Analysis of the effects of three commercially available supplements on performance, exercise induced changes and bio-markers in recreationally trained young males

Robert Cooper

A thesis is submitted in partial fulfilment of the requirements of the University of Greenwich for the Degree of Doctor of Philosophy

This research programme was carried out in collaboration with GlaxoSmithKline Maxinutrition division

December 2013

School of Science

University of Greenwich, Medway Campus

Chatham Maritime, Kent ME4 4TB, UK

DECLARATION

"I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any degree other than that of Doctor of Philosophy being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised the work of others".

Signed_____ Date_____

Mr Robert Cooper

(Candidate)

……

PhD Supervisors

Signed_____ Date_____

Dr Fernando Naclerio

(1st supervisor)

Signed_____ Date_____

Dr Mark Goss-Sampson

(2nd supervisor)

ACKNOWLEDGEMENTS

Thank you to my supervisory team, Dr Fernando Naclerio, Dr Mark Goss Sampson and Dr Judith Allgrove for their support and guidance throughout my PhD. Particular thanks to Dr Fernando Naclerio for his tireless efforts, guidance and support in developing the research and my own research and communication skills. Thank you to Dr Eneko Larumbe Zabala for the statistics support.

I would like to take this opportunity to thank my wonderful mother and sister who continue to give me the support and drive to succeed. Also on a personal level thank you to my amazing fiancée, Jennie Swift. I am eternally grateful for all you have done and put up with, your support has been invaluable.

Additionally I would like to thank Prof Martin Snowden for his guidance and support. Thank you to the entire Department of Sport Science especially Mr Mark Chapman and Ms Kelly Cooper.

Finally, I would like to acknowledge GlaxoSmithKline-Maxinutrition division for providing joint funding along with the University of Greenwich which made this project possible along with Prof Alfonso Jimenez who arranged the collaboration.

ABSTRACT

Commercially available multi-ingredient formulas are ingested by the recreationally trained population to optimise training outcomes; however, there remains no convincing evidence in regards to their effectiveness. Thus, the aim of this project was to analyse the effects of three different multi-ingredient supplements on the expected and marketed outcomes. It was hypothesised that the supplements would potentiate the desired body composition, performance and recovery outcomes as claimed.

Study 1 was conducted to analyse the effects of combining a 12 weeks resistance training programme with the ingestion of a commercially available carbohydrate-protein-creatine based multi-ingredient supplement on strength performance and body composition in recreationally trained males. It was hypothesised that the ingestion the multi-ingredient supplement would potentiate strength performance adaptations to a greater extent than a maltodextrin placebo. As a secondary hypothesis it was expected that ingesting the multi-ingredient supplement would benefit body composition outcomes in comparison to the placebo. Thirteen healthy male subjects were assigned to either a multi-ingredient formula (n=7) or a carbohydrate placebo (n=6). Both groups ingested the multi-ingredient supplement or placebo in the morning and immediately after training. Before and after the 12 weeks progressive resistance training; percentage body fat and fat free mass were determined. Maximum strength and repetitions to failure with 60% one repetition maximum on bench press and parallel squat were also assessed before and after the resistance training period. No significant increases in any of the performance or body composition variables were observed in either group. However, larger standardised effects sizes and magnitude-based inferences demonstrated that the addition of the multi-ingredient supplement to a 12 week progressive resistance training protocol could be effective to potentiate upper body maximum strength or muscular endurance performance, but not body composition outcomes.

Study 2 was undertaken in order to analyse the acute effects of a commercially available carbohydrate and caffeine gel on intermittent sprint performance in twelve recreationally trained males. It was hypothesised that the combination of carbohydrate and caffeine in gel form would attenuate fatigue and decrease perception of effort when compared to

the ingestion of carbohydrate gels alone and placebo gels. A secondary hypothesis postulated that the carbohydrate and caffeine gel would maintain blood glucose levels throughout the intermittent sprint test in regards to both the carbohydrate and placebo gels. Using a cross-over design, one 70 mL dose of gel containing either, 25 g of carbohydrate with or without 100 mg of caffeine or a non-caloric placebo was ingested on three occasions: one hour before, immediately prior and during the intermittent repeated sprint test. Blood glucose, rating of perceived exertion and fatigue index were analysed. The main finding was that ingesting the carbohydrate and caffeine gel one hour before, prior to and during an IST is effective at transiently reducing fatigue and RPE whilst maintaining higher glucose levels at the final stages of the exercise.

Study 3 was conducted in order to analyse the acute effects of a commercially available carbohydrate and protein based multi-ingredient recovery formula on the recovery process and muscle damage, in 10 recreationally trained males, after performing a bout of intermittent sprint exercise. It was hypothesised that the ingestion of a carbohydrate and protein based multi-ingredient supplement, before, during and after an acute bout of intermittent repeated sprint exercise would promote recovery estimated through the attenuation of neuromuscular fatigue and markers of muscle damage respect to the ingestion of carbohydrate only or a low caloric placebo. As a secondary hypothesis the ingestion of the multi nutrient formula would attenuate a decline in sprint performance during the intermittent sprint test when compared to the carbohydrate and placebo conditions. Using a cross-over design, one 500 mL dose of a multi-ingredient recovery beverage, a carbohydrate beverage or a placebo beverage was divided in to 4 equal servings of 125 mL and ingested before each of the 4 blocks of the intermittent sprint test. A second full serving was ingested with 20 minutes of completing the intermittent sprint test. 15m sprint times, creatine kinase, myoglobin, and interleukin-6 were assessed before (pre), immediately post (post), 1 hour and 24 hour after exercise. Total sprint time measured during the intermittent protocol was not different between conditions. 15m sprint time was slower at post, 1 hour and 24 hour compared to pre without differences between conditions. Creatine kinase at 24 hour was lower in the multi-ingredient compared to both carbohydrate and placebo. Myoglobin increased in all three conditions at post, and 1 hour compared to pre, showing lower values at 1 hour for the

carbohydrate and approached significance (p=0.060) for multi-ingredient compared to placebo condition. Interleukin-6 increased at both post and 1 hour compared to pre with no differences between conditions. Thus demonstrating, the ingestion of a multi-ingredient supplement before, during and immediately after a 90 min intermittent sprint test resulted in no effects on performance and fatigue. However, the accumulation of some biomarkers of muscle damage could be attenuated.

It would appear that manufacturers make use of the research available to formulate multi-ingredient supplements as the supplements that showed efficacy in studies 1 and 2 possess the most established research. Whereas investigations and knowledge in to the acute effects of a carbohydrate-protein based multi-ingredient supplement on recovery from an intermittent sprint test (study 3) still need to be determined.

ADDENDUM

Peer reviewed publications

Cooper R, Naclerio F, Allgrove J, Jimenez A: Creatine supplementation with specific view to exercise/sports performance: an update. *Journal of the International Society of Sports Nutrition 2012*, 9:33.

Cooper R, Naclerio F, Larumbe-Zabala E, Chassin L, Allgrove J, Jimenez A: Effects of a Carbohydrate Protein-Creatine Supplement on Strength Performance and Body Composition in Recreationally Resistance Trained Young Men. *Journal of Exercise Physiology online* 2013, 16:72-85.

Cooper R, Naclerio F, Allgrove J, Larumbe-Zabala E: Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males. *European Journal of Sport Science (Submitted:*2014).

Conference communication

Cooper R, Naclerio F, Allgrove J, Larumbe-Zabala E: (June 2013): Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males. 18th Annual Congress of the European College of Sport Science, Barcelona; oral presentation.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

%BF = percentage body fat

1RM = maximum strength

ATP = adenosine triphosphate

BT = bleep test

BM = body mass

BP = bench press

CHO = carbohydrate supplement

CHOCAF = carbohydrate-caffeine gel supplement (study 2)

CK = creatine kinase

CM = creatine monohydrate

CYC = carbohydrate-protein-creatine based multi-ingredient supplement (study 1)

d = Cohen´s *d*

EIMD = exercise induced muscle damage

ES = effect size

F = familiarisation

FFM = fat free mass

FI = fatigue index

FS = fasted sprint

GL = Glutamine

HMB = beta-hydroxy-beta-methylbutyrate

HMBCa = beta-hydroxy-beta-methylbutyrate mono-hydrated calcium salt

HMBFa = beta-hydroxy-beta-methylbutyrate acid

IRST = intermittent repeated sprint test

IST = intermittent sprint test

KIC = keto-isocaproate

LB = lower body

LIST = Loughborough Intermittent Shuttle Test

M = mean

MAS = maximum aerobic speed

Mb = myoglobin

MTN = carbohydrate-protein based multi-ingredient (Study 3)

PL = placebo

Post = post-assessment

Pre = pre-assessment

PRT = progressive resistance training programme

ROM = range of movement

RRT = recreationally resistance trained

RT = resistance trained

RTF60% = repetitions to failure with 60% maximum strength

RPE = rate of perceived exertion

S = standard deviation

SD = standard deviation

SGLT = intestinal sodium dependant glucose transporters

SQ = squat

SS = slowest sprint

UB = upper body

WP = whey protein

η^2 = eta squared

ω^2 = omega squared

Chapter 1
Overview

The terms supplement, dietary supplement or ergogenic aid in the sporting context relates to oral consumption of vitamins, minerals, herbal remedies, carbohydrates, proteins, amino acids and other substances [1]. Generally, dietary supplements are sold in the form of tablets, capsules, soft gels, liquids, powders, or bars [2]. In the UK, most dietary supplements are subject to the Trade Descriptions Act 1968, the general provisions of the Food Safety Act 1990, and the Food Labelling Regulations 1996, therefore it is not mandatory for manufacturers to prove the efficacy of their dietary supplements before marketing, nor are they subject to prior approval unless the dietary supplement is genetically modified or claimed to be new [3].

Dietary supplements have been researched across many age groups of both genders in a medical and sports/exercise context [4-7]. The use of dietary supplements also spans these populations [8-10] and a large proportion of consumers are that of the general population [2]. In 2009, the market value for dietary supplements and vitamins in the United Kingdom was worth more than £670 million and in 2010 the sports supplement market alone was worth £200 million [11]. According to a 2 year survey conducted from 2008-10, a quarter of adults aged 19-64 consumed at least 1 dietary supplement within a 4 day recording period, and an even higher proportion of participants reported taking at least one type of supplement during the previous year [12].

The efficacy, safety and regulatory status of dietary supplements can sometimes be unclear [13]. This is potentially due to a few factors such as individuals that are inadequately scientifically informed advising on supplement use, misinformation found on the internet, weak research based recommendations of supplement use and poor quality control [2]. With the rise in internet sales of dietary supplements [14] it is difficult to regulate their sale and use [15].

Dietary supplements are used by both professional and recreational athletes [16]. The rational for the use for some of these supplements is often lacking in solid evidenced based research [4]. Research into dietary supplementation has been carried out in vitro,

in animal models and in human studies raising questions as to the efficacy and safety of these supplements [17].

Dietary supplements have been associated with endocrine and metabolic disorders as well as hepatotoxicity [4, 15, 18]. For example the excessive or prolonged use of caffeine has been associated with restlessness, nervousness, tachycardia, arrhythmias and hypertension [19]. Although these negative aspects are usually only present in particularly sensitive individuals or with the ingestion of amounts >9 $mg \cdot kg^{-1}$ [20]. However, when combined with an appropriate training and nutritional programme dietary supplements can create a favourable metabolic environment and/or optimise training outcomes, which is well documented in the scientific literature [21-25]. Furthermore, the various combinations of supplements that are found as commercially available 'multi-ingredient formulas', in the sports supplement market, require further scientific evidence to assess the proposed efficacy and safety of these products.

Supplementation, just like nutrition and training, should be specific to the desired outcome. For example, a rugby player may wish to increase strength and power whereas a marathon runner seeks to increase endurance capacity. The training and nutritional strategies for these two specific objectives would be substantially different, therefore it is only fitting that the supplementation strategies should also consider each specific objective [26]. Although the same supplements may be used in each case, the amounts and timings of the supplementation could be different. A marathon runner, for example, would benefit from greater amounts of carbohydrate, especially around times of long duration endurance runs, than a rugby player would require during a match [24]. This is one of the reasons by which some commercially available products provide varying combinations of ingredients, aimed to satisfy the requirements of specific modes of exercise and sports.

The effectiveness of individual supplements, such as carbohydrate, protein, amino acids, and creatine, has been extensively studied. However, the more recent literature has supported the use of combinations of supplements for improving performance. While the positive effects from individual supplements such as whey protein, carbohydrate, creatine monohydrate, β-hydroxy-β-methylbutyrate, carnitine and to a lesser extent

possibly glutamine on exercise and sports performance are generally supported, the effect of multi-ingredient commercially available products with specific combinations is not well documented [27].

Given the strong body of evidence on the efficacy of some individual supplements, such as creatine monohydrate, to improve exercise performance, attenuate muscle damage and promote the recovery process, manufacturers have recently developed new multi-ingredient formulas. The ingredients in these formulas are usually designed to work synergistically towards the same predetermined goal, with the additional benefit of the convenience of different supplements being ingested in one intake.

Chapter 2
General introduction

2.1 Brief

Of the many commercially available dietary supplements only a few have a strong evidence base for their efficacy [2]. This literature review focuses on the supplements featuring in this project. The mechanisms and effects of each individual supplement will be reviewed, as well as the possible additive effects when in combination as a part of different multi-ingredient formulas.

2.2 Carbohydrate

Carbohydrate supplements can be useful to assist exercise practitioners to provide the required or optimal amount of carbohydrate in their diet. The human body has a limited supply of carbohydrate. Around 300 to 500 g and 75 to 100 g of glycogen is stored in the liver and muscles respectively [28]. With the depletion of glycogen glucose availability to exercising muscle and central nervous system will be limited. Low glycogen levels have been shown to negatively affect performance of prolonged (>90 min) sub-maximal or intermittent high intensity exercise [29]. Furthermore, muscle tissue break down and immune depression is associated with the depletion of glycogen [24].

In order to maintain appropriate glycogen stores, it has been recommended for general fitness practitioners, team sport players and endurance athletes to consume between 3-5 $g \cdot kg^{-1} \cdot day^{-1}$ to 5-8 $g \cdot kg^{-1} \cdot day^{-1}$ and up to 8-10 $g \cdot kg^{-1} \cdot day^{-1}$ of carbohydrate, respectively [25].

As exercise intensity rises, the rate of carbohydrate utilisation increases. When exercising at low to moderate intensity (50 to 65% of maximum oxygen uptake) the percentage energy contribution obtained from fatty acids will peak predominating over the energy obtained from carbohydrate. However, as the intensity continues to increase above 65% of maximum oxygen uptake, the utilisation of carbohydrate as an energy substrate (mainly from muscle glycogen stores) will further increase and progressively become the predominant energy substrate over fat and protein [30].

Several studies [24] have considered the ingestion of carbohydrate as one of the main strategies for heightening endogenous glycogen stores and maintaining serum glucose

and muscle glycogen levels; this strategy is predominately used to enhance long lasting endurance exercise. Glycogen stores can be significantly increased to a sports/event specific desired level. A practitioner of moderate intensity exercise lasting over 90 min could benefit from approximately 36 hours of high carbohydrate intake (10 – 12 $g \cdot kg^{-1} \cdot d^{-1}$) and rest [29]. Exercise practitioners involved in intense and long duration exercise training may consume concentrated carbohydrate supplements as beverages, bars and/or gels. These forms of carbohydrates have been recently proposed, due to the difficulty of ingesting high amounts of carbohydrate rich foods, to achieve daily requirements as well as to provide the required amounts of carbohydrate to attenuate a drop in performance during exercise [31]. However, for the general population enrolled in a typical low to moderate intensity endurance training programme (three to four times a week for 30 to 60 min), it would be not necessary as carbohydrate demands can typically be satisfied with a normal diet [25].

Carbohydrate ingestion one hour prior to exercise is controversial in terms of performance benefits. One possible reason for this controversy is based on the time (around four hours) necessary for storage, in the form of muscle or liver glycogen, to occur after the ingestion and digestion of a carbohydrate rich meal. It is highly accepted that in order to avoid possible digestive disturbances and enhance glycogen saturation, the last carbohydrate rich meal should be consumed about 4 to 6 hours prior to exercise [25].

Carbohydrate feeding during exercise may improve performance by maintaining blood glucose concentrations, increasing levels of glucose oxidation, sparing endogenous glycogen, synthesising glycogen during low intensity exercise [32], or a central effect mediated via carbohydrate receptors in the mouth stimulating areas of the brain linked with reward and motivation [33]. Consideration to the type of carbohydrate should be given, since fructose and galactose have to be first converted into glucose in the liver before they can be metabolised. When those types of monosaccharaides are consumed, a slower rate of carbohydrate utilisation will be produced in respect to when glucose is provided [34].

Carbohydrate feeding, during both short duration high intensity and prolonged low intensity exercise maintains blood glucose concentrations and has elicited performance benefits such as improved time trail time or increased time to exhaustion [34]. It is generally acknowledged that the human body can oxidise exogenous carbohydrate around 1 $g \cdot min^{-1}$ [24]. Thus the American College of Sports Medicine [35] has recommended that in order to maintain appropriate levels of glycaemia and attenuate glycogen depletion, carbohydrate in amounts of 0.7 $g \cdot kg^{-1} \cdot hour^{-1}$ should be ingested during exercise. This can be consumed as a solution (6-8%) as this will also support hydration, however, ingesting carbohydrate as a solid or a gel and consuming water would also be appropriate [35].

In well trained endurance athletes, the rate of exogenous carbohydrate oxidation can be increased to approximately 1.5 to 1.8 $g \cdot min^{-1}$ by ingesting a mix of carbohydrates [36]. This is because glucose absorption relies on an intestinal sodium dependent glucose transporter, which becomes saturated at about 1 $g \cdot min^{-1}$ whereas fructose is transported from the intestine using the sodium independent facilitative glucose transporter 5 [37]. This may result in an increased rate of appearance of glucose in the blood stream when glucose is provided in a 2:1 and 1:1 ratio with fructose [37], and sucrose [38] respectively compared to the ingestion of an isocaloric amount of glucose alone.

Carbohydrate mouth rinses of around 6.4%, have demonstrated an improvement in performance when running and cycling at approximately 75% of VO_2max [39] but not maximal strength or strength endurance related activities [40]. These positive effects appear to be greater after an overnight fast [39]. Receptors in the mouth detect the presence of carbohydrate, not sweetness, stimulating areas of the brain (including the anterior cingulate cortex and striatum) linked with reward, motivation and regulation of motor activity [41]. Continued research in this area is required to fully understand the separate taste transduction pathways of different carbohydrates [39].

Ingestion of 1.2 $g \cdot kg^{-1} \cdot hour^{-1}$ of carbohydrate within 30 min after a glycogen depleting exercise results in a higher rate of muscle glycogen re-synthesis compared to post exercise ingestion being delayed by two hours [36]. This is largely due to increased muscle insulin sensitivity [24]. Glycogen re-synthesis occurs to similar levels regardless of

the form (solid or liquid) of ingested carbohydrate [24]. Nevertheless, as fructose does not stimulate insulin secretion [42] lower levels of glycogen re-synthesis are observed in regards to other simple carbohydrates [24]. Furthermore, frequent feedings (every one to two hours) for four to six hours after glycogen depleting exercise should ensure muscle and liver glycogen re-synthesis [24].

In addition to the positive effects of carbohydrate on glycogen re-synthesis and glucose oxidation, carbohydrate consumption before, during and after exercise has been related with beneficial effects on anabolic and catabolic hormones, such as insulin, growth hormone and cortisol [43].

The ingestion of carbohydrate increases serum concentrations of insulin which can stimulate the cellular uptake of amino acids facilitating protein synthesis, and suppressing protein catabolism. Therefore, rises in insulin levels help to maintain a more anabolic cellular environment and support a better recovery process after exercise. Artificially induced high levels of growth hormone, with concurrent resistance training, have been shown to increase fat free mass and decrease fat mass via increases of amino acid transport and protein synthesis [43]. Hypoglycaemia, mediated by a rapid insulin response to the ingestion of a large bolus of carbohydrate or a high glycaemic index carbohydrate, can result in higher levels of growth hormone [44]. Therefore, carbohydrate mediated increases in growth hormone could potentiate hypertrophy outcomes from resistance training [25].

Cortisol is considered a general catabolic hormone. In the skeletal muscle, cortisol can suppress muscle protein synthesis and promote muscle protein break down in type I and to a greater extent type II muscle fibres. If cortisol is chronically elevated, it has the potential to result in sustained muscle protein breakdown and loss of performance in athletes and general exercise practitioners [43]. The ingestion of carbohydrate before, during and after prolonged endurance, intermittent or resistance exercise has resulted in attenuated cortisol levels [43, 45, 46]. This effect has been shown to be associated with an increase of insulin [43]. Hypoglycaemia initiates a rise in cortisol which significantly stimulates muscle protein catabolism, providing amino acids for supporting liver gluconeogenesis [43]. Therefore, providing carbohydrate before and during exercise

would maintain blood glucose levels, attenuating muscle and liver glycogen utilisation and reduce protein break down [45]. Additionally, cortisol elevations, from exhaustive exercise, can negatively affect the immune system via cytotoxic effect on lymphocytes, therefore carbohydrate supplementation could attenuate decreases in immune functioning [43].

Collectively, consuming carbohydrate around times of exercise, either alone or with protein or amino acids, has shown to optimise exercise performance, improve recovery and attenuate muscle damage [24].

2.3 Whey protein

Similar to carbohydrate, protein supplementation can be useful to optimise diets in regular exercise practitioners, particularly those enrolled in high intensity or strength and power related sports [25]. The turnover for the skeletal muscle protein is about 1 to 2% and is usually in a state of equilibrium between muscle protein breakdown and muscle protein synthesis. Generally, muscle protein synthesis will predominate over muscle protein breakdown when a protein containing meal is consumed [47].

The recommended daily allowance for the general population, children, adolescents and adults, is 0.8 $g \cdot kg^{-1} \cdot day^{-1}$. Research has shown that athletes undertaking intense training require more protein, in amounts of 1.5 to 2 $g \cdot kg^{-1} \cdot day^{-1}$, to maintain protein balance [25] or promote muscle hypertrophy (also dependent on training and energy intake) [48]. Consuming inadequate amounts of protein will result in increased protein catabolism, due to a negative nitrogen balance, which will slow recovery and over time could lead to muscle wasting resulting in negative training consequences [25]. The increased amount of protein required by the active population would result from an increase in intramuscular protein oxidation and breakdown caused by higher activity levels [49]. In addition, larger amounts of protein would be needed in order to complement intramuscular protein re-synthesis and attenuate proteolytic mechanisms that occur as a consequence of exercise [50].

In the athletic population, dietary protein can be utilised as a signal and a substrate for muscle protein synthesis. Therefore, timing, quality and quantity of protein intake in

relation to the training stimulus should be considered as an important factor for maximising training adaptations [47]. The use of protein and amino acid supplements, particularly in young males, has demonstrated positive physiological adaptations to exercise [51]. However, although it has been established that protein supplementation before, during or after exercise can promote adaptation and recovery, there is no clear consensus as to which timing, of protein supplementation, promotes the greatest adaptive response [47].

Whey protein, a commonly used commercially available nutritional supplement, is a high quality, rapidly absorbed milk protein rich in essential amino acids, which are necessary for stimulating and conducting muscle protein synthesis [51]. Whey protein is available in several forms, either as whey protein concentrate, isolate, hydrolysate and native whey which may contain between 29 and 89% total protein with the remaining fractions comprising of carbohydrate (predominately lactose) and lipid. The higher concentrations (≥70% protein) of whey protein concentrate may not significantly affect gastric emptying rate and amino acid absorption [51] and may actually be of benefit, due to the lipid content, on post exercise protein balance. Elliot *et al* [52] demonstrated that the post resistance exercise ingestion of fat free and whole milk may increase utilisation of available amino acids for protein synthesis when compared to fat free milk.

Supplementation with whey protein hydrosylate has been shown to produce a greater degree of muscle protein synthesis during the first three hours after ingestion, in respect to casein and soy protein, both at rest (93 and 18% respectively) and after resistance exercise (122 and 31% respectively) in young healthy males. It is speculated that these findings may hold true for whey protein isolate and concentrate [51].

Whey protein is rich in the branch chain amino acid leucine, which has been shown to increase skeletal muscle protein synthesis by acting as a signalling molecule in the mammalian target of rapamycin (mTOR), a central component of a complex signalling network responsible for regulation of cell growth and proliferation as well as organism size [53]. Amino acids, particularly leucine, can activate mTOR complex 1 signalling via inhibition of tuberous sclerosis proteins 1 and possibly 2 or, alternatively, via stimulation

of the GTP binding protein Ras homolog enriched in brain (Rheb), furthermore, it is possible that amino acids are sensed directly by mTOR complex 1 [54].

Although rapid leucinemia may activate protein synthesis, other essential amino acids may be required to sustain the anabolic response [47]. Around 0.75 g of leucine in combination with other essential amino acids will initiate maximal protein synthesis in young males [55]. However, a more sustained elevation of protein synthesis, after resistance exercise, has been achieved from a dose of whey containing both essential and non-essential amino acids, when compared to essential amino acids alone [55]. This presumably would lead to a greater hypertrophy response to resistance exercise, as a greater muscle protein accrual in elderly subjects has been observed from 15 g of whey protein when compared to a dose of its constituent 6.72 g dose of essential amino acids [51].

In young males, the ingestion of 20 g of protein after a bout of resistance exercise, has been shown to elicit a plateau of muscle protein synthesis, with an absence of any further increase up to 40 g. Furthermore, there was a marked stimulation of whole-body leucine oxidation above the 20 g threshold [56]. Similarly, a 10 g dose of essential amino acids, which is the equivalent to approximately a 25 g dose of intact protein such as whey, has been shown to maximally stimulate protein synthesis at rest [47]. Additionally, the ingestion of 20 g of whey before or after an acute bout of resistance exercise (at 80% of one repetition maximum) found no difference in the positive anabolic response between the two times of ingestion, suggesting that whey protein supplementation before or after exercise can maximally stimulate protein synthesis after an exercise bout [24].

It is suggested that high quality protein, such as whey, should be ingested as soon as possible following exercise as the muscle is prone to increased rates of muscle protein synthesis which are enhanced by feeding. The acute protein synthetic response to protein supplementation post exercise on endurance performance appears to be on myofibrillar proteins, much like the response to resistance exercise, therefore assisting in maintaining structural integrity and power generating ability [47]. In addition to the effects on protein synthesis, whey protein supplementation has been linked with positive effects on

immune function in animal studies most likely due to cysteine; an amino acid that is needed for glutathione (an antioxidant) production [49].

Collectively, it seems that whey protein supplementation can be of benefit, to both resistance and endurance exercise via mechanisms related to protein synthesis and attenuating muscle damage [24].

2.4 β-Hydroxy-β-Methylbutyrate (HMB)

HMB is a metabolite of the essential and branch chain amino acid leucine. HMB is produced through the transanimation of leucine to α-keto-isocaproate (KIC) by the enzyme branched chain amino acid transferase. KIC is then metabolised, in the mitochondria, by the enzyme α-ketoacid dehydrogenase to form isovaleryl-CoA (the primary metabolite of leucine) or in the cytosol by the enzyme α-ketoisocaproate dioxygenase to form HMB [57].

Endogenic synthesis of HMB ranges from 0.3 to 1.0 $g \cdot day^{-1}$ depending on the quantity of HMB containing foods (grapefruit, catfish, dairy and meat) ingested [58]. Approximately 5% of leucine is metabolised to form HMB, therefore 60 g of leucine would have to be consumed to achieve a 3 $g \cdot day^{-1}$ dose of HMB [57]. This would require the impractical ingestion of 600 $g \cdot day^{-1}$ of high quality protein to achieve this level of leucine consumption, therefore, supplementation with HMB is a more practical approach [59].

HMB is commercially available as a salt, monohydrated calcium salt (HMBCa), with the empirical formula Ca (HMB)2-H2O [59]. Recently, a new free-acid form of HMB, known as β-hydroxy-β-methylbutyric acid (HMBfa), has been investigated showing quicker and greater plasma levels (185%) and 25% faster plasma clearance rate, indicating improved tissue uptake and utilisation when compared to HMBCa [60]. Despite this, the majority of the research focuses on HMBCa [59].

HMB supplementation is suggested to possess anti-catabolic, anabolic and lipolytic effects [57]. The effects of HMB are presumed to be mediated through anti-catabolic action and therefore attenuation of muscle damage [61]. This rationale is due to the absence of ergogenic effects when HMB is consumed without exercise, in healthy participants [57]. However, the mechanisms of action are still being considered [58].

Hydroxymethylbutyrate, stored in the cytosol of liver and muscle cells, is converted to 3-hydroxy-3-methylglutarylcoenzyme A and used in the cellular synthesis of cholesterol, which may promote muscle growth by attenuating deficiencies of cholesterol during muscle cell hypertrophy [62]. Reduced cholesterol in the muscle cell may result in inadequate cholesterol for cell membrane synthesis, retarding the growth of the cell and/or causing inadequate cell membrane functioning [63]. The effect of HMB supplementation on the muscle cell membrane (sarcolemma) is thought to be the reason why HMB supplementation has been shown to attenuate exercise induced muscle proteolysis [63]. Therefore, a training programme would have to result in an increased amount of muscle damage through an increase activity and/or intensity of the current programme in order to potentially benefit from the supplementation of HMB [64]. This may be one of the reasons for the controversies on the efficacy of HMB supplementation [57].

It is likely that the training status of an individual and the training stimulus are interlinked as to the outcome of HMB supplementation. Muscle damage appears earlier in a training programme in untrained participants, compared to trained [65]. It is therefore more likely that untrained participants will demonstrate a benefit from HMB supplementation, especially when considering that the majority of studies on HMB are of a duration of 4 weeks or less [59].

The majority of the research on HMB supplementation has been performed on strength/power athletes and bodybuilders to improve performance and increase muscle hypertrophy [58]. Despite this, HMB supplementation has the potential to improve aerobic performance, fat loss and mitochondrial biogenesis [59]. The mechanisms of action for improving endurance performance and fat loss are poorly understood, however, it is thought to be mediated by an improvement in fatty acid oxidation, adenosine monophosphate kinase (an intracellular metabolic regulator), silent information regulator transcript 1 and silent information regulator transcript 3 activity (a class of deacetylases that modify the acetylation level of histones and protein) in 3T3-L1 adipocytes and in skeletal muscle cells [59] therefore improving mitochondrial biogenesis, fat oxidation, energy metabolism, and the reactive oxygen defence system. Increases in protein synthesis and skeletal muscle regeneration have been observed due

to HMB supplementation, the mechanisms for this are poorly understood but thought to be mediated through stimulation of mTOR and satellite cell activation. HMB supplementation has been previously observed to increase phosphorylation of mTOR and its downstream targets ribosomal protein S6 kinase and eukaryotic initiation factor 4 binding protein 1 [59].

2.5 Carnitine (L-3-hydroxytrimethylaminobutanoate)

The carnitine pool, in mammals, consists of nonesterified L-carnitine and many acylcarnitine esters [66]. Humans obtain 75% of carnitine through the diet, the remainder is synthesised from the body using the essential amino acids lysine and methionine [67] in amounts of approximately 1 to 2 $\mu mol \cdot kg^{-1} \cdot day^{-1}$ [66]. Carnitine is predominately sourced from red meat and dairy products [68]. In adults, although the daily maximal mucosal absorption is around 2 g, a daily intake of carnitine between 24 to 81 mg would be sufficient to allow for normal physiological functioning in its roles during energy production and fatty acid metabolism [69].

The biologically active stereoisomer of carnitine is L-carnitine which is absorbed from foods across enterocyte (intestinal cell) membranes and also defuses through the serosal membrane and into the circulation [66]. The bioavailability of L-carnitine is dictated by exogenous consumption. L-carnitine bioavailability is high in vegetarians, who are adapted to low carnitine diets (66 to 86%) but is lower in regular red meat consumers, who are adapted to high carnitine diets (54 to 72%) [66]. Humans can also synthesise carnitine in the kidneys, liver and brain from the essential amino acids lysine and methionine, although there are essential co-factors (ascorbic acid, ferrous iron, pyroxidine and niacin) and any deficiencies in these co-factors could result in carnitine deficiency [67].

Carnitine can be found in low concentrations in the plasma, which transports the carnitine to the heart and skeletal muscle where 98% of the bodies total carnitine (of around 27 g) is found [70]. Microorganisms of the large intestine mostly degrade any unabsorbed L-carnitine [66]. Carnitine is expelled from the body via renal excretion, however, this is usually in amounts of <5% as carnitine is efficiently reabsorbed depending on plasma concentrations [66].

The transport of long chain fatty acids across the outer and inner mitochondrial membranes (carnitine palmitoyl transferase I and II, respectively) relies on carnitine. This premise led to the theory that oral L-carnitine supplementation would enhance skeletal muscle concentrations, thus increasing transport and β-oxidation of fatty acids [68] and therefore the chronic supplementation would lead to a gradual loss of body fat [70]. This theory has been demonstrated as incorrect as muscle carnitine concentrations and lipid oxidation have not been increased from oral L-carnitine supplementation despite increased availability of serum carnitine [70, 71]. However, recent research has observed increases in muscle carnitine concentration, with favourable effects, due to the co-ingestion of L-carnitine L-tartrate with carbohydrate [72]; however, this will be reviewed in section 2.12.

Carnitine has also been investigated for its ability to attenuate hypoxic stress, muscle damage and promote exercise recovery [68]. Specifically, adenosine triphosphate is broken down, as a result of exercise. This causes an accumulation of adenosine diphosphate within the smooth muscle of the pre-capillary sphincter and activation of the enzyme adenylate kinase, which catalyses two adensosine diphosphate molecules to form adenosine triphosphate and adenosine monophosphate. Hypoxanthine is then formed, due to the accumulation of adenosine monophosphate, and diffuses out of the capillary endothelial cell. Exercise induced hypoxia causes an accumulation of intracellular calcium, due to shortage of adenosine triphosphate, interrupting normal functioning adenosine triphosphate dependant calcium pumps. Increased intracellular calcium results in the activation of calcium dependent proteases causing the proteolytic cleavage of a portion of xanthine dehydrogenase converting it to xanthine oxidase which acts as a catalyst for hypoxanthine to form xanthine, which is converted to uric acid. A superoxide radical is formed from these reactions, as molecular oxygen is used as an electron acceptor, and can combine with iron to produce reactive hydroxyl radical disrupting polyunsaturated fatty acids in cell membranes causing a chain of lipid peroxidation reactions. Aldehydes such as malondialdehyde, which can be measured in the plasma as a marker of free radical damage, are produced from lipid peroxidation. The damage inflicted on the cell membranes causes cytosolic proteins to leak out in to the plasma, which can be used as a measure of tissue damage. Intermediates formed from

superoxide radicals can attract neutrophils which further disrupt cellular membranes [68]. L-Carnitine supplementation increases serum carnitine concentration which enhances capillary endothelial function increasing blood flow, resulting in a reduction of exercise induced hypoxia, therefore, attenuating structural damage [68]. Additionally, Kraemer *et al* [73] suggests that more intact receptors, as a result of attenuated cell damage, will be available for hormonal interactions if a greater amount of undamaged tissue is present.

2.6 Glutamine

In humans, glutamine is the most abundant non-essential free amino acid in the skeletal muscle and plasma [74] and in the muscle tissue where it represents about 60% of the total free amino acids [75]. Glutamine is required for a number of physiological roles [74], such as the immune system [76], protein synthesis [77] and glycogen synthesis [78].

Theoretically, glutamine supplementation has the potential to affect exercise performance directly, by means of reduced fatigue [79], or indirectly through means of attenuating exercise induced immune depression [80] allowing for continued training and/or better quality training sessions in the absence of illness.

Exercise can cause decreases in plasma glutamine concentration [81], which have been linked with overtraining syndrome [76] and disruption of normal leukocyte activity [74]. This has led to the hypothesis that glutamine supplementation can attenuate exercise induced immune depression [80]. Although this has yet to be scientifically proven [82, 83].

Early studies in humans and animal models showed that glutamine was used in a number of physiological roles that could promote performance and muscle hypertrophy [77, 78, 84]. Currently, general consensus is that there is not any research that would justify the use of glutamine supplementation to directly enhance performance; however, it is recommended that further research is needed [25].

There is evidence for glutamine supplementation to have a positive effect on exercise performance by helping produce a favourable metabolic environment via mechanisms such as faster replenishment of citric acid intermediates [85], increases in plasma growth

hormone concentrations [86] and attenuation of exercise induced blood ammonia levels [79]. Despite this, it is regularly reported that as a result of glutamine supplementation, performance and body composition outcomes are not statistically different than that of the placebo group [75, 87]. In addition, glutamine supplementation has resulted in post exercise glycogen resynthesis without the stimulation of insulin [88].

2.7 Creatine

Creatine is one of the most popular and widely used and researched supplements. The majority of studies have focused on the effects of creatine monohydrate on performance and health such as in the treatment of various pathologies or disorders such as myopathies [89]. Many other forms of creatine exist and are commercially available in the sports nutrition/supplement market most of which have their specific manufacturer claims that are often unfounded [90].

Creatine is produced endogenously, predominately in the liver, kidneys, and to a lesser extent in the pancreas, at an amount of about 1 $g \cdot day^{-1}$. The remainder of the creatine available to the body is obtained through the diet at about 1 $g \cdot day^{-1}$ for an omnivorous diet. 95% of the bodies creatine stores are found in the skeletal muscle and the remaining 5% is distributed in the brain, liver, kidneys, and testes [91].

The majority of creatine in the human body is in two forms, either the phosphorylated form making up 60% of the stores or in the free form which makes up 40% of the stores. The average 70 kg young male has a creatine pool of around 120 to 140 g which varies between individuals [92, 93] depending on the skeletal muscle fibre type [91] and quantity of muscle mass [93]. The endogenous production and dietary intake matches the rate of creatinine production from the degradation of phosphocreatine and creatine at 2.6 and 1.1%·day respectively. In general, oral creatine supplementation leads to an increase of creatine levels within the body predominately in the skeletal muscle [89]. Creatine can be cleared from the blood by uptake into various organs and cells or by renal filtration [91].

Three amino acids (glycine, arginine and methionine) and three enzymes (L-arginine:glycine amidinotransferase, guanidinoacetate methyltransferase and methionine

adenosyltransferase) are required for creatine synthesis. Creatine ingested through supplementation is transported into the cells exclusively by creatine transporter 1 (CreaT1). However, there is another creatine transporter creatine transporter 2 (CreaT2), which is primarily active and present in the testes [94]. Creatine uptake is regulated by various mechanisms, namely phosphorylation and glycosylation as well as extracellular and intracellular levels of creatine. Crea T1 has shown to be highly sensitive to the extracellular and intracellular levels being specifically activated when total creatine content inside the cell decreases [94]. It has also been observed that in addition to cytosolic creatine, the existence of a mitochondrial isoform of Crea T1 allows creatine to be transported into the mitochondria. This indicates another intra-mitochondrial pool of creatine, which seems to play an essential role in the phosphate transport system from the mitochondria to the cytosol [95].

Many studies have shown supplementation with creatine to increase strength, fat free mass, and muscle morphology with concurrent heavy resistance training more than resistance training alone [21]. Creatine may be of benefit in other modes of exercise such as high intensity sprints or endurance training. However, it appears that the effects of creatine diminish as the length of time spent exercising increases [96]. Additionally, creatine supplementation could benefit recovery from injury [97], as well as muscle damage [98] and oxidative stress [99].

Even though not all individuals respond similarly to creatine supplementation [100], it is generally accepted that its supplementation increases creatine storage and promotes a faster regeneration of adenosine triphosphate between high intensity exercises [96]. These improved outcomes will increase performance and promote greater training adaptations [21]. More recent research suggests that creatine supplementation combined with resistance training improves training adaptations at a cellular and sub-cellular level [22, 23]. Current consensus indicates ingesting creatine as an oral supplement is considered safe and ethical. However the perception of safety cannot be guaranteed, especially when administered for long period of time to different populations [21].

2.8 Caffeine (1,3,7-trimethylxanthine)

Caffeine is the most consumed pharmacologic and psychoactive stimulating substance in the world [101] and has been used as an ergogenic aid to enhance cognitive and physical performance [102]. Caffeine is naturally present in a number of foods in varying amounts, coffee and tea contain approximately 60 to 150 mg and 40 to 60 mg of caffeine per cup, respectively [101].

Once ingested caffeine is absorbed through the gastrointestinal tract, it then passes through cellular membranes and circulated to tissue [20] and since caffeine is lipid soluble it can easily cross the blood brain barrier [103]. Through a process of enzymatic action, caffeine metabolised by the liver producing three metabolites: paraxanthine, theophylline, and theobromine [20]. Circulating caffeine levels peak between 30 to 75 min after ingestion [101] but elevated levels can be apparent after 15 min [20]. Caffeine has a half life of around four to five hours which can increase if the dose is above 300 mg [101]. Caffeine and its metabolites are excreted via the kidneys [20].

Caffeine has been successfully used to enhance endurance [104], strength [105] and team sports [106] performance, as well as to improve mood state [107] and cognitive functioning [108]. There are several mechanisms of action to explain the effects of caffeine including cognitive perception and habituation [101]. It is difficult to determine if the greatest effect of caffeine is on the nervous system or on the skeletal muscle because of how freely caffeine can move around the body [20]. Initially, it was thought that altered substrate metabolism was the primary ergogenic action of caffeine as it was demonstrated that caffeine could spare glycogen and increase fat oxidation [101]. However, more mechanisms have been demonstrated.

One of the primary mechanism is now thought to be at the adenosine receptor sites, where caffeine competes with adenosine [103] attenuating adenosines calming effect on the central nervous system [101] and increasing arousal [108]. Another mechanism is thought to be analgesic action, via increased secretion of β-endorphins, leading to altered pain/effort perception and subsequently enhancing endurance [109] and resistance [105] exercise performance. Muscle force production can also be enhanced from caffeine supplementation due to an increased intracellular Ca^{++} concentration and improved Na^{+}–

K^+ ATPase pump activity [110]. Caffeine can stimulate the release from intracellular Ca^{++}, via the caffeine sensitive ryanodine receptor acting as a Ca^{++} channel, which acts as a signal for various neuronal processes including neurotransmitter release [111]. With regard to improved cognitive performance, caffeine can increase reaction time and enhance attention [108] as well as improving alertness, even in a sleep deprived state [20].

Caffeine habituation can occur after five to six days of a moderate daily dose (6 $mg \cdot kg^{-1}$), which can affect individual responses to a given dose due to increased tolerance [101]. For example, Bell [104] observed the ergogenic effect of caffeine, from a moderate dose, to differ between habituated caffeine consumers and non-consumers as the effect of caffeine on endurance cycle ergometry was greater and of longer duration in the non-consumers.

High dose caffeine supplementation and intake late in the day can cause unwanted side effects, such as, jitteriness, nervousness, nausea, headache and insomnia which could lead to decreased performance [101]. Caffeine withdrawal can cause similar side effects, which peak between 28 to 48 h before decreasing to baseline values in four to seven days, although this does not occur in all individuals but again could lead to decreased performance [101]. To avoid symptoms of withdrawal in habituated caffeine consumers the dose should be gradually reduced over three to four days, instead of quitting abruptly [101]. The notion that caffeine supplementation can negatively affect hydration is unsupported in the literature as studies have failed to show changes in sweat rate, total water loss, or negative change in fluid balance that could negatively affect performance, even under conditions of heat stress [20].

It is necessary to consider the timing of an acute dose of caffeine, as the ergogenic effects of caffeine are observed one and three hours after caffeine ingestion (5 mg kg^{-1}) but not at six hours [104]. Taking into account time to peak plasma caffeine concentrations, it is therefore generally recommended to consume an acute dose of caffeine about one hour prior to prolonged endurance exercise or no more than three hours before power, sprint and short endurance events [101]. Additionally, caffeine ingestion in the anhydrous state (tablet or powder form) produces a greater ergogenic effect than caffeine from a cup of

coffee presumably due to derivatives of chlorogenic acids present in coffee that may have the potential for altering the affects of caffeine as an adenosine antagonist [20].

Finally, although the World Anti-Doping Agency does not view caffeine as a banned substance, the International Olympic Committee mandates a maximum of 12 µg of caffeine per mL of urine, which could result from 9 to 13 $mg \cdot kg^{-1}$ dose of caffeine around one hour prior to performance [20].

2.9 Bicarbonates

Bicarbonates are alkalinising agents ingested to improve anaerobic exercise endurance performance [2]. Arterial blood pH of the average resting human is around pH 7.4, however, this may fall to pH 7.1 and muscle pH to 6.8 due to strenuous exercise [112]. The reduction of adenosine triphosphate, during muscular contractions, causes the release of hydrogen ions into the cellular space. If there is adequate phosphocreatine present, the hydrogen ions will bind to it allowing for continued muscle contraction. However if phosphocreatine availability is reduced during high intensity anaerobic contractions, the hydrogen ions within the cell will build up and decrease intracellular pH levels therefore causing acidity [28]. This metabolic acidosis is involved with the fatigue process through inhibition of key glycolytic enzymes, interfering with calcium transport and binding, or by a direct effect on the actin–myosin interaction. It is possible to delay this fatigue process by induction of alkalosis prior to exercise, increasing muscle buffering capacity and increasing the efflux of hydrogen ions from the working muscles [76]. The hydrogen ions are absorbed by CO_2 (also buffered by bicarbonates) and then expelled by the lungs [25].

Bicarbonate, as a supplement, is commercially available predominately as sodium bicarbonate ($NaHCO_3$) [25] but is also available in other forms such as potassium bicarbonate (KHCO3) [113]. Bicarbonate supplements are usually ingested orally in solution or capsule form [76].

Supplementation with sodium bicarbonate, in amounts of around 300 $mg \cdot kg^{-1}$ ingested about 90 min before exercise, can transiently increase blood bicarbonate concentrations. This has been shown to enhance the muscle buffering capacity of hydrogen ions,

produced from anaerobic glycolysis, which accumulate and efflux from the working muscle. An increased blood buffering capacity has the potential to increase time to fatigue from high intensity exercise [114]. Chronic supplementation of lower doses, such as 5 g ingested twice a day for five days, has also been shown to reduce exercise induced acidosis and improve performance of short duration high intensity exercise [25].

Studies into sodium bicarbonate supplementation have shown an ergogenic effect for sports/activities involving one to seven minutes of sustained strenuous exercise and for prolonged sports/activities involving intermittent or sustained periods of high intensity work rates. In addition prolonged exercise, below the anaerobic threshold, could also benefit from sodium bicarbonate supplementation if bursts of increased intensity are present, such as a sprint to the finish in the final stage of an endurance run. In the same manner, modes of intermittent exercise which involve repeated bursts of intensity may be improved from sodium bicarbonate supplementation [114].

Hypertrophic resistance exercise has shown increased ability to perform repetitions, due to sodium bicarbonate supplementation, later on in the exercise session [115]. This phenomenon is typically seen from sodium bicarbonate supplementation, presumably due to a strong enough pH gradient not being established until later on in the exercise [115]. A recent meta-analysis found a potential for a moderate 1.7% (90% confidence limit ± 2.0%) increase in performance of a one minute sprint in male athletes, and an additional increase of approximately 0.5% for each 100 $mg \cdot kg^{-1}$ body mass of sodium bicarbonate supplementation or by the addition of five extra sprints. Reductions in the ergogenic effect were seen in amounts of 0.6% (± 0.9%) for each 10 fold increase in test duration (e.g. 1 to 10 min); reductions of 1.1% (±1.1%) with non-athletes and 0.7% (± 1.4%) with females.

Although not related directly to improving performance, bicarbonate supplementation has, in vitro and in animal models, shown to increase synthesis of proteoglycans, and improved bone mineral content and density, which therefore may improve bones and connective tissue [28]. Potassium bicarbonate may offer advantages over sodium bicarbonate in this regard. For example, urinary calcium retention was reduced from supplementation with potassium bicarbonate and not sodium bicarbonate, improving

calcium balance via enhancing renal calcium retention and/or skeletal calcium retention [116]. However, there is not enough available data to make an accurate recommendation [28].

Although bicarbonates are permitted for use by the World Anti-Doping Agency code [112], it should be considered that Individual tolerances to bicarbonate supplementation differ. It is frequently reported that gastrointestinal distress occurs, such as vomiting and diarrhoea, from relatively small doses of bicarbonate. This would greatly limit any performance improvements in susceptible individuals. However, these effects are not serious and there seem to be no long term adverse consequences of occasional use. Although tolerance should be tested before any competitive event, as there have been anecdotal reports of athletes using bicarbonates and being unable to compete due to the severity of gastrointestinal distress [76].

2.10 BioPerine (piperine)

BioPerine is a commercially available standardised black pepper extract that usually contains around 98% piperine [117]. Piperine (1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4, pentadienyl]piperidine), a non-polar molecule and alkaloid, is the main bioactive compound from the fruit of Piper nigrum and Piper longum. Chavicine, piperines stereoisomer, is also present in the fruit and converts to piperine upon storage. Piperine has been shown to possess immune modulatory, anti-oxidant, anti-asthmatic, anti-carcinogenic, anti-inflammatory, anti-ulcer, and anti-amoebic properties, based on using modern cell, animal and human studies. Piperine is used as a bioavailability enhancer for certain drugs, as piperine is a non-polar molecule which may form complexes with more polar drugs leading to increased absorption [118]. The bioavailability of certain nutrients can also be enhanced by piperine supplementation which will be the focus of this section [118]. This property, of increased bioavailability of nutrients, has led manufacturers of supplements to include piperine in many nutritional supplements [117].

Using rat and guinea pig models, both in vitro and in vivo, piperine supplementation has resulted in significantly enhanced pancreatic lipase activity and stimulated pancreatic amylase, trypsin, and chymotrypsin which may contribute to the recognised digestive stimulant action of piperine. Enhancement of digestion seems to be the main attribute

for the increased bioavailability of nutrients that piperine exerts. Piperine supplementation can cause an alteration in membrane lipid dynamics and conformation of enzymes in the intestine, as well as stimulate leucine amino peptidase and glycyl-glycine dipeptidase activity. Furthermore, microvilli length and free ribosomes and ribosomes on the endoplasmic reticulum in enterocytes have been increased due to piperine supplementation. Piperine, which is transported through transcellular pathways, is rapidly absorbed across the intestinal barrier and demonstrates a short absorption clearance and a high apparent permeability coefficient. [118].

Increased plasma levels, due to increased gastrointestinal absorption, of supplemented nutrients has been observed when concurrently ingested with as small an amount as 5 mg of piperine [119]. For example, the area under the curve for serum concentrations of coenzyme Q10 was found to be significantly 30% greater from 21 days of 120 mg of coenzyme Q10, ingested once a day, with the concurrent ingestion of 5 mg of piperine in regards to the same dose of coenzyme Q10 ingested with a placebo [119]. Similar findings have been reported for β-carotene supplementation with concurrent piperine ingestion [117].

Piperine ingested daily with nutrient(s) is effective and safe in a broad dose range (0.0004 – 0.15 $mg \cdot kg^{-1} \cdot d^{-1}$) [120]. As assessed by mice models, piperine supplementation did not induce male germ cell mutations, leading to the conclusion that piperine is a non-genotoxic chemical. Furthermore, no adverse effects on growth, food efficiency ratio, and organ weights; red and white blood cell count and differential counts; levels of blood constituents, such as, hemoglobin, total serum proteins, albumin, globulin, sugar, and cholesterol; levels of serum aminotransferases and phosphatases; and fat and nitrogen balance have been observed in rat models from doses 5 to 20 times normal human intake [118]. Despite this care should be taken if medications are being consumed, due to piperines effects on drug metabolism [121].

Although piperine supplementation has been shown to be effective for enhancing the absorption of β-carotene, vitamin B6, vitamin C, and selenium, it has not as yet been studied to assist with the absorption of carbohydrate, protein and creatine containing multi-ingredient supplements [119] Therefore, it appears that the justification for the

addition of piperine to multi-ingredient supplements is related to the previously observed effects of enhancing digestion.

2.11 Chromium

Chromium is an essential mineral that must be obtained through the diet. Trivalent chromium and hexavalent chromium are two common forms. Trivalent chromium is the form obtained from food and utilised by the body [28].

Chromium enhances glucose tolerance, protein and lipid metabolism, and serum cholesterol. It is suggested that chromium is part of an auto-amplification system for insulin signalling due to its ability to increase insulin sensitivity via its effect on tyrosine kinase activity on insulin activated insulin receptors. More specifically, insulin sensitive cells store apochromodulin, when insulin is bound to sensitive cells, due to increases in blood insulin, auto-phosphorylation of tyrosine residues on the internal side of the receptor occurs. This changes the receptor into an active tyrosine kinase which sends a signal from the insulin to the cell, moving chromium into the insulin sensitive cell from the blood causing the apochromodulin to be loaded with chromium forming a holochromodulin. It is thought that the binding of holochromodulin to the insulin receptor keeps the receptor in its active conformation. The receptor reverts back to its relaxed state due to a reduction in insulin levels causing the holochromodulin to exit the cell into the blood stream to be expelled in the urine [122].

Chromium is predominately commercially available as chromium picolinate, however, chromium nicotinate and chromium chloride supplements are also available. Additionally, it is thought that the organic compound picolinic acid that binds to chromium enhances absorption and transport [70].

It has been reported that practitioners of intense exercise, particularly that of resistance exercise, have increased acute losses of chromium, leading to the hypothesis that chromium is a potentially anabolic nutrient [123]. An initial study, split into two experiments, demonstrated that 200 $\mu g \cdot day^{-1}$ increased fat free mass without increasing fat mass and that chromium supplementation, of the same amount, resulted in a significant reduction in body fat. However, energy intake and dietary chromium were not

taken into account, furthermore, skin fold and limb circumference measurements were used to assess body composition which is not an ideal method [28]. Since this initial study similar findings, using more precise methods of measuring body composition such as dual energy X-ray absorptiometry and hydrostatic weighing, have not been replicated in other studies [25]. Furthermore, although more research is need, in vitro experiments on animal cells have shown chromium picolinate to accumulate in cells and cause chromosome damage. This has not been confirmed in human studies [70].

Although chromium supplementation appears ineffective for weight loss [70], there seems to be evidence that chromium supplementation in combination with a carbohydrate and protein supplement (as part of a controlled energy intake), can be effective for reducing body fat whilst maintaining fat free mass compared to a placebo group [124].

2.12 Supplement combinations

There are various combinations of ergogenic aids and nutritional components that are commercially available in sports supplement market. Due to the legislation in the UK, as described in chapter 1, it is not mandatory to prove the efficacy of dietary supplements unless a product is deemed new to the market. Research is currently being performed on multi-ingredient products [27, 125, 126]. Further evidence is required to assess the purported uses and efficacy of multi-ingredient supplements especially in regards to individual supplements.

Only a few studies have demonstrated beneficial effects from various combinations of supplements [127]. For example, L-carnitine supplementation alone has not resulted in the increase of muscle carnitine concentrations [70]. However, when two doses per day of 80 g of glucose polymer were combined with 2 g of L-carnitine L-tartrate a 21% increase in carnitine muscle concentration was observed after 24 weeks of supplementation [72]. Possibly the rise in insulin concentrations, elicited by the concurrent carbohydrate feeding, would have mediated this positive effect that was not observed when L-carnitine was administered alone. In addition, the above mentioned supplementation protocol elicited a greater work output in cycle ergometry performance resulting in an 11% improvement from baseline, and a 35% improvement in regards to

the control group. Substrate use was also modified during exercise at 50% VO_2max as muscle glycogen utilisation was reduced, indicating a possible increase in muscle lipid utilisation. Increased fat oxidation was previously thought unlikely from L-carnitine supplementation. However, the interpretation and possible application of the pervious results to another population need further analysis [70].

Combinations of carbohydrate and protein supplementation have been shown to elicit positive benefits with no major adverse effects in already healthy individuals [19]. The benefits of carbohydrate supplementation in combination with protein/amino acids seems to positively affect the recovery response after muscle damaging exercise [24]. With regards to endurance exercise, the addition of protein, in amounts of 0.2 to 0.5 $g \cdot kg \cdot day^{-1}$, to carbohydrate at a ratio of 1:3 respectively has been shown to stimulate glycogen re-synthesis to a greater extent than carbohydrate alone when ingested post exercise [24].

Specific to resistance exercise, supplementation with combinations of carbohydrate, protein/amino acids and/or creatine before and after a training session has been shown to promote a more anabolic environment, muscle hypertrophy and recovery compared to when similar amount of nutrients are ingested more than four to six hours before or after the training session [48, 128]. The addition of carbohydrate to protein causes a greater rise in insulin, suppressing muscle protein breakdown [47]. Adding creatine to a carbohydrate and protein supplement has been shown to further promote the adaptation response to resistance training in resistance trained males, due to the additive effects of creatine [127]. Supplementing with carbohydrate and the combination of carbohydrate and protein appears to increase the absorption of creatine, opposed to ingesting creatine alone, mediated through an insulin response from the pancreas. This has not been shown to have any performance effects but would promote a faster muscle creatine saturation rate [89].

The concurrent ingestion of carbohydrate and caffeine has demonstrated positive performance effects, such as improved sprints, jumps and agility, during high intensity performance when applied to an intermittent exercise test intended to simulate team sports [129, 130]. Furthermore, the simultaneous supplementation of carbohydrate and

caffeine has been shown to improve intestinal glucose absorption [131], increase rates of exogenous carbohydrate oxidation and improve steady state [132] and intermittent endurance [133] performance in respect to carbohydrate ingestion alone.

In order to stimulate greater training adaptations, improve recovery process and favour a more anabolic environment, some vitamins, minerals and amino acids or derivatives such as L-glutamine or and HMB, as well buffering substances such as bicarbonates, have been frequently added to carbohydrate and/or protein multi-ingredient formulas [125, 134, 135]. Various combinations of commercially available supplements have demonstrated efficacy for potentiating adaptation to exercise, improving performance and promoting recovery [127, 133, 136]. However, given the fact that there are currently many multi-ingredient supplements continuously appearing on the market, it would be beneficial to develop high quality research aimed to assess the efficacy of multi-ingredient supplements. The results from these investigations would provide essential and useful information to consumers and assist manufacturers in the development of effective products as well.

2.13 Rationale for the current research

In spite of recommendations and the extensive use of several multi-ingredient formulas, ingested to optimise training outcomes, there remains no convincing evidence in regards to their efficacy. Furthermore, the effects of these supplements on the average recreational exercise/sports practitioner, as opposed to a high performance or sports specific athlete, would allow for a greater general application of the potential results and represents a substantial proportion of the consumers from the supplement market [2].

It has been reported that recreational exercise practitioners ingest individually or mixtures of whey protein, carbohydrate, amino acids, creatine and caffeine to potentiate training outcomes, promote recovery and enhance performance [137]. The supplement choices depend on the exercise programme and the type of sport [9]. Another reason for a recreational exercise practitioners choice of supplements may be down to clever marketing strategies that portray supplements to produce unrealistic results in a short space of time [138].

2.14 Aims and hypothesis

The aim of this project was to analyse the effects of three different multi-ingredient supplements on the expected and marketed outcomes, performance and recovery when performing different types of resistance and intermittent endurance training in young adult male recreational exercise practitioners. It was hypothesised that the supplements would potentiate the desired body composition, performance and recovery outcomes as claimed. In order to achieve the proposed objective, three studies were designed.

The first study was conducted to analyse the effects of combining a 12 weeks resistance training programme with the ingestion of a carbohydrate-protein-creatine based supplement on strength performance and body composition in recreationally trained men. It was hypothesised that the ingestion of the multi-ingredient supplement would potentiate strength performance adaptations to a greater extent than a maltodextrin placebo. As a secondary hypothesis it was expected that ingesting the multi-ingredient supplement would benefit body composition outcomes in comparison to the placebo.

The second study was aimed to analyse the acute effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males. It was hypothesised that the ingestion of the combination of carbohydrate and caffeine in gel form would attenuate fatigue and decrease perception of effort when compared to the ingestion of carbohydrate gels alone and placebo gels. A secondary hypothesis postulated that the ingestion of the carbohydrate and caffeine gel would maintain blood glucose levels throughout the intermittent sprint test in regards to both the carbohydrate and placebo gels.

The third study was aimed to analyse the acute effects of a carbohydrate-protein based multi-ingredient recovery formula on the recovery process and muscle damage after performing a bout of intermittent sprint exercise. It was hypothesised that the ingestion of a carbohydrate, protein based multi-ingredient supplement, before, during and after an acute bout of an intermittent repeated sprint exercise would promote recovery estimated through attenuation of neuromuscular fatigue and markers of muscle damage respect to the ingestion of carbohydrate only or a low caloric placebo. As a secondary hypothesis the ingestion of the multi-ingredient formula would attenuate a decline in

sprint performance during the intermittent sprint test when compared to the carbohydrate and placebo conditions.

The information in table 1 shows the relevant manufacturer suggested effects from each of the three studies. A certificate of analysis was provided by the manufacture to verify the content of each supplement. Supplements were labelled with an alpha numeric code and distributed by an independent research technician to ensure double blinding of the experimental conditions.

Table 1 Relevant manufacturer claims for supplements used in studies 1, 2 and 3

Study	Relevant manufacturer suggested effects
1	Increase muscle mass Increase strength
2	Attenuate fatigue Provide fuel
3	Muscle recovery

Chapter 3

Study 1 - Effects of a carbohydrate-protein-creatine supplement on strength performance and body composition in recreationally resistance trained young men

3.1 Introduction

Traditional nutritional interventions in athletes have focused on carbohydrate, protein, amino acids, and other natural supplements such as creatine [139]. However, the more current literature has supported a combination of different nutrients as effective for improving performance [140, 141]. While the positive effects from individual supplements such as whey protein [51, 142], creatine monohydrate (CM) [21, 143], carbohydrate [144], HMB [145, 146] and to a lesser extent glutamine (GL) [139] on health and sports performance are generally supported, the effect of multi-ingredient products with specific combinations is not well documented. Also, research into the effects of nutritional supplements on the average gym user as opposed to a high performance or sports specific athlete would allow for a greater general application of the potential results.

Today, resistance training (RT) is one of the most popular physical activities recommended for people regardless of age. In fact, evidence exists to support the effectiveness of RT to improve strength, muscle mass, and physical performance (including daily living activities) [147]. Interestingly, only a few studies have examined the effects of a multi-ingredient supplements on the performance outcomes obtained from a high intensity RT programmes. Cribb *et al* [148] observed greater improvement on maximal strength, lean mass, fibre cross sectional area, and muscle contractile proteins after 10 wks of RT combined with a multi-ingredient carbohydrate, whey protein, and CM compared to an equivalent dose of only whey protein or carbohydrate. Schmitz *et al* [149] observed greater significant improvements in strength, muscle endurance, and body composition in a group of young males when RT was combined with a multi-ingredient supplement rather than a similar supplement containing identical quantities of CM, whey protein, and carbohydrate but lacking in other specific synergetic ingredients that are supposed to elicit positive synergistic affects to enhance training outcomes.

Meanwhile, Kraemer *et al* [136] observed positive effects of a popular multi-ingredient supplement for improving strength and power, and performance.

Based on the research findings and the expert recommendations, supplement manufacturers have developed various multi-ingredient supplements combining whey protein, CM, carbohydrate, and other anabolic or anti-catabolic agents such as HMB. These mixes should potentiate the benefits induced by RT workouts by favouring a more anabolic state of the body throughout the day mediated from the attenuation of increases in catabolic hormones and decreases of anabolic hormones as well as signalling and maintaining protein synthesis and limiting protein degradation [136, 150, 151]. Aside from the convenience of having multiple ingredients in one product, there is potential for the components to exert additive or synergistic effects when combined [149, 152]. To our knowledge no studies have assessed the effects of a multi-ingredient supplement providing >10 $g \cdot d^{-1}$ of CM combined with whey protein, carbohydrate, HMB, and GL on strength performance and body composition in recreational RT practitioners. Therefore, the aim of this study was to analyse the effects of the commercially available multi-ingredient supplement (CYC) that consists of CM, carbohydrate, whey protein, GL, and HMB on 12 wks of a progressive resistance training (PRT) programme on body composition, strength, and muscular endurance in recreationally resistance trained (RRT) young adult males.

It was hypothesised that the ingestion the multi-ingredient supplement would potentiate strength performance adaptations to a greater extent than a maltodextrin placebo. As a secondary hypothesis it was expected that ingesting the multi-ingredient supplement would benefit body composition outcomes in comparison to the maltodextrin placebo.

3.2 Methods

3.2.1 Participants

Thirteen healthy RRT males (24 ± 3 years, BM = 80 ± 13 kg, height = 179 ± 6 cm) were randomised to receive either CYC or PL in combination with a 12 wk PRT programme. The data in table 2 shows the group specific demographic characteristics.

Table 2. Baseline mean and SD values for age, height and BM in both groups

Characteristics	CYC (n = 7)	PL (n = 6)
Age (yrs)	22 ± 1	26 ± 2
Height (cm)	178 ± 5	180 ± 8
Body mass (kg)	73.7 ± 11.4	87.3 ± 11.7

All participants agreed to comply with the RT and supplementation protocol and provided written informed consent (see Appendix IV). Approval of the research proposal was granted by the Universities Research Ethics Committee, in accordance with the standards of the declaration of Helsinki. Participants were regular but recreationally RT practitioners with at least two years experience, possessed normal vital signs and were free from musculoskeletal limitations. Physical activity levels and health history were determined at baseline using a standardised questionnaire (see appendix V)[153].

Key criteria used for exclusion of the participants were: (a) history of various metabolic conditions and/or diseases; (b) concomitant use of a variety of medications, including but not limited to those with androgenic and/or anabolic effects; (c) use of nutritional supplements known to improve strength and/or muscle mass such as creatine, HMB, whey protein, GL and dehydroepiandrosterone within six weeks prior to the start of the study; (d) current use of tobacco products; and (e) the presence of any orthopedic limitations or injuries.

3.2.2 Experimental design

The study used a randomised, double blinded, placebo-controlled parallel design. Since the participants were recreationally experienced RT practitioners, only two days of familiarisation with the testing procedures and minimal correction of exercise techniques were needed to minimise any potential learning effects with the assessment methodology. After familiarisation, the participants were randomly assigned to a supplementation group: (CYC; n = 7) or a placebo group (PL; n = 6). Before (Pre) and after (Post) the 12 wk PRT period, all of the participants were assessed for body composition, maximal strength, and muscle endurance capabilities as depicted in Figure 1. The

participants were instructed to maintain the recommended dietary habits throughout the duration of the study.

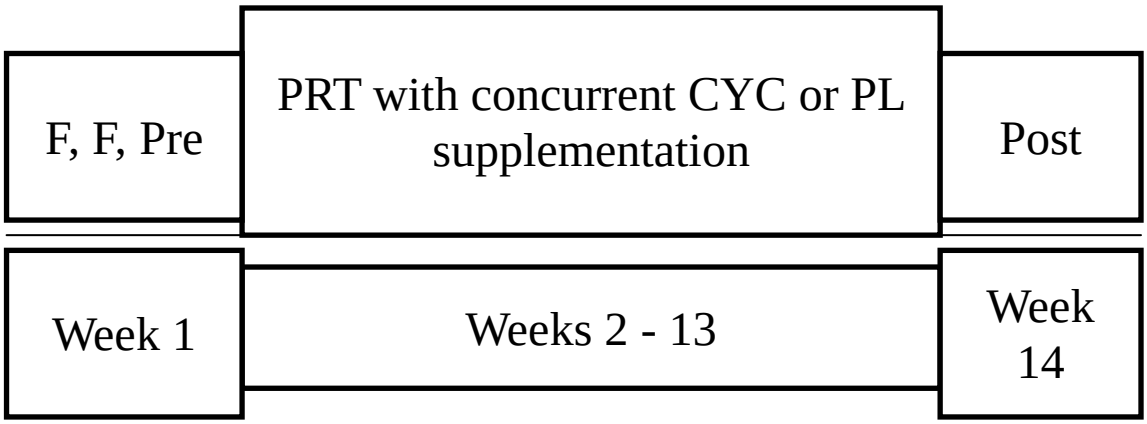

Figure 1. Schematic of study design

F = Familiarisation period; Pre: Pre assessment; Post: Post assessment

3.2.3 Procedures

Prior to any testing session, the participants were instructed to refrain from any vigorous activity for 48 h and avoid caffeine ingestion for at least 24 h.

Nutrition. A research nutritionist collected dietary habits (see appendix VI) and explained the proper procedures for recording dietary intake. The baseline diet of all participants (three consecutive days consisting of two weekdays, and one weekend day) was analysed using Dietplan 6 software to determine its energy and macronutrient content by a research nutritionist. In order to guarantee an adequate macronutrient intake throughout the 12 wk study intervention, a standardised nutritional diet plan was given to each participant. According to the American Colleges Sports Medicine [154] and the International Society of Sports Nutrition recommendation [155] and in order to promote optimal outcomes from the RT programme, 1.5 to 2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein, 5 to 6 g of carbohydrate $g \cdot kg^{-1} \cdot d^{-1}$ along with 25% to 30% of total caloric intake from fats had to be provided by the diets.

3.2.4 Experimental protocols

Exercise assessment. The bench press (BP) and parallel squat (SQ) exercises were

performed using Olympic bars and plates [156]. For the BP exercise, the participants had to maintain contact with the bench throughout the lift and perform each repetition with proper exercise technique by lowering the bar to touch the chest before returning the weight to the starting position. In order to standardise exercise technique for the SQ exercise, the participants were instructed to maintain a shoulder width stance and descend until the thighs were parallel to the floor.

The one repetition maximum (1 RM) and repetitions to failure at 60% of one repetition maximum (RTF60%) tests were performed for both the BP and SQ exercises. To minimise fatigue, the following assessment order was used: 1st 1 RM and 2nd RTF60%. To avoid any specific muscle group interaction, the order of testing for BP and SQ was randomised. All tests were carried out pre and post intervention at the same time of day specific to each participant. All testing sessions were started with a standardised, general warm up of three to five minutes, which consisted of light dynamic flexibility exercise involving the muscle(s) to be tested.

Resistance training programme. All participants were placed on a 4 $d \cdot wk^{-1}$ upper/lower split PRT programme that incorporated all the muscle groups for 12 wks as shown in table 3.

Table 3. Training schedule

Day	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Training	UB	LB	Rest	UB	LB	Rest	Rest

UB = Upper body exercises

LB = Lower body exercises

Training methods were standardised, again to eliminate as many confounding variables as possible. A progressive, 12 wk, hypertrophy, split training programme (four set per exercise of 6 to 12 repetition with 65 to 80% 1 RM range and two minute rest between sets) was designed based on previously published findings [147, 157]. The upper body (UB) and lower body (LB) routines were organised as follows:

- UB: Bench press; Bent over row; Shoulder press, Bicep curls, and Triceps extension

- LB: Squat, Stiff leg deadlift, Lunges, and Dynamic upright row

The exercise regime was based upon the 1 RM for BP and SQ of each participant:

Set 1: 12 repetitions at 65% 1 RM;

Set 2: 10 repetitions at 70% 1 RM;

Set 3: 8 repetitions at 75% 1 RM,

Set 4: 6 repetitions at 80% 1 RM.

The weight for the remaining exercises was adjusted, based on the resistance training experience of the participants, to allow for the required number of repetitions per set. Good form and technique had to be maintained at all times and with the exception of the fourth set, no failure achievement was allowed. As the participants perceived performance improvements, in order to maintain training stimulus, weight was added at 2.5% or 5% increments of the previously used weight for the upper and lower body, respectively.

3.2.5 Supplementation protocol

On each of the testing and training days the participants ingested the CYC or PL twice per day: one serving (60 g) with 350 – 400 mL of water at breakfast and another immediately (within 15 min) after the workout. On non-training days, the second intake was ingested in the afternoon at approximate the same hour of training. The data in table 4 provides the nutritional composition for one 70 g serving of Cyc and PL.

Table 4. Nutritional composition for CYC or PL

	Cyc (60 g serving)	PL (60 g serving)
Total Energy (Kcal)	230 Kcal	228 Kcal
Whey protein (g)	30	0
Carbohydrate (g)	21	56
Fat (g)	4.68	0
Creatine Monohydrate (g)	5.1	0
Glutamine (g)	5.1	0
HMB (g)	1.5	0
Potassium Bicarbonate (mg)	500	0
Sodium Bicarbonate (mg)	500	0
Bioperine (mg)	5	0
Chromium Picolinate (μg)	241	0

The PL supplement was virtually indistinguishable from the CYC supplement in taste, colour, and consistency. Both the CYC and PL supplements were prepared in powder form and packaged in coded generic sachets for double blind administration by an independent company (Maxinutrition). Compliance to the supplementation protocol was monitored by a researcher who contacted the study participants on a weekly basis. All participants were required to bring in their supplement sachets on the sixth week and the twelfth week for visual inspection by study personnel to assess compliance with the research protocol.

3.2.6 Measurements

Anthropometry. Body mass and height was assessed, on a standard scale and stadiometer according the methods described by Ross and Marfel-Jones [19]. Body composition was assessed by whole body densitometry using air displacement via the Bod Pod® (Life Measurements, Concord, CA). Dual-energy X-ray absorptiometry would have been the preferred method of assessing body composition, however in the absence of a qualified radiographer operator required to conform to the Universities regulations a Bod Pod® was used. All testing was done in accordance with the manufacturer instructions as detailed elsewhere [158]. Briefly, the participants were tested wearing only tight fitting clothing

(swimsuit or undergarments) and an acrylic swim cap. The participants wore the exact same clothing for all testing. Thoracic gas volume was estimated for all participants using a predictive equation integral to the Bod Pod® software. The calculated value for body density was based on the Siri equation to estimate body fat [159] (Eq. 1)

Equation 1. Siri equation

$$\%\, Body\ fat = \langle \frac{495}{body\ density} \rangle - 450$$

Measurements were performed twice and if the percentage of body fat (%BF) was within 0.05%, the two tests were averaged. If the two tests were not within the 0.05% agreement, a third test was performed and, then, the average of three complete trials was used for all body composition variables.

1 RM test. The 1 RM test was determined according to methodology proposed by Baechle *et al* [160]. In short, the participants performed a specific warm up set of eight repetitions at 50% of the perceived 1 RM followed by another set of three repetitions at 75% of the perceived 1 RM. Subsequent lifts were single repetitions of progressively heavier weights until reaching the 1 RM. This process was repeated until a maximum of five attempts. If the participants arrived at the fifth attempt, they were then asked to perform as many repetitions as possible. If more than one repetition was performed in the fifth attempt, the 1 RM value was calculated using the Brzycki equation [160] (Equation 2).

Equation 2. Brzycki equation

$$1RM\ (kg) = \frac{weight\ lifted(kg)}{[1.0278 - (0.0278 * repetitions\ achieved)]}$$

The test-retest intra-class reliability for the two exercise test was R >0.93 to <0.98 ($p<0.001$).

RTF60%. Muscle endurance for the BP and the SQ exercises was measured as the total repetitions completed during a single bout of maximum repetitions to failure, using 60% of the previous determined 1 RM. All participants were required to perform repetitions with correct form until voluntary exhaustion or failure of exercise form [161].

3.2.7 Statistical analyses

Mean and standard deviation (SD) are expressed as mean ± SD. Data normality of distribution for each group was assessed using the Shapiro-Wilk test and scrutinising the Q-Q plots. Series of factorial ANOVA, (2 × 2; time [pre vs. Post training] × group [CYC vs. PL]) were employed. The repeated measures were the pre and post treatment, and the treatment groups were CYC and PL. For all variables tested with the 2 x 2 factorial ANOVA, equality of covariance was checked with the box test of equality of covariance matrices while the Levene test was used to ascertain equality of variances. Independent samples *t* test were conducted to compare BM, height, %BF, and fat free mass (FFM).

Standardised effect sizes (ES) were calculated to determine the magnitude of an effect independent of sample size. Cohen´s effect sizes (d) were measured by the formula (Equasion 3):

Equation 3. Cohens d

$$Cohen's\ d = \frac{M2-M1}{SD}$$

Small effect sizes are considered $d \leq 0.2$, *moderate* effect sizes are $0.2 < d < 0.8$, and *large* effects sizes are $d \geq 0.8$ [162]. A multivariate analysis of effects was performed for the different treatment groups on all the dependent variables.

In addition to the use of statistical significance and standardised effect sizes, magnitude based inferences were used to determine the practical significance of 1 RM BP, 1 RM SQ, RTF60%, BP and RTF60% SQ performance. Using a Microsoft Excel spreadsheet designed for sports science research [163], mean effects and the 90% confidence limits were estimated to establish the percentage likelihood of each experimental condition having a positive/trivial/negative effect on performance. The smallest worthwhile improvement for 1 RM and RTF60% for both BP and SQ was considered to be an increase equivalent to 0.2 between participant standardised ES established from baseline performance [164], which were 2.2 kg (2.4%) and 4.1 kg (3.3%) for 1 RM BP and 1 RM SQ and 1.6 (7.5%) repetitions and 0.9 (4.0%) for BP RTF60% and SQ RTF60%, respectively. IBM SPSS Statistics software (version 19) was used to conduct the statistical analysis.

3.3 Results

3.3.1 Dietary analysis

There were no significant differences in dietary intake for the participants in either cohort, based on dietary diary evaluation ($p>0.05$). Dietary protein contents were between the expected range of >1.5 to 2.0 $g \cdot kg^{-1}$ for all of the participants regardless of the group. Means and SD for average daily consumption of carbohydrate, protein, fat and energy analysed from the three days diet diary for all participants was 5.3 ± 0.3, 1.8 ± 0.2, 1.2 ± 0.2 $g \cdot kg^{-1}$ and mean 39 ± 3 $Kcal \cdot kg^{-1}$ respectively.

3.3.2 Body composition

The data in table 5 shows the mean and SD values for the body composition variables.

Table 5. Mean and SD body composition values measured before (Pre) and after (Post) the training period

Group	Body composition			
	%BF		FFM (kg)	
	Pre	Post	Pre	Post
CYC	11.3 ± 5.4	13.2 ± 5.1	64.9 ± 6.6	66.9 ± 7.4
PL	18.4 ± 10.8	18.5 ± 10.4	70.4 ± 5.3	71.1 ± 5.7

*$p<0.05$ CYC vs PL

No significant statistical differences were observed at baseline for any of the variables ($p>0.05$). A main effect for time interaction was observed for BM ($F_{(1,11)}=14.98$, $p<0.005$, $\eta^2=<0.577$). The interaction between time and group was also significant ($F_{(1,11)}=5.75$, $p<0.05$, $\eta^2=<0.343$). However, no significant interaction effects was observed between the groups ($F_{(1,11)}=3.56$, $p>0.05$, $\eta^2=0.245$). The same approach was adopted in the analysis of the %BF and FFM. The time main effect was significant for FFM ($F_{(1,11)}=7.60$, $p<0.05$, $\eta^2=0.409$), but not for %BF ($F_{(1,11)}=3.42$, $p>0.05$, $\eta^2=0.237$). The group by time interaction and the group main effects were not significant for %BF ($F_{(1,11)}=2.78$, $p>0.05$), $\eta^2=0.201$), ($F_{(1,11)}=1.90$, $p>0.05$, $\eta^2=0.147$), respectively, and FFM ($F_{(1,11)}=1.91$, $p>0.05$, $\eta^2=0.148$), ($F_{(1,11)}=1.90$, $p>0.05$, $\eta^2=0.147$).

3.3.3 Performance

The data in table 6 shows the mean and SD values for the performance variables.

Table 6. Mean and SD strength related performance values measured before (Pre) and after (Post) the training period

Exercise	BP				SQ			
Test	1 RM (kg)		RTF60%		1 RM		RTF60%	
Group	CYC	PL	CYC	PL	CYC	PL	CYC	PL
Pre	88 ± 16	96 ± 30	18 ± 4	20 ± 2	111 ± 33	137 ± 43	22 ± 8	22 ± 9
Post	104 ± 22	105 ±24	17 ± 3	18 ± 3	150 ± 49	177 ± 46	31 ± 11	29 ± 15

*p<0.05 CYC vs PL

There was a significant time effect for 1 RM BP ($F(1,11)=16.99$, $p<0.05$, $\eta^2=0.611$); 1 RM SQ ($F(1,11)=46.20$, $p<0.001$, $\eta^2=0.81$) and SQ RTF60% ($F_{(1,11)}=9.41$, $p<0.05$, $\eta^2=0.46$). However, no significant effects for time interaction was observed for BP RTF60% ($F_{(1,11)}=1.315$, $p>0.05$, $\eta^2=0.107$). No significant differences were observed between CYC and PL for any of the performance variables. No group differences have been detected for 1 RM BP ($F_{(1,11)}=0.14$, $p>0.05$, $\eta^2=0.01$), 1 RM SQ ($F_{(1,11)}=0.22$, $p>0.05$, $\eta^2=0.02$) RTF60% BP ($F_{(1,11)}=1.32$, $p>0.05$, $\eta^2=0.11$) or RTF60% SQ ($F_{(1,11)}=0.02$, $p>0.05$, $\eta^2=0.002$).

3.3.4 ES analysis

The Standardised ES analysis, as depicted in figure 2, revealed large values for CYC in 1 RM BP (1); 1 RM SQ (1.2) and RTF60% SQ (1.1) while for the PL group only for 1 RM SQ (0.9). Moderate ES were observed for CYC in FFM (0.3). The PL group showed moderate ES for 1 RM BP (0.3) and RTF60% SQ (0.7). Inherently both groups showed a non-significant negative change in BP RTF 60%, but with larger ES in PL (-0.9) compared to CYC (-0.3).

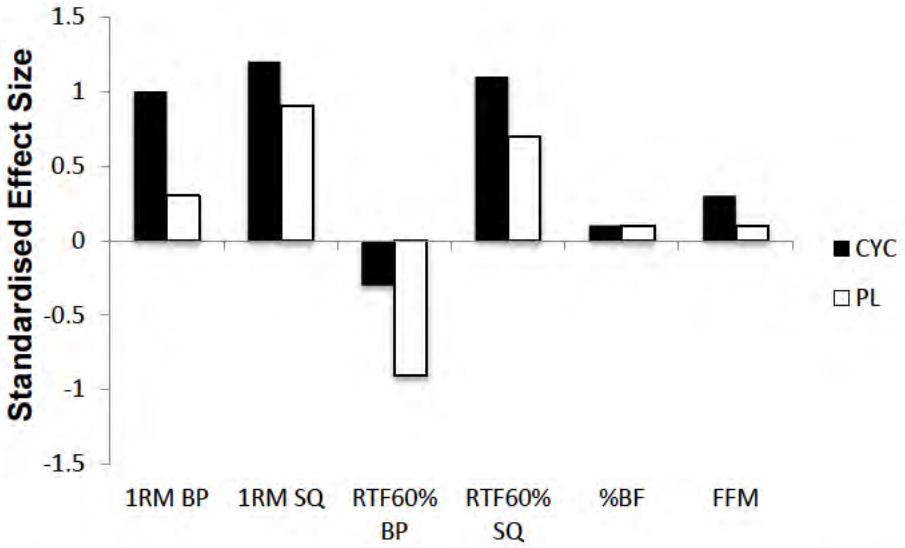

Figure 2. Comparison between the standardised effect sizes calculated for CYC and Pl group for the six analysed variables in the study

3.3.5 Magnitude-based inferences

The data in table 7 compares the performance improvements of CYC to the PL and the percentage likelihood of CYC having beneficial/trivial/negative effects. The CYC was associated with a 78%, 49%, and 49% likelihood of producing performance benefits compared to PL for 1 RM BP, RTF60% BP, and RTF60% SQ, respectively. However, CYC seems less effective than PL for improving 1 RM SQ.

Table 7. The change in 1 RM and RTF60% performance determined in CYC group from the baseline relative to the changes measured in PL group from baseline

Δ CYC - Δ PL: Raw difference ±90% confidence limits		Magnitude based inferences: Likelihood of CYC compared with PL of being		
		Positive	Trivial	Negative
1 RM BP (kg)	7.8 ± 11.1	78 likely	20 Unlikely	2 very unlikely
1 RM SQ (kg)	-1.4 ± 20.6	31 possible	28 Possible	41 possible
RTF60% BP (reps)	0.9 ± 4.7	49 Possible	26 Possible	25 possible
RTF60% SQ (reps)	1.6 ± 8.4	49 Possible	26 Possible	25 possible

Figure 3 depicts the Δ CYC - Δ PL Raw difference with 90% confidence limits and the actual raw change from CYC and PL groups.

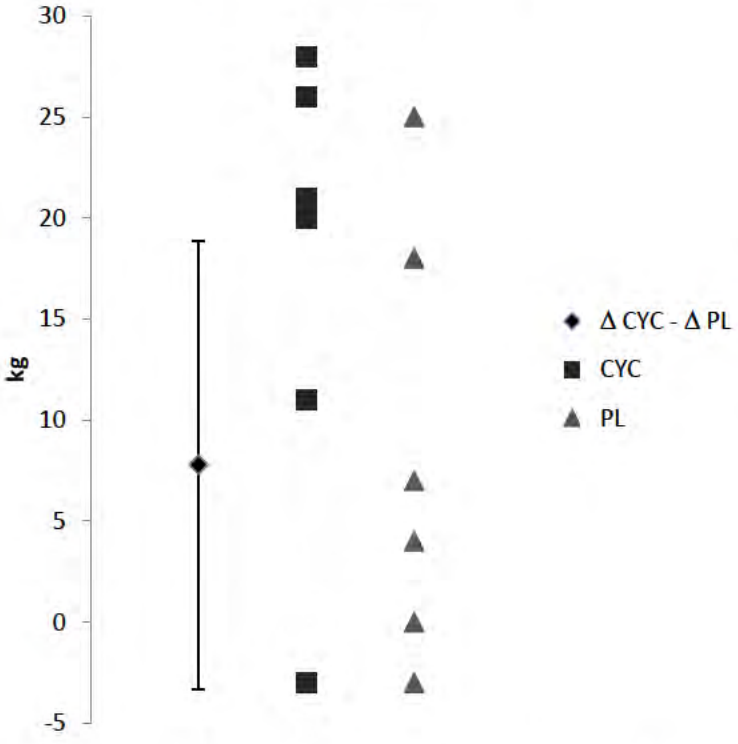

Figure 3. Δ CYC - Δ PL Raw difference with 90% confidence limits against the actual raw difference from CYC and PL groups

3.4 Discussion

The results of the present study show that both groups increased total BM and FFM as well as 1 RM and RTF60% regardless of the treatment. However, no significant differences ($p<0.05$) were observed between groups for body composition or performance related variables. As demonstrated by the standardised effects sizes (ES) values, training outcomes achieved at Post show a trend to be more favourable for CYC compared to PL (Figure 2). Additionally, magnitude based inferences suggest that in recreationally trained males, combining a multi-ingredient supplement containing CM, whey protein, carbohydrate, GL and HMB was 78% likely to improve upper body maximal strength, with 2% likelihood of a negative effect, when compared to PL. The same trend was observed for upper and lower body muscular endurance tests where CYC showed a 49% possibility to improve performance with a 25% chance of producing negative effects in respect to PL.

The larger standardised ES for FFM, 1 RM BP and RTF60% SQ were consistent with the results reported by other studies. Cribb *et al* [148] examined the effects of a whey protein-carbohydrate supplement containing 0.1 $kg \cdot d^{-1}$ of CM compared to the same amount of whey protein-carbohydrate supplement (without CM) during 10 wks of RT in recreational male bodybuilders. Although both supplements were similar in energy and nitrogen content, the group that received CM demonstrated greater gains in 1 RM strength, lean body mass, fibre cross sectional area, and contractile protein content. In a similar study, Cribb *et al* [165] observed greater maximal strength and hypertrophy responses when a 11 wk RT programme was combined with a carbohydrate, whey protein, and CM supplement compared to a carbohydrate supplement.

In spite of the fact that CYC supplementation did not show a significant effect on 1 RM SQ or 1 RM BP, a main effect for time was significant for both 1 RM BP and 1 RM SQ. This demonstrates the efficacy of the RT intervention alone. However, the lack of significance due to CYC supplementation does not mean the supplement was ineffective. In fact, CYC showed a 78% likelihood of being of greater benefit than PL for improving 1 RM BP. Although speculative, but based on the ES and magnitude based inferences analysis, the small sample size ($n = 13$) could have affected these results. Moreover, given that the

sample consisted of RRT practitioners, it is possible that the training alone may elicit larger performance outcomes compared to training with CYC [166]. Also, as pointed out in previous studies [96, 143, 167], high doses of CM supplementation alone or in combination with carbohydrate and protein, as administered in the present study (10 $g \cdot d^{-1}$), have shown significant and positive effects for improving strength performance and FFM in practitioners of RT.

In addition to the small sample size, another reason that may have influenced the lack statistical significance of CYC effect could be assigned to the supplementation protocol. The present study analysed the efficacy of the protocol suggested by the manufacturer: one intake at breakfast and other immediately after training. This protocol is different from others applied in previous studies, where significant improvements in strength and body composition have been observed after consuming the supplements just prior to and immediately after the workout [148, 150]. The International Society of Sports Nutrition suggests that the ingestion of whey protein, carbohydrate, and CM after a workout may potentiate expected adaptations to RT. It is also stated that ingestion of carbohydrate and protein before exercise may result in peak increases of protein synthesis [24]. In the present study, FFM showed a slight trend for a greater increase in the supplemented group.

Supplementation with HMB has been suggested to increase protein synthesis [57]. However, the alleged benefits of HMB supplementation appear to have conflicting evidences. A recent meta-analysis [61] concluded that HMB supplementation has: (a) small to a negligible effect on strength depending on the experience of the weightlifter; and (b) trivial effect on body composition in both untrained and trained weightlifters. The average intervention time from the meta-analysis was 5 ± 2 wks, which as previously mentioned may not be enough time for trained participants to experience the effects from HMB supplementation. It is presumed that HMB works through anti-catabolic action and attenuation of muscle damage [61]. Therefore, a training programme would have to stress the participant sufficiently to increase activity and/or intensity of the current programme in order to potentially benefit from the supplementation of HMB [64]. It is believed that the RT programme in the present study was of sufficient duration and intensity to benefit from HMB supplementation [61]. The combination of HMB and

creatine, as used in our study, has been shown to mediate greater increases in strength and FFM than HMB supplementation alone through additive effect [63]. This may have mediated the larger ES for 1 RM observed in this study. Speculation on HMB supplementation, especially in athletes, should be treated with caution as further research is needed before conclusions can be drawn [139].

Supplementation with GL is well tolerated, even in amounts up to 0.65 $g \cdot kg^{-1}$ of body mass. However, there is not enough evidence to recommend the use of GL as an effective supplement for improving body composition and strength performance [139]. For RTF60% BP both groups showed a trend to reduce performance, but CYC experienced less of a decrease compared to PL. This was possibly due to the RT programme having more emphasis on the lower body and less emphasis on the upper body than the RRT participants were accustomed to. The use of CYC would seem to attenuate this decrease in performance. In fact, the likelihood of CYC being of benefit to upper body muscular endurance compared to PL was 49% with a 25% possibility of being of no benefit.

Additional research with a larger sample size is needed to further the understanding of the effects of combined multi-ingredient supplements and RT on strength performance and body composition.

3.5 Conclusion

Based on the observed ES and magnitude based inferences analysis, it seems reasonable to conclude that combining a PRT programme with the ingestion of a natural multi-ingredient supplement such as CYC may be more effective than a maltodextrin placebo compound to potentiate the expected performance outcomes from a 12 wk progressive hypertrophy RT programme.

Chapter 4
Study 2 - Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males

4.1. Introduction

Several studies have reported improved performance following caffeine ingestion during varying intensities and modalities of exercise [20, 168]. Enhanced effects of caffeine on performance have been demonstrated for improving maximum voluntary strength and lower body endurance exercise [169]. The proposed mechanisms associated with the ergogenic effect of caffeine are related to central mechanisms or through facilitating muscle function [170]; reducing the perception of fatigue, enhancing central drive, and/or improving muscle fibre recruitment [171]. The stimulatory action of caffeine on the brain is probably mediated by an adenosine receptor blockade [172].

Early studies demonstrated that the ingestion of caffeine doses between 5 to 13 $mg \cdot kg^{-1}$ were effective for improving endurance performance [173]. However, more recent investigations have proved the effectiveness of lower doses of caffeine within the range of 1 to 2 or up to 6 $mg \cdot kg^{-1}$ for improving visual information [173], cognitive function [174] and intermittent endurance performance [20]. Conversely, higher doses of caffeine between 9 to 13 $mg \cdot kg^{-1}$, besides possessing the potential to be detrimental on performance [101], have been associated with negative symptoms, such as headaches, nervousness, gastrointestinal disturbances and dizziness [104].

Carbohydrate ingestion can also delay the onset of fatigue and enhance exercise performance [29, 34, 175]. The benefits of carbohydrate have been attributed to the maintenance of plasma glucose concentrations and higher rates of glucose oxidation later on in exercise when muscle and liver glycogen stores are low [24]. Furthermore, the presence of carbohydrate in the mouth has been shown to positively affect endurance performance by activating specific areas of the brain associated with reward and the regulation of motor activity [41] Therefore, exogenous carbohydrate ingestion could enhance performance by supporting vital glucose metabolism and by specific central nervous system stimulation. Additionally, caffeine could potentially enhance physical performance by influencing other alternative neuromuscular pathways e.g., improving

force production [102, 172], and reducing perception of effort via its actions as an adenosine antagonist [102].

Previous studies have observed positive additional effects when caffeine is added to a carbohydrate solution [131, 176]. Van Nieuwenhoven *et al* [131] reported a higher rate of intestinal glucose absorption after ingesting a solution providing 0.5 $g \cdot min^{-1}$ of glucose with 1.4 $mg \cdot kg^{-1}$ of caffeine compared with the ingestion of glucose alone. Yeo *et al* [132] observed a 26% increase in the rate of exogenous carbohydrate oxidation during two hours of cycling at 64% maximum oxygen uptake (VO_2 max) when glucose (0.8 $g \cdot kg^{-1} \cdot min^{-1}$) was ingested with caffeine (10 $mg \cdot kg^{-1}$) compared to the ingestion of glucose alone. Furthermore, a significant increased time to exhaustion has been observed after ingesting a caffeine and carbohydrate solution, containing 5.3 $mg \cdot kg^{-1}$ of caffeine, when compared to the ingestion of carbohydrate alone or placebo [176]. In addition, the ingestion of individual doses of both carbohydrate [24] and caffeine [20], as well as in combination [129] have been shown to be effective for enhancing intermittent sprint performance.

In recent years, the ingestion of carbohydrate in the form of a gel has become more prevalent, due in part to the ability to independently manipulate carbohydrate and fluid intake as well as to consume greater amounts of carbohydrate when they are ingested instead of in liquid forms [177]. Patterson and Gray [178] observed positive effects, from carbohydrate containing gels (0.7 $g \cdot kg^{-1} \cdot min^{-1}$), on the maintenance of blood glucose levels over five, 15 min blocks of intermittent endurance exercise. More recently, Phillips *et al* [179] observed significant increases on intermittent endurance performance after the ingestion of gels containing carbohydrate in adolescents team games players.

In summary, the co-ingestion of caffeine and carbohydrate has been shown to positively affect performance during predominantly aerobic, continuous, endurance [132, 180] and intermittent exercises [129]. However, to our knowledge there are no published studies that have investigated the effects of co-ingesting caffeine and carbohydrate gels on intermittent sprint performance. Therefore, the aim of the present investigation was to analyse the effects of carbohydrate gel with and without a moderate dose of caffeine

administered prior to and during an intermittent sprint test (IST) on the glycaemic response, rating of perceived exertion and fatigue.

Based on previous investigation, it was hypothesised that the combination of carbohydrate and caffeine in gel form (CHOCAF) would attenuate fatigue and decrease perception of effort when compared to the ingestion of carbohydrate gels (CHO) alone and placebo gels (PL). A secondary hypothesis postulated that CHOCAF would maintain blood glucose levels throughout the intermittent sprint test in regards to both CHO and PL.

4.2 Methods

4.2.1 Participants

Twelve healthy, recreationally trained males (age mean 23 ± 3 years, height mean 179 ± 6 cm, body mass mean 79 ± 10 kg) agreed to participate and provided written informed consent (see Appendix IV). Approval of the research proposal was granted by the Universities Research Ethics Committee, in accordance with the standards of the declaration of Helsinki. Participants were regular but recreationally trained team sports practitioners, possessed normal vital signs and were free from musculoskeletal limitations. Health history was determined at baseline by the use of a questionnaire (see appendix V). Additionally, in order to be accepted, participants had to achieve a minimum performance of level eight on the bleep test (multistage fitness test).

Key exclusion criteria included: a history of various metabolic conditions or diseases such as myopathies; the concomitant use of a variety of medications, including but not limited to those with androgenic and/or anabolic effects; the use of nutritional supplements (e.g., creatine, whey protein, essential or non-essential amino acids) within six weeks prior to the start of the study; the current use of tobacco products; and the presence of any orthopaedic limitations or injuries.

4.2.2 Experimental design

This study included a double blind, randomised, counter balanced, placebo controlled cross over design, in which three within participant conditions (CHOCAF, CHO and PL)

were considered. Each participant attended the laboratory on six occasions. The first visit was intended for body weight and height assessment, as well as for the determination of maximal aerobic speed (MAS). The second and third visits were used to familiarise participants with the IST protocol and the last three visits to perform the IST under the three assessed conditions: CHOCAF, CHO and PL. In order to avoid possible confounding trial order effects, the conditions were tested following a counter balanced randomised order. Seven to ten days were allowed between each of the testing sessions. Participants were asked to abstain from any unaccustomed or hard exercise during the 72 hours before each of the three main testing sessions.

4.2.3 Procedures

Diet diaries. Before the three main tests, participants were required to provide a three consecutive days' diet diary for two week days and one weekend day (see appendix VI). Diets were then analysed for carbohydrate, protein, fat and energy content using Dietplan6 software. Participants were required to maintain their specified habitual diet throughout the main trials and to abstain from caffeine consumption for four days prior to the study intervention.

Pre-exercise standardised meal. Two hours before arriving to the lab, participants were required to consume a standardised meal sourced from porridge oats and semi skimmed milk that provided 1 $g \cdot kg^{-1}$ of carbohydrate and 0.15 $g \cdot kg^{-1}$ protein.

4.2.4 Experimental protocols

Multistage fitness test. In a protocol first standardised by Léger and Lambert [181], participants ran a back and forth course (''shuttles'') between two lines placed 20 m apart from one another. The speed of the run started at 8.5 $km \cdot h^{-1}$, and increased by 0.5 $km \cdot h^{-1}$ every minute. Participants speed was governed by a recorded audible ''beep'' that sounded each time the participants were expected to reach a line to complete a shuttle. The number of shuttles per stage was coordinated with the speed of the beeps so that each stage was approximately a minute in length. Participants received verbal encouragement throughout the duration of the test. When a participant failed to complete two successive shuttles in the allotted time between ''beeps,'' the test was

terminated for that participant. Estimated MAS was determined from the last completed stage. This value was used as a reference to establish the pace speed for the IST.

Intermittent sprint test (IST). The IST, a modified version of the Loughborough Intermittent Shuttle Test [182], consisted of four blocks, each of which involved 11 cycles of three repetitions of 20 m of walking at below 60% of MAS; one repetition of a 15 m sprint; three repetitions of 20 m of running at 80% of MAS and three repetitions of 20 m of jogging at 60% of MAS. Therefore a total of 44 cycles were completed for each IRST, covering a total distance of 8,580 m at varying velocities. A standardised warm up, 10 min in duration, consisting of various paced shuttle runs and dynamic stretching was performed before the start of IST.

4.2.5 Supplementation

Participants ingested one 70 mL serving of a gel containing 25 g of carbohydrate and 100 mg of caffeine (CHOCAF), 25 g of carbohydrate and no caffeine (CHO) or a non-caloric placebo (PL) one hour before, immediately prior to the start and at the end of second block of the IST. Therefore, a total of three 70 mL servings of supplement or placebo were ingested in each condition. The data in table 8 provides the nutritional composition for one 70 mL serving of CHOCAF and CHO.

Table 8. Nutritional composition for CHOCAF and CHO

	CHOCAF (70 mL serving)	CHO (70 mL serving)
Total energy (kcal)	101	101
Carbohydrate Total (g)	25	25
as		
Maltodextrin (g)	15.4	15.4
Sucrose (g)	7.84	7.84
Dextrose (g)	1.05	1.05
Fructose (g)	0.56	0.56
Caffeine (mg)	100	0

The PL supplement was a low Kcal gel of the same colour and flavour as the CHOCAF and the CHO supplement.

4.2.6 Measurements

Sprint times. Every sprint time over the 15 m track during the IST was recorded, using infra red Brower Timing Systems Speed Trap 2 timing units.

Fatigue index (FI). Calculated using the following equation [183]:

Equation 4. *FI*

$$FI = 100\ X\ \frac{ss - fs}{fs}$$

In which SS is the time in s for the slowest 15 m sprint and FS is the time in s for the fastest 15 m sprint.

Rating of perceived exertion (RPE). Borg scale (6-20) [184] was used to determine the rating of perceived exertion at the end of each block and after completing the IST.

Blood glucose. A fingertip blood sample was obtained at specific time points: rest (immediately before ingesting first supplement serving and at the end of every block during IST. Blood was then analysed for glucose concentration using a Biosen C_line (EKF diagnostic). Figure 4 depicts the sequence of the study.

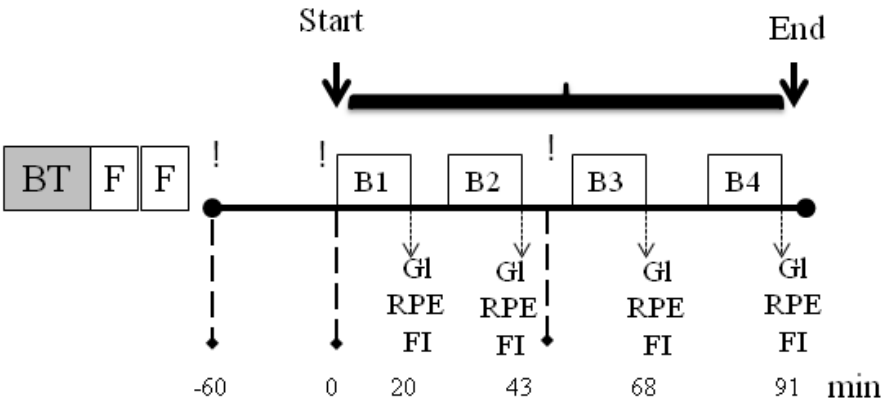

Figure 4. Schematic overview of study design

BT: Bleep test; F: Familiarisation sessions; IST: Intermittent sprint test involving four blocks of 11 sets of three repetitions of 60 m at <60%; 80% and 60% MAS plus 1 x 15 m sprint

RPE: Determination of the rating of perceived exertion at the end of each block

FI: Determination of fatigue index at the end of each block

Gl: Determination of glucose at the end of each block

!: CHOCAF; CHO or PL ingestion

4.2.7 Statistical analysis

Mean and standard deviation (SD) are expressed as mean ± SD. Mauchly's Test of Sphericity was used for testing the normality distribution of the data.

Repeated measures ANOVA were used to assess the differences in between the three tested conditions. In the case of finding significant differences, Bonferroni *post hoc* analysis was carried out. Eta squared (η^2) was used as a measure of standardised effect size, taking reference values of small (η^2=0.01), medium (η^2=0.06) and large (η^2=0.14) from Cohen [162]. Significance level was set at $p \leq 0.05$ for all calculations. IBM SPSS Statistics software (version 19) was used to conduct the statistical analysis.

4.3 Results

4.3.1 Dietary analysis

Means and SD for average daily consumption of carbohydrate, protein, fat and energy analysed from the three days diet diary for all participants was 4.2 ± 0.3, 1.5 ± 0.30, 1.2 ± 0.1 $g \cdot kg^{-1}$ and mean 32 ± 2 $Kcal \cdot kg^{-1}$ respectively.

4.3.2 Glucose response

Mean blood glucose values measured at the end of each of the four blocks during the IST are depicted in Figure 5. Resting blood glucose values (BB) were not statistically different between the supplement conditions ($F_{(1,11)}$=0.020, p=0.981, η^2=0.002). Main effects between conditions were found at the end of the first (B1) ($F_{(1,11)}$=7.862, p=0.010, η^2 =0.417), second (B2) ($F_{(1,11)}$=5.001, p=0.016 η^2=0.313), third (B3) ($F_{(1,11)}$=10.269, p=0.001 η^2=0.483) and fourth (B4) ($F_{(1,11)}$=5.030, p=0.016 η^2=0.314) blocks. *Post hoc* comparison showed that the CHOCAF condition resulted in significantly higher blood glucose levels (5.5 ± 0.9; 5.4 ± 0.8; 5.4 ± 0.9; 5.2 ± 0.8 $mmol \cdot L^{-1}$) compared with PL (4.5 ± 0.8 p=0.005; 4.7 ± 0.3 p=0.009; 4.4 ± 0.6 p=0.003; 4.4 ± 0.8 $mmol \cdot L^{-1}$ p=0.002) at B1, B2, B3, and B4 respectively. Meanwhile the CHO condition showed significantly higher blood glucose levels compared with the PL supplement at the end of B1 (CHO= 5.3 ± 0.6 vs PL= 4.5 ± 0.6 $mmol \cdot L^{-1}$ p=0.000), B2 (CHO= 4.7 ± 0.3 vs PL= 4.5 ± 0.8 $mmol \cdot L^{-1}$ p=0.008) and B3 (CHO= 5.4 ± 0.8 vs PL= 4.4 ± 0.6 $mmol \cdot L^{-1}$ p=0.001). No other significant differences were observed. η^2 values for each of the measured time points were considered large.

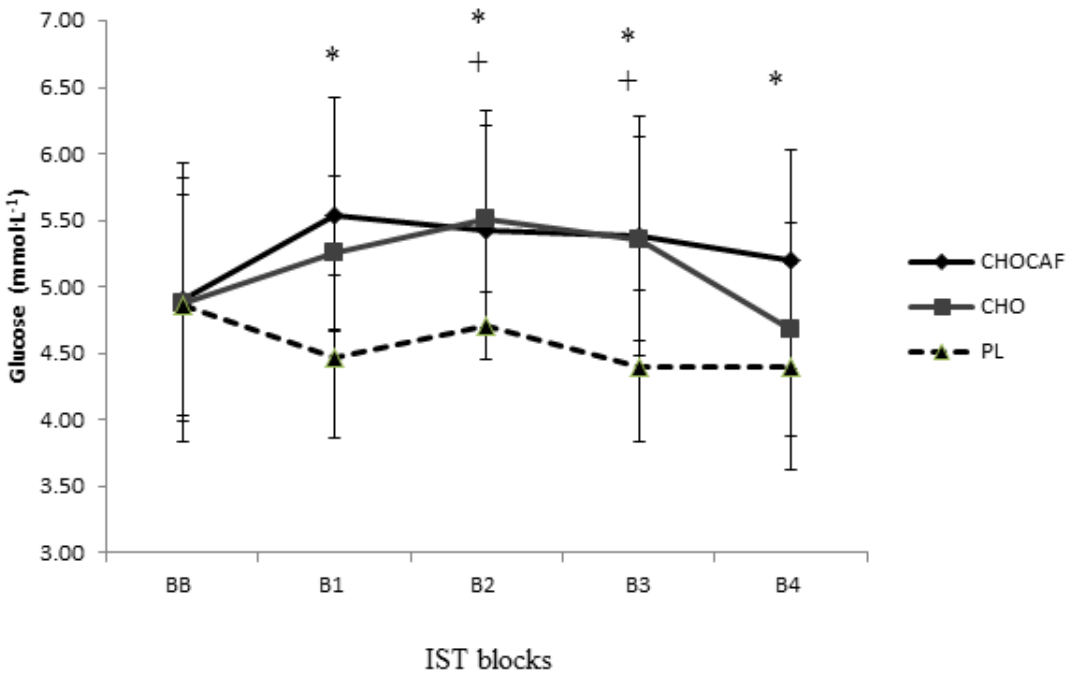

Figure 5. Mean and standard deviation of glucose value measured at the end of each block during the intermittent sprint test

*P≤0.05 from CHOCAF to PL

+P ≤ 0.05 from CHO to PL

4.3.3 Perception response (RPE)

The data in table 9 shows mean RPE values that increased throughout the exercise and showed significant increments over time regardless of the condition.

Table 9. Mean and standard deviation of RPE measured at the end of each block

	CHOCAF*	CHO^{+}	PL$^{\#}$
B1	12.8 ± 1.8	13.3 ± 2.4	12.5 ± 2.4
B2	13.9 ± 2	14.1 ± 2.4	14.2 ± 2.3
B3	14.2 ± 2.3	14.9 ± 2.3	15.3 ± 1.9
B4	16.3 ± 3	16.5 ± 3.1	17 ± 2.8

*F=23.438 , p=0.000 η^2=0.681

$^{+}$F=10.460 , p=0.004 η^2=0.487

$^{\#}$F=14.380 , p=0.001 η^2=0.567

As depicted in figure 6 main effects between conditions were found only in B3 ($F_{(1,11)}$=6.445, p=0.006, η^2=0.369). *Post hoc* comparison, as shown in figure 6, indicated that the CHOCAF condition showed a significantly lower RPE value (14.2 ± 2.3) compared with the PL condition (15.3 ± 1.9; p=0.003) and approached statistical significance when compared with the CHO condition (14.9 ± 2.3; p=0.056). No other significant differences for RPE values were observed between the conditions. Values of η^2 for both B1 and B3 were large; meanwhile, small and medium values were observed in B2 and B4 respectively.

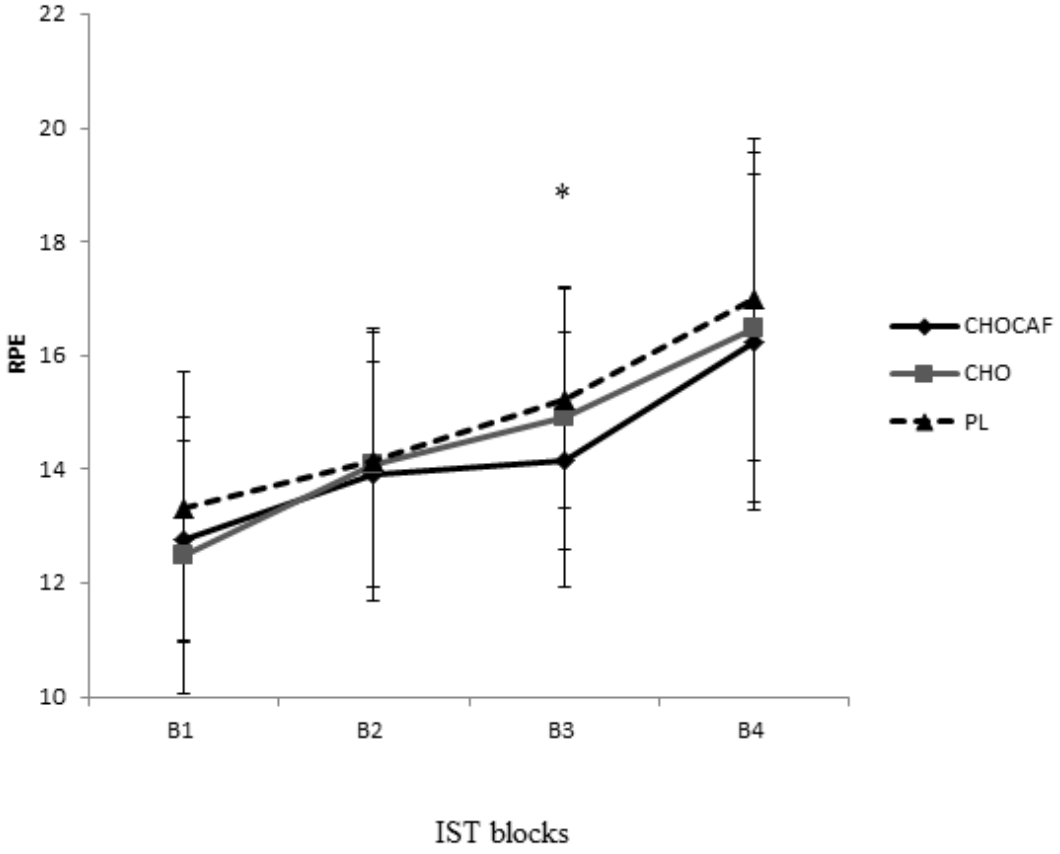

Figure 6. Mean and standard deviation of the RPE scores measured at the end of each intermittent sprint test blocks

*p=0.05 CHOCAF vs PL

4.3.4 Performance response

Sprint times. The data in table 10 shows that no significant differences were found between the best sprint times measured at each block of the IST between the three tested conditions.

Table 10. Mean and standard deviation of the best sprint times measured from each block of the IST

	CHOCAF (s)	CHO (s)	PL (s)
B1	2.03 ± 0.2	2.11 ± 0.1	2.12 ± 0.1
B2	2.06 ± 0.2	2.14 ± 0.1	2.16 ± 0.1
B3	2.09 ± 0.2	2.16 ± 0.1	2.17 ± 0.1
B4	2.09 ± 0.2	2.19 ± 0.1	2.19 ± 0.1

Fatigue index (FI). Mean FI values measured at the end of each of the four blocks during the IST are depicted in Figure 7. Significant main effects were found only in B3 ($F_{(1,11)}$=5.804, p=0.009, η^2=0.345). The CHOCAF condition showed significantly lower FI scores (5.0 ± 1.7) compared with both the CHO (7.6 ± 2.6; p=0.034) and PL (7.4 ± 2.4; p=0.006) conditions. No other significant differences were observed. η^2 values for B3 were large, meanwhile, a small value was found for B1 and B4 and a medium value for B2.

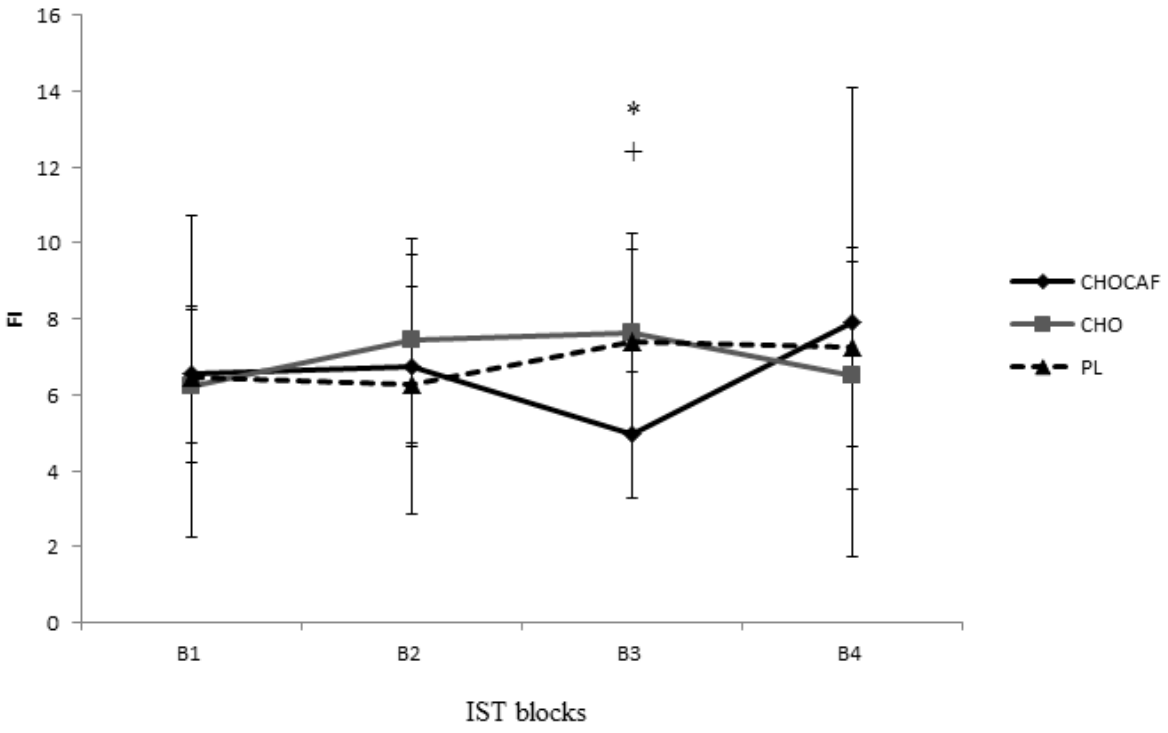

Figure 7. Mean and standard deviation of FI score measured at the end of each intermittent sprint test blocks

* p ≤ 0.05 CHOCAF vs PL

+ p ≤ 0.05 CHOCAF vs CHO

4.4 Discussion

The main finding of the present study was that the co-ingestion of caffeine (100 mg) and carbohydrate (25 g) on three occasions, one hour, immediately before and at the middle of a 90 min intermittent endurance test was effective in attenuating fatigue (in respect to both the CHO and the PL conditions) and reducing the rating of perceived exertion (in respect to the PL condition only) at the end of B3 (68 min of exercise). Furthermore, the likely hood of the CHOCAF condition to reduce the RPE value in respect to the CHO condition approached statistical significance (p=0.056). Conversely, ingestion of the CHO supplement seemed to have no effect in attenuating both fatigue and RPE during the IST when compared with the PL condition. As expected blood glucose levels were significantly higher in the CHOCAF and the CHO conditions compared with the PL condition, with the exception of B4 in the CHO condition, but no significant differences

were observed between the CHOCAF and the CHO conditions. However, in B4 the approaching of statistical significance and a large η^2 value (η^2=0.314) indicates that the CHOCAF supplement has the potential to produce higher levels of blood glucose compared with the CHO supplement. Performance benefits have been reported with the ingestion of relatively small amounts of carbohydrate (e.g. 16 $g \cdot h^{-1}$), but this positive effect is more apparent with larger amounts and especially when different carbohydrate sources (e.g. glucose and fructose) are ingested together [34] with a ratio of 2:1 for glucose and fructose respectively [37]. Although participants of the current study were fed with carbohydrates of different sources (maltodextrin, sucrose, dextrose, and fructose), the lack of significant differences in blood glucose levels between the CHOCAF and the CHO conditions could be explained by the amount of carbohydrate administered. Our participants ingested 75 g over 150 min (involving 90 min of exercise) or 0.5 $g \cdot min^{-1}$. This is far below the amount of carbohydrate needed to achieve the maximum ingestion rate of >1.2 $g \cdot min^{-1}$ required to saturate intestinal glucose transporters [37]. Therefore, intestinal glucose absorption may not have been limiting the rate of exogenous carbohydrate oxidation and that caffeine would only exert its effect once the glucose transporter is saturated [176]. In addition, under the CHO condition, participants showed significantly (p=0.005) higher serum glucose concentrations only at the end of B1, B2 and B3 with no significant effects on RPE or FI value compared to the PL condition. The η^2 value for B4 was considered large leading us to speculate that if the sample had been larger, a significant difference may have been reached between the CHO and PL conditions.

The results of this current study are in the line with previous investigations [129, 130, 180, 185]. Gant *et al* [129] reported positive ergogenic effects of adding 160 $mg \cdot L^{-1}$ (3.7 $mg \cdot kg^{-1}$) of caffeine to a 6% (1.8 $g \cdot kg^{-1}$) carbohydrate-electrolyte solution in male soccer players. Participants improved 15 m sprint time and vertical jump performance but not the rating of perceived exertion after ingesting the carbohydrate-caffeine supplement one hour before and every 15 min during a 90 min intermittent shuttle running test.

Although reduced RPE at a given workload is a well documented response to caffeine ingestion [102], discrepancies in the perception of exercise intensity have been reported by others [186]. Lower RPE values have been observed when participants ingested a

caffeinated carbohydrate solution during the fixed intensity phase of their protocol and similar RPEs during the self selected phase [186]. It could be possible that an altered perception of fatigue can be manifested in two ways: a) attenuation of the subjective perception of fatigue and b) enabling players to better maintain voluntary sprinting during the fatiguing stages of exercise [102].

When considering the average sprint performance measured at the end of each block, regardless of the FI and despite being unable to determine significant differences between conditions, under the CHOCAF condition participants performed faster sprint times than both the CHO (3.63, 3.74, 3.47 and 4.82%) and the PL (4.09, 4.63, 3.95 and 4.82%) for B1, B2, B3 and B4 respectively. Compared to the performance measured at B1, the PL condition showed a significant performance reduction in B2, B3 and B4, meanwhile under the CHOCAF and CHO conditions significant sprint performance reduction was observed at B3 and B4 but not at B2. This suggests an early attenuation of fatigue in the CHOCAF and CHO conditions due to the ingestion of carbohydrate with and without caffeine. This rationale would support the hypothesis that the CHOCAF gel attenuates fatigue and the perception of effort during intermittent exercise.

Participants in our study ingested a gel containing 100 mg of caffeine (1.1 to 1.54 $mg \cdot kg^{-1}$) on three occasions, one hour before, immediately prior and in the middle of the IST (45 min). Although speculative, if we consider that caffeine would peak in the plasma one hour after ingestion [173], the amount of caffeine in the plasma, under CHOCAF condition, would be expected to be increasing during B3 after completing the ingestion of a total of 300 mg of caffeine. However, even though the ergogenic effects of caffeine are observed between one to three hours after ingestion [101], no significant effects were found at the end of B4 (150 min after the first serving, 91 min after the second serving and 48 min after the third serving of the CHOCAF supplement). Furthermore, the η^2 values for RPE and FI in B4 were considered to be small to medium (0.032 and 0.02 respectively) opposed to the η^2 values being large in B3 (0.369 and 0.366 respectively) where a significant effect was observed. Therefore, the ergogenic effect provided by the ingestion of CHOCAF gels at B3 may not have been of enough magnitude to overcome the fatigue experienced later at B4 in IST.

Although mechanisms of action for carbohydrate and caffeine were not measured it could be assumed that the ergogenic effects of co-ingesting caffeine with carbohydrate are interlinked with feelings of fatigue and performance [101]. This is mirrored in the results of this current study, particularly observed at the end of B3 (68 min of exercise) when RPE and fatigue are attenuated. However, further research into the influence of carbohydrate and caffeine on intermittent endurance performance should include a greater sample size and differing methodologies, doses and times of supplementation.

4.5 Conclusion

The results of our study indicate that ingesting gels containing 100 mg of caffeine and 25 g of carbohydrate per serving one hour before, immediately prior to and in the middle (45 min) of a 90 min IST is effective for transiently decreasing perceived effort, attenuating fatigue (at 68 min) as well as maintaining higher blood glucose levels during the final stages of intermittent endurance exercise in recreationally trained athletes.

Chapter 5

Study 3 - Effect of a carbohydrate-protein multi-ingredient supplement on intermittent sprint performance and muscle damage in recreational athletes

5.1 Introduction

Exercise induced muscle damage (EIMD) is produced when an individual is exposed to a repeated bout of unaccustomed movement [187]. Damage can be variable and ranges from the isolated disruption of a few sarcomeres to tears in the sarcolemma basal lamina and supportive connective tissue, all of which may alter the function of contractile elements and the cytoskeleton. Damage to the myofibres, in addition to being associated with the alteration of the excitation contraction coupling system and sarcomere disruption, can also result in the release of intracellular proteins and influx of extracellular proteins leading to cell swelling, disturbance of extracellular matrix and mediation of the inflammatory response associated with further functional muscle impairment [187, 188]. Neuromuscular fatigue can also contribute to impaired muscle function and is defined as an exercise reduced reduction in the ability to exert force or power, regardless of whether the task can be sustained [189].

Structural damage to the muscle cell is accompanied by the leakage of proteins such as creatine kinase (CK) and myoglobin (Mb) out of the cell and into the circulation [190]. Thompson *et al* [191] observed significant increases of CK (379 % from baseline) that peaked at 24 h after performing a 90 min Loughborough Intermittent Shuttle Test (LIST) in 16 young males. Similarly, Twist and Easton [192] reported significant rises of CK values coupled with slower 10 m sprint times observed 24 h after performing a battery of sprint tests in 10 young male college athletes. Elevated plasma Mb after 30 min and CK levels throughout the 72 h recovery period were also reported after performing a 90 min LIST and a friendly match in football players [193, 194]. EIMD may also result in the release of several plasma cytokines into the circulation [195]. Elevated levels of skeletal muscle derived interlukin-6 (IL-6) into the blood appears to be the major alteration in cytokines observed during exercise [196]. Leeder *et al* [197] reported a decrease in vertical jump performance in trained young males together with increased levels of plasma CK and IL-6 peaking at 24 h and immediately after an intermittent exercise protocol.

Some protective strategies, including nutritional interventions, have been proposed to attenuate the negative consequences of the EIMD [198]. In fact, the consumption of carbohydrate alone and in combination with protein supplements has been shown effective for improving performance, attenuating fatigue, promotion of the recovery process and a reduction in markers of muscular damage when ingested before [199, 200], during [200] and after exercise [201-203]. With regards to intermittent exercise involving frequent changes of direction, acceleration and repeated sprints as seen during football or rugby, the consumption of natural supplements intended to attenuate performance decreases or enhance the recovery process have been well studied [204]. Alghannam [205] observed positve effects of ingesting a carbohydrate-protein supplement, providing 0.7 $g \cdot kg^{-1}$ and 0.3 $g \cdot kg^{-1}$ respectively, on intermittent running performance in six young male amateur football players. Other studies using intermittent exercise protocols, reported positive effects of L-glutamine or L-carnitine supplementation for attenuating oxidative stress, muscular damage and hence favour performance and optimise recovery after training. [79, 206]. The ingestion of 100 $mg \cdot kg^{-1}$ of L-glutamine has been shown to reduce the accumulation of blood ammonia and possibly attenuate peripheral and central fatigue in young professional football players [79]. On the other hand, 2 to 4 $g \cdot day^{-1}$ [72, 207] of L-carnitine L-tartrate has been shown to positively attenuate muscle damage and oxidative stress after performing a muscle damaging exercise protocol.

To summarise, the ingestion of carbohydrate alone or combined with protein or other nutrients such as L-carnitine or L-glutamine has shown the potential to positively influence performance and promote recovery from strenuous exercise. To our knowledge this is the first study on the effects of a carbohydrate-protein enriched multi-ingredient supplement on sprint performance over an acute bout of intermittent exercise, and recovery along a 24 h period. The aim of this study therefore, was to compare the effects of consuming a carbohydrate and protein based multi-ingredient supplement enriched with L-glutamine and L-carnitine L-tartrate (MTN) with carbohydrate only (CHO) and a low caloric placebo (PL) on endurance sprint performance, neuromuscular fatigue and muscular damage over a 24 h recovery period. Based on previous studies it was hypothesised that the ingestion of a carbohydrate, protein and amino acid multi-ingredient supplement, before, during and after an acute bout of an intermittent

repeated sprint exercise would promote recovery estimated through the attenuation of neuromuscular fatigue and markers of muscle damage respect to the ingestion of carbohydrate only or a low caloric placebo. As a secondary hypothesis the ingestion of the multi nutrient formula would attenuate a decline in sprint performance during the intermittent sprint test when compared to the carbohydrate and placebo conditions.

5.2 Methods

5.2.1 Participants

Ten healthy recreationally trained males (age 25 ± 4 years height 182 ± 7 cm, body mass 80 ± 10 kg) volunteered to participate in the study providing written informed consent (see Appendix IV). Approval of the research proposal was granted by the Universities Research Ethics Committee, in accordance with the standards of the declaration of Helsinki. Participants were regular but recreationally trained team sports practitioners, possessed normal vital signs and were free from musculoskeletal limitations. Health history was determined at baseline by the use of a questionnaire (see appendix V). Additionally, in order to be accepted, participants had to achieve a minimum performance of level eight on the bleep test (multistage fitness test).

Key exclusion criteria included: a history of various metabolic conditions or diseases such as myopathies; the concomitant use of a variety of medications, including but not limited to those with androgenic and/or anabolic effects; the use of nutritional supplements known to affect muscle damage or oxidative stress (e.g., creatine, L-carnitine, antioxidants) within six weeks prior to the start of the study and the current use of tobacco products.

5.2.2 Experimental design

This study included a double blind, randomised, counter balanced, placebo controlled cross over design, where three within-participant conditions: multi-ingredient (MTN), carbohydrate (CHO) and placebo (PL) were considered. Once considered eligible for the study, each participant was required to attend the laboratory on six different occasions. On the first visit participants were assessed for body mass, height and maximal aerobic speed (MAS). The next two visits were intended to familiarise participants with the 90

min intermittent sprint test (IST) protocol. The remaining visits required participants to perform the IST under the three assessed conditions: MTN, CHO and PL.

In order to maintain a suitable balance between all the possible orders of treatments and minimise any confounding effects, the order of the treatments was randomised in a controlled manner. Thus, a third of the participants started with treatment one, a third with treatment two, and the remaining third with treatment three. The same arrangement was used for the allocation of the second and third treatment sessions. Five to seven days were allowed between each of the three testing conditions.

5.2.3 Procedures

Diet diaries. Prior to the ISTs assessment, participants were required to provide a diet diary for three consecutive days consisting of two week days and one weekend day (see appendix VI). Participants were required to maintain their specified habitual diet throughout the assessed ISTs. Furthermore, participants were required to abstain from protein and carbohydrate supplements on days of testing.

Pre-exercise standardised meal. Participants consumed the same standardised meal in the same manner as described in section 4.2.3.

5.2.4 Experimental protocols

Multistage fitness test. A standard multistage 20 m shuttle run test was again used to estimate MAS, as described in section 4.2.4.

Intermittent sprint test (IST). The IST was a modified version of the LIST [182] as described in section 4.2.4. Prior to the IST and baseline performance measures, but after baseline biomarker measures, a standardised five minutes warm up was completed comprising shuttle runs of vary paces and dynamic stretching.

5.2.5 Supplementation

Participants ingested 500 mL water mixed with MTN, CHO or PL divided into four equal servings and administered; immediately prior to the first (B1), second (B2), third (B3) and

fourth blocks (B4) of the IST. The data in table 11 provides the nutritional composition for one 500 mL serving of MTN and CHO.

Table 11. Nutritional composition for MTN and CHO

	MTN (500 mL serving)	CHO (500 mL serving)
Total Energy (Kcal)	280 kcal	265 kcal
Carbohydrate (g)	53	69.5
Whey protein (g)	14.5	0
Fat (g)	1.2	0
Glutamine (g)	5	0
L-carnitine L-tartrate (g)	1.5	0

The PL was low kcal beverage of the same volume, colour and flavour as MTN and CHO. A second full serving was provided 20 min after the IST. A total of two full servings of MTN, CHO or PL were ingested in each condition. Therefore, the MTN supplement provided a total of 106 g of carbohydrate, 29 g protein, 2.4 g fat, 10 g glutamine, 3 g of L-carnitine L-tartrate and 560 Kcal, whilst the CHO supplement provided a total of 139 g of carbohydrate 530 Kcal.

5.2.6 Measurements

Neuromuscular fatigue 15 m sprint test. A 15 m sprint test was selected to specifically examine neuromuscular fatigue induced by the IST [208]. Each participant performed three 15 m sprints; each sprint time was measured using an infrared timing gate system (Brower Timing Systems). After walking back to the start of the sprint track, participants were required to rest for 30 s between sprints to allow for recovery, and the best of the three sprints was used for the analysis. The coefficients of variation for this test, calculated from reliability trials conducted in previous pilot studies, were between 0.5% and 1%.

Blood sampling and analysis. Vacutainer venous blood collection tubes were used to collect 10mL of heparinised blood and 10 mL of whole blood from the cubital fossa. Non-heparinised blood samples were inverted five times and allowed to stand for one hour prior to being centrifuged at 3500 revolutions per minute (RMP), at a temperature of 4

°C, for ten minutes, after which the plasma was aliquoted into eppendorfs and frozen at –70 °C for later analysis. Heparinised samples were inverted eight times in the vacutainer to ensure adequate mixing with the heparin. 32 µL of heparinised blood was pipetted out onto a test strip and analysed, for CK, using a colorimetric assay procedure (Reflotron Boehringer Mannheim, Germany) [209]. The remaining heparinised blood was centrifuged at 3500 RPM, at 4 °C, for ten minutes, after which the plasma was aliquoted into eppendorfs and frozen at –70 °C for later analysis. IL-6 (R & D Systems; HS600B, Abingdon, United Kingdom) and myoglobin (Abcam; ab108652, Cambridge, United Kingdom) were each assayed in duplicate using an ELISA in accordance with the assay kit instructions provided by the manufacturer.

Neuromuscular fatigue assessment and biomarker measures were determined before the IST (pre), immediately after IST (post), 1 hour after IST (1hr), and 24 hours after IST (24hr). Figure 8 depicts the general structure of study.

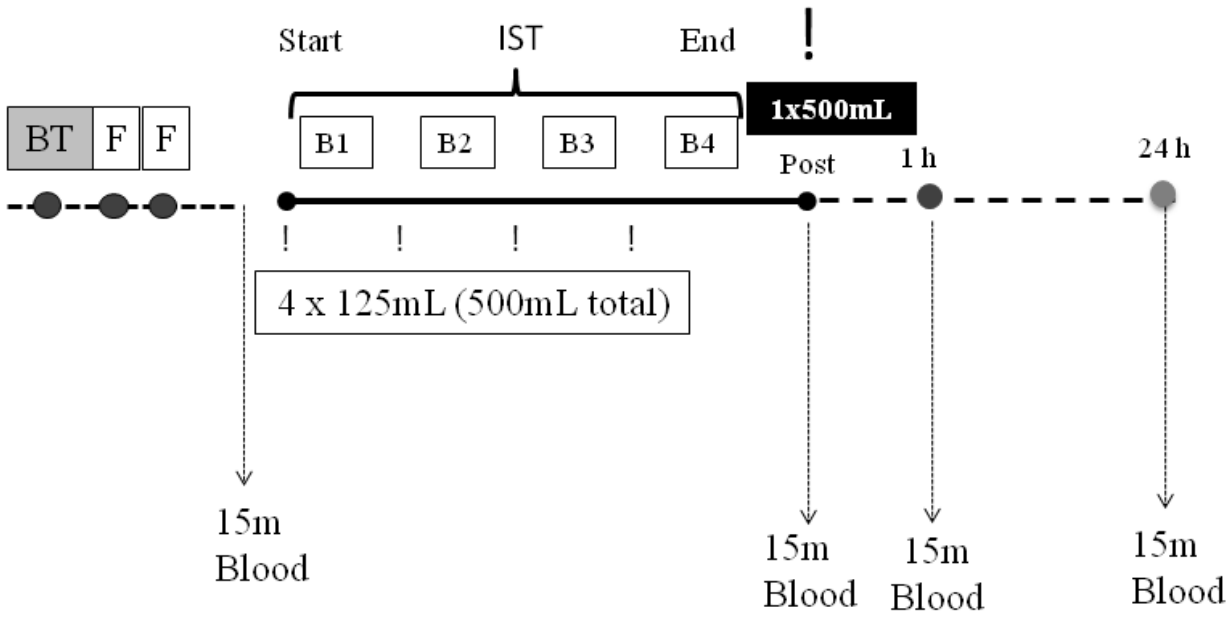

Figure 8. Schematic overview of the study design

BT, bleep test; F, familiarisation sessions; IST, intermittent sprint test involving four blocks of 11 sets of three repetitions of 60 m at 60 %; 80 % and 60 % MAS plus 15 m sprint

15m: sprint test

! MTN, CHO or PL ingestion

5.2.7 Statistical analysis

Mean and standard deviation (SD) are expressed as mean ± SD. Mauchly's Test of Sphericity was used for testing the normality distribution of the data.

Two-way repeated measures ANOVA (3 test conditions x 4 sprint blocks) was performed to analyse the sprint time performed at IST. The differences between conditions and time points were assessed using two-way repeated measures ANOVA (3 test conditions x 4 time points). Bonferroni adjusted *post hoc* analysis was performed for pairwise comparisons. To measure standardised effect size, omega squared (ω^2) was used. In absence of specific thresholds from the literature, reference values [small (η^2=0.01), medium (η^2=0.06) and large (η^2=0.14)] from Cohen [162] were considered. Significance level was set at P<0.05 for all tests. IBM SPSS Statistics software (version 19) was used to conduct the statistical analysis.

5.3 Results

5.3.1 Dietary analysis

Means and SD for average daily consumption of carbohydrate, protein, fat and energy analysed from the three days diet diary for all participants was 4.2 ± 0.2, 1.5 ± 0.2, 1.04 ± 0.2 $g \cdot kg^{-1}$ and mean 31 ± 2 $Kcal \cdot kg^{-1}$ respectively.

5.3.2 IST, repeated sprint performance

The sum of eleven 15 m sprint times obtained per each of the four blocks and for the 44 total sprints performed for entire IST was considered as indicators of sprint performance. The data in table 12 shows the total times summarised at B1; B2; B3 and B4 as well as for the total IST for each of the three analysed conditions (MTN; CHO: and PL). No significant main effects were observed when compared the sum of the total time for the entire IST per condition (MTN; CHO or PL) [$F(2,18)=0.12$, $p=0.891$, $\omega^2 \approx 0.00$].

Table 12. Total Sprint 15 m time (s) per block and all the entire IST

	Total (s)	B1 (s)	B2 (s)	B3 (s)	B4 (s)
MTN	106.81 ± 7.2	26.44 ± 1.7	26.57 ± 1.7	27.14 ± 2.4	26.66 ± 1.6
CHO	107.58 ± 7.6	26.31 ± 1.4	26.82 ± 2	27.23 ± 2.1	27.23 ± 2.3
PL	107.50 ± 8.4	26.71 ± 1.9	26.73 ± 2	26.93 ± 2	27.13 ± 2.7

Significant effect was observed when considering the time performed per block (one, two, three and four) [$F(3,27)=3.03$, $p=0.047$, $\omega 2 \approx 0.14$]. However, no significant interaction effects between conditions and time per block were determined [$F(6,54)=1.57$, $p=0.174$, $\omega 2 \approx 0.01$]. *Post hoc* analysis did not reveal any significant difference.

5.3.3 Neuromuscular fatigue 15 m sprint test

The data in table 13 shows the 15 m sprints performed at the four different times points (pre; post 1hr and 24hr) for the three analysed conditions (MTN; CHO: and PL)

Table 13. Specific neuromuscular variable: 15 m sprint times measured at pre; post, 1hr and 24hr after IST

	15 m (s)			
	Pre	Post	1hr	24 hr
MTN	2.44 ± 0.2	2.65 ± 0.2	2.63 ± 0.2	2.58 ± 0.2
CHO	2.40 ± 0.2	2.63 ± 0.2	2.67 ± 0.2	2.61 ± 0.1
PL	2.45 ± 0.15	2.62 ± 0.2	2.59 ± 0.1	2.59 ± 0.15

Significant main effects were observed for time (pre post, 1hr and 24hr), [$F(3,27)=20.21$, $p<0.001$, $\omega^2=0.20$], but not between conditions (MTN; CHO; PL) [$F(2,18)=0.10$, $p=0.904$, $\omega^2\approx0.00$]. Additionally, no significant interaction effects between time and conditions were determined [$F(6,54)=1.28$, $p=0.283$, $\omega^2\approx0.00$]. Post hoc analysis revealed significant ($p<0.05$) longer sprint times for all three conditions at either post; 1hr and 24hr compared to pre but not between the sprints times determined at the three post-times points (post; 1hr and 24hr).

5.3.4 Muscle damage markers

The data in table 14 shows the values determined for CK; Mb and IL-6 at the four different times points (pre; post 1hr and 24hr) for the three conditions (MTN; CHO: and PL). Significant main effects were observed for time (pre post, 1hr and 24hr), [$F(3,27)=20.21$, $p<0.001$, $\omega^2=0.20$], but not between conditions (MTN; CHO; PL) [$F(2,18)=0.10$, $p=0.904$, $\omega^2\approx0.00$]. Additionally, no significant interaction effects between time and conditions were determined [$F(6,54)=1.28$, $p=0.283$, $\omega^2\approx0.00$]. *Post hoc* analysis revealed significant ($p<0.05$) longer sprint times for all three conditions at either post; 1hr and 24hr compared to pre but not between the sprints times determined at the three post times points (post; 1hr and 24hr).

Table 14. Muscle damage markers determined at pre, post, 1hr and 24hr after IST

		MTN	CHO	PL
CK (UL/L)	Pre	230.4 ± 60.0	151.5 ± 61.4	147.4 ± 68.4
	Post	349.9 ± 140.5	269.6 ± 70.0	307.6 ± 134.1
	1hr	401.2 ± 196.7	313.0 ± 97.7	356.3 ± 172.7
	24hr	461.8 ± 271.8	606.6 ± 314.5	636.3 ± 344.6
Mb (ng/mL)	Pre	7.2 ± 10.9	3.5 ± 4.8	32.7 ± 51.9
	Post	181.9 ± 126.6	218.7 ± 145.1	162.8 ± 136.9
	1hr	211.4 ± 127.2	239.4 ± 103.8	484.6 ± 200.0
	24hr	4.7 ± 8.5	13.2 ± 18.2	88.1 ± 86.2
IL-6 (pg/mL)	Pre	1.2 ± 2.6	1.0 ± 2.1	1.1 ± 2.7
	Post	4.7 ± 2.5	4.1 ± 2.4	4.8 ± 3.0
	1hr	4.0 ± 2.3	3.9 ± 2.4	4.7 ± 2.8
	24hr	1.3 ± 2.4	1.1 ± 2.2	1.3 ± 2.7

Before performing the IST (pre), no significant differences between conditions (MTN, CHO and PL) were observed for the three analysed markers: CK [$F(2,16)=4.23$, $p=0.066$]; Mb [$F(2,16)=0.59$, $p=0.485$] and IL-6 [$F(2,16)=2.55$, $p=0.146$]. However, some differences and interaction effects were determined for the measurement obtained at post, 1hr and 24hr after performing the IST. Significant effect of time points (Pre, post, 1hr and 24hr)

[F(3,24)=26.0, p<0.001, ω^2=0.24] and interaction between conditions and times [F(6,48)=2.79, p=0.021, ω^2=0.02] were determined for CK. *Post hoc* analysis revealed significant (p<0.05) higher values of CK at 24hr compared to all other time points for CHO and PL conditions but not for the MTN condition as depicted in figure 9.

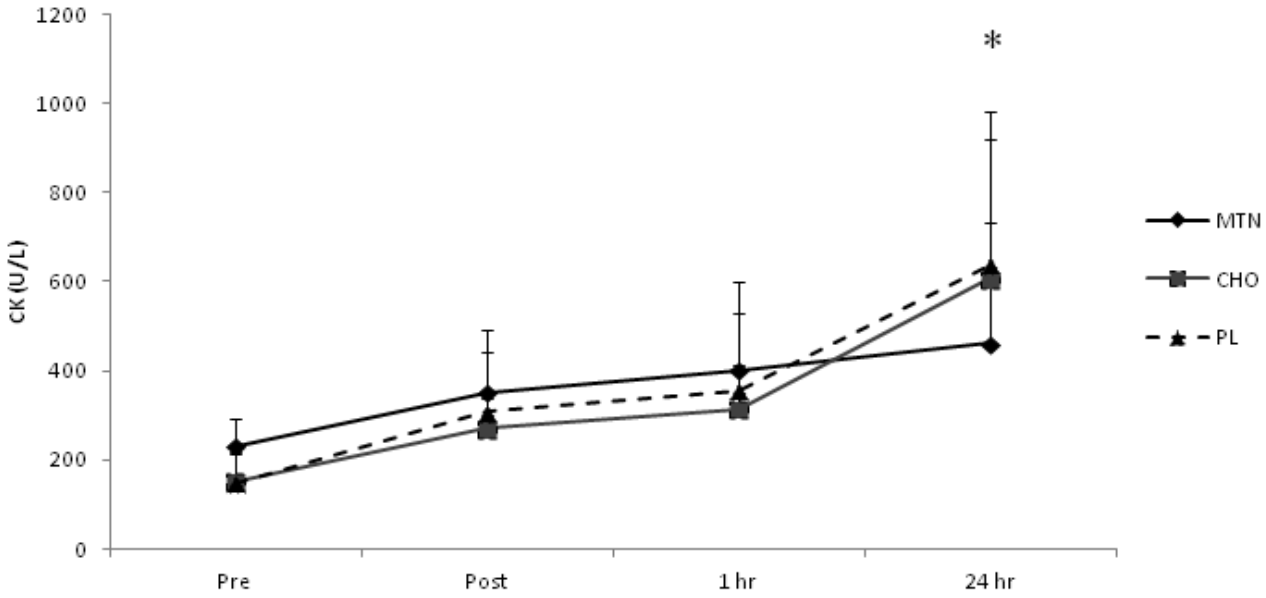

Figure 9. Serum CK levels determined at all times points for the three tested conditions
* p<0.05 from 24hr to pre, post and 1hr for CHO and PL conditions

Mb analysis showed significant differences between conditions (MTN; CHO and PL) [F(2,16)=5.17, p=0.019, ω^2=0.04], time (Pre, post, 1hr and 24hr) [F(3,24)=49.65, p<0.001, ω^2=0.49] and interactions effects between conditions and time [F(6,48)=5.79, p<0.001, ω^2=0.08].

Post hoc analysis revealed significantly higher Mb values for all three conditions at post (MTN p=0.024; CHO p=0.013; and PL p=0.028) and 1hr (MTN p=0.010; CHO p=0.001 and PL p=0.003) compared to pre. However, only PL showed significant higher values at 1hr compared to post (p=0.013). At 24hr Mb decreased for all three conditions, reaching significantly lower values than those measured at 1hr (MTN p= p=0.006; CHO p=0.001 PL p<0.001). However, MTN (p=0.015) and CHO (p=0.017), but not PL, showed significantly lower values at 24hr compared to those measured at post (Figure 10). In addition, significantly lower Mb values were determined at 1hr for CHO compared to PL (p=0.019)

and approached statistical significance when compared the values measured for MTN with those determined at PL (p=0.060). The same trends, shown in figure 11, were found for the 24hr values when compared the Mb produced after MTN (p=0.065) and CHO (p=0.057) with those measured after PL condition.

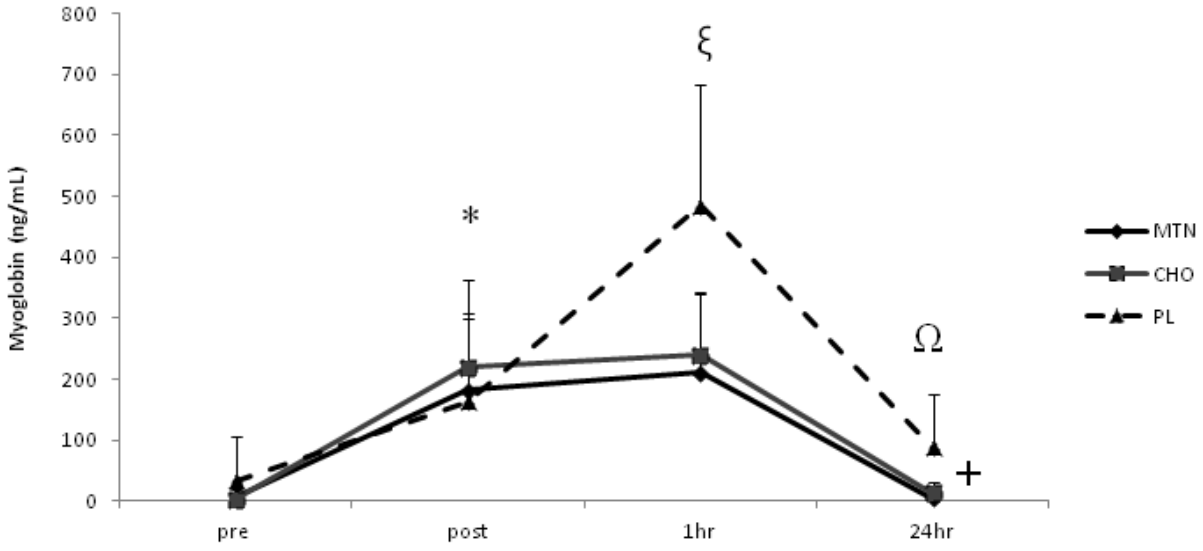

Figure 10. Serum Mb values determined at all times points for the three conditions

* p<0.05 to pre for the three conditions

ξ p<0.05 from 1hr to post only for PL condition

Ω p<0.05 to 1hr for all three conditions

+ p<0.05 to post for MTN and CHO

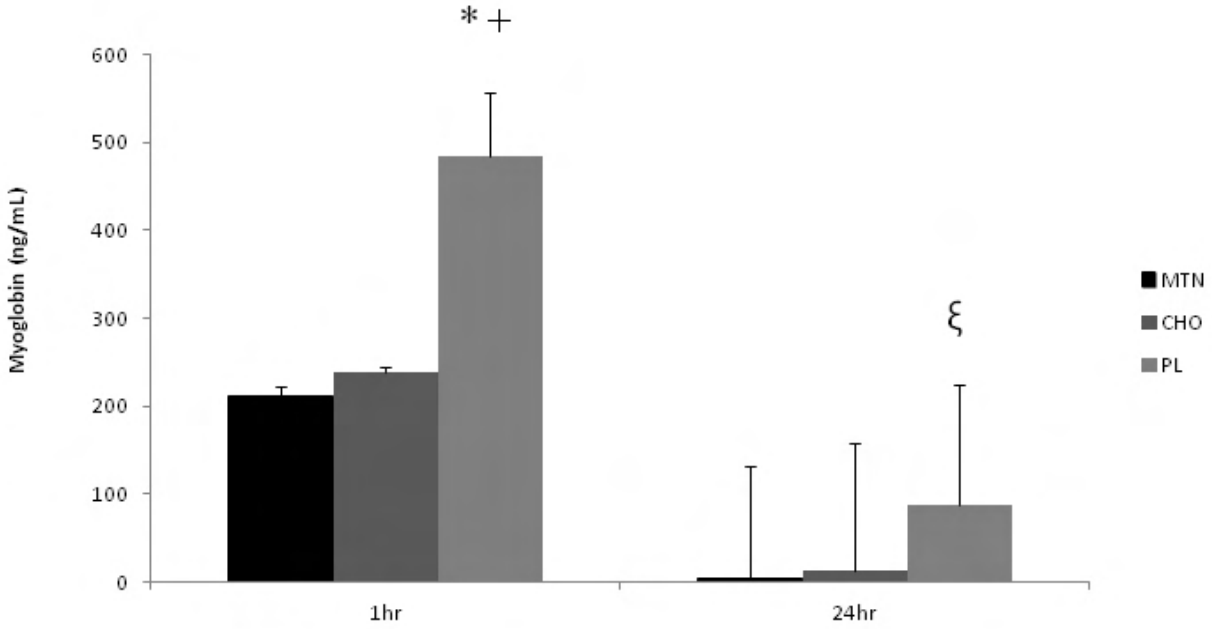

Figure 11. Comparison between the serum Mb levels determined for the three tested conditions at 1hr and 24hr after performing IST

*p<0.05 to CHO

+ p=0.060 to MTN

ξ approaching of statistical significance to CHO (p= 0.057) and to MTN (p=0.065)

IL-6 analysis showed a significant difference for time (Pre, post, 1hr and 24hr) [$F(3,24)=40.31$, $p<0.001$, $\omega^2=0.30$]. However, no differences were found for condition effect [$F(2,16)=1.81$, $p=0.196$, $\omega^2\approx0.00$] or its interaction with the time points [$F(6,48)=1.78$, $p=0.124$, $\omega^2\approx0.00$]. *Post hoc* analysis revealed higher IL-6 values for all three conditions at both post (MTN p=0.001; CHO p=0.001; PL p =0.0130) and 1hr (MTN p<0.001; CHO p= 0.001; PL p=0.005) compared to pre. In addition, at 24hr the IL-6 values seemed to approach baseline values, being significantly lower than those measured at both post (MTN p=0.001; CHO p= 0.001; PL p=0.010) and 1hr (MTN p=0.000; CHO p= 0.000; PL p=0.003) after performing the IST but not from those determined at pre as depicted in figure 12. No significant differences were observed when comparing the IL-6 values measured at 24hr with those obtained at pre for the three analysed conditions.

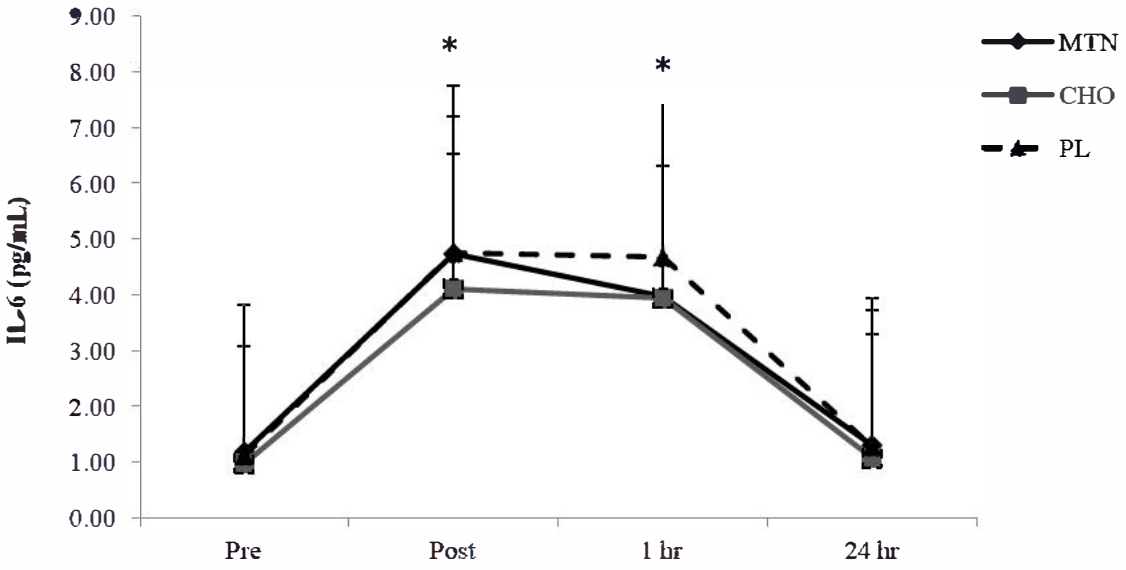

Figure 12. Serum IL-6 levels determined at all time points for the three tested conditions

* $p<0.05$ to pre and 24hr values

5.4 Discussion

The findings of this study demonstrate that there was no effect of ingesting a MTN or CHO supplement before, during and immediately after exercise on repeated sprint ability, neuromuscular fatigue (15 m sprint) or pattern of serum IL-6 responses measured at post, 1hr and 24hr after performing a 90 min IST in recreationally trained males. However, the ingestion of a MTN supplement appears to be effective in blunting the increase in CK observed 24hr after the IST. In addition, changes in Mb were observed following consumption of both MTN and CHO compared to PL.

These results are in contrast to a number of studies that have suggested positive effects of carbohydrate-protein mixtures, compared to the ingestion of carbohydrate alone or a low caloric placebo, for improving intermittent endurance performance [205] and the attenuation of neuromuscular fatigue [203, 210]. Although the total amount of protein and carbohydrate provided in this study were greater than those recommended as effective for attenuating fatigue [210] or to maximally stimulate muscle and albumin protein synthesis after performing a lower body resistance exercise [211], this did not positively affect either the IST performance or the 15 m sprint time measured over a 24 h recovery period. It has been proposed that the benefit induced by the ingestion of

protein-carbohydrate supplements is due to the increase in muscle protein synthesis and/or blunting protein degradation [203]. Moore *et al* [211] demonstrated that muscle protein synthesis is not further stimulated with intakes of egg protein above 20 g after performing four sets of 8 – 10RM for three lower body resistance exercises. Indeed, it has been suggested that 20 g of high quality protein represents an upper limit for the incorporation of amino acids into protein pools. This amount of protein would set an optimal value for achieving the maximal response beyond which a marked stimulation of whole-body amino acid oxidation, with no further increases in muscle protein synthesis, would occur in young resistance trained males [211].

The benefits of ingesting protein appears to be based on the efficacy to increase protein synthesis, attenuate protein degradation and protect against muscle membrane disruption [203], after an acute bout of resistance exercises [202, 203, 210-212]. For our participants, the consumption of a total amount of 29 g of high quality protein mixed with 106 g of CHO and enriched with 10 g of L-glutamine and 3 g of L-carnitine L-tartrate does not appear to provide sufficient amino acids to significantly stimulate muscle protein anabolism and protect muscle membranes following a 90 min IST. Even when total CK activity was significantly reduced in MTN compared with CHO and PL, both MTN and CHO showed lower Mb values at 1hr respect to PL with no difference between them. However total CK is a highly variable, indirect and nonspecific marker of exercise induced muscular damage [213] and Mb should also be used with caution [214]. In fact, the analysis used for the assay of both markers does not distinguish between skeletal or cardiac muscle isoforms. In addition, substantial variation between participants (table 14) together with the lack of homogeneity in the observed response for the three tested conditions makes it difficult to differentiate any muscular protective effects due to the ingestion of MTN or CHO alone and hence challenges their use as meaningful markers of exercise induced muscular damage. However, the significant increase of IL-6 observed immediately post and 1hr after exercise but not at 24hr (when Il-6 levels approach baseline) without differences between conditions, impede the elucidation of any further protective effects due to the ingestion of MTN or CHO compared to PL. Indeed, it has been suggested that the increase in IL-6 following exercise is not primarily related to muscle damage but with the exercise volume [215] and intensity [216]. During exercise, active muscles secrete IL-6

to influence and regulate substrate metabolism. IL-6 stimulates lipolysis and hepatic glycogenolysis as exercise progresses and muscular glycogen becomes progressively depleted [217]. Hence we would expect a blunted IL-6 response after MTN or CHO condition, however no effects where observed compared to PL. After exercise, circulating IL-6 should decrease approaching the baseline levels within a few hours. However, some degree of muscular damage can be inferred when a sustained elevation of circulating Il-6 is observed from six hours to several days after exercise [218].

The quantity of protein provided in the MTN condition was intended to saturate protein synthesis during recovery, and therefore maximise this specific nutrient effect on the cellular response to exercise. Leucine is the most important amino acid triggering muscle protein synthesis when a minimum amount of 0.75 [55] to 1 g or 2 g per meal is consumed by young or elderly people respectively [219, 220]. Hence, a sustained high leucine signal may promote a greater translation of new exercise response mRNA transcripts during recovery, compared with a low leucine signal. The whey protein content in the MTN administered in the present study are higher in leucine compared to other protein sources, also, essential amino acids comprised approximately half of the ingested protein [221]. Therefore, participants were ingesting a total of about 14.5 g of essential amino acids (3 g leucine) in the MTN condition. In the line with this rationale, Rowlands *et al* [222] observed no effect of ingesting carbohydrate-protein enriched bars on a cycling repeated sprint test performed 15 h after a long lasting high intensity exercise protocol. However substantial improvements, together with a notable reduction of CK activity were observed after 60 h when compared to the ingestion of carbohydrate-low protein bars control condition.

The result of the present study are also similar to those reported by Roberts *et al* [223] who found no effects of ingesting a solution providing 1.2 $g \cdot kg^{-1} \cdot h^{-1}$ of carbohydrate alone or combined with 0.4 $g \cdot kg^{-1} \cdot h^{-1}$ of high quality protein, before, during and after a rugby specific shuttle running protocol to attenuate muscular function and serum Mb accumulation measured immediately after exercise. In addition, similar to the present study, total CK peaked 24 h after exercise with no differences between the three analysed conditions. More recently, Cockburn *et al* [224] reported that consuming 500 mL of semi skimmed milk providing 13.6 g of casein, 3.5 g of whey proteins 24.5 g of CHO

and 8.5 g of fats, after an acute isokinetic concentric eccentric hamstring muscle damage protocol, is likely to be beneficial in limiting the loss of performance during an agility task, 15 m sprint and a 90 min repeated sprint test but not for attenuating the decrease of reactive strength and the increase of both serum Mb and CK measured over a 72 h period.

Differences in the exercise protocol used in the present study, which involves repeated running maximal acceleration and decelerations over a 90 min test, would have increased the need for a higher amino acids supply in order to determine a more noticeable effect on muscular function and markers of muscle damage. However, this is purely speculative as there is no conclusive evidence regarding the dose response relationship between the effects of ingesting protein-carbohydrate based multi-ingredient supplements and performance or muscle damage markers.

The addition of 5 g of L-glutamine and 1.5 g of L-carnitine L-tartrate per serving does not seem to be effective enough to acutely affect intermittent sprint performance or to attenuate the specific neuromuscular fatigue respect to the ingestion of CHO alone or PL nor to reduce serum Mb level respect to the CHO condition. The effect on CK measured at 24hr could be considered but it would not be possible to identify if this effect is due to the administration of protein, or because of the added L-glutamine and L-carnitine or a combination of different nutrients. Even though the available data respect to the effectiveness of the L-glutamine supplementation on performance are inconclusive (Kreider, 2010), some effects to prevent excessive muscle damage and neutrophil function suppression have been observed after several days of supplementation [225] but not after an acute intake as administered in the present study. With regard to L-carnitine, previous research demonstrated that 1 [226] or 2 $g \cdot d^{-1}$ [226] of L-carnitine L-tartrate administered for three weeks results in less metabolic stress and concentration of both Mb [226] and CK [207] measured after performing an acute bout of resistance exercise in young males. It is important to highlight that the above reported benefits obtained from the oral L-carnitine ingestion were observed after 21 days of regular administration and not after an acute intake. In addition as L-carnitine peaks in blood after three to six hours of ingestion [227], in order to promote its benefits, multiple daily doses where the last single intake should be administered around three hours before

exercise has been recommended [227]. Thus, it could be possible that the protocol used in our study where participants were supplemented during and after exercise, would not favour the positive effects of L-carnitine to acutely attenuate muscle damage as observed by others [207, 226].

Although speculative, even when MTN or CHO were not effective to improve repeated sprint ability during IST neither to reduce the neuromuscular fatigue estimated from the 15 m sprint time measured over a 24 h period, as suggested by others previous investigations [207, 226], the lower concentration of CK and Mb observed after exercise would be positively related with a faster recovery process in recreationally trained athletes.

A limitation of this study was that only three time points post-exercise were analysed (post, 1hr and 24hr) and therefore no further information about a longer recovery period (>24h) was obtained. Additionally, other markers were not analysed such as plasma aminoacidemia, glycaemia, serum glutamine and carnitine concentrations. The current study measured IL-6 released into the blood and not locally and given the paracrine and autocrine function of IL-6, future studies could use microdialysis techniques or muscle biopsies to better analyse this parameter. Lastly, even when acute studies can give a clear picture about the acute response resulted from different training and feeding protocols, in order to have more useful information about the potential benefit of natural supplements, more research conducted over a longer period of time (weeks or month) should be designed.

5.5 Conclusion

In conclusion the ingestion of a MTN (53 g of CHO, 14.5 g of whey protein, 1.5 g of L-carnitine and 5 g of L-glutamine) or CHO (69.5 g of maltodextrin), dosed during and immediately after a 90 min intermittent sprint exercise bout was not effective to increase performance, attenuate fatigue or alter the IL-6 responses measured at post, 1hr, and 24hr after exercise in recreationally trained males. However, supplementing with the MTN would be effective to blunt the increase in CK observed 24h after the IST with

respect to CHO and PL. Finally, both MTN and CHO seems to have similar positive effect to blunt the increase in Mb observed 1h after performing an IST.

Chapter 6
General summary

6.1 General discussion

The research presented in this thesis has focused on the expected and marketed outcomes of three commercially available multi-ingredient supplements in recreationally trained males. Two of the multi-ingredient supplements used in the this project consisted of different combinations of carbohydrate, protein and other compounds such as creatine monohydrate, L-carnitine, L-glutamine or β-Hydroxy-β-Methylbutyrate (HMB) (studies 1 and 3) while the other was a carbohydrate and caffeine containing gel (study 2). Based on documented effects of their individual components [27, 138], these supplements have been designed to benefit specific sports/modes of exercise, to which they are marketed. Antonio and Ciccone [228] stated that, although it has not yet been fully alluded to, it is apparent that nutrient timing and combination can affect the adaptive response to exercise. Further to this the amount of nutrients required can also vary depending on the mode and duration of exercise [24]. Therefore, the observed benefits from the individual supplements can be specific to the amount; timing and training protocol used in the experimental procedure and would not necessarily result in the same outcomes when applied to other amounts, timings, cohorts and/or modes of exercise. For example Volek *et al* [229] observed a significant increase in strength performance after a 12 wks creatine supplementation protocol with concurrent periodised heavy resistance training in a cohort of resistance trained young males. The creatine supplementation protocol consisted of a weeklong loading period of 25 $g \cdot d^{-1}$ followed by a 5 g maintenance dose for the remainder of the training. Whereas Bemben *et al* [230] reported no additional benefits of ingesting creatine, with and without whey protein, for improving strength and muscle mass after a progressive 14 wks (three days per week) resistance training programme in middle age and older men. The creatine supplementation protocol consisted of a loading period of 7 g administered three times per week for two weeks followed by 5 g consumed after each resistance training session. Volek *et al* [229] utilised a periodised resistance training programme, in which participants could progressively increase the training load as they progressed. Whereas in the study of Bemben *et al*, [230], participants had to follow a predetermined progressive

resistance training protocol that probably does not allow them to take advantage form the creatine mediated increased training capacity and therefore this specific limitation would have been hindrance the potential induced benefit commonly reported by the combination of creatine and resistance training [89]. The different methodologies used in these studies could probably explain the contradictory results.

Specifically to resistance training, the use of a multi-ingredient supplement, as analysed in study 1, could be of long term benefit to increase resistance exercise performance. This can be supported from previous research carried out on the effectiveness of the individual components included in the tested supplement and their known or assumed synergistic effects [25]. The multi-ingredient combinations used in the study 1 are thought to promote recovery by attenuating muscle damage, replenishing glycogen stores, promoting an anabolic environment within the body [24] and attenuate a rise in cortisol [43]. These effects would be potentiated by the addition of high quality protein such as whey [24].

Whey protein would support a positive net protein balance by stimulating muscle protein synthesis after exercise [55] . The addition of HMB may also promote cell membrane stabilisation [63], favouring the interaction of circulating anabolic hormones (growth hormone and testosterone) with cell membrane receptors [62, 73]. On the other hand, even though the positive effects of glutamine to enhance exercise adaptation remain still controversial [25], it would have some positive effects to attenuate fatigue [85, 231], blunt exercise induced immune depression [80] and contribute to replenishing glycogen stores [88].

The use of piperine within a multi-ingredient supplement is based on the speculation of its potential benefit for increasing the bioavailability of nutrients [118]. However, to date, the effects of piperine within a carbohydrate-protein-creatine multi-ingredient supplement has not been investigated. As limitation of study 1, the methods used would not allow us to speculate on the effects of piperine on a multi-ingredient formula to potentiate resistance training outcomes.

The addition of chromium picolinate is based on its theoretical positive effects on glucose tolerance, protein and lipid metabolism, serum cholesterol and avoid losses of chromium

picolinate due to strenuous exercise and thus support the increase of muscle mass [123]. However, these theoretical positive effects have not been supported by research [70]. Similarly the rational to include bicarbonates is based on its demonstrated effect for improving predominantly anaerobic-glycolitic performance [2], via mechanisms of attenuated acidosis [76]. Given the fact that the hypertrophy training performed by the participants of study 1 emphasises the glycolitic-energy pathway, it could be speculated that the addition of bicarbonate to the multi-ingredient supplement would benefit performance in conjunction with hypertrophy training. However, the total daily dose found in each serving of the multi-ingredient supplement would amount to only 2 $g \cdot d^{-1}$. This small amount has not been studied to show efficacy in improving resistance training performance. As categorised in the literature, a small effective dose for chronic bicarbonate supplementation shown to reduce exercise induced acidosis and improved performance of short duration high intensity exercise is in amounts of 5 g ingested two times per day for five days [25]. Therefore it is difficult to speculate on the efficacy of the addition of small amounts of bicarbonates to a multi-ingredient supplement such as that used in study 1.

The amount creatine monohydrate (10 $g \cdot d^{-1}$) present in the multi-ingredient formula administered in study 1 has been shown to allow for increased training capacity, via faster replenishment of adenosine triphosphate, as well as favouring a more anabolic environment by changing cellular osmolality [89]. Furthermore, creatine can act as an antioxidant attenuation muscle damage and promoting the recovery process [89]. The addition of creatine to a carbohydrate and protein beverage has previously demonstrated greater resistance training outcomes than carbohydrate and protein supplementation alone [232] and allowed for greater, insulin mediated muscular creatine uptake [89]. Despite the speculation on the effects of the individual supplements, the effect of the multi-ingredient supplement as a whole should be the main consideration.

The results obtained in study 1, in relation to the effects of a carbohydrate-protein-creatine multi-ingredient supplement on strength performance in recreationally trained males demonstrates potential effects to optimise adaptation and expected outcomes from regular resistance training. However, the small sample size used in this investigation would have impeded the ability to determine a significant difference between the

variables (fat free mass and strength related performance) measured in this study. In fact, when analysed, the ES was found to be larger, in the multi-ingredient supplemented group, for increases of fat free mass, strength and muscular endurance outcomes. The magnitude based inferences suggested similar outcomes, particularly for increased improvements in upper body maximum strength. This shows us that it is likely that with a larger sample the supplemented group would have significantly greater strength and muscular endurance performance outcomes. This speculated outcome support the work of Cribb *et al* [165, 232] who performed similar interventions with similar outcomes. However, unlike Cribb *et al* no lower body strength performance benefits due to the ingestion of the multi-ingredient supplement were observed in this study. This may be due to the sample of the previous study consisting of specifically recreational bodybuilders and not recreationally trained participants, as in our intervention, which may have performed other recreational activities such as football or rugby. This has the potential to hinder maximal strength outcomes [233]. Furthermore, as the participants were recreationally trained it is possible that the training programme elicited greater performance effects compared to training with the multi-ingredient supplement [166]. Although speculative, this rational can further be supported on the improved maximum strength performance observed for bench press and squat over the course of the intervention. Even when the findings of study 1 could be useful for advising supplementation strategy for recreationally trained individuals who combine resistance training with others intermittent activities such as football or rugby, our results should be considered with caution until more research involving larger samples size are successfully completed.

The use of the carbohydrate-caffeine gel tested in study 2 has shown some possible benefit for attenuating fatigue and reducing the rate of perceived excretion after 45 min of intermittent exercise. Although not previously studied in gel form, it is probable that the an altered perception of fatigue mediated by the co-ingestion of carbohydrate and caffeine can positively affect performance through attenuated subjective perception of fatigue which could enable a better or maintained voluntary effort especially during the fatiguing stages of exercise [102]. The presence of carbohydrate in the mouth could have stimulated areas of the brain linked with motivation and reward which may have

attenuated perception of effort [41] whereas the caffeine can form an adenosine receptor blockade which hinders adenosines' calming effect on the nervous system [101]. These effects could lead to altered perception of effort. However, a carbohydrate mouth rinse has recently been shown ineffective in enhancing intermittent exercise performance [234]. Additionally, carbohydrate ingested during exercise, both alone and with caffeine could maintain blood glucose concentrations, increase levels of carbohydrate oxidation and spare endogenous glycogen [32] especially when utilising multi-source carbohydrate ingestion [38]. The combination of carbohydrate and caffeine has the possibility to further increase carbohydrate oxidation and increase blood glucose concentrations through increased intestinal absorption [132].

Jentjens *et al* [37] previously observed increased carbohydrate oxidation rates in response to the combination of two monosaccharaides, glucose and fructose, at a ratio of 2:1 respectively, when ingested at a rate of 0.6 and 1.2 $g \cdot min^{-1}$ of fructose and glucose, respectively, over 120 min of cycling exercise. The same research group reported similar results from the ingestion a 1:1 ratio of glucose and sucrose (disaccharide) at a rate of 1.2 and 1.2 $g \cdot min^{-1}$ for glucose and sucrose respectively [38]. The carbohydrate combinations used in these above mentioned studies differ from the mixture of carbohydrate and rate of ingestion we have used in study 2, that combines an oligosaccharide (maltodextrin), a disaccharide (sucrose) and monosaccharaides (dextrose and fructose) ingested at a rate of 0.5 $g \cdot min^{-1}$ over a period of 150 min. Both carbohydrate and carbohydrate caffeine conditions tested in study 2 provided 30% of sucrose and 2% fructose. This carbohydrate combination has not been studied before and therefore further speculations about their effects on glucose oxidation rates cannot be made at this time. Despite the specific blend of carbohydrates, intestinal sodium dependant glucose transporters achieve maximum saturation at around an ingestion rate of 1 $g \cdot min^{-1}$ which can be increased to approximately 1.7 $g \cdot min^{-1}$ when ingesting a mix of carbohydrates [36] which is far above the rate of ingestion tested in our study and thus we can estimate that intestinal glucose absorption would not be a limiting factor. Additionally, increased oxidation is generally only acknowledged in well trained endurance athletes [35], and therefore may have not benefitted recreationally trained individuals as those used in study 2. Although study 2 demonstrated maintained levels of blood glucose in the carbohydrate-caffeine condition

and the carbohydrate condition with regards to the placebo condition in the first three blocks but not the fourth of the intermittent sprint test (IST). In the last block the carbohydrate condition did not produce a significantly higher level of blood glucose in respect to the placebo condition nor did the carbohydrate-caffeine condition produce a significantly higher blood level than the carbohydrate condition. However, there was a large ES and an α level that approached statistical significance which together indicted the potential of an additive effect of carbohydrate-caffeine to maintain higher blood glucose levels in respect to the ingestion of carbohydrate alone. Study 2 also demonstrates some interlinked perceptions of fatigue and performance. The ingestion of a gel containing 25 g of CHO and 100 mg of caffeine one hour, immediately before and at the middle of a 90 min intermittent repeated sprint exercise transiently attenuated fatigue and subjective perceived exertion, after 68 min of exercise. Even though a significant difference ($p \leq 0.05$) was not reached when compared the ratings of perceived exertion between carbohydrate-caffeine and carbohydrate alone, *post hoc* analysis revealed the α level that approached statistical significance and a large ES which is in line with other studies that have reported positive effects of combined caffeine and carbohydrate in liquid solution [129, 130, 176].

It is possible that due to an absolute amount of caffeine being provided, differing body masses and potential individual responses to caffeine, that participants reacted differently to supplementation [20]. This would also be considered a possible limitation of the one size fits all manufacturer approach to commercially available multi-ingredient supplements.

Despite the low amounts of carbohydrate found in each serving of carbohydrate-caffeine gel coupled with the staggered servings, which may have resulted in a lower caffeine peak than from a single ingestion, it was found that fatigue and perception of fatigue was transiently attenuated. Additionally higher levels of serum glucose concentrations were maintained which collectively demonstrates that doses provided in commercially available supplements, even when considered low from the scientific literature [20, 34], would have some effects in recreationally trained cohorts.

Although of serum glucose concentrations were found to be maintained, no effect on fatigue was found from the carbohydrate condition. Therefore, the inclusion of caffeine to a supplement containing carbohydrate appears to be beneficial for attenuating fatigue from an IST of around 90 min.

During study 3 the rate of carbohydrate ingestion was increased before and during the IST to 0.88 and 1.16 $g \cdot min^{-1}$ for the multi-ingredient supplement (maltodextrin and dextrose) and the carbohydrate supplement (maltodextrin) respectively. Similarly to study 2 no significant effects on fatigue or rate of perceive exertion was found, despite the increased amount of carbohydrate.

Collectively, the results of third study demonstrate that the ingestion of a multi-ingredient supplement containing carbohydrate, protein, L-glutamine and L-carnitine before, during and after exercise does not acutely affect repeated sprint ability performance nor does it attenuate neuromuscular fatigue estimated from the 15 m sprint performance measured immediately, 1 and 24 h after exercise in recreationally trained young males. Additionally the multi-ingredient was also not effective to alter the pattern of serum IL-6 responses measured over a 24 h recovery period. Despite the absence of performance effects, the ingestion of the multi-ingredient formula did attenuate an increase in creatine kinase (CK) observed 24 h after the IST. In addition, the carbohydrate condition significantly attenuated serum myoglobin (Mb) concentration measured at 1 h post exercise, whereas the multi-ingredient supplement demonstrated an α level that approached statistical significance (P=0.060).

The ingestion of carbohydrate has resulted in attenuated cortisol levels from intermittent exercise [45]. The addition of whey protein to carbohydrate can not only provide the amino acids required for protein synthesis but can also act as a signal, in the mammalian target of rapamycin (mTOR), to initiate skeletal muscle protein synthesis [53]. An amount of 0.75 g of leucine is required for this in a cohort of recreationally trained young males, whilst the other amino acids found in the whey protein will continue the anabolic response [47]. It is possible that the L-carnitine L-tartrate can contribute to an attenuation of muscle damage by reducing exercise induced hypoxia via mechanisms described in section 2.5 [68]. The glutamine contained in the multi-ingredient supplement

may aid glycogen resynthesis [78] and immune function [80], however as previously mentioned, to our knowledge there is no convincing evidence about the positive effects of L-glutamine supplementation on exercise performance [25].

Studies investigating the optimal amount of protein or amino acids required to maximally stimulate post exercise muscle protein synthesis and optimise the recovery process have mainly used resistance training protocols [211] and there is no conclusive dose response relationship reported for intermittent training exercise. However it is considered that the more intense the exercise the greater the protein catabolism or the lower the protein synthesis will be [50]. Consequently, a greater amount of protein would be required to attenuate catabolism and promote recovery [25]. Therefore, although speculative the dosing protocol used in study 3 may not have appropriate to attenuate the supposed larger amount of muscle damage elicited by a 90 min IST as used in study 3.

Although altered patterns of general biomarkers were observed from the ingestion of the multi-ingredient supplement from study 3, it cannot be eluded to any specific muscle damage. Furthermore serum IL-6 concentrations, a marker of progressively depleting glycogen, yielded no significant between group differences nor where there any significant effects on rating of perceived exertion between the three conditions tested in the study 3. As there is a linear relationship between muscle glycogen levels and rating of perceived exertion, this coupled with the unaffected IL-6 concentrations shows that the exercise stress between the three conditions was similar. Due to these observations it can only be concluded that the biomarkers were blunted by the ingestion of the multi-ingredient supplement. Despite this, it is possible that the altered concentrations of CK and Mb could be positively related to recovery of recreationally trained exercise practitioners from a bout of intermittent sprint exercise. Even when these findings do not support the acute positive effects of the tested multi-ingredient on repeated sprint ability and performance recovery outcomes from a bout of intermittent repeated sprint exercise in recreationally trained males it is possible that some benefit to protect muscle damage would be achieved.

Taken together, the results from the three studies included in this project highlight the importance of the correct application of commercially available supplements and their

potential benefits on resistance training outcomes (study 1), acute performance (study 2) and some possible acute effects on a 24 h recovery period (study 3). The combination of ingredients found in the first study seems to potentiate chronic performance adaptations from a resistance training programme, also the combination of carbohydrate and caffeine from the second study was affective to transiently positively affect fatigue and perception of fatigue. The third study showed that although there may be some potential positive effects on recovery, the multi-ingredient supplement was ineffective for maintaining sprinting performance and attenuation of neuromuscular fatigue over a 24 h recovery period. However, it would important to highlight that these results should be considered only under the specific mode of exercise, protocols and the methods of supplementation used in ours studies.

Evidence from the scientific literature and the results of the present project would support the following recommendations for recreationally trained young males:

a) The ingestion of a multi-ingredient supplement containing carbohydrate-protein-creatine enriched with L-glutamine, HMB and others nutrients would be an alternative to consider in order to potentiate resistance training outcomes.

b) The ingestion of carbohydrate-caffeine gels would be considered to acutely increase repeated sprint performance during the last part of a 90 min intermittent exercise bout.

c) The use of a carbohydrate-protein based multi-ingredient supplement enriched with L-glutamine and L-carnitine L-tartrate would not be necessary to acutely promote intermittent exercise performance and neuromuscular recovery. The use of carbohydrate only would be enough for possible attenuation of muscle damage after intermittent exercise.

6.2 Strengths and limitations

Study 1 utilised a double blind, randomised placebo-controlled parallel design, whereas studies 2 and 3 were of a double blind, randomised, counter balanced, placebo controlled cross over design in order to reduce experimenter and participant bias. As studies 2 and 3 were counter balanced this would help to minimise the chances of a learning effect along

with the familiarisation sessions provided before participants could partake in studies 1, 2 and 3.

Each study had a set of exclusion criteria in order to minimise the effects of any confounding variables, such as habituation to supplements, medication interactions and age effects, to achieve a homogenous sample.

The aim of this thesis was to determine the efficacy of commercially available supplements on popular activities for recreationally trained males. Specifically, the participants were young recreationally trained males who undertook a variety of exercise practices focused around recreational resistance training and team sport activities. Therefore the findings of this thesis should only be applied to this cohort.

In addition to utilising larger sample sizes to eliminate speculation based on α levels that approach statistical significance, large ES and magnitude based analysis, further measurements both in relation to measured time points and methods of measurement should be investigated. As the BOD POD® is a general measure of fat free mass it does not allude to specific muscle hypertrophy. Anthropometric measurements could be used in addition to the BOD POD® measurements or dual energy X-ray absorptiometry (see study 1) to indicate to if and where muscle hypertrophy has occurred. Muscle biopsies and plasma concentrations of the ingested supplements could also be measured to provide evidence of the supplements absorption and effects. This would have been useful across the three studies. For example, although glycaemia was measured in study 2 plasma caffeine levels were not. Therefore it is purely speculative that the ingested caffeine reached similar plasma levels at similar time points between participants. This could have impacted on the results obtained; however, as the research of this thesis focuses on the recreationally trained participants from the general population it would reflect real world scenarios.

Differing protocols of supplementation, based on the literature, should also be studied to maximise the potential for efficacy. The use of the supplement found in study 1 utilised a protocol of one serving in the morning and one serving after exercise, as recommended by the manufacturer. Supplementation with carbohydrate and whey protein before exercise is recommended to increase protein synthesis and after exercise, with the

addition of creatine to potentiate adaptation [24]. Furthermore, with regard to the third study, as stated along the discussion, in order to benefit from L-carnitine supplementation, multiple daily doses should be administered with the last daily dose ingested 3 h before exercise [227].

Study 3 demonstrated attenuation of CK and Mb despite not finding any significant differences on performance and neuromuscular recovery up to 24 h after exercise. This shows that there may be the possibility of a chronic effect. Therefore using studies of a greater longitudinal design would confirm or reject this hypothesis.

The main challenge in the use of larger cohorts for long term studies, on recreationally trained participants, lasting weeks if not months in duration is participant adherence to the investigation. General life activities will take precedence over the study, such as work and family commitments. Standardising experimental practices such as dietary and adherence exercise other than that demanded of the investigation can also be difficult, this leads to many drop outs and exclusions if participants cannot meet the experimental requirements.

6.3 Future work

The studies included in this thesis utilised recreationally trained participants, however, the use of supplements spans across other cohorts, such as various age groups and female exercise/sports practitioners [138], future research should also focus on these groups.

Supplement manufacturers can be quite vague in their recommendations regarding the mode of exercise the supplement may benefit. Consequently, and in addition to studies of greater duration and sample size, varying modes of exercise should be used to establish the specific effects of each commercially available supplement as different modes of exercise will not result in the same outcomes or have the same metabolic effects.

More generally, studies investigating the amount of nutrients required optimise exercise performance and adaptation in regards to intermittent sprinting exercise should be carried out in order to determine optimal requirements. For example, determining how

much protein is required to cover oxidative protein losses and enhance recovery in recreational intermittent endurance athletes would enable supplement manufactures to formulate products aimed at intermittent exercise/sports.

6.4 Conclusions

The results from the present project demonstrated that two of the three supplements tested had beneficial effects on exercise performance and adaptation; however, the supplement aimed at recovery did not display clear benefits over a 24 h period. It would appear that manufacturers make use of the research available to formulate multi-ingredient supplements as the supplements that showed efficacy (studies 1 and 2) possess the most established research. Whereas investigations and knowledge in to the effects of a carbohydrate-protein based multi-ingredient supplement on recovery from intermittent sprint exercise are unknown.

The findings from each of the three studies included in this thesis allow for the following conclusions to be drawn:

The ingestion of a carbohydrate-protein-creatine based multi-ingredient commercially available supplement enriched with L-glutamine and HMB (study 1) has the potential to optimise some of the expected strength performance outcomes but not body composition changes in a group of recreationally trained young males after 12 wks of regular resistance training.

The ingestion of a commercially available gel containing carbohydrate and caffeine (study 2) can positively transiently affect fatigue and the rate of perceived exertion, in recreationally trained young males while performing a 90 min intermittent sprint test.

The ingestion of a carbohydrate and protein based commercially available supplement enriched with L-glutamine and L-carnitine L-tartrate (study 3) was not effective to acutely enhance performance during a 90 min IST or attenuate neuromuscular fatigue over a 24 h recovery period in recreationally trained males. In addition, the biomarkers of CK and Mb were attenuated although the acute effects seem to be similar to the ingestion of only carbohydrates for Mb.

References

1. Petróczi A, Naughton DP, Mazanov J, Holloway A, Bingham J: **Limited agreement exists between rationale and practice in athletes' supplement use for maintenance of health: a retrospective study.** *Nutr J* 2007, **6:**34.
2. Maughan RJ, Greenhaff PL, Hespel P: **Dietary supplements for athletes: emerging trends and recurring themes.** *J Sports Sci* 2011, **29 Suppl 1:**S57-66.
3. Petróczi A, Naughton DP: **Supplement use in sport: is there a potentially dangerous incongruence between rationale and practice?** *J Occup Med Toxicol* 2007, **2:**4.
4. Zadik Z, Nemet D, Eliakim A: **Hormonal and metabolic effects of nutrition in athletes.** *J Pediatr Endocrinol Metab* 2009, **22:**769-777.
5. Dunn-Lewis C, Kraemer WJ, Kupchak BR, Kelly NA, Creighton BA, Luk HY, Ballard KD, Comstock BA, Szivak TK, Hooper DR, et al: **A multi-nutrient supplement reduced markers of inflammation and improved physical performance in active individuals of middle to older age: a randomized, double-blind, placebo-controlled study.** *Nutr J* 2011, **10:**90.
6. Martarelli D, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, Pompei P: **Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training.** *Curr Microbiol* 2011, **62:**1689-1696.
7. Kim HK, Suzuki T, Saito K, Yoshida H, Kobayashi H, Kato H, Katayama M: **Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial.** *J Am Geriatr Soc* 2012, **60:**16-23.
8. Denison HJ, Jameson KA, Syddall HE, Dennison EM, Cooper C, Aihie Sayer A, Robinson SM: **Patterns of Dietary Supplement Use Among Older Men and Women in the UK: Findings from the Hertfordshire Cohort Study.** *J Nutr Health Aging* 2012, **16:**307-311.
9. Bianco A, Mammina C, Paoli A, Bellafiore M, Battaglia G, Caramazza G, Palma A, Jemni M: **Protein supplementation in strength and conditioning adepts: knowledge, dietary behavior and practice in Palermo, Italy.** *J Int Soc Sports Nutr* 2011, **8:**25.

10. Heikkinen A, Alaranta A, Helenius I, Vasankari T: **Dietary supplementation habits and perceptions of supplement use among elite Finnish athletes.** *Int J Sport Nutr Exerc Metab* 2011, **21:**271-279.

11. **Supplements Who Needs Them?** [http://www.nhs.uk/news/2011/05May/Documents/BtH_supplements.pdf]

12. **National Diet and Nutrition Survey: Healine Results From Years 1 and 2 (Combined) of the Rolling Programme (2008/2009-2009/2010)** [https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/216484/dh_128550.pdf]

13. Jäger R, Purpura M, Shao A, Inoue T, Kreider RB: **Analysis of the efficacy, safety, and regulatory status of novel forms of creatine.** *Amino Acids* 2011.

14. Saldanha LG, Dwyer JT, Andrews KW, Bailey RL, Gahche JJ, Hardy CJ, Holden JM, Picciano MF, Roseland JM, Thomas PR, Wolf WR: **Online dietary supplement resources.** *J Am Diet Assoc* 2010, **110:**1426, 1428, 1430-1421.

15. Licata A, Macaluso FS, Craxì A: **Herbal hepatotoxicity: a hidden epidemic.** *Intern Emerg Med* 2012.

16. Nissen SL, Sharp RL: **Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis.** *J Appl Physiol* 2003, **94:**651-659.

17. Jerome-Morais A, Diamond AM, Wright ME: **Dietary supplements and human health: for better or for worse?** *Mol Nutr Food Res* 2011, **55:**122-135.

18. Stickel F, Kessebohm K, Weimann R, Seitz HK: **Review of liver injury associated with dietary supplements.** *Liver Int* 2011, **31:**595-605.

19. Ahrendt DM: **Ergogenic aids: counseling the athlete.** *Am Fam Physician* 2001, **63:**913-922.

20. Goldstein ER, Ziegenfuss T, Kalman D, Kreider R, Campbell B, Wilborn C, Taylor L, Willoughby D, Stout J, Graves BS, et al: **International society of sports nutrition position stand: caffeine and performance.** *J Int Soc Sports Nutr* 2010, **7:**5.

21. Buford T, Kreider R, Stout J, Greenwood M, Campbell B, Spano M, Ziegenfuss T, Lopez H, Landis J, Antonio J: **International Society of Sports Nutrition position stand: creatine supplementation and exercise.** *J Int Soc Sports Nutr* 2007, **4:**6.

22. Safdar A, Yardley N, Snow R, Melov S, Tarnopolsky M: **Global and targeted gene expression and protein content in skeletal muscle of young men following short-**

term creatine monohydrate supplementation. *Physiol Genomics* 2008, **32:**219-228.

23. Deldicque L, Atherton P, Patel R, Theisen D, Nielens H, Rennie M, Francaux M: **Effects of resistance exercise with and without creatine supplementation on gene expression and cell signaling in human skeletal muscle.** *J Appl Physiol* 2008, **104:**371-378.
24. Kerksick C, Harvey T, Stout J, Campbell B, Wilborn C, Kreider R, Kalman D, Ziegenfuss T, Lopez H, Landis J, et al: **International Society of Sports Nutrition position stand: nutrient timing.** *J Int Soc Sports Nutr* 2008, **5:**17.
25. Kreider RB, Wilborn CD, Taylor L, Campbell B, Almada AL, Collins R, Cooke M, Earnest CP, Greenwood M, Kalman DS, et al: **ISSN exercise & sport nutrition review: research & recommendations.** *J Int Soc Sports Nutr* 2010, **7:**7.
26. Pendergast DR, Meksawan K, Limprasertkul A, Fisher NM: **Influence of exercise on nutritional requirements.** *Eur J Appl Physiol* 2011, **111:**379-390.
27. Cooper R, Naclerio F, Larumbe-Zabala E, Chassin L, Allgrove J, Jimenez A: **Effects of a Carbohydrate-Protein-Creatine Supplement on Strength Performance and Body Composition in Recreationally Resistance Trained Young Men.** *JEPonline* 2013, **16:**72-85.
28. Haff GG: *Essentials of sports nutrition and supplements.* Springer; 2008.
29. Burke LM, Hawley JA, Wong SH, Jeukendrup AE: **Carbohydrates for training and competition.** *J Sports Sci* 2011, **29 Suppl 1:**S17-27.
30. Wildman R, Kerksick C, Campbell B: **Carbohydrates, Physical Training, and Sport Performance.** *Strength & Conditioning Journal* 2010, **32:**21-29 10.1519/SSC.1510b1013e3181bdb1161.
31. Mujika I, Burke LM: **Nutrition in team sports.** *Ann Nutr Metab* 2010, **57 Suppl 2:**26-35.
32. Jeukendrup AE: **Carbohydrate intake during exercise and performance.** *Nutrition* 2004, **20:**669-677.
33. de Salles Painelli V, Nicastro H, Lancha AH: **Carbohydrate mouth rinse: does it improve endurance exercise performance?** *Nutr J* 2010, **9:**33.
34. Jeukendrup A: **Carbohydrate feeding during exercise.** In *European journal of Sport Science*, vol. 8. pp. 77-86. London: Taylor & Francis; 2008:77-86.

35. Rodriguez NR, Di Marco NM, Langley S, Association AD, Canada Do, Medicine ACoS: **American College of Sports Medicine position stand. Nutrition and athletic performance.** *Med Sci Sports Exerc* 2009, **41:**709-731.

36. Cermak NM, van Loon LJ: **The Use of Carbohydrates During Exercise as an Ergogenic Aid.** *Sports Med* 2013.

37. Jentjens RL, Moseley L, Waring RH, Harding LK, Jeukendrup AE: **Oxidation of combined ingestion of glucose and fructose during exercise.** *J Appl Physiol* 2004, **96:**1277-1284.

38. Jentjens RL, Shaw C, Birtles T, Waring RH, Harding LK, Jeukendrup AE: **Oxidation of combined ingestion of glucose and sucrose during exercise.** *Metabolism* 2005, **54:**610-618.

39. Jeukendrup AE: **Oral carbohydrate rinse: placebo or beneficial?** *Curr Sports Med Rep* 2013, **12:**222-227.

40. Painelli VS, Roschel H, Gualano B, Del-Favero S, Benatti FB, Ugrinowitsch C, Tricoli V, Lancha AH: **The effect of carbohydrate mouth rinse on maximal strength and strength endurance.** *Eur J Appl Physiol* 2011, **111:**2381-2386.

41. Chambers ES, Bridge MW, Jones DA: **Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity.** *J Physiol* 2009, **587:**1779-1794.

42. Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, Townsend RR, Keim NL, D'Alessio D, Havel PJ: **Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women.** *J Clin Endocrinol Metab* 2004, **89:**2963-2972.

43. Haff GG, Lehmkuhl MJ, McCoy LB, Stone MH: **Carbohydrate supplementation and resistance training.** *J Strength Cond Res* 2003, **17:**187-196.

44. Markkanen HM, Pekkarinen T, Välimäki MJ, Alfthan H, Hämäläinen E, Stenman UH: **Comparison of two growth hormone stimulation tests and their cut-off limits in healthy adults at an outpatient clinic.** *Growth Horm IGF Res* 2013, **23:**165-169.

45. de Sousa MV, Madsen K, Simões HG, Pereira RM, Negrão CE, Mendonça RZ, Takayama L, Fukui R, da Silva ME: **Effects of carbohydrate supplementation on**

competitive runners undergoing overload training followed by a session of intermittent exercise. *Eur J Appl Physiol* 2010, **109:**507-516.

46. Maughan R: **The athlete's diet: nutritional goals and dietary strategies.** *Proc Nutr Soc* 2002, **61:**87-96.

47. Phillips SM, Breen L, Watford M, Burke LM, Stear SJ, Castell LM: **A to Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance: part 32.** *Br J Sports Med* 2012, **46:**454-456.

48. Stark M, Lukaszuk J, Prawitz A, Salacinski A: **Protein timing and its effects on muscular hypertrophy and strength in individuals engaged in weight-training.** *J Int Soc Sports Nutr* 2012, **9:**54.

49. Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, Antonio J: **International Society of Sports Nutrition position stand: protein and exercise.** *J Int Soc Sports Nutr* 2007, **4:**8.

50. Phillips SM: **Dietary protein requirements and adaptive advantages in athletes.** *Br J Nutr* 2012, **108 Suppl 2:**S158-167.

51. Hulmi JJ, Lockwood CM, Stout JR: **Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein.** *Nutr Metab (Lond)* 2010, **7:**51.

52. Elliot TA, Cree MG, Sanford AP, Wolfe RR, Tipton KD: **Milk ingestion stimulates net muscle protein synthesis following resistance exercise.** *Med Sci Sports Exerc* 2006, **38:**667-674.

53. Sarbassov DD, Ali SM, Sabatini DM: **Growing roles for the mTOR pathway.** *Curr Opin Cell Biol* 2005, **17:**596-603.

54. Wullschleger S, Loewith R, Hall MN: **TOR signaling in growth and metabolism.** *Cell* 2006, **124:**471-484.

55. Churchward-Venne TA, Burd NA, Mitchell CJ, West DW, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM: **Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men.** *J Physiol* 2012, **590:**2751-2765.

56. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM: **Ingested protein dose response of muscle and**

albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 2009, **89:**161-168.

57. Wilson GJ, Wilson JM, Manninen AH: **Effects of beta-hydroxy-beta-methylbutyrate (HMB) on exercise performance and body composition across varying levels of age, sex, and training experience: A review.** *Nutr Metab (Lond)* 2008, **5:**1.

58. Portal S, Eliakim A, Nemet D, Halevy O, Zadik Z: **Effect of HMB supplementation on body composition, fitness, hormonal profile and muscle damage indices.** *J Pediatr Endocrinol Metab* 2010, **23:**641-650.

59. Wilson JM, Fitschen PJ, Campbell B, Wilson GJ, Zanchi N, Taylor L, Wilborn C, Kalman DS, Stout JR, Hoffman JR, et al: **International Society of Sports Nutrition Position Stand: beta-hydroxy-beta-methylbutyrate (HMB).** *J Int Soc Sports Nutr* 2013, **10:**6.

60. Fuller JC, Sharp RL, Angus HF, Baier SM, Rathmacher JA: **Free acid gel form of β-hydroxy-β-methylbutyrate (HMB) improves HMB clearance from plasma in human subjects compared with the calcium HMB salt.** *Br J Nutr* 2011, **105:**367-372.

61. Rowlands DS, Thomson JS: **Effects of beta-hydroxy-beta-methylbutyrate supplementation during resistance training on strength, body composition, and muscle damage in trained and untrained young men: a meta-analysis.** *J Strength Cond Res* 2009, **23:**836-846.

62. Palisin T, Stacy JJ: **Beta-hydroxy-beta-Methylbutyrate and its use in athletics.** *Curr Sports Med Rep* 2005, **4:**220-223.

63. Jówko E, Ostaszewski P, Jank M, Sacharuk J, Zieniewicz A, Wilczak J, Nissen S: **Creatine and beta-hydroxy-beta-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program.** *Nutrition* 2001, **17:**558-566.

64. Hoffman JR, Cooper J, Wendell M, Im J, Kang J: **Effects of beta-hydroxy beta-methylbutyrate on power performance and indices of muscle damage and stress during high-intensity training.** *J Strength Cond Res* 2004, **18:**747-752.

65. Clarkson PM, Hubal MJ: **Exercise-induced muscle damage in humans.** *Am J Phys Med Rehabil* 2002, **81:**S52-69.

66. Rebouche CJ: **Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism.** *Ann N Y Acad Sci* 2004, **1033:**30-41.

67. Flanagan JL, Simmons PA, Vehige J, Willcox MD, Garrett Q: **Role of carnitine in disease.** *Nutr Metab (Lond)* 2010, **7:**30.

68. Kraemer W, Volek J, Spiering B, Vingren J: **L-Carnitine Supplementation: A New Paradigm for its Role in Exercise.** *Chemical Monthly* 2005, **136:**1383–1390.

69. Eizadi M, Bakhshi S, Payman A, Khorshidi D: **Carnitine, Metabolism, Supplementation and Exercise Performance.** *Indian Journal of Fundamental and Applied Life Sciences* 2011, **1:**376-385.

70. Jeukendrup AE, Randell R: **Fat burners: nutrition supplements that increase fat metabolism.** *Obes Rev* 2011, **12:**841-851.

71. Vukovich MD, Costill DL, Fink WJ: **Carnitine supplementation: effect on muscle carnitine and glycogen content during exercise.** *Med Sci Sports Exerc* 1994, **26:**1122-1129.

72. Wall BT, Stephens FB, Constantin-Teodosiu D, Marimuthu K, Macdonald IA, Greenhaff PL: **Chronic oral ingestion of L-carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel metabolism during exercise in humans.** *J Physiol* 2011, **589:**963-973.

73. Kraemer WJ, Spiering BA, Volek JS, Ratamess NA, Sharman MJ, Rubin MR, French DN, Silvestre R, Hatfield DL, Van Heest JL, et al: **Androgenic responses to resistance exercise: effects of feeding and L-carnitine.** *Med Sci Sports Exerc* 2006, **38:**1288-1296.

74. Gleeson M: **Dosing and efficacy of glutamine supplementation in human exercise and sport training.** *J Nutr* 2008, **138:**2045S-2049S.

75. Antonio J, Sanders MS, Kalman D, Woodgate D, Street C: **The effects of high-dose glutamine ingestion on weightlifting performance.** *J Strength Cond Res* 2002, **16:**157-160.

76. Maughan RJ, King DS, Lea T: **Dietary supplements.** *J Sports Sci* 2004, **22:**95-113.

77. Rennie MJ, Ahmed A, Khogali SE, Low SY, Hundal HS, Taylor PM: **Glutamine metabolism and transport in skeletal muscle and heart and their clinical relevance.** *J Nutr* 1996, **126:**1142S-1149S.

78. Varnier M, Leese GP, Thompson J, Rennie MJ: **Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle.** *Am J Physiol* 1995, **269:**E309-315.

79. Bassini-Cameron A, Monteiro A, Gomes A, Werneck-de-Castro JP, Cameron L: **Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way.** *Br J Sports Med* 2008, **42:**260-266.

80. Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R, Newsholme EA: **Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system.** *Med Sci Sports Exerc* 1992, **24:**1353-1358.

81. Kargotich S, Rowbottom DG, Keast D, Goodman C, Dawson B, Morton AR: **Plasma glutamine changes after high-intensity exercise in elite male swimmers.** *Res Sports Med* 2005, **13:**7-21.

82. Walsh NP, Blannin AK, Bishop NC, Robson PJ, Gleeson M: **Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise.** *Int J Sport Nutr Exerc Metab* 2000, **10:**39-50.

83. Krieger JW, Crowe M, Blank SE: **Chronic glutamine supplementation increases nasal but not salivary IgA during 9 days of interval training.** *J Appl Physiol* 2004, **97:**585-591.

84. Low SY, Taylor PM, Rennie MJ: **Responses of glutamine transport in cultured rat skeletal muscle to osmotically induced changes in cell volume.** *J Physiol* 1996, **492 (Pt 3):**877-885.

85. Bruce M, Constantin-Teodosiu D, Greenhaff PL, Boobis LH, Williams C, Bowtell JL: **Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in human skeletal muscle.** *Am J Physiol Endocrinol Metab* 2001, **280:**E669-675.

86. Welbourne TC: **Increased plasma bicarbonate and growth hormone after an oral glutamine load.** *Am J Clin Nutr* 1995, **61:**1058-1061.

87. Candow DG, Chilibeck PD, Burke DG, Davison KS, Smith-Palmer T: **Effect of glutamine supplementation combined with resistance training in young adults.** *Eur J Appl Physiol* 2001, **86:**142-149.

88. Bowtell JL, Gelly K, Jackman ML, Patel A, Simeoni M, Rennie MJ: **Effect of oral glutamine on whole body carbohydrate storage during recovery from exhaustive exercise.** *J Appl Physiol* 1999, **86:**1770-1777.

89. Cooper R, Naclerio F, Allgrove J, Jimenez A: **Creatine supplementation with specific view to exercise/sports performance: an update.** *J Int Soc Sports Nutr* 2012, **9:**33.

90. Spillane M, Schoch R, Cooke M, Harvey T, Greenwood M, Kreider R, Willoughby DS: **The effects of creatine ethyl ester supplementation combined with heavy resistance training on body composition, muscle performance, and serum and muscle creatine levels.** *J Int Soc Sports Nutr* 2009, **6:**6.

91. Persky A, Brazeau G: **Clinical pharmacology of the dietary supplement creatine monohydrate.** *Pharmacol Rev* 2001, **53:**161-176.

92. Bemben M, Lamont H: **Creatine supplementation and exercise performance: recent findings.** *Sports Med* 2005, **35:**107-125.

93. Brosnan JT, da Silva RP, Brosnan ME: **The metabolic burden of creatine synthesis.** *Amino Acids* 2011, **40:**1325-1331.

94. Snow RJ, Murphy RM: **Creatine and the creatine transporter: a review.** *Mol Cell Biochem* 2001, **224:**169-181.

95. Snow RJ, Murphy RM: **Factors influencing creatine loading into human skeletal muscle.** *Exerc Sport Sci Rev* 2003, **31:**154-158.

96. Branch J: **Effect of creatine supplementation on body composition and performance: a meta-analysis.** *Int J Sport Nutr Exerc Metab* 2003, **13:**198-226.

97. Op 't Eijnde B, Urso B, Richter EA, Greenhaff PL, Hespel P: **Effect of oral creatine supplementation on human muscle GLUT4 protein content after immobilization.** *Diabetes* 2001, **50:**18-23.

98. Bassit RA, Pinheiro CH, Vitzel KF, Sproesser AJ, Silveira LR, Curi R: **Effect of short-term creatine supplementation on markers of skeletal muscle damage after strenuous contractile activity.** *Eur J Appl Physiol* 2010, **108:**945-955.

99. Lawler JM, Barnes WS, Wu G, Song W, Demaree S: **Direct antioxidant properties of creatine.** *Biochem Biophys Res Commun* 2002, **290:**47-52.

100. Casey A, Greenhaff P: **Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance?** *Am J Clin Nutr* 2000, **72:**607S-617S.

101. Sökmen B, Armstrong LE, Kraemer WJ, Casa DJ, Dias JC, Judelson DA, Maresh CM: **Caffeine use in sports: considerations for the athlete.** *J Strength Cond Res* 2008, **22:**978-986.

102. Doherty M, Smith PM: **Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis.** *Scand J Med Sci Sports* 2005, **15:**69-78.

103. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE: **Actions of caffeine in the brain with special reference to factors that contribute to its widespread use.** *Pharmacol Rev* 1999, **51:**83-133.

104. Bell DG, McLellan TM: **Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers.** *J Appl Physiol* 2002, **93:**1227-1234.

105. Duncan MJ, Oxford SW: **Acute caffeine ingestion enhances performance and dampens muscle pain following resistance exercise to failure.** *J Sports Med Phys Fitness* 2012, **52:**280-285.

106. Schneiker KT, Bishop D, Dawson B, Hackett LP: **Effects of caffeine on prolonged intermittent-sprint ability in team-sport athletes.** *Med Sci Sports Exerc* 2006, **38:**578-585.

107. Duncan MJ, Oxford SW: **The effect of caffeine ingestion on mood state and bench press performance to failure.** *J Strength Cond Res* 2011, **25:**178-185.

108. Giles GE, Mahoney CR, Brunyé TT, Gardony AL, Taylor HA, Kanarek RB: **Differential cognitive effects of energy drink ingredients: caffeine, taurine, and glucose.** *Pharmacol Biochem Behav* 2012, **102:**569-577.

109. Laurent D, Schneider KE, Prusaczyk WK, Franklin C, Vogel SM, Krssak M, Petersen KF, Goforth HW, Shulman GI: **Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise.** *J Clin Endocrinol Metab* 2000, **85:**2170-2175.

110. Herrmann-Frank A, Lüttgau HC, Stephenson DG: **Caffeine and excitation-contraction coupling in skeletal muscle: a stimulating story.** *J Muscle Res Cell Motil* 1999, **20:**223-237.

111. McPherson PS, Kim YK, Valdivia H, Knudson CM, Takekura H, Franzini-Armstrong C, Coronado R, Campbell KP: **The brain ryanodine receptor: a caffeine-sensitive calcium release channel.** *Neuron* 1991, **7:**17-25.

112. Castell LM, Burke LM, Stear SJ, McNaughton LR, Harris RC: **BJSM reviews: A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance Part 5.** *Br J Sports Med* 2010, **44:**77-78.

113. Ceglia L, Harris SS, Abrams SA, Rasmussen HM, Dallal GE, Dawson-Hughes B: **Potassium bicarbonate attenuates the urinary nitrogen excretion that accompanies an increase in dietary protein and may promote calcium absorption.** *J Clin Endocrinol Metab* 2009, **94:**645-653.

114. Burke LM: **Practical considerations for bicarbonate loading and sports performance.** *Nestle Nutr Inst Workshop Ser* 2013, **75:**15-26.

115. Carr BM, Webster MJ, Boyd JC, Hudson GM, Scheett TP: **Sodium bicarbonate supplementation improves hypertrophy-type resistance exercise performance.** *Eur J Appl Physiol* 2013, **113:**743-752.

116. Lemann J, Gray RW, Pleuss JA: **Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men.** *Kidney Int* 1989, **35:**688-695.

117. Majeed M, Badmaev V, Rajendran R: **Use of piperine to increase the bioavailability of nutritional compounds.** Google Patents; 1996.

118. Meghwal M, Goswami TK: **Piper nigrum and Piperine: An Update.** *Phytother Res* 2013, **27:**1121-1130.

119. Badmaev V, Majeed M, Prakash L: **Piperine derived from black pepper increases the plasma levels of coenzyme Q10 following oral supplementation.** *J Nutr Biochem* 2000, **11:**109-113.

120. Dudhatra GB, Mody SK, Awale MM, Patel HB, Modi CM, Kumar A, Kamani DR, Chauhan BN: **A comprehensive review on pharmacotherapeutics of herbal bioenhancers.** *ScientificWorldJournal* 2012, **2012:**637953.

121. Koleva II, van Beek TA, Soffers AE, Dusemund B, Rietjens IM: **Alkaloids in the human food chain--natural occurrence and possible adverse effects.** *Mol Nutr Food Res* 2012, **56:**30-52.

122. Bailey MM, Sturdivant J, Jernigan PL, Townsend MB, Bushman J, Ankareddi I, Rasco JF, Hood RD, Vincent JB: **Comparison of the potential for developmental toxicity of prenatal exposure to two dietary chromium supplements, chromium picolinate and [Cr3O(O2CCH2CH3)(6(H2O)3]+, in mice.** *Birth Defects Res B Dev Reprod Toxicol* 2008, **83:**27-31.

123. Lukaski HC, Bolonchuk WW, Siders WA, Milne DB: **Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men.** *Am J Clin Nutr* 1996, **63:**954-965.

124. Kaats GR, Blum K, Pullin D, Keith SC, Wood R: **A randomized, double-masked, placebo-controlled study of the effects of chromium picolinate supplementation on body composition: A replication and extension of a previous study.** *Current Therapeutic Research* 1998, **59:**379-388.

125. Chromiak JA, Smedley B, Carpenter W, Brown R, Koh YS, Lamberth JG, Joe LA, Abadie BR, Altorfer G: **Effect of a 10-week strength training program and recovery drink on body composition, muscular strength and endurance, and anaerobic power and capacity.** *Nutrition* 2004, **20:**420-427.

126. Schmitz SM, Hofheins JE, Lemieux R: **Nine weeks of supplementation with a multi-nutrient product augments gains in lean mass, strength, and muscular performance in resistance trained men.** *J Int Soc Sports Nutr* 2010, **7:**40.

127. Cribb PJ, Williams AD, Hayes A: **A creatine-protein-carbohydrate supplement enhances responses to resistance training.** *Med Sci Sports Exerc* 2007, **39:**1960-1968.

128. Wilson J, Wilson GJ: **Contemporary issues in protein requirements and consumption for resistance trained athletes.** *J Int Soc Sports Nutr* 2006, **3:**7-27.

129. Gant N, Ali A, Foskett A: **The influence of caffeine and carbohydrate coingestion on simulated soccer performance.** *Int J Sport Nutr Exerc Metab* 2010, **20:**191-197.

130. Roberts SP, Stokes KA, Trewartha G, Doyle J, Hogben P, Thompson D: **Effects of carbohydrate and caffeine ingestion on performance during a rugby union simulation protocol.** *J Sports Sci* 2010, **28:**833-842.

131. Van Nieuwenhoven MA, Brummer RM, Brouns F: **Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine.** *J Appl Physiol* 2000, **89:**1079-1085.

132. Yeo SE, Jentjens RL, Wallis GA, Jeukendrup AE: **Caffeine increases exogenous carbohydrate oxidation during exercise.** *J Appl Physiol* 2005, **99:**844-850.

133. Cooper R, Naclerio F, Allgrove J, Larumbe-Zabala E: **Effects of a carbohydrate and caffeine gel on intermittent sprint performance in recreationally trained males.** *Eur J Sport Sci* 2013.

134. Beck TW, Housh TJ, Johnson GO, Coburn JW, Malek MH, Cramer JT: **Effects of a drink containing creatine, amino acids, and protein combined with ten weeks of resistance training on body composition, strength, and anaerobic performance.** *J Strength Cond Res* 2007, **21:**100-104.

135. Cramer JT, Housh TJ, Johnson GO, Coburn JW, Stout JR: **Effects of a carbohydrate-, protein-, and ribose-containing repletion drink during eight weeks of endurance training on aerobic capacity, endurance performance, and body composition.** *J Strength Cond Res* 2012.

136. Kraemer WJ, Hatfield DL, Spiering BA, Vingren JL, Fragala MS, Ho JY, Volek JS, Anderson JM, Maresh CM: **Effects of a multi-nutrient supplement on exercise performance and hormonal responses to resistance exercise.** *Eur J Appl Physiol* 2007, **101:**637-646.

137. El Khoury D, Antoine-Jonville S: **Intake of Nutritional Supplements among People Exercising in Gyms in Beirut City.** *J Nutr Metab* 2012, **2012:**703490.

138. Goston JL, Correia MI: **Intake of nutritional supplements among people exercising in gyms and influencing factors.** *Nutrition* 2010, **26:**604-611.

139. Kreider RB, Wilborn CD, Taylor L, Campbell B, Almada AL, Collins R, Cooke M, Earnest CP, Greenwood M, Kalman DS, et al: **ISSN exercise & sport nutrition review: research & recommendations.** *J Int Soc Sports Nutr* 2010, **7:**7.

140. Willoughby DS, Stout JR, Wilborn CD: **Effects of resistance training and protein plus amino acid supplementation on muscle anabolic, mass, and strength.** *Amino Acids* 2007, **32:**467-477.

141. Smith AE, Fukuda DH, Kendall KL, Stou JR: **The effects of a pre-workout supplement containing caffeine, creatine, and amino acids during three weeks of high-intensity exercise on aerobic and anaerobic performance.** *J Int Soc Sports Nutr* 2010, **7:10 doi:10.1186/1550-2783-7-10**.

142. Hulmi JJ, Kovanen V, Selänne H, Kraemer WJ, Häkkinen K, Mero AA: **Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression.** *Amino Acids* 2009, **37:**297-308.

143. Volek J, Rawson E: **Scientific basis and practical aspects of creatine supplementation for athletes.** *Nutrition* 2004, **20:**609-614.

144. Roy BD, Fowles JR, Hill R, Tarnopolsky MA: **Macronutrient intake and whole body protein metabolism following resistance exercise.** *Med Sci Sports Exerc* 2000, **32:**1412-1418.

145. Panton LB, Rathmacher JA, Baier S, Nissen S: **Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (hmb) during resistance training.** *Nutrition* 2000, **16:**734-739.

146. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC, Jr.: **beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors.** *J Nutr* 2000, **130:**1937-1945.

147. Medicine ACoS: **American College of Sports Medicine position stand. Progression models in resistance training for healthy adults.** *Med Sci Sports Exerc* 2009, **41:**687-708.

148. Cribb PJ, Williams AD, Hayes A: **A creatine-Protein-Carbohydrate supplementation enhances responses to resistance training.** *Med Sci Sports Exc* 2007, **39:**1960-1968.

149. Schmitz SM, Hofheins JE, Lemieux R: **Nine weeks of supplementation with a multi-nutrient product augments gains in lean mass, strength, and muscular performance in resistance trained men.** *J Int Soc Sports Nutr* 2010, **7:**40.

150. Cribb PJ, Hayes A: **Effects of supplement timing and resistance exercise on skeletal muscle hypertrophy.** *Med Sci Sports Exerc* 2006, **38:**1918-1925.

151. Walsh AL, Gonzalez AM, Ratamess NA, Kang J, Hoffman JR: **Improved time to exhaustion following ingestion of the energy drink Amino Impact.** *J Int Soc Sports Nutr* 2010, **7:**14.

152. Steenge G, Simpson E, Greenhaff P: **Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans.** *J Appl Physiol* 2000, **89:**1165-1171.

153. Burke L: *Practical Sports Nutrition.* Human Kinetics; 2007.

154. ACSMps: **Nutrition and Athletes performance.** *Med Sci Sports Exerc* 2009, **41:**709-731.

155. Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, Antonio J: **International Society of Sports Nutrition position stand: protein and exercise.** *J Int Soc sports Nutr* 2007, **doi:10.1186/1550-2783-4-8:**4:8 .

156. Baechle TR, Eaerle RW, Wathen D: **Resistance Training, Chapter 18.** In *Essential of Strength Training and Conditioning (NSCA).* 2º edition. Edited by Baechle TR, Earle RW. Champaing IL: Human Kinetics.; 2000: 395-425.

157. Mazzetti S, Douglass M, Yocum A, Harber M: **Effect of explosive versus slow contractions and exercise intensity on energy expenditure.** *Med Sci Sports Exerc* 2007, **39:**1291-1301.

158. Dempster P, Aitkens S: **A new air displacement method for the determination of human body composition.** *Med Sci Sports Exerc* 1995, **27:**1692-1697.

159. Siri WE: **Body composition from fluid spaces and density: analysis of methods.** In *Techniques for measuring body composition* Edited by Brozek J, Henschel A, Washington DC: National Academeny of Sciences, National Research Council 1961: 223-244

160. Baechle TR, Earle RW, Wathen D: **Resistance training, chapter 15** In *Essentials of strength training and conditioning.* 3rd edition. Edited by Baechle TR, Eaerle RW: Human Kinetics; 2008: 381-412

161. Izquierdo M, Ibañez J, González-Badillo JJ, Häkkinen K, Ratamess NA, Kraemer WJ, French DN, Eslava J, Altadill A, Asiain X, Gorostiaga EM: **Differential effects of strength training leading to failure versus not to failure on hormonal responses, strength, and muscle power gains.** *J Appl Physiol* 2006, **100:**1647-1656.

162. Cohen J: ***Statistical power analysis for the behavioral sciences*.** 2nd edition. Mahwah, NJ: Lawrence Erlbaum; 1988.

163. Hopkins WG, Batterham AM: **Making meaningful inferences about magnitudes** *Sportsci* 2005, **9:**6-13.

164. Hopkins WG: **Measures of reliability in sports medicine and science.** *Sports Med* 2000, **30:**1-15.

165. Cribb PJ, Williams AD, Stathis CG, Carey MF, Hayes A: **Effects of whey isolate, creatine, and resistance training on muscle hypertrophy.** *Med Sci Sports Exerc* 2007, **39:**298-307.

166. Kraemer WJ, Adams K, Cafarelli E, Dudley GA, Dooly C, Feigenbaum MS, Fleck SJ, Franklin B, Fry AC, Hoffman JR, et al: **American College of Sports Medicine position stand. Progression models in resistance training for healthy adults.** *Med Sci Sports Exerc* 2002, **34:**364-380.

167. Kreider RB: **Effects of creatine supplementation on performance and training adaptations.** *Mol Cell Biochem* 2003, **244:**89-94.

168. Burke LM: **Caffeine and sports performance.** *Appl Physiol Nutr Metab* 2008, **33:**1319-1334.

169. Warren GL, Park ND, Maresca RD, McKibans KI, Millard-Stafford ML: **Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis.** *Med Sci Sports Exerc* 2010, **42:**1375-1387.

170. Graham TE: **Caffeine and exercise: metabolism, endurance and performance.** *Sports Med* 2001, **31:**785-807.

171. Magkos F, Kavouras SA: **Caffeine and ephedrine: physiological, metabolic and performance-enhancing effects.** *Sports Med* 2004, **34:**871-889.

172. Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA: **Central nervous system effects of caffeine and adenosine on fatigue.** *Am J Physiol Regul Integr Comp Physiol* 2003, **284:**R399-404.

173. Hespel P, Maughan RJ, Greenhaff PL: **Dietary supplements for football.** *J Sports Sci* 2006, **24:**749-761.

174. Cox GR, Desbrow B, Montgomery PG, Anderson ME, Bruce CR, Macrides TA, Martin DT, Moquin A, Roberts A, Hawley JA, Burke LM: **Effect of different protocols of caffeine intake on metabolism and endurance performance.** *J Appl Physiol* 2002, **93:**990-999.

175. Coyle EF, Hagberg JM, Hurley BF, Martin WH, Ehsani AA, Holloszy JO: **Carbohydrate feeding during prolonged strenuous exercise can delay fatigue.** *J Appl Physiol* 1983, **55:**230-235.

176. Hulston CJ, Jeukendrup AE: **Substrate metabolism and exercise performance with caffeine and carbohydrate intake.** *Med Sci Sports Exerc* 2008, **40:**2096-2104.

177. Pfeiffer B, Cotterill A, Grathwohl D, Stellingwerff T, Jeukendrup AE: **The effect of carbohydrate gels on gastrointestinal tolerance during a 16-km run.** *Int J Sport Nutr Exerc Metab* 2009, **19:**485-503.

178. Patterson SD, Gray SC: **Carbohydrate-gel supplementation and endurance performance during intermittent high-intensity shuttle running.** *Int J Sport Nutr Exerc Metab* 2007, **17:**445-455.

179. Phillips SM, Turner AP, Sanderson MF, Sproule J: **Carbohydrate gel ingestion significantly improves the intermittent endurance capacity, but not sprint performance, of adolescent team games players during a simulated team games protocol.** *Eur J Appl Physiol* 2011.

180. Conger SA, Warren GL, Hardy MA, Millard-Stafford ML: **Does caffeine added to carbohydrate provide additional ergogenic benefit for endurance?** *Int J Sport Nutr Exerc Metab* 2011, **21:**71-84.

181. Léger LA, Lambert J: **A maximal multistage 20-m shuttle run test to predict VO2 max.** *Eur J Appl Physiol Occup Physiol* 1982, **49:**1-12.

182. Nicholas CW, Nuttall FE, Williams C: **The Loughborough Intermittent Shuttle Test: a field test that simulates the activity pattern of soccer.** *J Sports Sci* 2000, **18:**97-104.

183. Dawson B: **Repeated-sprint ability: where are we?** *Int J Sports Physiol Perform* 2012, **7:**285-289.

184. Borg GA: **Psychophysical bases of perceived exertion.** *Med Sci Sports Exerc* 1982, **14:**377-381.

185. Astorino TA, Terzi MN, Roberson DW, Burnett TR: **Effect of caffeine intake on pain perception during high-intensity exercise.** *Int J Sport Nutr Exerc Metab* 2011, **21:**27-32.

186. Cureton KJ, Warren GL, Millard-Stafford ML, Wingo JE, Trilk J, Buyckx M: **Caffeinated sports drink: ergogenic effects and possible mechanisms.** *Int J Sport Nutr Exerc Metab* 2007, **17:**35-55.

187. Schoenfeld BJ: **The use of nonsteroidal anti-inflammatory drugs for exercise-induced muscle damage: implications for skeletal muscle development.** *Sports Med* 2012, **42:**1017-1028.

188. Friden J, Lieber RL: **Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components.** *Acta Physiol Scand* 2001, **171:**321-326.

189. Conchola EC, Thompson BJ, Smith DB: **Effects of neuromuscular fatigue on the electromechanical delay of the leg extensors and flexors in young and old men.** *Eur J Appl Physiol* 2013, **113:**2391-2399.

190. Peake JM, Suzuki K, Wilson G, Hordern M, Nosaka K, Mackinnon L, Coombes JS: **Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation.** *Med Sci Sports Exerc* 2005, **37:**737-745.

191. Thompson D, Nicholas CW, Williams C: **Muscular soreness following prolonged intermittent high-intensity shuttle running.** *J Sports Sci* 1999, **17:**387-395.

192. Twist C, Eston R: **The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance.** *Eur J Appl Physiol* 2005, **94:**5-6.

193. Ascensao A, Rebelo A, Oliveira E, Marques F, Pereira L, Magalhaes J: **Biochemical impact of a soccer match - analysis of oxidative stress and muscle damage markers throughout recovery.** *Clin Biochem* 2008, **41:**841-851.

194. Magalhaes J, Rebelo A, Oliveira E, Silva JR, Marques F, Ascensao A: **Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters.** *Eur J Appl Physiol* 2010, **108:**39-48.

195. Pedersen BK: **Muscles and their myokines.** *J Exp Biol* 2011, **214:**337-346.

196. Thiago Gomes H, Ludwig MS, Pizzato Scomazzon S, Homem de Bittencourt Jr PI: **The Role of Heat Shock Proteins in Skeletal Muscle chapter 5** In *Skeletal Muscle - From Myogenesis to Clinical Relations.* Edited by Cseri J. Novi Sad: InTech Prepress; 2012: 105-122

197. Leeder JD, van Someren KA, Gaze D, Jewell A, Deshmukh NI, Shah I, Barker J, Howatson G: **Recovery and Adaptation from Repeated Intermittent Sprint Exercise.** *Int J Sports Physiol Perform* 2013.

198. Howatson G, van Someren KA: **The prevention and treatment of exercise-induced muscle damage.** *Sports Med* 2008, **38:**483-503.

199. Kerksick C, Harvey T, Stout J, Campbell B, Wilborn C, Kreider R, Kalman D, Ziegenfuss T, Lopez H, Landis J, et al: **International Society of Sports Nutrition position stand: Nutrient timing.** *J Int Soc Sports Nutr* 2008, **Oct 3**.

200. de Sousa MV, Madsen K, Fukui R, Santos A, da Silva ME: **Carbohydrate supplementation delays DNA damage in elite runners during intensive microcycle training.** *Eur J Appl Physiol* 2012, **112:**493-500.

201. Costa RJS, Walters R, Bilzon JLJ, Walsh NP: **Effects of immediate postexercise carbohydrate ingestion with and without protein on neutrophil degranulation.** *Int J Sport Nutr Exerc Metab* 2011, **21:**205-213.

202. Cockburn E, Hayes PR, French DN, Stevenson E, St Clair Gibson A: **Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage.** *Appl Physiol Nutr Metab* 2008, **33:**775-783.

203. Cockburn E, Stevenson E, Hayes PR, Robson-Ansley P, Howatson G: **Effect of milk-based carbohydrate-protein supplement timing on the attenuation of exercise-induced muscle damage.** *Appl Physiol Nutr Metab* 2010, **35:**270-277.

204. Nieman DC, Bishop NC: **Nutritional strategies to counter stress to the immune system in athletes, with special reference to football.** *J Sports Sci* 2006, **24:**763-772.

205. Alghannam AF: **Carbohydrate-protein ingestion improves subsequent running capacity towards the end of a football-specific intermittent exercise.** *Appl Physiol Nutr Metab* 2011, **36:**748-757.

206. Spiering BA, Kraemer WJ, Hatfield DL, Vingren JL, Fragala MS, Ho JY, Thomas GA, Häkkinen K, Volek JS: **Effects Of L-Carnitine L-Tartrate Supplementation On Muscle Oxygenation Responses To Resistance Exercise.** *J Strength Cond Res* 2008, **22:**1130-1135.

207. Volek JS, Kraemer WJ, Rubin MR, Gómez AL, Ratamess NA, Gaynor P: **L-Carnitine L-tartrate supplementation favorably affects markers of recovery from exercise stress.** *Am J Physiol Endocrinol Metab* 2002, **Vol 282:**474-482.

208. Nicol C, Avela J, Komi PV: **The stretch-shortening cycle : a model to study naturally occurring neuromuscular fatigue.** *Sports Med* 2006, **36:**977-999.

209. Horder M, Jorgensen PJ, Hafkenscheid JC, Carstensen CA, Bachmann C, Bauer K, Neuwald C, Rosalki SB, Foo AY, Vogt W: **Creatine kinase determination: a European evaluation of the creatine kinase determination in serum, plasma and whole blood with the Reflotron system.** *Eur J Clin Chem Clin Biochem* 1991, **29:**691-696.

210. Cockburn E, Robson-Ansley P, Hayes PR, Stevenson E: **Effect of volume of milk consumed on the attenuation of exercise-induced muscle damage.** *Eur J Appl Physiol* 2012, **112:**3187-3194.

211. Moore RD, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM: **Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men.** *Am J Clin Nutr* 2009, **89:**161-168.

212. Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR, Phillips SM: **Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men.** *Appl Physiol Nutr Metab* 2007, **32:**1132-1138.

213. Brancaccio P, Lippi G, Maffulli N: **Biochemical markers of muscular damage.** *Clin Chem Lab Med* 2010, **48:**757-767.

214. Sorichter S, Mair J, Koller A, Calzolari C, Huonker M, Pau B, Puschendorf B: **Release of muscle proteins after downhill running in male and female subjects.** *Scand J Med Sci Sports* 2001, **11:**28-32.

215. Phillips MD, Mitchell JB, Currie-Elolf LM, Yellott RC, Hubing KA: **Influence of commonly employed resistance exercise protocols on circulating IL-6 and indices of insulin sensitivity.** *J Strength Cond Res* 2010, **24:**1091-1101.

216. Ostrowski K, Schjerling P, Pedersen BK: **Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise.** *Eur J Appl Physiol* 2000, **83:**512-515.

217. Pedersen BK, Febbraio MA: **Muscle as an endocrine organ: focus on muscle-derived interleukin-6.** *Physiol Rev* 2008, **88:**1379-1406.

218. Lancaster GI: **Exercise and cytokines, Chapter 10.** In *Immune function in sport and exercise.* Edited by Gleeson M. New York (2006): : Elsevier; 2006: 205-220

219. Norton LE, Layman DK, Bunpo P, Anthony TG, Brana DV, Garlick PJ: **The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats.** *J Nutr* 2009, **139:**1103-1109.

220. Breen L, Phillips SM: **Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance'of ageing.** *Nutr Metab (Lond)* 2011, **8:**2-11.

221. Reitelseder S, Agergaard J, Doessing S, Helmark IC, Lund P, Kristensen NB, Frystyk J, Flyvbjerg A, Schjerling P, van Hall G, et al: **Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion.** *Am J Physiol Endocrinol Metab* 2011, **300:**E231-242.

222. Rowlands DS, Rossler K, Thorp RM, Graham DF, Timmons BW, Stannard SR, Tarnopolsky MA: **Effect of dietary protein content during recovery from high-intensity cycling on subsequent performance and markers of stress, inflammation, and muscle damage in well-trained men.** *Appl Physiol Nutr Metab* 2008, **33:**39-51.

223. Roberts SP, Stokes KA, Trewartha G, Hogben P, Doyle J, Thompson D: **Effect of combined carbohydrate-protein ingestion on markers of recovery after simulated rugby union match-play.** *J Sports Sci* 2011, **29:**1253-1262.

224. Cockburn E, Bell PG, Stevenson E: **Effect of milk on team sport performance after exercise-induced muscle damage.** *Med Sci Sports Exerc* 2013, **45:**1585-1592.

225. Sasaki E, Umeda T, Takahashi I, Arata K, Yamamoto Y, Tanabe M, Oyamada K, Hashizume E, Nakaji S: **Effect of glutamine supplementation on neutrophil function in male judoists.** *Luminescence* 2013, **28:**442-449.

226. Spiering BA, Kraemer WJ, Vingren JL, Hatfield DL, Fragala MS, Ho JY, Maresh CM, Anderson JM, Volek JS: **Responses of criterion variables to different supplemental doses of L-carnitine L-tartrate.** *J Strength Cond Res* 2007, **21:**259-264.

227. Rebouche CJ: **Kinetics, pharmacokinetics, and regulation of l-carnitine and acetyl -carnitine metabolism.** *ANNals of the New York Academy of Sciences* 2004, **1033**.

228. Antonio J, Ciccone V: **The effects of pre versus post workout supplementation of creatine monohydrate on body composition and strength.** *J Int Soc Sports Nutr* 2013, **10:**36.

229. Volek J, Duncan N, Mazzetti S, Staron R, Putukian M, Gómez A, Pearson D, Fink W, Kraemer W: **Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training.** *Med Sci Sports Exerc* 1999, **31:**1147-1156.

230. Bemben MG, Witten MS, Carter JM, Eliot KA, Knehans AW, Bemben DA: **The effects of supplementation with creatine and protein on muscle strength**

following a traditional resistance training program in middle-aged and older men. *J Nutr Health Aging* 2010, **14:**155-159.

231. Newsholme P, Krause M, Newsholme EA, Stear SJ, Burke LM, Castell LM: **BJSM reviews: A to Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance--part 18.** *Br J Sports Med* 2011, **45:**230-232.

232. Cribb PJ, Williams AD, Hayes A: **A creatine-protein-carbohydrate supplement enhances responses to resistance training.** *Med Sci Sports Exerc* 2007, **39:**1960-1968.

233. Jones TW, Howatson G, Russell M, French DN: **PERFORMANCE AND NEUROMUSCULAR ADAPTATIONS FOLLOWING DIFFERING RATIOS OF CONCURRENT STRENGTH AND ENDURANCE TRAINING.** *J Strength Cond Res* 2013.

234. Dorling JL, Earnest CP: **Effect of carbohydrate mouth rinsing on multiple sprint performance.** *J Int Soc Sports Nutr* 2013, **10:**41.

Appendices

8.1 Appendix I – Participant information sheet: Effects of a creatine-carbohydrate-protein supplement on strength performance in recreationally trained young men

Research Information Sheet for Participants Involved in the University of Greenwich Nutritional supplement Study – The effect on exercise performance and body composition due to supplementation

Firstly thank you for agreeing to take part in this research project. I hope you find the information given here useful and adequately informative for your participation in the project. The research aims to monitor differences in performance and body composition due to supplementation with a commercially available product.

Procedures

You will initially attend the laboratory at the University of Greenwich at Medway campus for an explanation of the study and to familiarise yourself with the equipment used. This will take ~2 h and will involve:

- Assessing your suitability to the study and to complete the health questionnaire and informed consent form.
- Familiarising you with the exercise tests - lifts (to assess maximal strength and strength endurance) and Cybex testing machine (to assess concentric muscular force of the leg muscles).
- Assessing body composition
- To instruct basic nutrition, diet analysis and diet recording

.

The following day you will return to the laboratory to perform another set of familiarisations on the equipment used for testing. This session will take approximately ~1 h.

Two days later you will return to the laboratory for the first set of assessments. This will take ~1hr.

The following day you will again return to the laboratory for the second set of assessment. This will take ~1hr.

Following this you will then commence a standardised 12 week training programme consisting of 4 X ~1 h resistance training sessions per week. This will be performed independently of supervision.

After 6 weeks and 12 weeks of training you will attend the laboratory to perform the assessments again. There will be four of these sessions lasting ~1.5 h.

During the first 6 weeks and the final 6 weeks you will complete a 5 day diet diary (Monday – Friday).

<u>Measurements</u>

Measurements will be performed 3 times before, during (half way point) and at the end of the study:

- 1 repetition maximum (1RM) of 3 exercises (squat, deadlift and bench press) to assess muscle strength using the National Strength and Conditioning Association (NSCA) protocol (Found in Essentails of Strength Training and Conditioning by Baechle and Earle 2008)
- Number of repetitions performed at 60% of 1RM of 3 exercises (squat, deadlift and bench press) to assess strength endurance
- Cybex HUMAC isokinetic dynamometer will be used to assess concentric force of the leg muscles.
- Air-displacement plethysmography (BODPOD) will be used to assess body composition (body weight, lean body mass, fat mass, and fat mass percentage).
-

Requirements

On the days of testing, participants are requested not to consume caffeine-containing drinks, sports drinks or food four days prior to testing. Participants are instructed to abstain from any vigorous and unaccustomed physical activity 72 hours before testing with no exercise 24 hours before testing and arrive in the laboratory in a rested state. 500mL of water should be consumed during the 1 h prior to testing.

Possible Risks/Discomforts

The risks involved in the study are minimal. Prior to the study you will have filled out a health questionnaire to assess your suitability for participation in the study. This study does involve strenuous exercise, but providing you perform in regular physical activity the sensation you experience will not be unfamiliar. Further, you will be instructed to exercise until volitional fatigue.

You are free to withdraw from the study at any time.

Benefits

You will increase your knowledge of nutrition in relation to exercise, how to analyse and record your diet. You will also gain insight in to the effects of a structured resistance exercise programme on your performance and appearance.

Confidentiality

Your contact information and testing results (held anonymously) will be stored on a USB memory stick which will be stored in a secure location. This information will be deleted at the end of the study.

I look forward to seeing you, if there are any problems please do not hesitate to call me on the email or telephone number on the next page:

Investigator: Rob Cooper

Email; cr86@gre.ac.uk

Tel: 07531903224

8.2 Appendix II – Participant information sheet: Effects of a carbohydrate and caffeine gel (Viper Boost, Maxinutrition) on intermittent endurance exercise performance

Research Information Sheet for Participants Involved in the University of Greenwich Nutritional supplement Study – The effect carbohydrate and caffeine supplementation on exercise performance

Firstly thank you for agreeing to take part in this research project. I hope you find the information given here useful and adequately informative for your participation in the project. The research aims to monitor differences in exercise performance due to supplementation with commercially available products.

Procedures

Initial interview and bleep test – 40 minutes

Familiarisation session X 2 – ~30 minutes each

Intermittent endurance testing X 3 – 2.5 hours each

You will initially attend the laboratory at the University of Greenwich at Medway campus for an explanation of the study and to perform a bleep test. This will take around 40 minutes:

- Assessing your suitability to the study and to complete the health questionnaire and informed consent form
- Initial interview to discuss what you can expect from the project
- A bleep test

Two further visits of around 30 minutes each will be required to familiarise you with the exercise protocol in which you will perform 1 block of the exercise protocol.

You will then be required to attend the laboratory a further three times separated by around one week to perform the actual exercise protocol under one of 3 different supplement conditions;

- Condition 1; Viper Boost supplement – carbohydrate and caffeine
- Condition 2; Viper Active – carbohydrate
- Condition 3; calorie free placebo

Measurements

Measurements will be performed taken throughout the exercise protocol and during a 15 minute recovery period. The measurements are as follows:

- Every 15 meter sprint time
- Heart rate
- Rate of perceived exertion (RPE) scale
- Stomach comfort
- Blood lactate
- Saliva

Requirements

Four days before testing, participants are requested not to consume caffeine-containing drinks and foods. In addition no sports drinks or food three hours prior to testing, except for the standardised pre exercise meal. Participants are instructed to abstain from any vigorous and unaccustomed physical activity 72 hours before testing with no exercise 24 hours before testing and arrive in the laboratory in a rested state.

Possible Risks/Discomforts

The risks involved in the study are minimal. Prior to the study you will have filled out a health questionnaire to assess your suitability for participation in the study. This study does involve strenuous intermittent endurance exercise, but providing you perform in

regular physical activity the sensation you experience will not be unfamiliar. Furthermore, you will be instructed to exercise until volitional fatigue. Up to 300 mg of caffeine will be consumed in one of the experimental conditions. This amount of caffeine is suggested to be a moderate dose.

You are free to withdraw from the study at any time.

Benefits

You will increase your knowledge of nutritional supplementation in relation to exercise performance as well as information into your physical capabilities.

Confidentiality

Your contact information and testing results (held anonymously) will be stored on a USB memory stick which will be stored in a secure location. This information will be deleted at the end of the study.

I look forward to seeing you, if there are any problems please do not hesitate to email or telephone number below:

Investigator: Rob Cooper Supervisor: Dr Fernando Naclerio

Email: cr86@gre.ac.uk Email: f.j.naclerio@gre.ac.uk

Tel: 07531903224

8.3 Appendix III – Participant information sheet: Effects of a carbohydrate-protein multi-nutrient formula on acute recovery response from intermittent endurance activity

Research Information Sheet for Participants Involved in the University of Greenwich Nutritional supplement Study – Effects of a carbohydrate-protein multi-nutrient formula on the acute recovery response from intermittent endurance activity

Firstly, thank you for taking an interest in this research project. I hope you find the information given here useful and adequately informative for your participation in the project. The research aims to monitor differences in performance and recovery due to supplementation with the commercially available product 'Recovermax'. The primary ingredients in Recovermax are; whey protein, maltodextrin, dextrose, glutamine and carnitine.

Procedures

You will initially attend the laboratory at the University of Greenwich at Medway campus for an explanation of the study and a bleep test. This will take approximately 40 minutes and will involve:

- Assessing your suitability to the study and to complete the health questionnaire and informed consent form.
- A bleep test.
- This is also an opportunity for you to ask any questions that you may have. The following meeting, should you agree\be eligible to take part in the study, will see you return to the laboratory, on two occasions, to perform familiarisation sessions (on separate days) on the intermittent endurance exercise protocol and assessment procedures. You will then attend the laboratory again for a base line assessment (performance, saliva and blood sampling) and a single bout of

intermittent endurance exercise (duration ~90 minutes) followed by visit 24 hours after the end of the intermittent endurance exercise. Recovery (performance testing, saliva and blood sampling) will be assessed at the following time points post intermittent endurance exercise;

1. Immediately after exercise bout
2. 1 hour after exercise bout
3. 24 hours after exercise bout

The above will be repeated a further 2 times in order to assess performance and recovery under 3 conditions; once under the Recovermax condition, once under the carbohydrate condition and once under the placebo condition. Around 7 days should be left between testing sessions i.e. one testing session per week.

Measurements

Performance

15 metre acceleration test (sprint test)

Blood tests

Inflammation, Oxidative stress, muscle damage and Immune function

Requirements

On the day of testing a standardised meal should be consumed 2-3 hours prior to the exercise and up to 500mL of water. Eating and drinking should then be stopped until 1.5 hours after the intermittent endurance exercise test. Ergogenic aids (creatine, beta alanine etc) are to be stopped at least 6 weeks prior to the study and abstained from during the course of the study except for those provided by the researchers. Caffeine is to be avoided 24 hours prior to testing. Participants are instructed to abstain from any vigorous and unaccustomed physical activity 72 hours before testing with no exercise 24 hours before testing and arrive in the laboratory in a rested state. Furthermore, smokers are not considered eligible for this project.

Possible Risks/Discomforts

The risks involved in the study are minimal. Prior to the study you will have filled out a health questionnaire to assess your suitability for participation in the study. This study does involve strenuous exercise, but providing you perform in regular physical activity the sensation you experience will not be unfamiliar. Further, you will be instructed to exercise until volitional fatigue. All supplements contained in the commercially available multi-nutrient formula 'Recovermax' are dosed within recommended amounts.

You are free to withdraw from the study at any time.

Benefits

You will increase your knowledge of nutrition in relation to exercise knowledge of exercise, and the experimental process. Furthermore you will increase your knowledge of experimental protocols. You will also receive a cheque for £100 after completion of the study (cheques will take approximately 2 weeks to process).

Confidentiality

Your contact information and testing results (held anonymously) will be stored on a USB memory stick which will be stored in a secure location. This information will be deleted at the end of the study.

I look forward to seeing you, if there are any problems please do not hesitate to contact me on the email below:

Investigator: Rob Cooper

Email: cr86@gre.ac.uk

Supervisor 1: Dr Fernando Naclerio

Email: f.j.naclerio@gre.ac.uk

8.4 Appendix IV – Participant consent form

Participant Consent Form

To be completed by the participant	
1. I have read the information sheet about this study	YES/NO
2. I have had an opportunity to ask questions and discuss this study	YES/NO
3. I have received satisfactory answers to all my questions	
4. I have received enough information about this study	YES/NO
5. I understand that I am free to withdraw from this study:	YES/NO
• At any time	
• Without giving a reason for withdrawing	
• (If I am, or intend to become, a student at the University of Greenwich) without affecting my future with the University	YES/NO YES/NO
• Without affecting any medical or nursing care I may be receiving.	YES/NO
6. I agree to take part in this study	YES/NO
	YES/NO
Signed (participant)	Date
Name in block letters	
Signature of investigator	Date

This project is supervised by: Dr Fernando Naclerio
Contact details (including telephone number and e-mail address): f.j.naclerio@gre.ac.uk Alternatively telephone or email Rob Cooper: Cr86@gre.ac.uk 07531903224

8.5 Appendix V – Pre-test health and physical activity questionnaire

CONFIDENTIAL

Pre-test Health & Physical Activity Questionnaire

Date :	Sex : .....
Name:	Address: .....
D.O.B.	.....
Tel. No:	.....
Fax No:	.....
E. Mail:	Postcode: .....

Please circle when appropriate

1. Do you, or have you ever smoked? Yes/No

If yes please state the number/day ..... or when stopped .....

2. Do you drink alcohol regularly? Yes/No

If yes how many units/week? (1/2 pint = 1 units) .....

3. Have you consulted your general practitioner with the last 3 months?
Yes/No

If yes please give details .....

4. Are you on any medication at present? Yes/No

If yes please state which and for what .....

.....

5. When was the last time you had a medical check-up?.....

6. Have you ever suffered from:-

Any heart condition Yes/No

If yes please specify .....

High blood-pressures (>140/90) Yes/No Fainting Yes/No

Heart or chest pains Yes/No Anaemia Yes/No

Family history of heart of vascular disease Yes/No

If yes please specify .....

High blood cholesterol (>5.2mmol/L) if known Yes/No

Any blood condition Yes/No

If yes please specify .....

HIV, Hepatitis A, B or C, Venereal Disease, Haemophilia, Any other

Respiratory problems (asthma, bronchitis, etc.)

Yes/No

If yes please specify .....

Diabetes - NIDDM or IDDM (please circle)

Yes/No

Epilepsy

Yes/No

Cancer Yes/No

If yes please specify .....

7. Are you currently injured

Yes/No

If yes please specify .....

8. Have you been ill within the last 3 weeks?

Yes/No

If yes please specify .....

9. Have you ever “over-reached”, had overtraining syndrome

Yes/No

or chronic fatigue Syndrome?

If yes please specify .....

10. To your knowledge are there any health related reasons for not undergoing the tests that have been explained to you? Yes/No

If yes please specify .....

11. How many times do you exercise every week .....

12. Do you weight train? (Frequency –number of times per week?) .....

play games? (Frequency –number of times per week &which) .....

swim, run or cycle? (Frequency, which & how long for each time?) .....

13. Are you out of breath during exercise; (always?) 10----------5----------0 (never)

14. Are there any relevant factors? .....

.....

15. Height (metres) ..... Weight (kg) .....

Signature of participant : ..... Date .....

Signature of researcher: ..... Date .....

RISK ASSESSMENT (ACSM Guidelines)

No. of Cardiopulmonary signs / symptoms ……

No of Risk Factors ……

Recommendation ……

Assessors signature ……

8.6 Appendix VI –Diet diary record sheet

`

Name and Address

_____
_____
_____
_____
_____
_____

Food Record Diary

Please record everything that you eat and drink over a 3 day period.

Please bring this completed food diary record with you to testing sessions

Instructions for using the food diary

Everything that you eat and drink over a 3 day period should be recorded below. Please provide the weights and volumes of the food and drink. Many food packaging has the weight on the labelling so if you have half a pack, then record half the weight etc. This will enable the researcher to analyse your diet. Please record the cooking method (boiled/grilled/fried etc) and whether the food is fresh, canned, frozen or dried.

DATE:

Time	Description of food eaten	Cooking method	Weight/portion size/volume