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Effects of Post-Exercise Recovery Drink Composition on Subsequent Performance in Masters Class Athletes

Erica Goldstein
University of Central Florida

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EFFECTS OF POST-EXERCISE RECOVERY DRINK COMPOSITION ON SUBSEQUENT PERFORMANCE IN MASTERS CLASS ATHLETES

by

ERICA R. GOLDSTEIN
B.S. University of North Florida, 2013
M.S. Florida Atlantic University, 2009
B.A. Elon University, 1997

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ABSTRACT

Carbohydrate (CHO) and carbohydrate-protein coingestion (CHO-P) have been shown to be equally effective for enhancing glycogen resynthesis and subsequent same-day performance when CHO intake is suboptimal (≤0.8 g/kg). Few studies have specifically examined the effect of isocaloric CHO vs CHO-P consumption on subsequent high-intensity aerobic performance with limited time to recover (≤2 hours) in masters class endurance athletes. Participants (n = 22) were assigned to consume one of three beverages during a 2-hour recovery period: PLA (electrolytes and water), CHO (1.2 g/kg bm), or CHO-P (0.8 g/kg bm CHO + 0.4 g/kg bm PRO). All beverages were standardized to one liter (~32 oz.) of total fluid volume regardless of treatment group. One liter of a standard ready to drink sports beverage contains ~58 g of CHO. CHO powder was weighed in grams via a digital food scale and added to the existing liter of fluid to reach the total amount of CHO needed if a participant required more than 58 g of CHO. During Visit#1, participants completed graded exercise testing (VO2peak; cycle ergometer). Familiarization (Visit#2) consisted of 5 x 4 min intervals at 70-80% of peak power output [PPO, watts] with 2 min of active recovery at 50W, followed by time to exhaustion [TTE] at 90% PPO. The same high-intensity interval protocol with TTE was conducted pre-and post-beverage consumption on Visit#3. The ANCOVA indicated a significant difference among the group means for the posttest TTE ($F_{2,18} = 6.702$, $p = .007$, $\eta^2 = .427$) values after adjusting for the pretest differences. Both CHO and CHO-P were effective in promoting an increase in TTE performance with limited time to recover in this sample of masters class endurance athletes.

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CHAPTER ONE: INTRODUCTION

The rate of participation by masters class athletes (MCAs) in organized endurance and ultra-endurance (>6 hours) events has increased exponentially over the past 10-15 years (Lepers et al., 2013; Lepers & Stapley, 2016). Carbohydrate and protein are key nutritional factors that promote glycogen and protein synthesis, support recovery and allow for repetitive high intensity efforts. Few studies, however, have examined short-term (2-6 hours) post-exercise recovery nutrition specific to MCAs (Doering, Jenkins, et al., 2016; Doering et al., 2017). MCAs are individuals generally classified as ≥ 35 years of age who meticulously train for and compete in organized sport (Fien et al., 2017; Lepers & Cattagni, 2012; Lepers & Stapley, 2016; Ransdell et al., 2009). MCAs participation in endurance sport reaches beyond the recreational athlete as the first and second place winners of multiple international Ironman races in 2016 were between 40-45 years old (Lepers & Stapley, 2016). MCAs equated to 48% and 56% of total male finishers between 2007-2010 for Ironman Switzerland and Hawaii, respectively (Lepers et al., 2013; Stiefel et al., 2014). The mean age of male finishers between the studied period 1998-2010 was 46.5 ± 10.8 years for Lauf Biel', a Switzerland based 100-km (62 miles) ultra-marathon (Knechtle et al., 2012). While impressive, this is just a small sample of international MCA involvement in endurance sport.

MCAs as a group are an exceptional example of healthy aging; nevertheless, MCAs exhibit age-related declines in strength, aerobic capacity, and power even with regular training (Peiffer et al., 2008; Sultana et al., 2012). MCA cyclists maintain weekly mileage of approximately 250 – 350 miles per week, with no significant difference between younger (35-44 years) and older (≥ 55) age groups (Macgregor et al., 2013; Peiffer et al., 2008). That said, the absolute rate of decline of VO_2max beginning from age 20 to 70 years had been suggested to be between -4.2 to -5.4 ml/kg/min per decade from a baseline of between 79.4-80.7 ml/kg/min, respectively (Sultana et al., 2012). A decline in VO_2max has been reported in several studies with the oldest groups (45-54; ≥ 55 years) showing the greatest decline in comparison to younger (<44 years) MCAs (Peiffer et al., 2008; Sultana et al., 2012). The decline in peak power output (watts) is similar to VO_2max with the older age groups showing a significantly greater decline than the younger age group (Peiffer et al., 2008). For example, Peiffer et al. (2008) reported peak power output was significantly lower in the ≥ 55 age group in comparison to both the 45-54 and 35-44 age groups.

The only studies examining substrate utilization in MCAs have been carried out in endurance-trained athletes greater than 60 years of age and less than 30 (Dubé et al., 2016; Malone et al., 2019). However, it is MCAs in their 40s who are successfully competing in elite-level endurance-based events. Endurance exercise at this level of competition requires the prolonged high-intensity activity of ~70-90% VO_2max, which relies quite heavily on carbohydrate oxidation (Romijn et al., 1993a; Spriet, 2007). Competitive athletes rely on their ability to both store and mobilize glucose from muscle. Typical glycogen depleting protocols in studies that have examined the difference between carbohydrate (CHO) ingestion or carbohydrate-protein (CHO-P) co-ingestion have been running or cycling to fatigue at 70% VO_2max or intermittent exercise at 70%-90% VO_2max. These protocols have been sufficient for depleting muscle glycogen content to ~100 – 200 mmol/kg/dw (Alghannam et al., 2016; Dahl et al., 2020; R. L. P. G. Jentjens et al., 2001; Tsintzas et al., 1995). Conversely, both CHO (1.2 g/kg) and CHO-P co-ingestion (0.8/0.4 g/kg) were found to be equally effective for promoting glycogen resynthesis during acute recovery periods of 3, 4, and 5 hours (Alghannam et al., 2016; Dahl et al., 2020; R. L. P. G. Jentjens et al., 2001; van Loon et al., 2000b).

As stated, MCAs in the 45-54-year-old age group tend to have the lowest weekly mileage as compared to younger and older age groups, likely due to the demands of family and professional responsibilities (Macgregor et al., 2013). MCAs often have demanding schedules, frequently training twice per day or in the evening and the next morning. Nutrient intake during the immediate post-exercise recovery period is essential and insufficient nutrient intake due to time constraints may compromise the recovery of muscle and liver glycogen stores, along with subsequent performance (Cintineo et al., 2018). Consuming CHO immediately post-exercise has been shown to increase the rate of glycogen resynthesis by three times as compared to delaying CHO for 2 hours (Ivy, Katz, et al., 1988). Ivy et al. (1988a) demonstrated the rate of glycogen synthesis to be 45% slower when delaying CHO intake by 2 hours. Consequently, muscle glycogen concentration was also significantly less when CHO intake was delayed by 2 hours following high-intensity intermittent exercise (Ivy, Katz, et al., 1988). A similar pattern was shown for leg protein synthesis when a CHO-P supplement (10 g protein; 8 g carbohydrate; 3 g fat) was provided immediately post-exercise in comparison to 3 hours later. Leg uptake of glucose and amino acids was higher immediately post-exercise, which resulted in significantly greater protein synthesis (3x higher) and net deposition in comparison to delaying energy intake by 3 hours (Levenhagen et al., 2001).

Two different isoenergetic options have been shown to effectively promote glycogen resynthesis post-exercise: CHO only (1.2 g/kg) or CHO-P co-ingestion (0.8 g/kg/0.4 g/kg). Several studies have demonstrated an equivalent increase in muscle glycogen content (Alghannam et al., 2016; R. L. P. G. Jentjens et al., 2001; van Loon et al., 2000b) when these recovery beverages were consumed beginning immediately and 30-min post-exercise as well as at 3, 4, and 5-hour recovery periods, or an increase in favor of CHO-P when consumed immediately-post and at 1-hour intervals during the first 2 hours (J. Berardi et al., 2006). The term isoenergetic must be underscored because CHO-P is not substantially more useful for promoting glycogen resynthesis when protein is added to CHO in the amount of 1.2 g/kg (Betts & Williams, 2010; Howarth et al., 2009; R. L. P. G. Jentjens et al., 2001). Therefore, CHO availability refers to the consumption of CHO in the amount of approximately 1.2 g/kg bm or the addition of protein during the recovery period when CHO intake is insufficient and/or time is limited, and athletes are unable to match nutrient demand with intake.

MCAs may train twice per day, or in the evening and next morning, with limited time between bouts of exercise, therefore an effective recovery strategy is paramount. Three previous studies have shown that CHO-P was associated with either an increase in performance or an attenuated decrement to performance during a subsequent bout of endurance exercise (J. M. Berardi et al., 2008; Dahl et al., 2020; Goldstein et al., 2022). A recent investigation reported time to exhaustion performance increased by 35% during a second bout of exercise in college-aged male and females following consumption of a CHO-P (0.8 g/kg and 0.4 g/kg) beverage and a 2-hr recovery period (Goldstein et al., 2022). In comparison, performance decreased by 14% and 31% with CHO (1.2 g/kg) and PLA (electrolytes and water), respectively (Goldstein et al., 2022). Dahl et al. (2020) reported participants were able to cycle 8.4 ± 1.8 min longer following a 5-hour recovery period, in favor of CHO-P as compared to an isoenergetic CHO only beverage despite similar glycogen synthesis between groups (Dahl et al., 2020). Berardi et al. (2008) reported a decrease in power for both CHO-P co-ingestion and CHO only groups during a subsequent bout of exercise (i.e., morning exercise, 6-hour recovery period, afternoon exercise);

however, the performance decrement was significantly less with CHO-P in comparison to CHO, 3.86 watts vs. 16.5 watts, respectively (J. M. Berardi et al., 2008). To reiterate, while these results are promising, all studies examining CHO-P have been examined in athletes under the age of 30. As such, a critical gap in the literature examining post-exercise nutrition exists for high-performance MCAs.

Appropriate post-exercise nutrient intake is vital for MCAs as several studies have reported slower recovery from muscle-damaging exercise, even when moderate amounts of protein are consumed. Doering et al. (2016) reported significantly lower fractional synthetic rates of myofibrillar protein synthesis and slower afternoon cycling TT performance in MCAs as compared to younger similarly trained athletes (~ 53 vs. 27 years of age) (Doering, Jenkins, et al., 2016). In that study, both groups consumed a recovery beverage containing 1 g/kg of carbohydrate and 20 g of whey protein immediately post-exercise. They were required to consume protein at meals in the amount of 0.3 g/kg for the 3-day trial (30-min downhill run and 20-km time trial at 10, 24, 48 hours post-run). Doering et al. (2017) also examined nutrient intake and recovery over an 8-hour recovery period, where MCAs in a crossover fashion performed isokinetic dynamometry of the knee extensors and a downhill run in the morning followed by repeated isokinetic dynamometry and a cycling time trial in the afternoon. While MCAs consumed in total 5.9/1.05 g/kg of CHO-P in the moderate group and 5.0/1.95 g/kg in the high group, the protein was provided in 0.3 g/kg and 0.6 g/kg boluses in the moderate and high groups, respectively. No significant effect for time trial performance was reported; however, the high protein intake attenuated reductions in force during afternoon peak isometric torque of the knee extensors to a greater degree than moderate protein (-3.6% vs. -8.6%, respectively) (Doering et al., 2017). Taken together, age-specific nutrient recommendations may be necessary for MCAs to improve the rate of recovery from prior exercise, support adaptation, and thus performance in a subsequent bout of training.

Protein promotes skeletal muscle remodeling and repair, adaptation to training, and replenishment of energy stores (Doering, Reaburn, Phillips, et al., 2016; Moore et al., 2014). Therefore, protein intake post-exercise should be of equal priority to that of CHO. Formative studies aimed at determining the appropriate amount of protein for MCAs have centered around an absolute dose of 20-30 g of protein, which for the most part, has been recommended to all athletes, regardless of age (Areta et al., 2013; Moore et al., 2009; Witard et al., 2014). When examined in response to resistance exercise and an energy deficit, supplementing with 30 g of protein immediately post-exercise appears to be useful for promoting muscle protein synthesis (Areta et al., 2014). A higher dose of protein is, therefore, likely to be beneficial for the glycogen depleted endurance athlete with limited time to match the demands of exercise with nutrient intake. Moore et al. (2015) have suggested that relative to body mass, older men require 0.40 g/kg of protein to maximally stimulate muscle protein synthesis, whereas young men require 0.24 g/kg (Moore et al., 2015). This higher protein intake is supported by additional MCA specific nutrition and recovery related data (Doering, Reaburn, Phillips, et al., 2016; Louis et al., 2019; Reaburn et al., 2015).

Post-exercise nutrient intake in a group of Australian (endurance) MCAs was reported to be well below the suggested amount for CHO (~0.7 g/kg) and slightly below the recommended threshold for protein (~0.3 g/kg) (Doering, Reaburn, Cox, et al., 2016). Furthermore, the MCAs surveyed in this study demonstrated poor or no knowledge of post-exercise nutritional practices for both CHO and protein intake (Doering, Reaburn, Cox, et al., 2016). It has been suggested that while endurance athletes are familiar with the need for CHO in the diet, the importance of meeting total daily protein needs as well as the incorporation of protein within the immediate-post recovery period may be less well understood (Vitale & Getzin, 2019). Inadequate nutrient intake in the acute post-exercise recovery period may compromise the rate of both glycogen and protein synthesis (Doering, Jenkins, et al., 2016; Ivy, Katz, et al., 1988; Levenhagen et al., 2001). On a cellular level, this may negatively affect skeletal muscle remodeling and glycogen storage, which in turn can promote fatigue, and poor training outcomes and performance (Breen et al., 2011; Ivy, Katz, et al., 1988; Kruseman et al., 2020). As previously discussed, two recent studies in MCAs have demonstrated slower recovery between bouts of exercise in the short term (8 and 10 hours), while a higher protein intake co-ingested with CHO (0.6 g/kg vs. 0.3 g/kg) was shown to be beneficial for supporting the recovery process especially following muscle damaging activity (Doering et al., 2017; Doering, Jenkins, et al., 2016).

Exercise-induced dehydration can impair thermoregulation and promote cardiovascular strain during a bout of prolonged endurance exercise (Sawka, 1992). Both heart rate variability (HRV) and heart rate recovery (HRR) have been used as measures to examine cardiac autonomic function during recovery from exercise (Borges et al., 2017). Some studies have specifically examined the effect of consuming water or an isotonic solution to promote vagal reactivation upon exercise cessation. (Moreno et al., 2013; Peçanha, Paula-Ribeiro, et al., 2014; Vanderlei et al., 2015). HRR is a valid and simple measure that is easily calculated at the cessation of exercise as the difference between peak heart rate (beats per min) and the heart rate 1-, 2-, 3-, and 5-min later (Adabag & Pierpont, 2013). The heart rate recovery index is a novel index that accounts for total work (i.e., HRR relative to work) and can be useful in exercise trials where total work varies between participants, such as with time to exhaustion (Sterkowicz-Przybycień et al., 2019). To our knowledge the effect of isocaloric recovery drinks on HRR relative to total work in male masters class endurance athletes has not been examined.

In conclusion, the rate of MCA participation in elite-level endurance competition is exponentially increasing. In contrast, the level of nutritional knowledge and recovery practices was reportedly poor in one group of surveyed endurance MCAs. Additionally, a critical gap in post-exercise recovery research exists specific to MCAs. Even less research exists specific to the MCA and short-term (2 hours or less) recovery. This is a problem as slower rates of recovery between subsequent bouts of exercise have been demonstrated, and higher protein needs post-exercise have been indicated to promote muscle remodeling. A unique opportunity exists to examine CHO-P in a 2-hour recovery period to promote optimization of post-exercise glycogen resynthesis and protein remodeling in support of successive bouts of training and competition. The hypothesis is performance in a subsequent bout of high-intensity glycogen depleting exercise will not be significantly different regardless of post-exercise recovery drink (carbohydrate vs. carbohydrate and protein vs. placebo).

Purpose of the Study

The purpose of this investigation is to examine the effects of carbohydrate vs. carbohydrate-protein co-ingestion vs. placebo on a subsequent bout of performance following high-intensity exercise and a 2-hour recovery period in Masters Class Athletes.

Research Question

Does carbohydrate improve performance in Masters Class Athletes measured during a subsequent bout of time to exhaustion?

And,

Does carbohydrate and protein improve performance in Masters Class Athletes measured during a subsequent bout of time to exhaustion?

And,

Does placebo improve performance in Masters Class Athletes measured during a subsequent bout of time to exhaustion?

And,

Does carbohydrate and protein improve performance in Masters Class Athletes significantly better than carbohydrate measured during a subsequent bout of time to exhaustion?

Hypotheses

We hypothesize that carbohydrate (CHO) will improve subsequent performance significantly better than the placebo post-exercise in male Masters Class Athletes.

And,

Carbohydrate and protein (CHO-P) will improve subsequent performance significantly better than PLA post-exercise in male Masters Class Athletes.

And,

Carbohydrate and Protein (CHO-P) will improve subsequent performance equally to CHO post-exercise in male Masters Class Athletes.

CHAPTER TWO: REVIEW OF LITERATURE

While research describing the effect of post-exercise nutrition on short term recovery (3-6 hours) in young endurance athletes is widely available, few studies have examined recovery nutrition in masters class athletes (MCAs). The masters class athlete (MCA) has been categorized as an individual 40 years or older, who meticulously trains for and competes in organized sport (Fien et al., 2017; Lepers & Stapley, 2016). This population has merit and warrants attention not only for the increased rates of participation in both endurance and ultra-endurance (>6 hours duration) events but also for their elite performance (Lepers & Stapley, 2016). In 2016, winners of both the New-Zealand and Austria Ironman triathlons were 43 and 40 years of age, respectively (Lepers & Stapley, 2016), the 2nd place winner of the Austria Ironman was 43 years old (Lepers & Stapley, 2016). Data from Ironman Switzerland indicates an increased rate of participation among male MCAs (≥40 years old), who represented 48% of total male finishers between the period 2007-2010 (Stiefel et al., 2014). Male MCAs (>40 years of age) represented 56% of total finishers in the Hawaii Ironman (2010) (Lepers et al., 2013). Lauf Biel', a Switzerland based 100-km (62 miles) ultra-marathon, had male and female master runner representation of approximately 73% for the studied period 1998-2010.; During this time, the mean age of male finishers was 46.5 ± 10.8 years, with greatest participation among men 40 to 49 years (Knechtle et al., 2012). It is worth mentioning that Lauf Biel' also saw a significantly increased rate of participation from the 50-59-year age group. Meanwhile, the participation of endurance athletes younger than 40 years of age for these same events decreased (Knechtle et al., 2012; Lepers et al., 2013; Stiefel et al., 2014).

Physiological Characteristics of Masters Class Athletes

A group of 156 athletes (age range = 40 – 79 years) from the 2014 Pan Pacific Masters Games (Gold Coast, Australia) helps to characterize this population. Of the 156 athletes, approximately 78% (121) reported no prescribed medications (Fien et al., 2017). For those participants who reported having a chronic disease, hypertension (n = 22) and diabetes mellitus (n = 14) were the most common; therefore the most prescribed medications were for these two medical conditions (Fien et al., 2017). No participants were described as sarcopenic (for all age groups), due to the high level of lean muscle mass as assessed by bioelectrical impedance analysis; however, a significant decline in handgrip strength was reported (Fien et al., 2017).

Loss of strength is, of course, a concern for MCAs. However, Louis et al. (2012) demonstrated an ability for masters cyclists to increase strength (i.e. maximal voluntary contraction torque; MVC) following a 3-week strength training program with three training sessions per week, consisting of knee extensions at 70% 1RM (Louis et al., 2012). In a separate study, knee extensor and flexor MVC was not shown to be different between a group of endurance-trained masters and young triathletes (Sultana et al., 2012). Training for triathlon is endurance-based, and strategies for improving triathlon performance involve technique such as cadence selection, developing power output, and drafting during a race can be of benefit to an endurance athlete (Hausswirth & Brisswalter, 2008). Training specificity could likely explain the lack of difference in strength between young and masters endurance athletes(Sultana et al., 2012). In the population studied, the primary mode of exercise training was less likely to promote strength development. Conversely, Easthope et al. (2010) found a significant difference in MVC between masters and young athletes trained in trail running (Easthope et al., 2010). Long-distance trail running involves traversing mountainous terrain and promotes a high degree of eccentric muscle action during the downhill segments of the race-course (Easthope et al., 2010).

VO_2max is one of the central physiological mechanisms to be affected by the aging process (Lepers & Stapley, 2016; Tanaka & Seals, 2008). Factors that contribute to this decline are maximal heart rate (maxHR) and stroke volume; arterio-venous O2 difference is likely affected more by delivery than skeletal muscle extraction (Lepers & Stapley, 2016; Tanaka & Seals, 2008). The absolute rate of decline of VO_2max beginning from age 20 to 70 years has been suggested to be between -4.2 to -5.4 $ml·kg·min^{-1}$ per decade from a baseline of between 79.4-80.7 $ml·kg·min^{-1}$, respectively (Sultana et al., 2012). This was demonstrated by Peiffer et al. (2008), where trained cyclists grouped into 10-year age brackets (35-44; 45-54; ≥55) completed a maximal graded exercise test. Results confirmed an age-related decline in VO2max, peak power output (PPO), and maxHR per decade (Peiffer et al., 2008). For example, PPO was significantly reduced in the 45-54-year age group, as compared to the youngest cyclists, and PPO for the ≥55 group was significantly less than both younger age groups. Remarkably, the first and second ventilatory threshold (percent of VO_2max) was greater in the ≥55 group, as compared to all younger groups. Sultana et al. (2012) reported similar results for MCAs (52.4 ± 10 years) when running speed at the first ventilatory threshold was expressed as a percentage of VO_2max (Sultana et al., 2012). Both central and peripheral factors are diminished as a result of the aging process, but additional studies have indicated that attenuation and adaption can occur as a result of continued high-intensity aerobic activity throughout adulthood (Dubé et al., 2016; Power et al., 2010).

When examining training frequency of MCA cyclists in Australia, Macgregor et al. (2013) reported no significant difference between weekly mileage for any of the 10-year age brackets studied (35-44; 45-54; ≥55). Mileage ranged between 251 ± 112 km/week for the youngest group and 245 ± 87 km/week for the oldest cyclists (Macgregor et al., 2013). Peiffer et al. (2008) report even higher weekly mileage in cyclists ranging from 301 ± 99 for the youngest to 359 ± 120 for the oldest. In a study of MCAs (64 ± 3 years), who ran >45 km/week and had been actively training for 30+ years, the estimated number of motor units (MU) of the tibialis anterior did not differ between young (37 ± 3 years) recreational adults (150 ± 43 MU) and MCAs (140 ± 53 MU), respectively (Power et al., 2010). However, the estimated number of motor units were significantly less (91 ± 22 MU) for the sedentary age-matched group (66 ± 3 years) as compared to both master runners and young adults (Power et al., 2010),suggesting that endurance training through the life stages may be protective against age-related loss of functional motor units.

Muscle characteristics, Substrate Utilization and Endurance Athletes

Lifelong aerobic training also influences physiological effects related to muscle characteristics and substrate use. MCAS (64.8 ± 4.9 years), with a self-reported history of ~35-40 years of endurance training, composed of 6 training sessions/week of predominantly running and cycling were compared to younger adults (27.8 ± 4.9 years) in a study that examined skeletal muscle phenotype, oxidative capacity, and substrate storage (Dubé et al., 2016). Similar to other studies, VO_2peak, was approximately 25% higher in the younger athletes (70.53 ± 9.08 ml·min·kg of fat free mass^{-1}), as compared to MCAs (55.82 ± 7.22 ml·min·kg of fat free mass^{-1}). Peak power output was also significantly reduced for MCAs as compared to the younger group, 201.9 ± 13.4 watts vs. 285.7 ± 20.3 watts, respectively. However, there was no difference between groups for fat-free mass, fat mass, or percent body fat (Dubé et al., 2016). As expected, MCAs had a significantly higher proportion of type I fibers and lower type IIa but higher content of intramuscular triglyceride in each fiber type. Data from Dubé et al. (2016) indicated MCAs had significantly lower glycogen reserves, and relied less on carbohydrate oxidation at moderate intensities, in comparison to younger athletes. In contrast, younger athletes had significantly greater skeletal muscle glycogen content, type IIa fibers, and rate of carbohydrate oxidation at moderate intensities; thus, younger endurance-trained adults are likely more suited to higher intensity activity (Dubé et al., 2016). A critical gap in the research exists as several evaluations related to substrate utilization have been carried out in athletes older than 60 and less 30 years of age (Dubé et al., 2016; Malone et al., 2019). Indeed, the average age of male finishers at the Lauf Biel' ultra-marathon race during 1998-2010 was 46.5 ± 10.8 years. Endurance exercise at this level of competition requires prolonged high-intensity activity of ~70-90% VO_2max, which relies quite heavily on carbohydrate oxidation (Romijn et al., 1993b; Spriet, 2007). Therefore, during high-intensity endurance-based training and competition athletes rely on their ability to mobilize glucose from muscle and subsequently resynthesize glycogen stores.

A recent meta-analysis examining factors that affect muscle glycogen at rest and during exercise reported a resting skeletal muscle glycogen content of the vastus lateralis in males with a VO_2max of 53 ± 8 ml/kg/min and normal carbohydrate availability to be 462 ± 132 mmol/kg dry mass (Areta & Hopkins, 2018). Normal carbohydrate availability was defined as not being on a low or high carbohydrate diet (high = ≥6 g/kg of carbohydrate per day for ≥3 days or ≥7 g/kg for ≥2 days). High carbohydrate availability resulted in a moderate increase in resting glycogen content of 102 ± 47 mmol/kg (Areta & Hopkins, 2018). Low carbohydrate availability was defined as glycogen depleting exercise followed by ≥1 day of low carbohydrate intervention (varied by researcher) and resulted in quite a large decrease of resting skeletal muscle glycogen content of -253 ± 30 mmol/kg (Areta & Hopkins, 2018). Cycling was found to utilize muscle glycogen to a greater extent than running, and continuous exercise at ~70% of VO_2max depletes levels of resting muscle glycogen to ~60% of baseline (assuming normal carbohydrate availability), or to ~250 mmol/kg dry mass (Areta & Hopkins, 2018). Rates of glycogen depletion as reported by Areta and Hopkins (2018) are slightly higher than other studies of similar intensity and duration of exercise (Dahl et al., 2020; Tsintzas et al., 1995, 1996; van Loon et al., 2000a). Of course, variance exists among athletes - a more aerobically fit individual with a greater muscle oxidative capacity will rely less on carbohydrate at the same relative intensity as a less fit individual. Finally, intermittent exercise at approximately 70% VO_2max as compared to continuous exercise at the same intensity was shown to have a trivial effect on glycogen depletion (Areta & Hopkins, 2018).

Glycogen can provide enough glucose to sustain high-intensity exercise for approximately 90 minutes to two hours (Hermansen et al. 1967; Bergström and Hultman, 1967; O'Brien et al. 1993). Tsintzas et al. (1995) found that when young recreational runners (~29 years old; ~54.5 ml/kg/min) consumed water and completed a 60-min treadmill run at 70% of VO_2max muscle glycogen of the vastus lateralis was depleted by ~ 66% in type I fibers, as compared to ~38% when a 5.5% carbohydrate-electrolyte (CHO-E) beverage was consumed immediately before and during the run (Tsintzas et al., 1995). Tsintzas et al. (1996) replicated this study so that participants were now running to exhaustion at 70% of VO_2max. When participants consumed the CHO-E solution, they were able to run ~27% longer as compared to when running with water: 132.4 ± 12.3 min vs. 104.3 ± 8.6 min, respectively (Tsintzas et al., 1996). Glycogen depletion was specific to the type I muscle fibers at the point of exhaustion regardless of water or CHO-E consumption (~32 vs. 28 mmol/kg/dry weight, respectively)(Tsintzas et al., 1996). From these studies, it is clear that young (~29 years-old) endurance-trained men oxidize carbohydrates at moderately high intensities and that consumption of additional carbohydrates is necessary to maintain this level of duration and intensity.

Carbohydrate Availability, Glycogen Synthesis, and Performance

Masters class endurance athletes tend to be well-educated and of higher socioeconomic status (Guthrie & Erickson, 2016; Stiefel et al., 2014). This likely yields access to high-end equipment, technology, coaching, and nutritional strategies that support their athletic success (Stiefel et al., 2014). Masters level cyclists in the 45-54-year-old age group tend to have the lowest weekly training volume as compared to younger and older cyclists likely due to demands of their family and professional lives (Macgregor et al., 2013). Triathletes in particular may train twice per day for multiple events (swim, run, bike). Insufficient nutrient intake due to time constraints may compromise muscle and liver glycogen stores, along with subsequent exercise performance (Cintineo et al., 2018). In fact, out of 212 male and female masters cyclists, only 38% reported using a CHO-P mix following training or competition, and 29% reported using high-glycemic foods within 30 minutes (Reaburn et al., 2013). Consumption of CHO immediately post-exercise is essential to promote glycogen resynthesis, which occurs in two phases. The first phase is rapid, occurs for 30-60 minutes, and is insulin-independent, stimulated by muscle contraction (Ivy & Kuo, 1998; R. Jentjens & Jeukendrup, 2003; Murray & Rosenbloom, 2018). During the process of resynthesis, there is an increase in GLUT4 number and translocation to the cellular membrane, increase in membrane permeability to glucose, and an initial increase in G6P and glycogen synthase activation, which is known to be rate-limiting to glycogen synthesis (Ivy & Kuo, 1998; Lai et al., 2010; Murray & Rosenbloom, 2018).

The second phase of resynthesis is much slower and insulin dependent. Skeletal muscle glycogen content, CHO availability, and plasma insulin concentration positively influence GLUT4, glycogen synthase activity, and glucose uptake. (Burke et al., 2017; Ivy & Kuo, 1998; R. Jentjens & Jeukendrup, 2003; Lai et al., 2010). Taking advantage of the rapid phase with oral glucose ingestion soon after exercise promotes 3 times greater rates of glycogen resynthesis in comparison to delaying nutrient intake by 2 hours. (Ivy, Katz, et al., 1988, 1988; R. Jentjens & Jeukendrup, 2003). In fact, when a carbohydrate-protein supplement (10 g protein; 8 g carbohydrate; 3 g fat) was provided immediately post-exercise in comparison to 3 hours later, leg uptake of glucose and amino acids were greater immediately post exercise, which resulted in significantly greater protein synthesis (3x greater) and net deposition in comparison to delaying energy intake by 3 hours (Levenhagen et al., 2001).

Endurance athletes who train at an intensity and duration that substantially depletes muscle glycogen are encouraged to consume carbohydrates at a rate and amount that a) promotes glycogen resynthesis and b) sufficiently restores exercise capacity for subsequent workouts. The amount of CHO to consume immediately post-exercise and throughout the first 4-6 hours has been widely researched and discussed (Burke et al., 2017; R. Jentjens & Jeukendrup, 2003). Just as important as the amount of CHO to consume is the timing of consumption. Not only is taking advantage of the rapid phase of glycogen resynthesis beneficial, but regular intervals appear to promote glycogen resynthesis to a greater degree than one large bolus of CHO within the 2-hour post-exercise period (Burke et al., 2017; van Loon et al., 2000a). A seminal study by van Loon et al. (2000) demonstrated that when trained cyclists consumed CHO in the amount of 1.2 g/kg, every 30 min over 5 hours muscle glycogen content was significantly increased to a greater degree than 0.8 g/kg of carbohydrate (362 ± 46 vs. 272 ± 54 µmol glycosol units/g dry muscle weight).

It has been relatively difficult to compare studies in this area because of the varying methods used to examine post-exercise glycogen resynthesis. Regardless of the type of athlete (team sport vs endurance) the consensus appears to be a post-exercise recommendation of 1.0-1.2 g/kg of CHO in small feedings within the first 4 hours of glycogen depleting exercise (Betts & Williams, 2010; Burke et al., 2017; R. Jentjens & Jeukendrup, 2003). The upper end of this range appears to be sufficient for maximally restoring muscle glycogen, with no additive effect when consuming beyond 1.5 g/kg (Ivy, Lee, et al., 1988). Dependent upon the mode duration and intensity of exercise, a daily target of 5-10 g/kg of CHO is sufficient for restoring and maintaining muscle glycogen stores over 24 hours (Vitale & Getzin, 2019). Even when team sport or endurance-based athletes are not participating in twice per day workouts, it is still a practical recommendation to consume 1.2 g/kg of CHO within the first hours after exercise to maximize rates of glycogen resynthesis to ensure athletes can meet the demands of training on successive days (Murray & Rosenbloom, 2018).

A third treatment was included in the van Loon et al. (2000) study, which was a combination of CHO (0.8 /kg) plus protein (0.4 g/kg). The co-ingestion of CHO-P at 30-minute intervals post-exercise also significantly increased muscle glycogen synthesis to a greater degree than CHO alone (0.8 g/kg), 351 ± 39 vs. 272 ± 54 µmol glycosol units/g dry muscle weight) (van Loon et al., 2000a). It is important to emphasize the association between CHO-P coingestion and CHO availability. When post-exercise CHO is adequate (i.e., 1.0-1.2 g/kg), the addition of protein does not further enhance muscle glycogen resynthesis (Betts & Williams, 2010; Howarth et al., 2009; R. L. P. G. Jentjens et al., 2001). Instead, when treatments are isoenergetic, and the CHO content is reduced, then muscle glycogen resynthesis is either enhanced in favor of CHO-P co-ingestion (J. Berardi et al., 2006) or similar between treatments (Alghannam et al., 2016; van Loon et al., 2000a). Similar rates of glycogen resynthesis between groups or an increase in resynthesis was demonstrated in studies, where participants received isoenergetic CHO-P (0.8 g/kg/ + 0.4 g/kg) and CHO (1.2 g/kg) beverages beginning immediately post-exercise and at 30-min or 1-hour intervals during 2-, 3-, 4-, and 5-hour recovery periods (Alghannam et al., 2016; J. Berardi et al., 2006; van Loon et al., 2000a).

Not all studies have reported an improvement in performance even when glycogen resynthesis was either enhanced by CHO-P co-ingestion or equivalent to an isoenergetic CHO beverage (Alghannam et al., 2016; J. Berardi et al., 2006). However, recently Dahl et al. (2020) reported a significant increase in time to exhaustion performance following glycogen depleting exercise in favor of CHO-P co-ingestion (0.8 g/kg and 0.4 g/kg) as compared to an isoenergetic CHO only beverage (1.2 g/kg). In a randomized cross-over fashion, endurance-trained males consumed either beverage within 15 min of completing exhaustive exercise and at 30-min intervals for the first two hours of a 5-hour recovery period. Participants were able to cycle 8.4 ± 1.8 min longer, with the CHO-P condition, even though no difference in glycogen synthesis was reported between groups (Dahl et al., 2020).

Berardi et al. (2008) reported a performance decrement during a subsequent afternoon exercise session, which followed morning exercise and a 6-hour recovery period. Treatments for both groups included a CHO-P (0.8 g/kg and 0.4 g/kg) or isoenergetic CHO beverage (1.2 g/kg). The decrease in power for the CHO-P group was significantly less than the carbohydrate-only group, 3.86 watts vs. 16.5 watts, respectively (J. M. Berardi et al., 2008). Glycogen depletion and resynthesis were not examined in this study (J. M. Berardi et al., 2008). The attenuation of a performance decrement whether power output (watts) or time trial performance (time) is a critical factor for MCAs. Improving the rate of recovery from prior exercise can positively affect adaptation and thus performance in a subsequent bout of training or competition (Fell & Williams, 2008). However, all studies examining CHO-P co-ingestion and performance have been carried out in endurance athletes under the age of 30 years.

Protein Consumption and Short-Term Recovery

Carbohydrate recommendations are the same for both young and MCAs, which can be as low as 2-3 g/kg for low activity days and as high as 7-10 g/kg for days requiring greater intensity and exertion (Burke et al. 2001; Reaburn et al. 2015; Louis et al. 2019). However, protein intake, especially post-exercise, should be of equal importance for MCAs to support recovery and to promote skeletal muscle protein remodeling and repair, adaptation to training, and replenishment of energy stores (Doering, Reaburn, Phillips, et al., 2016; Moore et al., 2014). Few studies have investigated protein needs specific to MCAs as compared to their younger competitors. Meredith et al. (1989) examined the daily needs of highly trained endurance athletes that were, on average, 52, and 27 years of age. It was determined that both groups of athletes would require approximately 1.3 g/kg/day to be in positive nitrogen balance (Meredith et al., 1989).

More recently, Doering et al. (2016) reported interesting results regarding protein synthesis following muscle-damaging exercise in MCAs vs. younger (~ 53 vs. 27 years of age) highly trained endurance athletes. Participants completed a 30-min downhill run and over the next 48-hours performed three 20-km cycling time trials (TT; 10, 24, 48 hours post-run). Immediately after exercise, participants received a recovery beverage that contained 20 g whey protein and 1 g/kg CHO. Participants were required to consume daily protein intakes of 1.6-1.7 g/kg (each meal contained 0.3 g/kg/d) and carbohydrate intake of 6-7 g/kg. Results indicated that MCAs had significantly lower fractional synthetic rates of myofibrillar protein synthesis, as compared to the younger athletes (Doering, Jenkins, et al., 2016). When examining the change in time trial performance from baseline to 10 hours, there was no significant difference; however, MCAs appeared to exhibit a slower recovery as indicated by the moderate between-group effect size (*d* = 0.51). Overall, results suggest short-term recovery between bouts of exercise was slower in MCAs (Doering, Jenkins, et al., 2016).

Doering et al. (2017) examined the effect of moderate (0.3 g/kg) vs. high (0.6 g/kg) protein intake immediately post and 2-hour post damaging exercise in MCAs (52 ± 2.1 years) (Doering et al., 2017). The protein was co-ingested with 1.3 and 1.0 g/kg of CHO, respectively. In total, MCAs consumed 5.9/1.05 g/kg of CHO-P in the moderate group and 5.0/1.95 g/kg in the high group during the 8-hour recovery period. Participants performed isokinetic dynamometry of the knee extensors then performed a downhill run in the morning. In the afternoon, the participants performed repeated isokinetic dynamometry and a cycling TT (work equating to 7kJ/kg completed in the quickest possible time). In terms of perceived recovery, high protein intake resulted in significantly less subjective fatigue than the moderate intake (Doering et al., 2017). While there was no significant effect for TT performance, high protein intake attenuated reductions in force during afternoon peak isometric torque of the knee extensors to a greater degree than moderate protein intake (-3.6% vs -8.6%, respectively; *d* = 0.66) (Doering et al., 2017). Taken together, it appears that recent MCA-specific studies indicate decrements to performance between bouts of morning and afternoon exercise and that current protein recommendations do not appear to be sufficient for maximizing recovery from muscle-damaging exercise (Doering et al., 2017; Doering, Jenkins, et al., 2016).

Slower recuperation from muscle-damaging exercise (i.e., increase in contraction time, decrease in peak twitch at 48 and 72 hours) following a 55-mile trail run in MCAs in comparison to younger similarly trained athletes, has been reported previously (Easthope et al., 2010). Collectively, these studies (Doering et al., 2017; Doering, Jenkins, et al., 2016) indicate a need for age-specific nutrition-based recovery options as MCAs could benefit from quicker recovery and the ability to train and perform at peak capacity. Two studies in young endurance athletes showed increases in muscle protein synthesis (MPS) following endurance exercise and CHO-P coingestion immediately post-exercise and in 30-min increments thereafter (Breen et al., 2011; Rowlands et al., 2015). More recently Goldstein et al. (2022) reported a 35% increase in TTE performance following CHO-P consumption and a 2-hr recovery period in recreationally active college-age males and females. The provision of CHO-P co-ingestion post-exercise may serve to promote muscle remodeling and, thus, accelerate recovery in the endurance athlete (Moore et al., 2014).

It has been suggested that MCAs exhibit a diminished muscle protein synthetic response to a given dose of protein; in the general aging population, this is referred to as anabolic resistance (Doering, Reaburn, Phillips, et al., 2016; Moore et al., 2015; Morton et al., 2018). A formative 2013 publication (Areta et al.) demonstrated significantly higher rates of MPS throughout a 12-hour recovery period when young males consumed 20 g of protein every 3 hours as opposed to smaller, more frequent or larger less frequent doses (Areta et al., 2013). This publication seemed to form the basis for the 20-30 g of protein (per meal) recommendation commonly provided to athletes. In support, Moore et al. (2009) and Witard et al. (2014) reported a plateau in MPS at 20 g following an acute bout of resistance exercise, with larger doses of protein supplementation (40 g) promoting amino acid catabolism (Moore et al., 2009; Witard et al., 2014). However, in a state of energy deficit, it appears that a larger dose (30 g) of protein supplementation immediately post resistance exercise was effective for promoting MPS (Areta et al., 2014). A >20 g dose of protein may therefore prove beneficial for a glycogen depleted endurance athlete with limited time to recover. Taken together Moore et al. (2015) has reported that relative to body mass, older men require 0.40 g/kg of protein to maximally stimulate MPS, whereas younger men require 0.24 g/kg (Moore et al., 2015). While some publications (Desbrow et al., 2019) do not find it necessary to suggest higher per meal doses of protein for masters class (endurance) athletes, others are in support of greater protein needs (Doering, Reaburn, Phillips, et al., 2016; Louis et al., 2019; Reaburn et al., 2015).

Sports Nutrition Knowledge and Recovery Practices Among Endurance Athletes

Doering et al. (2016b) surveyed Australian triathletes (n = 110), and both MCAs and young athletes demonstrated poor or no knowledge of post-exercise nutritional practices for both carbohydrate and protein. “I don’t know” was the response when asked about recommended carbohydrate and protein intake (43.1% and 43.9 %, respectively) – frequency of responses is pooled for both MCAs and young athletes as a group. Carbohydrate consumption was considerably more in line with post-exercise recommendations for young athletes (1.1 g/kg) in comparison to MCAs (~0.7 g/kg). MCAs were slightly under the age-specific recommended threshold for post-exercise protein consumption (~0.3 g/kg), whereas protein intake was ~0.4 g/kg for young athletes. Finally, absolute post-exercise protein consumption was as follows: 19.6 ± 13.5 g and 26.4 ± 15.8 g, for masters and young athletes, respectively. “Own previous knowledge” was the most frequently selected response (17.3%) for primary source of information regarding post-exercise nutrition for recovery, followed by accredited sports dietitian (12.7%), and friends/teammates (10.0%) . It appears that MCAs surveyed in this study lack the essential knowledge regarding post-exercise nutritional practices to maximize recovery. (Doering, Reaburn, Cox, et al., 2016).

Additional studies have described post-exercise nutritional practices in both amateur and elite endurance athletes. Results from McLeman et al. (2019) indicated that in a group of young (30 ± 13) amateur runners, 50% of participants (n = 82) consumed 1.1 ± 0.8 g/kg of CHO and 75% of participants consumed 0.25 g/kg of protein within 60 min of exercise completion (McLeman et al., 2019). Of the 100 runners who completed the sports nutrition knowledge portion of the questionnaire, only 1% and 4% correctly identified post-exercise CHO and protein recommendations, respectively (McLeman et al., 2019). Eighty-nine percent of the sample responded “I don’t know” or provided an incorrect answer choice for post-exercise nutrient intake recommendations. The answer choice “internet and websites” was the primary (33%) source of sports nutrition information for this group of amateur runners in the UK (McLeman et al., 2019).

The Momentum 94.7 (km) Cycle Challenge is an annual cycling road race in South Africa, with annual participation rates between 27,000 and 35,000. In 2013, 30,640 cyclists registered for the Momentum 94.7 and 2,550 of those race participants responded to a survey that assessed sports nutrition knowledge related to CHO intake, nutritional supplementation, and hydration practices (Sparks et al., 2018). Of the 2,550 respondents, 66% (1682) were between the age of 36 and ≥56 years. Sixty-one percent of respondents indicated use of a nutritional supplement, with recovery as the most frequently selected (45%) reason for supplementation.

The most frequently selected ingredients were CHO, caffeine, whey protein, amino acids, magnesium, and vitamin C (Sparks et al., 2018). Recommendation by a friend was the most frequently cited (35%) reason for cyclists to use a sports nutrition supplement (Sparks et al., 2018).

Post-exercise nutrient intake during a week of altitude training was examined in a group of young male (27.7 ± 4.5 years) and female (25.8 ± 2.9 years) elite middle- and long-distance runners (Heikura et al., 2017). Training sessions were based on type, duration, and rating of perceived exertion and then categorized as either “hard” or “recovery”. Sixty percent of males (9 of 15) and 40% of females (9 of 23) met the recommended CHO intake of 1 g/kg of body mass following “hard” training sessions (Heikura et al., 2017). Protein consumption was substantially less following “hard” training sessions, as 33% of males (5 of 15) and only 17% (4 of 23) of females met the recommended intake of 0.25 g/kg of body mass (Heikura et al., 2017). Poor compliance with post-exercise recovery was reported in an earlier study of Australian Olympic athletes (25 ± 5 years) (Burke et al., 2003). Elite endurance athletes consumed an adequate amount of CHO (≥0.8 g/kg of CHO) within the first hour of exercise but only for 67% of training sessions completed within a 7-day period. (Burke et al., 2003). Taken together results from studies (Burke et al., 2003; Doering, Reaburn, Cox, et al., 2016; Heikura et al., 2017; McLeman et al., 2019; Sparks et al., 2018) that have examined post-exercise nutrient intake in athletes demonstrate an inconsistency in the ability of different groups of endurance athletes (young vs old; amateur vs elite; male vs female) to meet post-exercise CHO and protein recommendations. Furthermore, post-exercise nutritional recommendations from an accredited professional are lacking among both young and masters-level endurance athletes.

Heart Rate Recovery

Heart rate variability (HRV) can be used as a measure of cardiac autonomic function, or the interplay between the sympathetic and parasympathetic nervous systems, during rest and exercise (Deus et al., 2019). Aging is associated with autonomic nervous system changes that can negatively impact cardiovascular health (Wichi et al., 2009). An example of age-associated autonomic modulation is a decrease in parasympathetic activity and HRV, and increase in sympathetic activity and heart rate (Wichi et al., 2009). Chronic exercise, however, can exert a positive influence by acting to preserve cardiac autonomic function throughout the aging process (Deus et al., 2019; Wichi et al., 2009). Wichi et al. (2009) report, “HRV changes are directly related to the intensity, duration, and frequency of aerobic training” referring to greater impact of exercise on HRV with increased intensity, duration, and frequency of cardiovascular activity.

Deus et al. (2019) examined the difference in HRV during 15 minutes of rest in master endurance (53.6 ± 8.6 years) and sprinter (51.8 ± 11.1 years) athletes in comparison to two groups of age-matched (47.5 ± 6.0 years) and younger (25.4 ± 3.9 years) sedentary controls. The MCAs in this study were highly trained, with a 15-year history competing in either sprinting or endurance-based sport, such as marathon and triathlon (Deus et al., 2019). Results indicated HRV measured in the time domain was a) significantly improved in MCAs, as compared to the age-matched controls, and b) similar to the younger sedentary control group (Deus et al., 2019). Additionally, HRV was significantly decreased for the middle-aged sedentary group, in comparison to the younger control group (Deus et al., 2019).

Heart rate recovery (HRR) is a measure that is easy to assess at the cessation of exercise and is calculated as the difference between peak heart rate (beats per min; bpm) and the heart rate one min later (Adabag & Pierpont, 2013; Suzic Lazic et al., 2017). HRR can be calculated at additional time points postexercise (e.g., 2-, 3-, and 5-min) and is a valid and simple measure that has been applied in clinical practice as an indicator of autonomic function (Adabag & Pierpont, 2013; Peçanha, Silva-Júnior, et al., 2014; Suzic Lazic et al., 2017). It has been suggested that 1- and 5-min postexercise are representative of the fast and slow phases of HRR, respectively. The rapid decline in heart rate during the first minute of recovery is typical of parasympathetic reactivation while the gradual decay later into recovery (i.e., ≥ 2 min) is more characteristic of the interaction of parasympathetic reactivation and sympathetic withdrawal (Peçanha, Silva-Júnior, et al., 2014).

Exercise-induced dehydration can impair thermoregulation and promote cardiovascular strain during a bout of prolonged endurance exercise (Sawka, 1992). Therefore, HRV has been used to study the effect of fluid balance on autonomic regulation of cardiac function during exercise and recovery. Moreno et al. (2013) reported no significant influence of isotonic drink consumption (i.e. Gatorade®) during exercise on indices of HRV, in comparison to no fluid intake during 90 min of submaximal exercise in young active males (Moreno et al., 2013). In contrast, during the 60-min recovery period, continual isotonic drink consumption resulted in rapid recovery of several indices of parasympathetic activity, as analyzed in both the time and frequency domains. Interestingly, in a state of hypohydration these same indices (i.e., markers of parasympathetic activity) did not return to resting levels throughout the recovery period. In support, Vanderlei et al. (2015) reported an improvement in autonomic modulation during recovery from submaximal exercise when participants were hydrated, regardless of treatment (water vs. isotonic beverage). The absence of fluid during the 60-min recovery period resulted in an increase in sympathetic activity, relative to when participants consumed either water or an isotonic beverage (Vanderlei et al., 2015).

Conclusion

In conclusion, MCAs are a unique group of athletes. The opportunity exists to promote optimization of post-exercise glycogen resynthesis and protein remodeling in support of successive bouts of training and competition. Including protein in a recovery drink may increase protein intake and help MCAs meet daily protein needs while promoting recovery. Moreover, consumption of a recovery beverage within the short-term recovery period will assist with fluid intake, which may in turn promote the rehydration process and improve cardiac autonomic modulation at rest. The purpose of this study is to demonstrate that performance in a subsequent bout of high-intensity glycogen depleting exercise will not be significantly different regardless of post-exercise recovery drink (carbohydrate vs. carbohydrate and protein vs. placebo).

CHAPTER THREE: METHODS

Experimental Design

This study utilized a randomized, double-blind, placebo-controlled between-subject design to examine the effects of CHO and CHO-P supplementation on short-term recovery following aerobic interval exercise and time to exhaustion testing in MCAs. The primary independent variable was the treatment group (CHO vs. CHO-P vs. PLA), and the secondary independent variables were level of sports nutrition knowledge, and total daily carbohydrate and protein intake. The primary dependent variables were time to exhaustion (TTE) test, HRR, and fluid loss.

Participants completed a total of three visits to the Physiology of Work and Exercise Response (POWER) Laboratory at the University of Central Florida. During the first visit (Visit #1), participants completed paperwork and maximal oxygen consumption testing (VO_2peak). Visit #2 consisted of familiarization with the high-intensity aerobic interval exercise (INT) and TTE protocol. During Visit #3, participants completed pre-intervention INT and TTE followed by a two-hour recovery period. Participants were required to consume one of three treatments within the first 90 minutes of the two-hour recovery period. Following the recovery period, participants completed the post-intervention INT and TTE protocol and then rested for an additional 30 minutes. Fluid loss was determined immediately post INT and TTE testing, while HRR was determined post-exercise.

During Visit #1, participants were assessed for body weight, standing height, and body composition. Height and body weight were assessed using a stadiometer and scale (Health-o-meter Professional Patient Weighing Scale, Model 500 KL, Pelstar, Alsip, IL, USA), and body composition was assessed using bio-electrical impedance spectroscopy analysis (SOZO, Carlsbad, CA, USA). If time permitted, and after consent was provided, participants completed a graded exercise test to determine VO_2peak. Participants were provided with the option to return to the laboratory on a separate date for VO_2peak testing. Forty-eight to 72 hours after Visit #1, or VO_2peak assessment, participants returned to the laboratory for familiarization with the INT exercise and TTE protocol (Visit #2). Both the familiarization and the experimental protocol occurred at approximately the same time of day for each participant. Participants were instructed to avoid exercise for 24 hours prior to Visit #3 (experimental protocol).

Within five to nine days after completion of Visit #2, participants returned to the laboratory for Visit #3 to complete INT exercise and TTE, a 2-hour passive recovery period including the consumption of one of three treatments, followed by repeated INT exercise and TTE, measurement of body weight to assess fluid loss, and HRR. Participants also completed the Post-Exercise Nutrition and Recovery Practices survey (PENRP) during the 2-hour recovery period. Before the first exercise trial, participants were randomly assigned to one of three treatments by the primary investigator via a web-based random assignment generator (Urbaniak & Plous, 2013). Only the primary investigator had access to the random assignment of treatments, and the primary investigator was responsible for preparing each recovery beverage.

During the 2-hour recovery period (Visit #3) participants consumed either placebo (PLA; Gatorade® Zero Thirst Quencher Orange, Chicago, IL, USA), CHO (6% carbohydrate-electrolyte solution; Gatorade® Thirst Quencher Orange, Chicago, IL, USA) in the amount of 1.2 g/kg of body weight, or CHO with whey protein isolate powder (CHO-P; 6% carbohydrate-electrolyte solution Gatorade® Thirst Quencher Orange, Chicago, IL, USA; BiPro Elite, Agropur Inc., Appleton, WI, USA) in the amount of 0.8 g/kg of body weight and 0.4 g/kg of body weight, respectively. If necessary, Gatorade® Thirst Quencher Orange powder (Chicago, IL, USA) was weighed in grams via a digital food scale and added to the existing liter of fluid to reach the total amount of CHO needed. Fat free mass (kg) was used to calculate the CHO or CHO-P needs for any participant with a measured body fat percentage that was greater than or equal to 20%. The total fluid volume of each recovery drink regardless of treatment was 32 ounces or approximately 1 liter. Participants were allowed sips of water as needed during the 2-hr recovery period. The 32 ounces of fluid provided as part of the treatment in addition to sips of water were recorded for each participant as total fluid consumed during the 2-hour recovery period. To disguise appearance, all three recovery beverages were prepared in the same dark red Nalgene water bottles (Nalge Nunc International Corporation, Rochester, NY, USA). The consistent orange flavor and the opacity of the bottle resulted in effective blinding to the participant. In a pilot study done in our lab using a between subject design, there were approximately 42.1% correct guesses when participants consumed all three recovery beverages (Goldstein et al., 2022).

Participants were instructed to consume the treatment beverage in its entirety within 90 minutes from the start of the recovery period. During Visit #3, participants were weighed in their cycling bib shorts immediately before and after the first and second bouts of INT exercise and TTE testing. Fluid loss was determined based on the difference between pre- and post-weight changes for each bout of exercise (Casa, 2019). After the second bout of INT and TTE, participants were seated for 30 minutes while wearing a heart rate monitor (Polar H10, Polar Electro Oy, Kempele, Finland). Heart rate was recorded during this period via the Elite HRV® smartphone app. The difference in beats per minute from peak heart rate at the cessation of exercise to 1-, 2-, and 5-min post-exercise was used as an indicator for recovery of the autonomic nervous system (HRR) from the high-intensity aerobic intervals and TTE testing. HRR index was calculated as the last 3-sec peak heart rate value (beats per min; bpm) postexercise plus the heart rate (bpm) recorded 1 min postexercise, divided by total work (Sterkowicz-Przybycień et al., 2019). Total work was calculated as TTE in sec x 90% PPO divided by 1000 (kilojoules).

The calculation of sample size (G*Power 3.1.9.4) was based on pilot data in moderately trained young men and women (22.0 + 2.4 years) collected in our lab using the same protocol (90% PPO TTE) as outlined in this study. The total sample size was determined to be a total of 18 (6 in each group) based on a one-way ANCOVA, power of 0.90, and an alpha of 0.05. However, due to potential dropout rates, a total of 30 participants (10 per group) were recruited with the goal of completing 18.

Thirty-one male MCAs between the ages of 35-59 years old were recruited for this study, Eligibility criteria required MCAs to be regularly engaged in endurance exercise (running, cycling, swimming) for a minimum of three years, a weekly training volume of 5-10 hours, and a minimum maximal oxygen uptake of 45.00 $ml \cdot kg^{-1} \cdot min^{-1}$ (Deus et al., 2019; Doering et al., 2017; Doering, Jenkins, et al., 2016; Knechtle et al., 2012; Lepers et al., 2013; Lepers & Cattagni, 2012; Peiffer et al., 2008; Stiefel et al., 2014). A minimum oxygen uptake of 45.00 $ml \cdot kg^{-1} \cdot min^{-1}$ was chosen because this was shown to be the lowest average VO_2max in available studies of MCAs (Doering et al., 2017; Doering, Jenkins, et al., 2016; Peiffer et al., 2008; Sultana et al., 2012). Participants provided written informed consent before being screened for participation in the study, and the primary investigator was responsible for reviewing all paperwork and study enrollment. This study was approved by the UCF IRB (STUDY00002618) and listed on ClinicalTrials.gov (NCT04859491). Participants were recruited locally from the central Florida area via recruitment flyers, social media, and verbal recruitment by the primary investigator and co-investigators.

Participants were screened for health conditions that might prevent them from participating in the study, such as muscle or joint injuries or heart conditions. Following an explanation of all procedures, risks, and benefits, each participant was asked to complete the American College of Sports Medicine Exercise Preparticipation Health Screening Questionnaire for Exercise Professionals (PHSQEP), Physical Activity Readiness Questionnaire for Everyone (PAR-Q+), and Medical Health and Activity Questionnaire. The PHSQEP was utilized to screen for cardiovascular, metabolic, and renal disease and determine if a medical clearance is necessary and/or if the participant is ready to continue engaging in moderate or vigorous-intensity exercise. The PAR-Q+ was utilized as an additional level of screening to assess for issues with muscle or joint injury that may preclude an individual from immediate physical activity or require medical clearance. Exclusion criteria included any recent musculoskeletal injuries or surgeries, signs or symptoms suggestive of cardiovascular, metabolic or renal disease as outlined in the American College of Sports Medicine preparticipation screening algorithm (e.g. shortness of breath at rest or with mild exertion; known heart murmur, unusual fatigue or shortness of breath with usual activities), or a chronic illness that requires continuous medical care (Magal & Riebe, 2016).

Determination of VO_2peak

All participants performed a graded exercise test (GXT) to volitional exhaustion on a cycle ergometer (Lode, Corival cpet, Groningen, The Netherlands) to determine VO2peak and peak power output. Prior to testing, each participant was fitted with a Polar heart rate monitor (chest strap and sensor; Polar H10, Polar Electro Oy, Kempele, Finland) to record heart rate. Seat height was set based on feedback from each participant then recorded and kept constant throughout the remainder of the study. Participants then completed a five-minute warm-up on the cycle ergometer at a self-selected intensity and cadence. The test consisted of 2-minute stages, beginning at an initial workload of 50 watts (W), then100W, then 150W followed by an increase of 30W every 2 minutes until the participant could no longer maintain 60 revolutions per minute (rpm) (McCarthy & Spriet, 2020). Open-circuit spirometry was used to estimate VO_2peak ($L \cdot min^{-1}$) by sampling and analyzing the breath-by-breath expired gases with a calibrated metabolic cart (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Sandy, UT). Ventilation and expired gases were continuously recorded and averaged every 15s to determine VO2peak, which was determined as the highest peak value achieved during the last completed stage of the test coinciding with at least two of the following three parameters: heart rate (HR) within 10% of age-predicted maximal HR; respiratory exchange ratio (RER) of 1.15 or higher; a plateau in oxygen consumption despite an increase in exercise intensity. The highest power output achieved during the last completed 2-min stage was recorded as peak power output (PPO) in watts.

High-Intensity Aerobic Intervals and Time to Exhaustion

All participants performed a warm-up on a cycle ergometer for 3-minutes at 50W, 2-minutes at 100W, and 1-minute at 75W followed by a 3-minute rest (McCarthy & Spriet, 2020). Participants then performed 5 x 4-minute high-intensity aerobic intervals at 70-80% of individual PPO, as determined during the familiarization trial. During the familiarization trial, each person attempted to complete all five intervals at 80% of individual PPO followed by the TTE trial at 90% of PPO. If a participant was unable to complete the intervals at 80% of individual PPO or was able to complete the intervals but not the TTE trial, then the power was be decreased by 5% (to a minimum of 70% PPO) until the participant could perform the entire series of aerobic intervals and TTE protocol (Goldstein et al., 2022). Two-minutes of low-intensity cycling at 50W separated each aerobic interval. Immediately following the fifth interval, participants cycled at a work rate (watts) that corresponded to 90% PPO until volitional exhaustion or when rpm fell below 60 for 10 seconds. Following the endurance trial (INT and TTE), participants cycled with no resistance for 5 minutes to cool down. Revolutions per minute were the only performance measure visible to participants, and minimal verbal encouragement was provided throughout the aerobic intervals and time to exhaustion. Ratings of perceived exertion (RPE) were recorded using the Borg 10 scale during the last 30 seconds of each aerobic and active rest interval. Gas exchange data was collected with a metabolic analyzer (Trueone 2400, Parvo Medics, Utah, USA) throughout the experimental protocol for both bouts of INT and TTE testing.

Heart Rate Recovery

Heart rate (beats per minute; bpm) was recorded via the heart rate monitor and Elite HRV® smartphone app. Heart rate was continuously recorded every three seconds and downloaded using a laptop with commercially available heart rate variability analysis software (Kempele, Finland; Kubios HRV Analysis v 3.3, Kuopio, Finland). The Heart Rate Recovery index (HRRi) was calculated as the last 3-sec peak heart rate value (bpm) post-exercise plus the heart rate (bpm) recorded 1 min post-exercise, divided by total work. The HRRi was adapted from a previously published formula used by others (Sterkowicz-Przybycień et al., 2019). Total work was calculated as TTE in sec x 90% PPO divided by 1000 (kilojoules). HRRi was calculated 1-, 2-, and 5-min post-exercise. A smaller HRRi value indicated improved recovery.

$$HRRi = \frac{\text{Final peak heart rate (bpm)} + \text{Heart Rate1min (bpm)}}{\text{TTE (sec) x 90\% PPO}}$$

Dietary Analysis

To understand if MCAs are meeting carbohydrate and protein intake recommendations, participants were asked to complete the ASA24$^{®}$. The ASA24$^{®}$ is a validated, automated self-administered 24-hour dietary assessment tool developed by the National Cancer Institute (Bethesda, Maryland) (Park et al., 2018). Each participant was instructed on the use of the ASA24® during Visit #1, by a registered dietitian. Following completion of the familiarization trial, participants were emailed a sample of a detailed diet recall and an individual username and password to access the ASA24$^{®}$ system. They were asked to report a 24-hour intake that is typical for days that they train. The sample diet recall demonstrated a level of detail that includes all foods and fluids consumed upon waking until bedtime, including foods and/or supplements consumed pre, during, and immediately post-exercise. Participants were asked to complete the electronic diet recall during the 5-9-day period between familiarization (Visit #2) and the experimental protocol (Visit #3). After data collection, the dietary intake was analyzed for total energy (kilocalorie [kcal]) and macronutrient distribution (carbohydrate, protein, and fat). An Excel spreadsheet of each participant's macro- and micronutrient analysis was downloaded from the ASA24$^{®}$ website.

Post-Exercise Nutrition and Recovery Practices Survey

The Nutritional Recovery Practices, Knowledge and Beliefs of Australian Triathletes survey is an instrument previously designed to assess knowledge of postexercise nutritional recommendations and recovery practices in endurance athletes (Doering, Reaburn, Cox, et al., 2016). Participants completed the survey electronically during the 2-hour recovery period (Visit #3). MCAs in this study were asked to complete a series of 34 total questions assessing their postexercise nutrition knowledge and recovery practices. Questions 1 through 10 were omitted as the current study was not specific to triathletes.

Statistical Analysis

All statistical analyses were conducted via the Statistical Package for Social Science (SPSS) software for Windows version 28 (SPSS Inc., Chicago, IL). Descriptive statistics were calculated to determine group demographics. Before analysis, all data was tested for normality (Shapiro-Wilks), homogeneity of variance (Levene's test of equality of error variance), and homogeneity of slopes. Data was statistically analyzed using separate 1-way analysis of covariance (ANCOVA) for TTE and HRRi. The pre-test and the pos-ttest values were used as a covariate and dependent variable, respectively. If ANCOVA assumptions, such as homogeneity of slopes, were not met, then Quade's nonparametric analysis was used. Differences in total energy intake, macronutrients, and fluid loss were assessed with a one-way ANOVA. Fisher's LSD Post-Hoc analysis was used to determine group differences. For effect size (ES), the partial eta squared statistic was reported, with 0.01, 0.06, and 0.14 representing small, medium, and large ES, respectively (Green et al., 2000). The significance level was set at $p \leq 0.05$.

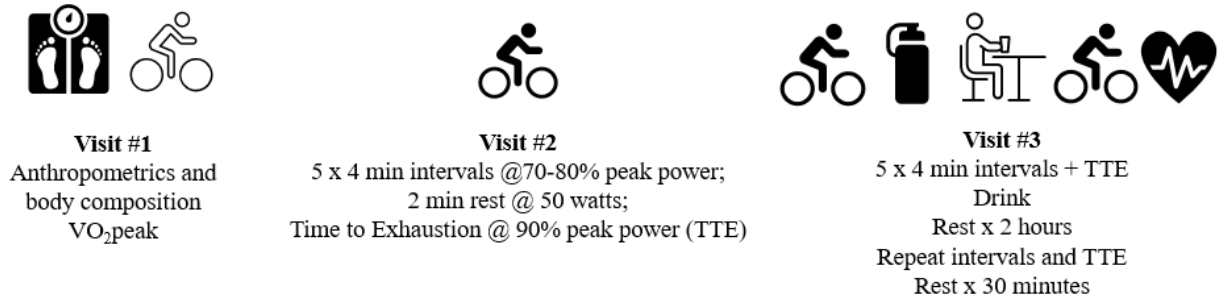


Fig. 1 Experimental design of the study; anthropometrics and body composition, graded exercise test, high-intensity interval and time to exhaustion testing, recovery beverage, 2-hour recovery period, heart rate recovery

Figure 1 Timeline of Activities and Testing

CHAPTER FOUR: RESULTS

Participants

Data from twenty-two trained male MCAs (49.1 ± 6.9 years old) were used in this research investigation (Table 1). Thirty-one athletes were recruited however three were withdrawn from the study due to illness (i.e., COVID, flu); two discontinued participation after expressing discomfort with the mask required for metabolic testing; and one was withdrawn due to scheduling issues. Data for three athletes were omitted from analysis due to the following: noncompliance with activity 24 hours prior to the experimental protocol, inability to complete TTE testing following the second bout of aerobic intervals, and extended time between visits (i.e., ≥ 21 days). The MCAs in this study met the eligibility criteria of a minimum of three years participating in endurance sport. Nineteen participants reported engaging in regular endurance activity: eighteen participants reported cycling 7.7 ± 2.0 hours per week, six reported running 2.5 ± 2.3 hours per week, and two participants reported swimming 2.0 ± 1.4 hours per week. The average VO_2peak was 48.6 ± 6.7 $mL \cdot kg^{-1} \cdot min^{-1}$. Nine of 22 total participants had a body fat percentage greater than 20%; three participants were in the CHO group, one in the CHO-P group, and five in the PLA group. Therefore, fat free mass (kg) was used to calculate the nutrient needs for four (18.1%) participants.

Table 1. Mean and SD values for Demographic Data

	Total (n = 22)
Age (years)	49.1 ± 6.9
Height (centimeters)	175.8 ± 4.8
Weight (kilograms)	80.7 ± 8.6
Body fat (%)	19.1 ± 5.8
VO_2peak ($mL•kg^{-1}•min^{-1}$)	48.6 ± 6.7
Peak Power Output (watts)	291.8 ± 39.5
	(n = 19)
Cycling (hr/wk)	7.7 ± 2.0 (n = 18)
Running (hr/wk)	2.5 ± 2.3 (n = 6)
Swimming (hr/wk)	2.0 ± 1.4 (n = 2)

Blinding

Blinding results were available for 19 of 22 total participants with 31.6% (n = 6) correctly identifying which treatment beverage they consumed.

High-Intensity Aerobic Intervals and Time to Exhaustion

Table 2 shows the group means (±SD) for the pretest and posttest values. Figure 2 shows the group means (±SE) for the posttest TTE (sec) values adjusted for the initial differences in the pretest TTE. There was homogeneity of regression slopes as the interaction term was not statistically significant ($F_{2,16}$= 2.756, *p* =.094). Assumptions were met for normality and there was homogeneity of variance for the TTE values, as assessed by Levene's test for equality of variances (*p* = .324). The ANCOVA indicated a significant difference ($F_{2,18}$= 6.702, *p* =.007, η^2 =.427) among the group means for the posttest TTE values after adjusting for the pretest differences. The strength of the association (i.e., effect size, η^2) indicated that the treatment groups (PLA, CHO, and CHO-P) accounted for 43% of the variance of the posttest TTE values.

Fisher's LSD pairwise comparisons indicated that the posttest TTE was greater in CHO (*p* <.002) and CHO-P (*p* =.028) when compared to PLA group with no differences between CHO and CHO-P (*p* =.265).

Table 2. Mean and SD values for TTE (sec) at pretesting and post-testing for each group.

TTE		**PLA (n = 8)**	**CHO (n = 7)**	**CHO-P (n = 7)**
Pretest	Mean	165.00	218.57	231.00
	SD	79.82	138.70	133.60
Posttest	Mean	132.75	311.14	283.29
	SD	64.28	167.90	158.90

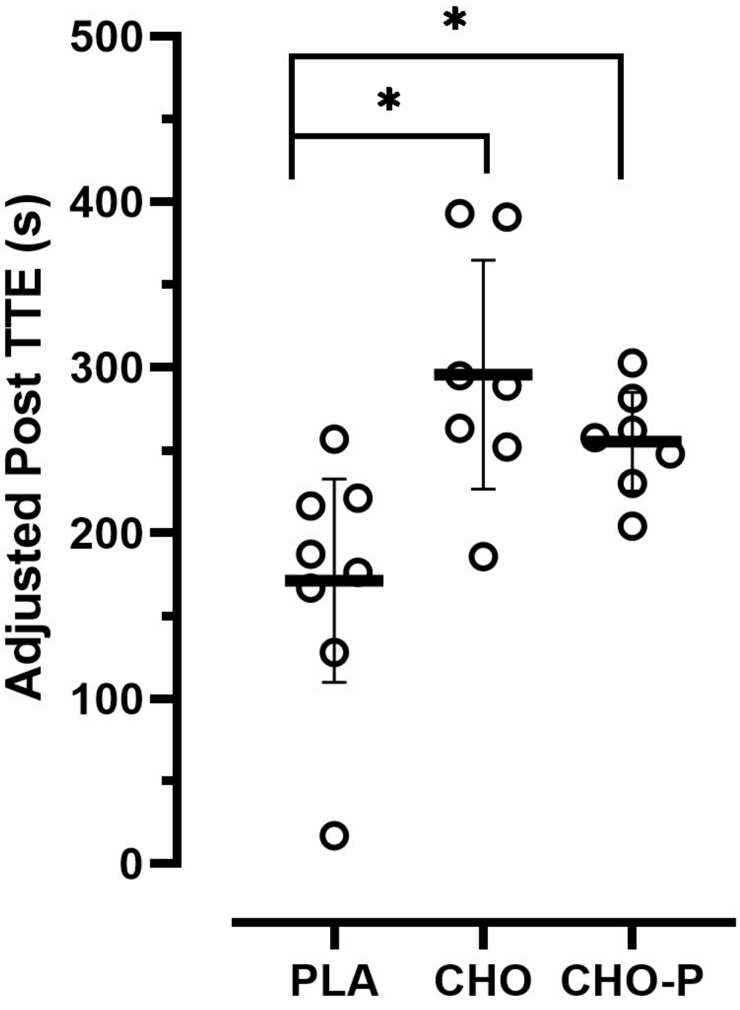


Figure 2 Group mean values (±SE) for posttest TTE scores adjusted for the initial differences in the pretest TTE (covariate). The adjusted pretest value was 203.05 sec. *Indicates significant difference compared to PLA.

Heart Rate Recovery

Table 3 shows the group means (±SD) for the pretest and posttest HRRi values at three separate time points. The assumption of normality and equality of variance was not met therefore the Quade nonparametric ANCOVA was used and indicated a significant difference among the group means for the post-testing HRRi values at 1-min ($F_{2,17}$=6.715, p=.007), 2-min ($F_{2,17}$=5.720, p=.013), and 5-min ($F_{2,17}$=6.438, p=.008). Fisher's LSD pairwise comparisons indicated the posttest HRRi was significantly different between PLA and CHO at 1-min (p=.003), 2-min (p=.005), and 5-min (p=.003) post-testing. Post hoc testing indicated the posttest HRRi was significantly different between PLA and CHO-P at 1-min (p=.026), 2-min (p=.029), and 5-min (p=.025) post-testing. There were no differences in posttest HRRi values between the CHO and CHO-P group at any of the three post-testing time points: 1-min (p=.469), 2-min (p=.628), 5-min (p=.549).

Table 3. Mean and SD Values for HRRi (bpm) at pretesting and post-testing for each group.

HRRi		**PLA (n = 8)**	**CHO (n = 7)**	**CHO-P (n = 5)**
	1-min			
Pretest	Mean	9.77	6.95	8.26
	SD	5.18	3.0	8.33
Posttest	Mean	13.54*†	5.10	7.66
	SD	10.85	2.84	9.10
Delta Score (Post-Pre)	Mean	3.77	-1.86	-0.59
	SD	8.37	1.99	1.01
	2-min			
Pretest	Mean	9.13	6.64	7.86
	SD	4.80	2.93	8.05
Posttest	Mean	12.73*†	4.75	7.26
	SD	10.10	2.66	8.65
Delta Score (Post-Pre)	Mean	3.60	-1.89	-0.61
	SD	7.60	1.93	0.94
	5-min			
Pretest	Mean	8.84	6.43	7.63
	SD	4.70	2.81	7.93
Posttest	Mean	12.39*†	4.59	7.08
	SD	10.10	2.57	8.60
Delta Score (Post-Pre)	Mean	3.55	-1.84	-0.55
	SD	7.64	1.99	1.00

*Significant difference between PLA and CHO groups.
†Significant difference between PLA and CHO-P groups.

Dietary Analysis

Table 4 contains the mean and standard deviation values for total energy intake (kilocalories), protein, fat, and carbohydrate among the three groups (PLA, CHO, CHO-P) of participants. Due to issues with compliance, data is only reported for 59% (13 of 22) of the participants: 5 of 8 participants in the PLA group; 4 of 7 participants in the CHO group; 4 of 7 participants in the CHO-P group. Assumptions were met for normality and there was homogeneity of variance for total calories (*p* = .921), protein (*p* = .437), fat (*p* = .485), and carbohydrate (*p* = .958) as assessed by Levene's test for equality of variances. A one-way ANOVA revealed no significant differences (*p* > .05) between total energy intake, protein, fat, or carbohydrate.

Table 4. Total energy intake and macronutrient values are reported for 13 of 22 participants. Values are expressed as mean ± standard deviation.

	Total (n = 13)	**PLA (n = 6)**	**CHO (n = 4)**	**CHO-P (n = 4)**
Weight (kilograms)	80.0 ± 7.7	77.6 ± 5.4	76.7 ± 2.9	86.3 ± 10.6
Total energy intake (kcal)	3011.9 ± 1063.2	2788.9 ± 1153.2	2916.2 ± 1123.7	3386.2 ± 1097.2
Total energy intake (kcal) (g/kg)	37.6 ± 12.9	36.1 ± 15.0	37.8 ± 13.6	39.4 ± 13.2
Protein (g)	142.7 ± 51.1	138.3 ± 65.6	136.8 ± 34.8	154.0 ± 57.0
Protein (g/kg)	1.8 ± 0.6	1.8 ± 0.9	1.8 ± 0.4	1.8 ± 0.7
Fat (g)	119.3 ± 49.1	105.6 ± 48.4	121.7 ± 66.6	134.0 ± 38.3
Fat (g/kg)	1.5 ± 0.6	1.4 ± 0.6	1.6 ± 0.8	1.6 ± 0.4
Carbohydrate (g)	338.5 ± 122.7	319.3 ± 135.8	333.7 ± 117.1	367.3 ± 142.0
Carbohydrate (g/kg)	4.3 ± 1.6	4.2 ± 1.8	4.3 ± 1.4	4.3 ± 1.8

Fluid Loss

Table 5 contains the mean and standard deviation values for participant body mass (kg) measured at the start of and immediately following the first bout of exercise (i.e., INT and TTE), and at the conclusion of the 2-hour recovery period at the start of the second bout of exercise (INT and TTE). The assumption of normality was met for the PLA and CHO-P groups. The Shapiro-Wilk test indicated a significant departure from normality for the CHO group at each of the three time points (W = .705, *p* = .004, W = .710, *p* = .005, W = .700, *p* = .004). There was homogeneity of variance at the start of bout 1 (*p* = .967), immediately following bout 1 (*p* = .965), and at the start of bout 2 (*p* = .965). The deviation from normality for the CHO group was reviewed and the one-way ANOVA was conducted. Results indicated no significant difference (*p* > .05) in participant body mass (kg) as measured at the beginning and end of the first bout of exercise, and at the start of the second bout of exercise.

Table 5. Participant fluid loss pre-post exercise and total volume consumed during the 2-hour recovery period. Values are expressed as mean ± standard deviation.

	Total (n = 22)	**PLA (n = 8)**	**CHO (n = 7)**	**CHO-P (n = 7)**
Exercise Bout 1: *Pre*-bike weight (kilograms)	80.7 ± 8.8	78.5 ± 7.7	80.5 ± 10.2	83.5 ± 8.9
Exercise Bout 1: *Post*-bike weight (kilograms)	80.1 ± 8.6	77.8 ± 7.6	79.8 ± 10.1	82.8 ± 8.7
Total Weight Loss *Bout 1*	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
Exercise Bout 2: *Pre*-bike weight (kilograms)	80.6 ± 8.6	78.3 ± 7.5	80.4 ± 9.9	83.5 ± 8.8
Volume of Fluid Consumed: Treatment beverage + sips of water (liters)	1.1 ± 0.2	1.0 ± 0.1	1.1 ± 0.3	1.1 ± 0.2

Post-Exercise Nutrition and Recovery Practices Survey

Nineteen MCAs completed the PENRP survey. The top three self-reported levels of participation were described as: competing as a competitive age grouper (7; 36.8%), competing as a top age-grouper (4; 21.0%), and competing to maintain fitness (6; 32.0%). Two athletes (10.5%) reported competing for fun and social interaction. The response to the question, "How many grams of CHO (g/kg bm) should an endurance athlete consume in the post-exercise snack/meal to optimize recovery?" varied as 0.7 g/kg bm was the lowest participant suggested amount and 20.0 g/kg bm the largest participant suggested amount. The average participant response was 7.9 ± 6.8 g/kg bm CHO. The response was similar for the question "How many grams of protein (in total) should an endurance athlete consume in the post-exercise snack/meal to optimize recovery?" with the lowest participant suggested amount reported as 10.0 g and 65.0 g as the largest participant suggested amount. The average response was 28.9 ± 13.9 g of protein. The two most frequently cited responses to the question, "How important do you feel post-exercise nutrition is to your recovery after training" were *very important* (52.6%; 10/19) and *important* (26.3%; 5/19).

Approximately 63.0% (12/19) responded *yes* and 36.8% (7/19) responded *no* to the question, "Do you specifically plan your immediate post-racing nutrition?". Eight respondents (42.1%) reported consuming a supplement within 30 min from the end of training, one participant reported within 45 min, and six (31.6%) reported consuming a supplement within 1 hour of exercise completion.

The most frequently cited response to the question, "Are there any situations where you intentionally avoid high carbohydrate containing foods after training? was *I always consume high carbohydrate containing foods after training* (57.9%; 11/19). Approximately 37% (7/19) of respondents reported that they *sometimes avoid high carbohydrate containing foods after training*. Assisting with weight management (13.3%; 2/15), training sessions not long enough to justify carbohydrate intake (13.3%; 2/15), fueling adequately before (13.3%; 2/15) and throughout training sessions (13.3%; 2/15) were the most frequently cited responses for sometimes avoiding high carbohydrate containing foods after training. Additional free text responses were, “I don’t avoid” (26.7%; 4/15), “I eat carbs after training” (1/15), and “I don’t know why” (1/5). One MCA selected the response, “I avoid high carbohydrate containing foods because this will enhance my exercise adaptation to endurance training”.

Thirteen (72.2%; 13/18) MCAs reported intentionally including high protein containing foods after training, while four (22.2%) athletes cited the response, “Unsure, or I may unintentionally consume high protein containing foods after training”. One athlete reported intentionally avoiding high protein containing foods after training, and two athletes cited the response, “*I avoid high protein containing foods because endurance athletes do not require high amounts of protein*” as the primary reason to avoid high protein containing foods after training. Two separate MCAs selected the following reasons for avoiding high protein containing foods after training: “*I avoid high protein containing foods because it compromises my carbohydrate intake*” and “*I avoid high protein containing foods because I don’t tolerate anything substantial immediately after exercise*”. One participant stated the following (i.e., free text response) as a primary reason for including high protein containing foods after training, “*I don’t avoid high protein foods after training. I do take about 30 grams of protein minimum to begin muscle recovery after training*”, while a different athlete stated, “*I eat protein after training to help maintain and grow muscle*”. An additional response by a separate athlete was, “*Depends on the* *training, heavy work will have high protein, easy/regular workouts not focused on increased protein*".

Assist with muscle repair/hypertrophy after strength/resistance training sessions (37.5%; 6/16) and assist with muscle repair after all training sessions (31.3%; 5/16) were the two most frequently cited explanations by MCAs for including high protein containing foods after training. One athlete responded, "*I include high protein containing foods to assist muscle repair as I am an older athlete*". The two remaining responses were, "I include high protein containing foods to assist muscle repair after extended/long endurance training sessions" (18.8%; 3/16) and "I include high protein containing foods to assist muscle repair after hard running/high impact training sessions" (1/16).

Own previous knowledge (27.8%; 5/18) was the most frequently cited response when asked the question, "What is the primary source of your information about post-exercise nutrition for recovery?". The second and third most frequently cited responses were friends/teammates (16.7%; 3/18) and fellow athletes (16.7%; 3/18), respectively. The remaining responses for primary source of information were triathlon/cycling/running magazines (9.6%; 2/21), internet (911.1%; 2/18), and other (books; podcasts; Trainer Road podcast) (16.7%; 3/18).

CHAPTER FIVE: DISCUSSION

The purpose of this investigation was to examine the effectiveness of three different beverages (CHO, CHO-P, and PLA) on short-term recovery from repeated bouts of exhaustive exercise in male endurance MCAs. The results support our hypotheses that CHO-P (0.8 g/kg bm CHO + 0.4 g/kg bm PRO) was equivalent to CHO (1.2 g/kg bm) for promoting an increase in time to exhaustion (TTE) performance following a 2-hour recovery period. Both CHO and CHO-P were superior to PLA (electrolytes and water) for supporting short-term recovery in masters class endurance athletes. The other main finding of this investigation was that both CHO and CHO-P appeared to be equally effective and significantly better than PLA in promoting HRRi.

A similar study with younger participants (Goldstein et al., 2022) demonstrated an improvement in TTE performance (225.54 ± 20.12 sec) during a repeated bout of exercise following CHO-P (0.8 g/kg bm CHO + 0.4 g/kg bm PRO) consumption. Consumption of the CHO (1.2 g/kg bm) beverage attenuated the decrement in subsequent TTE performance (137.41 ± 18.90 sec) but was not significantly different from the PLA group (111.37 ± 22.01). In the current study, TTE performance in the second bout of exercise improved for both the CHO (295.48 ± 24.90) and CHO-P (255.08 ± 25.07 sec) groups. The water and electrolyte solution was not sufficient for restoring TTE performance in the PLA group (171.13 ± 23.71 sec). Therefore, in the current study, the consumption of a CHO and CHO-P beverage resulted in improved TTE performance by 30% and 19%, respectively. Whereas TTE performance declined by 20% in the PLA condition.

On an individual level, sixty-three percent (5 of 8) of participants in the PLA group demonstrated a decline in TTE performance averaging a -68 sec change from the first to second bout of exercise, with a range of -16 to -185 sec. In comparison, only one of seven participants in the CHO group had an 18-sec decrease in TTE performance in the subsequent exercise bout, with the range of *improvement* between 48 to 190 sec. Results were similar for the CHO-P group where one participant cycled 74 sec in both the first and second bout of exercise, and all other performance times *increased* between 27 and 101 sec. The individual participant response is valuable for understanding the potential translation of findings from laboratory to the field (Close et al., 2019).

The MCAs who completed this study identified with a specific sport (i.e., primarily cycling but also running), which is in contrast to the heterogenous sample of young college-aged adults in our previously published research (Goldstein et al., 2022). In comparison, the sample of young adults in that investigation participated in numerous modes of exercise at a moderate-to-vigorous intensity (Goldstein et al., 2022). The homogenous sample of MCAs actively engaged in endurance sport that requires periods of sustained power output may partly explain the difference in results such that in the current investigation both CHO and CHO-P treatments promoted an increase in TTE performance.

Three previously published studies that have examined post-exercise isocaloric CHO (1.2 g/kg bm) and CHO-P (0.8 g/kg bm CHO + 0.4 g/kg PRO) and recovery from exhaustive exercise in young endurance-trained males have demonstrated that glycogen resynthesis postexercise is not necessarily the primary determinant of endurance-based performance outcomes (Alghannam et al., 2016; J. Berardi et al., 2006; Dahl et al., 2020). For example, Berardi et al. (2006) reported no group differences in a subsequent time trial performance even though the amount of muscle glycogen resynthesized in the vastus lateralis was significantly greater with the CHO-P condition as compared to both CHO and PLA. Alghannam et al. (2016) reported a similar increase in muscle glycogen concentration with isocaloric CHO-P and CHO drinks and a 4-hr recovery period, with no significant difference in performance when participants performed a second run to exhaustion (Alghannam et al., 2016).

The experimental protocol used in the present study was adapted from McCarthy and Spriet (2020), who estimated muscle glycogen depletion to be between approximately 55% to 65% following the first bout of high-intensity aerobic intervals and time to exhaustion. While glycogen synthesis was not measured in the present study, results were similar to the performance outcomes reported for Berardi et al. (2006) and Alghannam et al. (2016). TTE at 90% PPO, while significantly improved during a subsequent bout of exercise relative to PLA, was not different between the CHO and CHO-P groups. Therefore, while CHO-P was not superior to CHO for improving acute TTE performance during a subsequent bout of high-intensity exercise, the addition of protein to a recovery beverage may have longer term benefits. Doering et al. (2016) reported that a group of masters triathletes consumed 0.3 ± 0.2 g/kg bm of protein in the post-exercise recovery meal, which is slightly less than the recommended amount of 0.4 g/kg bm that has been found to maximally stimulate muscle protein synthesis in older adults (Moore et al., 2015). In support, Churchward-Venne et al. (2020) reported 30 g of protein (0.49 g protein/kg) co-ingested with 45 g of carbohydrate was found to maximally stimulate rates of myofibrillar protein synthesis in young adult males (27 ± 1 years) following 90 min of continuous cycling at 60% of maximal workload capacity. In the current study, the addition of whey protein to the CHO-P recovery beverage in the amount of 0.4 g/kg bm was approximately 32 ± 2.5 g, which is in line with current recommendations for post-exercise nutrient intake (Churchward-Venne et al., 2020; Moore et al., 2015).

Additionally, Churchward-Venne et al. (2020) reported whole-body net protein balance was *negative* when participants consumed a carbohydrate-only beverage (45 g). A *negative* nitrogen balance with a carbohydrate-only beverage was also a primary finding for both Rustad et al. (2016) and Dahl et al. (2020) when endurance-trained participants consumed energy matched recovery drinks and performed repeated bouts of high-intensity exercise and time to exhaustion cycling. Conversely, consumption of the CHO-P beverage resulted in a *positive* nitrogen balance in addition to improved TTE performance (Dahl et al., 2020; Rustad et al., 2016). While the current study examined the acute response of CHO-P ingestion, additional benefits of chronic protein supplementation and endurance training have been reported. These benefits include: upregulation of mechanistic target of rapamycin which may have important implications for skeletal muscle protein synthesis in response to endurance training (i.e., repair of acutely damaged proteins) (Alghannam et al., 2020) and favorable changes in body composition such as an increase in lean body mass and a decrease in fat mass (Knuiman et al., 2019; Lin et al., 2021).

The current investigation utilized a novel index to examine HRR relative to total work. Results indicated an improved recovery for both the CHO and CHO-P conditions at each of the three time points postexercise, with no difference between conditions. In comparison, the PLA condition was inadequate for promoting HRR at 1-, 2-, and 5-min postexercise as evidenced by a significantly higher HRRi value. The findings of an improved recovery for the CHO and CHO-P conditions are similar to the findings of Moreno and colleagues (2013) and others (Vanderlei et al., 2015). In the study of Moreno et al., (2012), consumption of 1.4 ± 0.5 L of an isotonic solution (i.e., Gatorade®) during 90 min of exercise at 60% VO_2peak and throughout the 60 min recovery period enhanced recovery of parasympathetic activity by 25 min of exercise cessation

(Moreno et al., 2013). In the current study, the average change in body mass as a result of fluid loss during the first bout of aerobic intervals and TTE for all participants was 0.7 ± 0.2 kg. Weight either approached or returned to baseline for all participants following consumption of 1.1 ± 0.2 liters of fluid during the 2-hour recovery period. In support, Moreno et al. (2013) reported lack of fluid intake during exercise and recovery resulted in approximately 2.0 ± 0.6% loss of body weight, as compared to -0.2 ± 0.7% with the recovery beverage. Consequently, indices of vagal activity as assessed by heart rate variability did not fully recover by the end of the 60-min recovery period when participants received no fluid intake (Moreno et al., 2013).

Even though in the current study PLA provided similar amounts of water and electrolytes it is possible that HRR may not be a suitable indicator for assessing recovery of parasympathetic activity either with fluid alone (i.e., water), within the first 5 min of exercise cessation, or following high-intensity exercise (Borges et al., 2017; Peçanha, Paula-Ribeiro, et al., 2014). Additionally, a delay in parasympathetic reactivation (Buchheit et al., 2007) and sympathetic withdrawal (Michael et al., 2017) during the first 10 min of recovery from high-intensity exercise has been demonstrated following repeated sprints (15-m sprints with 17s passive recovery) and continuous exercise at 90%-95% of heart rate reserve. It is possible that HRR becomes a more sensitive indicator when calculated relative to total work (HRRi) and when the HRRi is used in conjunction with high-intensity exercise, though additional research is warranted to support these findings. Therefore, the high-intensity aerobic interval and TTE protocol (20 min at 70-80% PPO plus TTE at 90% of PPO) employed in this study may necessitate caloric intake in the form of rapidly digestible CHO or CHO-P intake during a 2-hour recovery period to maximize HRR post exhaustive exercise, or the postexercise recovery period may need to extend beyond five min to fully evaluate HRR from exhaustive exercise.

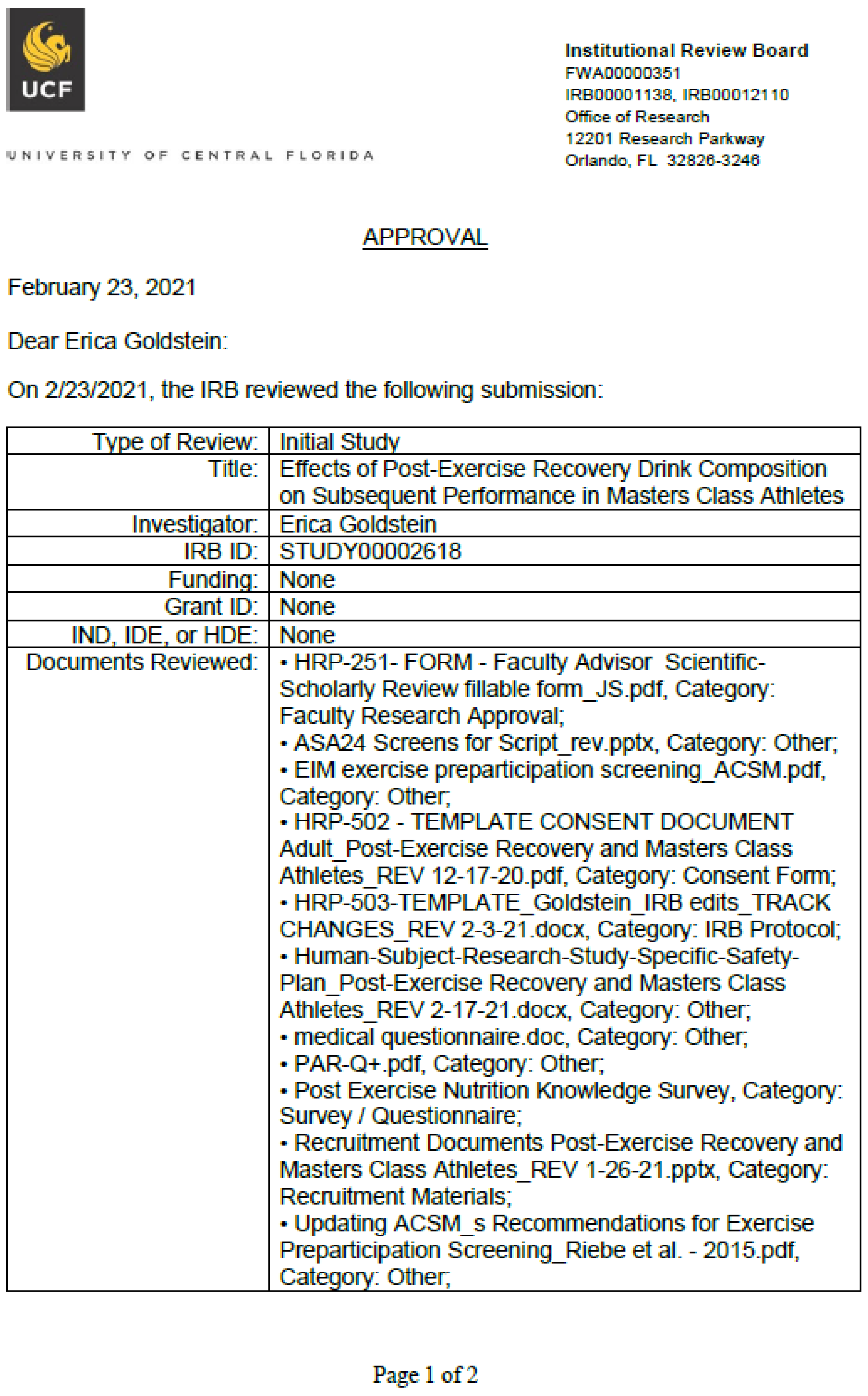
Approximately 30% of a sample of Australian masters triathletes correctly answered 1.0-1.2 g/kg bm as the amount of CHO that should be included in a postexercise meal (or snack) to optimize recovery, which is in contrast to the highly variable response of 7.9 ± 6.8 g/kg bm cited by the group of older athletes surveyed in this study (Doering, Reaburn, Cox, et al., 2016). Twenty-five percent of the Australian masters athletes correctly identified 20-25 g as the recommended amount of protein to optimize recovery in the postexercise meal/snack, in comparison to the average response of 28.9 ± 13.9 g of protein in the current study. *Own previous knowledge* was the most frequently cited response when asked about primary source of postexercise nutrition for recovery for both young and older Australian triathletes (17.3%) and the present sample of MCAs (27.8%; 5/18). Accredited sports dietitian was the second most frequently cited (12.7%) source of information by Australian athletes, however, none of the MCAs in this study reported dietitian as a source of information. According to Doering et al. (2016) Triathlon magazines (11.8%) and friends/teammates (10.0%) were the next most frequently cited sources of information regarding postexercise nutrition for recovery. Results were similar for the current study as friends/teammates (16.7%; 3/18) and triathlon/cycling/running magazines (9.6%; 2/21) were frequently cited sources of postexercise nutrition for recovery. Knowledge of postexercise nutrition for recovery is lacking, especially in regard to carbohydrate and protein needs, according to results of two different groups of masters endurance athletes.

There are several limitations to this study, including poor compliance in completing the electronic 24-hour dietary assessment. In addition, dietary intake was not standardized among participants prior to the experimental protocol; however, statistical analysis revealed no significant differences in energy intake or macronutrient distribution between the three treatment groups for those athletes that did comply. We did not standardize dietary intake on the day of the experimental protocol nor was the high-intensity exercise examined in a fasted state. We also did not quantify or restrict caffeine intake 24 hours prior to testing. The goal of this investigation was to examine recovery from high-intensity exercise in an applied manner which allowed for exercise testing without a drastic change to the participant's habitual diet or nutritional pre-exercise practice (Close et al., 2019). Alternatively, participants were instructed to keep their dietary intake consistent on both the day of familiarization and the experimental protocol. Additionally, it can be argued that restricting caffeine intake in a habitual user may unnecessarily interfere with acute testing outcomes and may also lack application in an applied setting. It has been suggested that caffeine consumption following an acute period of withdrawal functions to relieve the negative symptoms of caffeine abstinence thereby positively influencing performance (Guest et al., 2021). Due to the exhaustive nature of the high-intensity exercise protocol many participants chose to terminate the cool down period prior to 5 min. Future research using the high-intensity aerobic interval and TTE protocol employed in this study may consider a standardized cool down following TTE testing (i.e., mandatory 3-5 min of self-paced cycling without resistance) followed by 15-30 min in the seated or supine position for assessment of HRR.

This is the first study to examine the effect of an isocaloric CHO vs CHO-P drink on performance during a repeated bout of exhaustive exercise following a 2-hour recovery period in masters class endurance athletes. The primary novel outcome of this study was that CHO-P was equally effective as CHO for enhancing performance in a subsequent bout of high-intensity exercise with a limited time to recover. The results of the current study along with prior research findings can be used to inform postexercise nutritional recovery practices, especially considering

a) CHO-P co-ingestion has not been found to hinder recovery from repeated exhaustive exercise b) the inclusion of protein to a recovery drink has been shown to support recovery in an important physiologic manner other than glycogen synthesis and c) knowledge of post-exercise recovery practices including protein intake appears to be lacking in masters class endurance athletes.

APPENDIX:
IRB APPROVAL LETTER



UNIVERSITY OF CENTRAL FLORIDA

Institutional Review Board
FWA00000351
IRB00001138, IRB00012110
Office of Research
12201 Research Parkway
Orlando, FL 32826-3246

APPROVAL

February 23, 2021

Dear Erica Goldstein:

On 2/23/2021, the IRB reviewed the following submission:

Type of Review:	Initial Study
Title:	Effects of Post-Exercise Recovery Drink Composition on Subsequent Performance in Masters Class Athletes
Investigator:	Erica Goldstein
IRB ID:	STUDY00002618
Funding:	None
Grant ID:	None
IND, IDE, or HDE:	None
Documents Reviewed:	• HRP-251- FORM - Faculty Advisor Scientific-Scholarly Review fillable form_JS.pdf, Category: Faculty Research Approval; • ASA24 Screens for Script_rev.pptx, Category: Other; • EIM exercise preparticipation screening_ACSM.pdf, Category: Other; • HRP-502 - TEMPLATE CONSENT DOCUMENT Adult_Post-Exercise Recovery and Masters Class Athletes_REV 12-17-20.pdf, Category: Consent Form; • HRP-503-TEMPLATE_Goldstein_IRB edits_TRACK CHANGES_REV 2-3-21.docx, Category: IRB Protocol; • Human-Subject-Research-Study-Specific-Safety-Plan_Post-Exercise Recovery and Masters Class Athletes_REV 2-17-21.docx, Category: Other; • medical questionnaire.doc, Category: Other; • PAR-Q+.pdf, Category: Other; • Post Exercise Nutrition Knowledge Survey, Category: Survey / Questionnaire; • Recruitment Documents Post-Exercise Recovery and Masters Class Athletes_REV 1-26-21.pptx, Category: Recruitment Materials; • Updating ACSM_s Recommendations for Exercise Preparticipation Screening_Riebe et al. - 2015.pdf, Category: Other;

The IRB approved the protocol on 2/23/2021.

In conducting this protocol, you are required to follow the requirements listed in the Investigator Manual (HRP-103), which can be found by navigating to the IRB Library within the IRB system. Guidance on submitting Modifications and a Continuing Review or Administrative Check-in are detailed in the manual. When you have completed your research, please submit a Study Closure request so that IRB records will be accurate.

If you have any questions, please contact the UCF IRB at 407-823-2901 or irb@ucf.edu. Please include your project title and IRB number in all correspondence with this office.

Sincerely,

Racine Jacques, Ph.D.
Designated Reviewer

REFERENCES

Adabag, S., & Pierpont, G. L. (2013). Exercise heart rate recovery: Analysis of methods and call for standards. *Heart*, *99*(23), 1711–1712. https://doi.org/10.1136/heartjnl-2013-303863

Alghannam, A. F., Jedrzejewski, D., Bilzon, J., Thompson, D., Tsintzas, K., & Betts, J. A. (2016). Influence of Post-Exercise Carbohydrate-Protein Ingestion on Muscle Glycogen Metabolism in Recovery and Subsequent Running Exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(6), 572–580. https://doi.org/10.1123/ijsnem.2016-0021

Alghannam, A. F., Templeman, I., Thomas, J. E., Jedrzejewski, D., Griffiths, S., Lemon, J., Byers, T., Reeves, S., Gonzalez, J. T., Thompson, D., Bilzon, J., Tsintzas, K., & Betts, J. A. (2020). Effect of carbohydrate–protein supplementation on endurance training adaptations. *European Journal of Applied Physiology*, *120*(10), 2273–2287. https://doi.org/10.1007/s00421-020-04450-1

Areta, J. L., Burke, L. M., Camera, D. M., West, D. W. D., Crawshay, S., Moore, D. R., Stellingwerff, T., Phillips, S. M., Hawley, J. A., & Coffey, V. G. (2014). Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *American Journal of Physiology-Endocrinology and Metabolism*, *306*(8), E989–E997. https://doi.org/10.1152/ajpendo.00590.2013

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., Broad, E. M., Jeacocke, N. A., Moore, D. R., Stellingwerff, T., Phillips, S. M., Hawley, J. A., & Coffey, V. G. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of Physiology*, *591*(9), 2319–2331. https://doi.org/10.1113/jphysiol.2012.244897

Areta, J. L., & Hopkins, W. G. (2018). Skeletal Muscle Glycogen Content at Rest and During Endurance Exercise in Humans: A Meta-Analysis. *Sports Medicine*, *48*(9), 2091–2102. https://doi.org/10.1007/s40279-018-0941-1

Berardi, J. M., Noreen, E. E., & Lemon, P. W. (2008). Recovery from a cycling time trial is enhanced with carbohydrate-protein supplementation vs. Isoenergetic carbohydrate supplementation. *Journal of the International Society of Sports Nutrition*, *5*(1), 24. https://doi.org/10.1186/1550-2783-5-24

Berardi, J., Price, T., Noreen, E., & Lemon, P. (2006). Postexercise Muscle Glycogen Recovery Enhanced with a Carbohydrate-Protein Supplement. *Medicine & Science in Sports & Exercise*, *38*(6), 1106–1113. https://doi.org/10.1249/01.mss.0000222826.49358.f3

Bergström, J., & Hultman, E. (1967). A Study of the Glycogen Metabolism during Exercise in Man. *Scandinavian Journal of Clinical and Laboratory Investigation*, *19*(3), 218–228. https://doi.org/10.3109/00365516709090629

Betts, J. A., & Williams, C. (2010). Short-Term Recovery from Prolonged Exercise. *Sports Medicine*, *40*(11), 941–959. https://doi.org/10.2165/11536900-000000000-00000

Borges, N. R., Reaburn, P. R., Doering, T. M., Argus, C. K., & Driller, M. W. (2017). Autonomic cardiovascular modulation in masters and young cyclists following high-intensity interval training. *Clinical Autonomic Research*, *27*(2), 83–90. https://doi.org/10.1007/s10286-017-0398-6

Breen, L., Philp, A., Witard, O. C., Jackman, S. R., Selby, A., Smith, K., Baar, K., & Tipton, K. D. (2011). The influence of carbohydrate–protein co-ingestion following endurance exercise on myofibrillar and mitochondrial protein synthesis. *The Journal of Physiology*, *589*(16), 4011–4025. https://doi.org/10.1113/jphysiol.2011.211888

Buchheit, M., Laursen, P. B., & Ahmaidi, S. (2007). Parasympathetic reactivation after repeated sprint exercise. *American Journal of Physiology-Heart and Circulatory Physiology*, *293*(1), H133–H141. https://doi.org/10.1152/ajpheart.00062.2007

Burke, L. M., Cox, G. R., Cummings, N. K., & Desbrow, B. (2001). Guidelines for Daily Carbohydrate Intake: Do Athletes Achieve Them? *Sports Medicine*, *31*(4), 267–299. https://doi.org/10.2165/00007256-200131040-00003

Burke, L. M., Slater, G., Broad, E. M., Haukka, J., Modulon, S., & Hopkins, W. G. (2003). Eating Patterns and Meal Frequency of Elite Australian Athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *13*(4), 521–538. https://doi.org/10.1123/ijsnem.13.4.521

Burke, L. M., van Loon, L. J. C., & Hawley, J. A. (2017). Postexercise muscle glycogen resynthesis in humans. *Journal of Applied Physiology*, *122*(5), 1055–1067. https://doi.org/10.1152/japplphysiol.00860.2016

Casa, D. (2019, August 11). *Hydration*. Korey Stringer Institute. https://ksi.uconn.edu/prevention/hydration/

Churchward-Venne, T. A., Pinckaers, P. J. M., Smeets, J. S. J., Betz, M. W., Senden, J. M., Goessens, J. P. B., Gijsen, A. P., Rollo, I., Verdijk, L. B., & van Loon, L. J. C. (2020). Dose-response effects of dietary protein on muscle protein synthesis during recovery from endurance exercise in young men: A double-blind randomized trial. *The American Journal of Clinical Nutrition*, *112*(2), 303–317. https://doi.org/10.1093/ajcn/nqaa073

Cintineo, H. P., Arent, M. A., Antonio, J., & Arent, S. M. (2018). Effects of Protein Supplementation on Performance and Recovery in Resistance and Endurance Training. *Frontiers in Nutrition*, *5*. https://doi.org/10.3389/fnut.2018.00083

Close, G. L., Kasper, A. M., & Morton, J. P. (2019). From Paper to Podium: Quantifying the Translational Potential of Performance Nutrition Research. *Sports Medicine*, *49*(S1), 25–37. https://doi.org/10.1007/s40279-018-1005-2

Dahl, M. A., Areta, J. L., Jeppesen, P. B., Birk, J. B., Johansen, E. I., Ingemann-Hansen, T., Hansen, M., Skålhegg, B. S., Ivy, J. L., Wojtaszewski, J. F. P., Overgaard, K., & Jensen, J. (2020). Co-ingestion of protein and carbohydrate in the early recovery phase improves endurance performance despite like glycogen degradation and AMPK phosphorylation. *Journal of Applied Physiology*. https://doi.org/10.1152/japplphysiol.00817.2019

Desbrow, B., Burd, N. A., Tarnopolsky, M., Moore, D. R., & Elliott-Sale, K. J. (2019). Nutrition for Special Populations: Young, Female, and Masters Athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *29*(2), 220–227. https://doi.org/10.1123/ijsnem.2018-0269

Deus, L. A., Sousa, C. V., Rosa, T. S., Filho, J. M. S., Santos, P. A., Barbosa, L. D., Silva Aguiar, S., Souza, L. H. R., & Simões, H. G. (2019). Heart rate variability in middle-aged sprint and endurance athletes. *Physiology & Behavior*, *205*, 39–43. https://doi.org/10.1016/j.physbeh.2018.10.018

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 & Science in Sports & Exercise*, *48*(8), 1613–1618. https://doi.org/10.1249/MSS.0000000000000935

Doering, T. M., Reaburn, P. R., Borges, N. R., Cox, G. R., & Jenkins, D. G. (2017). 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*, *27*(1), 76–82. https://doi.org/10.1123/ijsnem.2016-0079

Doering, T. M., Reaburn, P. R., Cox, G., & Jenkins, D. G. (2016). Comparison of Postexercise Nutrition Knowledge and Postexercise Carbohydrate and Protein Intake between Australian Masters and Younger Triathletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(4), 338–346. https://doi.org/10.1123/ijsnem.2015-0289

Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Postexercise 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. https://doi.org/10.1123/ijsnem.2015-0102

Dubé, J. J., Broskey, N. T., Despines, A. A., Stefanovic-Racic, M., Toledo, F. G. S., Goodpaster, B. H., & Amati, F. (2016). Muscle Characteristics and Substrate Energetics in Lifelong Endurance Athletes. *Medicine & Science in Sports & Exercise*, *48*(3), 472–480. https://doi.org/10.1249/MSS.0000000000000789

Easthope, C. S., Hausswirth, C., Louis, J., Lepers, R., Vercruyssen, F., & Brisswalter, J. (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. *European Journal of Applied Physiology*, *110*(6), 1107–1116. https://doi.org/10.1007/s00421-010-1597-1

Fell, J., & Williams, A. D. (2008). The Effect of Aging on Skeletal-Muscle Recovery From Exercise: Possible Implications for Aging Athletes. *Journal of Aging and Physical Activity*, *16*, 97–115.

Fien, S., Climstein, M., Quilter, C., Buckley, G., Henwood, T., Grigg, J., & Keogh, J. W. L. (2017). Anthropometric, physical function and general health markers of Masters athletes: A cross-sectional study. *PeerJ*, *5*, e3768. https://doi.org/10.7717/peerj.3768

Goldstein, E., Stout, J., Starling-Smith, T., & Fukuda, D. (2022). Carbohydrate-Protein Coingestion Enhances Cycling Performance with Minimal Recovery Time between Bouts of Exhaustive

Intermittent Exercise. *Journal of Exercise and Nutrition*, *5*(2). https://doi.org/10.53520/jen2022.103125

Green, S. B., Salkind, N. J., & Akey, T. M. (2000). *Using SPSS for Windows; Analyzing and Understanding Data* (2nd ed.). Prentice Hall PTR.

Guest, N. S., VanDusseldorp, T. A., Nelson, M. T., Grgic, J., Schoenfeld, B. J., Jenkins, N. D. M., Arent, S. M., Antonio, J., Stout, J. R., Trexler, E. T., Smith-Ryan, A. E., Goldstein, E. R., Kalman, D. S., & Campbell, B. I. (2021). International society of sports nutrition position stand: Caffeine and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), 1. https://doi.org/10.1186/s12970-020-00383-4

Guthrie, S. K., & Erickson, S. R. (2016). Masters Swimmers Use More Dietary Supplements Than a Large National Comparison Population in the United States. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(2), 123–127. https://doi.org/10.1123/ijsnem.2015-0043

Hausswirth, C., & Brisswalter, J. (2008). Strategies for Improving Performance in Long Duration Events: Olympic Distance Triathlon. *Sports Medicine (Auckland, N.Z.)*, *38*, 881–891. https://doi.org/10.2165/00007256-200838110-00001

Heikura, I. A., Burke, L. M., Mero, A. A., Uusitalo, A. L. T., & Stellingwerff, T. (2017). Dietary Microperiodization in Elite Female and Male Runners and Race Walkers During a Block of High Intensity Precompetition Training. *International Journal of Sport Nutrition and Exercise Metabolism*, *27*(4), 297–304. https://doi.org/10.1123/ijsnem.2016-0317

Hermansen, L., Hultman, E., & Saltin, B. (1967). Muscle Glycogen during Prolonged Severe Exercise. *Acta Physiologica Scandinavica*, *71*(2–3), 129–139. https://doi.org/10.1111/j.1748-1716.1967.tb03719.x

Howarth, K. R., Moreau, N. A., Phillips, S. M., & Gibala, M. J. (2009). Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology*, *106*(4), 1394–1402. https://doi.org/10.1152/japplphysiol.90333.2008

Ivy, J. L., Katz, A. L., Cutler, C. L., Sherman, W. M., & Coyle, E. F. (1988). Muscle glycogen synthesis after exercise: Effect of time of carbohydrate ingestion. *Journal of Applied Physiology*, *64*(4), 1480–1485. https://doi.org/10.1152/jappl.1988.64.4.1480

Ivy, J. L., & Kuo, C.-H. (1998). Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise. *Acta Physiologica Scandinavica*, *162*(3), 295–304.

Ivy, J. L., Lee, M. C., Brozinick, J. T., & Reed, M. J. (1988). Muscle glycogen storage after different amounts of carbohydrate ingestion. *Journal of Applied Physiology*, *65*(5), 2018–2023. https://doi.org/10.1152/jappl.1988.65.5.2018

Jentjens, R., & Jeukendrup, A. E. (2003). Determinants of Post-Exercise Glycogen Synthesis During Short-Term Recovery. *Sports Medicine*, *33*(2), 117–144. https://doi.org/10.2165/00007256-200333020-00004

Jentjens, R. L. P. G., van Loon, L. J. C., Mann, C. H., Wagenmakers, A. J. M., & Jeukendrup, A. E. (2001). Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. *Journal of Applied Physiology*, *91*(2), 839–846. https://doi.org/10.1152/jappl.2001.91.2.839

Knechtle, B., Rüst, C. A., Rosemann, T., & Lepers, R. (2012). Age-related changes in 100-km ultra-marathon running performance. *Age (Dordr)*, *34*(4), 1033–1045. https://doi.org/10.1007/s11357-011-9290-9

Knuiman, P., van Loon, L. J. C., Wouters, J., Hopman, M., & Mensink, M. (2019). Protein supplementation elicits greater gains in maximal oxygen uptake capacity and stimulates lean mass accretion during prolonged endurance training: A double-blind randomized controlled trial. *The American Journal of Clinical Nutrition*, *110*(2), 508–518. https://doi.org/10.1093/ajcn/nqz093

Kruseman, M., Lecoultre, V., & Gremeaux, V. (2020). Nutrition for long-distance triathletes: Facts and myths. *Deutsche Zeitschrift Für Sportmedizin/German Journal of Sports Medicine*, *71*(10), 229–235. https://doi.org/10.5960/dzsm.2020.461

Lai, Y.-C., Zarrinpashneh, E., & Jensen, J. (2010). Additive effect of contraction and insulin on glucose uptake and glycogen synthase in muscle with different glycogen contents. *Journal of Applied Physiology*, *108*(5), 1106–1115. https://doi.org/10.1152/japplphysiol.00401.2009

Lepers, R., & Cattagni, T. (2012). Do older athletes reach limits in their performance during marathon running? *Age*, *34*(3), 773–781. https://doi.org/10.1007/s11357-011-9271-z

Lepers, R., Rüst, C. A., Stapley, P. J., & Knechtle, B. (2013). Relative improvements in endurance performance with age: Evidence from 25 years of Hawaii Ironman racing. *Age*, *35*(3), 953–962. https://doi.org/10.1007/s11357-012-9392-z

Lepers, R., & Stapley, P. J. (2016). Master Athletes Are Extending the Limits of Human Endurance. *Frontiers in Physiology*, *7*. https://doi.org/10.3389/fphys.2016.00613

Levenhagen, D. K., Gresham, J. D., Carlson, M. G., Maron, D. J., Borel, M. J., & Flakoll, P. J. (2001). Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *American Journal of Physiology-Endocrinology and Metabolism*, *280*(6), E982–E993. https://doi.org/10.1152/ajpendo.2001.280.6.E982

Lin, Y.-N., Tseng, T.-T., Knuiman, P., Chan, W. P., Wu, S.-H., Tsai, C.-L., & Hsu, C.-Y. (2021). Protein supplementation increases adaptations to endurance training: A systematic review and meta-analysis. *Clinical Nutrition*, *40*(5), 3123–3132. https://doi.org/10.1016/j.clnu.2020.12.012

Louis, J., Hausswirth, C., Easthope, C., & Brisswalter, J. (2012). Strength training improves cycling efficiency in master endurance athletes. *European Journal of Applied Physiology*, *112*(2), 631–640. https://doi.org/10.1007/s00421-011-2013-1

Louis, J., Vercruyssen, F., Dupuy, O., & Bernard, T. (2019). Nutrition for Master Athletes: Is There a Need for Specific Recommendations? *Journal of Aging and Physical Activity*, *28*(3), 489–498. https://doi.org/10.1123/japa.2019-0190

Macgregor, C., Climstein, M., & Reaburn, P. R. (2013). The Effect of Age on Training Practices in Veteran Cyclists: An Australian Study. *Medicine and Science in Sports and Exercise*, *45*(5S), 85–85. https://doi.org/10.1249/01.mss.0000433622.74297.42

Magal, M., & Riebe, D. (2016). New preparticipation health screening recommendations: What exercise professionals need to know. *ACSM's Health & Fitness Journal*, *20*(3), 22–27. https://doi.org/10.1249/FIT.0000000000000202

Malone, J. J., Bassami, M., Waldron, S. C., Campbell, I. T., Hulton, A., Doran, D., & MacLaren, D. P. (2019). Carbohydrate oxidation and glucose utilisation under hyperglycaemia in aged and young males during exercise at the same relative exercise intensity. *European Journal of Applied Physiology*, *119*(1), 235–245. https://doi.org/10.1007/s00421-018-4019-4

McCarthy, D. G., & Spriet, L. L. (2020). Performance Effects of Carbohydrate Ingestion Between Bouts of Intense Aerobic Interval Exercise. *International Journal of Sports Physiology and Performance*, *15*(2), 262–267. https://doi.org/10.1123/ijspp.2019-0239

McLeman, L. A., Ratcliffe, K., & Clifford, T. (2019). Pre- and post-exercise nutritional practices of amateur runners in the UK: Are they meeting the guidelines for optimal carbohydrate and protein intakes? *Sport Sciences for Health*, *15*(3), 511–517. https://doi.org/10.1007/s11332-019-00537-1

Meredith, C. N., Zackin, M. J., Frontera, W. R., & Evans, W. J. (1989). Dietary protein requirements and body protein metabolism in endurance-trained men. *Journal of Applied Physiology*, *66*(6), 2850–2856. https://doi.org/10.1152/jappl.1989.66.6.2850

Michael, S., Jay, O., Graham, K. S., & Davis, G. M. (2017). Higher exercise intensity delays postexercise recovery of impedance-derived cardiac sympathetic activity. *Applied Physiology, Nutrition & Metabolism*, *42*(8), 834–840. https://doi.org/10.1139/apnm-2017-0049

Moore, D. R., Camera, D. M., Areta, J. L., & Hawley, J. A. (2014). Beyond muscle hypertrophy: Why dietary protein is important for endurance athletes. *Applied Physiology, Nutrition, and Metabolism*, *9*, 987. https://doi.org/10.1139/apnm-2013-0591

Moore, D. R., Churchward-Venne, T. A., Witard, O., Breen, L., Burd, N. A., Tipton, K. D., & Phillips, S. M. (2015). Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men. *The Journals of Gerontology: Series A*, *70*(1), 57–62. https://doi.org/10.1093/gerona/glu103

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., Prior, T., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *The American Journal of Clinical Nutrition*, *89*(1), 161–168. https://doi.org/10.3945/ajcn.2008.26401

Moreno, I. L., Pastre, C. M., Ferreira, C., de Abreu, L. C., Valenti, V. E., & Vanderlei, L. C. M. (2013). Effects of an isotonic beverage on autonomic regulation during and after exercise. *Journal of the International Society of Sports Nutrition*, *10*(1), 2. https://doi.org/10.1186/1550-2783-10-2

Morton, R. W., Traylor, D. A., Weijs, P. J. M., & Phillips, S. M. (2018). Defining anabolic resistance: Implications for delivery of clinical care nutrition. *Current Opinion in Critical Care*, *24*(2), 124–130. https://doi.org/10.1097/MCC.0000000000000488

Murray, B., & Rosenbloom, C. (2018). Fundamentals of glycogen metabolism for coaches and athletes. *Nutrition Reviews*, *76*(4), 243–259. https://doi.org/10.1093/nutrit/nuy001

O'Brien, M. J., Viguie, C. A., Mazzeo, R. S., & Brooks, G. A. (1993). Carbohydrate dependence during marathon running. *Medicine and Science in Sports and Exercise*, *25*(9), 1009–1017.

Park, Y., Dodd, K. W., Kipnis, V., Thompson, F. E., Potischman, N., Schoeller, D. A., Baer, D. J., Midthune, D., Troiano, R. P., Bowles, H., & Subar, A. F. (2018). Comparison of self-reported dietary intakes from the Automated Self-Administered 24-h recall, 4-d food records, and food-frequency questionnaires against recovery biomarkers. *The American Journal of Clinical Nutrition*, *107*(1), 80–93. https://doi.org/10.1093/ajcn/nqx002

Peçanha, T., Paula-Ribeiro, M., Campana-Rezende, E., Bartels, R., Marins, J. C. B., & Lima, J. R. P. de. (2014). Water Intake Accelerates Parasympathetic Reactivation After High-Intensity Exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, *24*(5), 489–496. https://doi.org/10.1123/ijsnem.2013-0122

Peçanha, T., Silva-Júnior, N. D., & Forjaz, C. L. de M. (2014). Heart rate recovery: Autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clinical Physiology and Functional Imaging*, *34*(5), 327–339. https://doi.org/10.1111/cpf.12102

Peiffer, J. J., Abbiss, C. R., Chapman, D., Laursen, P. B., & Parker, D. L. (2008). Physiological Characteristics of Masters-Level Cyclists. *Journal of Strength and Conditioning Research*, *22*(5), 1434–1440. https://doi.org/10.1519/JSC.0b013e318181a0d2

Power, G. A., Dalton, B. H., Behm, D. G., Vandervoort, A. A., Doherty, T. J., & Rice, C. L. (2010). Motor Unit Number Estimates in Masters Runners: Use It or Lose It? *Medicine & Science in Sports & Exercise*, *42*(9), 1644–1650. https://doi.org/10.1249/MSS.0b013e3181d6f9e9

Ransdell, L. B., Vener, J., & Huberty, J. (2009). Masters Athletes: An Analysis of Running, Swimming and Cycling Performance by Age and Gender. *Journal of Exercise Science & Fitness*, *7*(2, Supplement), S61–S73. https://doi.org/10.1016/S1728-869X(09)60024-1

Reaburn, P. R., Doering, T., & Borges, N. R. (2015). Nutrition Issues for the Masters Athlete. In *Clinical Sports Nutrition* (5 edition). McGraw-Hill Education / Australia.

Reaburn, P. R., Macgregor, C., & Climstein, M. (2013). Poor use of post-exercise recovery strategies in veteran cyclists: An Australian study. *Medicine and Science in Sports and Exercise*, *45*(5S), 85–85. https://doi.org/10.1249/01.mss.0000433622.74297.42

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993a). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism*, *265*(3), E380–E391. https://doi.org/10.1152/ajpendo.1993.265.3.E380

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993b). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism*, *265*(3), E380–E391. https://doi.org/10.1152/ajpendo.1993.265.3.E380

Rowlands, D. S., Nelson, A. R., Phillips, S. M., Faulkner, J. A., Clarke, J., Burd, N. A., Moore, D., & Stellingwerff, T. (2015). Protein–Leucine Fed Dose Effects on Muscle Protein Synthesis after Endurance Exercise. *Medicine & Science in Sports & Exercise*, *47*(3), 547–555. https://doi.org/10.1249/MSS.0000000000000447

Rustad, P. I., Sailer, M., Cumming, K. T., Jeppesen, P. B., Kolnes, K. J., Sollie, O., Franch, J., Ivy, J. L., Daniel, H., & Jensen, J. (2016). Intake of Protein Plus Carbohydrate during the First Two Hours after Exhaustive Cycling Improves Performance the following Day. *PLOS ONE*, *11*(4), e0153229. https://doi.org/10.1371/journal.pone.0153229

Sawka, M. N. (1992). Physiological consequences of hypohydration: Exercise performance and thermoregulation. *Medicine & Science in Sports & Exercise*, *24*(6), 657–670.

Sparks, I. M., Rensburg, D. C. J. van, Fletcher, L., & Rensburg, A. J. van. (2018). A cross-sectional study of 2550 amateur cyclists shows lack of knowledge regarding relevant sports nutrition guidelines. *South African Journal of Sports Medicine*, *30*(1), Article 1. https://www.ajol.info/index.php/sasma/article/view/170610

Spriet, L. L. (2007). Regulation of Substrate Use During the Marathon: *Sports Medicine*, *37*(4), 332–336. https://doi.org/10.2165/00007256-200737040-00015

Sterkowicz-Przybycień, K., Fukuda, D. H., & Franchini, E. (2019). Meta-Analysis to Determine Normative Values for the Special Judo Fitness Test in Male Athletes: 20+ Years of Sport-Specific Data and the Lasting Legacy of Stanisław Sterkowicz. *Sports*, *7*(8), 194. https://doi.org/10.3390/sports7080194

Stiefel, M., Knechtle, B., & Lepers, R. (2014). Master triathletes have not reached limits in their Ironman triathlon performance: Master triathletes improved their performance. *Scandinavian Journal of Medicine & Science in Sports*, *24*(1), 89–97. https://doi.org/10.1111/j.1600-0838.2012.01473.x

Sultana, F., Abbiss, C. R., Louis, J., Bernard, T., Hausswirth, C., & Brisswalter, J. (2012). Age-related changes in cardio-respiratory responses and muscular performance following an Olympic triathlon in well-trained triathletes. *European Journal of Applied Physiology*, *112*(4), 1549–1556. https://doi.org/10.1007/s00421-011-2115-9

Suzic Lazic, J., Dekleva, M., Soldatovic, I., Leischik, R., Suzic, S., Radovanovic, D., Djuric, B., Nesic, D., Lazic, M., & Mazic, S. (2017). Heart rate recovery in elite athletes: The impact of age and exercise capacity. *Clinical Physiology and Functional Imaging*, *37*(2), 117–123. https://doi.org/10.1111/cpf.12271

Tanaka, H., & Seals, D. R. (2008). Endurance exercise performance in Masters athletes: Age-associated changes and underlying physiological mechanisms. *The Journal of Physiology*, *586*(1), 55–63. https://doi.org/10.1113/jphysiol.2007.141879

Tsintzas, O. K., Williams, C., Boobis, L., & Greenhaff, P. (1995, November 15). *Carbohydrate ingestion and glycogen utilization in different muscle fibre types in man.* The Journal of Physiology. https://doi.org/10.1113/jphysiol.1995.sp021046

Tsintzas, O. K., Williams, C., Boobis, L., & Greenhaff, P. (1996). Carbohydrate ingestion and single muscle fiber glycogen metabolism during prolonged running in men. *Journal of Applied Physiology*, *81*(2), 801–809. https://doi.org/10.1152/jappl.1996.81.2.801

Urbaniak, G. C., & Plous, S. (2013). *Research Randomizer (Version 4.0) [Computer software]*. Research Randomizer. http://www.randomizer.org/

van Loon, L. J., Saris, W. H., Kruijshoop, M., & Wagenmakers, A. J. (2000a). Maximizing postexercise muscle glycogen synthesis: Carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *The American Journal of Clinical Nutrition*, *72*(1), 106–111. https://doi.org/10.1093/ajcn/72.1.106

van Loon, L. J., Saris, W. H., Kruijshoop, M., & Wagenmakers, A. J. (2000b). Maximizing postexercise muscle glycogen synthesis: Carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *The American Journal of Clinical Nutrition*, *72*(1), 106–111. https://doi.org/10.1093/ajcn/72.1.106

Vanderlei, F., Moreno, I., Vanderlei, L., Pastre, C., Abreu, L., & Ferreira, C. (2015). Comparison of the Effect of Hydration With Water or Isotonic Solution on the Recovery of Cardiac Autonomic Modulation. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(2), 145–153. https://doi.org/10.1123/ijsnem.2014-0004

Vitale, K., & Getzin, A. (2019). Nutrition and Supplement Update for the Endurance Athlete: Review and Recommendations. *Nutrients*, *11*(6), 1289. https://doi.org/10.3390/nu11061289

Wichi, R. B., De Angelis, K., Jones, L., & Irigoyen, M. C. (2009). A brief review of chronic exercise intervention to prevent autonomic nervous system changes during the aging process. *Clinics*, *64*(3), 253–258. https://doi.org/10.1590/S1807-59322009000300017

Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., & Tipton, K. D. (2014). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *The American Journal of Clinical Nutrition*, *99*(1), 86–95. https://doi.org/10.3945/ajcn.112.055517