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Post Exercise Ingestion of Plant vs Animal Protein Enhances Exercise Performance Outcomes Similarly

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POST EXERCISE INGESTION OF PLANT VS ANIMAL PROTEIN ENHANCES
EXERCISE PERFORMANCE OUTCOMES SIMILARLY

(Thesis format: Integrated Article)

by

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Abstract

Recently, the ingestion of a carbohydrate-protein supplement after exercise has garnered considerable interest among athletes and scientists. However, debate exists about whether the type of protein consumed, animal vs plant, affects exercise performance differently. Thus, it is the purpose of this dissertation to compare performance outcomes following carbohydrate (CHO)-plant or CHO-animal protein ingestion utilizing a variety of exercise conditions (acute bouts of aerobic exercise, 10 weeks of strength training and six weeks of concurrent training).

Study 1 demonstrated that following a glycogen depleting bout of cycling, dairy chocolate, soy chocolate, hemp chocolate or dairy milk ingestion all enhanced the performance of a subsequent 20km cycling time trial relative to that of a placebo drink ($p = 0.02$). Further because the drinks were matched for energy and total liquid consumed these data suggest that a post exercise CHO-plant protein drink is as effective as a post exercise CHO-animal protein drink with respect to same day 20km time trial performance following glycogen depleting exercise.

Study 2 demonstrated that regardless of protein type (hemp, dairy milk or whey protein isolate) post bout CHO-protein supplementation increased isokinetic bicep ($p = 0.03$), quadriceps ($p = 0.04$ and isometric bicep strength ($p = 0.05$) vs a carbohydrate only drink over 10 weeks of strength training. Thus, post bout CHO-protein ingestion appears to be as effective for strength development over 10 weeks of training whether the protein source is vegetable (hemp) or animal (milk, whey).

Study 3 demonstrated that post training session CHO - hemp protein ingestion produced greater improvements in time trial ($p = 0.01$), peak Wingate power ($p = 0.001$)

and 1-RM strength (p = 0.01) with 6 weeks of concurrent training vs carbohydrate only. Six weeks of concurrent training, when supplemented with hemp based protein-CHO drink post exercise, appears to be effective at enhancing aerobic and anaerobic performance outcomes similarly to what has been previously found with a CHO- animal protein supplement.

Together, these studies demonstrate significant and similar improvements with plant or animal protein-CHO post exercise supplementation for strength or concurrent training and for time trial performance following glycogen depleting exercise.

Key Words: hemp protein, post exercise supplementation, concurrent training, time trial performance, high intensity interval training, strength training

Co-Authorship Statement

All of the data collected in this dissertation was interpreted by the author, Adam N. Upshaw, under the supervision of Dr. Peter W.R. Lemon. The first author on all manuscripts presented is Adam N. Upshaw.

The paper based on Chapter 3 is co-authored by Tiffany Lam, Arash Bandegan and Peter W.R. Lemon and is in preparation for submission.

The paper based on Chapter 4 is co-authored by Sarah B. Wilkinson, William. J. Booth, Arash Bandegan, Omar Choque, Greg D. Marsh and Peter W.R. Lemon and is in preparation for submission.

The paper based on Chapter 5 is co-authored by John G. Huggard, Omar Choque and Peter W.R. Lemon and is in preparation for submission.

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DISCUSSION

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Abbreviations

ACSM   American College of Sports Medicine

AMPK   adenosine monophosphate activated protein kinase

AKT   protein kinase B

BCAA   branched chain amino acid

BF   body fat

BMI   body mass index

BV   body volume

BM   body mass

CHO   carbohydrate

CM   chocolate milk

CR   carbohydrate replacement

CSEP   Canadian Society for Exercise Physiology

CT   concurrent training

FAO   Food and Agriculture Organization

FFA   free fatty acids

HIIT   high intensity interval training

HR   heart rate

IAAO	indicator amino acid oxidation
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
NB	nitrogen balance
N.m	newton.metre
NPU	net protein utilization
PDCAAS	protein digested corrected amino acid score
PER	protein efficiency ratio
PGC-1	Peroxisome proliferator-activated receptor-gamma coactivator-1
PI3 k	phosphoinositide 3 kinase
RDA	recommended daily allowance
ROI	region of interest
RPM	revolution per minute
SIT	sprint interval training
ST	strength training
TE	echo time
TR	repetition time
TT	time trial

TTE	**time to exhaustion**
UWO	**University of Western Ontario**
VO$_2$max	**maximal rate of oxygen consumption**
VO$_2$peak	**greatest rate of oxygen consumption achieved during incremental exercise test**

Chapter 1

General Introduction

1.1 Introduction

The manipulation of exercise strategies and nutrition regimens are the cornerstones to any good training program. Specifically, body composition, strength, power and/or speed, endurance and, recovery rate, can all be affected positively with exercise training and nutrition programs. Most athletes utilize a variety of methods to train efficiently, perform optimally and recover quickly and fully, including daily nutrition manipulation; particularly, carbohydrate and protein intake, before, during and after exercise. With the correct balance of nutrition timing and type, as well as sport specific exercise regimes, both endurance and strength athletes, can optimize their training adaptations and as a result, their performance. As demonstrated by a myriad of metabolic investigations, adequate carbohydrate intake before, during, and after exercise has been shown to be critical to provide optimal energy to the exercising muscles as well as the brain during both moderate intensity long duration and intermittent high intensity exercise (Coggan & Coyle. 1987; Costill et al. 1981; Coyle et al. 1999; Fielding et al. 1985; Koopma et al. 2007; Lambert et al. 2003; vanLoon et al. 2003; Zawadzki et al. 1992). Daily intakes between 5-10g carbohydrate/kg of body mass, generally referred to as carbohydrate loading, delays exercise fatigue by prolonging the use of glucose from glycogen stores and/or by providing glucose directly to muscle (Burke et al. 2001). Dietary protein, on the other hand, although a potential energy source, especially in cases of low carbohydrate intake, is primarily utilized for lean tissue repair and recovery (Biolo et al. 1997). Daily protein requirements for athletes remain somewhat controversial in the nutrition and exercise research community, however, it is believed that a daily intake between 1.2 - 1.7g protein/kg is sufficient to meet daily needs as well as maximize rates

of muscle protein synthesis – even for the most elite athlete (Lambert et al. 2003). This is about 150 to nearly 220% of recommendations for sedentary individuals.

Although dietary carbohydrate and protein totals are both important, the timing with which these macronutrients are consumed relative to the exercise bout may be most critical for both performance and adaptation. For example, prior to both endurance and strength exercise, carbohydrates should be consumed to ensure adequate quantities of muscle glycogen are present (Haff et al. 2003) because depleted glycogen stores impact performance negatively (Burke et al. 2004). Further, maintenance of blood glucose concentration and reduced muscle glycogen utilization can be achieved with carbohydrate ingestion during exercise (Burke et al. 2004). Moreover, with the goal of restoring muscle and liver glycogen following an exercise bout for subsequent exercise, research has focused mainly on post exercise carbohydrate intake. An intake of 1.2g carbohydrate.kg^{-1}.h^{-1} of easily digestible carbohydrate post exercise for at least 2h is considered to be an effective dose to restore exercise depleted glycogen stores (Burke et al. 2004). Specifically, there appears to be a 'window' of opportunity shortly following glycogen depleting exercise (perhaps as brief as 15-20 minutes) where consumption of ~1.2g.kg^{-1} of CHO will replenish muscle glycogen more completely resulting in enhanced performance in subsequent exercise bouts (Ivy et al. 2001). This 'window' may be because muscle enzymes (glycogen synthase for example) are most responsive to CHO intake shortly after muscle glycogen is depleted.

The addition of protein to CHO post exercise supplementation has garnered considerable interest in recent years, as well. As Ivy et al. (2001) noted, protein consumption increases circulating insulin and activates several of the same enzymes

responsible for cellular carbohydrate uptake and thus the addition of protein to CHO post exercise might be most beneficial. For strength and endurance athletes, post exercise protein intakes of 20-30g every 90-120min (Moore et al. 2009) totaling about 1.2 - 1.7 g.kg^{-1} of body mass per day (Lambert et al. 2003), seems to promote muscle protein maximally. However, there is some disagreement within the nutrition research community whether or not there is sufficient data to establish definitively a protein intake recommendation. This will be discussed further in Chapter 2 (pages 29-32)**.**

Although adequate protein and CHO intake are critical post exercise, it is possible that CHO and protein type may also play a key role in muscle recovery. For CHO, it is well established that high glycemic CHO (rapidly absorbed into the bloodstream) is best for rapid glycogen storage compared to low glycemic CHO (Blom et al. 1987; Burke et al. 1993; Parkin et al. 1997). Protein type has also been investigated quite extensively with investigations on whey protein, casein protein, branched chain amino acids (BCAA), dairy milk and soy protein. Generally, animal protein is considered better suited to deliver the necessary amino acids for protein synthesis than plant proteins due to a more complete complement of indispensable amino acids (Hartman et al. 2007; Lord et al. 2007; Wilkinson et al. 2007) but this may not be the critical factor (to be discussed below). To date, most investigations have focused on soy protein as the plant protein source and either whey or dairy milk as the animal protein comparison. Wilkinson et al. (2007) noted that soy protein appears to be inferior to dairy protein in terms of building muscle mass perhaps related to differences in rate of digestion/absorption (slow for milk and rapid for soy). Subsequently, these findings were substantiated in a chronic strength training study by Hartman et al. (2007). However, very little research has examined plant

proteins other than soy protein, probably because of low quality rating that other plant proteins usually receive. This is unfortunate because hemp protein for example may have a greater absorbability vs soy protein (Wang et al. 2007) and, it contains a full complement of the important branched chain amino acids (Calloway. 2007). At present, there is little information on hemp protein ingestion and exercise, thus investigations into whether hemp protein supplementation can enhance the exercise response are warranted.

Further and importantly, there is a dearth of research about the benefits of any protein and/or CHO supplement following concurrent training. Concurrent training, which is utilized traditionally by endurance athletes to enhance strength and power quantities, involves the incorporation of both strength and endurance training into an athlete's regular regime often within the same training session or at least on the same day. Considerable controversy about this type of training exists ever since Hickson (1980) first described an apparent interference effect (an incompatibility of both forms of training on molecular adaptations in the muscle), yet more recent research has demonstrated that concurrent training can produce great benefits for both endurance and strength athletes (Balabinis et al. 2003; Glowacki et al. 2004; Gorostiaga et al. 1999; Jackson Davis et al. 2008; Johnston et al. 1997; Wong et al. 2010). Others criticize concurrent training because of potential fatigue or over-training adverse effects (Dudley & Djamil. 1985; Kraemer et al. 1995; Nader. 2006). To date, several strategies have been utilized in an attempt to overcome these barriers including order of exercise training, i.e. strength before or after the aerobic training, the addition of a prolonged rest period between the two training types, more time for recovery between training days and varying the exercise intensity from prolonged endurance to high intensity training. High intensity, short

duration training in lieu of endurance training has been shown to produce similar physiological adaptations with a much reduced time-commitment (Gibala & McGee. 2008). This may decrease the risk of overtraining and/or minimize the chronic fatigue experienced commonly by endurance athletes (Kuipers & Keizer. 1988). Few however, have investigated whether proper diet manipulation can be effective with concurrent training. Specifically, if the addition of a CHO-protein supplement following bouts of concurrent training might alleviate any fatigue or overtraining adverse effects. As demonstrated by Romano-Ely et al. (2006) and Hall et al. 2013, the addition of a CHO - animal protein mixture can improve physical (muscle damage) recovery, and improve perceptions of fatigue (ratings of perceived exertion) following aerobic exercise, respectively. The benefits of a CHO protein drink has also been observed following strength training (Rasmussen et al. 2000). Therefore, it is reasonable to assume that CHO - protein ingestion may also be beneficial following a training session which incorporates endurance and strength training, i.e., concurrent training. Regardless, investigations into this topic are prudent.

As stated, clearly nutrient manipulation is important for maximizing exercise performance in a variety of activities (strength, power, aerobic or anaerobic). Although CHO - animal protein supplements have been considered optimal pre- and post exercise nutrients for athletes, certain plant proteins in combination with CHO may be equally effective. While it is unlikely that any one supplemented nutrient (animal protein or otherwise) would be responsible solely for significant performance enhancements, any benefit that can be gained potentially by the ingestion of a plant based protein on

glycogen supercompensation, on strength development with training, or with concurrent strength and aerobic training is unknown.

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Chapter 2

Literature Review

2.1 Physiological adaptations of strength, endurance and concurrent training on performance

Traditional endurance training as well as interval training have long been used by athletes to improve exercise performance. Similarly, strength training enhances muscle adaptations leading to improvements in power, speed and ultimately, exercise performance. Although both modes of training provide the necessary stimulus for physiological adaptations to occur, the incorporation of both these exercise modes into a single training program, termed concurrent training (CT), is not as well accepted.

In 1980, Hickson introduced the concept of an interference effect, whereby the addition of an aerobic training regimen to a strength training program would impact negatively the potential strength gains achievable by the power athlete. This concept was based on the idea that myofibril and mitochondrial molecular adaptations cannot occur simultaneously and that any mode of training that targets both adaptations would fail or at least be suboptimal. However, clearly the addition of a strength component to the training program of an endurance athlete could be beneficial for increasing aerobic performance if aerobic adaptations remain unaffected and if fatigue and overtraining (Nader. 2006) can be eliminated.

2.1.1   Interference effect with CT

Endurance training induces both central and peripheral adaptations which lead to increases in VO_2max, time trial performance and time to fatigue (Lepretre. 2004). Specifically, central changes include pulmonary oxygen diffusion capacity, cardiac output and hemoglobin affinity. Peripheral changes include increases in myoglobin affinity, capillary density, mitochondrial content and size (Gollnick et al. 1973) and, PGC-1α protein content (Russell et al. 2003). Strength training too involves central and

peripheral adaptations however in this case, central adaptations are neural while peripheral adaptations are at the level of the contractile proteins (Cannon and Caferelli. 1987). Specifically, neural adaptations involve greater motor recruitment, greater muscle fibre recruitment, increases in frequency of neural firing/connections and better synchronization of motor unit activation (Docherty & Sporer. 2000). The peripheral adaptations involve increases in muscle cross sectional area due to increased myofibrillar protein synthesis (Docherty & Sporer. 2000).

Incorporating both strength and aerobic training into a single program has long been utilized for rehabilitation and by some athletes to improve these central and peripheral components (Docherty and Sporer. 2000). Despite anecdotal benefits of such training among athletes, the idea that strength and aerobic training are incompatible proposed by Hickson (1980) remains gospel for many scientists. One of the most common findings in the literature is that strength is compromised significantly when athletes include endurance training compared to strength training alone (Bell et al. 1997; Kraemer et al. 1995). One explanation for this interference is overtraining (Nader. 2006). Overtraining is often defined as an insufficient recovery and typically increases the risk of both injury and psychological staleness (Nader. 2006). Although it can reach chronic status, short term overtraining, often defined as overreaching, is far more common (Armstrong & VanHeest. 2004). Symptoms include increased sympathetic tone at rest, increased heart rate and blood lactate with submaximal exercise, loss of body mass, impaired immune function, fatigue, lower circulating growth hormone and testosterone concentrations and decreased appetite (Armstrong & VanHeest. 2004). As Fry et al. (1994) and Halson et al. (2002) have shown, an exercise program with little to no rest

between bouts, can affect both strength and endurance performance adversely. Another possible mechanism for interference is impaired or fatigued motor unit recruitment (Fry et al. 1994).

One of the more obvious molecular interference effects involves protein turnover rates. Specifically, with concurrent training the cell must manage both muscle growth (hypertrophy) and aerobic (mitochondrial) adaptations (Kraemer et al. 2005). For example, an increase in strength is the result of increases in myofibril proteins (actin and myosin) from increases in mRNA translation rates (Pain. 1996). The mTOR pathway, which is activated by enzymes such as PI3 K and Akt, is the primary stimulus for protein synthesis (Bolster. 2002; Sarbassov et al. 2001). In contrast, increases in aerobic capacity are a result of an increase in mitochondrial protein biogenesis (Gollnick et al. 1969; Holloszy. 1967). Increased AMPK activation plays a major role in this response however, an increase in AMPK, which occurs during aerobic exercise as a result of a depleting glycogen concentration, has also been shown to inhibit mTOR activity (Hardie et al. 1998). Perhaps skeletal muscle is unable to adapt, molecularly, to simultaneous endurance and strength training but this has not translated consistently into observed decrements in performance among athletes utilizing concurrent training programs.

Although the interference data have been inconsistent over the years, recent data support a performance benefit, albeit minor, for endurance athletes who incorporate strength training into their regular aerobic training. For example, Sillanpaa et al. (2008 & 2009) reported no mechanical interference effect when strength and aerobic training was performed concurrently. In fact, they noted an increase in aerobic performance with the addition of strength training sessions. Similarly, Donges et al. (2012) reported no

molecular interference effects from concurrent exercise training noting that myofibril and mitochondrial protein synthesis increased following such training without a decrement in protein signaling or mRNA expression. Additionally, both explosive and plyometric training has been shown to decrease 5km time trial even when 30% of the endurance training was replaced with strength training (Paavolainen et al. 1999). A possible explanation could be the improved joint stability following concurrent training which would assist with strength and power output (Docherty et al. 2000).   Also, Tao et al. (2010) noted that although AMPK is usually thought of as a mTOR inhibitor, the enzyme alone can stimulate mTOR and protein synthesis independently by increasing activity of P13k and Akt. Although these seem like plausible explanations, other areas of interest should be explored in order to gain a better understanding and in turn an ability to create an optimal CT program. This would include investigating whether high intensity interval training (HIIT) is best to combine with strength training based on the understanding that both strength training and HIIT primarily utilize similar fiber types and thus would be expected to produce less molecular interference.   Further, little is known about any possible benefits of nutritional supplementation between and following training sessions on fatigue or overtraining.

2.1.2   The addition of high intensity training with CT

It is well established that endurance athletes can benefit by including bouts of HIIT into their programs (Gibala & McGee. 2008; Weston et al. 1996). As there appears to be an upper limit on duration of training at which point there is no further increase in endurance performance or physiological biomarkers such as creatine kinase, testosterone

or cortisol (Costill et al. 1991), incorporating shorter but more intense workouts into one's program can have a positive effect on several physiological adaptations seen with endurance training. These adaptations include increases in the liver and muscle glycogen stores, increased muscle free fatty acid (FFA) utilization, reduced exercise glycogen use, improved heat dissipation, and being able to utilize a larger percentage of VO_2max for longer periods (Laursen. 2002). Further, as HIIT stimulates peripheral adaptations and increases type 2 fiber utilization, there could be a carryover with strength training. At present, only a handful of studies have investigated the benefits of CT when HIIT is part of the training.   Helgerud et al. (2011) observed that when elite European footballers incorporated strength training into their aerobic training sessions, improvements in speed, aerobic capacity, power and strength were found. However, the strength training utilized was of low volume, thus, this may not present a clear picture of any 'interference' that could occur when max efforts are performed over the course of an entire training periodization program. Similarly, combining both high intensity strength training and HIIT in an endurance program has been observed to enhance multiple short and moderate distance running speed tests (Wong et al. 2010). However, this concurrent training contained strength sessions at times that were separated from aerobic training bouts (both endurance and HIIT) by several hours (Wong et al. 2010). This extended recovery time may not be representative of the needs of endurance athletes who train multiple times per day (swimmers, ultra-triathletes, marathon runners, soccer players, professional hockey players, track athletes etc.). Completing an intra-session (training within the same session) aerobic and strength concurrent program would allow athletes to continue with their aerobic volume while at the same time possibly benefiting from the strength

training. Chtara et al. (2005) reported that concurrent intra-session aerobic training followed by strength training produced greater improvements in aerobic performance than when either was performed separately or in reverse order. However, they did not include a HIIT session as part of the aerobic training and thus such an investigation remains warranted.

2.1.3   *Nutrition, fatigue and overtraining*

Molecular interference may play a key role in limiting the benefits of CT but as Nader (2006) speculated, poor nutrition and general fatigue could also be major factors. For example, maintaining glycogen stores is essential for both endurance athletes (to prolong the effort) and strength athletes (to maximize efforts and maintain elevated protein synthesis rates). Depleting muscle glycogen with exhaustive endurance exercise impairs subsequent exercise (Balsom et al. 1999), including maximum strength (Haff et al. 1999). Further, in addition to the importance of glycogen stores for endurance (Saltin et al. 1967), strength (Tesch et al. 1988) or concurrent training (Reed et al. 2013), the incorporation of dietary protein into an athlete's diet remains a priority because of the reported benefits of protein ingestion with exercise (Hall et al. 2013; Holwerda et al. 2013; Pasiakos et al. 2014; van Loon. 2013). Therefore, it is reasonable to assume that proper nutrition is important for any concurrent training program as well.

2.2 The importance of protein supplementation following endurance or strength training

It is understood that CHO ingestion shortly following an exhaustive bout of either endurance or strength exercise is necessary for rapid and optimal restoration of muscle

glycogen – a critical factor for athletes competing in multiple events within a 24h period (Haff et al. 2000; Ivy et al. 2002; Jentjens et al. 2003; vanLoon et al. 2000). As reported by van Loon et al. (2000), a CHO supplement consumed within 2h post exercise, and preferably at 30 minute intervals beginning immediately after exercise, can increase glycogen synthesis rates by 170 percent. The increased glucose availability and resulting increased insulin concentrations post ingestion are key factors in the heightened glycogen synthesis response. Further, it has been demonstrated that the addition of protein to a CHO supplement can enhance the insulin response further, thus promoting a greater uptake of glucose into the cell (van Loon et al. 2000). However, this is likely only the case when there is less than optimal intake of CHO post exercise, i.e, an intake below $1.2 g.kg^{-1}.h^{-1}$ (Jentjens et al. 2001). Thus not only does protein intake enhance protein synthesis post strength training exercise, it is beneficial for both endurance and strength exercise because it enhances glycogen resynthesis, as well.

2.2.1   Endurance exercise and protein supplementation

Muscle glycogen stores can become very low following exhaustive bouts of endurance exercise making subsequent bouts, especially within a 24h period, more difficult without proper post exercise CHO supplementation (Nicholas et al. 1995). Specifically, it has been demonstrated consistently that CHO supplementation within 15-30min of the fatiguing exercise at a dose of at least $1.2 g.kg^{-1}.h^{-1}$ is effective at restoring muscle glycogen concentration more rapidly than if the intake is delayed or is at a lower dose (Van Hall et al. 2000 & van Loon et al. 2000). The synergistic effects of insulin following the ingestion of CHO and protein has led investigators to assess whether together, these two macronutrients can increase glycogen stores even further. In a study

by van Loon et al. (2000), a greater insulinemic response was achieved with the ingestion

of a CHO- animal protein drink of ~0.8g.kg^{-1} .h^{-1} of carbohydrate to 0.4g.kg^{-1}. h^{-1} of

protein vs a CHO only drink.   In fact Ivy et al. (2002) noted the greatest insulinemic

effect and subsequent improvements in endurance performance would occur with the

ingestion of a drink containing approximately 4 parts carbohydrate to 1 part protein. In

their study, subjects completed a time to exhaustion bout that alternated intensities

between 45% and 75% VO$_2$max for 3 h and then 85% VO$_2$max until fatigue. They

compared three conditions, a flavoured aspartame-sweetened placebo supplement to a

CHO supplement (7.75%) and to a 4:1 CHO + protein supplement (7.75% CHO + 1.94%

protein). Both drinks contributed to an improved endurance performance vs the placebo

however, the CHO-animal protein further enhanced the effect. Unfortunately, the drinks

were not matched for energy and thus it cannot be stated with certainty that the improved

performance was due to the drink type vs the energy provided. Others have reported

ergogenic effects with lower CHO: protein ratios (Berardi et al. 2006; Millard-Stafford et

al. 2005; Romeno-Ely. 2006). For example, Williams et al. (2003), Berardi et al. (2008),

and Thomas et al. (2009) all reported increases in performance following either

exhaustive exercise or increases in rates of glycogen resynthesis following the ingestion

of a carbohydrate-protein drink at ratios of 3:1, 2:1 and 4:1, respectively. Importantly, the

type of protein used within the mixture has not been established. The amino acids

leucine, phenylalanine and tyrosine may be optimal for increasing the insulinemic

response, while arginine, glutamine and intact casein ingestion appear to be ineffective

(van Loon et al. 2000). As a result, researchers began to investigate CHO-protein

mixtures that contained high glycemic carbohydrates and relatively high quantities of

proteins that contained the amino acids described above (Thomas et al. 2009; Williams et al. 2003). Thomas et al. (2009) utilized a chocolate milk supplement for the CHO-protein supplement because it contains high glycemic CHO, dairy proteins (leucine) and a 4:1 ratio of CHO to protein. Although Thomas et al. (2009) was one of the first researchers to investigate comprehensively the effects of chocolate milk on muscle glycogen recovery, others (Karp et al. 2006; Fergusson-Stegall et al. 2010; Pritchett et al. 2009) completed similar investigations with varying results. However, all of these investigations found that a chocolate milk recovery beverage was at least as effective as an isoenergetic CHO supplement. Perhaps the variance is due to the possibility that post exercise CHO-protein supplementation is only effective when CHO concentrations fall below the recommended dose of at least $1.2g.kg^{-1}.h^{-1}$. As Jentjens et al. (2001) noted, following a glycogen depleting bout of exercise, the addition of protein to a CHO drink during recovery did not increase rates of glycogen synthesis when CHO dose was at least $1.2g.kg^{-1}.h^{-1}$. Thus, although CHO ingestion in the form of high glycemic CHO is necessary following glycogen lowering exercise for rapid replenishment of glycogen stores, the necessity of protein remains somewhat controversial – especially when the CHO intake is $> 1.2g.kg^{-1}.h^{-1}$. What is more firmly established however, is that dietary protein, when consumed in conjunction with strength training exercise, is required to maximize muscular adaptations.

2.2.2   Strength exercise and protein supplementation

The optimal dose of protein ingestion post strength training exercise is difficult to establish given the numerous methodologies that have been used to assess protein requirements, as discussed previously. However, considerable data indicate that protein

supplementation following strength training can enhance muscle protein synthesis, muscle hypertrophy and strength development (Cribb et al. 2006; Pitkanen et al. 2003; Rennie et al. 1991; Tipton et al. 2003). Most of the underlying rationale centers on the theory that with exercise there is an increase in amino acid oxidation and a greater conversion of amino acids to glucose thereby reducing the availability of amino acids for muscle repair and protein synthesis post exercise (Rennie et al. 1991). Additionally, protein ingestion post strength training can attenuate muscle catabolism which occurs naturally following such exercise. As Pitkanen et al. (2003) found, strength exercise in the fasted state can increase muscle breakdown for as long as 195 minutes post exercise. Moreover, it has been reported that ingestion of a CHO-protein mix post exercise enhances protein synthesis even more. This might be the indirect result of enhanced glycogen stores post exercise with CHO ingestion (Koopman et al. 2006) or as a result of the increased insulin response resulting from protein ingestion alone. In theory, an increase in insulin can reduce protein degradation by inhibiting autophagy via the mTOR activation pathway (Sacheck et al. 2004) or suppress the breakdown of proteins by inactivating transcriptional factors (Sacheck et al. 2004). Further, AMPK, a known mTOR inhibitor (Dreyer et al. 2006) may be activated in a glycogen depleted state (Wojtaszewski et al. 2002). Thus, the ingestion of a CHO-protein mix post strength exercise could potentially increase protein synthesis while decreasing rates of protein breakdown.

In contrast, a recent study by Staples et al. (2011) suggested that muscle protein synthesis was not increased nor was muscle protein breakdown decreased with ingestion of a mixed CHO-protein drink containing adequate protein, 25g of whey protein,

following acute strength exercise. Thus, it can be stated that a CHO induced insulin response may play only a minor role in protein turnover rates when adequate protein is ingested. Nevertheless, numerous studies (Burke et al. 2001; Cribb et al. 2006; Hartman et al. 2006; Paddon-Jones et al. 2003; Tipton et al. 2003; Tipton et al. 2004) investigating post strength training supplementation have found that muscle strength and hypertrophy are increased significantly with ingestion of a CHO-protein mix. This effect is especially significant when participants are in the fasted state. Apparently, the more glycogen and energy depleted the individual, the more dramatic the effect of the CHO-protein supplement on muscle protein synthesis and muscle protein breakdown.

In summary, post exercise protein is required to maximize the performance of endurance and strength athletes. Frequently, the benefit of protein ingestion on aerobic and strength performance outcomes is observed when mixed with CHO; however this is not without its controversy. Perhaps part of the confusion involves the absence of a clear consensus regarding the daily protein requirements for all athletes.

2.3 Daily protein Requirement

Given the numerous factors that are implicated in daily protein requirements (including training status, CHO availability, age and gender), there exists some controversy in the literature with respect to daily protein requirements for both strength and endurance athletes. For the most part among sport nutritionists since the mid 1980s it is has been accepted that athletes require a greater daily dietary intake of protein than sedentary individuals due to their need to maintain a larger muscle mass, repair damaged muscle tissue and maintain plasma protein for other physiological functions (Lemon et al.

1992; Lemon et al. 1997; Tarnopolsky et al. 1988). However, these requirements were established using the nitrogen balance technique primarily and this methodology has several limitations as will be discussed in section 2.4.1 (Humayun et al. 2007). Nevertheless, the current Recommended Daily Allowance (RDA) for protein (0.8g/kg of body mass for adults 18 years and older) is based largely on nitrogen balance techniques and includes no allowance for those who exercise regularly (Trumbo et al. 2002). Phillips (2006) suggests that strength and endurance athletes do not require a greater daily protein intake and, in fact, some have suggested that their requirement may even be less than current recommendations. This assertion is based on the idea that the reincorporation of intracellular amino acids decreases the protein requirements rather than elevating it, as chronic strength training increases the efficiency of use of protein. Others (Coleman, 2012; Poortmans et al. 2012; Tarnopolsky et al. 2004) argue that systematically trained strength and endurance individuals require on average a daily intake in the range of 1.2 - 1.7 $g.kg^{-1}. d^{-1}$ to maintain muscle mass and repair damaged tissues. As reported by Tarnopolsky et al. (2004), elite endurance athletes may require up to 1.6 - $1.7g.kg^{-1}.d^{-1}$. Further, routinely, strength athletes and endurance athletes consume well above the current RDA (Phillips. 2004). In fact, many athletes consume much more protein with intakes reaching ~$3.7g.kg.^{-1} d^{-1}$(Grandjean et al. (1989). Initially, it was believed that protein intake in excess of the RDA would have negative health implications, including kidney disorders (Metges and Barth. 2000) and osteoporosis (Barzel & Massey. 1998), however more recent evidence suggests that there is little health risk even when intakes reach 3.5 $g.kg.^{-1}.d^{-1}$ (Poortmans et al. 2007). Thus, the possibility of suffering from serious health implications is low; similarly, the benefit of

ingesting protein at a dose of 3.5g.kg^{-1}.d^{-1} for protein synthesis may be minimal.

However, more concerning is that high intakes of protein could compromise the intake of

another critical macronutrient (CHO for example) for those athletes looking to maintain

strict daily caloric intakes. Carbohydrates, although usually thought of as a key fuel for

endurance exercise, are also important for strength performance (Dryer et al. 2006). In

the presence of a high protein diet, CHO intake may be reduced intuitively as athletes

adhere to a strict daily-total energy intake. Thus, it is crucial for athletes to adhere to

dietary protein guidelines of 1.2g.kg^{-1} -1.7g.$^{-1}$ kg which may in turn allow for an optimal

rate of protein synthesis, while avoiding any reduction in CHO consumption.

In summary, daily protein intake should include 1.2g.kg^{-1} -1.7g.$^{-1}$ kg of protein,

consumed every 3-4 waking hours with 10 grams originating from indispensable amino

acids, and 2-2.5 of those grams being derived from the amino acid leucine (Moore et al.

2009). Additionally, more slowly releasing proteins such as the dairy protein casein, can

be consumed before bed to minimize the rates of muscle protein breakdown throughout

the sleep cycle (Res et al. 2012).

Although strides have been made toward estimating protein requirements for

sedentary as well as strength and aerobically trained individuals, the various scientific

technologies and methodologies used has led to much controversy. Given the challenges

of previous research methodologies, new techniques continue to be employed in an

attempt to offer greater insight into daily protein requirements.

2.4 Determination of protein requirements

2.4.1   *Nitrogen balance*

Protein requirement is defined as the intake of dietary protein that is sufficient to achieve body nitrogen equilibrium, that is, whole body nitrogen balance (NB). Nitrogen is a main body component and is required for, among many other functions, protein synthesis (Brody. 1998). Since nitrogen is contained primarily in proteins, any change in total protein content within the body, especially skeletal muscle proteins, would result in a shift in nitrogen balance (Brody. 1998). The common approach to NB calculations involves an adaptive period on a diet of various protein content (above and below the requirement) followed by a NB measurement (Lemon et al. 1992). Specifically, NB would be calculated from measured nitrogen intake as well as excretion (typically, urinary, fecal, perspiration, and estimated miscellaneous nitrogen losses). Several problems can arise with this method as described by Pencharz & Ball (2003) including ensuring NB calculations are precise (losses in fecal collection) and, the five to seven day adaption period to the changing amino acid intake which is not representative of day-to-day life of a sedentary individual or athlete. As a result of some of these inadequacies, NB tends to overestimate nitrogen intake and underestimate nitrogen excretion – both leading to a false positive NB (Millward. 2001). Although this method of evaluation of protein requirements has been considered the Gold standard, its use has decreased in recent years given its many inadequacies.

2.4.2   *Isotope tracers*

Isotope labeling is a technique to track the movements of an isotope through the numerous metabolic pathways. A stable isotope is a variant of a particular chemical

element (charge and reaction with other molecules remain constant) however because

they differ in the number of neutrons, their mass is different and thus can be measured

using mass spectroscopy techniques (Brody. 1998). Following isotope infusion or

ingestion, subsequent tracer incorporation into muscle protein or oxidation over time are

possible (Phillips et al. 1997).   The choice of tracer depends on three critical factors: the

amino acid must be indispensable, the carboxyl group must be oxidized irreversibly and

the amino acid must be directly partitioned to oxidation or synthesis (Millward. 1998).

However, as mentioned, this method requires constant tracer infusion or ingestion and is

only suitable for <u>acute</u> whole body and muscle protein synthesis assessments in a

laboratory setting (Millward. 1998).

2.4.3   *Indicator amino acid Oxidation Technique*

Young et al. (1989) and Zello et al. (1990) noted that nitrogen balance studies

often have underestimated protein requirements. Further, Gasier et al. (2010) found that

intravenous infusion of a labeled amino acid is invasive, does not allow for cumulative

assessments of fractional protein synthesis rates, faces challenges related to the

measurement and interpretation of the precursor/product labeling ratio, and finally, is

impractical for real world scenarios. Although not used routinely with exercise

treatments, a non-invasive method (indicator amino acid oxidation technique; IAAO) has

been used to determine daily protein requirements for children and adults (Elango et al.

2008). The IAAO relies on the concept that when one indispensable amino acid is

deficient for protein synthesis, then all other amino acids, including the indicator amino

acid will be oxidized (i.e., not incorporated into protein synthesis) (Elango et al. 2008). If

the intake of the limiting amino acid is increased progressively, catabolism of the labeled

indicator amino acid will diminish because the indicator amino acid is now being incorporated into protein (Pencharz & Ball. 2003). Once the requirement is met for the limiting amino acid, there will be no further change in the oxidation of the indicator amino acid (Elango et al. 2008). The rate of oxidation can be monitored by using a safe stable isotope, a carbon-13 labeled amino acid, usually phenylalanine (l-[1-^{13}C]phenylalanine), via breath sample collections (Elango et al. 2008). To simplify, the more label found in breath, the more the label is being oxidized and the less is being used for protein synthesis. As noted by Elango et al. (2008), "the IAAO method is robust, rapid, and reliable; it has been used to determine amino acid requirements in different species, across the life cycle, and in diseased populations". Unfortunately, the IAAO method is quite expensive and, to date, has not been used to assess protein requirements among athletes but holds significant potential.

Although all the techniques identified here have their advantages and disadvantages, the protein requirements established from each thus far, point to an intake of 1.0 up to 3.0g.kg^{-1}.d^{-1} (Norton et al. 2009) for athletes. Thus, at this point, it is reasonable to assume a daily protein intake within this range would be sufficient to cover protein requirements of most physically active men and women.

2.5 Protein supplementation by sex

Although women, on average, have less muscle mass than men, daily protein requirements per unit body mass are similar (0.8g.kg^{-1}) as per current RDA recommendations. Further, protein requirements throughout the menstrual cycle do not appear to affect protein needs despite changes in estrogen and progesterone production (Miller et al. 2006). Few studies have compared men and women in response to exercise

and supplementation either alone and or in combination, however a study by Tarnopolsky et al. (1997) revealed that when evaluating rates of glycogen re-synthesis, men and women do respond similarly to a carbohydrate-protein drink post exercise. For protein synthesis, there is little sex difference in muscle protein synthesis rates following either an overnight fast (Fugita et al. 2006), following high intensity leg exercise (Dreyer et al. 2010), during a hyperinsulinemic-hyperaminoacidemic-euglycemic state (Smith et al. 2009), and/or following a single bout of exercise paired with a whey protein supplement (West et al. 2012). Given the nature and scope of these studies, it appears there are no sex based differences in muscle protein metabolism at rest, in the fasted and protein supplemented state, or following bouts of exercise. Women are able to hypertrophy muscle at a similar relative rate as men despite hormonal differences and absolute muscle mass (Cureton et al. 1988, Abe et al. 2000). Although the literature substantiates the claim that no differences appear to exist in protein turnover rates between young men and women, recent research indicates that elderly women have a reduced capacity to hypertrophy as well as a reduced ability to respond to protein supplementation (Smith et al. 2008). Nevertheless, in light of the current state of the research, little and what appears to be insignificant differences exists in protein synthesis rates between men and women, at least in in young adults. Whether these findings can be applied to long term molecular adaptations remains to be investigated.

2.6 Protein Intake Timing

Currently, protein supplement intake timing is an area of focus in the exercise nutrition field. Some research groups have used whole food proteins whereas other groups use supplements such as individual amino acids or combinations of several amino

acids and these experimental differences have led to a disagreement relative to the optimal protein ingestion timing in order to maximize muscle protein synthesis. For example, studies by Tipton et al. (2001) and Rasmussen et al. (2000) noted that the provision of indispensable amino acids before exercise resulted in greater muscle protein synthesis rates following exercise than when the same indispensable amino acids were given at different time intervals post exercise. In this study, six healthy young males consumed an amino acid supplement immediately before and immediately after strength training exercise. Following confirmation of amino acid enrichment, the authors noted that amino acid delivery to the exercising leg muscles was much greater following pre-exercise amino acid ingestion compared to post.   Similarly, Tipton et al. (2001) found that indispensable amino acids ingestion prior to exercise produced a greater increase in net protein balance vs whole protein (whey) ingestion. However, these observations made by Tipton et al. (2001) and others were later challenged by a follow up study by Tipton et al. (2007) in which the authors concluded that participants who were subjected to an acute bout of exercise and consumed a whey protein supplement either before or after exercise, showed no difference in amino acid uptake. Further, Levenhagen et al. (2002) demonstrated that whole body and skeletal muscle homeostasis was achieved optimally following post exercise ingestion of an amino acid supplement. Fujita et al. (2008) reported that indispensable amino acids + CHO ingestion before strength exercise did not enhance post exercise muscle protein synthesis rates compared to exercise where there was no pre-exercise supplementation. Finally, a more recent study by Cockburn et al. (2010) noted that intact whole proteins, in the form of dairy milk, were most beneficial for attenuating muscle damage and increasing strength performance when consumed

immediately post exercise vs pre- exercise. The proposed benefit of post exercise protein supplementation derives from the idea that stimulatory effects of increased amino acid availability on protein synthesis are enhanced following exercise and thus an improvement in muscle balance occurs by enhancing synthesis and limiting degradation (Cockburn et al. 2010; Phillips et al. 1997). Investigations by Hartman et al. (2009) and Wilkinson et al. (2007) demonstrated that dairy milk ingestion immediately following acute and chronic strength training exercise stimulated muscle protein synthesis to a greater extent than a CHO placebo given at the same time. Similarly, Cribbs and Hayes (2006) noted that immediate post exercise protein plus CHO supplementation increased lean muscle tissue and limb cross sectional area significantly. As Kumar et al. (2009) observed, muscle protein synthesis is greatest immediately after strength exercise in the range of 100-150%.

Although the research groups of Hartman, Wilkinson and Cribbs & Hayes all proposed that immediate post exercise supplementation is optimal for enhancing protein synthesis, others including Wojcik et al. (2001) and White et al. (2008) concluded that the ingestion of a CHO-protein supplement either immediately before or immediately after exercise, has no significant effects on strength, muscle damage, or soreness. Specifically, Wojcik et al. (2001) and White et al. (2008) reported that young untrained men who were fed a CHO-protein beverage either before or after performing a fatiguing acute bout of eccentric leg exercise, experienced no significant difference in leg muscle voluntary contraction strength or muscle inflammation and that there was no influence from post exercise beverage timing on muscle performance, function or soreness. Further, Phillips et al. (2011) concluded that the benefits of protein supplementation timing post

exercise is poorly understood and may in fact not be that critical because muscle protein

synthesis is enhanced for at least 24 hours post exercise and perhaps even for 48h. As

such, if the required amino acids are being transported to the muscle at a rate at which

they are utilized optimally by the muscle (within 24h of exercise), timing of ingestion

(that is immediately pre- or post exercise) may not be critical. This conflicting

information may stem from the various methodological differences. One key factor

however that must not be ignored, is the fasted state, in which investigators usually have

their subjects prior to exercise and subsequent supplementation. It is common for protein

metabolism investigators to instruct their participants to attend the testing laboratory in

the fasted state to control for amino acid availability. However this is problematic

because a post exercise protein supplement will be more beneficial following exercise

done in a fasted state as net amino acid balance is negative vs participants that are in the

fed state prior to exercise. Further, a state of hyper-aminoacidemia can linger for several

hours following ingestion, usually well through the exercise bout making any additional

amino acid availability incidental for protein synthesis. As Aragon and Schoenfeld

(2013) hypothesized, when there is a net negative amino acid balance in the pre- exercise

state, as one would see following an overnight fast, a continued negative amino acid

balance would have been seen in the post exercise state. Thus, it would be at this point

where post exercise supplementation may be the most beneficial converting a catabolic

state into an anabolic state. Taken together, immediate post exercise supplementation

may not increase rates of muscle protein synthesis significantly when one is fed,

assuming intake meets daily protein requirements. Regardless, in the absence of any

conclusive evidence on protein timing, immediate post exercise supplementation should ensure that rates of muscle protein synthesis are maximized.

It appears that the timing of protein intake may not be the most important determinant of muscle protein synthesis as long as essential amino acids remain available during states of protein synthesis following exercise (Schoenfeld et al. 2013). On the other hand, protein type warrants further investigation as it is unclear whether plant based protein supplements are as effective for protein synthesis as animal based supplements.

2.7 Animal vs plant protein supplementation

In addition to serving as structural components of muscle and other tissues in the body, proteins are used to produce hemoglobin, enzymes and energy (Brody. 1998). In order to be utilized within the body, protein needs to be broken down into amino acids; 20 total directly encoded by the universal genetic code (Brody. 1998). Indispensable amino acids (9 in total) are those amino acids that cannot be manufactured in the body and thus must be consumed (Brody. 1998). The absence of any one of these amino acids will result in the inability of the body to properly produce, repair and maintain muscle tissue. Animal proteins contain all indispensable amino acids as do all plant proteins, however most plant proteins have insufficient amounts of one or more indispensable amino acid for human needs (Young and Pellet. 1994). Soy protein is a complete protein (Velasquez & Bhathena. 2007), meaning it contains all indispensable amino acids in sufficient quantities. However hemp protein for example, is deficient in lysine for human needs and as a result is classified as incomplete (Tang. 2006), even though all 20 amino acids can be found in this protein (Calloway. 2004). It should be noted that the effectiveness of a dietary protein to stimulate protein synthesis maximally depends not

only on a complete amino acid profile of the test protein, but also on the digestibility and absorption of the amino acids in that protein (Schaafsma. 2012). Fortunately, there are several protein assessment tools to evaluate the amino acid profile, rate of digestibility and absorption for both animal and plant proteins.

2.7.1   *Quality of proteins*

The evaluation of protein quality has changed over the years and each method has had quite a significant impact on subsequent understanding on the quality of protein. As noted, proteins with a 'complete' profile of indispensable amino acids that can be absorbed and utilized by the body are considered to be most optimal. One protein assessment tool is the protein efficiency ratio (PER) which determines the effectiveness of a protein through mass gained by an animal divided by the intake of a particular protein (Chapman et al. 1959). This technique involved rats being fed a test protein and then weighed to determine the mass gained which was compared to that established when casein protein was fed. Although this method is valuable at establishing the protein requirements of rats, it likely does not provide a strong indicator into the needs for humans as the protein requirements of a young growing rat are not the same as the protein requirements necessary for maintenance in adult humans (Whitney & Whitney. 2009)

Similarly, biological value (BV) has long been utilized to assess the true digestibility of a protein in that the method is based on the amount of nitrogen retained following intake of a protein source – with the belief that what is retained (not excreted in feces or urine) is being utilized by tissues in the body (Shrikantia. 1981). Generally, animal proteins receive a high value whereas most plant proteins score lower due to

insufficient amounts of one or more indispensable amino acids (Hoffman. 2004).

Moreover, there are several inherent problems with the BV method in that it does not take

into account factors which influence digestion and absorption such as anti-nutritional

factors and amino acid loss to bacteria in the large intestine. Net protein utilization (NPU)

method is another method used and is similar to BV method however it differs in that the

NPU measures nitrogen retention based on nitrogen ingested, whereas BV, as mentioned,

is based on nitrogen absorbed (Hoffman. 2004).

The current and most widely used method for the evaluation of protein quality is

the protein digestibility corrected amino acid Score (PDCAAS) (Schaafsma. 2000). The

PDCAAS is believed to the most accurate way to evaluate protein quality for humans,

because it uses humans as opposed to rat amino acid requirements to calculate the amino

acid score (Marks & Lister. 2008). This method is based on a scoring profile of 1 or less,

as it compares the amino acid profile of a protein with the essential amino acid

requirements for humans according to The Food and Agriculture Organization (FAO). It

is calculated by taking the limiting amino acid of the test protein divided by the same

amount of the reference protein, multiplied by fecal true digestibility percentage (Marks

& Lister. 2008). When a protein meets the requirement, it gets a score of 1.0 or greater.

If it does not, a score below 1 is provided. Proteins having a score greater than 1 receive

a truncated score of 1 as scores above 1 are considered an indication that the protein

contains indispensable amino acids equal to human needs. As a result, animal proteins

(especially egg and dairy proteins) receive on average, a greater score than most plant

proteins (Marks & Lister. 2008). The exception is soy protein which along with whey and

egg protein, receives a score of 0.9, while hemp protein receives a score of 0.66

(Calloway. 2004). Hemp protein receives a lower score on the PDCAAS because it is deficient in lysine (Young and Pellet. 1994). So using the PDCAAS, hemp protein and in fact, most plant proteins, especially grains, would be considered lower quality vs animal protein at maximizing the body's ability to utilize these proteins for tissue production, repair and maintenance. However, as with PER, BV, and NPU, the PDCAAS has inherent limitations (Schaafsma et al. 2005). Ileal digestibility is one such limitation in that amino acids that pass to the distal digestive tract may be consumed by bacteria and as a result not be excreted in the feces and thus not be calculated in the PDCAAS (Schaafsma et al. 2005). As a result, some amino acids believed to be used towards protein synthesis may be unavailable (Schaafsma et al. 2005). Further, the PDCAAS method does not take into account the effects of processing on protein quality or the effects of anti-nutritional factors on protein absorption (Marks & Lister. 2008). For example, soy protein which has, along with egg and whey protein, the highest PDCAAS value possible, a value of 1.0, is not necessarily an optimal source of protein because soy protein contains a trypsin serine protease inhibitor which reduces the nutritional value of the protein by limiting the enzyme's ability to act on peptide bonds (Schaafsma et al. 2005). Hemp protein does not contain such anti-nutritional factors (Liener. 2009) so its actual quality may be underestimated with the PDCAAS. Regardless, soy protein, primarily because of its PDCAAS, is often used as the plant protein of choice in research studies investigating the difference between the effectiveness of protein type on muscle protein synthesis and performance (Anthony et al. 2007; Brown et al. 2004; Hartman et al. 2008; Nikawa et al. 2002; Tang et al. 2009; Wilkinson et al. 2007). Additionally, although PDCAAS is utilized currently to evaluate protein quality, all methods

mentioned here, have inherent limitations in that these methods measure single proteins

and humans, rarely consume a single protein. None of the methods discussed here

evaluate the abundance of amino acids contained within traditional foods or meals which

is critical. As suggested by Young and Pellet (2004), the assessment of quality of

individual proteins is relatively meaningless considering the adult human diet which

contains numerous proteins. Consequently, the ideal protein quality assessment tool

would need to go beyond measuring individual ingredients. Thus, many proteins,

whether plant or animal, consumed post exercise could promote protein synthesis.

2.7.2   Plant vs animal on performance and protein synthesis

As discussed earlier, protein supplementation post exercise may be a necessary

step to maximize rates of myofibril and myofilament protein synthesis. It is believed that

that protein synthesis is maximized within the first few hours post exercise (Kumar et al.

2009) and because whey protein is considered a fast absorptive protein (Dangin et al.

2001), it is thought to be one of the most beneficial proteins to consume post exercise to

maximize muscle protein synthesis. This is due to its relatively high content of branched

chain amino acids (BCAA), especially leucine (Ha & Zemel. 2003). The BCAA, leucine,

the other two being valine and isoleucine, is considered the most powerful regulator of

the mTOR and P70-S6K pathways and has thus been shown to enhance the rate of

muscle protein synthesis (Anthony et al. 2001). Burke et al. (2001) found that adults who

consumed, in 4 equal servings, $1.2g.kg^{-1}.d^{-1}$ of whey protein over a 6 week strength

training program, experienced a two fold increase in strength and muscle hypertrophy

compared with an isoenergetic CHO supplement. Similar gains have been found not only

in performance, strength and muscle mass measures, but at the cellular level as well, in

both type 1 and type 2 muscle fibers. For example, Willoughby et al. (2007) found

greater increases in myosin heavy chain I and II gene expression following 10 weeks of

strength training with a pre – or post exercise whey protein supplement compared to a

CHO supplement. These results are consistent with those of Anderson et al. (2005) who

observed a greater hypertrophic response in both type I and type II muscle fibers with a

pre – and post exercise protein drink following 14 weeks of strength training. Moreover,

often in combination with a CHO supplement, protein ingestion post exercise increases,

among others, mTOR signaling pathways vs control following a bout of exhaustive

exercise (Willoughby et al. 2007). However, typically the nature and type of protein have

been considered to affect the anabolic response of muscle tissue differently. Investigators

have observed that animal protein, most commonly in the form of whey protein, is more

beneficial at maximizing protein synthesis than plant proteins, particularly soy protein.

For example, following 12 weeks of split body strength training, Hartman et al. (2008)

observed that dairy milk protein ingestion at a dose of 25g immediately and 1h post

exercise increased the rate of muscle hypertrophy vs an isonitrogenious soy protein.

These results are consistent with Tang et al. (2009) who found that mixed muscle protein

fractions were increased acutely following whey hydrolysate ingestion compared to a soy

protein isolate supplement. The exact mechanism for the inferior response rates

following the ingestion of soy proteins has not been clearly described. However, it may

be that the difference in BCAA profile and the anti-nutritional factors in soy, as

discussed, are involved. Further, whey protein is a rapidly absorbed protein vs slow

acting proteins such as casein and most plant proteins (Dangin et al. 2001). The more

quickly the amino acids enter the blood, the greater the rate of protein synthesis, acutely.

(West et al. 2011). Soy protein is also rapidly absorbed, but may be slightly slower than whey protein which could explain the observed lower rates of protein synthesis following its ingestion compared to whey (Tang et al. 2009). Although plasma amino acid kinetics have been identified for whey protein, soy protein and even rice protein, unfortunately, to date, little is known regarding the appearance of plasma amino acids following the ingestion of hemp protein isolate.

Nevertheless, the plant based protein hemp seed, has a leucine content of approximately 2.2g per 30g serving (Calloway. 2004) of protein powder that, according to Moore et al. (2009), would provide sufficient leucine (2.0g – 2.5g of leucine per 30g of protein) to stimulate muscle protein synthesis maximally in young men. Hemp protein and its effects following exercise have not been investigated to date and in fact, very little is known about the quality of hemp protein isolate. What is known however, is that the bioavailability of hemp protein is at least an adequate source of protein, especially compared with soy protein (Wang et al. 2008) (Table 2.1).

Table 2.1 – Protein profile of Whey, Hemp and Soy proteins

	PDCAAS	BCAA (g/100g)	LEUCINE (g/100g)
WHEY	1.0	24.5	10.7
HEMP	0.7	17	7.4
SOY	1.0	18	8.1

(Calloway. 2004)

That being said, the protein type that is consumed following exercise may be of little importance as long as the protein delivers sufficient indispensable amino acids. Although the wealth of nutrition science research indicates that whey protein has a more profound effect on protein synthesis immediately following exercise than most other protein sources, the net gain of protein over a 48h post exercise period – the time that

muscle protein synthesis can be optimally stimulated - may not be influenced by protein type but rather the availability of BCAA and the rate at which they become available. As Biolo et al. (1997) noted, hyper-aminoacidemia from exogenous protein consumption immediately following exercise increased rates of muscle protein synthesis and amino acid transport mechanisms more than when protein was delayed. However, the acute effects of hyper-aminoacedemia will not dictate completely chronic strength and muscle mass developments (Atherton & Smith. 2012). In other words, the immediate availability of amino acids which can be derived following animal protein ingestion may not be as crucial as the daily totals of amino acid availability over the long term. Thus, the ingestion of animal protein specifically, immediately post exercise, may have no greater effect on muscle protein synthesis or rate of glycogen re-synthesis over weeks and months than any other protein consumed as part of a regular diet. Since hemp protein has an amino acid profile that is sufficient to maximize protein synthesis rates, it might be used effectively as an immediate post exercise supplement in addition to a regular diet. Taken together, it is worth investigating whether an animal protein supplement post-endurance, strength and/or concurrent training is any more effective at improving performance and enhancing strength, muscle hypertrophy and aerobic capacity vs a hemp protein supplement.

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Chapter 3

Time Trial Performance 4h following Glycogen-Depleting Exercise is Enhanced Similarly with Recovery Non-Dairy Chocolate Beverages vs Chocolate Milk

3.1 Introduction

Post exercise nutrition is an important factor for re-fuelling depleted muscle CHO stores following exercise. Specifically, consuming CHO immediately after exercise is very effective at restoring skeletal muscle glycogen to pre-exercising quantities (Shirreffs et al. 2007). Further, it is generally accepted that nutritional interventions leading to increased glycogen storage during recovery would produce a substantial performance benefit for subsequent high intensity exercise, assuming the exercise bout is of a sufficient duration and intensity for glycogen to be limiting (Berardi, Noreen & Lemon. 2008). Results of several studies comparing a CHO-protein supplement with a CHO only supplement have found the former to have a greater influence at restoring muscle glycogen vs the latter (Ivy et al. 2003; Niles et al. 2001; Saunders et al. 2004). Relative to exercise performance, Berardi et al. (2008) found that a 2:1 CHO-protein (0.8g/kg CHO + 0.4g/kg protein) post exercise drink enhanced subsequent cycling time trial performance when compared to an isoenergetic CHO drink (1.2g/kg). Further, the CHO-protein drink produced an enhanced glycogen storage over the following 6 h likely explaining the performance effect (Berardi et al. 2008). In contrast, Ivy et al. (2003) suggested that the optimal ratio of CHO to protein for improving endurance performance is approximately 4 parts CHO to 1 part protein. Subjects completed a time to exhaustion bout that varied at intensities between 45% and 75% VO_2max for 3 h and then a separate bout at 85% VO_2max until fatigued. Researchers compared a flavoured aspartame-sweetened placebo supplement, with a CHO supplement (7.75%) and 4:1 CHO + protein supplement (7.75% CHO + 1.94% protein). Both contributed to an improved endurance

performance vs the placebo treatment, however, the CHO-protein enhanced the effect further. This performance benefit was attributed to the observed greater glycogen synthesis with the CHO- protein treatment compared to CHO only treatment. These findings are consistent with studies by Ivy et al. (2002) and Saunders et al. (2004), however, the latter study could not establish whether the CHO – protein ratio was responsible as the treatment beverages provided post exercise were not matched for energy.

Nevertheless, chocolate milk, with an energy content similar to many commercially available sport drinks (Pritchett et al. 2009), a 4:1 of CHO-protein, and both whey and casein (proteins known to stimulate both protein synthesis and insulin response) could be an effective post exercise supplement. In light of this, Karp et al. (2006) examined the effects of chocolate milk, a fluid replacement drink (Gatorade), and a CHO replacement drink (containing matched energy and CHO as the chocolate milk treatment) on time to exhaustion (70% VO_2max) during an endurance bout following a depleting bout of exercise and a subsequent recovery period. Carbohydrate content was equivalent for chocolate milk (CM) and CHO replacement drink (CR) (1.0 g/kg) and all three drinks were isovolumetric. Subjects cycled 49% longer with CM in comparison to CR. Although the three drinks were not isoenergetic, these data are intriguing because Thomas et al. (2009) found similar results with isoenergetic drinks. Consequently, it may be that a CHO-animal protein drink, with a favourable 4:1 CHO-protein ratio, can enhance endurance performance following glycogen depleting exercise. However, to our knowledge no one has investigated whether a CHO-plant protein beverage can elicit comparable results.

Therefore, the purpose of the current study was to establish whether two non-dairy varieties of chocolate milk, chocolate hemp and chocolate soy milk, are as effective as dairy chocolate milk for post-exercise recovery.   A secondary purpose was to assess whether a 4:1 CHO-protein mix is optimal for recovery compared to 6:1 (chocolate hemp), 4:1 (chocolate soy) and 1.5:1 mix (dairy milk).   Essentially then, the data were used to determine whether the benefits of drinking chocolate milk post exercise are due to its carbohydrate to protein content, its protein type or simply, its total energy. It was hypothesized that given all protein drinks provided adequate carbohydrate and total energy, the type of protein within each of drinks would not have a meaningful effect on subsequent recovery performance.

3.2 METHODS

Participants

Ten trained male cyclists between the ages of 18-30 y (Table 3.1) were recruited to participate in this 5 week, crossover, counterbalanced, repeated-measure trial. Participants were recruited from the University of Western Ontario (UWO) Triathlon club and/or undergraduate Kinesiology program. Exclusion criteria included symptoms or medication for respiratory or cardiovascular disease, use of heart rate or blood pressure medications, and an allergy or sensitivity to dairy. Upon initial arrival at the laboratory, participants were informed of the purpose and associated risks, before written informed consent was obtained according to a protocol approved by the Research Ethics Review Board of Western University. Participants completed a 3-day food record before each

trial and duplicated all food and fluid intake prior to all sessions to control for any

differences in dietary intake among the trials.

Table 3.1 – Participant Characteristics

	Mean ± SD
Age (y)	21.8 ± 2.3
Height (m)	1.7 ± 0.1
Mass (kg)	73.4 ± 10.5
Body Mass Index (Kg/m²)	24.5 ± 2.6
VO$_2$max(ml•kg^{-1} •min^{-1})	61.2 ± 1.4

Experimental Design (Figure 3.1)

Familiarization Procedures

Each participant performed an introductory familiarization session at the Exercise

Nutrition Research Laboratory at Western prior to any data collection. This was designed

to minimize any learning effects.

Baseline Tests

Each participant arrived at the laboratory after an 8-h overnight fast (water was

allowed) and having abstained from caffeine, alcohol and high intensity exercise for a

minimum of 24 h. Participants consumed a small standardized breakfast (125ml orange

juice, 2 eggs, 250ml of plain low fat yogurt) no less than 60 minutes prior to testing.

VO$_2$peak was determined during incremental exercise to volitional fatigue using

the Velotron Coaching Software (Racer Mate, Seattle, U.S.A). Starting at 125 watts (W),

the workload was increased by increments of 50W every minute (duration was 10.8 ±

1.1min). Seat height and handlebars were adjusted based on individual preference and

were kept standard across trials. Participants were fitted with a Polar RS200TM heart rate

(HR) transmitter (Polar RS200sd RED USA/CAN) for all testing. Oxygen consumption was assessed using an online breath by breath gas collection system (Vmax Legacy Sensory Medics, California, U.S.A) or a metabolic cart (Parvo Medics' TrueOne 2400, Sandy, Utah), Both systems were calibrated before testing by using gases of known concentration and, where necessary, a 3 L syringe. A silicon face mask (8940 Series, Hans Rudolph Inc, Kansas City MO USA) positioned on the subject's face with strapping was used after being checked for leaks. VO_2peak was determined at the point where participants could no longer maintain a continuous cadence of 50 RPM.

Glycogen Depletion Trial

Each session began with a VO_2 peak test identical in nature to the baseline testing to accommodate for day-to-day variability of peak power output. Participants then performed an exercise bout designed to lower glycogen stores substantially as described elsewhere (Jentjens & Jeukendrup. 2003). In brief, participants cycled in 2 minute intervals alternating between workloads of 90% and 50% of maximal power output, respectively until they were unable to continue at a self-selected cadence between 75 and 85 rpm. Once this occurred, power output was reduced to 80% maximal power output and the participant continued alternating between 80% and 50% max power output each 2 minutes, as above. When the participant was unable to continue at the appropriate cadence, the power output was reduced to 70% max power output and alternated between 70% and 50% maximal power output. Then, if the participant was unable to continue at the appropriate cadence, the test was completed. Participants consumed water ad libitum and the amount ingested was recorded (649ml ± 166ml).

Recovery Period

Recovery drinks were consumed immediately following the glycogen depletion trial and at 30 minute intervals for the first 2 hours of a 4 hour recovery period. The order of trials was randomized and counter balanced. Participants consumed either 1% Chocolate Milk (CHOC) (*Beatrice Chocolate Milk Beverage, Parmalat Canada Inc.*), low fat 1% Milk (MILK) (*Beatrice 1% Milk, Parmalat Canada Inc.),* Hemp Chocolate Milk (HEMPCHOC) (*Chocolate Hemp Bliss, Manitoba Harverst, Winniped Manitoba Ca*), Soy Chocolate Milk (SOYCHOC) (*Silk Chocolate Soy Milk, White Wave Services Inc.)* or a low calorie placebo (PLACEBO) (*Crystal Light Lemon Lime, Kraft Canada Inc*.). All drinks, except the PLACEBO were isoenergetic (504 ± 64 kcal) and all chocolate flavoured drinks provided at least 1g carbohydrate•kg body mass^{-1}•h^{-1} (Table 3.2). Fluid intake across CHOC treatments was equalized (1796 ± 498 ml) by ingesting appropriate quantities of water (in separate bottles) based on milk intake. All drinks were provided in opaque bottles and taste of drinks was chocolate flavoured. Participants remained in the laboratory during this 2 h time period. For the remaining 2 hours of recovery they were allowed to engage in normal daily leisure activities outside the lab such as going to the library and/or a class but no food or drink (other than water) were consumed.
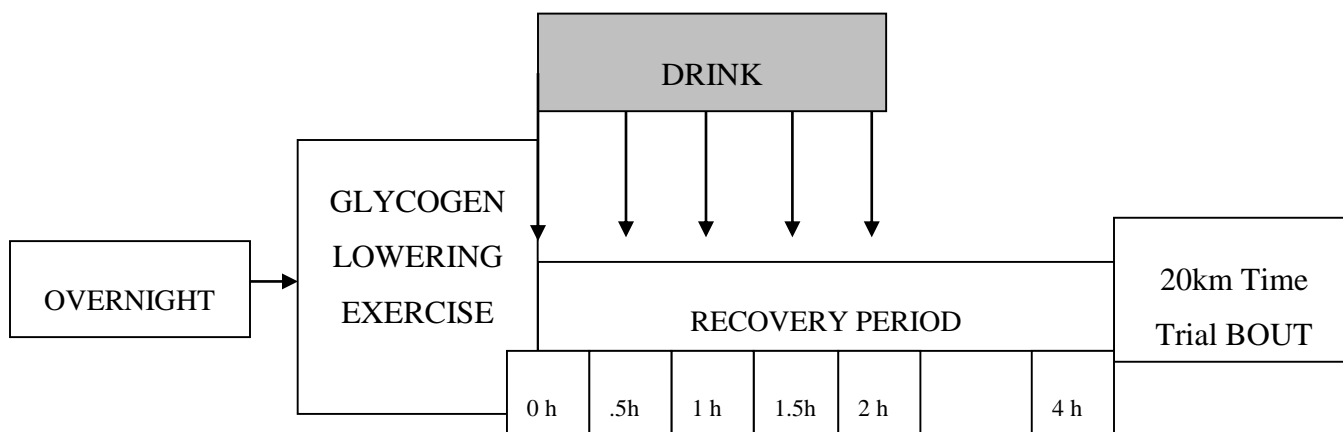
Table 3.2 - Macronutrient Composition

Trial Beverage	Carbohydrate (g)	Protein (g)	CHO-PRO ratio	Fat (g)	Energy (kJ)	Drink Volume (ml)	Water Consumed (ml)
CHOC	77.5 ± 9.7	17.6 ± 2.2	4:1	13.7±1.1	2107	1260 ± 162	1000 ± 16
SOYCHOC	80.11 ± 10.2	20.1 ± 2.5	4:1	11.5±1.4	2107	1669 ± 214	595 ± 21
HEMPCHOC	71.2 ± 8.7	11.5 ± 2.1	6:1	19.5±1.2	2107	1917 ± 243	345 ± 24
MILK	54.4 ± 7.1	39.4 ± 5.7	1.5:1	14.3±2.1	2107	2262 ± 299	0
PLACEBO	14.5 ± 1.5	0	n/a	0	247	2262 ± 290	0

Values are expressed as means ± SD

Post Recovery Bout

Four h following the glycogen depletion exercise bout, participants were reweighed and completed a best effort 20km time trial on a simulated computer race course at a self-selected pace on a cycle using the Velotron 3D software. The bicycle was interfaced to a laboratory computer and monitor allowing for real-time visual feedback. Participants were able to track their performance with a virtual cyclist whose speed was controlled by the subject's actual efforts on the bicycle, and a virtual opponent that was programmed to be 1-5 seconds distance ahead (not used as a pacer). This was to control for variability in how subjects' performances responded to pacers. No other feedback was provided. All participants were given verbal encouragement every 5km (5km, 10km and 15km) and were reminded that the test was a maximal effort to be completed as quickly as possible.

Figure 3:1 Protocol Timeline

3.3 Statistical Analysis

Descriptive characteristics were computed for the participants and the

macronutrient composition of drinks (Table 3.2). A 1-way ANOVA for repeated

measures was used to compare time trial performance among the drink treatments. Tukey

post-hoc tests were used in the case of a significant *(p < 0.05) F* ratio to locate the

differences within the ANOVA. All statistical analyses were performed using the

Statistical Package for the Social Sciences for Windows software, v. 19.0 (SPSS Inc.).

All data were reported as means ± SD.

3.4 Results

Every participant completed all trials in full. The average number of workloads during the depletion phase was 12-14 and did not differ between trials ($p = 0.78$). Thus, all time trials were performed following similar glycogen depleting exercise. Water intake during the recovery was similar for each bout ($p = 0.18$). A significant group x time interaction ($p = 0.02$) was noted with Tukey post hoc test revealing a significant difference for all milk drinks (CHOC, SOYCHOC, HEMPCHOC, MILK) from PLACEBO ($p < 0.05$) (Figure 3.2). Further, there were no differences in HRavg (choc = 171 ± 7.02; soychoc = 172 ± 7.8; hempchoc = 169 ± 3.5; milk = 170 ± 170; CHO = 172 ± 5.4; $p = .440$) during any of the time trials.

20Km Time Trial Performance (min)
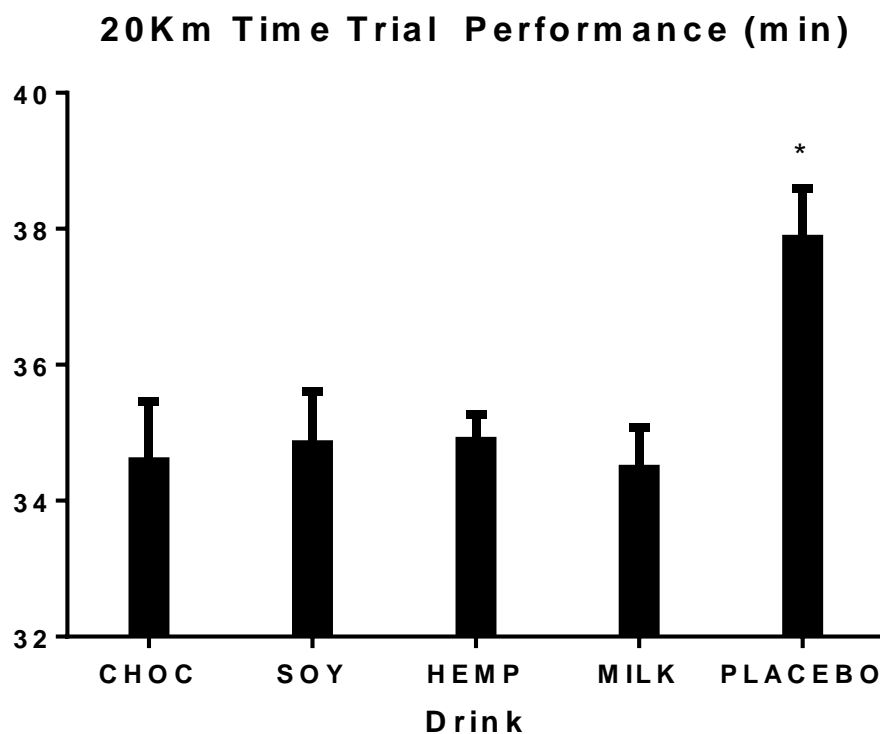
Figure 3.2- Time required to complete a computer simulated 20km time trial when participants ingested a dairy chocolate milk, a soy chocolate drink, a hemp chocolate drink, a dairy only drink and a low calorie placebo during a 4 h recovery period following a glycogen lowering cycling effort. Bars are means ± SD. $p < 0.05$ placebo vs CHO-protein drinks.

3.5 Discussion

The aim of this study was to assess the effect of four isoenergetic beverages with different carbohydrate-protein ratios and protein source vs placebo, consumed during recovery following glycogen-depleting exercise, on a subsequent post recovery cycling time trial. All recovery drinks were matched in total energy except the placebo and the chocolate drinks provided at least $1.0g.kg^{-1}.h^{-1}$ of CHO. The carbohydrate : protein ratio

of each varied with CHOC and SOYCHOC having 4:1 ratios, HEMPCHOC with a 6:1 ratio and MILK with a 1.5:1 ratio. Following a depletion bout of intense cycling, across all subjects, there was no difference in subsequent 20km time trial performance for any of the treatment drinks and all drinks produced significantly faster times than the placebo. These data suggest that the CHO and protein content of all treatment drinks was sufficient to maximize performance during this same day 20 km time trial.

Recently, dairy chocolate milk has been reported to be an effective post exercise drink due to its apparent ability to restore energy stores and, therefore, to enhance performance following a recovery period (Karp et al. 2006 & Thomas et al. 2009). Ferguson-Stegall et al. (2011) reported that dairy chocolate milk intake during a recovery period following high intensity endurance exercise produced a faster post recovery 40km time trial, a greater rate of glycogen synthesis, and a significant increase in intracellular signaling stimulus for protein synthesis when compared to an isoenergetic matched carbohydrate drink or a placebo. The current 20km time trial data are consistent with the findings of Ferguson-Stegall (2011) in that all chocolate beverages produced significantly faster time trial times than the placebo drink. However, the novel information with the present study is that there were no differences between the CHO-protein beverages even though they differed in both protein type (dairy, soy or hemp) and carbohydrate to protein ratios (1.5:1 to 6:1).

As noted, the current study used drinks with varying protein to carbohydrate ratios. With chocolate milk having what is considered generally to be the optimal carbohydrate-protein ratio of 4:1, it was expected that the time trials following CHOC consumption would produce significantly faster time trials than the other drinks.

However, no such difference was found in this study. Despite previous work by Ivy and colleagues (2002 & 2003) who found that ingestion of a carbohydrate-protein solution with a 4:1 ratio, enhanced cycling performance significantly among 9 trained male cyclists, other studies comparing a carbohydrate-protein supplement with a carbohydrate only supplement on performance have found no such benefit. For example, Romano-Ely (2006) reported that a carbohydrate-protein (animal protein with a 4:1 of CHO to protein) drink consumed during and after exercise did not enhance time-to-fatigue cycling performance compared with an isoenergetic carbohydrate drink. Further, Millard-Stafford et al. (2005) reported that following a long intense run and a subsequent recovery period, the ingestion of a carbohydrate-protein (~4:1) beverage vs an isoenergetic carbohydrate only beverage did not significantly affect subsequent performance bouts. The discrepancies found in the studies of Millard-Stafford et al. (2005) and Romeno-Ely (2006), make it difficult to conclude that a 4:1 carbohydrate-protein ratio has the greatest impact on recovery when provided post exercise. In fact, several studies report benefits with alternative carbohydrate-protein ratios. For example, Berardi et al. (2008) found that a 2:1 carbohydrate-protein supplementation drink enhanced cycling time trial performance compared to a carbohydrate drink containing the same energy content. Consequently, the carbohydrate-protein ratio appears to be unimportant as long as the CHO and protein are sufficient.

Further, the present study indicates that performance is increased regardless of the type of protein found in these drinks. Previous research has highlighted the benefits of dairy milk on protein synthesis including a sustained rise in blood amino concentration (Wilkinson et al. 2007), the phosphorylation of mTOR pathway (Wilkinson et al. 2007)

and activation of glycogen synthase (Ivy et al. 2008). If dairy protein was critical, the CHOC treatment should have exceeded all other chocolate flavoured drinks because none of the other drinks contained dairy proteins. However, there was no significant differences between any of drinks suggesting that the enhanced 20km time trial cycling performance was similar with dairy, soy and/or hemp protein post exercise beverages ingestion. Although Wilkinson et al. (2007) observed that the consumption of dairy milk resulted in greater increases in fat free mass than either soy protein or control groups, to date, few studies have reviewed the effects of soy protein with endurance exercise. Similarly, hemp protein, has garnered even less attention on either strength or endurance performance. The current time trial data (times, HR and VO_2peak) suggest that both vegetarian proteins combined with CHO were comparable to dairy chocolate milk as a recovery nutritional supplement following glycogen depleting exercise.

The lack of any significant changes in time trial performance between recovery supplements including milk is perhaps more an indication that, once adequate CHO and protein are available, the provision of sufficient energy is the key rather than the type of protein or the ratio of carbohydrate-protein; a theory which is supported by the work of Betts et al. (2007), Gilson et al. (2010), Jentjens et al. (2001), Van Essen et al. (2006) and Watson et al. (2008).

Although we matched for total energy and volume of liquid provided and consumed throughout each of the trials for all participants, we did not match for caffeine in all drinks which has been shown to have ergogenic effects (Bruce et al. 2000; Ivy et al. 2009; Spriet. 1995). However, this is unlikely to be important because the total caffeine consumed from the chocolate beverages (8-12mg) falls well below the recommended 3-

9mg/kg body weight (Spriet. 1995) which has been shown to increase time trial performance (Bruce et al. 2000; Ivy et al. 2009).

This study supports previous findings by Karp et al. (2006) and Thomas et al. (2009) that chocolate milk is an effective recovery beverage following glycogen depleting exercise. Additionally however, the current study highlights that with respect to aerobic time trial performance following glycogen depleting exercise, as long as adequate protein and carbohydrate are available, the energy content of a glycogen repletion post exercise beverage may be more important than whether the protein source is animal or vegetable.

3.6 References

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Chapter 4

Strength training gains are enhanced similarly with post exercise plant (hemp) or animal protein (whey and dairy milk) ingestion

4.1 Introduction

The importance of maintaining adequate muscle mass for the purpose of increased resting metabolism, fat loss and physical strength is well known. Although strength training provides powerful stimuli for protein synthesis, dietary supplementation with protein has been shown to be an added benefit (Biolo et al. 1997). In particular, whey protein isolate has been found to be an effective stimulus for protein anabolism (Burke et al. 2001; Katsanos et al. 2008). It appears the benefits of whey protein are due primarily to the high content of branched chain amino acid (BCAA) and the rapid and more transient rise in plasma amino acid concentration that occurs following its ingestion (Wilkinson et al. 2007). Conversely, casein, the other major protein found in dairy milk, has also been shown to be an effective protein supplement for building muscle mass and strength, to a similar (Tipton et al. 2004) or perhaps a slightly lesser extent than whey (Cribb et al. 2006 & Hall et al. 2003). When investigating the combined effects of whey and casein ingestion in the form of dairy milk, Hartman et al. (2007) found that following 12 weeks of strength training exercise (5 d/wk), the consumption of dairy milk immediately and 1 hr post workout, produced significant increases in muscle cross sectional area. Further, Wilkinson et al. (2007) found that compared with a soy protein based drink, dairy milk provided a greater increase in protein accretion following strength training exercise. Kerksick et al. (2006) observed that post exercise supplementation with casein and whey proteins throughout a 10 week, 4 day a week split body strength training program, increased strength and lean mass vs a whey + branched chain amino acid supplement and a carbohydrate placebo supplement. Thus, Phillips (2010) proposed that consuming either whey protein or dairy milk (whey + casein) proteins post-exercise in sufficient quantities, promotes muscle protein synthesis, muscle strength and

hypertrophy. However to date, there has been no direct comparison between the ingestion of dairy milk vs whey protein isolate immediately following bouts of supervised and chronic strength training.

In addition to whey and dairy proteins, the plant protein soy has been studied relative to strength effects (Anthony et al. 2007; Evans et al. 2007; Wilkinson et al. 2007). Compared with whey and diary milk, post exercise ingestion of soy protein appears to be inferior for stimulating muscle protein synthesis and strength (Hartman et al. 2007; Wilkinson et al. 2007). However, another plant protein, hemp protein isolate with its relatively high content of branched amino acids (Callaway et al. 2004) and high digestibility (Wang et al. 2008) may be a more optimal source of plant protein than soy. If so, hemp protein could provide an alternate source of protein for vegetarians looking for a post exercise supplement (Borrione. 2009). However, to date there are no studies which have investigated its potential to increase muscle mass and strength. Therefore, the purpose of the current study was to examine whether post exercise hemp protein ingestion can enhance muscle mass and strength following 10 weeks of strength training vs whey protein or low fat dairy milk in women. Given the amino acid profile and bioavailability of hemp protein, it was hypothesized that hemp protein ingestion would enhance muscle mass and strength following strength training.

4.2 Methods

Participants

Sixty young (18-39y) untrained women were recruited and forty five completed all study components. Women were excluded from the study if they had a body mass

index (BMI) under 35 kg/m², ingested a protein supplement for 4 week period prior to the

start of the supplementation, had strength trained over the previous three months,

abstained from any nutritional supplement that may affect muscle growth or the ability to

train intensely for the duration of the study, and/or had a history of muscle, tendon or

joint injuries. All were informed of the study risks and benefits prior to signing an

informed consent document approved by the Research Ethics Board of the University of

Western Ontario and the Research Ethics Committee of Humber College. Following a

familiarization session of all procedures and subsequent baseline testing, participants

were matched for age and lean muscle mass and then randomly assigned to one of four

groups (three treatment or the placebo).

Body Composition Testing

Body composition was measured via densitometry using the Bod Pod® (software

version 1.69), as outlined by the manufacturer. Before testing, the scale was calibrated

using two 10-kg weights, and the Bod Pod® chamber using a cylinder of known volume.

The participant's height was measured using a stadiometer to the nearest 0.1cm and each

subject was weighed wearing only a tight-fitting swimsuit or undergarments and a lycra

swim cap. Participants sat in the chamber and body volume measurements were taken.

This measurement was done in duplicate, with each test lasting approximately 40 s. If

both measures were within 150 mL of each other, the mean was taken and used in

subsequent calculations. If the two measurements differed by > 150 mL, a third

measurement was performed. If two of the three measurements were within 150 mL of

each other, the mean of those two were taken and used, but if the three measurements

were not within 150 mL of each other, the entire process, including the calibration steps,

was repeated. The resultant body density was used in the Siri equation (BF = (4.95/ρ −

4.50) × 100; Siri. 1961) to estimate body composition. All calculations were done using

the system software. This testing was completed pre- and post training.

Hypertrophy -Magnetic Resonance Imaging (MRI)

Magnetic resonance images were acquired via serial axial plane in a 3.0 Telsa

magnet. For the thigh, proton density images were acquired using the following

parameters: repetition time (TR) of 2000ms, echo time (TE) of 19ms, 256x256 matrix,

250x250 field of view, slice thickness of 4mm with no separation. Participants lay

supine, feet first, with the mid-thigh iso-centered to the bore of the magnet. Two series

of 35 slices each were obtained imaging the entire musculature of the thigh.

For the arm, proton density images were acquired using the following parameters:

repetition time (TR) of 2000ms, echo time of 19ms, matrix of 192x192, 192x192 field

of view, 4mm slice thickness with no separation (arm iso-centered to the bore of the

magnet). Three series of 17 slices each were obtained imaging the entire musculature of

the arm.

Using the acquired images, muscle cross-sectional areas were calculated pixel-

wise via a combination of manual and semi-automated techniques using open-source

OsiriX image processing software (version 3.7, Geneva, Switzerland). Briefly, a

region of interest (ROI) was outlined manually on the slice with the greatest cross-

sectional area with the brush tool and repeated every five slices; missing ROI were

automatically generated. With the ROI outlined, all pixels outside the ROI were set

to zero. A 3D threshold-growing tool was used to ensure only muscular tissue was

included in the ROI (excluding non-contractile tissue and septal spaces). Any errors

produced by the automatic generation were corrected manually. Cross-sectional area

was calculated automatically by the software program. This procedure was repeated

using the same muscle segment pre- and post-training.

Strength Testing - Biodex

Individual leg and arm isometric and isokinetic strength was assessed using a

Biodex Multi-Joint System 3 Dynamometer and accompanying software. Following body

composition testing, participants were seated on the Biodex dynamometer chair with their

non-dominant leg or arm aligned with the appropriate axis of the dynamometer. For

isokinetic leg measurements, participants were seated against a back support with

stabilizing straps positioned across the chest and the distal one third of the tested leg. The

axis of rotation of the dynamometer lever arm was positioned with the lateral femoral

condyle and a resistance pad was secured over the distal anterior one third of the thigh.

The dynamometer was set in the concentric mode for knee extension/flexion at an

angular velocity of 60 degrees per second. Range of motion consisted of movement from

90 degrees to 170 degrees of knee flexion. Participants were instructed to perform knee

extension and flexion as fast and as hard as possible for 5 consecutive repetitions. The

highest peak torque was obtained during the 5 repetitions for extension and flexion.

Following 5 minutes of rest, participants performed isometric contractions on the same

leg. The dynamometer set up remained the same for these trials. Participants were

instructed to perform three maximal isometric leg contractions for 5 seconds for 3

repetitions with 5 second rest between each repetition. Peak torque was obtained during

the 3 repetitions for extension and flexion. Following isokinetic and isometric leg

testing, participants were given 10 minutes rest before proceeding to isokinetic arm

measurements. For isokinetic arm measurements, participants were positioned in the Biodex chair with their nondominant shoulder in the anatomical neutral position, elbow flexed to 90º and forearm supinated. The axis of rotation of the dynamometer lever was positioned at the lateral epicondyle of the humerus. The dynamometer was set in the concentric mode for elbow extension/flexion at an angular velocity of 60 degrees per second. Participants were instructed to perform an arm extension and flexion as fast and as hard as possible for 5 consecutive repetitions. The highest peak torque was obtained during the 5 repetitions for extension and flexion. Following 5 minutes of rest, participants performed isometric contractions on the same arm. Isometric contractions by grasping a handle and pushing against the stationary lever arm were then performed. Participants then performed three maximal isometric elbow contractions for 5 seconds for 3 repetitions with 5 second rest between each repetition.

Strength Testing - 1 Repetition Maximum (RM)

1 RM prediction equations were used to determine training intensity as the participants were novice lifters and multiple 1RM's were collected over the 10 week training program. As noted by Dohoney et al. (2002), 1 RM prediction equations for lower and upper body using 4-6 reps is an effective and efficient method for predicting the 1 RM of novice lifters. Briefly, participants were asked, on week 1, 3, 6 and 9, to perform maximum repetitions of leg curls, leg extension, bicep curls and tricep extension exercise with the mass prescribed by their personal trainer who followed 1 RM mass predictions according to ACSM guidelines (ACSM, 2005b). Briefly, following familiarization with the movement, participants performed a light warm-up of 5 minutes.

An estimated weight was added and the participants performed the movement no more than 5 times. If the participant was successful at lifting the weight, 5 minutes of active (walking) recovery was allotted and then participants lifted a heavier weight using the same sequence until a failed attempt occurred. A maximal of 5 attempts was allowed during a single session. Participants returned 48h later if a 1RM could not be determined during the first session. All lifts were supervised by certified personal trainers and were recorded, by the trainers, in training journals.

Strength Training Program

All sessions were supervised by a Can Fit Pro or Canadian Society of Exercise Physiology (CSEP) certified personal trainer (CPT) in a one-on-two or one-on-three fashion. The strength training program began 1 to 2 weeks following baseline testing and was a 3 day whole body routine consisting of 6-7 exercises per workout with equal volume distributed to the biceps and triceps and similarly, to the quadriceps and hamstrings (Table 4.1). The sequence and order and tempo (2/0/4/0 – 2 sec concentric phase, no pause, 4 sec eccentric phase, no pause; repeat) of exercises were maintained for the entire 10 week training period. All participants worked at intensities of 75-85% of their estimated 1RM with 4 sets; sets 1 to 3 were performed with 8-10 repetitions and 90 second rest intervals while set 4 was performed at approximately 90-95% of 1RM and to exhaustion. 1RM prediction measurements were taken at week 1, week 3, week 6 and week 9. All participants were provided a personal logbook which the trainers used to chart progress.

Table 4.1- Training Program

Week 1-10, 4 sets w/ 3 sets @ 80-85% 1-RM, 10-12 reps & 4th @ 90% 1-RM to failure

MONDAY	WEDNESDAY	FRIDAY
Hack Squats	Leg Press	Leg Extension
Leg Curls	Hack Squats	Leg Curls
Leg Extension	Zottman Curls	Leg Press
Seated Angled Curls	Cable Tricep Extension	Seated Bicep Curls
Seated Eccentric Bicep Curls	DB Lateral Raises	Cable Tricep Extension
Seated Overhead Tricep Ext	Standing DB Bicep Curls	Lat Pull Down
Abdominal Crunches	Abdominal Crunches	Abdominal Crunches

Protein Supplement

Treatment groups included whey protein (W) (*Ergogenics New Zealand Whey Protein Isolate, BC, Canada*), low fat dairy milk (with added whey protein) (MILK) (*Beatrice Chocolate Milk Beverage, Parmalat Canada Inc.*), hemp protein (H) (*Manitoba Harvest Hemp Pro 70, Winnipeg Manitoba*) and a carbohydrate (maltodextrin) group (MD) (*Maltodextrin, QSE Gold Medal Carb)*. All treatment drinks were isoenergetic, isovolumetric and all three protein drinks contained at least 21g of protein (Table 4.2). The supplement dose was based on previous estimates of maximum protein intake for a young male population (Moore et al. 2009). The Whey and Hemp powders were tested independently (Agri-Food Laboratories, Guelph, Ontario Canada) for correct labeling of protein content (Table 4.3). Individuals not associated with the exercise training program of the participants were responsible for all drink making and distribution to participants in unmarked, identical and sealed containers to ensure that a double blind protocol was maintained. Participants were given their drinks immediately following the completion of each exercise training session and were monitored to ensure that all contents of the drink were consumed within 15 minutes of completing their final exercise. Further,

participants confirmed throughout the study that they did not ingest any other protein for

at least 3 hours after the protein drink and did not take any additional protein powder or

protein bar supplement at any time throughout the entire 10 week training program.

Table 4.2 - Drink Composition

	Energy (kJ)	Carbohydrate (g)	Protein (g)	Fat (g)	Volume (ml)
Milk	820	28	21	0	471
Whey	820	10	34	0	471
Hemp	820	18	21	5	473
MD	820	49	0	0	479

Table 4.3 - Nutritional Analysis of Whey and Hemp protein powders

Source (per gram of powder)	Protein Content - %
Whey protein	82
Hemp protein	67

Food record

Daily nutritional intake was recorded using a 3 day food intake record which was

provided to all participants prior to the start of the study and during week 5 and 10.

Records were analyzed using Nutrition Pro Software. All participants were given explicit

instructions on how to complete the questionnaire from an experienced nutritionist.

4.3 Statistical Analysis

All data were analyzed by a two way ANOVA using SPSS for Windows version

19.0 software. If a significant group interaction was observed, least significant

differences (LSD) post-hoc analyses were performed to locate the pair-wise differences between means. Data are presented as means ± SDs and a p value set at < 0.05.

4.4 Results

Participant Characteristics

At baseline there were no differences in age, height, mass or percent body fat (Table 4.4). Forty five participants completed all post study measures (MD, n = 8; Milk, n = 10; Hemp, n = 12; Whey, n =15)

Table 4.4   Baseline Characteristics of all groups

Participant Characteristics	MD N = 13	Milk N = 13	Hemp N =17	Whey N =17
Age (y)	24 ± 6	24 ± 3	25 ± 4	23 ± 2
Mass (kg)	68 ± 10	72 ± 11	65 ± 15	70 ± 15
Height (cm)	160 ± 6	165 ± 7	162 ± 6	166 ± 6
Body Fat (%)	31 ± 5	32 ± 7	30 ± 7	31 ± 8

Values are means ± SD.

Dietary Assessments

No difference in daily dietary energy (MD 6445 ± 133; MILK 7310 ± 91; H 6403 ± 71; W 6870 ± 65 kJ.d^{-1}, p = 0.25) or protein intake (MD 0.9 ± 0.02; M 1.1 ± 0.3; 1.0 ± 0.3; 1.1 ± 0.2 g.kg^{-1}.d^{-1}, p = 0.09) was detected throughout the study. Note that only 33 of 45 respondents (Groups: W = 9, MILK = 8, H = 8, MD = 8) had their nutritional data analyzed due to some participants failing to provide all three 3 day food diaries (n=10) and because 2 were not completed appropriately.

Body Composition

No interaction effect in body fat % was detected (p = 0.56) however the average

decrease in body fat percentage over the course of training was 2.1%. A main effect of

training for lean mass percentage was observed with increases from 69.1 ± 2.4 (mean,

SD) to 73.2 ± 3.2 (p < 0.001) over the course of 10 weeks (Table 4.5) again however, no

group (p >0.05) or interaction effect (p > 0.05) was observed.

Table 4.5 Body composition across all groups.

Variable	PRE-	POST
Body Fat (%)	30.9 ± 6.8	28.7 ± 6.6
Lean Mass (%)	69.1 ± 2.4	73 ± 3.2
Body Mass (kg)	68 ± 14	69 ± 15

Values are means ± SD
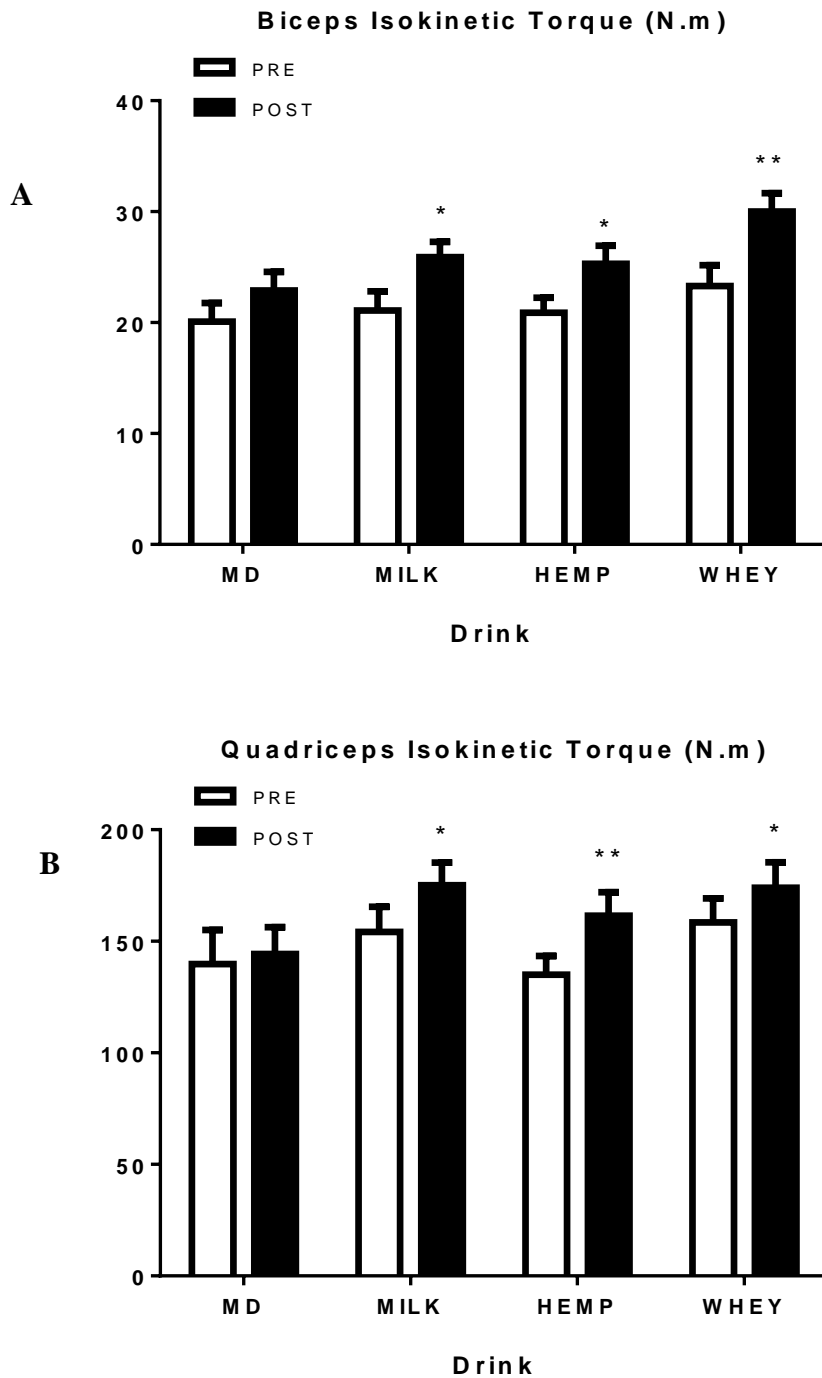
Strength

Data from the Biodex revealed main effects of time for isometric strength

(p < 0.001, p < 0.01), isokinetic strength (p < 0.001, p <0.001) and isokinetic power

(p < 0.001, p < 0.001) in both arms and legs, respectively. Significant group x time

interaction effects were observed after 10 weeks of training, whereby post hoc analysis

showed strength was greater compared to MD in all three protein groups for bicep

isometric strength (p = 0.007) (figure 4.1) and both bicep (p = 0.03) and quadriceps (p =

0.05) isokinetic strength (figure 4.3). Further, least significant post hoc analysis revealed

that bicep isokinetic torque following W consumption was greater (30 N.m ± 5.3) than

both H (25.3 N.m ± 4.9) and MILK (25.9 ± 4.4) (p = 0.04) after 10 weeks of training.

Further, the H group had a greater increase in quadriceps isokinetic torque (133.3 ± 51 to

161.5 ± 33.2; p = 0.05) than both W (158.3 ± 32 to 174 ± 36.1) and MILK (154.3 ± 34 to

175.1 ± 32.2) groups. (Figure 4.2)
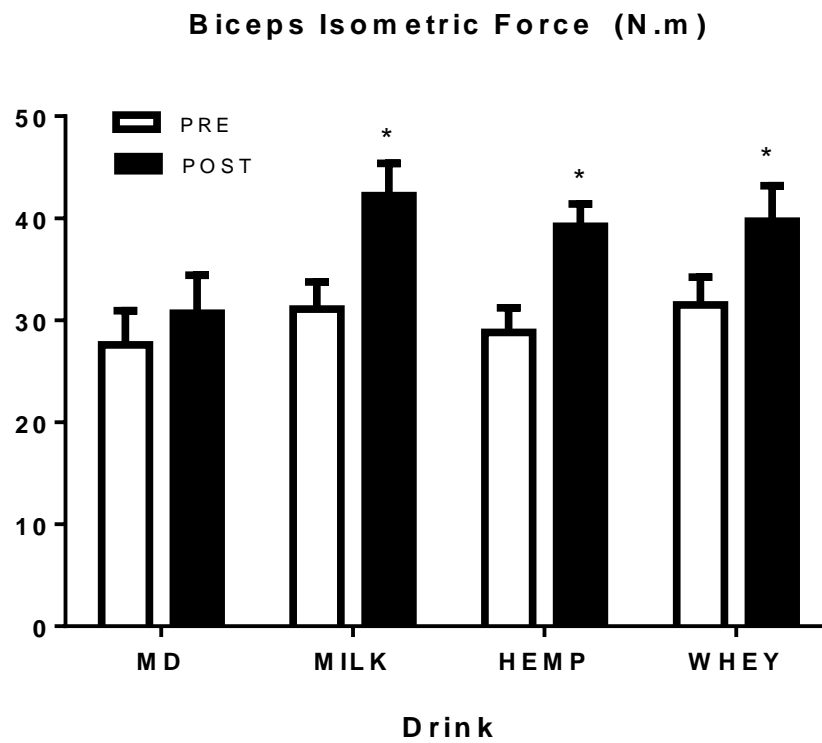
Figure 4.1 Isometric force (N•m) of non-dominant biceps pre- and post strength training for 10 weeks in all groups. Group x time interaction (p = 0.007). * Significantly different from MD for same condition (p < 0.05). Data are means ± SD.

Figure 4.2 Isokinetic torque of the non-dominant Biceps brachii (A) and Quadriceps (B) pre- and post strength training for 10 weeks in all groups. Group x time interaction for A (p = 0.05) and B (p = 0.03). *Significant difference from MD for same condition (p <0.05). ** Significantly different from MILK and HEMP in A (p < 0.05) and from MILK and WHEY in B (p < 0.05). Data are means ± SD.

Further, after training, both bicep isokinetic power (p = 0.001) and quadriceps isokinetic

power (p = 0.02) were greater in all protein groups vs MD with no significant difference

between protein groups (Figure 4.3).
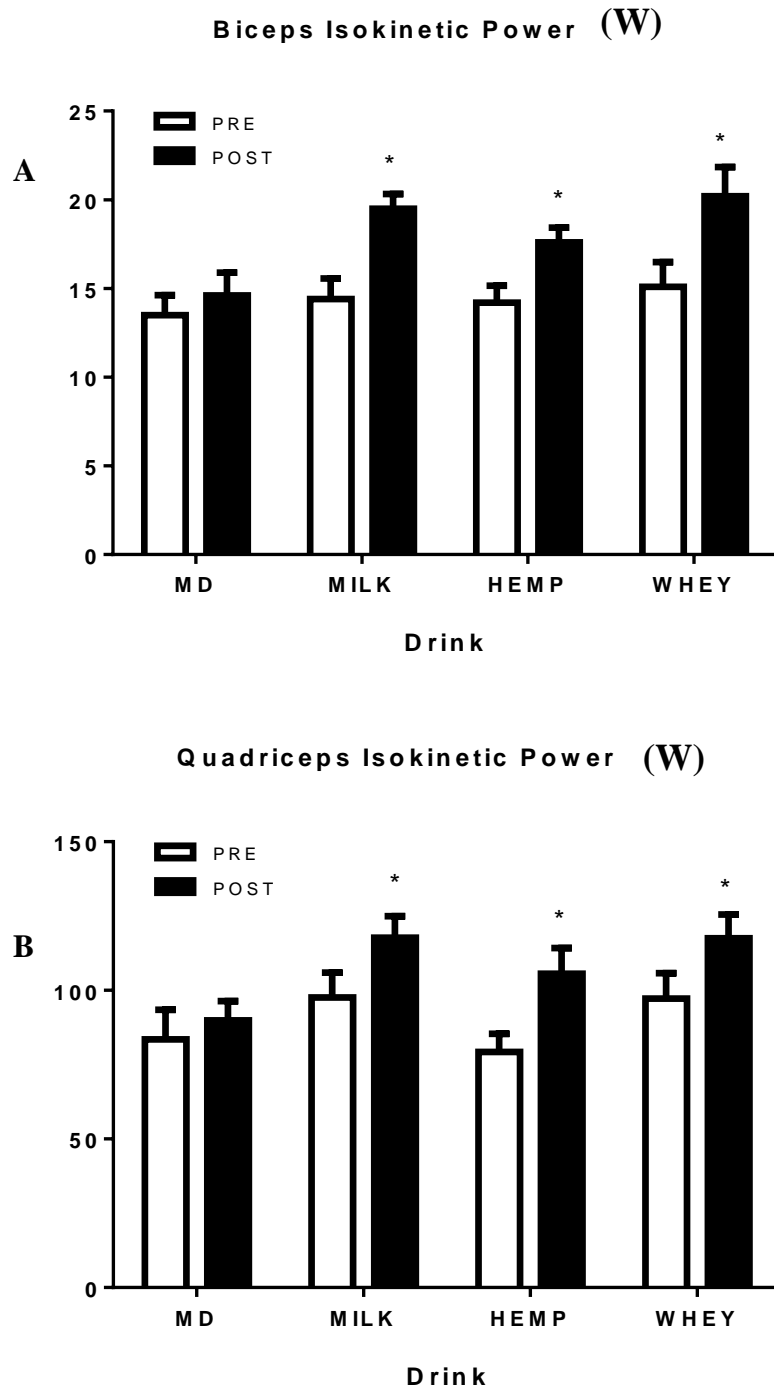
Figure 4.3 - Isokinetic power of non-dominant biceps (A) and quadriceps (B) pre- and post strength training for 10 weeks in all groups. Group x time interaction in A (p = 0.001) and B (p = 0.02). *Significant difference from MD for same condition in A (p <0.05) and B (p < 0.05). Data are means ± SD.

A main effect of time was observed for predicted 1-RM scores over the 10 weeks ($p = 0.01$) with no interaction effects ($p < 0.05$). 1RM data were collected for all exercises throughout the study, however only bicep curl and leg press are reported. Predicted 1RM increases for both biceps (figure 4.5a) and quadriceps (figure 4.5b) are displayed below.

Predicted 1RM - Bicep Curl (kg)

A

Predicted 1RM - Leg Press (kg)

B
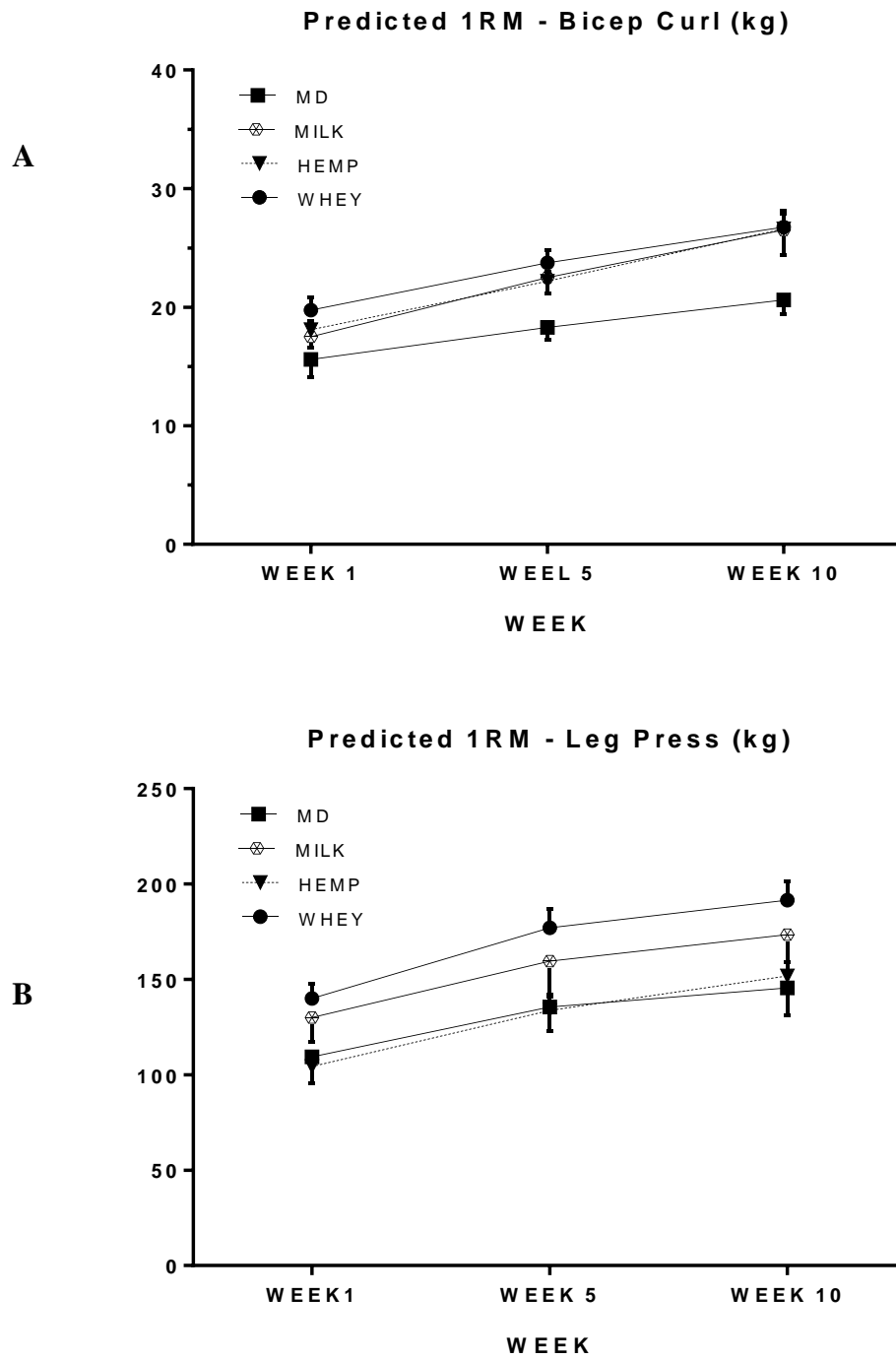
Figure 4.4 **A)** Predicted 1 RM biceps data from week 1 of training to week 10. Data are means ± SD. **B)** Predicted 1 RM quadriceps data from week 1 of training to week 10. Data are means ± SD.

Muscle Area

A main effect of time was observed (p = 0.01) indicating a training effect occurred; however, there was no significant group x time interaction (p > 0.05). Cross sectional area (CSA) (Figure 4.5) increases in cm^2 were 8% and 7.3% for arm and thigh musculature, respectively (Table 4.6). Further, the increase in the thigh musculature was not significantly different from the increase in the arm (p > 0.05).

Table 4.6 – Arm and Thigh CSA

	Arm and Thigh CSA (cm²)	
	Arm	**Thigh**
Pre-	26.2 ± 5.8	117 ± 22.4
Post	28.25 ± 6.2	126 ± 24.6
Values are presented as means ± SD.		

Figure 4.5 – MRI CSA Image of Arm and Thigh Pre and Post Training

Representative MR images from one participant pre- & post-
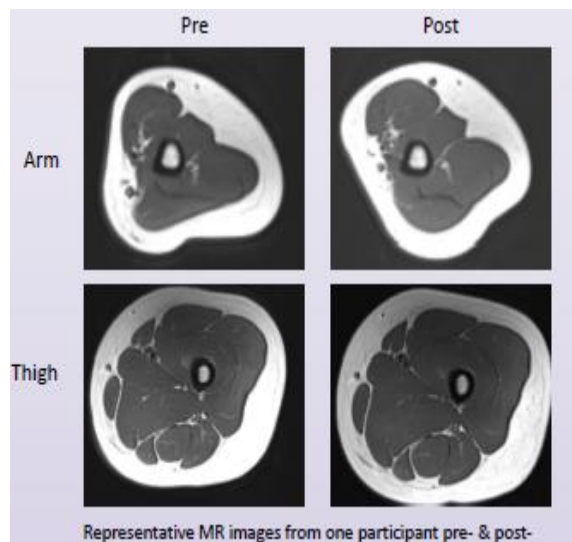
4.5 Discussion

Results of the present study indicate that post exercise CHO-protein supplementation in young untrained but physically active women enhances strength and muscle hypertrophy over 10 weeks of strength training compared to an isoenergetic CHO placebo. Additionally, protein type, whether milk, whey or hemp, appears to have little influence on the degree to which participants increased strength and muscle size when sufficient amounts of protein were provided chronically.

Importantly, the present study indicates that the plant based CHO-hemp protein, increased arm and leg muscle strength similarly to CHO-whey protein and dairy milk. The absence of meaningful between-group differences between plant proteins and animal proteins have not been well supported by previous research examining the effects of soy protein vs. whey protein supplementation following strength training. For example, following an acute bout of exercise (Wilkinson et al. 2007) and 12 weeks of strength training (Hartman et al. 2009), a soy protein drink immediately following exercise did not produce similar strength increases or rates of muscle protein synthesis compared to a whey protein supplement. However, in the current study, little difference could be found between CHO-hemp protein isolate, CHO-whey protein isolate and dairy milk. This may be because, as reported by Wang et al. (2008), the digestibility of hemp protein isolate, *in vitro*, is superior compared to soy protein isolate and, hemp protein has a proportion of essential amino acid to total amino acid that is greater than soy protein. When compared to whey protein and even dairy milk, hemp protein does not have the same amino acid profile, especially the BCAA content (Calloway et al. 2006). However, as noted by Moore et al. (2009), only 2.5g of leucine are required to stimulate muscle protein

synthesis maximally in young active men. Given that the participants in the hemp group in the present study were female, with a lower body mass on average than those in the study by Moore et al. (2009), and, were receiving about 2.2g of leucine per serving, it appears that the hemp protein provided a significant stimulus for protein synthesis. This suggests that protein amount and, subsequent amount of amino acid availability, and not protein type per se, is of prime importance for muscle accretion.

Participants in the current study had daily protein intakes that were in line with current recommendations. Based on the completed food intake records, our participants had a daily mean protein intake of 1.0g/kg of body mass, not including their treatment beverage, which is greater than the current protein RDA (0.8g /kg per day) for adults. Additionally, the protein drinks were provided in dosages of no less than 20g, an amount that is stated by Moore et al. (2009), to be the maximum absorbed amount per ingestion for young healthy men. Typically, women have a lower daily absolute protein requirement (Rand et al. 2003) due to a greater BMI and reduced muscle mass, so the amount provided in a bolus drink immediately following exercise in this study, should have been adequate to maximize the acute effects.

The addition of CHO-protein immediately following exercise training increased strength and muscle size quite consistently in all groups vs maltodextrin. Similar muscle gains have been observed previously in women with strength training (Candow et al. 2006; Josse et al. 2010). Of course, it has long been known that strength training is beneficial for increasing strength and muscle size in young females (Staron et al. 1991), however much less is understood about the potential interacting effects of protein supplementation in women engaged in vigorous strength training exercise. However,

Josse et al. (2010) found that young healthy women who consumed dairy milk immediately following strength training sessions, improved muscle strength and size with similar results to studies investigating men. Despite the fact that the etiology of this effect was not examined in our study, the results provide further support that protein supplementation following bouts of strength training is beneficial for women.

One important control within our study was that all drinks, regardless of protein type, were matched for energy, including the MD. Unfortunately, previous studies evaluating the effects of protein supplementation on strength training have often failed to match the different groups for energy in the supplementation drink, possibly affecting the outcomes (Cribb et al. 2007; Gater et al. 1992). Each of these studies show an improvement in muscle mass and size; however, the observed gains cannot be attributed to the supplemented protein-strength interaction because the experimental supplements or the comparable placebo controls were not matched for energy. As reported by Todd et al. (1984), increasing energy intake in participants who are exercising and taking protein supplements, also increases nitrogen balance. If supplements are not matched for energy, it becomes impossible to assess the effects of the protein supplement on muscle changes. As shown in table 4.2, all the supplement drinks were isoenergetic. Of course, matching energy can affect the matching of other macronutrients when using commercial products as supplements and thus, protein intake varied as well. The hemp protein and dairy milk drinks each contained 21g of protein while the milk + added whey drink had 34g of protein. However this difference likely had little impact on the results based on current knowledge of protein intake amounts that will stimulate protein synthesis maximally immediately post exercise. As noted earlier, Moore et al. (2009) reported that after

investigating the effects of the ingestion of 5g, 10g, 20g and 40g of protein in young men on muscle protein synthesis (MPS) following a bout of exercise, any amount in excess of 20g is likely to be oxidized and not contribute to MPS. Thus, it was more important to match for energy in each drink once the minimal amount of 20g per experimental drink was obtained.

The strength training program used was designed to emphasize muscle strength and size. Participants exercised 3 times per week for approximately 50min each session by following a whole body routine (Table 4.1) that targeted leg and arm musculature primarily. The entire program was followed for 10 weeks which was, as noted by Chilibeck et al. (1997), sufficient to elicit significant lean tissue mass increases in young untrained females. It was important that the strength training program emphasized high intensity (75-85% 1RM) and multiple exercises for the same muscle group in order to target primary, secondary and stabilizing muscles.

Interestingly, the results of the present study demonstrate that dairy protein intake following a bout of strength training does not appear to reduce fat mass. This is in contrast to some research (Hartman et al. 2007; Josse et al. 2010). Following 12 weeks of strength training both Hartman et al. (2007) and Josse et al. (2010) reported losses in body fat with Hartman et al. (2007) reporting a 5% decline in fat mass for participants who ingested dairy milk within a few minutes of their session vs only a 1.5% decline for those ingesting a soy protein drink. Increased calcium ingestion from dairy milk can accelerate loss of adipose tissue by reducing intracellular calcium concentration and thus promoting lypolysis and inhibiting lipogenesis (Zemel. 2004). However, several other studies are consistent with the results of the present study which found no fat reducing

benefit from consuming dairy products. Rankin et al. (2004) noted that following 12 weeks of strength training, ingestion of a CHO only or an isoenergetic dairy milk drink pre – and post training, reported a similar loss of 8% body fat in both groups. Further, Harvey-Berino et al. (2005) reported no significant difference in body mass or fat loss between subjects who consumed 3 servings of dairy per day as compared to those who consumed less than one serving per day. The results of the present study indicated a decrease of approximately 2.1% body fat for all groups, thus, likely the reduction in body fat was a result of the training program with no significant influence from drink type. Although actual intake was not assessed, our participants were instructed to avoid dairy products, especially dairy milk, on non-training days throughout the study. Of course, on training days however, participants in the dairy groups had 2 and 3 servings of dairy, respectively. This intake is similar to those used by Gunther et al. (2005); Harvey-Barino et al. (2005) & Phillips et al. (2003), who all found no significant interaction between dairy milk and a reduction in body fat.

Although fat mass did not change significantly between groups, as mentioned, significant muscle size changes were apparent from pre- to post training with no differences between groups. Contrary to previous research (Calder et al. 1994; Chilibeck et al. 1997), the increase in cross sectional area (CSA) between thigh and arm musculature was not significantly different. A less than 5% increase in thigh CSA vs an approximate 10% increase in arm CSA following strength training was reported by these groups. It was noted that the arm musculature is often less 'trained' than thigh musculature and thus the response to an exercise stimulus would be greater in the arm.

Although the women in our study were untrained, we did not observe a relative difference in limb CSA responses.

One of the limitations of the study was the unanticipated drop rate of our participants. As mentioned, fifteen participants did not complete the study for various reasons including pain or injury to the back, knee or shoulder. Although a progressive lifting program based on 1RM data was initiated for all participants in the present study, perhaps a two to three week pre-study introductory period may be required to train the participants so that they are ready to handle optimal lifting loads during the study period. Regardless, 75% of the participants completed the training either with minimal, short-lived periods of discomfort other than that experienced typically with strength training. Another reason for the dropout rate was the failure of 7 of the 15 dropout participants to complete the post testing in a timely fashion due to other personal and scholarly priorities. The ten week training program (three times per week for one hour) did elicit significant muscle strength and size increases. However, Kraemer et al. (2004) found that young women who followed a 24 week strength training periodized program continued to show lean tissue mass increases for the entire 24 week period so a longer training program may have been even better.

In summary, the consumption of a protein drink, regardless of protein type, animal vs plant, immediately following high intensity strength exercise, resulted in significant increases in arm and leg muscle strength, with an insignificant effect on body fat percentage. Changes in muscle hypertrophy were also seen over the course of 10 weeks of training regardless of drink consumed. This investigation is the first to examine the effects of the plant (hemp) protein-CHO post training supplement and its effects on

muscle strength and hypertrophy in females. Although the mechanisms of action were not identified in this investigation, it is clear that hemp protein warrants further consideration as a protein supplement alternative to animal and soy protein following strength training sessions.

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Chapter 5

**Hemp protein-carbohydrate drink post concurrent strength-HIIT improves
10km cycling time trial performance in young women and men**

5.1 Introduction

Many triathletes, cyclists and marathoners desire to include multiple strength building exercises into what is primarily, an aerobic training regimen. Although some data suggest that concurrent training (CT – the use of strength and endurance training as part of one program) among athletes would compromise either strength or endurance development due to a so called 'interference' effect (Hickson. 1980), more recent research has shown both a physical and performance benefit for such training, especially for endurance performance (Cantrell et al. 2014). Sillanpaa et al. (2008 & 2009) reported no molecular interference effect when strength and endurance training was performed concurrently and in fact noted an increase in endurance performance with the addition of strength training sessions. Similarly, Donges et al. (2012) reported no molecular interference effects from concurrent exercise noting that both myofibril and mitochondrial protein synthesis increased following such training without a decrement in protein signaling or mRNA expression. Further, Chtara et al. (2005) reported that concurrent intra-session (training within the same session) endurance training followed by strength training produced greater improvements in endurance performance than when performed separately or in reverse order. Other negative factors associated with CT include general muscle fatigue, soreness and poor recovery (Leveritt et al. 1999; Nader. 2006). Muscle glycogen is an important energy substrate for prolonged endurance exercise (Saltin & Karlsson. 1971), high intensity exercise (Jacobs et al. 1982), and strength training (Creer et al. 2005) and because chronically low muscle glycogen has been shown to reduce muscle strength, endurance performance (Karlsonn & Saltin. 1971) and muscle protein synthesis (Lemon et al. 1980), muscle glycogen likely plays a significant role in the fatigue, soreness and recovery of the concurrently trained muscle.

Importantly, the ingestion of a CHO-whey protein supplement following either strength or endurance training can increase protein synthesis (Beelan et al. 2008; Tang et al. 2007), muscle recovery (decreased catabolism) (Borsheim et al. 2003) and glycogen re-synthesis (Berardi et al. 2006).   Thus, the provision of a CHO-protein supplement following CT has the potential to offset the detrimental consequences of CT as outlined above.   Although most research to date examining the effects of protein supplementation post exercise has focused on the use of animal protein or, more specifically whey protein, the result of Chapter 4 would suggest that hemp protein, when provided as a supplement post exercise, achieves similar strength benefits to a whey protein supplement. Therefore, perhaps the provision of hemp protein-carbohydrate drink following a bout of CT will be advantageous.

Recently, high intensity interval training (HIIT), has garnered considerable interest among elite endurance athletes as it has become clear that HIIT produces similar cardiovascular and performance benefits as endurance training with a much reduced time commitment and subsequently, less chance of developing chronic fatigue (Burgomaster et al. 2005; Gibala et al. 2006; MacDougall et al. 1998). In fact, it has been suggested that the intensity of endurance exercise is a greater determinant of VO_2max and performance than the duration, and that increases in duration cannot compensate for reductions in intensity (Laursen & Jenkins, 2002; Wenger et al. 1986).

Thus, the purpose of this study was to identify whether a plant protein + carbohydrate supplement following intra-session bouts of CT (strength and aerobic exercise performed during the same workout) can enhance performance in physically active men and women compared to a carbohydrate placebo. An animal protein +

carbohydrate supplement was unnecessary in this study because it was previously shown (unpublished data – Chapter 3 and 4) that plant and animal protein + carbohydrate supplements enhance recovery similarly. A second purpose of the study was to investigate whether the use of HIIT as part of CT sessions (HIIT + strength training), would produce greater strength, power and endurance outcomes vs either method alone. Due to similar neuromuscular recruitment patterns between HIIT and high intensity strength training, it was hypothesized that this mode of training would not create 'interference' effects and thus would not be detrimental to training outcomes. Further, it was hypothesized that the inclusion of a plant protein (hemp) + carbohydrate supplement post CT exercise would be more beneficial for aerobic, anaerobic and strength performance vs a carbohydrate only beverage.

5.2 Methods

Fifty three participants were recruited to participate in a 6 week CT study (n = 43 women; 10 men). Two women dropped out prior to data collection due to the required time commitment. Participants were matched for baseline aerobic capacity (VO_2peak) and 10km cycle time trial (TT) performance and randomized in one of four groups: CT (Wingate + strength training) with a carbohydrate-Hemp protein supplement (CT-P; 12 females and 4 males; n=16), CT (Wingate + strength training) with a carbohydrate only supplement (CT-CHO; 9 females and 3 males; n=12), Wingate training only with carbohydrate-Hemp protein (W; 11 females and 2 males; n=13) and Strength with carbohydrate-Hemp protein (S; 9 females and 1 male; n=10). This grouping enabled an evaluation of post concurrent training session ingestion of CHO-hemp vs CHO only as

well as whether Wingate + strength training as part of CT was a better training approach vs either Wingate or strength training alone. Participants were recruited from Humber College Fitness and Health Promotion program and the University of Guelph-Humber Kinesiology program (Table 5.1). Exclusion criteria included symptoms of respiratory or cardiovascular disease, use of heart rate or blood pressure medications, an allergy or sensitivity to dairy and pregnancy. Upon arrival, participants were fully informed of the purpose and associated risks, and written informed consent was obtained according to a protocol approved by the Research Ethic Review Board of Western University and Humber College.

Table 5.1 Baseline Participant Characteristics

	Female n = 43	Male n = 10
Age (y)	20.7 ± 1.1	20.9 ± 0.5
Body Mass (kg)	60.8 ± 4.2	74.1 ± 6.1
Fat Mass (kg)	15.4 ± 4.1	10.8 ± 2.9
VO_2peak (ml.kg^{-1}.min^{-1})	36.1 ± 6.1	47 ± 1.6
10km Time Trial (min:sec)	22:38 ± 0:45	20:14 ± 1:12

Experimental Design

Prior to the six weeks of training, participants visited the laboratory for a familiarization session with all equipment that would be used for pre-post testing and for the training program. Within one week of the familiarization session, participants began 4 days of pre-testing which included body composition testing (Bodpod®) on day 1, maximum aerobic capacity (VO_2peak) and a 30sec Wingate cycle test on day 2; a 10km cycling time trial (TT), max 1-RM leg press and vertical jump on day 3 and lactate testing during a time to exhaustion (TTE) trial on day 4. Each testing day was separated

by at least 48h. Following pre-testing, participants began 6 weeks of training (3 days a week) in their respective groups. Following each training session, participants were provided an isoenergetic post exercise drink, either a carbohydrate-hemp protein mixture or a carbohydrate only drink. Within three days of the final training session, post testing was initiated in the same order as pre-testing for each participant.

A variety of tests were utilized to assess body composition, leg strength, leg power, speed, endurance and metabolic adaptations to exercise. Specifically, body composition was assessed using a Bod Pod® (software version 1.69), as outlined by the manufacturer. A maximum continuous incremental aerobic test was performed on a Monark cycle ergometer. Oxygen consumption was assessed using an online breath by breath gas collection system (Vmax Legacy Sensory Medics, California, USA) which was calibrated before testing by using gases of known concentration and a 3 L syringe. Initial resistance was set at 0.5kp for women and 1kp for men and was increased by 0.5kp every two minutes for the first eight minutes while participants maintained a pedaling cadence of 70RPM. The data were averaged over 15 second intervals and the greatest value was taken as VO_2 peak. A maximum anaerobic Wingate test was performed 20min (10min of active recovery + 10min of passive recovery) after participants had completed their max aerobic test. For the Wingate, the Monarch cycle ergometer was connected electronically to a laptop utilizing Monarch Anaerobic Test software – 849E. To begin, participants were advised to pedal at their preferred cadence with a resistance of 0.5kp for 5 minutes as a warm-up prior to beginning the actual test. They were then instructed to pedal as fast as possible while staying seated immediately prior to a resistance equaling 7.5% of their individual body mass being added. Once the resistance was engaged,

participants completed an all-out effort for 30 seconds. Maximum anaerobic power, fatigue index ([maximum – minimum / maximum] X 100) and average anaerobic power were recorded.

On day 3, a 10km TT was performed on a simulated computer race course at a self-selected pace on a Computrainer® bike using the Velotron 3D® software. Briefly, the bicycle was interfaced to a laboratory computer and monitor allowing for real-time visual feedback. Participants were able to track their performance with a virtual cyclist whose speed was controlled by the individual's actual efforts on the bicycle, and a virtual opponent that was programmed to be 1-5 seconds distance ahead or behind (not used as a pacer). This was to control for variability in how subjects' performances responded to pacers. All other displayed data such as cycle speed, distance traveled, subject position relative to the virtual opponent, work output (current watts and average watts), heart rate (via a heart rate monitor attached over the heart), and revolutions per minute were available to the participant. All were given verbal encouragement every 5 minutes and were reminded that the test was an all out effort to be completed as quickly as possible.

Additionally, a 1-RM leg press test and vertical jump were performed on Day 3. The 1-RM testing was performed in accordance with the protocol suggested by Kraemer et al. (1995). Briefly, participants were given a warm-up of 5-10 reps at an absolute mass that was estimated to be well below their maximum. Following a 4-5 minute break, a mass was selected that was estimated to challenge each participant to perform approximately no more than 5 repetitions. If subjects could press 3-4 times, they were stopped immediately, given 5 minutes to rest and additional mass was added. This continued until only 1 leg press could be performed or until participants had reached a 5

set effort. Whenever 5 sets were reached, participants returned 48h later to attempt another 1-RM trial. Ongoing encouragement and communication between tester and testee were used to obtain maximum efforts. The vertical jump was performed with a Vertec as described elsewhere (Burr et al. 2007; Leard et al. 2007). Briefly, participants were measured for maximum standing height with the Vertec device wearing shoes and with one arm outstretched over their head. To ensure maximum standing reach height was attained, the tester pulled upward on the participants forearm. Participants were then advised that they had 3 attempts to jump as high as possible with the highest jump being recorded for the calculation of leg power using the validated and reliable Sayers leg power prediction equation ([60.7 X jump height cm] + [45.3 X mass kg] – 2055) (Lara et al. 2006). Participants were instructed to squat to a knee angle of ~90 degrees while simultaneously placing their arms behind them. Following a second pause, participants were advised to jump vertically and tap the highest possible vane attainable with the dominant outstretched hand. A maximum of 3 attempts was permitted with at least 3 minutes rest between each trial.

Blood lactate was measured during an incremental time to exhaustion cycle test on day 4. Participants cycled on the Monarch ergometer with a starting resistance of 0.5kg at an RPM of 70 with incremental increases of 0.5kg every 4min (Coyle. 1994). Participants were advised to maintain a cadence of 70 rpm and continued pedaling until volitional fatigue. Participants in the Strength group (S) did not perform this test as it has been shown previously that strength training will have a negligible effect on blood lactate (Bishop et al. 1999). Using a lancet, capillary fingertip blood samples were taken at rest, during the final 30sec of every subsequent workload and at 2min and 5min post exercise.

All samples were analyzed within 30 sec of collection using the highly reliable and validated Lactate Pro Analyzer (Pyne et al. 2000).

Training Programs

Three separate training programs (strength alone, Wingate alone, 2 concurrent training groups [Wingate + Strength training) were designed for the four groups with each program beginning 1 week following baseline testing. All training sessions were supervised by a Can Fit Pro or Canadian Society of Exercise Physiology (CSEP) certified personal trainer (CPT) in a one-on-two or one-on-three fashion. The progressive resistance strength training (ST) program was designed to increase muscle strength maximally. It was a 3 day whole body routine consisting of 7 exercises per workout with a primary focus on leg strength (Table 5.2). The sequence and order of exercises were maintained for the entire six week training period. All participants completed a total of 4 sets; sets 1 to 3 included 8-10 repetitions at 80-85% of 1 repetition maximum (RM) and 90 second rest intervals while set 4 was performed at ~85-90% of 1RM to exhaustion. All participants were provided a personal logbook which the trainers used to chart progress.

Table 5.2- Strength Training Protocol

Week 1-6, 4 sets (3 sets @ 80-85% 1-RM, 10-12 reps & the 4th @ 85-90% 1-M to failure)

MONDAY	WEDNESDAY	FRIDAY
Leg Press	Leg Extension	Leg Curls
Jump Squats	Lunges	Box Step-Ups
Seated back Row	Standing DB Bicep Curl	Lat Pull down
Standing Bicep Curl	Zottman Curls	Deep Body Squats
Push-ups	Cable Tricep Extension	Seated Bicep Curls
Abdominal Crunches	DB Lateral Raises	Cable Tricep Extension
	Abdominal Crunches	Abdominal Crunches

The Wingate group (W) trained 3 times a week utilizing repeat cycle Wingates (Gibala & Little. 2010) as well with at least 48h recovery between each session. The group began with four Wingates per session in week 1 with four min recovery between each and increased this workload by 1 repetition every four training sessions until a maximum of ten Wingates was completed during a single session. The resistance for each Wingate was held constant at 0.075kg/kg BM. Each 30 second "all-out" effort Wingate was performed while seated.

Both CT groups utilized the same program as the W group, however, the final Wingate was followed by a 15min rest period and then a strength workout that comprised the same leg exercise (Leg Press, Leg Extension, Leg Curl, Jump Squats, Lunges, Deep Body Squats and Box Step Ups) as the S group (Figure 5.1). Therefore, both CT groups participated in the same training program however, one of the groups received a carbohydrate-hemp protein drink following each training session while the other received an isoenergetic carbohydrate only beverage.

Figure 5.1 Protocol Timeline
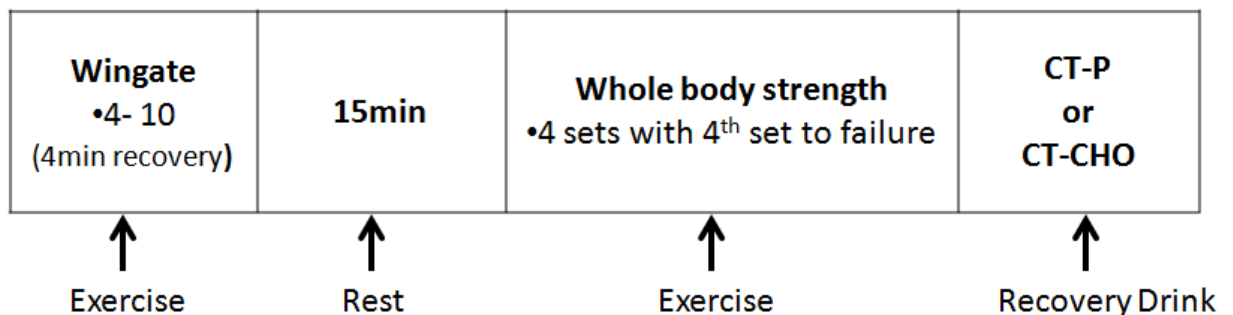
Drinks

Recovery drinks were consumed immediately following each of the training

programs in the presence of the trainers. As mentioned, the CT-P, S and W group

consumed a hemp protein - carbohydrate drink (*Manitoba Harvest Hemp Pro 70,*

Winnipeg Manitoba & Maltodextrin, QSE Gold Medal Carb) while the CT-carbohydrate

group consumed a carbohydrate only drink (*Maltodextrin, QSE Gold Medal Carb).* All

drinks were isoenergetic (~1,345 kJ), chocolate flavoured and isovolumetric (325 ml) and

were provided in opaque bottles. The hemp protein -carbohydrate supplement dose

contained a 1:2 ratio of protein to carbohydrate (21g Hemp protein per serving) (Table

5.3). This protein supplement dose was based on previous estimates of required protein

intake to maximize rates of protein synthesis in young men (Moore et al. 2009).

Individuals not associated with the exercise training program were responsible for all

drink making and distribution to participants to ensure the double blind protocol was

maintained. Participants confirmed throughout the study that they were not taking any

additional protein supplements or ergogenic aids.

Table 5.3 – Drink Composition

Drinks	Ingredients	Amount	Protein (g)	Carbohydrate (g)	Fat(g)	Energy kcal	Energy kJ
Protein DRINK	almond milk	250ml	1	20	3	**111**	**464**
	hemp protein	60g	20	4.5	4.5	**139**	**582**
	Maltodextrin	45g	0	18	0	**72**	**301**
	Water	75ml	0	0	0	**0**	**0**
						TOTAL: 322	**1347**
Carbohydrate DRINK	almond milk	125ml	0.5	10	1.5	**55**	**229**
	Maltodextrin	175g	0	70	0	**280**	**1172**
	Water	200ml	0	0	0	**0**	**0**
						TOTAL: 335	**1402**

Three day food records (2 weekdays & 1 weekend day) were collected at the beginning of training, at week 3 of training and within 1 week after all training was completed. Further, participants documented their nutritional intake on each of the pre-testing days so that the same diet could be replicated on post testing days.

5.4 Statistical Analysis

Statistical analysis included standard descriptive statistics, two-way [2 x 2 (CT-P and CT-CHO); and 3 x 2 (CT-P, W and S)] ANOVA (group x time) with repeated measures for all variables. The location of significant differences was determined using the Tukey-Kramer post hoc procedure. The assumption for repeated measure ANOVA (test of homogeneity) was tested for each variable. Ninety percent participant training compliance was required for the participant's data to be included in the analysis. Statistical significance was set at a p value of < 0.05. Values are expressed as mean $\pm$ SD. All analyses were performed using the Statistical Package for Social Sciences (SPSS v21).

5.5 Results

Mean ± SD from 6 weeks of CT-P, CT-CHO, Wingate and Strength on body

composition and physical performance are summarized in Table 5.4

Table 5.4 - Effects of 6 weeks of CT-P, CT-CHO, Wingate and Strength on body composition and physical performance

	CT-P		CT-CHO		Wingate		Strength	
	Pre-	*Post*	*Pre-*	*Post*	*Pre-*	*Post*	*Pre-*	*Post*
Body fat (%)	25.6 ± 2.1	25.7 ± 2.1	25.3± 2.5	27.5 ± 2.5	29.4±4	28.7± 8	30.3±3	30.0 ± 3.5
Mass (kg)	67.4±5.0	65.5±4.6	59.2±5.1	61.2±6.7	65.2±7	66.0± 6.8	65.9±9	66.4±9.0
Vertical Jump (cm)	44 ± 10.5	49 ± 11.6[1]	34 ± 7.8	39 ± 7.9[1]	41 ± 11	44± 13.3	36 ± 11	39 ± 12.1
30s Wingate - (Watts)	754 ± 48	895 ± 47[1,2,4]	663 ± 48	699 ± 55[1]	769± 60	868± 51[1,2]	502±84	504 ± 76
Wingate - Fatigue (%)	50.2 ± 9.1	57.7 ± 7.4	50.5± 6.7	53.2±6.6	56 ± 7.7	49 ± 7.0	56±14	48.4 ± 9.3
1-RM Leg Press (kg)	224 ±19.5	371 ± 23[1,3,4]	171 ± 20	272 ± 31[1]	282 ±29	383 ± 49[1]	219±26	363 ± 29[1,3]
TT- Time (min)	21.51 ± 0.36	20.17± 0.27[1,2,4]	21.41 ± 0.39	21.06 ± 0.47[1]	21.68 ± 0.26	20.95 ± 0.35[1,2]	21.85 ± 0.49	21.51 ± 0.38
TT - Average Power (Watts)	141 ± 45	164 ± 44	143 ± 33	148 ± 32	143± 63	154 ±70	139±19	147 ± 38
VO$_2$peak (ml.kg^{-1}.min^{-1})	42.9 ± 1.9	46.5 ± 1.8[1]	41.1± 2.3	44.6±2.1[1]	40.7±2	42.7± 1.9[1]	40.2±3	40.6 ± 1.7

Values are mean ± SD.
[1]Significant difference between pre- and post tests at $p< 0.05$
[2]Significantly different from Strength at $p < 0.05$
[3]Significantly different from Wingate at $p < 0.05$
[4]Significantly different from CT-CHO at $p < 0.05$
TT = 10km Time Trial;

Blood Lactate and Time to Exhaustion Cycling test

Blood lactate concentration at the same absolute intensity (2.5kg) decreased by 28%, from 5.1 ± 1.7 mmol l^{-1} to 4.7 ± 2.3 mmol l^{-1} in the CT-P group, by 27%, from 4.6 ± 1.5 mmol l^{-1} to 3.6 ± 1.7 mmol l^{-1} in the CT-CHO group and by 21%, from 5.8 ± 2.1 mmol^{-1} to 4.8 ± 1.1 mmol^{-1} in the W group with no significant difference between groups ($p = 0.33$) (Figure 5.2). Although heart rate values were lower on average at all power outputs in the post test compared to the pre- test, no main effect of time ($p = 0.12$) or group x time interaction ($p = 0.21$) was observed.
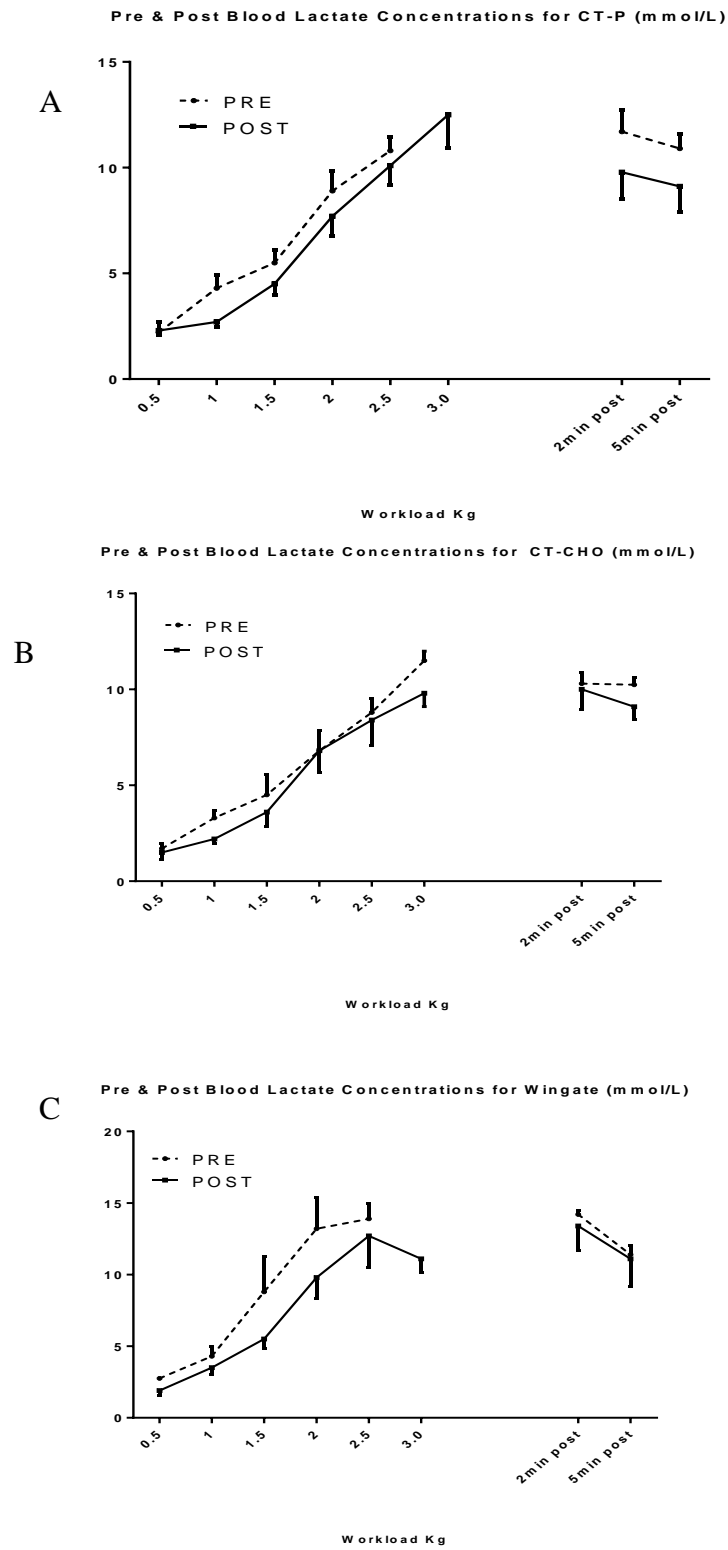
Figure 5.2 Blood lactate response to incremental cycling exercise from pre- to post testing. (A) CT-P, (B) CT-CHO, (C) W group. No significant main effect of time ($p = 0.09$) or interaction ($p = 0.33$) effect.

CT-P vs Wingate vs Strength

Time Trial Performance

A main effect of time ($p < 0.05$) was observed for 10km TT performance. An interaction effect was also noted with post hoc analysis revealing both CT-P and W ($p = 0.04$, $p = 0.04$; respectively) performed better compared to the S group (Figure 5.3).

10Km Time Trial Performance (min)
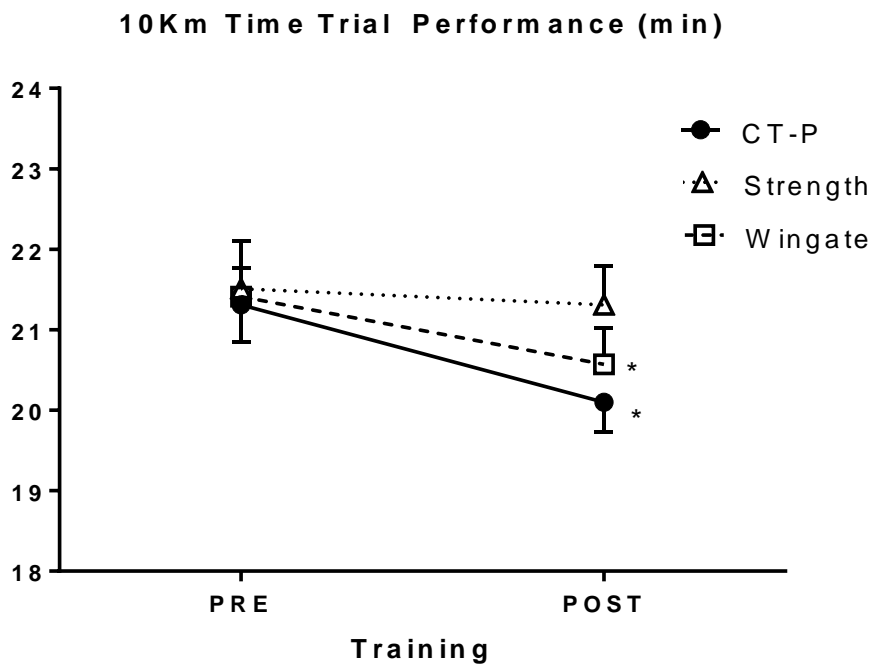
Figure 5.3 10 km Cycling Time trial completion times. The graph shows group means ± SD completion times before and after training. Completion time was significantly faster in both the CT-P (21.51 ± 0.36 to 20.17 ± 0.27, $p = 0.04$) and W groups (21.68 ± 0.26 to 20.95 ± 0.35, $p = 0.04$, respectively)

VO$_2$peak

For VO$_2$peak there was a significant main effect ($p = 0.01$) of time suggesting that overall the training enhanced aerobic capacity. No interaction effect was observed ($p > 0.05$).

30sec Wingate Peak Power

A main effect of training was noted for peak power output ($p = 0.04$). A significant interaction was also observed ($p = 0.01$) with post hoc testing noting an increase in the CT-P (754 ± 48 to 895 ± 47; +18.7%) and W (769 ± 60 to 868 ± 51; +12.8%) ($p = 0.04$, $p = 0.02$; respectively), while no change was observed in the S group (502 ± 84 to 504 ± 76; +0.4% ; $p > 0.05$). A main effect of time for fatigue index (percentage) was also observed ($p = 0.03$).
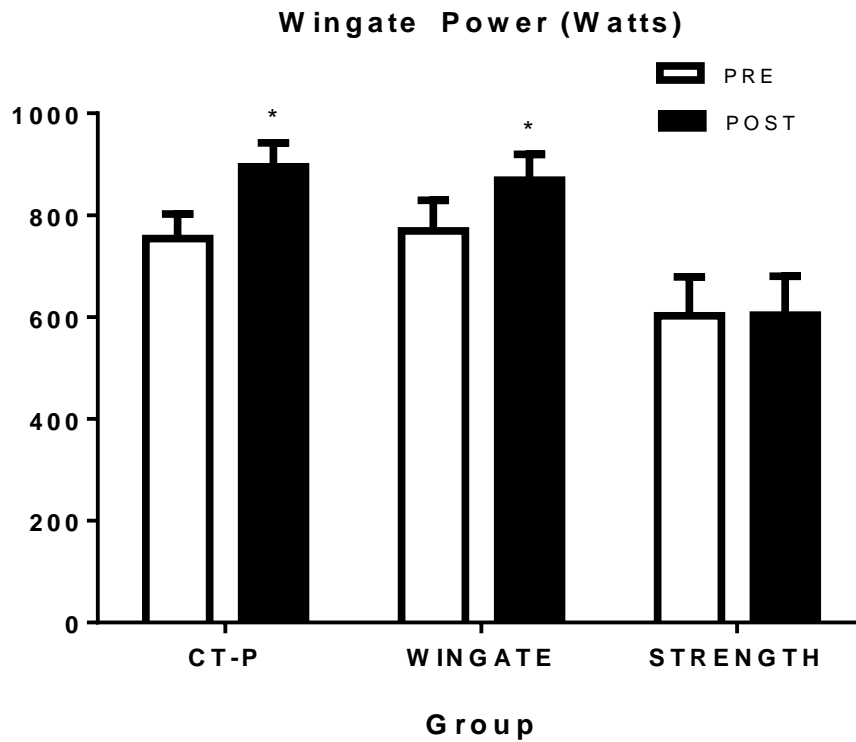
Figure 5.4 Peak Power Output during 30 sec Wingate test. *Significantly different from Strength ($p < 0.05$). All values are means ± SD.

1-RM

A main effect of time was observed (p = 0.001). A significant group x time interaction was observed in 1RM leg press (p = 0.04). CT-P (224 ± 19.5 to 371 ± 23), S (219 ± 26 to 363 ± 29), and W (282 ± 29 to 383 ± 49) all increased from pre – to post training with post hoc testing revealing 1 RM leg press following CT-P and S was greater than W (p < 0.05) (Figure 5.5).
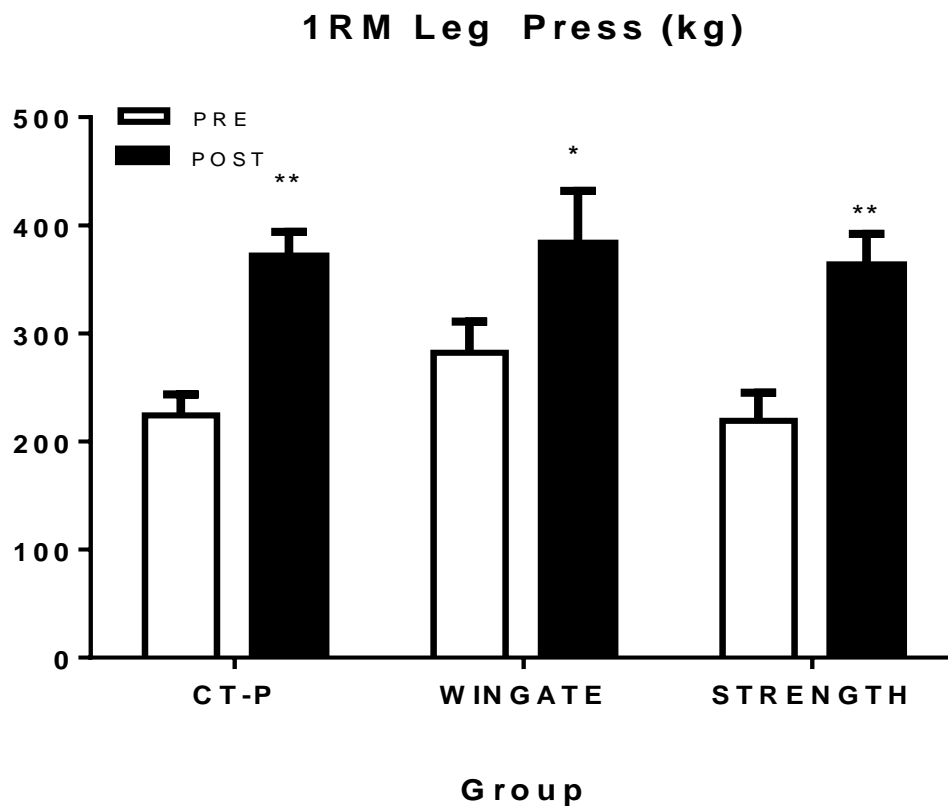
1 R M L e g P r e s s (k g)

Figure 5.5 1 RM Leg Press between CT-P, Wingate and Strength groups. *Significantly different from pre measure (p = 0.01). **Significantly different than W for same condition (p <0.05). All values are expressed as means ± SD.

Vertical Jump and Peak Leg Power

There was no significant group x time interaction (p > 0.05) however there was a significant main effect of time in leg power as predicted by jump height (p = 0.001). The percent change increases were 2%, 3% and 5% for CP-P, W and S, respectively.

CT-P vs CT-CHO

Time Trial and Wingate Peak Power and Wingate Fatigue

A main effect of time (p = 0.001) was noted for time to complete the simulated 10km TT as well as a significant interaction (p = 0.01) with decreases in the CT-P vs the CT-CHO of 6% and 3% Δ, respectively (Figure 5.6a). A main effect of time was observed for Wingate peak power (p = 0.001) in addition to a significant group x time interaction (p = 0.001) with increases in the CT-P group (754 ± 48 to 895 ± 47) vs the CT-CHO group (663±48 to 699±55 (Figure 5.6b). There was a main effect of time (p = 0.03) in fatigue index with no significant interaction effect (p=0.47) with both CT-P and CT-CHO increasing in rates of fatigue from 50.2% to 57.7% and 50.5% to 53.2%, respectively.
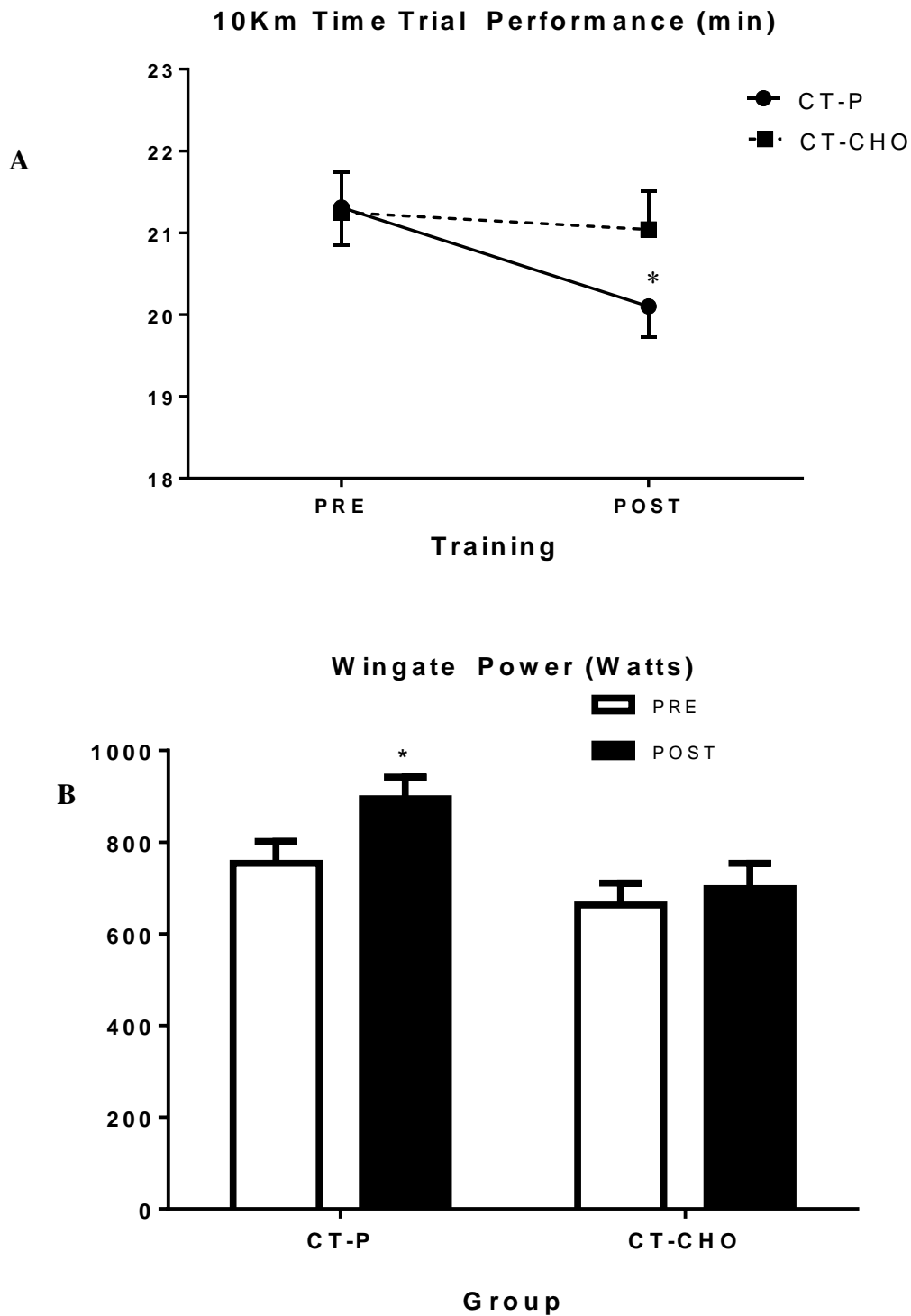
Figure 5.6 10 km Cycling Time trial completion times (A) and Wingate Peak Power (B). The upper and lower graph shows group means ± SD completion times before and after training. Completion time (A) was improved (reduced) in CT-P over the CT-carbohydrate group (p = 0.01). Wingate peak power (B) increased from pre to post testing in the CT-P group only (p = 0.001). All values are expressed as means ± SD.

Leg Power and 1-RM

There was a significant main effect of time in leg power (based on jump height) (p = 0.01) with no group x time interaction (p > 0.05).   A main effect of time was noted for 1RM leg press (p = 0.001). A significant interaction was also observed (p = 0.01) with CT-P having a greater increase than CT-CHO for the same condition (14%Δ; p = 0.04) (Figure 5.7).
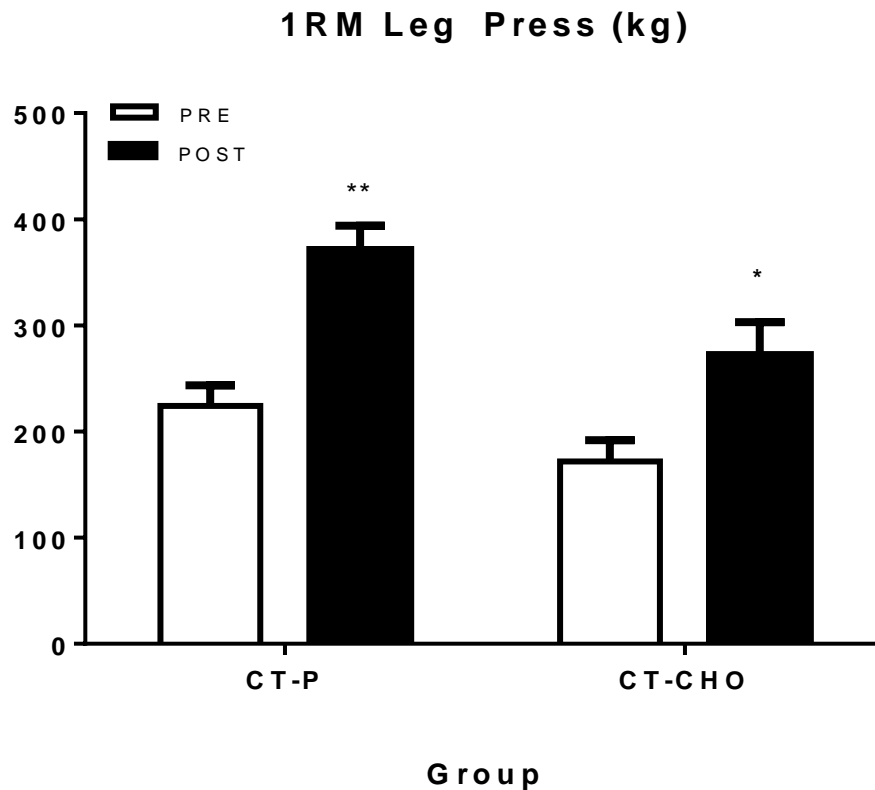
1 R M L e g P r e s s (kg)

Figure 5.7 1-RM Leg Press following 6 weeks of CT-P or CT-CHO training. *Group x time interaction (p = 0.01) such that both groups showed increases in strength as a result of training. **Significantly different from CT-CHO for same condition (p = 0.04). All values are express as mean ± SD.

<u>VO$_2$peak</u>

There was a main effect of time in VO$_2$peak over the course of training (p < 0.001) indicating that training increased VO$_2$peak with no difference observed between groups (Table 5.4).

Dietary Analysis

Total energy carbohydrate and protein intakes pre – and post – study are shown in Table 5.5. Main effects were observed for total protein (p = 0.02) and carbohydrate (p = 0.05) intake with increases in consumption of both protein and CHO from pre – to post study. No group x time interaction effects were noted.

Table 5.5 Dietary Analysis

	CT-P		CT-CHO		WINGATE		STRENGTH		
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	Sig / Pre and Post
kJ/d	6208 ± 275	6482 ± 184	5923 ± 184	6307 ± 165	6482 ± 111	6954 ± 163	6115 ± 113	6132 ± 112	NS
Protein (g/kg)	0.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.86 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	0.78 ± 0.1	0.9 ± 0.1	p = 0.02
CHO (g/d)	195 ± 56	202 ± 19	201 ± 46	214 ± 23	189 ± 19	199 ± 39	200 ± 29	211 ± 14	p = 0.05

NS = Not significant

5.6 Discussion

The primary findings of this study indicate that a 6 week intra-session CT program with a post training hemp protein-carbohydrate drink improved maximum anaerobic power and time trial performance significantly compared to a similarly trained CT group who received a carbohydrate only drink. That is, the addition of a plant protein to the recovery beverage increased time trial average power output (↑25% vs ↑5%; p= 0.003) and reduced 10km time trial time (Δ 7%, 21.51 ± 0.36 to 20.17 ± 0.27 vs Δ 2%, 21.41 ± 0.39 to 21.06 ± 0.37 min; mean±SD; p= 0.01). The increase in peak Wingate

power, average Wingate power and average power output in the CT-CHO group for the

time trial was also significantly less from pre- to post compared to the CT-P group.

Although it has been well documented that the addition of a protein-carbohydrate drink

post exercise will improve strength, power and aerobic performance, few have

investigated the benefits of post CT drink and whether the inclusion of such a drink,

would minimize some of the negative aspects of CT which include fatigue and poor

recovery. Saenz (2011) investigated the effects of supplementing active men with a

carbohydrate-animal protein drink during a CT session and found that the animal protein

-carbohydrate drink provided no greater strength or power benefit than the water placebo.

However, participants in Saenz's study (2011) were provided the protein-carbohydrate

drink or water placebo following only one bout of CT and thus long term adaptations,

which are observed typically in protein-carbohydrate supplemented studies, could not be

ascertained. Further, acute muscle damage and not performance, was the primary

outcome measure with no significant difference between the two drinks. In contrast,

following endurance training a protein-carbohydrate supplement provided greater aerobic

outcome measures than a carbohydrate only supplement was provided (Gibala et al.

2008). Similarly, Highton et al. (2013) found that sprint related activities were

significantly improved following ingestion of a protein-carbohydrate mixture vs a

carbohydrate only drink.   Given that current research indicates that the addition of

animal protein to a carbohydrate drink providing 1.2g carbohydrate/kg body mass (Gibala

et al. 2008), did not affect rates of glycogen re-synthesis, it was hypothesized that the

benefits of a protein-carbohydrate drink post exercise, likely involved decreased rates of

muscle catabolism, soreness and fatigue thereby improving subsequent bouts of exercise

(Luden et al. 2007). Thus, the increase in time trial performance in the CT-P over the CT-carbohydrate in the current study could have been due to a decrease in muscle soreness following each bout of exercise and in turn an increase in effort on subsequent exercise days. This is possibly also the case for the improved peak and average Wingate power of the CT-P and W group over the CT-carbohydrate group however no measurement of muscle soreness was taken during this investigation. Although the CT-P and CT-carbohydrate group performed an identical number of Wingates each and every exercise session, the intensity of each Wingate could have differed. Unfortunately, average power was not measured during each Wingate training session to verify uniformity of work; however, each participant was encouraged to maximize their effort at the required intervals (30sec work to 4min rest). Future studies utilizing Wingate training as part of the program should quantify exercise intensity of each Wingate for every exercise session.

Additionally, it was observed that the 6 week CT program enhanced aerobic and anaerobic performance to a similar degree as anaerobic training alone and greater than strength training was performed alone. When CT-P was compared to the W and S groups, that is, the effect of combined or individual modes of training, it was observed that the CT-P and W group increased both aerobic capacity and time trial performance significantly compared with the S group with no significant difference between CT-P and W outcomes.

It has been reported that CT interferes with optimal developments in strength (Hennessy & Watson, 1994), particularly when endurance training and strength training are performed using the same muscle groups (Hennessy & Watson. 1994; Hickson. 1980;

Kraemer et al. 1995). Further, strength training may increase muscle hypertrophy to an extent that may become an impediment to endurance performance (Bishop et al. 1999). The addition of strength training to endurance training, however, usually does not interfere with gains in aerobic capacity (Campos et al. 2002; Izquierdo et al. 2005; Millet et al. 2002; Paavolainen et al. 1999; Steras et al. 2002; Storen et al. 2008;) Although CT appears to enhance VO_2max in some cases (Campos et al. 2002; Sale et al. 1990) as observed in the current study, it has been shown to diminish in others (Campos et al. 2002; Hickson. 1980; Kraemer et al. 1995; Millet et al. 2002; Storen et al. 2008). However, the differences in aerobic adaptation between CT and aerobic training or CT and the controls in these studies were non-significant, but rather, noted as trends only. Thus the data of the present study suggest that the addition of strength training to Wingates (HIIT) does not appear to diminish gains in aerobic capacity because the W and CT-P group had similar VO_2peak increases.

The non-significant change in VO_2peak in the S group, agrees with others suggesting that the effect of strength training on VO_2peak in the absence of endurance training is minimal. In one case, strength training increased VO_2peak independently of endurance training; however, this effect could have been due to the fact that the participants were young and untrained (Sale et al. 1990). Much of the available research indicates that strength training has no effect on VO_2max (Campos et al. 2002; Hennessy & Watson. 1994, Hickson. 1980, Kraemer et al.. 1995, Loveless et al. 2005).

Even without increasing VO_2max, the addition of strength training may improve endurance performance, i.e., work economy, time to exhaustion, time trials, race times, etc., in untrained subjects (Loveless et al. 2005), especially when maximal or explosive

strength training is employed (Aagaard & Anderson. 2010; Sunde et al. 2010).

Moreover, it has been suggested that maximal ($\geq$ 85% 1RM) and explosive ST methods

may increase muscular performance via neuromuscular factors rather than hypertrophic

changes. Such changes might be advantageous for endurance athletes (Aagaard et al.

2011; Storen et al. 2008).

The beneficial aerobic effects of CT are largely based on endurance training being

performed in combination with strength training. For example, Millet et al. (2002) found

that following 14 weeks of CT strength and endurance training, both running economy

and performance of conditioned triathletes were enhanced to a greater degree compared

with athletes who performed either alone. Further, Ronnestad (2010) highlighted that 12

weeks of endurance + strength CT enhanced cycling performance (40min all-out effort)

significantly over endurance training alone. Our findings are consistent with these results

in that the 10km simulated cycling time trial was performed with more power in the CT-P

group over the S group but not the W group. Bouts of Wingates as part of a CT program

have not been studied extensively in the literature; however, the inclusion of high

intensity interval training (HIIT) vs endurance training has several advantages. First,

HIIT, Wingates for example, requires less time than traditional endurance training to

acquire similar aerobic benefits. A study by Gibala et al. (2006) examined six sprint

interval training sessions consisting of four to six 30-second Wingate tests at ~250%

VO_2peak interspersed with 4-minute rest intervals completed over the course of 2 weeks.

They found that HIIT offered a viable and time-efficient alternative to high-volume

endurance training due to similarities in muscular and performance adaptations between

the two modes of training. Second, interval training can increase strength and power by

enhancing neural adaptations while no such adaptations occur with traditional endurance training. Further, a study by Burgomaster et al. (2005) found that HIIT consisting of four to seven maximal 30-second Wingate tests interspersed with 4-minute rest intervals completed over the course of 2 weeks had no effect on VO_2peak, however it did increase citrate synthase activity by 38% and doubled endurance cycling capacity at ~80% VO_2peak in recreationally active participants. Finally, while traditional long duration, moderate to high intensity endurance exercise relies significantly on fat oxidation for energy (Burgomaster et al. 2007; Holloszy. 1973), elite endurance athletes competing in events such as triathlons, 10km runs, 10km cycling road races and marathons, require a greater percentage of carbohydrate to fuel their bouts (Coggan et al. 1987; Coyle et al. 1983, Coyle et al. 1986). Repeated bouts of HIIT have been shown to improve carbohydrate and fat metabolism during and following exercise. For example, Perry et al. (2008) found increases in whole body fat oxidation and decreases in carbohydrate utilization during submaximal exercise following 6 weeks of HIIT, suggesting that it can have favourable effects on fuel utilization for moderate duration and high intensity performance bouts beyond that of endurance training. Thus, faster time trial performance in all our groups having a HIIT component, and the lack of significant difference between the CT-P and W group (both receiving the post exercise hemp protein-carbohydrate drink), may be the result of the 6 weeks of HIIT influencing mitochondrial oxidative capacity, i.e., increasing oxidative metabolism, and not the result of additional strength training. However, none of these variables were measured directly in the current study so a conclusive statement on this matter is not possible at this time.

Robinson et al. (1995) reported significant increases in 1 RM leg press in a HIIT group but no significant changes in vertical jump between HIIT and strength groups after 5 weeks of training. Our study found increases in leg press strength with training, but observed no significant differences between any groups. Further, our study did not observe a significant change in leg power as estimated by vertical jump data. These findings are consistent with a meta-analysis conducted by Wilson et al. (2011) that concluded CT and strength groups improved maximal leg strength similarly and that no significant gains in vertical jump occur with strength training alone. However, a few studies have found improvements when plyometric training was included in the strength program (Harries et al. 2012; Markovic. 2007). This could explain why there were no changes in vertical jump results in any of the groups in the present study because there was not a significant amount of plyometric training.

Whole body lactate accumulation decreased at every time point in the post TTE test for all 3 groups (CT-P, CT-CHO and W) with no significant between or within subject differences. It is well established that the lactate threshold is increased with aerobic training –both endurance and interval training – and the results of the present study are in line with those data, however, it appears that protein supplementation had no meaningful effect on lactate threshold. Ferguson-Stegall et al. (2011) noted the lactate threshold is likely unaffected by the addition of a post exercise carbohydrate or carbohydrate-protein supplement. The strength group of the current study did not perform the lactate test during either pre- or post testing as it has been established in previous research that no significant improvements in lactate threshold can be expected following a strength training program (Bishop et al. 1999; Jung, 2003).

Combining endurance and strength exercise within the same session was important in order to reflect more accurately the training programs of many endurance athletes, especially triathletes who perform multiple workouts per day and more specifically, those that include a strength and aerobic workout into the same session. The sequencing of exercise mode used within this intra-session workout was established utilizing the findings of Chtara et al. (2005) who found that when 48 soccer players engaged in 12 weeks of CT exercise, those who performed aerobic exercise prior to strength training improved cycling time and aerobic capacity significantly vs those who performed similar exercise in reverse order or alone. In contrast, the decision to conduct all Wingate sessions prior to strength training in both CT groups was made based on Cadore et al. (2012) who found that intra-session exercise order had no impact on endurance performance measures in elderly men who exercised 3 times per week for 12 weeks. Consequently, our results may be due to training mode and drink supplementation rather than exercise order, although more study is needed to be sure.

In conclusion, the ingestion of a plant based protein (hemp)-carbohydrate drink immediately following CT is more effective at increasing aerobic, anaerobic and strength performance measures following 6 of weeks of training than an isoenergetic carbohydrate only drink. Further, the inclusion of HIIT as part of the CT program provides a more powerful aerobic and strength stimulus than either HIIT or strength training performed alone. Future studies should focus on the molecular adaptations that may occur as a result of ingestion of hemp protein-carbohydrate drink post CT session and, the inclusion of HIIT as part of CT. Both investigations will provide important insight for the endurance athlete looking to maximize their training and performance.

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Discussion

6.1	Clearly, the nutritional status of an athlete is of upmost importance in maximizing

performance. Although all three macronutrients contribute to energy production and

important metabolic processes, adequate carbohydrate and protein ingestion following

exercise training bouts are thought to be critical in maximizing recovery and future

exercise performance. Animal proteins and high glycemic, easily digestible

carbohydrates taken together, have been shown to improve recovery and thereby to better

prepare an athlete for subsequent training or performance. However, what is less

understood is whether the type of protein, that is, animal vs plant, is an essential part of

this equation, i.e., whether an ingested plant protein + carbohydrate supplement can

equally maximize aerobic, strength and CT adaptations compared with animal protein +

CHO. In this dissertation, a series of experiments were designed to assess possible

performance benefits from the ingestion of a plant (in the form of hemp or soy) protein-

carbohydrate supplement following aerobic, strength and CT exercise.

Study 1 demonstrated that following a glycogen lowering bout of cycling, well

trained cyclists are able to recover and perform similarly in a subsequent same day 20km

simulated cycling TT with the post exercise ingestion of a plant (soy or hemp) –

carbohydrate supplement compared to animal (dairy milk) -carbohydrate supplement.

Specifically, whether the well trained cyclists consumed isoenergetic soy protein -

carbohydrate, hemp protein-carbohydrate, dairy chocolate milk protein-carbohydrate

drink or a dairy only drink, TT performance improved similarly and significantly vs the

low energy placebo. Our findings which concur with reports by Millard-Stafford et al.

(2005), Romeno-Ely. (2006) and Berardi et al. (2008), extend our understanding by

indicating that a 4:1 carbohydrate-protein ratio which is commonly touted as being the ideal carbohydrate-protein ratio, was in fact not critical for 20 kmTT performance as carbohydrate-protein ratios in our drinks ranged from 1.5:1 to 6:1. The lack of any significant differences in time trial performance between isoenergetic, isovolumetric and isocarbohydrate recovery supplements, is an indication that the provision of a plant protein-carbohydrate is as effective as an animal protein-carbohydrate drink for recovery and that the total energy provided is critical.

Study 2 demonstrated that the ingestion of a hemp protein + carbohydrate supplement post strength training exercise, can increase muscular strength, hypertrophy and performance to a similar degree as whey protein + carbohydrate and/or dairy milk. The increases in strength in both the arm and thigh were greater in all groups vs an energy matched carbohydrate only drink. The duration of the strength training program (10 wk) and the intensities used were sufficient to elicit strength and non-significant hypertrophic gains. The results of this study confirm what was hypothesized by Atherton et al. (2012) that the type of protein provided immediately after exercise may not be the determining factor in maximizing protein synthesis, but rather, what matters more, is the provision of adequate amino acids. Hemp protein provided 2.2g per serving of leucine; an amount which falls within the range necessary to maximize protein synthesis in young men (Moore et al. 2009). Further, one's amino acid pool is not only made up of amino acid ingested but also amino acids from muscle protein breakdown. Thus the ingestion of a number of amino acids post exercise may increase protein synthesis. In our study, the ingestion of a hemp protein + carbohydrate drink was sufficient to produce similar strength and muscle hypertrophy gains as the milk protein beverage and the whey protein

beverage following 10 weeks of high intensity strength training among college aged females. It is worth noting that a significant difference in fat loss or gains in lean mass was not apparent between protein groups which is in contradiction to some research suggesting benefits of dairy (Hartman et al. 2007; Josse et al. 2010) but supported by others (Gunther et al. 2005; Harvey-Barino et al. 2005; Rankin et al. 2004). Recently a meta-analysis by Dougkas et al. (2011) concluded that the consumption of dairy milk proteins do not alter total body mass or total body fat without a corresponding energy restriction.

Study 3 demonstrated that post training session ingestion of a protein (hemp)-carbohydrate drink is more effective at eliciting training improvements in performance vs a carbohydrate only drink. Further, it was found that the use of HIIT as part of a CT program produces greater strength, power and aerobic benefits than when a CT program utilizes either method alone. Specifically, following 6 weeks of training, the CT-P and the HIIT group (both receiving a hemp protein-carbohydrate drink post exercise) achieved significant improvements in cycling TT performance and peak Wingate power. It appears from these data, that a hemp protein-carbohydrate supplement will produce greater increases in performance parameters compared to a carbohydrate only supplement, thus confirming that hemp protein-carbohydrate supplementation post exercise is effective at improving recovery. Further, it appears that a CT program which incorporates HIIT will produce significantly greater increases in aerobic and anaerobic performance measures than either a HIIT or strength training alone.

6.2 Summary

Together these studies demonstrate that, regardless of protein type (animal or plant), the provision of a post exercise session carbohydrate-protein supplement will produce greater improvements in several performance measures than isoenergetic carbohydrate supplementation. These data are in contrast to a number of studies touting dairy protein (especially whey) as the best post exercise supplement. A possible explanation for these findings could be that dietary amino acids from many food sources, combined with the intracellular amino acid pool, provide a sufficient stimulus to increase muscle protein synthesis and/or decrease muscle protein breakdown. Therefore, a plant based protein, specifically hemp or soy protein, could be used instead of the more common animal based protein, whey protein, for the provision of energy and amino acids following exercise. More studies are recommended in order to establish the metabolic pathways by which plant proteins may elicit beneficial performance enhancing effects over longer term training and supplementation periods.

6.3 Limitations

The type of protein which elicits the most favourable muscular and performance enhancing effects is discussed routinely in the sport supplement industry. Although this dissertation demonstrates a clear benefit of plant proteins, a variety of confounding variables must be noted.

In study 1 a glycogen depleting protocol that has been established in the literature as being effective was used but no measure of muscle glycogen was taken. As a result, the participants may have experienced varying degrees of glycogen depletion. Although not thought to be a substantial limitation, this may have influenced glycogen resynthesis rates. Future studies should assess both depletion and resynthesis of glycogen via Nuclear Magnetic Resonance (NMR) or needle biopsy techniques.

In study 2, the participant number was less than planned. This was due to the observed drop rate of our participants. Fifteen participants did not complete the study due to injuries and/or time limitations. The most common concern expressed was soreness/injury to the back, knee or shoulder. This was likely a result of novice strength trainers progressing too quickly. A two-three week pre-study period is recommended for future studies so the participants are ready to handle the lifting loads required in these types of studies. Despite the concern, 75% of the participants completed the training with minimal or no discomfort. Further, although ten weeks of training, three times per week for one hour elicited significant strength increases, the results may not have been maximized as the study may have been too short. Kraemer et al. (2004) found that young women who followed a 24 week strength training periodized program continued to show lean tissue mass increases for the entire 24 week period.

In study 3, it was impossible to match participants/groups on all variables measured. The decision was made to match participants according to aerobic capacity and time trial performance as our primary outcomes of interest focused on aerobic benefits obtained following CT. However, had 1-RM strength been matched for as well,

the reduced variability likely would have resulted in a statistically significant finding for strength.

6.4 Future studies

Although the current studies do demonstrate a benefit of plant protein-carbohydrate supplements as a post exercise supplement, several questions still remain.

1. Whole body protein requirements of elite endurance and aerobic athletes

The protein dosage used in this study was based on estimated optimal dosage identified by Moore et al. (2009) at 20-25g per dose in a single bolus. These studies involved a single acute exercise bout and it is still unclear how adaptation over weeks of supplementation and exercise training might affect these results. Future research using methods that determine both optimal as well as practical intakes of protein over the course of a training study, i.e., the indicator amino acid technique, are recommended.

2. The bioavailability of Hemp protein

To confirm the possible benefits of hemp protein specifically, future research should examine its in vivo bioavailability through the use of plasma kinetic measures following ingestion. These studies could help document whether hemp protein is effective at increasing recovery and protein synthesis following bouts of aerobic exercise and strength training, respectively. Moreover, adding molecular measures to performance data are recommended with future studies to explain the underlying mechanisms responsible for the observed effects.

3. Long term protein-carbohydrate intervention

It is known that both glycogen resynthesis and protein synthesis are influenced by nutrition. Consequently, it is hypothesized that the addition of a carbohydrate + protein supplement would be most beneficial for elite athletes where recovery time between workouts is significantly reduced (perhaps <8hr). Thus, an investigation into the benefits of a carbohydrate + protein supplement following bouts of training over the course of weeks and even months with elite endurance and strength athletes, although logistically difficult, would be enlightening.

4. Plant protein-carbohydrate supplement and muscle damage following CT

It was hypothesized in the CT study that the addition of protein to the carbohydrate beverage improved rates of recovery and thus performance outcomes. A future study should determine whether a carbohydrate + protein supplement following CT improves rates of recovery by examining muscle damage and soreness immediately post exercise and hours to days later. This might utilize needle biopsies (although not ideal), blood markers (not ideal), MRI, soreness scales, and/or functional strength testing. If a carbohydrate + protein supplement is going to be promoted than detailed information on its potential effectiveness, that is, enhancing recovery, needs to be determined.

6.5 Conclusion

Plant protein (especially soy and hemp) has become a popular protein supplement for athletes looking to eliminate animal products from their diets. The fitness and bodybuilding industry has been promoting the ingestion of hemp protein isolate in lieu of whey protein as an alternative to an animal based protein. However, few data exist in the scientific literature on the beneficial effects of hemp protein ingestion as it relates to

muscle protein synthesis and/or performance. The results of the present studies highlight the importance of a post exercise protein-carbohydrate drink while at the same time, note that plant based protein is effective at improving aerobic, anaerobic and strength adaptations following either aerobic, strength, HIIT or CT programs. Although more work is needed in this specific area, as mentioned above, the plant based protein may offer similar performance benefits to the exercising individual who is looking for an animal based protein alternative.

6.6 References

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Curriculum Vitae

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EDUCATION AND TRAINING

PhD (c) – Sports Nutrition and Exercise Metabolism, The University of Western Ontario
Completion: August 2014

Masters of Education – Adult Education and Curriculum Development, University of Toronto; August 2010

Masters of Health Science (Cardiovascular Physiology and Fitness), York University – Toronto, ON; December 2004

 Bachelor of Arts (Health Science) Honours Kinesiology, York University – Toronto, ON;   August 2003

PUBLICATIONS IN PEER REVIEWED JOURNALS

A) First Authorships:

1. **Upshaw, Adam**. A 'sweet' take on obesity. Health Science Inquiry. 2012. Volume 3, Issue 1. p. 72

B) Manuscripts to be submitted:
1. **Upshaw, A.,** Lam, T., Bandegan, A., Lemon, P.W.R. Time Trial Performance 4 h following Glycogen-Depleting Exercise is Enhanced Similarly with Recovery Non-dairy Chocolate Beverages vs. Chocolate Milk.

2. **Upshaw, A.,** Wilkinson. S.B., Booth, W., Bandegan, A., Choque, O., Marsh, G., Lemon, P.W.R. Strength training gains are enhanced similarly with post exercise plant (hemp) or animal protein (whey and dairy milk) ingestion

ABSTRACTS PRESENTED AT SCIENTIFIC CONFERENCES

A) First Authorships:
1. **Upshaw, A.,** Huggard, J., Choque, O., Lemon, P.W.R. Hemp protein-carbohydrate drink post concurrent strength-HIIT improves 10km cycling time trial performance in young women.
   ASCM Annual Conference, Orlando Fl., 2014

2. **Upshaw, A.,** Lam, T., Bandegan, A., Lemon, P.W.R. Time Trial Performance 4 h following Glycogen-Depleting Exercise is Enhanced Similarly with Recovery Non-dairy Chocolate Beverages vs. Chocolate Milk.
   ASCM Annual Conference, San Francisco Ca., 2012.

3. **Upshaw, A.,** Wilkinson. S.B., Booth, W., Bandegan, A., Choque, O., Marsh, G., Lemon, P.W.R. Strength training gains are enhanced similarly with post exercise plant (hemp) or animal protein (whey and dairy milk) ingestion.
   CSEP Annual Conference, Regina Saskatchewan Canada, 2012.

B) Co-Authorships:
1. Booth, W**.,** **Upshaw, A**., Wilkinson, S.B, Lemon, P.W.R., Marsh, G. Resistance training induces similar Hypertrophy in the arm and thigh musculature of young women.
ASCM Annual Conference, San Francisco Ca., 2012

Fourth Year Undergraduate student Abstracts:

1. Joseph Vecchione, Manuel Quinones, Omar Choque, **Adam N. Upshaw**. Acute effect of two aerobic exercise modes on subsequent strength and power in young endurance trained subjects. *Bodies of Knowledge Conference – University of Toronto. June 2014*

2. Choque, O. Huggard, J.G., Lee, J., and **Upshaw, A.** The addition of strength training to high-intensity interval training does not have an effect on blood lactate levels in active young women. *Ontario Exercise Physiology* Conference. 2014

3. Choque O., **Upshaw, A**., Lemon, P. Post training session carbohydrate-hemp protein ingestion does not affect submaximal exercise blood lactate decreases resulting from six weeks of concurrent training. *Canadian Nutritional Society Conference. 2014*

4. Quinones, MD., Vecchione, J. and **Upshaw, A.** Endurance and High Intensity Interval Training similarly affect subsequent leg power and strength in young trained men. *Ontario Exercise Physiology Conference. 2014*

5. Vecchione, J., Quinones, M., and **Upshaw, A**. Strength and power following endurance and high intensity interval training are independent of gender. *Ontario Exercise Physiology Conference. 2014*

SEMINAR PRESENTATIONS

Humber Professional Showcase, Humber College   June 2010
▪ Health and Nutrition Presentation
▫ Seminar open to all Humber staff and students
▫ Present up-to-date, information of whole-vegetarian nutrition for recreational athletes
▫ Summarized information into workshop handouts including fitness and nutrition tips and instruction

Wellness Day – Professional Workshop, College of Physician and Surgeons of Ontario   September 2005
▪ Weight Management Presentation
▫ Delivered to staff which included physicians, nurses and other College staff.
▫ Presented up-to-date, researched advice for general nutritional and exercise.
▫ Summarized information into workshop handouts including fitness and nutrition tips and instruction.

PROFESSIONAL MEMBERSHIPS

Canadian Society of Exercise Physiology – CEP (Certified Exercise Physiologist)

Ontario College of Kinesiologists – Certified Kinesiologist

TEACHING EXPERIENCE

Sessional Lecturer, Guelph Humber University   September 2013-current
• Natural Health Products – 4th year undergraduate Kinesiology Program course

Professor, Humber College & Guelph Humber University   Sept. 2009 -current
• Part-time Instructor in the Fitness and Health Promotion and Kinesiology program
• Instructor for various courses including:
   o Sports Nutrition, Anatomy, Exercise Physiology, Exercise Prescription

- Completed a "teaching effectiveness certificate" program offered through Humber College (May/10).
- Participated in professional development seminars (whiteboard, PowerPoint, blackboard –online course forum)

Lab Supervisor/Grader, Humber College   Sept. 2008 – Jan. 2009
- Supervised and graded students who were in their 3rd semester of their Health and Fitness Promotion program, conduct practical fitness assessments of their individual personal training clients
- I supervised a total of 3 sessions – 1 in the September/08, 1 in December/08 and 1 in January/09. Each session had a total of four 9 hour days of supervision and grading.

Instructor, York University   Sept. 2003 – May 2005
- Taught fitness assessment/exercise prescription course to first year Kinesiology undergraduate students
- Prepared and delivered lectures to lab students on topics that cover the areas of anatomy, biomechanics, physiology, exercise physiology and health promotion

Teaching Assistant, York University   Sept. 2003 – May 2005
- Assisted with the lab component of the 3rd year Fitness Consulting and Personal Fitness Training course
- Provided one-on-one tutoring to students in need of extra instruction
- Supervised students laboratory work

Academic Advisor, York University   Jun. 2004 – July 2004
- Advised 1st year Kinesiology students on academic planning, on how to choose a major, and developing a program of study
- Advised students on academic standing and strategies/guidelines for continued academic success
- Advised students on how to transition from High School to Post-Secondary life

FITNESS INDUSTRY EXPERIENCE

Fitness Appraiser Supervisor, York University Fitness   Sept. 2003 - current
- Supervise and train new staff on techniques to test physical activity and performance levels.
- Administer fitness tests for the Ontario Fire Fighters, Ontario Forest Fire Fighters, Toronto Maple Leafs NHL entry draft prospects and members of the National Figure Skating Team.
- Work directly with a multidisciplinary team of health professionals to administer fitness tests.

Kinesiologist, Pro-Santé / Pfizer Canada   Aug. 2007 – current
- Administration of fitness tests to Pfizer Executives and Employees
    o Sub-max VO2, Strength Tests, Flexibility, Anthropometric Measurements

Kinesiologist, Cardiac Rehab Therapist   Jun. 2008 – Sept. 2009
- Prescribed exercise counseling, exercise routines, weight management and healthy behaviours to patients who suffered a cardiac event
- Responsible for networking with other hospital departments to ensure smooth flow and transitions of patients from critical care to outpatient

Exercise Physiologist/Personal Trainer, Home Business   Oct. 2006 – Sept. 2009
- Fitness counselling to physically fit individuals and athletes including recreational marathon runners, hockey players, a RMC applicant and a Military Officer.
- Physical Fitness seminar presentations for various organizations.
    - Health and Wellness Day – College of Physicians and Surgeons of Ontario

Recreational Director, York University   Jun. 2004 – July 2004
- Temporary Contract position - Summer
- Created and presented fitness seminars.
- Organized recreational activities for business executives attending seminars at the Schulich School of Business.
- Performed personalized training sessions with business executives.

MENTORSHIP
- 2010 – current   I have advised eleven 4th year Kinesiology undergraduates through their 4th year independent research projects (literature reviews or research studies)

- 2004 – 2005   During my Master's at York University I was recruited to volunteer as an academic advisor for Kinesiology undergraduates students.