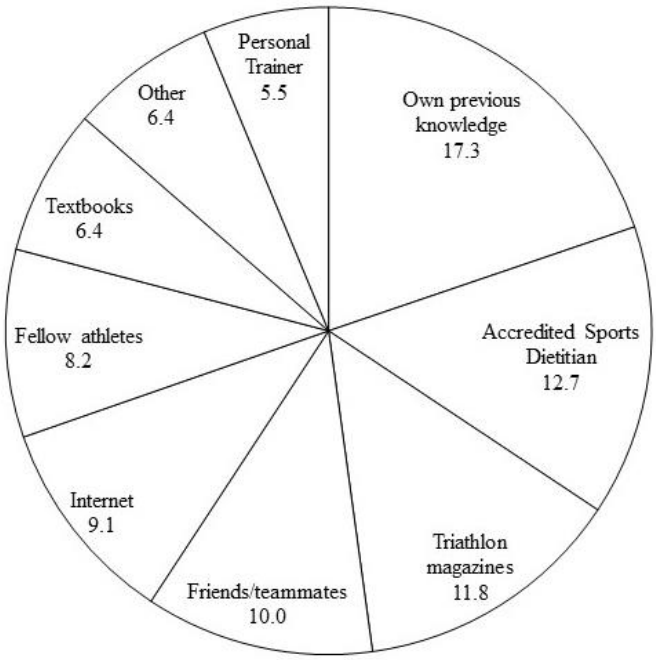

Figure 3.3: Frequency of response (%) by triathletes as a group (n=110) when asked what is the primary source of their information about post-exercise nutrition for recovery.

Figure 3.4 shows masters triathletes consumed significantly less carbohydrate (0.7±0.4 g·kg^{-1}, n=27) in their most frequently consumed post-exercise meal than younger (1.1±0.6 g·kg^{-1}, n=16) triathletes (p=0.01). Masters triathletes also consumed significantly less carbohydrate in their post-exercise meal than the lower range of sport nutrition recommendations (1.0 g·kg^{-1}; p<0.001). Younger triathletes met post-exercise recommendations (1.0-1.2 g·kg^{-1}) for carbohydrate intake. There was no significant difference in carbohydrate intake between male (0.7±0.4 g·kg^{-1}, n=18) or female (0.6±0.3 g·kg^{-1}, n=9) masters triathletes (p=0.65), or between male (0.9±0.3 g·kg^{-1}, n=3) and female (1.1±0.6 g·kg^{-1}, n=13) younger triathletes (p=0.54).

There were no significant difference in the total amount of protein consumed in the most frequent post-exercise meal between masters (19.6±13.5 g, n=27) and younger (26.4±15.8 g, n=15) triathletes (p=0.15). However, relative to body mass, masters triathletes consumed

Table 3.2: Response rates for different food options used post-exercise by masters and younger athletes.

	Never		Rarely		Sometimes		Often		Always		p-value*
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	
Eat nothing, or consume water only											
Masters	15	33.3	17	37.8	11	24.4	2	4.4	0	0.0	0.16
Younger	4	15.4	12	46.2	5	19.2	4	15.4	1	3.8	
Eat a scheduled meal (i.e. breakfast, lunch or dinner)											
Masters	2	4.4	7	15.6	11	24.4	18	40.0	7	15.6	0.57
Younger	1	3.8	1	3.8	7	26.9	14	53.8	3	11.5	
Eat/drink prepared snacks or snack foods/drinks readily available at supermarkets, bakeries, cafes, service stations or convenience stores											
Masters	10	22.2	16	35.6	12	26.7	7	15.6	0	0.0	0.37
Younger	3	11.5	10	38.5	5	19.2	7	26.9	1	3.8	
Eat/drink a sports supplement (i.e. sports drinks, protein-carbohydrate drinks, protein shake, protein bars)											
Masters	2	4.4	6	13.3	17	37.8	18	40.0	2	4.4	0.34
Younger	5	19.2	3	11.5	10	38.5	7	26.9	1	3.8	
Eat/drink a combination of snack foods and sports supplements (i.e. muesli bar + protein shake)											
Masters	3	6.7	15	33.3	16	35.6	11	24.4	0	0.0	0.39
Younger	4	15.4	10	38.5	6	23.1	5	19.2	1	3.8	

*p-value from chi-square analysis.

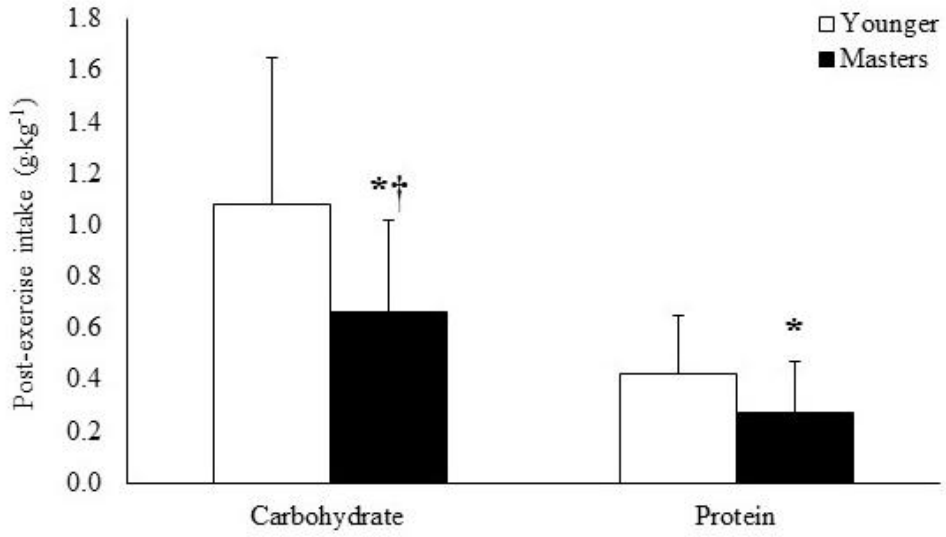

Figure 3.4: Typical post-exercise carbohydrate and protein intake of masters and younger triathletes in their most frequently consumed post-exercise meal (mean ± SD). *Significantly different to younger triathletes. †Significantly different to sports nutrition recommendations.

significantly less protein (0.3±0.2 g·kg^{-1}, n=27) than younger (0.4±0.2 g·kg^{-1}, n=15) triathletes (p=0.03) (Figure 3.4). Furthermore, when compared to currently suggested protein intakes to maximize muscle protein synthesis (MPS) in older adults, masters triathletes consumed significantly less protein than recommended (0.40 g·kg^{-1}; p=0.002) (Moore et al., 2015). There was no significant difference in protein intake between male (0.2±0.1 g·kg^{-1}, n=17) and female (0.3±0.3 g·kg^{-1}, n=10) masters triathletes (p=0.32), or between male (0.6±0.2 g·kg^{-1}, n=3) and female (0.4±0.2 g·kg^{-1}, n=12) younger triathletes (p=0.13).

There was no significant difference in absolute energy intake post-exercise between masters (1707.1±847.3 kJ, n=28) and younger (2143.0±1008.1 kJ, n=15) triathletes (p=0.15). However, relative to body mass, masters triathletes consumed significantly less energy post-exercise (22.7±11.7 kJ·kg^{-1}, n=27) than younger (37.8±19.2 kJ·kg^{-1}, n=16) triathletes (p=0.01). Masters triathletes more frequently consumed post-exercise meals within 30 minutes of

exercise (72%), in contrast to younger triathletes who were more likely to consume their post-exercise meals within 60 minutes (69%; p=0.01).

Discussion

This study is the first to compare knowledge of post exercise nutrition recommendations and post-exercise nutritional intakes between masters and younger triathletes. The novel findings from this study were that, 1) regardless of age, triathletes have poor knowledge of post-exercise nutrition recommendations for dietary carbohydrate and protein; 2) masters triathletes consumed significantly less carbohydrate post-exercise than current sport nutrition recommendations, and younger triathletes; and 3) despite meeting post-exercise sport nutrition recommendations for protein intake, masters triathletes may not consume adequate protein to ensure maximal rates of muscle repair and remodeling.

Several recent studies have assessed general nutrition knowledge among various athletic cohorts (Devlin & Belski, 2014; Spendlove et al., 2012); however, none have specifically assessed knowledge of post-exercise nutritional recommendations among triathletes. We found Australian amateur triathletes, regardless of age, have a poor understanding of the amount of carbohydrate and protein that should be consumed after exercise.

Given no other studies have assessed knowledge of post-exercise nutrition recommendations, comparisons to previous research are difficult. However, Devlin and Belski (2014) recently administered the General Nutrition Knowledge Questionnaire (GNKQ) and a sport nutrition knowledge questionnaire to a group of professional Australian rules footballers (23.5±2.8 years, n=46). As a group, professional footballers correctly answered 60.5% and 61.7% of questions relating to general, and sport-specific nutrition knowledge, respectively (Devlin & Belski, 2014). Similar findings were reported by Spendlove et al. (2012) who

administered the GNKQ to elite Australian athletes (18.9±4.9 years, n=175). These elite athletes correctly answered 65.4% of questions relating to knowledge of dietary recommendations (Spendlove et al., 2012). In comparison, our findings suggest that amateur athletes are poorly educated in the area of sport nutrition.

Devlin and Belski (2014) found that 98% of Australian Rules footballers identified the dietitian as their first source for nutritional advice. In contrast, only 13% of triathletes in the present study reported that an accredited sports dietitian was the most commonly used source to obtain nutritional information, with the most frequent response being "own previous knowledge". It's likely that accredited sports dietitians are not as readily accessible, nor are seen as a cost-effective option for amateur athletes. Therefore, the promotion of publicly available and easily interpretable information ("Recovery," 2012), including dietary recommendations and food suggestions may improve dietary knowledge and practices of amateur athletes.

Post-exercise carbohydrate consumption and subsequent glycogen replenishment is suggested to be a primary determinant of the duration required to recover from prior exercise (Beelen et al., 2010). In the present study, masters triathletes consumed significantly less carbohydrate in their most frequently consumed post-exercise meal/snack, than current sport nutrition recommendations (Beelen et al., 2010), and in comparison to younger triathletes. Furthermore, when post-exercise energy intake was expressed relative to body mass, masters triathletes consumed significantly less energy post-exercise. This is in line with the well-documented decrease in total daily energy intake observed with aging (Morley, 2001).

Limited research has investigated post-exercise carbohydrate intakes of masters athletes. However, Beshgetoor and Nichols (2003) analysed the dietary practices of supplementing and non-supplementing female masters runners and cyclists via four-day diet records. Supplementing and non-supplementing athletes reported daily carbohydrate intakes of

269±112 and 277±43 g, respectively (Beshgetoor & Nichols, 2003). Despite body mass being unreported, we suggest these values would be considerably low in comparison to current carbohydrate recommendations (Beelen et al., 2010). Importantly, current carbohydrate recommendations are presented relative to body mass, and do not account for differences in body composition that may exist between older and younger athletes (Wroblewski, Amati, Smiley, Goodpaster, & Wright, 2011).

While post-exercise food options were not different between younger and masters triathletes in this study, typically food options used by masters triathletes may help explain the lower nutrient and total energy intakes observed. For example, 40% of masters triathletes (vs. 27% of younger triathletes) reported that they "often" use sports supplements post-exercise. These findings are similar to those of Guthrie and Erickson (2015) who recently found masters swimmers are high users of supplements in comparison to the general population. The common use of supplements by masters athletes may limit post-exercise energy and nutrient intake in comparison to consuming nutrients from food sources. The present findings of low post-exercise carbohydrate intakes among masters triathletes are supported by the limited data available and may be a function of the lower total energy intake with aging, or poor meal/snack choices post-exercise.

The intake of adequate post-exercise protein is important for the repair and remodeling of muscle proteins following exercise (Moore, 2015). A finding of the present study was that masters triathletes consume significantly less protein (0.3±0.2 $g \cdot kg^{-1}$) in their post-exercise meal/snack compared to younger triathletes (0.4±0.2 $g \cdot kg^{-1}$). Despite meeting current sport nutrition recommendations for post-exercise protein intake (20 g) (Beelen et al., 2010), this dose has been suggested to be insufficient for aging athletes (Doering et al., 2016). Indeed, Robinson and colleagues (2013) have shown that protein doses of 36 g elicit significantly

greater rates of MPS than doses of 24 g among middle-aged (59±2 years) healthy men, of similar age to the masters athletes in this study. Furthermore, Moore and colleagues (2015) recently suggested that, based on the available dose-response evidence, healthy elderly adults (71±1 years) require protein doses in the range of 0.40±0.19 $g \cdot kg^{-1}$ to maximize MPS (Moore et al., 2015). This finding suggests that despite reaching current sport nutrition recommendation for protein intake based on absolute doses, masters triathletes may be compromising post-exercise MPS rates, and consequently post-exercise muscle protein remodeling to support favorable longer-term adaptation (Moore et al., 2014). The effect this may have on same-day exercise recovery (≥8 h), and longer-term exercise adaptation is currently unknown and requires further investigation.

Finally, the timing of post-exercise feeding is important when subsequent exercise is to be performed within 8 h (Moore, 2015). The present results suggest that despite lower intakes of carbohydrate and protein, masters triathletes more commonly consume their post-exercise meal/snack within 30 minutes of exercise, compared to younger triathletes who most commonly consume a meal/snack within 60 minutes. Importantly, post-exercise rates of glycogen synthesis are highest within the hour after exercise, given the translocation of GLUT-4 transporters to the cell membrane and the increased activity of glycogen synthase (Jentjens & Jeukendrup, 2003). Thus, acute consumption of carbohydrate after exercise is vital to maximize glycogen storage; however, whether consuming carbohydrate within 30 or 60 minutes would lead to any practical advantage in subsequent exercise would be dictated by the timing of the subsequent exercise (Moore, 2015). In summary, despite differences in the timing of nutrient intake among younger and masters triathletes, this may have minimal consequence to younger triathletes unless a subsequent training session was to be commenced in the acute post-exercise period (Moore, 2015).

We acknowledge several limitations of performing dietary analysis of self-reported data, including underreporting food intake and the potential inaccuracy of data analysis (Braakhuis, Meredith, Cox, Hopkins, & Burke, 2003; Schoeller, 1995). Thus, these data should be interpreted concomitantly with the typical post-exercise food options reported.

Masters athletes should seek professional advice to establish a post-exercise food option that is both convenient and in accordance with current sports nutrition recommendations. Future research should examine the recovery of masters vs. younger triathletes in response to current post-exercise nutrition recommendations. Furthermore, research to determine whether higher intakes of protein are indeed beneficial for older athletes is required.

Conclusion

In conclusion, the findings of the present study suggest that, regardless of age, triathletes have poor knowledge of post-exercise nutritional recommendations. However, this lack of knowledge does not compromise post-exercise dietary practices of younger triathletes. In contrast, the current data suggests that masters triathletes have poor post-exercise dietary practices, consuming lower than recommended carbohydrate intakes after exercise. Finally, the present findings suggest masters triathletes consume doses of protein post-exercise that may be insufficient to maximize muscle protein synthesis and thus muscle protein remodeling with advancing age.

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Chapter 4 preamble

The findings from the peer-reviewed published paper presented in Chapter 3 suggest that masters athletes typically consume doses of protein (19.6±13.5 g; ~0.3 g·kg^{-1}) in line with current sport nutrition recommendations for endurance athletes (~20 g) of all ages. However, whether or not these doses of protein consumed post-exercise are sufficient for masters athletes to achieve rates of muscle protein synthesis in line with those of younger athletes is unknown, particularly when recovering from a muscle-damaging exercise bout. Furthermore, given the paucity of laboratory-controlled studies comparing recovery rates of masters and younger athletes following muscle-damaging exercise, a dual purpose study examining both the recovery of cycling performance in masters compared to younger athletes, while also assessing muscle protein synthesis rates between these groups in response to typical training and currently recommended carbohydrate and protein feedings was warranted.

Therefore, the purpose of study 2 (Chapter 4) was to compare the myofibrillar fractional synthetic rates of masters and younger well-trained, long-distance (Half-Ironman and Ironman distance) triathletes, over a 72-hour period inclusive of a downhill run and three cycling time trials, while current sport nutrition recommendations for protein (20.0 g post-exercise, 0.30 g·kg^{-1}·meal^{-1}, and 1.6-1.7 g·kg^{-1}·day^{-1}) and carbohydrate intake (1.0 g·kg^{-1} post-exercise, and 6.0-7.0 g·kg^{-1}·day^{-1}) were met. Potential age-related differences in the recovery of endurance cycling performance were also assessed following the muscle-damaging run using multiple cycling time trials at 10, 24 and 48 h post-run. In the present study, masters triathletes were classified as 50-60 years of age, and younger triathletes were classified as 18-30 years of age. All triathletes in the present study were currently competitive and in the pre-competition or competition phase of training.

DECLARATION OF CO-AUTHORSHIP AND CONTRIBUTION

Title of Paper	Lower integrated muscle protein synthesis in masters compared to younger athletes
Full bibliographic reference for Journal/Book in which the Paper appears	Doering, T. M., Jenkins, D. G., Reaburn, P. R., Borges, N. R., Hohmann, E., & Phillips, S. M. (2016). Lower integrated muscle protein synthesis in masters compared to younger athletes. *Medicine and Science in Sports and Exercise, 48*(8), 1613-1618.
Status	Accepted and In Press ✓ Published

Nature of Candidate's Contribution

Thomas M. Doering co-conceived the research idea, designed the experiment, collected all performance data and assisted in biological tissue collection, undertook muscle analysis, statistically analysed the data, drafted the manuscript in full, edited the manuscript based on co-author feedback, submitted the manuscript, and responded to reviewer comments.

Nature of Co-Authors' Contributions

David G. Jenkins and Peter R. Reaburn co-conceived the research idea, refined the experimental design, reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscript, and reviewed responses to reviewer comments.

Nattai R. Borges contributed to data collection and Erik Hohmann took all skeletal muscle biopsies. Both reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscript, and reviewed responses to reviewer comments.

Stuart M. Phillips refined the experimental design, contributed to muscle analysis, reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscript, and reviewed responses to reviewer comments.

Candidate's Declaration

I declare that the publication above meets the requirements to be included in the thesis as outlined in the Publication of Research Higher Degree Work for Inclusion in the Thesis Procedures

Signature: 

Date: 08/11/2016

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Chapter 4: Lower integrated muscle protein synthesis in masters compared with younger athletes

This chapter is an exact copy of the manuscript that has been published in *Medicine and Science in Sports and Exercise*.

Doering, T. M., Jenkins, D. G., Reaburn, P. R., Borges, N. R., Hohmann, E., & Phillips, S. M. (2016). Lower integrated muscle protein synthesis in masters compared with younger athletes. *Medicine and Science in Sports and Exercise, 48*(8), 1613-1618. doi: http://dx.doi.org/10.1249/MSS.0000000000000935. Impact Factor: 3.98; Quartile 1 in Sport Science (2014).

Referencing format has been altered to conform to APA 6th edition. As this manuscript has been published, the references included in this manuscript are not also contained in the final references list of this thesis, but appear at the end of this chapter. Figure and Table numbers in this manuscript have been altered to also align with chapter numbers.

Abstract

Purpose: To compare the integrated muscle protein synthesis (MPS) rates of masters and younger triathletes over three consecutive days of intense endurance training. Recovery of cycling performance, following muscle-damaging running, was also compared between groups. *Methods:* Five masters (age, 53±2 y, $\dot{V}O_{2max}$, 55.7±6.9 $mL \cdot kg^{-1} \cdot min^{-1}$) and six younger (age, 27±2 y, $\dot{V}O_{2max}$, 62.3±1.5 $mL \cdot kg^{-1} \cdot min^{-1}$) trained triathletes volunteered for the study. Baseline skeletal muscle and saliva were initially sampled, following which a 150 mL bolus of deuterium oxide (70%) was consumed. Participants then completed a 30 min downhill run; three 20 km cycling time trials were completed 10, 24 and 48 h following the run. Saliva was collected each morning and skeletal muscle was again sampled 72 h following the run; both were used for MPS analysis. Diet was controlled throughout the study. *Results:* Over the three days, masters triathletes showed a significantly lower myofibrillar fractional synthetic rate (1.49±0.12%·d^{-1}) compared to the younger (1.70±0.09%·d^{-1}) triathletes (p=0.009, *d*=1.98). There was also a trend for masters triathletes to produce a slower cycle time trial (-3.0%, *d*=0.46) compared to younger triathletes (-1.4%, *d*=0.29) at 10 h post-run, in comparison to baseline. The between-group comparison of change was moderate (*d*=0.51), suggesting slower acute recovery among masters triathletes. *Conclusions:* The present data show lower MPS rates in well-trained masters triathletes over three days of training and this likely contributes to poorer muscle protein repair and remodeling. Furthermore, acute recovery of cycle time trial performance tended to be poorer in the masters triathletes.

Keywords

Aging, muscle damage, recovery, protein.

Introduction

Increasing participation rates and improving performances of masters endurance athletes (Lepers, Rüst, Stapley, & Knechtle, 2013) warrant an improved understanding of potential differences in post-exercise recovery between younger and older athletes (Easthope et al., 2010; Sultana et al., 2012). A better understanding of recovery after intense exercise, and the underlying mechanisms, is likely to inform more effective nutritional and recovery practices for masters athletes and in turn, further improve training adaptation and competition performance. The limited available data suggest that masters endurance athletes recover at similar rates to younger athletes following fatiguing cycle exercise (Borges, Reaburn, Driller, & Argus, 2016; Fell, Haseler, Gaffney, Reaburn, & Harrison, 2006). However, it appears that older athletes take longer to recover when compared to younger athletes following running, which is associated with greater muscle damage (Doering, Reaburn, Phillips, & Jenkins, 2016; Easthope et al., 2010).

Following exercise-induced muscle damage, longer recovery durations required by masters compared to younger athletes may be due to age-related impairments in the repair/remodeling mechanisms in skeletal muscle. In older untrained adults there is an age-related "anabolic resistance" following both resistance training (Kumar et al., 2009) and protein feeding (Pennings et al., 2012; Robinson et al., 2013), with lower muscle protein synthesis (MPS) rates often observed in response to these stimuli compared to younger adults. Despite suggestions that regular physical activity might attenuate age-related decrements in MPS by reinstating anabolic sensitivity to aminoacidemia (Burd, Gorissen, & van Loon, 2013), this has yet to be confirmed. In the present investigation, we chose to study older well-trained masters triathletes as a model of highly active aging, as their habitual high levels of regular exercise should, in theory, offset age-related anabolic resistance.

The aim of this study was to compare the myofibrillar fractional synthetic rate (FSR) of masters and younger well-trained triathletes over a 72-hour period of intense endurance training following a downhill run. We also, as an applied test of the capacity for recovery, compared potential age-related differences in the recovery of endurance cycling performance following the muscle-damaging run using multiple cycling time trials (TT) at 10, 24 and 48 h post-run. We hypothesized that masters triathletes would exhibit lower myofibrillar FSR in comparison to younger triathletes, and that masters triathletes would recover their performance at a slower rate than younger triathletes.

Methods

Participants

Six young (27±2 y) and five masters (53±2 y) well-trained triathletes participated in the present study. All reported training ≥10 $h \cdot wk^{-1}$ for at least eight weeks prior to the study. Table 4.1 outlines participant characteristics of each group. The study was approved by Central Queensland University's Human Research Ethics Committee and conformed to all international standards: Helsinki declaration and the Canadian Tri-Council policy on the participation of humans in research. All participants gave written informed consent prior to participation in the study.

Experimental design

Participants visited the Exercise Physiology laboratory at Central Queensland University for preliminary testing ($\dot{V}O_{2max}$ testing) and 48 h later, completed a baseline 20km cycling TT. At least five days later, participants completed the three-day experimental trial. Baseline testing was conducted at the same time of day as morning sessions of the experimental

Table 4.1: Participant characteristics.

	Masters	Younger
Age (y)	53 (52-56)*	26 (25-29)
Height (cm)	181.5 (168.5-181.9)	172.6 (170.7-176.6)
Body mass (kg)	82.2 (68.6-86.5)	73.7 (65.3-76.5)
Body fat (%)	12.5 (8.6-16.7)*	7.7 (6.8-9.6)
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	56.3 (48.7-61.9)*	63.4 (56.4-66.0)
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	4.4 (3.6-5.4)	4.5 (4.1-5.0)
Years in triathlon (y)	8 (3-17)*	3 (1-6)
Typical duration between daily training (h)	9 (5-10)	10 (3-12)
Weekly training volume (h)	14 (10-15)	12 (10-16)
Habitual protein intake ($g \cdot kg^{-1} \cdot day^{-1}$)	1.7 (1.2-2.6)	2.4 (1.5-3.4)

Data are medians (range). *Significantly different to younger triathletes ($p<0.05$).

trial (0400-0800). All exercise bouts were completed under standard laboratory conditions (22-24°C, 60% RH).

Preliminary testing

Participants completed a pre-exercise screening, a training questionnaire, anthropometric measures, and a $\dot{V}O_{2max}$ test. Skinfolds were obtained from eight sites using calibrated skinfold callipers (Harpenden, Baty International, West Sussex, UK), and body fat percentage calculated (Yuhasz, 1974). Subsequently, $\dot{V}O_{2max}$ testing was completed on a motorized treadmill (TMX428, Trackmaster, Newton, KS) commencing at a speed of 8 $km \cdot h^{-1}$ and increased at a rate of 1 $km \cdot h^{-1} \cdot min^{-1}$ until volitional exhaustion. Expiratory gas was continuously analysed via a metabolic cart calibrated to manufacturer's instructions (Trueone 2400, Parvomedics, Sandy, UT). $\dot{V}O_{2max}$ was determined as the highest rate of oxygen consumption recorded over an averaged 15 s period. Participants were subsequently

familiarized with all experimental protocols, including a truncated (60%) 20 km cycling TT under experimental conditions.

Trial overview

A schematic overview of the experimental trial can be found in Figure 4.1. On day one, participants reported to the laboratory following an overnight fast. Baseline and resting skeletal muscle, and saliva were sampled. Blood was sampled for the analysis of creatine kinase (CK) activity, and participants consumed a 150 mL bolus of deuterium oxide (D_2O). One hour later, and following a six min warm-up, participants completed a 30 min downhill run (-10%) on a motorized treadmill at a speed corresponding to 70% of $\dot{V}O_{2max}$ during flat running (Braun & Dutto, 2003). Participants returned to the laboratory 10 h post-run and provided a blood sample for CK analysis. They then completed a standardized six min warm-up on a cycle ergometer before completing a 20 km cycling TT. This afternoon testing format was repeated at 24 and 48 h following the downhill run. Saliva was collected upon arrival to the laboratory each morning, and skeletal muscle was sampled at 72 h following the initial biopsy. Both were used in the assessment of MPS.

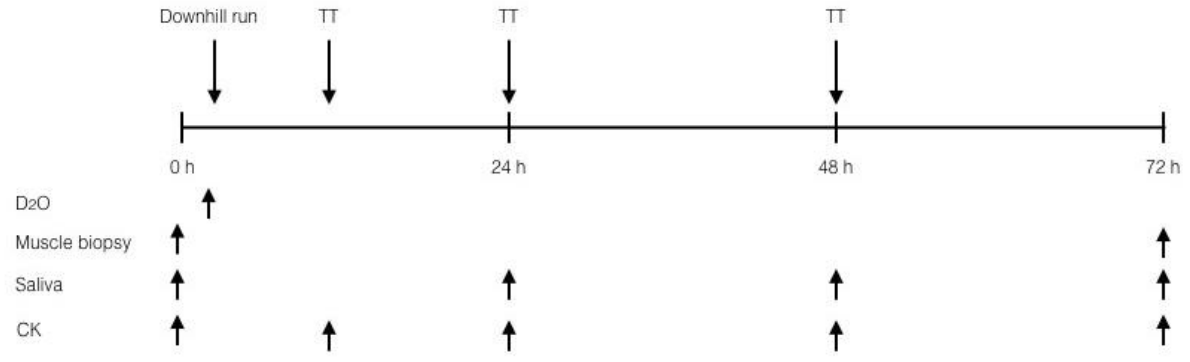

Figure 4.1: Experimental trial overview.

Diet and exercise standardisation

Participants completed a three-day diet record prior to testing. Based on the data provided, individualized diets for the 24 h preceding the baseline TT, and for the period spanning 24 h pre-trial until the final muscle biopsy at 72 h were prescribed (Foodworks 7.0®, Xyris, Brisbane, Australia), and all food consumed was recorded. Prescribed carbohydrate intake was 6 $g \cdot kg^{-1} \cdot d^{-1}$ (7 $g \cdot kg^{-1} \cdot d^{-1}$ on experimental day one), and protein intake was ~1.6 $g \cdot kg^{-1} \cdot d^{-1}$ (~1.7 $g \cdot kg^{-1} \cdot d^{-1}$ on experimental day one) with each meal containing 0.3 $g \cdot kg^{-1}$ of protein. Water was consumed ad libitum throughout the study, however was not recorded. Immediately following each exercise bout, participants were provided with an individualized protein-carbohydrate beverage consisting of 20 g protein (Whey Protein Isolate 894, Fonterra, Australia) and 1 $g \cdot kg^{-1}$ carbohydrate (1:1 of maltodextrin/glucose) to consume over the first hour of recovery. Following all morning exercise bouts, a second carbohydrate-only beverage (1 $g \cdot kg^{-1}$) was provided to participants; they were instructed to consume this beverage over the second hour of recovery. Participants were required to abstain from caffeine intake in the 12 h prior to each exercise bout and to refrain from vigorous exercise in the 48 h prior to both the baseline testing and experimental trial, and all exercise in the 24 h prior to testing. Only exercise associated with the experimental trial was completed in the three-day trial period.

Muscle sampling

Participants arrived at the laboratory after an overnight fast and rested supine for 15 min. Local anaesthetic (1% lidocaine) was administered to the lateral aspect of left *vastus lateralis*, and muscle was sampled using a 14 gauge percutaneous needle biopsy with cannula (Achieve®, CareFusion, Seven Hills, NSW, Australia). Approximately 80 mg of muscle was sampled, snap frozen in liquid nitrogen and stored at -80°C until subsequent analysis. 72 h samples were obtained from the contralateral leg.

^{2}H labelling

Immediately following baseline skeletal muscle and saliva sampling, each participant consumed a single 150 mL bolus of 70% D_2O (Cambridge Isotope Laboratories, Tewksbury, MA). This bolus has been shown to label body water at ~0.2 atom percent excess (APE) by 24 h post-ingestion, and shown to result in a linear incorporation of ^{2}H into muscle bound alanine over a period of ≤4 days (Wilkinson et al., 2014). Saliva samples were obtained daily, at least 30 min after consumption of any food or drink, for measurement of ^{2}H in body water. ^{2}H body water enrichment was used as a surrogate for plasma alanine ^{2}H labelling (Bell, Seguin, Parise, Baker, & Phillips, 2015; Wilkinson et al., 2014).

Myofibrillar fractional synthetic rate

Skeletal muscle (~50 mg) was homogenized in ice-cold buffer and the myofibrillar fraction separated as previously described (Burd et al., 2010). Analysis by gas chromatography pyrolysis isotope ratio mass spectrometry was conducted (Metabolic Solutions, Nashua, NH) as described elsewhere (Bell et al., 2015). The rate of myofibrillar fractional synthetic rate ($\%\cdot d^{-1}$) was calculated by the standard precursor-product method (Wilkinson et al., 2014). Briefly,

$$\text{FSR } (\%\cdot d^{-1}) = \left[\frac{(EAla2 - EAla1)}{(EBW \cdot t)}\right] \cdot 3.7 \cdot 100$$

Where: E_{Ala} is the enrichment of muscle bound alanine (APE) at each respective time point (1 – baseline and 2 – post-trial); E_{BW} is the mean ^{2}H enrichment of body water (APE) between time points (Wilkinson et al., 2014); t is the tracer incorporation time in days; 3.7 is the mean number of ^{2}H atoms incorporated into alanine (MacDonald et al., 2013; Wilkinson et al., 2014); and multiplication by 100 converts the fraction to a percentage.

Cycling time trial

On each occasion (10, 24, 48 h post-run), participants completed a 20 km cycling TT (Velotron Dynafit Pro, RaceMate, Seattle, WA). The 20km TT has been shown to be highly reproducible (CV=0.7%) in trained cyclists (Sporer & McKenzie, 2007). Feedback regarding distance covered at 25, 50, 75 and 90% of completion was provided.

Creatine kinase activity

CK activity was measured in capillary blood with a clinical chemistry system (Reflotron Plus, Roche Diagnostics, Almere, The Netherlands) as per manufacturer's instructions. This system has been reported to have high within-series and between-day precision with a CV of 3.1% and ≤3.0%, respectively (Hørder et al., 1991).

Statistical analysis

Data are presented as mean ± standard deviation (SD) unless otherwise stated. Data were analysed using SPSS (Version 22.0, SPSS, Armonk, NY). Normality was assessed by skewness and kurtosis z-scores. Independent t-tests were used to compare between-group differences for demographic data and myofibrillar FSR. TT performance and CK activity were examined over time using repeated measures ANOVA. Within-group, between time-point comparisons of TT performance were assessed by multiple paired t-tests with Bonferroni corrections. Alpha was set at 0.05. Cohen's *d* effect sizes were also determined for all analyses, and used to compare the magnitude of change in TT performance. Threshold values for small, moderate and large effect sizes were 0.2, 0.5 and 0.8, respectively (Sullivan & Feinn, 2012).

Results

^{2}H labelling

Figure 4.2 shows saliva ^{2}H enrichment (APE) decayed in a manner that was well described by linear models over the three-day trial.

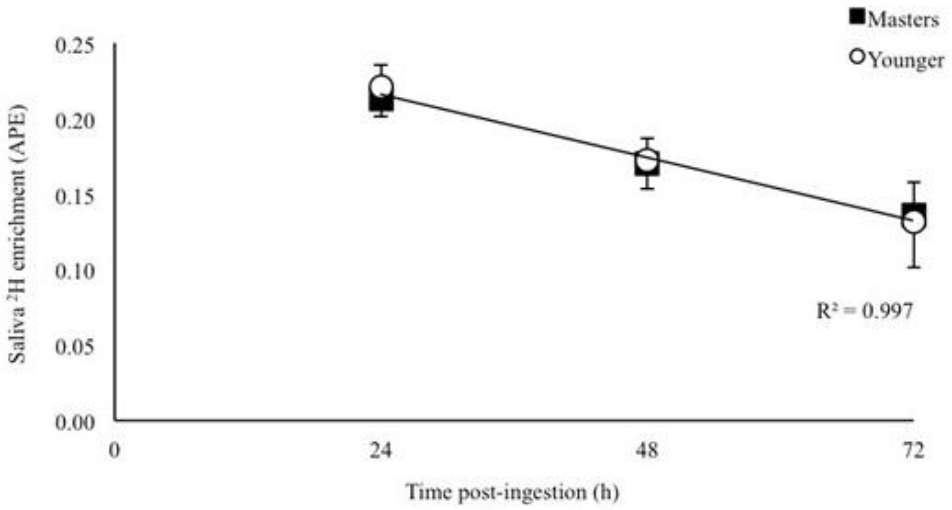

Figure 4.2: Saliva ^{2}H enrichment (APE) at 24, 48 and 72 h following D_2O ingestion in masters and younger triathletes (mean ± SD). The linear trend line is based on the mean ^{2}H enrichment of both groups.

Myofibrillar fractional synthetic rate

Figure 4.3 shows, (A) myofibrillar FSR (%·d^{-1}) over the three-day period, and (B) muscle ^{2}H enrichment (APE) at 72 h, in masters and younger triathletes. Masters triathletes had significantly lower muscle ^{2}H enrichment ($p=0.03$) and myofibrillar FSR compared to younger triathletes (1.49±0.12%·d^{-1} and 1.70±0.09%·d^{-1} respectively; $p=0.009$; $d=1.98$).

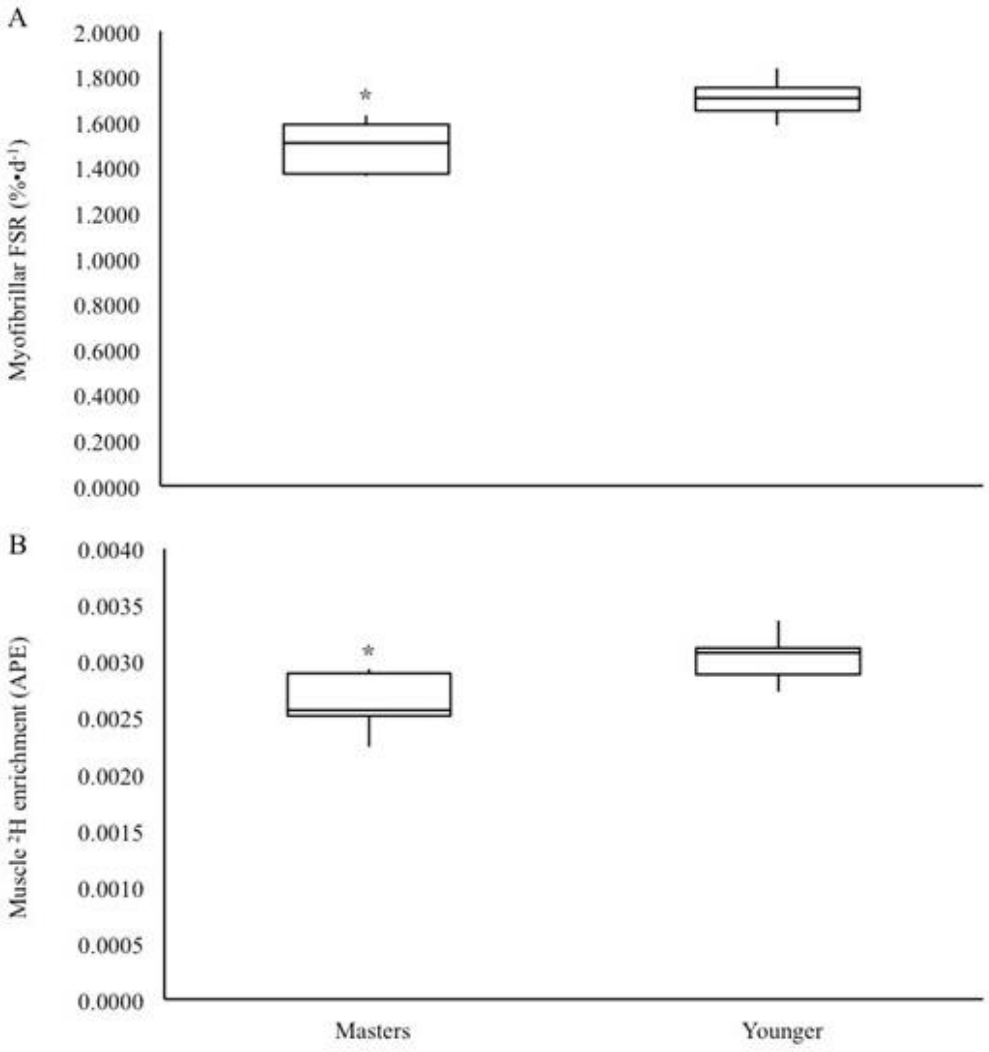

Figure 4.3: (A) Myofibrillar fractional synthetic rate (%·d^{-1}) over the three-day period, and (B) muscle ^{2}H enrichment (APE) at 72 h following D_2O ingestion in masters and younger triathletes. *Significantly different from younger triathletes.

Cycling time trial

Figure 4.4 shows the change in TT performance time (s) relative to baseline performance, at 10, 24 and 48 h following the downhill run. Though there were no statistical differences ($p>0.05$), there was a trend for masters triathletes to recover more slowly, indicated by a moderate between-group effect (d=0.51) when comparing change in performance time

between baseline and the 10 h TT. Between-group effects for the change in performance time between baseline and the 24 h TT, and baseline and the 48 h TT, were small (*d*=0.20) and trivial (*d*=0.08), respectively, and likely due to high variability in TT performance among masters triathletes.

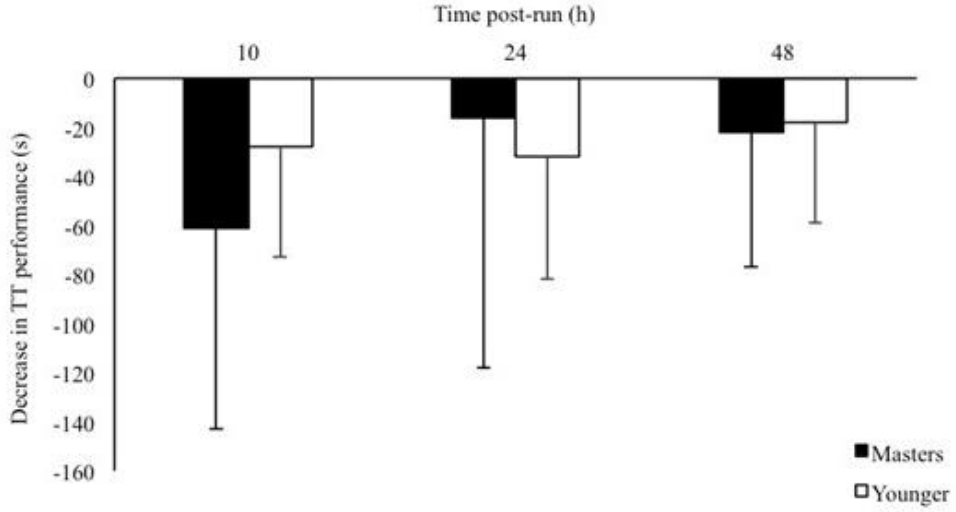

Figure 4.4: Change in time trial performance time (s) from baseline at 10, 24, and 48 h following the downhill run in masters and younger triathletes (mean ± SD).

Creatine kinase activity

Figure 4.5 displays CK activity ($U \cdot L^{-1}$) for masters and younger triathletes at baseline, and at 10, 24, 48 and 72 h post-run. CK activity was not different between groups, suggesting similar muscle damage over the three days. No group or interaction effect was observed ($p>0.05$) for CK activity, however a significant effect of time ($p<0.001$) was observed.

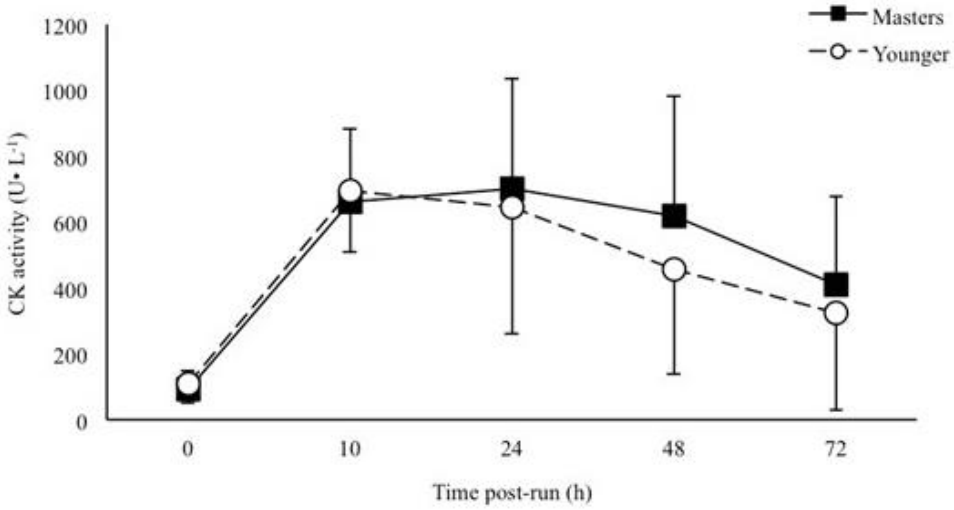

Figure 4.5: Creatine kinase activity ($U \cdot L^{-1}$) in whole blood measured at baseline, 10, 24, 48 and 72 h following the downhill run in masters and younger triathletes (mean ± SD).

Discussion

This study is the first to compare integrated MPS rates and exercise performance of well-trained masters and younger endurance athletes over three days of consecutive training following a muscle-damaging bout of running. Our data shows that masters triathletes had a lower myofibrillar FSR compared to younger triathletes when 20 g of dietary protein was provided post-exercise, and protein was prescribed during the study period at doses suggested to maximize MPS among younger athletic cohorts ("Recovery," 2012). We also observed a trend, as suggested by a moderate between-group effect size, for masters triathletes to experience greater decrements in afternoon cycling performance following a morning muscle-damaging run compared to younger triathletes.

The use of D_2O to ^2H isotope label newly synthesised muscle proteins has several favourable benefits over traditional acute stable isotope infusions. For example, the ability to measure MPS over multiple exercise bouts and days (Wilkinson et al., 2014), incorporating

normal feeding practices (McGlory & Phillips, 2014), provide a "real world" measure. To date, no other studies have evaluated MPS rates in well-trained athletes, nor investigated the effect of age on MPS in well-trained athletes by use of this method. Therefore, comparison of our data to prior studies is difficult. However, studies utilising D_2O for analysis of MPS in older untrained adults in response to aerobic exercise have reported comparable myofibrillar FSR to those in the present study (Bell et al., 2015). Furthermore, Wilkinson et al. (2014) present similar ^2H enrichments in body water 24 h post-consumption of D_2O, with rates of decay similar to the present study over the initial three-day period. Therefore, our data appears to be in line with previous studies utilising this approach to measure MPS.

In the present study, we observed that masters triathletes exhibited lower myofibrillar FSR over three days of consecutive training. Though resting MPS rates were not measured, these data have potential implications for the recovery of older athletes following exercise. Previous research has suggested that elevations in myofibrillar FSR may be a response to unaccustomed exercise (Wilkinson et al., 2008), and may be an important response to repair damaged muscle (Moore, 2015; Moore, Camera, Areta, & Hawley, 2014), and to potentially facilitate a more rapid recovery following exercise (Moore, 2015). Despite the fact that age-related neuromuscular factors may contribute to slower recovery in older athletes (Easthope et al., 2010), the lower myofibrillar FSR, and thus likely slower repair and remodeling of myofibrillar proteins observed in the present study may contribute to a slower recovery in masters compared to younger athletes following eccentric muscle damage, as observed here, and elsewhere (Easthope et al., 2010). However, we cannot discount that a lower force production during the cycling time trials by masters athletes may have lessened the mechanical stimulus for anabolic signaling, and thus reduced the measured MPS response, given this MPS rate is inclusive of these three days training. Nevertheless, attenuated cumulative elevations in

MPS, as observed in masters athletes, could represent an attenuated adaptation to training (Moore, 2015; Moore et al., 2014).

The lower myofibrillar FSR observed among masters triathletes in the present study occurred despite post-exercise and daily protein (per meal) consumed in accordance with current sport nutrition guidelines ("Recovery," 2012). Specifically, our triathletes ingested 20 g of high quality, leucine-rich, whey protein following each exercise bout in line with current sport nutrition recommendations (Beelen, Burke, Gibala, & van Loon, 2010; "Recovery," 2012); furthermore, each main meal contained 0.3 $g \cdot kg^{-1}$ of protein, a dose suggested to maximize MPS among young healthy cohorts post-exercise (Phillips, 2014). Importantly, current sport nutrition recommendations do not differentiate between masters and younger athletes. Therefore, masters athletes are currently recommended to, and report consuming protein boluses in line with those recommended for younger athletes (Doering, Reaburn, Cox, & Jenkins, 2015); these protein doses were therefore implemented in the present study. Our findings suggest that aging, or the inability to produce youth-like force into later age, and not merely lower habitual physical activity, may attenuate MPS rates. However, there appears some potential for higher post-exercise protein intakes to offset attenuated MPS response in masters athletes (Pennings et al., 2012; Robinson et al., 2013; Yang, Breen, et al., 2012; Yang, Churchward-Venne, et al., 2012).

Collectively, studies that have examined the effect of post-exercise protein feeding among young endurance athletes suggest that aminoacidemia significantly increases myofibrillar FSR, in a dose response manner (Breen et al., 2011; Rowlands et al., 2015). However, in younger athletes, this dose-response relationship has a ceiling at doses of protein of approximately 20-25 g (Rowlands et al., 2015), with higher doses merely elevating amino acid oxidation (Witard et al., 2014).

In contrast, data from acute stable isotope infusions suggest that older, untrained adults show a dose-response between protein and MPS up to protein doses of 40 g (Yang, Breen, et al., 2012). For example, Robinson and colleagues (2013) have shown healthy middle-aged men (59±2 y), of similar age to the masters triathletes in this study, showed a continued increase in myofibrillar FSR with consumption of 36 g of protein post-exercise and at rest, in comparison to boluses of 24 g or less, when consumed as beef. Similarly, Yang and colleagues (Yang, Churchward-Venne, et al., 2012) have shown that elderly men (71±5 y) elicit significantly greater MPS rates in response to 40 g of whey protein, compared with 20 g of whey protein. Given the lower myofibrillar FSR observed in masters compared to younger triathletes in the present study, we suggest that habitually high levels of physical activity do not offset the need for higher age-appropriate doses of high quality protein; this may be one strategy to counteract age-related attenuations in MPS and facilitate recovery and adaptation processes in masters athletes. Future research comparing MPS rates of masters athletes in response to habitual and higher protein intakes relative to baseline measures, in the presence and absence of exercise, should be investigated.

The present study also found that masters triathletes tended to have poorer same-day (10 h post-run) cycling performance (-3.0%, d=0.46) following morning muscle-damaging exercise compared to younger triathletes (-1.4%, d=0.29), as evidenced by a moderate between group effect when comparing change in performance from baseline. Few studies have examined recovery of exercise performance among masters compared to younger athletes following exercise-induced muscle damage. However, Sultana and colleagues (2012) compared the recovery of physiological parameters among masters (52±10 y) and younger (28±6 y) well-trained triathletes following an Olympic distance triathlon. The researchers observed that masters triathletes had a significantly reduced run speed at ventilatory threshold 24 h post-race, while younger triathletes had recovered to baseline run speeds (Sultana et al.,

2012). It is reasonable to speculate that this would likely have translated into poorer endurance exercise performance, similar to the trend found in the present study. Importantly, the recovery durations investigated in the present study, and that by Sultana and colleagues (2012) are similar to the normal training practices of competitive triathletes.

In summary, the present study has shown that well-trained masters triathletes have age-related attenuations in myofibrillar FSR compared to younger triathletes during a period of intense endurance training following muscle-damaging exercise. This finding was accompanied by a trend for masters triathletes to recover cycling performance at slower rates compared to younger triathletes. Future research examining the effects of higher protein intakes post-exercise on MPS, and recovery of exercise performance following muscle-damaging exercise among masters athletes are warranted.

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Conflicts of interest and source of funding

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Chapter 5 preamble

The findings from the peer-reviewed published paper presented in Chapter 4 suggest that masters triathletes exhibit lower rates of muscle protein synthesis (%·d^{-1}) compared to younger triathletes over a three-day period of training, when the current sport nutrition recommendations are met (Thomas et al., 2016). Furthermore, the findings of the previous study show that the greatest differences in the recovery between masters and younger triathletes occur in the afternoon following a morning muscle-damaging run. Given the findings of the previous study, it appears that masters athletes display "anabolic resistance" to protein feedings and exercise concomitantly. Thus, dietary protein strategies, as highlighted in Chapter 2, may be needed by masters athletes in order to overcome anabolic resistance. Despite these strategies being theoretically likely to maximise muscle protein synthesis in masters athletes, it is unknown whether these dietary protein strategies might translate to improved post-exercise recovery in masters athletes, particularly over a relatively short recovery period where mechanisms outside of elevated muscle protein synthesis may more heavily govern recovery.

Therefore, the purpose of study 3 (Chapter 5) was to examine whether repeated intakes of "higher" doses of protein (0.6 g·kg^{-1}), compared to doses of protein currently recommended by sport nutrition guidelines (0.3 g·kg^{-1}), led to enhanced same-day recovery of peak muscle function, perceptions of recovery, and afternoon cycling performance in well-trained, standard (Olympic) and Half-Ironman distance masters triathletes aged 50 years or older, following muscle-damaging exercise. All triathletes in the present study were currently competitive and in the pre-competition or competition phase of training.

DECLARATION OF CO-AUTHORSHIP AND CONTRIBUTION

Title of Paper	The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes
Full bibliographic reference for Journal/Book in which the Paper appears	Doering, T. M., Reaburn, P. R., Borges, N. R., Cox, G. R., & Jenkins, D. G. (2016). The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes. *International Journal of Sport Nutrition and Exercise Metabolism* (In Press).
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Nature of Candidate's Contribution

Thomas M. Doering co-conceived the research idea, designed the experiment, collected all data, undertook blood analysis, statistically analysed the data, drafted the manuscript in full, edited the manuscript based on co-author feedback, submitted the manuscript, and responded to reviewer comments.

Nature of Co-Authors' Contributions

Peter R. Reaburn, Nattai R. Borges, Gregory R. Cox and David G. Jenkins co-conceived the research idea, refined the experimental design, reviewed manuscript drafts and provided feedback for inclusion, reviewed the final manuscripts, and reviewed responses to reviewer comments.

Candidate's Declaration

I declare that the publication above meets the requirements to be included in the thesis as outlined in the Publication of Research Higher Degree Work for Inclusion in the Thesis Procedures

Signature: 

Date: 08/11/2016

5

Chapter 5: The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes

This chapter is an exact copy of the manuscript that has been accepted and is In Press in the *International Journal of Sport Nutrition and Exercise Metabolism*.

Doering, T. M., Reaburn, P. R., Borges, N. R., Cox, G. R., & Jenkins, D. G. (2016). The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes. *International Journal of Sport Nutrition and Exercise Metabolism* (In Press). doi: http://dx.doi.org/10.1123/ijsnem.2016-0079. Impact Factor: 2.44; Quartile 2 in Sport Science (2014).

Referencing format has been altered to conform to APA 6th edition. As this manuscript has been published, the references included in this manuscript are not also contained in the final references list of this thesis, but appear at the end of this chapter. Figure and Table numbers in this manuscript have been altered to also align with chapter numbers.

Abstract

Following exercise-induced muscle damage (EIMD), masters athletes take longer to recover than younger athletes. The purpose of this study was to determine the effect of higher than recommended post-exercise protein feedings on the recovery of knee extensor peak isometric torque (PIT), perceptions of recovery, and cycling time trial (TT) performance following EIMD in masters triathletes. Eight masters triathletes (52±2 y, $\dot{V}O_{2max}$, 51.8±4.2 $mL \cdot kg^{-1} \cdot min^{-1}$) completed two trials separated by seven days in a randomised, double-blind, crossover study. Trials consisted of morning PIT testing and a 30 min downhill run followed by an eight-hour recovery. During recovery, a moderate (MPI; 0.3 $g \cdot kg^{-1} \cdot bolus^{-1}$) or high (0.6 $g \cdot kg^{-1} \cdot bolus^{-1}$) protein intake (HPI) was consumed in three bolus feedings at two hour intervals commencing immediately post-exercise. PIT testing and a 7 $kJ \cdot kg^{-1}$ cycling TT were completed post-intervention. Perceptions of recovery were assessed pre- and post-exercise. The HPI did not significantly improve recovery compared with MPI ($p>0.05$). However, comparison of within-treatment change shows the HPI provided a moderate beneficial effect (d=0.66), attenuating the loss of afternoon PIT (-3.6%, d=0.09) compared to the MPI (-8.6%, d=0.24). The HPI provided a large beneficial effect (d=0.83), reducing perceived fatigue over the eight-hour recovery (d=1.25) compared to the MPI (d=0.22). Despite these effects, cycling performance was unchanged (HPI=2395±297 s vs. MPI=2369±278 s; d=0.09). In conclusion, doubling the recommended post-exercise protein intake did not significantly improve recovery in masters athletes; however, HPI provided moderate to large beneficial effects on recovery that may be meaningful following EIMD.

Keywords

Exercise-induced muscle damage; fatigue; cycling performance; nutrition.

Introduction

Following muscle-damaging exercise, masters athletes appear to take longer to recover muscle function than younger athletes (Easthope et al., 2010). Age-related differences in recovery have also been reported following exercise induced muscle damage (EIMD) incurred by downhill running in a laboratory trial, and are most pronounced when comparing differences in same-day recovery (Doering, Jenkins, et al., 2016). Together, these data suggest a slower rate of muscle repair and remodelling (Doering, Reaburn, Phillips, & Jenkins, 2016). Accelerating muscle repair following EIMD is critical to ensure optimal performance in subsequent training (Twist & Eston, 2005). However, few studies have focused on age-related factors that may influence recovery rates and thus recovery practices in masters athletes.

Appropriate post-exercise nutrition is widely accepted to accelerate recovery from prior exercise (Beelen, Burke, Gibala, & van Loon, 2010). Recently, protein intake has emerged as a vital component of post-exercise nutrition to assist both short and longer-term repair and remodelling of skeletal muscle (Moore, 2015; Moore, Camera, Areta, & Hawley, 2014). Current sport nutrition recommendations suggest protein feedings of 20 g post-exercise are sufficient to maximise muscle protein synthesis (MPS) in young cohorts (Beelen et al., 2010; Witard et al., 2014). Moreover, this dose repeated approximately four times per day (~3 h apart) is likely to maximise daily rates of MPS for maximal muscle protein repair and remodelling (Areta et al., 2013).

It is likely this pattern of protein feeding will also maximise daily MPS rates among masters athletes. However, current recommendations regarding the doses of protein to be consumed post-exercise do not account for age-related changes in protein metabolism (Doering, Reaburn, et al., 2016). We have recently shown well-trained masters triathletes (53±2 y) exhibit lower myofibrillar fractional synthetic rates compared to younger triathletes

over a three-day period of endurance training, with timed protein feedings in accordance with current sport nutrition recommendations (Doering, Jenkins, et al., 2016). Given these findings, and prior studies suggesting protein doses of 36 g (Robinson et al., 2013) and 40 g (Yang et al., 2012) are necessary to maximise MPS post-exercise in middle-aged and older adults, it is reasonable to suggest that higher doses of protein may offset the attenuated rates of MPS observed in masters compared to younger athletes. However, whether the delivery of greater doses of protein designed to maximise MPS in masters athletes will have any effect on same-day recovery of muscle function, or exercise performance following EIMD, is unknown. Recovery interventions targeting this time-frame are important, given the greatest difference in recovery rates between masters and younger athletes have been reported to be in the afternoon following morning EIMD (Doering, Jenkins, et al., 2016).

The aim of the present study was to compare the effect of repeated "high" (3×0.6 g·kg^{-1}) versus "moderate" (3×0.3 g·kg^{-1}) protein intakes following morning muscle-damaging running on recovery of afternoon peak isometric torque (PIT), perceptions of recovery, and cycling time trial (TT) performance in masters triathletes. It was hypothesised that the higher protein intake would accelerate recovery, and thus reduce the decrement in afternoon PIT, improve perceptions of recovery assessed in the afternoon, and improve afternoon cycling TT performance.

Method

Participants

Eight well-trained male masters triathletes participated in the study. All participants were currently competing and training ≥10 h per week for at least 12 weeks prior to participation. Participant characteristics can be found in Table 5.1. The study was approved by The University of Queensland's Human Research Ethics Committee.

Table 5.1: Participant characteristics.

Age (y)	52.1±2.1
Height (cm)	175.9±5.1
Body mass (kg)	74.6±5.2
Cycling PPO (W)	337.0±45.2
Treadmill $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	51.8±4.2
Training volume ($h \cdot week^{-1}$)	12.6±2.6
Duration of current training load (weeks)	15.9±5.5
Years in triathlon (y)	6.5±2.7
Typical duration between twice daily training (h)	7.9±2.3

Data are mean ± SD.

Experimental design

Participants completed two preliminary testing sessions separated by 48 h. Seven days later, the first of two experimental trials were commenced in a randomised, crossover, double-blind manner. Trial two was completed seven days later.

Preliminary testing

Participants completed one of two sessions in a randomised order involving either: 1) treadmill $\dot{V}O_{2max}$ testing, and familiarisation of PIT testing and the downhill run protocol; or 2) cycle peak aerobic power output testing, and familiarisation of perceptual measures of recovery and the cycling TT. Incremental treadmill testing commenced at 8 $km \cdot h^{-1}$ for five min followed by a ramp protocol increasing at a rate of 1 $km \cdot h^{-1} \cdot min^{-1}$ until volitional exhaustion (Saturn, h/p/cosmos, Nussdorf-Traunstein, Germany). Expiratory gas was continuously analysed using a calibrated metabolic cart (Trueone 2400, Parvomedics, Sandy, UT). $\dot{V}O_{2max}$ was determined as the highest 15 s value recorded over the duration of the test. Incremental cycle testing commenced at 100 W for five min, followed by a ramp protocol increasing at a rate of 25 $W \cdot min^{-1}$ until volitional exhaustion (Excalibur Sport, Lode, Groningen, Netherlands).

Trial overview

Participants reported to the laboratory between 5:30 and 8:00am after 24 h of dietary and 48 h of exercise standardisation. Upon arrival, baseline perceptual measures of recovery were recorded. Venous blood (8 mL) was sampled from an antecubital vein for the later analysis of serum myoglobin. Following a cycling warm up consisting of three, two-min intervals at 1.0, 1.5 and 2.0 $W \cdot kg^{-1}$, participants completed PIT testing. They then completed a standardised six-min warm up on a level treadmill before completing a 30-min downhill run. Immediately post-run, venous blood was sampled, and perceptual measure of recovery recorded. Participants were then provided with all food and fluids to be consumed over the next eight hours before returning to the laboratory that same afternoon to repeat the PIT testing and complete a cycling TT. The standardised cycle warm up was conducted prior to each test. Blood and perceptual measures were collected immediately before and after the afternoon session.

Dietary and exercise standardisation

A standardised diet consisting of 7.5 g of carbohydrate, 1.4 g of protein, and 200 kJ per kg of body mass was provided to participants for the 24 h before each trial. Athletes refrained from all exercise in the 24 h prior to each trial, and avoided muscle-damaging exercise in the 48 h prior to each trial. A training diary was maintained for seven days leading into trial 1, and replicated prior to trial 2.

Dietary intervention

In the recovery period of each trial, either a high protein intake (HPI) or moderate protein intake (MPI) was provided in three isocaloric liquid boluses administered immediately (0), 2 and 4 h post-exercise, in a randomised, double blind, crossover manner. At 0 and 2 h post-exercise, beverages were consumed containing either: 1.0/0.6 or 1.3/0.3 $g \cdot kg^{-1}$

carbohydrate/protein, for the HPI and MPI, respectively. At 4 h post-exercise, a standardised lunch was provided (1.0/0.3 $g \cdot kg^{-1}$ of carbohydrate/protein), with a blinded beverage containing either 0.3 $g \cdot kg^{-1}$ of protein or carbohydrate for the HPI and MPI, respectively. Regardless of experimental diet, a standardised pre-exercise meal was provided 2 h prior to the afternoon TT (6 h) consisting of 2.0 $g \cdot kg^{-1}$ and 40 $kJ \cdot kg^{-1}$ of carbohydrate and energy, respectively. In total, the HPI provided 5.0/1.95 $g \cdot kg^{-1}$ carbohydrate/protein, while the MPI provided 5.9/1.05 $g \cdot kg^{-1}$ carbohydrate/protein. All supplements contained whey protein isolate (Fonterra, Whey Protein Isolate 894, New Zealand), and maltodextrin (GPC, Maltrin QD-M585 Maltodextrin, IA) and glucose (iNova Pharmaceuticals, Glucodin, Australia) in a 1:1 ratio. Total quantities of each supplemental powder were corrected for macronutrient content in each powder. Non-caloric flavouring blinded participants to taste differences.

Downhill run

Participants completed a 30-min downhill (-10%) run at an intensity corresponding to 70% of $\dot{V}O_{2max}$ to induce EIMD (Braun & Dutto, 2003). Downhill run speed at 70% of $\dot{V}O_{2max}$ was determined during familiarisation under constant gas analysis. This run speed was utilised in trial 1 and 2. Expiratory gas, rating of perceived exertion (RPE; 6-20), and heart rate (RS800CX, Polar Electro, Finland) were monitored to ensure trial-to-trial standardisation.

Peak isometric torque

PIT (N·m) of the knee extensors was measured using isometric dynamometry (System 4 Pro, Biodex, Shirley, NY). Participants were secured in a seated position, and the dynamometer arm secured to the dominant ankle. Knee angle was fixed at 90°, and the dynamometer position was digitally recorded and replicated on all subsequent occasions. Participants completed three, three-second maximal isometric contractions of the knee

extensors with one-min passive recovery between repetitions. The highest peak torque value obtained was recorded for data analysis.

Perceptual measures of recovery

Subjective measures of fatigue (0 to 5), motivation (1 to 4), and Total Quality of Recovery (TQR; 6-20) (Kentta & Hassmen, 1998) were obtained, and perception of leg muscle soreness was measured on a 100 mm visual analogue scale as previously described (Fell, Reaburn, & Harrison, 2008).

Myoglobin concentration

Venous blood (8 mL) was sampled prior to, and immediately following all exercise bouts and serum stored at -80°C until further analysis. Serum myoglobin concentration ($ng \cdot mL^{-1}$) was measured as a marker of muscle damage via sandwich enzyme-linked immunosorbent assays (Abnova, Taipei City, Taiwan).

Cycling time trial

Participants completed a cycling TT in which work equating to 7 $kJ \cdot kg^{-1}$ was completed in the quickest possible time with the ergometer set in linear mode (Excalibur Sport, Lode, Groningen, Netherlands). Participants were notified when they had completed 25, 50, 75 and 90% of the total TT, and were blinded to all other data. RPE were recorded at these time points and a one-min expiratory gas sample was obtained at 25, 50 and 75% of TT completion. Blood lactate (Lactate Scout Plus, EKF Diagnostics, Germany) was measured pre- and post-exercise, and at 50% of TT completion. Heart rate was measured continuously throughout each TT.

Statistical analysis

To detect a 3.3% change in TT performance as previously found with dietary interventions (Stearns, Emmanuel, Volek, & Casa, 2010), with an 80% probability, and a

within-subject CV of 2% (Zavorsky et al., 2007), 8 cyclists were required for this study (Hopkins, 2006). Data were analysed using SPSS (Version 22.0, SPSS, Armonk, NY) and are reported as mean ± standard deviation (SD) or with 95% confidence interval (CI). Normality was assessed by skewness and kurtosis z-score. Repeated measures ANOVA were used to compare interventions, and main effects of order. TT performance, within-treatment change, and comparison of within-treatment changes were also analysed with paired t-tests (Hopkins, 2003) with Bonferroni corrections. Alpha was accepted at $p<0.05$. Cohen's *d* effect sizes were also calculated for within-treatment changes and their comparisons to determine magnitudes of change. Threshold values for small, moderate and large effects were 0.2, 0.5 and 0.8, respectively (Sullivan & Feinn, 2012).

Results

There were no significant differences in any variable recorded during the downhill run in trials 1 and 2 ($p>0.05$). In both trials, $\dot{V}O_2$ and RPE increased throughout the run ($p<0.05$). Randomisation resulted in five of eight participants consuming the MPI on their first trial. However, there was no effect of order ($p>0.05$) for any variable considered. At the completion of this study, 50% of athletes correctly identified the HPI, and no athlete was certain of trial order.

Peak isometric torque

Figure 5.1 displays mean and individual PIT values prior to the downhill run, and following each dietary intervention. There was no treatment*time interaction ($p=0.21$), or main effect for treatment ($p=0.95$). However, PIT was reduced following downhill running ($p=0.008$). The HPI attenuated reductions in afternoon PIT (-3.6%, $d=0.09$, $p=0.31$) to a greater extent than the MPI (-8.6%, $d=0.24$, $p=0.006$). Comparison of changes shows the HPI provided a moderate beneficial effect ($d=0.66$, $p=0.29$).

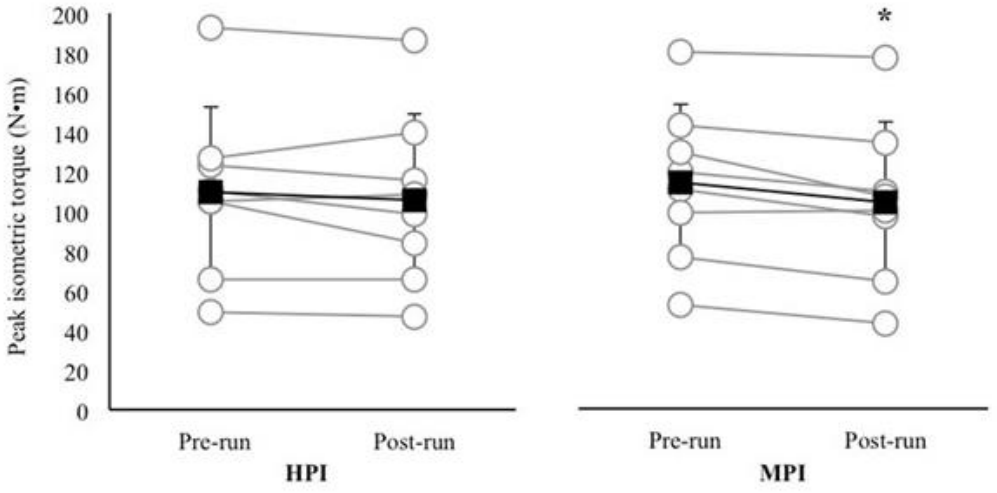

Figure 5.1: Peak isometric torque (N·m) of the knee extensors prior to downhill running and following the HPI and MPI for each participant. Mean values are represented by black squares (mean ± SD). *Mean significantly different from morning value.

Perceptual measures of recovery

Table 5.2 displays all perceptual measures data over the course of each trial. There was no treatment*time interaction ($p>0.05$), or main effect for treatment ($p>0.05$) for any perceptual measure. A significant effect of time was observed for all measures ($p<0.05$), except motivation ($p=0.43$). When examining the direct effect of the intervention (post-run to pre-TT), the HPI reduced perceived fatigue ($d=1.25$, $p=0.02$), to a greater extent than the MPI ($d=0.22$, $p=0.45$). Comparison of changes shows the HPI provided a large beneficial effect ($d=0.83$, $p=0.17$).

Myoglobin concentration

Figure 5.2 displays serum myoglobin concentration ($ng \cdot mL^{-1}$) throughout each trial. There was no treatment*time interaction ($p=0.56$), or main effect for treatment ($p=0.09$). Myoglobin concentration increased across each trial ($p<0.001$).

Table 5.2: Perceptual measures of recovery over the course of each experimental trials.

		Pre-run	Post-run	Pre-TT	Post-TT
Fatigue (0-5)	HPI	1.7±1.3	3.0±0.8	2.0±0.8	3.4± 0.7
	MPI	1.6±1.1	2.6±1.1	2.4±0.7	3.0±1.2
Motivation (1-4)	HPI	3.3±0.5	3.3±0.5	3.4±0.5	3.1±0.6
	MPI	3.3±0.7	3.4±0.5	3.4±0.5	3.3±0.7
TQR (6-20)	HPI	16.3±1.6	13.0±3.2	13.9±2.3	12.8±2.9
	MPI	15.4±2.3	14.3±2.0	14.5±1.3	14.0±1.8
Muscle soreness (0-100 mm)	HPI	16.9±10.8	31.0±9.5	28.0±13.6	40.9±22.9
	MPI	19.3±18.0	39.2±23.2	40.5±19.4	52.3±29.7

Data are mean ± SD. HPI = high protein intake, MPI = moderate protein intake, TQR = total quality of recovery.

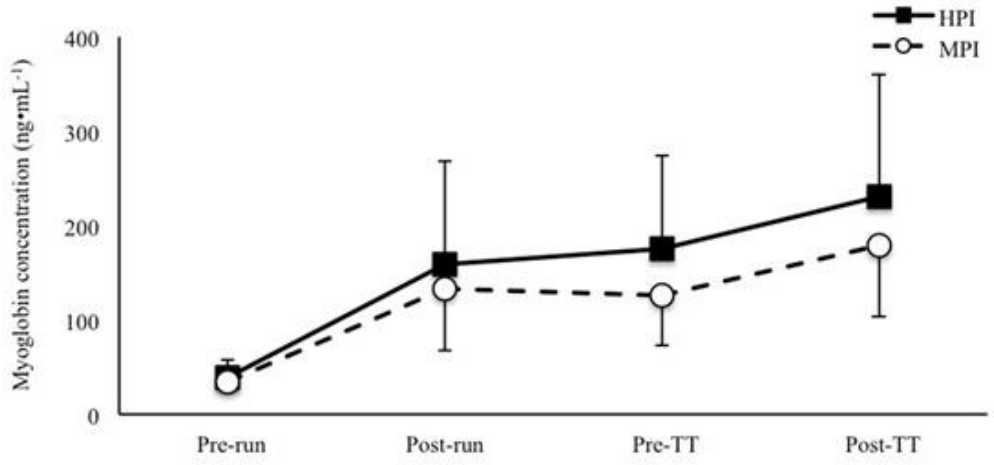

Figure 5.2: Concentration of serum myoglobin ($ng \cdot mL^{-1}$) throughout each experimental trial (mean ± SD).

Cycling time trial

Figure 5.3 shows mean and individual TT performance times (s) during both dietary treatments. There was no significant difference in TT performance between the HPI (2395±297 s) and MPI (2369±278 s; -26 s, 95% CI: -119 to 66 s, p=0.52, *d*=0.09).

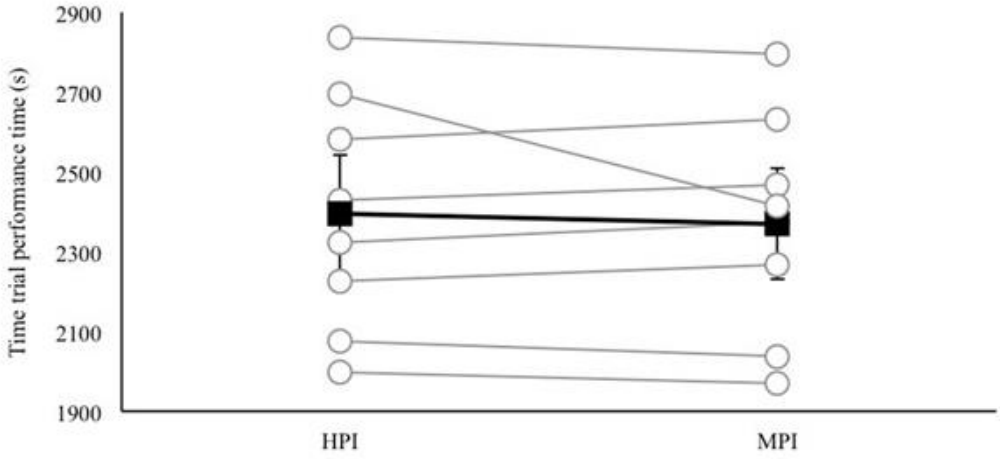

Figure 5.3: Time trial performance time (s) following the HPI and MPI for each participant. Mean performance times are represented by black squares (mean ± SD).

There was no treatment*time interaction or effect of treatment on RPE, blood lactate, heart rate, or $\dot{V}O_2$ throughout each TT ($p>0.05$). All variables increased across the TT ($p<0.001$), except $\dot{V}O_2$ ($p=0.73$). Mean TT heart rate was not different between trials (HPI=150±15 vs. MPI=151±14).

Discussion

This is the first study to examine the effects of higher than recommended (double) post-exercise protein feedings on same-day recovery following muscle-damaging endurance exercise in masters triathletes. The findings from this study were that repeated intake of higher protein feedings (3×0.6 g·kg^{-1} versus 3×0.3 g·kg^{-1}) following EIMD had: 1) a moderate beneficial effect on attenuating loss of PIT production; and, 2) a large beneficial effect on reducing perceptions of fatigue. Despite these differences, a higher than recommended protein intake during the recovery period following EIMD had no beneficial effect on afternoon cycling TT performance in masters triathletes.

Masters athletes have previously been shown to take longer than younger athletes to recover muscle function following EIMD (Easthope et al., 2010). This delayed recovery may, at least partially, be caused by slower muscle protein repair and remodelling (Doering, Reaburn, et al., 2016). We have shown masters triathletes exhibit attenuated rates of MPS in comparison to younger triathletes during a three-day period of training, with protein feedings equivalent to the MPI in the present study (Doering, Jenkins, et al., 2016). These results suggest the need for a higher, age-specific, post-exercise protein intake in masters athletes, as implemented in the present study, and suggested necessary for older untrained adults (Churchward-Venne, Holwerda, Phillips, & van Loon, 2016). However, the effect that higher protein doses may have on acute recovery was unknown.

In the present study a HPI post-exercise provided a moderate beneficial effect compared to the MPI, in attenuating the loss of PIT associated with prior EIMD in masters triathletes. Taken with the similar serum myoglobin concentrations in each trial, showing the amount of muscle damage incurred was the same, these findings show that the HPI post-exercise accelerated recovery of muscle function in masters athletes. These findings are supported by a previous study that showed a single high protein bolus (100 g) increased the rate of recovery of PIT, while a placebo treatment resulted in the reduction of PIT for 48 h in active young (21 ± 1 y) males (Etheridge, Philp, & Watt, 2008). In the present study, the attenuation of PIT loss, or the accelerated recovery of PIT with the HPI may be due to elevated rates of muscle repair/remodelling; however, this requires future investigation. Nonetheless, a HPI consumed over consecutive meals following morning exercise may be beneficial to masters athletes completing afternoon training requiring high strength/power outputs, such as high intensity interval training (Twist & Eston, 2005).

Despite the HPI providing a moderate beneficial effect in the present study, a previous review by Pasiakos, Lieberman, and McLellan (2014) concluded that post-exercise protein

supplementation appears to have limited effects on recovery of acute muscle function in younger (<40 years) adults and athletes. However, the review by Pasiakos et al. (2014) utilised a broad range of studies with differing designs, varying exercise modes, dietary protocols, and recovery periods (Pasiakos et al., 2014). In contrast, the present study used recovery periods typically used by endurance athletes when training twice daily, and was conducted in a cohort of masters triathletes with a mean age of 52 years. Furthermore, protein supplementation in the present study was not a single bolus, rather repeated intakes as shown necessary to maximise muscle protein remodelling (Areta et al., 2013). In summary, the present study is unique in many aspects, particularly the cohort tested. These differences make comparisons to research in younger cohorts difficult.

In the present study, the HPI post-exercise provided a large beneficial effect in lowering perceptions of fatigue, compared to MPI, over the eight-hour recovery. Previous work by Fell and colleagues (2008) reported that well-trained masters cyclists (45±6 y) perceive greater levels of fatigue post-exercise compared to younger (24±5 y) cyclists, over three consecutive days of high intensity cycling. Therefore, interventions that provide a reduction in perceived fatigue in masters athletes appear valuable. Similar to our findings, Rowlands and colleagues (2007) have previously shown a HPI (~2.9 $g \cdot kg^{-1}$) administered in regular boluses over four hours post-exercise, reduces perceptions of tiredness and leg soreness during exercise the following day, when compared to a low protein intake (~0.4 $g \cdot kg^{-1}$) in younger (35±10 y) cyclists. In summary, the present data shows there may be potential for a HPI post-exercise to lower perceptions of fatigue in masters athletes. Future research is warranted to examine perceptions of recovery in masters athletes in larger cohorts.

Despite beneficial effects observed on recovery of both PIT and perceptions of fatigue, the HPI provided no effect to afternoon cycling performance. These findings appear in line

with those observed in cohorts <50 years of age (McLellan, Pasiakos, & Lieberman, 2014). However, given the potential of the HPI to attenuate loss of muscle force, or accelerate recovery of PIT after EIMD in masters athletes, appropriate periodisation of this intervention may be beneficial to cycle training requiring high power outputs, or following repeated bouts of damaging exercise; however, this remains to be determined. Future research should examine the effect of HPI post-exercise on these exercise modes, in larger samples to decrease the likelihood of type two statistical errors.

Conclusion

In summary, the present findings suggest that a higher than recommended protein intake consumed over three meals during the post-exercise recovery period has a moderate effect on attenuating the loss of PIT, and a large effect on reducing perceptions of fatigue associated with EIMD; however, this feeding strategy had no effect on same-day cycling TT performance, nor other perceptions of recovery in well-trained masters triathletes.

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6

Chapter 6: General summary

The present thesis is comprised of an introduction chapter, a published literature review and three published manuscripts from three independent but closely linked studies. Each study is presented as a separate chapter (three though five), with each contributing original knowledge to the fields of both sport nutrition and protein metabolism specific to masters athletes. Below, a general summary of each study/chapter is provided with conclusions based on this series of studies subsequently presented. Practical implications of the series of studies are then offered. The final section of the current chapter will present future research directions based on the findings and the limitations of the present thesis.

Study 1: Comparison of post-exercise nutrition knowledge and post-exercise carbohydrate and protein intake between Australian masters and younger triathletes

Study 1 has been published in the *International Journal of Sport Nutrition and Exercise Metabolism*. The purpose of the study 1 (Chapter 3) was to compare the post-exercise nutritional practices of masters triathletes to both younger triathletes and current sport nutrition recommendations. The main finding of study 1 was that masters triathletes typically consume significantly lower amounts of carbohydrate post-exercise (0.7±0.4 $g \cdot kg^{-1}$) compared to younger triathletes (1.1±0.6 $g \cdot kg^{-1}$; p=0.01), and currently recommended carbohydrate intakes (1.0 $g \cdot kg^{-1}$; p=0.001). However, post-exercise protein intakes of masters triathletes (20±14 g) are commensurate with intakes of younger triathletes (26±16 g) and current recommendations (~20 g) when considered as absolute doses. Conversely, on a relative (per kilogram of body mass) basis, masters triathletes consumed significantly less protein (0.3±0.2 $g \cdot kg^{-1}$) than younger triathletes (0.4±0.2 $g \cdot kg^{-1}$; p=0.03).

Limitations of self-report dietary data aside, and the potential for athletes to lose focus over a 20-minute survey, it was concluded that the low post-exercise carbohydrate intakes of masters triathletes may contribute to slowed recovery following fatiguing endurance exercise. However, low carbohydrate intakes are not unusual among athletes in "weight conscious" sports such as triathlon (Burke et al., 2003), or among female athletes (Burke et al., 2001), and are unlikely to explain the age-related discrepancies in recovery following muscle-damaging exercise (Easthope et al., 2010), but not following fatiguing non-damaging exercise in older individuals (Borges et al., 2016; Fell et al., 2006). Given the dose of dietary protein consumed post-exercise has a major influence on skeletal muscle protein synthesis (MPS), and thus muscle remodelling following exercise-induced muscle damage (Churchward-Venne, Holwerda, Phillips, & van Loon, 2016), it was deemed important to examine whether the amount of protein currently recommended and typically consumed by masters triathletes post-exercise (~20 g), equally elevated MPS in masters compared to younger triathletes over a period of laboratory-controlled endurance training.

Study 2: Lower integrated muscle protein synthesis in masters compared with younger athletes

Study 2 has been published in *Medicine and Science in Sports and Exercise*. The purpose of study 2 (Chapter 4) was to: 1) compare the myofibrillar fractional synthetic rate (FSR) between masters and younger triathletes over a 72-hour period of endurance training following a muscle-damaging run, with timed protein and carbohydrate feedings consistent with current sport nutrition recommendations; and, 2) compare the recovery of endurance cycling performance following a muscle-damaging run between masters and younger triathletes at 10, 24 and 48 h following a downhill run. The main findings of this study were that: 1) masters triathletes displayed a significantly lower ($p=0.009$; $d=1.98$) myofibrillar FSR

(1.49±0.12%·d^{-1}) compared to younger triathletes (1.70±0.09%·d^{-1}) over the three-day period of training and dietary standardisation; and, 2) masters triathletes tended to recover their cycling performance at a slower rate than younger triathletes following muscle-damaging running, as evidenced by a greater decrement in cycling performance at 10 h post-run (compared to baseline) in masters (-3.0%, *d*=0.46) compared to younger triathletes (-1.4%, *d*=0.29). Comparison of these decrements in cycling performance revealed a moderate between-group effect (*d*=0.51).

The findings of study 2 are the first to demonstrate that, despite chronic high volume endurance training, masters triathletes exhibit lower myofibrillar FSR compared to younger triathletes when the current sport nutrition recommendations for both carbohydrate and protein intake, post-exercise and per meal, are met. This response was evident despite high quality, fast absorbing, leucine rich (~2.3 g per dose) whey protein isolate being administered post-exercise. This study also showed that in a laboratory-controlled environment, well-trained masters triathletes recover cycling performance more slowly than younger triathletes following a muscle-damaging endurance run.

It should be noted that a limitation to the present study was the inability to measure muscle protein breakdown, the other contributing factor (in addition to muscle protein synthesis) to muscle protein remodelling/turnover. Given this omission, it is unknown how rates of muscle protein breakdown vary between masters and younger athletes post-exercise, and if altered rates of muscle protein breakdown contributed to the poorer rates of muscle recovery among older athletes. Regardless, based on the findings from study 2, it was concluded that higher doses of dietary protein post-exercise may be warranted for masters triathletes, to maximise MPS rates. This suggestion is also supported by previous research that reliably demonstrates that elderly untrained adults retain the ability to produce MPS responses in line with younger adults, provided they consume higher than recommended (>20 g) doses

of dietary protein (Moore et al., 2015; Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009). However, whether or not alleviating this discrepancy of approximately 0.2% per day in myofibrillar MPS between masters and younger athletes would provide any substantial benefit to acute recovery is unknown. Furthermore, from the present data, the degree of disparity in rates of mixed muscle (sarcoplasmic/mitochondrial) protein synthesis between masters and younger triathletes are also unknown. However, it is indeed likely that over time, this observed disparity would lead to reduced muscle protein repair and contribute to poorer muscle remodelling.

Regardless, based on findings of study 2, it was concluded that recovery interventions targeting same-day recovery (i.e., morning and afternoon training) are required by masters athletes given the poorer recovery of performance at this time-point compared to younger athletes. Despite higher post-exercise dietary protein intakes being likely to facilitate increased myofibrillar protein synthesis, it had yet to be determined whether higher than recommended post-exercise protein intakes influenced same-day recovery of exercise performance, following morning muscle-damaging exercise in masters triathletes.

Study 3: The effect of higher than recommended protein feedings post-exercise on recovery following downhill running in masters triathletes

Study 3 has been accepted for publication in the *International Journal of Sport Nutrition and Exercise Metabolism*. The purpose of study 3 (Chapter 5) was to examine whether repeated post-exercise intakes of higher doses of protein (0.6 $g \cdot kg^{-1}$) were superior to doses of protein currently recommended (0.3 $g \cdot kg^{-1}$) following muscle-damaging exercise to enhance: 1) same-day recovery of peak muscle function (peak isometric torque); 2) perceptions of recovery; and, 3) afternoon cycling time trial performance in well-trained masters triathletes. The main findings of this study were that, compared to repeated post-exercise intakes of 0.3

g·kg^{-1}, repeated intakes of "high" post-exercise protein doses (0.6 g·kg^{-1}) provided: 1) a moderate beneficial effect (*d*=0.66) to attenuate loss of peak isometric torque production; and, 2) a large beneficial effect (*d*=0.83) to reduce perceptions of fatigue. Despite these benefits, a higher than recommended protein intake during the recovery period following downhill running did not affect afternoon cycling time trial performance in masters triathletes (*d*=-0.09). This study concluded that post-exercise protein intakes of doses double those currently recommended may be beneficial to masters athletes completing both morning and afternoon training sessions, particularly when morning training is likely to induce muscle damage, and afternoon training requires high strength and/or power outputs.

Given that recent research has also shown that recovery of strength following exercise-induced muscle damage is dependent on elevated MPS (Baumann et al., 2016), and that maximising daily rates of MPS may facilitate training adaptations among athletes (Moore, 2015; Moore, Camera, Areta, & Hawley, 2014), the present study provides evidence to suggest that masters athletes would benefit from both acute and chronic consumption of higher, age-specific, post-exercise protein boluses compared to those currently recommended. A further interesting outcome of this study was that higher protein intakes reduced perceptions of fatigue, despite no significant changes in afternoon cycling performance. Indeed, others have shown changes (decreases) in perceptions of recovery, despite no alterations in physiological recovery in masters athletes (Fell et al. 2008). The present study provides novel findings to suggest that elevated protein intakes (additional 1.0 g·kg^{-1}) may alleviate this phenomenon of heightened perceptions of fatigue in masters athletes, which may help older athletes to train more regularly than they otherwise would.

Despite downhill running being atypical of regular training, it may approximate muscle-damage induced by high intensity running, or an Olympic distance triathlon, in older

athletes. A recent study by Rowsell, Reaburn, Toone, Smith, and Coutts (2014) has shown that 7 × 5-minute running intervals on a treadmill elicited a myoglobin concentration of 83±46 $ng \cdot mL^{-1}$ measured 9 h post-run in young, highly-trained (VO_{2peak}, 73.46±10.2 $mL \cdot kg^{-1} \cdot min^{-1}$) triathletes. Furthermore, myoglobin concentrations of 360±296 $ng \cdot mL^{-1}$ have been reported following an Olympic distance triathlon in non-elite triathletes (37±7 years) (Park, Kim, Han, Ji, & Kwak, 2014). Finally, Neubauer, Konig, and Wagner (2008) have shown mean myoglobin concentration to rise to approximately 2000 $ng \cdot mL^{-1}$ following an Ironman triathlon. Considering the downhill run protocol used in the present study elicited a mean (of both trials) myoglobin concentration of 151±77 $ng \cdot mL^{-1}$ at 8 h post-run in older athletes (>50 years), this protocol may not be as extreme as it's perceived to be.

Finally, a limitation to this study is the cross-over nature of the study design. Indeed, the repeated bout effect suggests participants may become accustom to the downhill run after the first trial, which may dampen the muscle-damaging effect on the second trial. Randomisation of dietary interventions was implemented to lessen the likelihood of the repeated bout effect influencing results. Nonetheless, this is a limitation of the present study, and given the available number of well-trained masters triathletes, this was unavoidable.

Conclusions

In conclusion, the presented series of studies has shown that the dietary practices of masters triathletes differ to those of younger triathletes, and that these practices may contribute to the slower recovery of older athletes following demanding exercise. Moreover, findings of the present thesis suggest that chronic endurance training into middle/older age appears not to offset age-related anabolic resistance in masters athletes who routinely maintain high volumes of endurance-type exercise. Indeed, this finding may explain, in part, the slower recovery of

masters compared to younger athletes following muscle-damaging exercise, as elevations in MPS are required to facilitate muscle repair (Baumann et al., 2016; Hill et al., 2003); higher post-exercise protein doses may likely offset these attenuated rates of MPS, given that a number of recent studies have shown that older untrained adults can exhibit MPS rates equivalent to younger adults when fed high enough doses of protein (Churchward-Venne et al., 2016; Moore et al., 2015). Despite being unable to draw correlations between recovery of performance and MPS rates from the present data, due to the integrated mean measurement of MPS, this thesis also demonstrates that masters triathletes recover cycling performance at a slower rate than younger triathletes following muscle-damaging exercise in a laboratory-controlled environment. This age-related difference in the recovery of cycling performance is most evident at 10 h following a muscle-damaging run. Importantly, this schedule of training and between-session recovery duration is typical of athletes training twice a day. Finally, this thesis has shown that higher than recommended protein intakes post-exercise facilitate the recovery of peak muscle function (peak isometric torque) of the knee extensors, and reduce perceptions of fatigue over an eight hour period in masters triathletes. Furthermore, the lower carbohydrate intake (5.0 $g \cdot kg^{-1}$) associated with the higher protein intakes appear not to impede afternoon cycling performance in masters triathletes.

Practical implications

The present research suggests a number of practical implications to sport nutrition and training practices of masters athletes specifically, as well as physical activity recommendations for older adults in general.

1. It is recommended that masters endurance athletes consult a sports dietitian to determine convenient and appropriate post-exercise dietary options that contain whole-food options with optimal macronutrient contributions for differing training scenarios (i.e., one versus

two training sessions per day), and adequate protein intakes to maximise muscle protein repair and remodelling.

2. Masters athletes completing two training sessions per day should maximise the duration of the recovery period (i.e., early morning and late afternoon). Alternatively, following exercise that results in exercise-induced muscle damage, it should be expected that exercise performance will be reduced for up to 24 h.
3. When completing two training sessions per day, masters athletes should avoid high intensity training following exercise that involves high volumes of eccentric muscle actions, such as morning prolonged/undulating running or resistance training.
4. Masters athletes should consider implementing age-specific dietary protein strategies. Specifically, increasing their post-exercise protein intake to ~0.4-0.6 $g \cdot kg^{-1}$, and consuming high quality, leucine-rich whey protein. This may be particularly important if prior exercise has resulted in muscle-damage; however, this may not be necessary following non-damaging exercise bouts.
5. Masters athletes should consider implementing the above dietary protein strategies, namely increasing the doses of protein consumed at main meals, and ensuring breakfast also contains high quality sources of protein from whole-food sources to optimise daily MPS rates for muscle protein remodelling. This is particularly important if muscle repair and adaptation to training are of priority; however, total protein and energy intake should be manipulated with consideration of body composition goals.
6. Masters athletes should incorporate resistance training (Garber et al., 2011) and/or high intensity interval training (Bell, Seguin, Parise, Baker, & Phillips, 2015) to their training programs. Both resistance and high intensity interval training appear to raise MPS to significantly higher rates, and for significantly longer durations, than those achieved following an aerobic training bout in older adults (Bell et al., 2015). Given the present

thesis shows masters athletes exhibit attenuated rates of MPS in response to endurance training compared to younger athletes, the addition of these types of physical training may facilitate a prolonged and heightened elevation of MPS. This may be important to both acute muscle remodelling and the maintenance of lean mass into older age, which may be compromised with endurance training alone.

7. Older untrained adults should be encouraged to complete resistance-type training or high intensity interval training into older age, given that chronic endurance training at high weekly volumes (>10 $h \cdot wk^{-1}$) of over four times that currently recommended (2.5 $h \cdot wk^{-1}$) appears to not offset age-related anabolic resistance to exercise and/or protein feeding.

Future direction for research

Given the major findings of the present thesis, together with limitations to the present series of studies, future investigations could:

- Examine gender-related differences in carbohydrate and protein intake in larger, and different, cohorts of masters athletes.
- Examine differences in carbohydrate and protein intake between more experienced (>10 years) younger and masters athletes.
- Investigate the reasons or beliefs underpinning the poor post-exercise nutritional intakes of masters athletes.
- Investigate the underpinning mechanisms of anabolic resistance in masters compared to younger endurance athletes by examining the phosphorylation statuses of key mTORC1 signalling protein in response to a single exercise bout, and protein feeding of ~20 g or 0.25 $g \cdot kg^{-1}$.
- Investigate other potential mechanisms of anabolic resistance such as microRNA (miRNA) expression. miRNA's are known to inhibit protein translation by targeted degradation of

messenger RNA's (Guller & Russell, 2010). Preliminary studies have shown that miRNA species within the let-7 family of miRNA's may be related to age-related impairments in muscle repair/remodelling (Drummond et al., 2011; Zacharewicz et al., 2014), which deserves further investigation.

- Investigate other potential mechanisms of anabolic resistance such as impaired translational capacity due to reduced ribosomal biogenesis. Recently, Stec, Mayhew, and Bamman (2015) have shown that 24 h following a resistance training bout, only young adults possess an elevated expression of 45S precursor rRNA (precursor to ribosomal RNA) and a concomitant elevated transcription initiation factor 1α. Further, only young adults tended to increase ribosomal proteins S3 and S6. These results suggest older adults possess a blunted ribosomal biogenesis response to exercise, which deserves future attention as a potential mechanism contributing to anabolic resistance.
- Investigate other potential mechanisms of anabolic resistance such as chronic, low grade inflammation. It has been proposed that normal aging is characterised by chronic low grade inflammation, termed by some as "inflammaging" (Franceschi, Garagnani, Vitale, Capri, & Salvioli, 2016). Cuthbertson et al. (2005) have previously shown that nuclear factor (NF)-κB is substantially elevated in older compared to younger adults, and it's been proposed that elevated levels of NF-κB, tumor necrosis factor α, and interleukin-6 may interfere with mTOR signalling and thus contribute to anabolic resistance (Haran, Rivas, & Fielding, 2012), which deserves further investigation.
- Investigate the dose-response of post-exercise dietary protein feedings at doses of 0.25, 0.40 and 0.60 $g \cdot kg^{-1}$ and resulting MPS rates of well-trained masters endurance athletes compared to age-matched sedentary adults in response to increasing protein feedings in isolation, and following a standardised relative intensity exercise bout (i.e., post-exercise).

Such research may elucidate any effect of chronic endurance exercise training on retaining anabolic sensitivity to amino acids into older age. These protein doses are important to investigate given: 1) the lower dose of 0.25 $g \cdot kg^{-1}$ is the currently recommended protein intake for athletes; 2) the middle dose of 0.40 $g \cdot kg^{-1}$ is currently suggested, based on a compilation of current research, to be required to maximise MPS in older, but untrained adults; and, 3) the highest dose of 0.60 $g \cdot kg^{-1}$ is of importance given, to date, no studies have identified a protein-feeding induced ceiling to MPS in older adults/athletes.

- Examine the above dose-response (post-exercise dietary protein – MPS) relationship in well-trained masters strength/power athletes compared to younger similarly-trained athletes in response to increasing protein feedings in isolation, and following a standardised relative intensity exercise bout (i.e., post-exercise). Such research may elucidate any effect of exercise mode on retaining anabolic sensitivity to amino acids into older age.
- Examine the above dose-response (post-exercise dietary protein – MPS) relationship over a range of ages from 50-80 years of age, in trained and/or untrained adults, in response to increasing protein feedings in isolation, and/or following a standardised relative intensity exercise bout (i.e., post-exercise). Such research may identify the required protein dose to maximise MPS in progressive aging, rather than broadly classify athletes/adults as "older" and "younger".
- Examine the above dose-response (post-exercise dietary protein – MPS) relationships, or similar, using whole-food options, which may provide differing results given differing amino acid compositions to milk-derived protein powers, or similar. Such data will be of importance to prescribe "real-world" food options to both athletes and older individuals.

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Appendices

Appendix 1: Ethics approval for study 1

Secretary, Human Research Ethics Committee
Ph: 07 4923 2603
Fax: 07 4923 2600
Email: ethics@cqu.edu.au

A/Prof Peter Reaburn and
Mr Thomas Doering
School of Medical and Applied Sciences

16 May 2014

Dear A/Prof Reaburn and Mr Doering

HUMAN RESEARCH ETHICS COMMITTEE ETHICAL APPROVAL PROJECT: H14/04-071 *NUTRITIONAL RECOVERY PRACTICES, KNOWLEDGE AND BELIEFS OF AUSTRALIAN TRIATHLETES*

The Human Research Ethics Committee is an approved institutional ethics committee constituted in accord with guidelines formulated by the National Health and Medical Research Council (NHMRC) and governed by policies and procedures consistent with principles as contained in publications such as the joint Universities Australia and NHMRC *Australian Code for the Responsible Conduct of Research.* This is available at http://www.nhmrc.gov.au/publications/synopses/_files/r39.pdf.

On 16 May 2014, the Deputy Chair of the Human Research Ethics Committee considered your application under the Low Risk Review Process. This letter confirms that your project has been granted approval under this process, pending ratification by the full committee at its June 2014 meeting.

Please forward a copy of the survey when it has been finalised, so that it can be sighted before implementation.

The period of ethics approval will be from 16 May 2014 to 30 December 2014. The approval number is H14/04-071 please quote this number in all dealings with the Committee. HREC wishes you well with the undertaking of the project and looks forward to receiving the final report.

The standard conditions of approval for this research project are that:

(a) you conduct the research project strictly in accordance with the proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee;

(b) you advise the Human Research Ethics Committee (email ethics@cqu.edu.au) immediately if any complaints are made, or expressions of concern are raised, or any other issue in relation to the project which may warrant review of ethics approval of the project. *(A written report detailing the adverse occurrence or unforeseen event must be submitted to the Committee Chair within one working day after the event.)*

(c) you make submission to the Human Research Ethics Committee for approval of any proposed variations or modifications to the approved project before making any such changes;

(d) you provide the Human Research Ethics Committee with a written "Annual Report" on each anniversary date of approval (for projects of greater than 12 months) and "Final Report" by no later than one (1) month after the approval expiry date; *(A copy of the reporting pro formas may be obtained from the Human Research Ethics Committee Secretary, Sue Evans please contact at the telephone or email given on the first page.)*

(e) you accept that the Human Research Ethics Committee reserves the right to conduct scheduled or random inspections to confirm that the project is being conducted in accordance to its approval. Inspections may include asking questions of the research team, inspecting all consent documents and records and being guided through any physical experiments associated with the project

(f) if the research project is discontinued, you advise the Committee in writing within five (5) working days of the discontinuation;

(g) A copy of the Statement of Findings is provided to the Human Research Ethics Committee when it is forwarded to participants.

Please note that failure to comply with the conditions of approval and the *National Statement on Ethical Conduct in Human Research* may result in withdrawal of approval for the project.

You are required to advise the Secretary in writing within five (5) working days if this project does not proceed for any reason. In the event that you require an extension of ethics approval for this project, please make written application in advance of the end-date of this approval. The research cannot continue beyond the end date of approval unless the Committee has granted an extension of ethics approval. Extensions of approval cannot be granted retrospectively. Should you need an extension but not apply for this before the end-date of the approval then a full new application for approval must be submitted to the Secretary for the Committee to consider.

The Human Research Ethics Committee wishes to support researchers in achieving positive research outcomes. If you have issues where the Human Research Ethics Committee may be of assistance or have any queries in relation to this approval please do not hesitate to contact the Secretary, Sue Evans or myself.

Yours sincerely,

Professor Phillip Ebrall
Chair, Human Research Ethics Committee

Cc: Project file

Approved

Appendix 2: Study 1 survey distributed (online) by *Triathlon Australia*

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Participant Information Sheet

Investigators:

Thomas Doering
Dr Peter Reaburn
Dr David Jenkins
Dr Gregory Cox

Contact:

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Introduction:

The multi-sport nature of triathlon typically results in high daily training loads. Therefore, optimising recovery from physical training and racing appears important. Post-exercise nutrition strategies are an important aspect of the recovery process following both training and racing. The aim of this research study is to determine the current post-exercise nutritional practices, the knowledge of current sports nutrition recovery guidelines, and the beliefs that determine nutritional choices in Australian triathletes following exercise.

Participants:

Triathletes aged 18 years or older, who have competed in the sport of triathlon in the 2013/14 season, or

are consistently training for the 2014/15 season, are invited to participate in this online survey. Participants must only complete this survey once.

Overview:

In order to identify and understand nutritional recovery strategies in Australian triathletes, this survey consists of several sections. Questions will be asked regarding your:

1. Demographic,
2. Training history,
3. Post-exercise nutrition,
4. Recovery strategies used.

The information obtained from this survey will be used by CQUniversity in conjunction with Triathlon Australia for several purposes. Data will be used to identify what Australian triathletes are using for nutrition following exercise, and whether a greater awareness and dissemination of nutritional guidelines are required among Australian triathletes. Information obtained will be used in a PhD thesis, peer-reviewed publications, and conference presentations in several topic areas.

Full completion of this survey is anticipated to take ~20 minutes. You may withdraw from this research project at any stage prior to submitting the survey without penalty or prejudice. Due to all survey information being non-identifiable, once submitted, the survey data cannot be withdrawn as individual responses cannot be identified. Data collection for this survey will commence on the 9th of October 2014. Please complete all responses by the 1st of December 2014.

You must read and understand this information sheet in full prior to commencing this survey. Prior to answering survey specific questions, you must confirm that all inclusion criteria as outlined in the Participants section are met, that you consent for data to be used in a PhD thesis, and in any peer-reviewed publications, or conference presentations that may stem from this survey.

Risk:

Minimal risk is involved in this research project. In no way will any personal or identifying data be collected in this survey. Therefore all data collected is 100% confidential.

Benefits:

Upon successful completion of this survey, you will be redirected to a second SurveyMonkey webpage that is separate from the survey data you will provide. If you wish to go into the draw to win one of six, $50 gift cards for Coles Group & Myer, you may follow the prompts on this page, and enter your details to do so. The personal information you enter on the second SurveyMonkey webpage can in no way be connected to the data you provide in the “Nutritional recovery practices, knowledge and beliefs of Australian triathletes” survey.

Confidentiality & Informed Consent:

As no personal identifying data is collected within the survey, all data provided is 100% unidentifiable, and in no way able to be traced to the provider of the data. By checking 'Yes' to the check-boxes of questions 1-4 of the survey 'Nutritional recovery practices, knowledge, and beliefs of Australian triathletes', you are acknowledging that you have read and understood this information sheet, and consent to your data being used as specified above.

*** 1.**

i) I have read and understood the information provided, and consent to my unidentifiable data being used in a PhD thesis, peer-reviewed publications, and conference presentations,

ii) I am 18 years of age or older,

iii) I have competed in the 2013-14 triathlon season, or I am training regularly for the 2014-15 season,

iv) I acknowledge that this survey must only be completed once by myself.

- ◯ Yes
- ◯ No

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Demographic Information

*** 2. Please provide your age (years):**

*** 3. Gender:**

- ○ Male
- ○ Female

*** 4. Please provide your body mass (kg):**

*** 5. Please provide your height (cm):**

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Training History

This section aims to assess your training history and current level of competitiveness.

*** 6. How many years have you been competing in the sport of triathlon?**

*** 7. Please provide your personal best times for the following triathlon distances you have completed:**

	Hours	Minutes
Sprint distance (750m/20km/5km)		
Olympic/Standard distance (1500m/40km/10km)		
Half Ironman (70.3) distance (1900m/90km/21km)		
Ironman (140.6) distance (3800m/180km/42km)		

*** 8. What triathlon distance are you currently training for?**

- ◯ Sprint distance (750m/20km/5km)
- ◯ Olympic/Standard distance (1500m/40km/10km)
- ◯ Half Ironman (70.3) distance (1900m/90km/21km)
- ◯ Ironman (140.6) distance (3800m/180km/42km)
- ◯ Other (please specify below)

Other (please specify)

*** 9. How close is your next targeted event?**

- ◯ Less than 6 weeks away
- ◯ Between 6-10 weeks away
- ◯ Between 11-15 weeks away
- ◯ Between 16-20 weeks away
- ◯ More than 20 weeks away

*** 10. In this upcoming event, how would you describe your participation?**

- ◯ Competing for fun & social interaction
- ◯ Competing to maintain fitness
- ◯ Competing as a competitive age-grouper
- ◯ Competing as a top age-grouper
- ◯ Competing as a professional triathlete (hold a professional licence)

*** 11. In a typical week, how many sessions and total hours do you spend:**

	Sessions	Hours
Swimming		
Cycling		
Running		
Other (please specify below)		

Please specify your other activities, i.e. resistance training

*** 12. How often do you train multiple times per day?**

◯ Never ◯ Rarely ◯ Sometimes ◯ Often ◯ Always

*** 13. How important do you feel post-exercise nutrition is to your recovery after training?**

◯ Very Unimportant ◯ Unimportant ◯ Neither Important or Unimportant ◯ Important ◯ Very Important

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Post-exercise nutrition (training)

This section aims to determine your current post-exercise nutritional practices following training.

*** 14. In trying to understand your immediate post-training nutrition habits (i.e. the first thing you eat or drink after training), please indicate how often you would typically:**

	Never	Rarely	Sometimes	Often	Always
Eat nothing, or consume water only	○	○	○	○	○
Eat a scheduled meal (i.e. breakfast, lunch or dinner)	○	○	○	○	○
Eat/drink prepared snacks or snack foods/drinks readily available at supermarkets, bakeries, cafes, service stations or convenience stores	○	○	○	○	○
Eat/drink a sports supplement (i.e. sports drinks, protein-carbohydrate drinks, protein shake, protein bars)	○	○	○	○	○
Eat/drink a combination of snack foods and sports supplements (i.e. muesli bar + protein shake)	○	○	○	○	○

*** 15. If you eat a scheduled meal immediately after training (i.e. this meal is the first thing you will eat/consume after training, apart from water), which of the following meals would it typically be?**

- ○ I never use my next scheduled meal as a post-training nutrition option
- ○ Breakfast
- ○ Lunch
- ○ Dinner

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 16. What food groups would you typically include in this meal?**

- [ ] Fruits
- [ ] Grains and cereals
- [ ] Lean meats/proteins (Tofu)
- [ ] Dairy
- [ ] Vegetables and legumes

*** 17. Please describe this meal (breakfast, lunch or dinner + drinks consumed) that you typically consume after training, using household measures with as much detail as possible.**

For example, your typical post-exercise breakfast may consist of 4 Weet-Bix with 1½ cups of 99% (Pura) fat free milk, 1 large banana, and 1 cup (250 mL) of no-added sugar orange juice. Or, your typical post-exercise dinner may consist of 1 cup of cooked white rice, with 200 grams of lean beef strips, ½ a cup of broccoli, 1 medium carrot, ½ a medium zucchini, with ½ a cup of Kantong stir-fry sauce, and 1 cup of Cottee's cordial.

Please be as specific as possible (grams, pieces, brands, amount of spread, cups of drink, etc.) to enable accurate nutritional analysis of this meal.

*** 18. Typically, how long after the end of training do you consume this meal?**

- ◯ Within 30 minutes
- ◯ Within 1 hour
- ◯ Within 2 hours
- ◯ Within 3 hours
- ◯ Within 4 hours
- ◯ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 19. If you eat/drink prepared snacks, or readily available snack foods/drinks immediately after training (foods/drinks readily available at supermarkets, bakeries, cafes, service stations or convenience stores), what food groups would you typically include in this snack?**

- [ ] I never use prepared snacks, or readily available snack foods/drinks as a post-training nutrition option
- [ ] Fruits
- [ ] Grains and cereals
- [ ] Lean meats/proteins (Tofu)
- [ ] Dairy
- [ ] Vegetables and legumes

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 20. If you eat/drink prepared snacks, or readily available snack foods/drinks immediately after training, please describe this snack with as much detail as possible.**

For example, your snack may consist of 1 Uncle Toby's yogurt covered muesli bar + 1 row of seaweed flavoured rice crackers with a 1 glass of (99%) reduced fat milk and 2 heaped teaspoons of Milo.

Please be as specific as possible (grams, pieces, brands, amount of spread, cups, etc.) to enable accurate nutritional analysis of this meal.

*** 21. Typically, how long after the end of training do you consume this snack?**

- ○ Within 30 minutes
- ○ Within 1 hour
- ○ Within 2 hours
- ○ Within 3 hours
- ○ Within 4 hours
- ○ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 22. Are there occasions when you eat/drink ONLY sports supplements (i.e. carbohydrate/protein/sports drinks, powders, bars or similar) immediately after training?**

- ◯ Yes
- ◯ No

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 23. Please describe this sports supplement with as much detail as possible, including brand name and serving size.**

For example, your post-exercise sports supplement may consist of 600 mL of pre-made Blue-Bolt Gatorade, 1 x 60 g chocolate Powerbar performance bar, or 1 serve (30 g of powder) of Musashi lean whey protein isolate in 300 mL of water.

Please be as specific as possible (grams, pieces, brands, amount of spread, cups of drink, etc.) to enable accurate nutritional analysis of this meal.

*** 24. Typically, how long after the end of training do you consume this supplement?**

- ○ Within 30 minutes
- ○ Within 1 hour
- ○ Within 2 hours
- ○ Within 3 hours
- ○ Within 4 hours
- ○ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 25. If you eat/drink a COMBINATION of snacks/drinks and sports supplements immediately after training (i.e. 1 Carman's original muesli bar + 1 serve (50 g of powder) of Musashi Sports Protein-Carb Matrix in 300 mL of 99% fat free milk), what food groups would you typically include in this snack and sports supplement combination?**

- [ ] I never use a COMBINATION of snacks/drinks and sports supplements as a post-training nutrition option
- [ ] Fruits
- [ ] Grains and cereals
- [ ] Lean meats/proteins (Tofu)
- [ ] Dairy
- [ ] Vegetables and legumes

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 26. If you eat/drink a COMBINATION of snacks/drinks and sports supplements immediately after training, please describe this snack and sports supplement with as much detail as possible, including brand name and serving size.**

For example, your post-exercise snack and sports supplement may consist of 1 Carman's original muesli bar + 1 serve (50 g of powder) of Musashi Sports Protein-Carb Matrix in 300 mL of 99% fat free milk.

Please be as specific as possible (grams, pieces, brands, amount of spread, cups of drink, etc.) to enable accurate nutritional analysis of this meal.

*** 27. Typically, how long after the end of training do you consume this snack and sports supplement combination?**

- ◯ Within 30 minutes
- ◯ Within 1 hour
- ◯ Within 2 hours
- ◯ Within 3 hours
- ◯ Within 4 hours
- ◯ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Post-exercise nutrition (racing)

This short section will ask questions relating to your nutrition after racing only.

*** 28. How important do you feel post-exercise nutrition is to your recovery after racing?**

◯ Very Unimportant ◯ Unimportant ◯ Neither Important or Unimportant ◯ Important ◯ Very Important

*** 29. Do you specifically plan your immediate post-racing nutrition?**

◯ Yes

◯ No

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 30. What food groups would you typically include in this post-race meal/snack?**

- [ ] Fruits
- [ ] Grains and cereals
- [ ] Lean meats/proteins (Tofu)
- [ ] Dairy
- [ ] Vegetables and legumes

*** 31. Please describe your typical post-race meal or snack/drink/sports supplement in as much detail as possible using house-hold measures/commercial brands and quantities.**

Please be as specific as possible (grams, pieces, brands, amount of spread, cups of drink, etc.) to enable accurate nutritional analysis of this meal.

*** 32. How long after finishing the race do you consume this meal/snack?**

- ◯ Within 30 minutes
- ◯ Within 1 hour
- ◯ Within 2 hours
- ◯ Within 3 hours
- ◯ Within 4 hours
- ◯ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Post-exercise nutrition guidelines and knowledge of macronutrient content in foods

This section will provide information regarding your knowledge of sports nutrition guidelines, macronutrient content in foods, and beliefs that may influence food choices.

It is essential that you answer these questions to the best of your knowledge, without seeking external help.

*** 33. How many grams of carbohydrate (per kilogram of body mass) should an endurance athlete consume in their post-exercise snack/meal to optimise their recovery?**

*** 34. How many grams of protein (in total) should an endurance athlete consume in their post-exercise snack/meal to optimise their recovery?**

*** 35. To the best of your knowledge, please provide an estimate of the amount of carbohydrate and protein (grams) that is in the following snacks/meals, and whether each snack/meal meets sports nutrition guidelines for post-exercise carbohydrate and protein intake for a 70 kg athlete.**

	Amount of carbohydrate	Amount of protein	Meets guidelines?
⅔ of a cup of toasted muesli, 1 medium banana, 200 g tub of yoghurt, and 1 cup (200 mL) of milk			
⅓ of a cup of almonds (35 g), 200 g tub of yoghurt and 1 glass (250 mL) of milk with Milo (2 teaspoons)			
600 mL of Coca-Cola soft drink and 1 Mars bar			
1 Whey Protein Isolate (30 g) powder made with 300 mL of milk			
2 chicken sushi rolls and 600 mL of diet soft drink			
1 Hamburger (150 g lean beef mince patty, ½ an avocado, ½ a tomato, and lettuce) on a medium sized multi-grain bun and 600 mL of flavoured milk			
600 mL of Powerade sports drink, ½ a banana and 2 slices of watermelon			
1 Uncle Toby's Crunchy muesli bar			

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Post-exercise nutrition behaviours

It is essential that you answer these questions honestly, and to the best of your knowledge, without seeking external help.

*** 36. Are there any situations where you intentionally avoid high carbohydrate containing foods after training?**

- ◯ No, I always consume high carbohydrate containing foods after training
- ◯ Yes, I sometimes avoid high carbohydrate containing foods after training

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 37. What is the primary reason you sometimes avoid high carbohydrate containing foods after training?**

- ◯ I avoid high carbohydrate containing foods because I don't need that much carbohydrate/ I follow a low carbohydrate diet
- ◯ I avoid high carbohydrate containing foods because I eat adequately before exercise
- ◯ I avoid high carbohydrate containing foods because I fuel adequately throughout exercise
- ◯ I avoid high carbohydrate containing foods because my sessions aren't long enough to justify eating immediately after
- ◯ I avoid high carbohydrate containing foods because this will enhance my exercise adaptation to endurance training
- ◯ I avoid high carbohydrate containing foods because this will assist in weight management
- ◯ I avoid high carbohydrate containing foods because this will change how I burn fat after exercise
- ◯ I don't know why
- ◯ Other (please specify)

*** 38. Do you intentionally include high protein containing foods after training?**

- ◯ Yes, I intentionally include high protein containing foods after training
- ◯ No, I intentionally avoid high protein containing foods after training
- ◯ Unsure, or I may unintentionally consume high protein containing foods after training

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 39. What is the primary reason you avoid high protein containing foods after training?**

- ○ I don't know why
- ○ I avoid high protein containing foods because it compromises my carbohydrate intake
- ○ I avoid high protein containing foods because endurance athletes do not require high amounts of protein
- ○ I avoid high protein containing foods because I don't want to put on weight
- ○ I avoid high protein containing foods because I don't want to 'bulk up'
- ○ I avoid high protein containing foods because I don't tolerate anything substantial immediately after exercise
- ○ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 40. What is the primary reason you include high protein containing foods after training?**

- ○ I include high protein containing foods to assist muscle repair/hypertrophy after strength/resistance training sessions
- ○ I include high protein containing foods to assist muscle repair after extended/long endurance training sessions
- ○ I include high protein containing foods to assist muscle repair after hard running/high impact training sessions
- ○ I include high protein containing foods to assist muscle repair after all training sessions
- ○ I include high protein containing foods to assist in weight management
- ○ I include high protein containing foods to assist muscle repair as I am an older athlete
- ○ I don't know why
- ○ Other (please specify)

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

Post-exercise recovery strategies

This section will provide information on the additional post-exercise strategies that you commonly use following training and racing.

*** 41. Do you use other forms of recovery after training? (i.e. active recovery, foam roller, etc.).**

- ◯ Yes
- ◯ No

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 42. Please select all that apply (you may select more than one).**

- [ ] Dietary supplements
- [ ] Contrast therapy (hot/cold)
- [ ] Massage (remedial/sports)
- [ ] Stretching
- [ ] Self massage (foam roller/massage ball)
- [ ] Compression garments
- [ ] Ice bath
- [ ] Passive recovery (complete rest)
- [ ] Hydrotherapy/water immersion
- [ ] Active recovery
- [ ] Other (please specify)

*** 43. Do you use other forms of recovery after racing? (i.e. active recovery, foam roller, etc.).**

- ( ) Yes
- ( ) No

Nutritional recovery practices, knowledge and beliefs of Australian triathletes

*** 44. Please select all that apply (you may select more than one).**

- [ ] Dietary supplements
- [ ] Massage (remedial/sports)
- [ ] Self massage (foam roller/massage ball)
- [ ] Ice bath
- [ ] Hydrotherapy/water immersion
- [ ] Other (please specify)
- [ ] Contrast therapy (hot/cold)
- [ ] Stretching
- [ ] Compression garments
- [ ] Passive recovery (complete rest)
- [ ] Active recovery

*** 45. Finally, what is the primary source of your information about post-exercise nutrition for recovery?**

- ◯ Own previous knowledge
- ◯ Triathlon magazines
- ◯ Other magazines (please specify)
- ◯ Internet (please specify)
- ◯ Friends/teammates
- ◯ Fellow athletes
- ◯ Textbooks
- ◯ Scientific journals
- ◯ Nutrition store attendant
- ◯ Nutritionist
- ◯ Naturopath
- ◯ Personal Trainer
- ◯ Accredited Dietitian
- ◯ Accredited Sports Dietitian
- ◯ Accredited Exercise Physiologist
- ◯ Accredited Sports Scientist
- ◯ Other (please specify)

Other (please specify)

Appendix 3: Ethics approval for study 2

Secretary, Human Research Ethics Committee
Ph: 07 4923 2603
Fax: 07 4923 2600
Email: ethics@cqu.edu.au

A/Prof Peter Reaburn
Mr Thomas Doering
School of Medical and Applied Science

12 August 2014

Dear A/Prof Reaburn and Mr Doering

HUMAN RESEARCH ETHICS COMMITTEE OUTCOME MODIFICATION TO PROJECT: H14/05-106, *EFFECT OF THE RECOMMENDED POST-EXERCISE PROTEIN-CARBOHYDRATE SUPPLEMENTATION ON PERFORMANCE RECOVERY AND MUSCLE PROTEIN RESPONSE IN YOUNG AND MASTERS TRIATHLETES: A COMPARATIVE STUDY*

The Human Research Ethics Committee is an approved institutional ethics committee constituted in accord with guidelines formulated by the National Health and Medical Research Council (NHMRC) and governed by policies and procedures consistent with principles as contained in publications such as the joint Universities Australia and NHMRC *Australian Code for the Responsible Conduct of Research.* This is available at http://www.nhmrc.gov.au/publications/synopses/_files/r39.pdf.

On 27 May 2014, the committee met and considered your application. The project was assessed as being greater than low risk, as defined in the National Statement. On 5 June 2016, the committee acknowledged compliance with the revisions requested to be made to your research project *Effect of the recommended post-exercise protein-carbohydrate supplementation on performance recovery and muscle protein response in young and masters triathletes: a comparative study* (Project Number H14/05-106) and it is now **APPROVED**. On 24 June 2014, the Chair approved your request to amend the timing of the biopsy protocol from four hours (post exercise) to 1 hour (post exercise). On 12 August 2014, the Chair approved your request to include a maximal voluntary isometric contraction test, as outlined in your modification form.

The period of ethics approval will be from 5 June 2014 to 1 March 2016. The approval number is H14/05-106; please quote this number in all dealings with the Committee. HREC wishes you well with the undertaking of the project and looks forward to receiving the final report and statement of findings.

The standard conditions of approval for this research project are that:

(a) you conduct the research project strictly in accordance with the proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee;
(b) you advise the Human Research Ethics Committee (email ethics@cqu.edu.au) immediately if any complaints are made, or expressions of concern are raised, or any other issue in relation to the project which may warrant review of ethics approval of the project. *(A written report detailing the adverse occurrence or unforeseen event must be submitted to the Committee Chair within one working day after the event.)*

(c) you make submission to the Human Research Ethics Committee for approval of any proposed variations or modifications to the approved project before making any such changes;

(d) you provide the Human Research Ethics Committee with a written "Annual Report" on each anniversary date of approval (for projects of greater than 12 months) and "Final Report" by no later than one (1) month after the approval expiry date; *(A copy of the reporting pro formas may be obtained from the Human Research Ethics Committee Secretary, Sue Evans please contact at the telephone or email given on the first page.)*

(e) you accept that the Human Research Ethics Committee reserves the right to conduct scheduled or random inspections to confirm that the project is being conducted in accordance to its approval. Inspections may include asking questions of the research team, inspecting all consent documents and records and being guided through any physical experiments associated with the project

(f) if the research project is discontinued, you advise the Committee in writing within five (5) working days of the discontinuation;

(g) A copy of the Statement of Findings is provided to the Human Research Ethics Committee when it is forwarded to participants.

Please note that failure to comply with the conditions of approval and the *National Statement on Ethical Conduct in Human Research* may result in withdrawal of approval for the project.

The Human Research Ethics Committee is committed to supporting researchers in achieving positive research outcomes through sound ethical research projects. If you have issues where the Human Research Ethics Committee may be of assistance or have any queries in relation to this approval please do not hesitate to contact the Ethics and Compliance Officer or myself.

Yours sincerely,

Dr Tania Signal
Chair, Human Research Ethics Committee

Cc: A/Prof David Jenkins (co-supervisor), Dr Erik Hohmann (partner researcher) Project file

APPROVED

Appendix 4: Information sheet and informed consent form for study 2

Effect of post-exercise PRO CHO supplementation on performance recovery and muscle protein response in masters and young triathletes

Investigators

Thomas Doering
Dr Peter Reaburn
Dr David Jenkins

Location

School of Medical and Applied Sciences
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Introduction

Masters triathletes are over the age of 40 years and are the most rapidly expanding group of age group triathletes, particularly in long course events. In addition, athletes over the age of 45 years have shown the greatest improvement in performance over the past 25 years in the Hawaii Ironman.

Despite significant improvements in triathlon performance, masters athletes have been shown to take longer to recover from training or racing. Research has shown that older athletes require longer durations to recovery maximal strength following muscle damage from long duration running, in comparison to young similarly trained athletes. Furthermore, older athletes have been shown to perceive their recovery to

be poorer, perceive higher levels of fatigue and higher perceptions of muscle soreness following repeated endurance cycling sessions.

Correct recovery nutrition following training and racing is vital to refuel and repair muscles. Carbohydrate and protein are vital components of effective recovery nutrition. However, recent research has shown lower protein synthesis in older adults, suggesting muscle repair following hard exercise may be slower in older athletes. This factor may contribute to the impaired recovery of strength and higher levels of muscle soreness reported in older athletes.

Currently, masters athletes are recommended to consume the same post-exercise nutrition (protein and carbohydrate) as young athletes. However, given recent research suggesting impairments to muscle protein metabolism, these recommendations may not be sufficient to maximise subsequent performances in older athletes.

Therefore, the purpose of this study is to compare the recovery of endurance time trial performance and muscle protein metabolism responses between masters and similarly-trained younger triathletes.

Participants

Male triathletes between the ages of 18 and 30, and over 50 years of age are invited to participate in this research project. Participants will be required to undergo preliminary testing to identify suitability to this research project. Preliminary testing will include:

- Pre-exercise screening
- Training load consistency questionnaire
- VO_{2max} testing

Participants must report a minimum weekly training volume of 10 h per week, consistently for 12 weeks. In addition, athletes must meet minimum VO_{2max} criteria of 50 mL.kg.min^{-1} to ensure physiologically matched groups.

Requirements

Participants must be able to attend the Exercise Physiology laboratory at CQUniversity on six separate occasions. Your involvement in this study will span approximately 14 days. During this time you must be willing to:

1. Undertake body measures including skin-folds for determination of body fat percentage,
2. Undertake a treadmill VO_{2max} test,
3. Complete a three-day diet diary, and replicate the foods reported (quantities may be slightly altered) in your diary for four days during the study,

4. Complete one morning (downhill run of 30 min) and one afternoon (cycling time trial ~30 min) exercise bout separated by 10 h, with two small, thigh muscle biopsies to be taken before, and one hour following the morning exercise bout. Previous research has shown this procedure to be relatively painless,
5. Consume 150 mL of 'heavy water' for muscle protein synthesis analysis,
6. Complete a further two cycling time trials (~30 min) on the two subsequent mornings, at 24 and 48 h following the first exercise bout,
7. Undertake a total of two venous blood samples (venepuncture), and 14 finger-prick blood samples for determination of muscle damage and blood lactate concentration over the course of the study,
8. Undertake three (total) fine needle muscle biopsy procedures conducted by an orthopaedic surgeon (no incision, no stitches, merely a Band-Aid to cover).

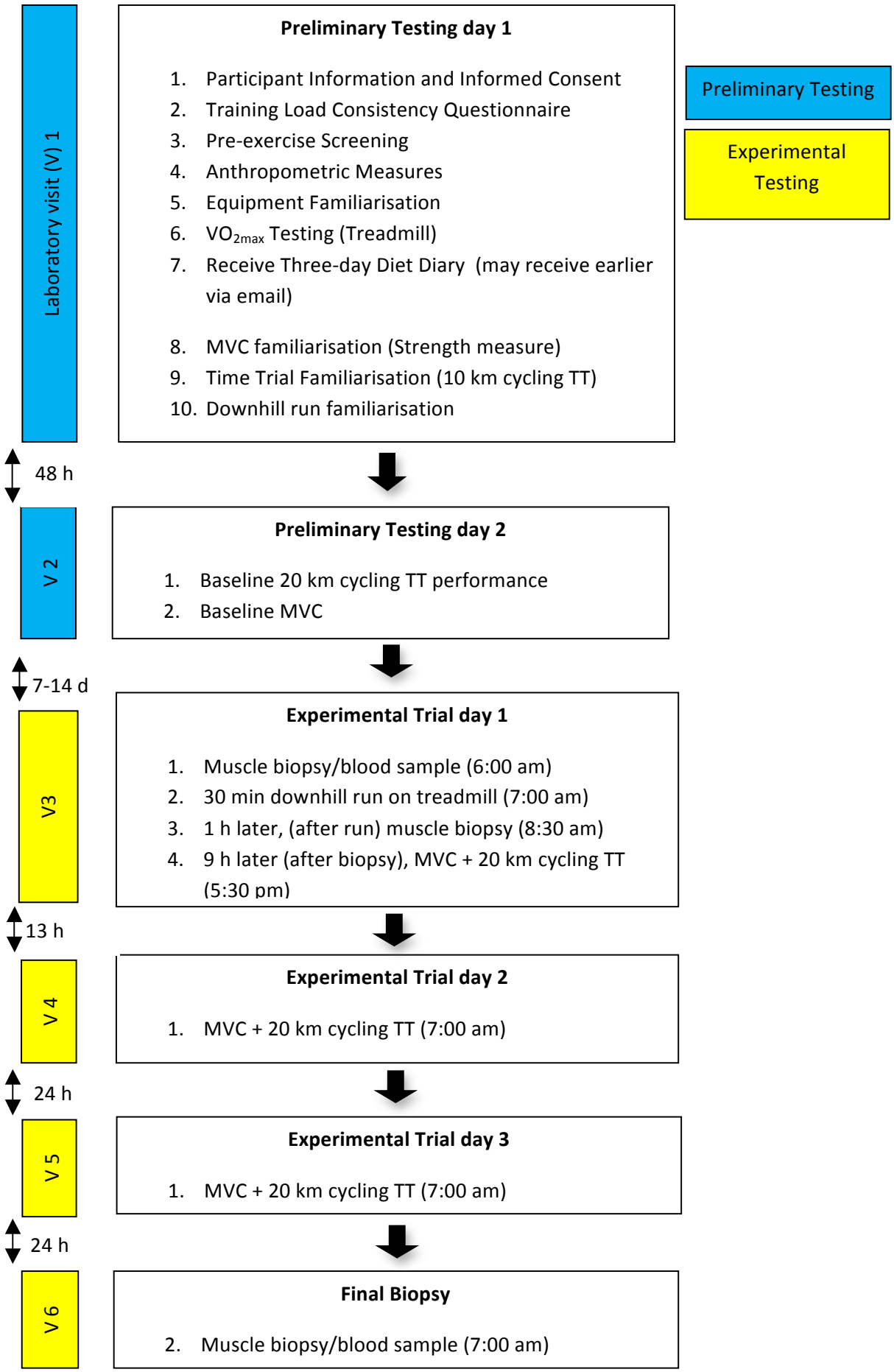
Study Overview

Participants must be able to attend the Exercise Physiology laboratory at CQUniversity Rockhampton Sport Centre (beside Bruce Highway) on six separate occasions. On the following page is an overview of the six visits, and approximate times they may be commenced. These times may be negotiated depending on work commitments, etc.

Supplementation

Currently, all athletes regardless of age are recommended to consume the same post-exercise recovery nutrition. Research suggests that the addition of protein to a carbohydrate beverage at a dose of 0.2-0.4 g/kg of body mass, or 20 g, provides adequate protein for muscle building and repair, and lowers the amount of carbohydrate needed to refuel the body. Therefore, the protein carbohydrate beverage to be consumed post-exercise will consist of 20 g and 1 g/kg of protein and carbohydrate respectively. Protein-carbohydrate supplementation will occur immediately following all exercise bouts, and a carbohydrate only beverage will be provided in the second hour to comply with SDA recommendations.

In addition to protein-carbohydrate beverages consumed in this study, participants will also ingest 150 mL of deuterium oxide (^2H_2O) immediately following the initial muscle biopsy sample. ^2H_2O is water, however in the hydrogen atoms (H) in this water are of higher weight (^2H) than normal, and hence can be differentiated from 'normal weight' hydrogen when analysed with specialist equipment. ^2H_2O consumption is safe, and frequently used in research for investigation of both energy expenditure and muscle protein synthesis rates. In this study, ^2H_2O will be consumed with the aim that ^2H will be incorporated into body water (~0.2%), and allow determination of muscle protein synthesis via determination of ^2H in muscle protein.


Laboratory visit (V) 1
Preliminary Testing day 1
1. Participant Information and Informed Consent
2. Training Load Consistency Questionnaire
3. Pre-exercise Screening
4. Anthropometric Measures
5. Equipment Familiarisation
6. VO_{2max} Testing (Treadmill)
7. Receive Three-day Diet Diary (may receive earlier via email)
8. MVC familiarisation (Strength measure)
9. Time Trial Familiarisation (10 km cycling TT)
10. Downhill run familiarisation
Preliminary Testing
Experimental Testing
48 h
V 2
Preliminary Testing day 2
1. Baseline 20 km cycling TT performance
2. Baseline MVC
7-14 d
V3
Experimental Trial day 1
1. Muscle biopsy/blood sample (6:00 am)
2. 30 min downhill run on treadmill (7:00 am)
3. 1 h later, (after run) muscle biopsy (8:30 am)
4. 9 h later (after biopsy), MVC + 20 km cycling TT (5:30 pm)
13 h
V 4
Experimental Trial day 2
1. MVC + 20 km cycling TT (7:00 am)
24 h
V 5
Experimental Trial day 3
1. MVC + 20 km cycling TT (7:00 am)
24 h
V 6
Final Biopsy
2. Muscle biopsy/blood sample (7:00 am)

Muscle Biopsy

Muscle biopsy samples will be collected on three separate occasions by orthopaedic surgeon, Dr Erik Hoemann. A fine needle biopsy will be used, removing the requirement for skin incision and stitching. This method of biopsy is now routinely used when small muscle samples are required for analysis (the size of each biopsy is approximately the size of half a pea – thought to amount to ~1/10,000th of your total muscle mass in your thigh).

This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle which is ~1.5 mm in diameter. This procedure involves two injections. First, the area over the muscle to be sampled is cleaned with an antiseptic solution and a small amount (1.5-3 mL) of local anesthetic is injected into and under the skin over the vastus lateralis (outer thigh) muscle. Second, a sterile probe covered in a polymer cannula sleeve is inserted through the anesthetized skin and through the fascia, which covers the muscle. The probe is removed leaving the hollow plastic cannula in place. The sterile biopsy needle is inserted/injected into the cannula. A small piece of muscle (~20mg) will quickly be obtained and then the needle will be removed. The muscle is quickly removed from the needle over a sterile work area nearby. The needle remains sterile during this process and is then re-inserted into the cannula for a 2nd small sample. This procedure is repeated 2 more times for a total of 4 small snips. These samples comprise a single biopsy time point and require 1 to 2 minutes in total. The total muscle removed is approximately 80mg (smaller than an eraser at the end of a pencil). During the time that each sample is being taken (~2-3 sec), the sensation of pressure in the muscle is felt, and on some occasions this is moderately uncomfortable for a few seconds (similar to a muscle twitch of cramp). However, the discomfort passes quickly and the participant is quite capable of getting up and moving around.

Following the removal of the sample, the biopsy site is covered with a sterile Band-Aid and wrapped with a tensor bandage. Once the anesthetic wears off, the leg may feel tight in the local area of the sampling and often there is the sensation of a bruise or cramp. The participant should not take any aspirin-based medicine for 24 h following the experiment as this can promote bleeding in the muscle. It is also beneficial, but not required, to keep the limb elevated when you are sitting, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day, the muscle may feel slightly ‘bruised’ upon movement, such as going down stairs. Any tightness in the muscle usually disappears within 1-2 days. In order to allow the incision to heal properly and minimize any risk of infection, the subject should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided. After a shower the incision area should only be tapped dry. The Band-Aid can be changed at any time if needed and removed after 3-4 days.

Blood Sampling

Venous blood samples will be obtained from the forearm anticubital vein by venepuncture procedure. Phlebotomists trained to industry standard will conduct all venepunctures within a controlled, sterile area designated for blood collection. Blood collection will occur before the downhill run bout on day one, and upon the sixth visit to the laboratory after all exercise is complete. A 5 mL sample of blood will be obtained, via a 21G needle inserted just below the elbow. Following blood collection, a cotton ball will be placed over the site and pressure will be applied to minimise risk of bruising. A sterile Band-Aid will subsequently be placed over the site.

Diet and Activity

Throughout participation in the experimental trial (the day before visit 3, and days of visit 3, 4, and 5), both diet and physical activity will be standardised. This is to ensure differences observed in performance and muscle metabolism between masters and young groups are attributable to experimental variables.

Participants will be provided with a three-day diet diary upon their first contact with the researcher. Prior to visiting the laboratory for baseline time trial testing, participants should record three full days of dietary intake in the provided diary. Preferably, diet should be recorded over one day of the weekend and two weekdays. This information will be utilised when prescribing the standardised diet to follow throughout the experimental trial (the day before visit 3, and days of visit 3, 4, and 5). Standardised macronutrient contributions (g/kg) will be prescribed based on body mass by an accredited sports dietician for optimal exercise performance and protein synthesis. Foods reported in the participant's diet diary will be manipulated (amounts of foods may be changed) in order to standardise the amount of carbohydrate and protein consumed by each participant. Participants are asked to consume the foods, and amounts prescribed, the night before visit 3, and days of visit 3, 4, and 5. No food is to be eaten before the final muscle biopsy is taken on visit 6.

Physical activity will also be standardised throughout the experimental period. Participants will be asked to refrain from vigorous, and all forms of physical activity for the 48 h, and 24 h respectively preceding each visit to the laboratory, apart from that inherent in the study. In addition, no additional physical activity is to be undertaken during the experimental trial (final four days).

Benefits

Participation in this research project will provide several benefits to the participant. Briefly, following study completion, all participants will receive:

1. VO_{2max} data, including identification of ventilatory threshold one and two, and VO_{2max},

2. Personalised heart rate and run speed training zones,
3. Body composition testing,
4. Summary of skin-fold data, including total body fat calculations,
5. Five supervised high intensity exercise sessions of approximately 30 minutes,
6. Your own muscle protein synthesis data once samples are analysed,
7. Full access to an Accredited Exercise Physiologist throughout your involvement in the study.

Associated Risks

Muscle Biopsy

This biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion, a small lump of fibrous tissue may form under the site of the cannula insertion, but this normally disappears within 2-3 months. As with any cannulation, there is also a slight risk of infection; however, this risk is virtually eliminated through proper cleansing of the area and daily changing of the Band-Aid. If the incision does not heal within a few days or you are in any way concerned about swelling and redness, please contact us immediately. In very rare occasions, there can be damage to a superficial sensory nerve, which will result in temporary numbness in the area. There is also an extremely remote chance (< 0.5%) that you will be allergic to the local anesthetic.

Blood sampling

Participants may experience faintness, slight bruising or infection as a result of blood sampling. Participants will lie down during blood collection and firm pressure will be applied to the blood collection site to reduce the risk of bruising. The strict adherence to all appropriate health and safety guidelines (the use of sterile, disposable needles and the proper handling of blood, use of gloves etc.) will minimise the chance of infection. Trained personnel will carry out all blood sampling.

Strenuous exercise

The side effects of high intensity exercise are minimal in a highly trained, athletic population but may include the following: light headedness, dizziness, nausea, leg cramps, muscle pain, chest discomfort and cardiac arrest. The intensity of the exercise sessions will be similar to that experienced during a race, which all participants will be familiar with. First Aid qualified personnel will be present at all testing sessions. Participant's heart rate, oxygen uptake, blood lactate concentration, and perceived effort (RPE) will be monitored during each exercise session.

Confidentiality

All of your test results will remain confidential and will be coded with a number, which will be kept separate from your personal details. Records will be kept in a

locked cabinet file at Central Queensland University, and all electronic data will be stored in password-protected files. Five years after the completion of this study, all raw data will be destroyed by shredding.

You will be provided with a summary of your results upon completion of the study and in the event of significant findings over the course of the study, which might affect you personally, you will be informed immediately. Results of this study may be used in peer-reviewed publication, conference proceedings, thesis material, and other forms of communication. Data will be presented so that participants can in no way be identified.

Upon your entry into the study, you will receive copies of the signed Participant Information sheet and Consent Form to keep. Your participation in this study is at all times voluntary, which means that you may withdraw at any time before, during, or after your participation, without any prejudice; employment, personal or otherwise. If you do want to withdraw you may also request that any responses or data collected from you be withdrawn also in which case this data will not be included in any publications that may arise from this study.

Should you have any questions regarding anything about this study feel free to contact the investigators Thomas Doering on (07) 4930 9793, or t.doering@cqu.edu.au

Please contact CQUniversity's Office of Research (Tel +61(7) 4923 2603, email: ethics@cqu.edu.au) should there be any concerns about the nature and/or conduct of this research project. This research project has been approved by the Central Queensland University Human Research Ethics Committee – Project number H14/05-106.

Effect of post-exercise PRO-CHO supplementation on performance recovery and muscle protein response in masters and young triathletes

CONSENT FORM

I consent to participation in this research project and agree that:

1. An Information Sheet has been provided to me that I have read and understood;
2. I have had any questions I had about the project answered to my satisfaction by the Information Sheet and any further verbal explanation provided;
3. I understand that my participation or non-participation in the research project will not affect my academic standing or my employment;
4. I understand that I have the right to withdraw from the project at any time without penalty;
5. I understand the research findings will be included in the researcher's publication(s) on the project and this may include conferences and articles written for journals and other methods of dissemination stated in the Information Sheet;
6. I understand that to preserve anonymity and maintain confidentiality of participants that fictitious names may be used any publication(s)<<unless I have expressly granted permission as outlined below>>;
7. I am aware that a Plain English statement of results will be available on the web address provided in the Information Sheet;
8. I agree that I am providing informed consent to participate in this project.

Signature: _____ Date: _____

Name (please print): _____

Where relevant to the research project, please check the box below:

	YES	NO
1. I wish to have a Plain English statement of results posted to me at the address I provide below.		
2. I give permission for photographs and digital images of me to be used in any publication(s) from the research project.		

Postal Address: _____

E-mail Address: _____

Appendix 5: Ethics approval for study 3 – CQUniversity

Secretary, Human Research Ethics Committee
Ph: 07 4923 2603
Fax: 07 4923 2600
Email: ethics@cqu.edu.au

A/Prof Peter Reaburn and
Mr Thomas Doering
School of Medical and Applied Sciences

19 June 2015

Dear A/Prof Reaburn and Mr Doering

HUMAN RESEARCH ETHICS COMMITTEE OUTCOME PROJECT: H14/07-159, *A DOSE-RESPONSE STUDY OF THE EFFECT OF INCREASING POST-EXERCISE DIETARY PROTEIN CONSUMPTION ON 24 HOUR PERFORMANCE RECOVERY AND ACUTE MUSCLE MARKERS OF MUSCLE PROTEIN SYNTHESIS IN MASTERS TRIATHLETES*

The Human Research Ethics Committee is an approved institutional ethics committee constituted in accord with guidelines formulated by the National Health and Medical Research Council (NHMRC) and governed by policies and procedures consistent with principles as contained in publications such as the joint Universities Australia and NHMRC *Australian Code for the Responsible Conduct of Research.* This is available at http://www.nhmrc.gov.au/publications/synopses/_files/r39.pdf.

On 29 July 2014, the committee met and considered your application. The project was assessed as being greater than low risk, as defined in the National Statement. On 12 August 2014, the committee acknowledged compliance with the revisions requested to be made to your research project *A dose-response study of the effect of increasing post-exercise dietary protein consumption on 24 hour performance recovery and acute muscle markers of muscle protein synthesis in masters triathletes* (Project Number H14/07-159) and it is now **APPROVED**. On 18 March 2015, the Chair approved your request to decrease the levels of invasiveness and time burden on participants, and CONDITIONALLY approved the expansion of recruitment to include A/Prof Reaburn's triathlon contacts, using University of Queensland facilities. On 17 June, the Chair acknowledged receipt of evidence of University of Queensland ethics approval, and therefore the relocation to Brisbane is now fully approval.

The period of ethics approval will be from 12 August 2014 to 1 March 2016. The approval number is H14/07-159; please quote this number in all dealings with the Committee. HREC wishes you well with the undertaking of the project and looks forward to receiving the final report and statement of findings.

The standard conditions of approval for this research project are that:

(a) you conduct the research project strictly in accordance with the proposal submitted and granted ethics approval, including any amendments required to be made to the proposal by the Human Research Ethics Committee;

(b) you advise the Human Research Ethics Committee (email ethics@cqu.edu.au) immediately if any complaints are made, or expressions of concern are raised, or any other issue in relation to the project which may warrant review of ethics approval of the project. *(A written report detailing*

the adverse occurrence or unforeseen event must be submitted to the Committee Chair within one working day after the event.)

(c) you make submission to the Human Research Ethics Committee for approval of any proposed variations or modifications to the approved project before making any such changes;

(d) you provide the Human Research Ethics Committee with a written "Annual Report" on each anniversary date of approval (for projects of greater than 12 months) and "Final Report" by no later than one (1) month after the approval expiry date; *(A copy of the reporting pro formas may be obtained from the Human Research Ethics Committee Secretary, Sue Evans please contact at the telephone or email given on the first page.)*

(e) you accept that the Human Research Ethics Committee reserves the right to conduct scheduled or random inspections to confirm that the project is being conducted in accordance to its approval. Inspections may include asking questions of the research team, inspecting all consent documents and records and being guided through any physical experiments associated with the project

(f) if the research project is discontinued, you advise the Committee in writing within five (5) working days of the discontinuation;

(g) A copy of the Statement of Findings is provided to the Human Research Ethics Committee when it is forwarded to participants.

Please note that failure to comply with the conditions of approval and the *National Statement on Ethical Conduct in Human Research* may result in withdrawal of approval for the project.

The Human Research Ethics Committee is committed to supporting researchers in achieving positive research outcomes through sound ethical research projects. If you have issues where the Human Research Ethics Committee may be of assistance or have any queries in relation to this approval please do not hesitate to contact the Ethics and Compliance Officer or myself.

Yours sincerely,

A/Prof Tania Signal
Chair, Human Research Ethics Committee

Cc: A/Prof David Jenkins, Prof Erik Hohmann (co-supervisors) Project file

APPROVED

Appendix 6: Ethics approval for study 3 – The University of Queensland

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	A Dose-Response Study of the Effect of Increasing Post-Exercise Dietary Protein Consumption on 24 Hour Performance Recovery and Acute Muscle Markers of Muscle Protein Synthesis in Masters Triathletes
Chief Investigator:	A/Prof David Jenkins, Mr Thomas Doering, A/Prof Peter Reaburn
Supervisor:	A/Prof David Jenkins, A/Prof Peter Reaburn
Co-Investigator(s):	Prof Erik Hohman
School(s):	UQ HMS; School of Medical and Applied Sciences, CQU
Approval Number:	2015000517
Granting Agency/Degree:	Health Collaborative Research Network PhD scholarship funding
Duration:	1st February 2016

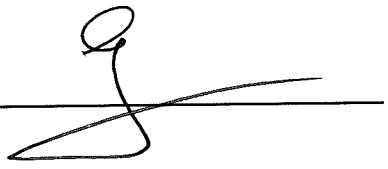
Comments/Conditions:

Expedited review on the basis of approval from the Central Queensland University HREC dated 12/08/2014 & 23/03/2015

Note: if this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
Medical Research Ethics Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Professor Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Signature  Date 8 Apr 2015

Appendix 7: Information sheet and informed consent form for study 3

Effects of 'high' or 'low' protein diets on muscle recovery and subsequent exercise performance in masters triathletes

Investigators

Thomas Doering
Associate Professor Peter Reaburn
Associate Professor David Jenkins

Contact

Thomas Doering
PhD Candidate
t.doering@cqu.edu.au
(07) 4930 9793

Introduction

Masters triathletes are over the age of 35 years and are the most rapidly expanding cohort of age-group triathletes, particularly in long-course events. Indeed, athletes over the age of 45 years have shown the greatest improvements in performance over the past 25 years in the Hawaii Ironman.

Despite improvements in triathlon performance, masters athletes have been shown to take longer to recover from training and racing. Research has shown that older athletes require longer to recover maximal strength following training and competition, in comparison to young, similarly trained athletes. Furthermore, older athletes have been shown to perceive their recovery to be poorer, perceive higher levels of fatigue, and have higher perceptions of muscle soreness following repeated endurance cycling sessions.

Correct nutrition following training and racing is vital to refuel and repair muscles. Carbohydrate and protein are both central components of effective recovery nutrition. Recent research has shown lower rates of muscle repair (protein synthesis) in older adults following exercise and currently recommended protein intakes. This suggests muscle repair following intense or high impact exercise may be slower in

older athletes. This may contribute to the poor recovery of strength and exercise performance reported in older athletes.

Recent research has shown that older adults require up to 40 grams of protein after exercise to maximise muscle growth and repair. This is in contrast to younger adults that require ~20 grams of protein after exercise. Currently, it is unknown if older trained athletes will similarly benefit from higher doses of protein post-exercise to induce higher rates of muscle protein synthesis (growth and repair), and subsequently benefit the recovery of exercise performance.

Therefore, the purpose of this study is to determine if higher than currently recommended protein intakes (0.6g/kg) in the 8 h following muscle-damaging running improves afternoon cycling time trial performance in comparison to lower protein intakes (0.3g/kg) among well-trained, masters triathletes.

Participants

Male triathletes aged 45 years of age and older are invited to participate in this research project. You will be required to undergo preliminary testing to identify suitability to this research project. Preliminary testing will include:

- Pre-exercise screening questionnaire
- Training load consistency questionnaire
- VO_{2max} testing

To participate in this study, you must be training at least 10 hours per week, for the past 6 weeks. In addition, you must have a VO_{2max} of 50 $mL.kg^{-1}.min^{-1}$ or greater.

Requirements

You will need to attend the Exercise Physiology laboratory at The University of Queensland on four separate days (two times per day on days 3 and 4 = six visits) over a two-week period. During this time you will:

1. Complete a three-day diet diary,
2. Undertake two VO_{2max} tests (one on a treadmill and one on a cycle ergometer), and equipment/protocol familiarisation sessions on two days separated by ~48 h (Day 1 and 2 – Preliminary testing),
3. At least three days later, complete an 'experimental trial' consisting of a morning downhill run (30 min) and strength test, and 8 hours later, a 20 km cycling time trial (~30 min) and strength test (Day 3),
4. Seven days later, repeat the experimental trial as of point 3 (Day 4),
5. Provide a small (~6-8 mL) venous blood sample (forearm) before and after the downhill-run and cycling time trial on each occasion (total of 8 blood draws in the study),

6. Consume a provided diet in the 24 h before each downhill run (2), and between the downhill run and the cycling time trial. This includes experimental protein/carbohydrate beverages of unknown content (i.e. you are blinded – won't know what you are drinking – it will be either the 'high' or 'low' protein beverage)

See below (Figure 1) for a diagrammatic overview of the experimental trial day. You will complete this trial twice, seven days apart. The only difference between the two trials will be the content of the beverages at 0, 2 and 5 h post-run.

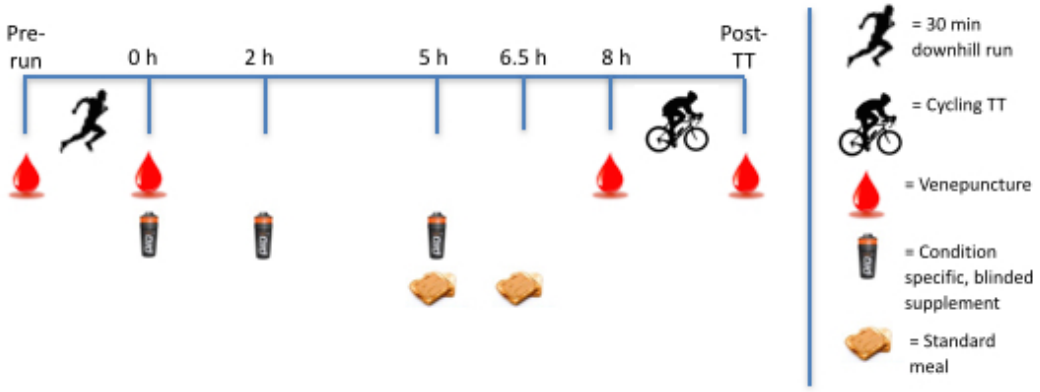


Figure 1: *Trial overview*

Diet and Supplementation

You will be provided with pre-packaged meals for the 24 h prior to the downhill run, and for the period between the morning downhill run and afternoon cycling time-trial, on two occasions. In the period between the run and cycling bout, experimental beverages will be provided to you and consist of either 0.6 g/kg of protein + 1.0 g/kg of carbohydrate ('high' protein), or 0.3 g/kg of protein + 1.3 g/kg of carbohydrate ('low' protein). At the time, you will not know what beverage you are consuming. Both beverages will contain the same total energy content. You must be willing to consume all foods and drinks provided.

Training Standardisation

Throughout the research project your training will be standardised. This is to ensure differences observed in recovery/performance following diets of differing protein contents are attributable to this experimental variable (the diet) and not differences in training leading into the testing sessions.

You will be asked to refrain from all exercise in the 24 h preceding each visit to the laboratory. Furthermore, you will be asked to refrain from vigorous training (and running) in the 48 h preceding each visit to the laboratory.

Benefits

Participation in this research project will provide several benefits to you. Briefly, following study completion, all participants will receive:

1. VO_{2max} data, including identification of ventilatory thresholds to help prescribe training intensities for both cycling and running,
2. Personalised heart rate training zones for both cycling and running,
3. Written report of findings,
4. Supervised and monitored high intensity exercise sessions of approximately 30 minutes each,
5. Access to an Accredited Exercise Physiologist throughout your involvement in the study.

Associated Risks

Blood sampling
You may experience faintness, slight bruising or infection as a result of blood sampling. The strict adherence to all appropriate health and safety guidelines (the use of sterile, disposable needles and the proper handling of blood, use of gloves etc.) will minimise the chance of infection. Trained personnel will carry out all blood sampling.

Strenuous exercise
The side effects of high intensity exercise are minimal in a well trained, athletic population but may include the following: light headedness, dizziness, nausea, leg cramps, muscle pain, chest discomfort and cardiac arrest. The intensity of the exercise sessions will be similar to that experienced during a race or hard training, which you will be familiar with. First Aid qualified personnel will be present at all testing sessions. Heart rate, oxygen uptake, blood lactate concentration, and perceived effort (RPE) will be monitored during each exercise session.

Confidentiality

All of your test results will remain confidential and will be coded with a number, which will be kept separate from your personal details. Records will be kept in a locked cabinet file at Central Queensland University, and all electronic data will be stored in password-protected files. Five years after the completion of this study, all raw data will be destroyed.

You will be provided with a summary of your results upon completion of the study and in the event of significant findings over the course of the study, which might affect you personally, you will be informed immediately. Results of this study may be used in peer-reviewed publication, conference proceedings, thesis material, and

other forms of communication. However, data will be presented so that participants can in no way be identified.

Upon your entry into the study, you will receive copies of the signed Participant Information and Informed Consent form to keep. Your participation in this study is at all times voluntary, which means that you may withdraw at any time before, during, or after your participation, without any prejudice; employment, personal or otherwise. If you do want to withdraw you may also request that any responses or data collected from you be withdrawn also in which case this data will not be included in any publications that may arise from this study.

Should you have any questions regarding anything about this study feel free to contact the investigators Thomas Doering at t.doering@cqu.edu.au.

Please contact CQUniversity's Office of Research (Tel +61(7) 4923 2603, email: ethics@cqu.edu.au) should there be any concerns about the nature and/or conduct of this research project. This research project has been approved by the Central Queensland University Human Research Ethics Committee – Project number H14 07-159, and The University of Queensland's Ethics Committee – Project number 2015000517.

Effects of a "high" protein diet on muscle recovery and subsequent exercise performance in masters triathletes

CONSENT FORM

I consent to participation in this research project and agree that:

1. An Information Sheet has been provided to me that I have read and understood;
2. I have had any questions I had about the project answered to my satisfaction by the Information Sheet and any further verbal explanation provided;
3. I understand that my participation or non-participation in the research project will not affect my academic standing or my employment;
4. I understand that I have the right to withdraw from the project at any time without penalty;
5. I understand the research findings will be included in the researcher's publication(s) on the project and this may include conferences and articles written for journals and other methods of dissemination stated in the Information Sheet;
6. I understand that to preserve anonymity and maintain confidentiality of participants that fictitious names may be used any publication(s)<<unless I have expressly granted permission as outlined below>>;
7. I am aware that a Plain English statement of results will be available on the web address provided in the Information Sheet;
8. I agree that I am providing informed consent to participate in this project.

Signature: _____ Date: _____

Name (please print): _____

Where relevant to the research project, please check the box below:

	YES	NO
1. I wish to have a Plain English statement of results posted to me at the address I provide below.		
2. I give permission for photographs and digital images of me to be used in any publication(s) from the research project.		

Postal Address: _____

E-mail Address: _____