Effect of various whey protein supplements on recovery from prolonged endurance exercise in trained cyclists

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Nutritional Science

at Massey University, Palmerston North,
New Zealand.

Dean Mathew Rankin

2005

ABSTRACT

Background:

Protein-containing recovery beverages are proposed to support an athlete's recovery from exercise through stimulation of insulin release, promoting the restoration of muscle glycogen stores, and stimulation of protein synthesis and muscle protein restoration.

Objective:

The present study aimed to determine, (1) whether there is an insulinotropic effect of whey proteins, when consumed in addition to carbohydrate, which is assumed to enhance muscle glycogen resynthesis and (2) whether a blend of hydrolysate and intact protein, when consumed in addition to carbohydrate, will enhance the athlete's recovery from exercise.

Design:

Twelve trained top level cyclists repeated a protocol on four consecutive weeks, during which either a control beverage (Carb) or three beverages containing whey protein (carbohydrate and intact protein (Carb + I); carbohydrate and protein hydrolysate (Carb + H; carbohydrate and intact protein : protein hydrolysate mix (Carb + M)) were consumed during recovery from exhaustive endurance exercise. The beverages were formulated to supply 1.2 g/kg/hour carbohydrate and 0.4 g/kg/hour protein. Subjects followed a controlled diet two days before each experimental day. On the experimental day the athletes each performed a glycogen-depleting exercise programme, then received the designated dietary beverage every 30 minutes for the first two hours post-exercise. The progress of recovery was monitored via the measurement of cardiovascular recovery, and the appearance and relative concentration of metabolites in blood (15 samples over a four hour period, obtained via an indwelling cannula) and urine samples (13 samples over a seven hour period) collected sequentially during the post-exercise recovery period.

Results:

Plasma albumin concentrations were significantly lower following consumption of beverages containing whey protein (Carb + H, $p<0.01$; Carb + M, $p<0.05$) compared to

that observed with the Carb beverage. Urine output was significantly higher after consumption of the Carb beverage than with any of the three-protein containing beverages (Carb + I, $p<0.01$; Carb + H, $p<0.05$; Carb + M, $p<0.05$) during the period of controlled fluid consumption. Heart rate recovery was found to be significantly greater following consumption of the three protein-containing beverages (Carb + I, $p<0.001$; Carb + M, $p<0.001$, Carb + H, $p<0.01$) than following consumption of the Carb beverage. The Carb + M beverage produced increased heart rate recovery ($p<0.001$) compared to that observed following consumption of the other two protein-containing beverages (Carb + I, Carb + H). Following correction of the data for haematocrit, to account for the hydration status of the athletes, a significant difference ($p<0.05$) in the ratio of plasma insulin to plasma glucose concentrations was found following consumption of any of beverages containing whey protein (Carb + I, Carb + H, Carb + M) compared to that observed for the Carb beverage. Consumption of the Carb + I beverage resulted in significantly higher concentrations of urinary nitrogen excretion as urea ($p<0.05$) and ammonia ($p<0.01$), and significantly higher plasma concentrations of the amino acids Valine, Leucine, Isoleucine, Phenylalanine, Tryptophan, and Tyrosine ($p<0.05$).

Conclusions:

The addition of whey protein to a carbohydrate-containing beverage stimulated enhanced recovery from exercise. A major factor in the improved recovery was increased rehydration following consumption of the protein-containing beverages, mainly due to the high sodium content of these beverages. This increased rehydration was shown to influence results for plasma insulin and plasma glucose concentrations where, after accounting for the hydration status of athletes, a difference between consumption of the Carb beverage and that observed for any of the three protein-containing beverages was observed. The results also allude to a potential benefit of protein hydrolysates over intact protein on protein recovery. Consuming a protein mix (Carb + M) also appears to improve heart rate recovery compared to consuming either intact (Carb + I) or hydrolysed (Carb + H) proteins individually. The results of this study highlight the importance of dietary protein on enhancing recovery from endurance exercise.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisors: Dr Alison Darragh (for her involvement right throughout this extensive project in all aspects of its planning, implementation, analysis and thesis writing); Dr Roger Lentle (for his involvement in the experimental trial, statistical analysis and thesis writing); and to Associate Professor Hugh Morton for his statistical help during the initial stages of the study. Their advice, guidance and encouragement throughout the study was excellent. I would also like to thank them for both their patience and their understanding of my other commitments outside of this thesis, which prolonged this project.

I would also like to thank Chris Booth for her time and efforts in the planning and implementation of this study, especially the time she put in during the experimental trial in the processing of samples. In addition, thanks also to Lena Lückel (from Germany) for volunteering her time and effort while in New Zealand to help out both prior to and during the experimental trial.

Thanks also to Fonterra Co-operative Group Ltd for the financial backing to allow this trial to occur. Thanks to all those at Fonterra who contributed their time and effort in supporting this study, especially Angela Rowan (for her continued support for the study both in gaining funding and overseeing it for Fonterra), Arron Harford (for his work with the proteins used in this study) and Roxanne Brassington (for her work in the masking of the dietary beverages).

Thanks must also go to all the subjects that participated in this trial. Their willingness and outstanding commitment to this trial to ensure its success was greatly appreciated. It was great privilege to have such highly talented and committed subjects on this trial.

I would also like to thank my current employer, Universal College of Learning (UCOL), for their assistance in allowing me to pursue post-graduate study and to the people there who, over the years, supported me during my studies.

And finally, a very special thanks to my wife, Paula, and two children, Dominic and Abby, for all of their patience, support and understanding throughout the entire duration of this study. Without your support, completion of this would not have been possible.

Approval for this research has been obtained from both the Massey University Ethics Committee and the Manawatu-Whanganui Ethics Committee for the experiments described in this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 3 RESULTS		Page Number
Figure 3.1.	Example graph of VO_2 output from a pre-experimental trial fitness test. The five-second interval for determining VO_{2peak} is highlighted on the right hand side of the graph. The result for this athlete was recorded at 4.73 L/min or 71.77 ml/kg/min.	73
Figure 3.2.	Mean (± SEM) haematocrit (%) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.	78
Figure 3.3.	Mean (± SEM) plasma albumin concentrations (g/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.	80
Figure 3.4.	Mean (± SEM) total urine volumes expressed as ml/kg BWT for the first 240 minutes (fluid intake controlled during this period) in a post-exercise recovery study. *Similar letter designations implies non-significance, non-similar implies significance ($p<0.05$).*	81
Figure 3.5.	Mean (± SEM) urine outputs (g/30minutes) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.	83
Figure 3.6.	Mean (± SEM) plasma insulin concentrations (mmol/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.	88
Figure 3.7.	Mean (± SEM) plasma glucose concentrations (mmol/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.	88
Figure 3.8.	Linear regression analysis results of mean post-exercise pulse rate recovery (bpm) (n=4900) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H), intact whey protein (Carb + I), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M) in a 240 minute period following exercise and recovery beverage consumption.	89

LIST OF ABBREVIATIONS

Carb	Carbohydrate Only
Carb + H	Carbohydrate + Whey Protein Hydrolysate
Carb + I	Carbohydrate + Intact Whey Protein
Carb + M	Carbohydrate + 50% Whey Protein Hydrolysate + 50% Intact Whey Protein
ACSM	American College of Sports Medicine
ADA	American Dietetic Association
ANP	Atrial Natriuretic Peptide
BWT	Body Weight
DEB	Dietary Electrolyte Balance
DM	Dry Matter
DOC	Dietitians of Canada
GLUT	Glucose Transporter Carrier Proteins
FAO	Food and Agriculture Organization of the United Nations
ISAK	International Society for the Advancement of Kinanthropometry
TCA	Tricarboxylic Acid
UNU	United Nations University
VO_{2peak}	Peak Oxygen Uptake
W_{max}	Maximum Power Output
WHO	World Health Organisation

GENERAL INTRODUCTION

Athletes around the world continually strive for that elusive edge that will give them victory over their fellow competitors. This search for the perfect performance is epitomised by the foundations of the modern Olympic Games and Olympism. The International Olympic Committee defines Olympism as…

> *"... a philosophy of life, exalting and combining in a balanced whole the qualities of body, will and mind. Blending sport with culture and education, Olympism seeks to create a way of life based on the joy found in effort, the educational value of good example and respect for universal fundamental ethical principles." (International Olympic Committee, 2004, p.9)*

An athlete's performance is influenced by a multitude of factors, ranging from their genetic makeup and their physical fitness, through to motivational levels and the desire to succeed. Nutrition is another such factor which, while a relatively new science in terms of sports nutrition, now has a strong focus within the sport science research community. With the commercialism of sport, athletes have a lot to gain from the spoils of victory, ranging from the personal satisfaction gained years of training and effort, through to financial rewards. This has led to the development of a 'win at all costs' attitude, which has often taken athletes down the path of performance enhancing ergogenic aids, many of which are banned by the International Olympic Committee and the World Anti-Doping Agency. With nutrition, however, there are many dietary factors that have the potential to provide the athlete with an ergogenic, or performance-enhancing effect. Dietary protein definitely falls into this category, and its potential has recently been further enhanced through the development of manufacturing processes which produce protein forms that are well suited to an athletes physiological needs. While protein intake is traditionally associated with strength-based athletes, it is now evident that the protein requirement of endurance athletes is also increased due to a combination of exercise-induced muscle damage and the use of protein as a fuel source.

Throughout history, athletes have trialled nutritional strategies, focused on dietary protein, that have resulted in both in enhancement and detriment of athletic performance. One of the most famous accounts of food consumption relates to Milos, a five-time Olympic Cretan wrestler champion (552-516 B.C.), who had a reported daily meat intake of 10kg per day (Maughan & Burke, 2000). Following the 1936 Berlin Olympics, it was stated that athletes frequently focused on the intake of meat and on average took in nearly half a kilogram of meat per day, with pre-event meals consisting of steak and eggs, supplemented with meat-juice (Schenk, 1936, cited in McArdle, Katch & Katch, 1999). This large consumption of protein by athletes, in the form of meat, has more recently been replaced with specially formulated protein supplements, which provide the athlete with a more convenient and healthier option.

Dietary protein supplementation is now common place both amongst athletes and the wider population, with a focus related to both exercise and weight loss. It also represents a major part of a world-wide, multi-million dollar supplement industry. In relation to athletes, there is a common belief that protein consumption has significant benefits on both performance and recovery from exercise. Yet uncertainty still remains with regard to the best form of dietary protein to use and when is the optimal time for its consumption (generally related to an exercise session). To date, nutritional research has been focused around both pre-exercise and during exercise nutritional strategies for maximising performance. However, due to the demands of modern sport, nutritional strategies focused on maximising recovery from exercise are of increased importance. It is during this post-exercise recovery period that the true benefits of protein consumption are becoming more evident. The potential benefit of protein to the recovering athlete relates to achieving maximal training adaptations, in addition to ensuring that exercise performance is not impaired due to any lingering effects from a previous exercise session.

There is still a lot to learn about the true extent that dietary protein intake may have on the recovering athlete. This study looks to extend the current knowledge related to the effect of dietary protein in exercise recovery, with a focus on the use of different forms of whey protein (intact and hydrolysed) on post-exercise recovery of endurance athletes.

Chapter 1

LITERATURE REVIEW

1.1 INTRODUCTION

There is no doubt that exercise places the human body under a large amount of stress. The demands of exercise necessitate maintenance of normal body homeostasis, while ensuring the working muscles receive an adequate blood supply. The nature of endurance exercise means that the body must try to maintain homeostasis over a prolonged period while the athlete pushes themselves to their physical limits. In the past, most research has focused on optimising nutritional practices before and during exercise with little emphasis placed on the post-exercise recovery phase. Due to the demands of modern sport, however, the importance of maximising an athlete's recovery from exercise is receiving greater recognition. Many athletes are in a situation where they compete in multiple events throughout the competitive season, in addition to the demands of their high training levels. The faster an athlete recovers from one bout of exercise the better able they will be to cope with subsequent exercise bouts. If an athlete does not complete their recovery they will find themselves beginning exercise in an already depleted state, which will limit athletic performance. Selection of an optimal nutrition programme for recovery will depend on the type of exercise (e.g. resistance, high intensity / anaerobic, or endurance exercise) they have been performing. The focus of this review will be on the metabolic and nutritional aspects of recovery from endurance exercise in man and nutritional strategies for maximising the rate of recovery.

1.2 NUTRITIONAL STRATEGIES DURING EXERCISE

In order to understand the important nutritional strategies relevant for an athlete's recovery it is necessary to understand the effect exercise has on an athlete's metabolism. For the purposes of this review, this will be discussed in three sections: Fluids and Electrolytes; Carbohydrate; and Protein.

1.2.1. FLUIDS & ELECTROLYTES

During exercise, the metabolic heat that is produced must be dissipated in order to prevent hyperthermia. Sweating is the major mechanism for heat loss, with as much as 75% of the heat lost during exercise achieved via this mechanism (Hargreaves, 2000). Sweat rates vary widely between individuals and between different modes of exercise, with environmental conditions also imparting a significant effect. Rates of sweating increase when either heat production is accelerated through an increase in metabolic rate, or heat loss via evaporation is decreased by a hotter, more humid or less windy environment (Dennis *et al.*, 1997). In hot, dry environments, evaporation of sweat accounts for more than 80% of metabolic heat loss (American College of Sports Medicine, American Dietetic Association & Dietitians of Canada, 2000[1]). It could be expected that an endurance cyclist may lose between 1.0-1.25 kg Body Weight (BWT)/hour under normal conditions, with endurance runners expecting to lose between 1.0-1.7 kg BWT/hour (Rehrer & Burke, 1996; Dennis *et al.*, 1997), with the majority of this weight loss being fluid lost through sweating. This is a significant decrease in body weight, especially as performance has been shown to decrease following fluid loss of approximately 1-2% loss of body weight (Sawka *et al.*, 2000). In the advanced stages of dehydration, an increase in serum sodium concentration and osmolality has been shown to cause a reduction in skin blood flow and sweating (Dennis *et al.*, 1997). Although fluid

[1] Herewith ACSM, ADA & DOC (2000).

consumption may limit the rise in serum osmolality and maintain sufficient skin blood flow for maximum heat loss, it will not prevent dehydration from occurring.

During endurance exercise, the athlete's requirement to replace fluids, along with maintaining a supply of glucose to the body, has led to the development of carbohydrate-based sports drinks. The general recommendation during exercise is for the carbohydrate content of any drinks consumed to be lower than 10% when water absorption is a priority (Coombes & Hamilton, 2000). The two major reasons for this relate to the effect carbohydrate content has on gastric emptying rate, and the osmolality of the drink. In a study by Noakes *et al.* (1991, cited in Coombe & Hamilton, 2000) it was reported that solutions containing up to 8% carbohydrate appear to have little effect on the rate of gastric emptying. Similarly, it is known that carbohydrate concentration influences the amount of water in the small intestine. Thus, when the carbohydrate concentration is greater than 10%, fluid enters the intestine from the vascular space (a phenomenon called reverse osmosis), which may promote dehydration (Gisolfi & Duchman, 1995; Maughan & Leiper, 1999). A negative correlation has been reported between osmolality of luminal contents and water absorption (Coombes & Hamilton, 2000). Hypertonic solutions (osmolality greater than that of body fluid) will result in a decrease in water absorption in the intestines with a greater secretion of water into the gastrointestinal lumen, which in turn may enhance the risk of dehydration. Therefore, both isotonic or hypotonic solutions can promote fluid absorption from the intestine. In the past, carbohydrate-based beverages usually contained a combination of glucose and fructose. The development of synthetic polymer maltodextrins, also known as glucose polymers, now allows for the manufacture of beverages with higher carbohydrate concentrations, because they allow carbohydrate content to be increased without a resultant increase in osmolality (Coombes & Hamilton, 2000). In a review by Jeukendrup & Jentjens (2000) of the oxidation rate of different types of carbohydrate during exercise it was evident that fructose

appeared to be oxidised at relatively low rates (approximately 25% lower oxidation compared with glucose) whereas glucose, sucrose, maltose, maltodextrin and soluble starch tended to be oxidised at relatively high rates during exercise (approximately 1g/min). Galactose was also stated as being oxidised at low rates similar to fructose. However, only one study, by Leijssen *et al.* (1995, cited by Jeukendrup & Jentjens, 2000) had been done on galactose at this time. Due to the different transport mechanisms available for different types of carbohydrates across the intestinal wall, Shi, *et al.* (1995, cited in Jeukendrup & Jentjens, 2000) suggested that the inclusion of different carbohydrates in a beverage may increase both water and carbohydrate absorption despite an increase in osmolality. Recent research has confirmed the use of carbohydrate mixes with the oxidation rates of either (1) a glucose/fructose mix (Jentjens, *et al.,* 2004a) or (2) a glucose/sucrose mix (Jentjens, *et a.,* 2004b) found to be higher (approximately 1.25-1.3 g/min), than that found following consumption of glucose alone.

The volume of the fluid consumed is also an important factor in determining the rate of gastric emptying, regardless of the composition of the ingested fluid (Maughan & Leiper, 1999). As the volume of fluid in the stomach increases, the stomach stretches up to a certain point. When maximal distension is reached, the pressure inside the stomach increases with the resultant rate of gastric emptying increasing initially and then decreasing as the volume is reduced (Jeukendrup & Gleeson, 2004). Therefore, a greater volume of fluid in the stomach may offset, to some degree, the effects on gastric emptying of consuming beverages with higher osmolality.

During endurance exercise there is also the potential for sodium losses through sweat to be significantly greater than the athlete's ability to replace it through food or fluid intake (Figure 1.1). The mechanism for this increased loss during exercise is that although sweat glands reabsorb sodium by active transport, their ability to reabsorb sodium does not increase in line with an increase in sweat

rate (Sawka *et al.*, 2000). Therefore, at high sweat rates the concentration of sodium in sweat increases. This has significant implications for the athlete because with its osmotic drive, sodium plays an important role in the distribution of fluids throughout the body.

The recommendation for athletes to replace large amounts of fluids during exercise has given rise to a new problem known as 'hyponatraemia' (also referred to as 'water intoxication'). Hyponatraemia is a disorder in fluid-electrolyte balance which results from an abnormally low plasma sodium concentration (Murray *et al.*, 2003). In general, a significant consumption of water, and potentially dilute sports drinks, can act to dilute the body's sodium concentration, a situation that may be potentially fatal as in the case of hyponatraemic encephalopathy (Noakes, 2003).

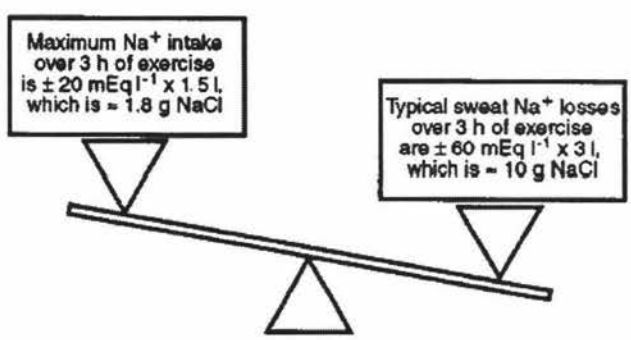

Figure 1.1. Estimated balance of Na^+ intake and Na^+ loss during 3 h of exercise at marathon pace. *Note:* 1 mmol of NaCl weighs 58.5 mg (Lambert, *et al*, 1997).

Despite the caution required when replacing fluids during exercise, the fact remains that consumption of fluids containing carbohydrate and sodium, as opposed to consuming solids, is vital as evidenced by the redistribution of

blood flow that occurs during exercise. During exercise, there is an increase in blood flow to the heart and working muscles, with a significant decrease to relatively inactive organs such as non-exercising muscles, skin, kidneys, and splanchnic organs (McAllister, 1998). Therefore, with this decrease in blood flow to the stomach and intestines, an athlete's ability to cope with concentrated fluids or solid foods decreases during exercise, and consumption of these may potentially lead to gastrointestinal distress. Splanchnic and renal blood flows have been found to be reduced in proportion to relative exercise intensity (McAllister, 1998). It appears, however, that splanchnic and renal blood flows were reduced by less during acute exercise following a period of endurance training (McAllister, 1998). Interestingly, this appears to suggest the adaptations to endurance training may also increase an athlete's tolerance to fluids and foods during exercise.

Following endurance exercise, the athlete will, to some extent, be in a dehydrated state. Correction of this dehydrated state must take into consideration the athlete's continued fluid loss following exercise and that sodium may also be required to aid in fluid absorption, fluid balance, and prevention of hyponatraemia. Inclusion of nutrients in a recovery beverage, namely carbohydrate and protein, is also important for stimulating carbohydrate and protein recovery as soon possible following cessation of exercise.

1.2.2. CARBOHYDRATE

The importance of carbohydrate as an energy source during endurance exercise is emphasised by the reported link between muscle glycogen depletion and early fatigue (Dennis *et al.*, 1997). In response to this, and the body's limited ability to store carbohydrates compared to fat and protein (Table 1.1), strategies have been developed to maximise carbohydrate stores and to ensure that carbohydrate supply for energy metabolism is maintained for longer during

endurance exercise. The body normally has sufficient energy stored as fat (as adipose tissue and within muscle) to exercise for extended periods of time (Table 1.1), meaning that the amount of energy stored as fat is not likely to be a liming factor in endurance exercise. One of the body's main adaptations to endurance exercise is an increased ability to utilise fat for energy and, therefore, preserve carbohydrate (Dennis, *et al.* (1997). This type of adaptation is beneficial due to the normally abundant stores of fat in our bodies and the potential sparing effect on carbohydrate use for energy during exercise.

Table 1.1. Endogenous fuel sources in man, (Lambert *et al.*, 1997).

	Approximate fuel reserve		Estimated days for which store would provide energy (*n*)		
Tissue fuel store	g	KJ	Starving	Walking	Running
Adipose Tissue	9000	337 000	34.00	10.80	3.85
Glycogen (liver)	90	1 500	0.15	0.05	0.02
Glycogen (muscle)	350	6 000	0.60	0.20	0.07
Triacylglycerol (muscle)	400	15 048	1.50	0.48	0.17
Glucose (blood)	20	320	0.03	0.01	>0.01
Protein	8800	150 000	15.00	4.80	1.29

'Carbohydrate loading' before exercise is designed to increase carbohydrate stores to levels above that normally present in an athlete through a combination of a high carbohydrate diet (>70% of total energy from carbohydrate) and the tapering of exercise in the days prior to competition. Maximising carbohydrate stores prior to exercise may increase time to fatigue by extending the time taken to deplete muscle glycogen stores during exercise. The ingestion of carbohydrates before and during exercise does increase overall reliance on carbohydrate oxidation, even at low exercise intensities (50-55% VO_{2peak}) (Lambert *et al.*, 1997). This could be counterproductive in reducing carbohydrate sparing, although this increase should be offset by the resultant

increase in carbohydrate stores and an increased ability to maintain exercise at higher intensities.

During exercise, the consumption of a carbohydrate-based sports drink, normally containing a glucose-maltodextrin mix, is used in order to maintain the supply of carbohydrates to the working muscle. Current recommendations are for endurance athletes to consume between 40-60 grams of carbohydrates per hour of exercise (ACSM, ADA & DOC, 2000).

Glucose can be stored as liver glycogen or as muscle glycogen (Table 1). The main role of liver glycogen is to maintain a stable blood glucose level, for once glucose enters the muscle it is effectively trapped through phosphorylation to glucose-6-phosphate (Jentjens & Jeukendrup, 2003). Glucose transport into skeletal muscle occurs primarily via facilitated diffusion using glucose transporter carrier proteins (GLUT). While there are different forms of this GLUT transporter, it appears that in the cell membrane of adult muscle fibres only the GLUT-4 isoform is expressed in significant levels, indicating its importance in glucose uptake (Gaster *et al.*, 2000). Translocation of GLUT4 transporters from the intracellular pool to the plasma membrane occurs in response to stimulation from insulin and/or muscle contraction (Ryder *et al.*, 2001). The effect of these two stimulating mechanisms on muscle glucose uptake appears to be additive. Carbohydrate ingestion during exercise decreases the conversion of liver glycogen to glucose, thus sparing liver glycogen and preventing the risk of hypoglycaemia occurring late in exercise as glycogen stores become depleted. However, it does not measurably slow the rate of muscle glycogen utilisation until muscle glycogen concentration falls below 70 $mmol.kg^{-1}$ (Lambert *et al.*, 1997). Sparing liver glycogen may aid in delaying the onset of fatigue due to the role of liver glycogen in maintaining blood glucose levels during the latter stages of endurance exercise, and as a result, also ensure an ongoing supply of glucose to the working muscle for energy.

During exercise, energy is produced from glucose via glycolysis. Carbohydrate may be involved in both anaerobic and aerobic metabolic pathways. In glycolysis, glucose-6-phosphate is either derived from glycogen or glucose and is broken down to lactate under anaerobic conditions and pyruvate under aerobic conditions (Maughan *et al.*, 1997). There is an inherent benefit in gaining glucose-6-phosphate from glycogen due to the conversion of glucose to glucose-6-phosphate requiring the use of an ATP molecule. The pyruvate produced from glycolysis can then be converted to Acetyl CoA and completely oxidised through the TCA cycle under aerobic metabolism. The interaction in metabolism of the three energy yielding substrates (carbohydrate, lipid and protein – Figure 1.2) shows that the hydrolysis of lipids or the catabolism of protein, mainly under aerobic conditions, are also important energy sources during exercise. While each of these energy substrates provides energy for the exercising muscle, the predominant use of one energy substrate during exercise will act to decrease the use of the other.

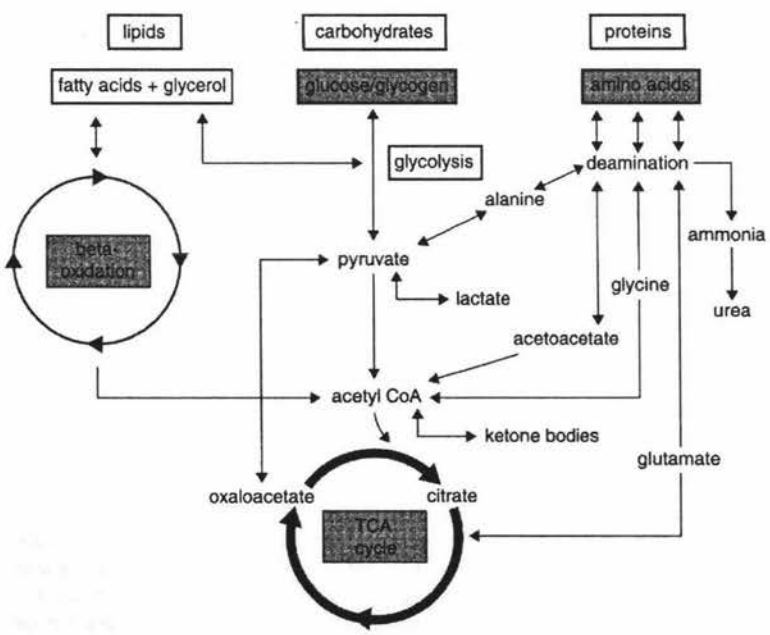

Figure 1.2. Summary of the main pathways of energy metabolism using carbohydrate, lipid, and protein as energy sources (Maughan *et al.*, 1997, p16).

During endurance exercise, the greater an athlete's ability to utilise fat at higher intensities of exercise the more muscle glycogen stores will be spared (Dennis *et al.*, 1997). Key adaptations to endurance exercise training act to increase the contribution of fat to oxidative energy metabolism, with a corresponding decrease in the contribution of carbohydrate, during submaximal exercise. Factors that may contribute to this adaptive response are increased density of mitochondria in skeletal muscle, proliferation of capillaries within skeletal muscle (enhances fatty acid delivery to muscle and oxygen supply for aerobic metabolism), an increase in carnitine transferase (facilitates uptake of fat into mitochondria), and an increase in fatty acid binding proteins to enhance fatty acid transport (Horowitz & Klein, 2001). In addition, the activity of lipid-mobilising and lipid-metabolising enzymes are also increased, such as the activity of lipoprotein lipase in the capillary endothelium of trained muscle and an enhanced capacity for beta-oxidation of free fatty acids within the mitochondria (Maughan, *et al.,* 1997). However, one of the main problems associated with the utilisation of lipid as a fuel source is not the physical availability of fat, but the rate at which it can be taken up and oxidised by the muscle. This limitation effectively means fat oxidation can only supply ATP at a rate sufficient to maintain exercise at an intensity of about 65% VO_{2max}, with fat oxidation being suppressed at higher intensities (Horowitz & Klein, 2000). In order to maintain exercise at higher intensities, carbohydrate must be utilised. Because of this, depletion of the body's carbohydrate stores will increase reliance on lipid to fulfil the energy demands of exercise, leading to a forced decrease in exercise intensity and athletic performance.

Carbohydrate intake before and during endurance exercise will aid in maintaining carbohydrate availability for the working muscle and as a result should support sustained exercise intensity. Regardless of what strategy the athlete employs before and during exercise, however, the athlete will usually complete their competition with depleted carbohydrate stores, making

carbohydrate replacement a key nutritional requirement during post-exercise recovery.

1.2.3. PROTEIN

During endurance exercise, there is an increase in the use of protein as an energy source, mainly towards the end of exercise when carbohydrate stores become depleted. It is estimated that protein may provide, through gluconeogenic pathways, between 3-6% of the total energy expenditure for contracting muscles, depending on the athlete's nutritional state (Hargraves & Snow, 2001). There may be cases where the energy contribution of amino acids is higher, perhaps up to 10%, when, for example, initial carbohydrate stores are low or when stores are depleted during exercise (Gibala, 2001).

The carbon skeletons of the glucogenic amino acids contribute to glucose synthesis through gluconeogenesis (synthesis of glucose from non-carbohydrate sources) (Maughan *et al.*, 1997). Amino acids can also be metabolised to either pyruvate or to intermediates of the TCA cycle and used for either glucose synthesis or metabolised for energy (Figure 1.3). The liver has the ability to oxidise most of the 20 amino acids while human skeletal muscle can only oxidise six, which are the three branched chain amino acids (leucine, isoleucine and valine), glutamate, aspartate and arginine (Figure 1.4). The involvement of amino acids in energy metabolism during exercise is not only as a direct fuel for energy production but also as a precursor for the synthesis of TCA-cycle intermediates and glutamine (Wagenmakers, 2000).

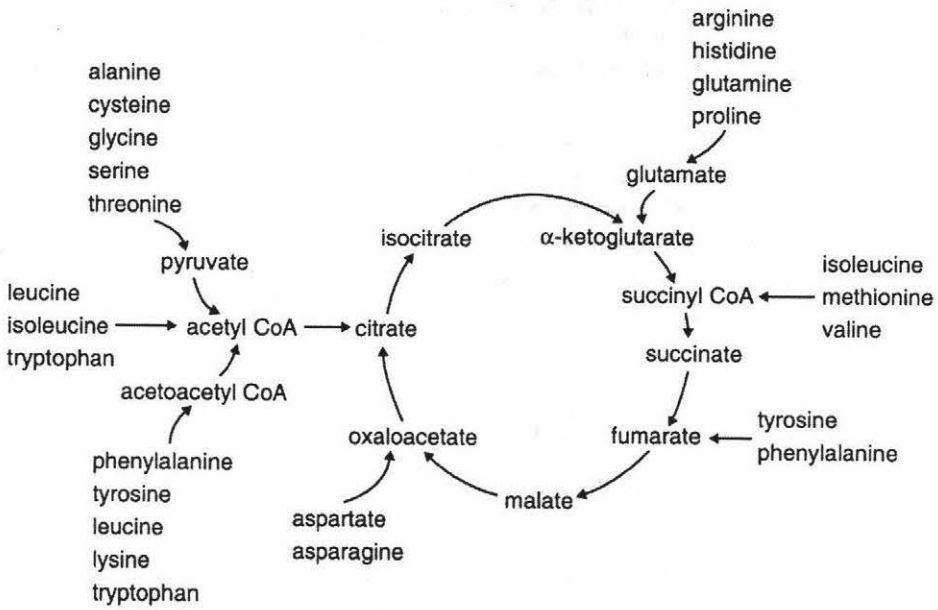

Figure 1.3. Amino acids are degraded to pyruvate, acetate, or to intermediates of the TCA cycle (Maughan *et al.*, 1997, p124).

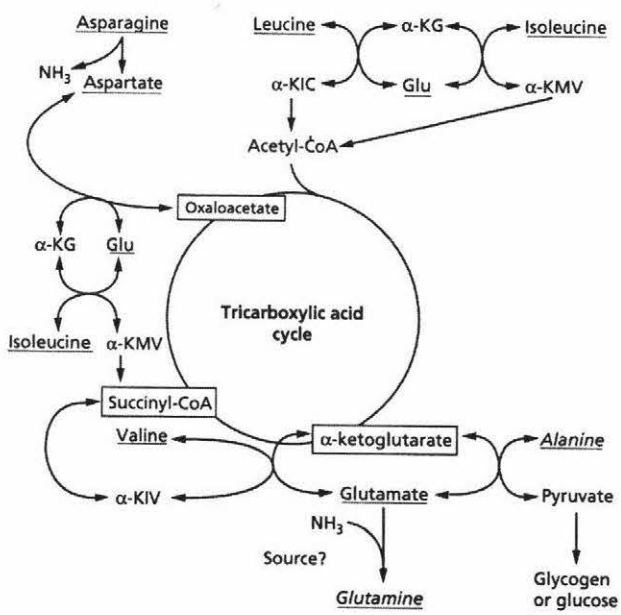

Figure 1.4. Amino acid metabolism in the muscle. (Wagenmakers, 2000, p120)..

A key consideration in the metabolism of amino acid carbon skeletons for energy is how the muscle metabolises the remaining nitrogen-containing amino groups. As shown in Figure 1.5, the amino group of all six amino acids within muscle can be removed from the muscle as either alanine or glutamine (Wagenmakers, 2000). Within the glucose-alanine cycle, pyruvate used for alanine production is derived from either glycolysis or other muscle protein derived amino acids. Alanine is then released into the blood and converted to glucose via gluconeogenesis in the liver. Formation of glutamine within the muscle also provides a mechanism for the muscle to maintain low ammonia levels. Ammonia is captured as amide nitrogen in glutamine and provides a non-toxic carrier of ammonia from the muscle, through the blood, to be excreted as urinary urea (Wagenmakers, 2000). The majority of the nitrogen resulting from degradation of amino acids ends up as urea, which is classed as metabolically inert, and can be excreted by the kidneys without altering the acid-base balance (Maughan, *et al.,* 1997).

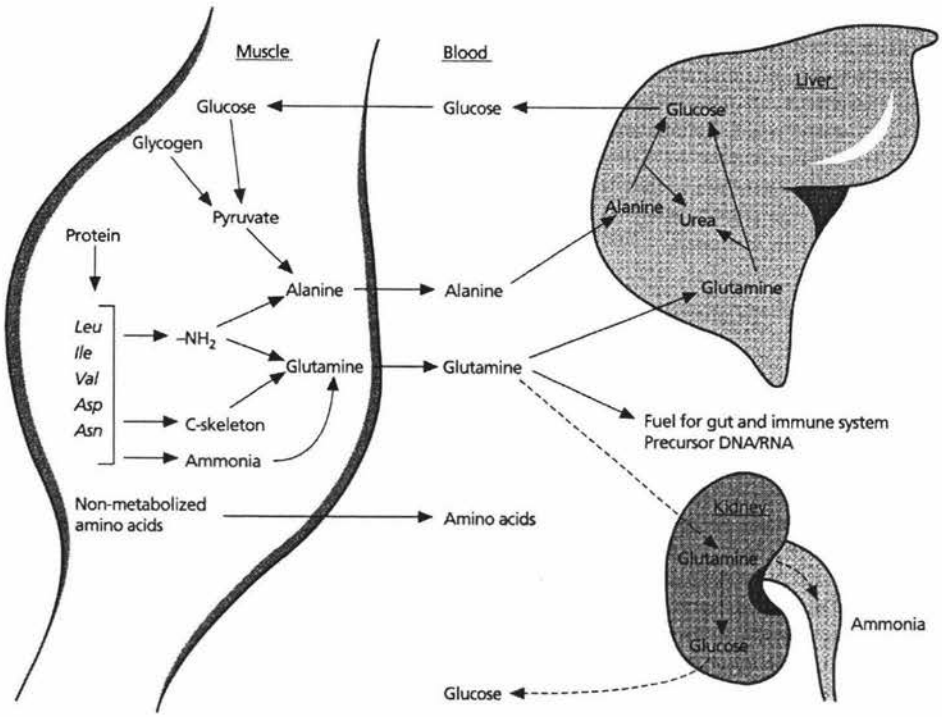

Figure 1.5. Interorgan relationship in the handling of amino acids. Dashed arrow, prolonged starvation only. (Wagenmakers, 2000, p122)..

The metabolism of protein for energy during endurance exercise is evidenced by an increased rate of branched-chain amino acid (leucine, isoleucine and valine) oxidation. When there is a reduced availability of glucose and free fatty acids in the blood during exercise, leucine oxidation increases markedly (Rennie & Tipton, 2000; Hargreaves & Snow, 2001). This suggests that although the use of leucine as a fuel source is important during exercise, its use can be suppressed by the use of other fuel sources (carbohydrate and lipids) if sufficient amounts of these other fuels are available.

The measurement of protein synthesis and protein breakdown during exercise is inherently difficult due to the dynamic nature of these metabolic processes, and literature available on the effect exercise has on protein metabolism is often

conflicting. If exercise is intense enough and/or long enough, there is some suggestion that protein synthesis will decrease and concurrently protein breakdown will increase during exercise (Rennie & Tipton, 2000). A reported increase in leucine oxidation during exercise, resulting in a negative net leucine balance, certainly suggests an increase in net protein breakdown (Tipton & Wolfe, 1998). While protein breakdown may increase during exercise it appears that urea production is not elevated, indicating that increased protein breakdown during exercise should provide a source of amino nitrogen for later use in protein synthesis (Tipton & Wolfe, 1998).

Supplying dietary protein during exercise is generally not recommended due to the demands placed on the gastrointestinal tract for its digestion and absorption. Thus an athlete competing endurance exercise is likely to be in a state of net protein breakdown. Minimising protein breakdown and stimulating protein synthesis through nutritional strategies will enhance the athlete's recovery following exercise.

1.3 NUTRITIONAL STRATEGIES POST-EXERCISE

A combination of substantial training outputs of endurance athletes, together with the demands of their intensive competition season, necessitates the maximisation of recovery to ensure the athlete can meet their future exercise demands. According to Burke (2000), nutrition-related issues for recovery from exercise include:

a. Replacement of fluid and electrolytes lost in sweat;
b. Restoration of muscle and liver glycogen stores;
c. Regeneration, repair and adaptation processes following the catabolic stress and damage caused by the exercise.

Fluid replacement following exercise is accepted as an important nutritional practice in order to correct for fluid lost during exercise. Post-exercise nutrition research has also extensively focused on carbohydrate replacement and its effect on the recovery of the athlete through muscle glycogen resynthesis. However, the importance of protein on the recovery of an athlete is now being recognised particularly for the athlete who is going to compete in the near future. Priority must be given to prompt restoration of circulating fluid volume and this may be a case for restoring circulatory fluid volume prior to intracellular fluid volume.

1.3.1. FLUIDS & ELECTROLYTES

Often fluid replacement in athletes is linked inextricably with carbohydrate replacement due to the effect carbohydrate intake can have on water absorption. Moreover, a recovery beverage containing carbohydrate will fulfil the athlete's requirement for recovery from dehydration and immediate replenishment of muscle and liver carbohydrate stores. As such it is difficult to discuss these as separate strategies. However, specifically for fluid replacement, the consumption of volumes equal to sweat losses has been found to result in only 50-70% rehydration, based on body weight restoration, over 2-4 hours of recovery (Burke, 2000). This finding may be partially due to sodium losses and the limited amount of sodium replaced, because sodium replacement is inherently linked to water replacement and rehydration. Nevertheless, it is recommended that athletes consume up to 150% of the weight loss during an exercise session in order to recover from losses through sweat and obligatory urine production (ACSM, ADA & DOC, 2000).

It is also recommended that the recovery beverage contains some form of sodium in order to replace losses incurred during exercise through sweating and to reduce the risk of hyponatraemia that may occur if only water is consumed. Sodium also aids the rehydration process by maintaining plasma osmolality and therefore the athlete's desire to drink (ACSM, ADA & DOC, 2000).

While it is important to replace fluids post-exercise especially as fluid loss continues after exercise has ceased, the risk of dehydration occurring is decreased due to reduced metabolic heat production and, as a result, a decreased activity of the mechanisms for thermostatic control, such as sweating. While increasing the carbohydrate content of a beverage (to greater than 10%) has been shown to increase carbohydrate availability, as discussed in previous section, it is generally not recommended during exercise as the higher carbohydrate content may decrease water absorption (Coombes & Hamilton,

2000). However, during the post-exercise recovery period, a beverage containing a higher carbohydrate concentration may be suitable due to the decreased risk of dehydration.

Decreased urine production occurs following exercise, most likely due to dehydration, but due to the redistribution of blood flow that occurs during exercise. During exercise, the magnitude of reduction in splanchnic and renal blood flows appears to be directly related to relative exercise intensity (McAllister, 1998). Renal sympathetic nerve activity may be a mechanism for increased angiotensin II through greater release of renin from the kidneys (McAllister, 1998). The result of these mechanisms would be a reduction in urine production during exercise, which continues during the early stages of recovery as the body redistributes blood flow and acts to maintain plasma volume. With the redistribution of blood flow back to the kidneys following exercise, possibly due to the reduced sympathetic drive, urine production will increase as waste products from energy and muscle metabolism are cleared from the body. The results of a study by Neumayr *et al.,* (2003) suggested that reduced renal perfusion is the mechanism responsible for slight impairment of renal function following exhaustive marathon cycling (in the absence of systemic dehydration and significant muscle damage). This seemed to be the result of stress-induced sympathetic overdrive responsible for the redistribution of blood flow during exercise. Neumayr *et al.,* (2003) also highlighted, however, that in well-hydrated athletes the high demands of marathon cycling only influenced renal function on a minimal scale.

1.3.2. CARBOHYDRATES

Replenishment of carbohydrate stores depleted during exercise occurs mainly via ingested carbohydrate, although there is some contribution from gluconeogenic pathways (Jentjens & Jeukendrup, 2003). As discussed previously, glucose transport into skeletal muscle primarily occurs via facilitated diffusion, in response to insulin or muscle contraction, using a GLUT4 isoform of the glucose transporter carrier proteins (Kuo *et al.*, 1999).

Supplying carbohydrates as part of a recovery beverage fulfils an athlete's requirement for the replacement of both energy and fluids. In terms of an athlete's ability to tolerate carbohydrates following exercise, the redistribution following exercise of blood flow back towards the splanchnic and renal organs will increase an athlete's ability to tolerate higher carbohydrate concentrations compared to during exercise. The inclusion of carbohydrates in a recovery beverage is important to ensure sufficient supply of glucose for muscle glycogen synthesis, via increased muscle glycogen synthesis rates following exercise. In a review by Jentjens & Jeukendrup (2003) low rates of muscle glycogen synthesis were observed when no carbohydrate was ingested after exercise (7-12 mmol/kg dry weight (dw)/h), whereas when a carbohydrate supplement is taken immediately following exercise the rate appears to be in the range of 20-50 mmol/kg dw/h.

The pattern of muscle glycogen synthesis following glycogen depleting exercise appears to occur in a biphasic manner with rapid and slow phases (Jentjens & Jeukendrup, 2003).

a) RAPID PHASE

This phase occurs immediately following exercise and lasts between 30-60 minutes (Jentjens & Jeukendrup, 2003; Price *et al.*, 2000). This phase proceeds independently of insulin and it is suggested that it only occurs when muscle glycogen is depleted and carbohydrate is supplied immediately following exercise. The rate of muscle glycogen resynthesis during this phase is approximately 12-30 mmol/L/h (Price *et al.*, 2000). (Note that unfortunately it is not possible to compare values for muscle glycogen resynthesis across studies due to unit differences, as the writer did not provide enough information to allow conversion).

A potential mechanism for this rapid phase is increased glycogen synthase activity immediately following exercise (Jentjens & Jeukendrup, 2003). Both muscular contraction and insulin have been shown to increase glycogen synthase activity. However, it now appears that muscle glycogen concentration is the more potent regulator of glycogen synthase activity, with an inverse relationship evident between muscle glycogen concentration and glucose transport stimulated by both insulin and muscular contraction (Jentjens & Jeukendrup, 2003).

With this phase depending on the availability of carbohydrate to the muscle, it is important that immediately following exercise the athlete consumes some form of carbohydrate that can be quickly absorbed and readily available for incorporation into the replenishing muscle glycogen stores.

b) SLOW PHASE

This phase of muscle glycogen synthesis occurs following the fast phase and requires the presence of both carbohydrate and elevated levels of insulin (Price *et al*, 2000; Jentjens & Jeukendrup, 2003). It is believed that this phase could last for several hours provided that carbohydrate supply is maintained, and is characterised by an increase in the cells' sensitivity to insulin, which in turn maintains ongoing glucose uptake and glycogen synthesis. The rate of muscle glycogen resynthesis during this phase has been observed at approximately 3 mmol/L/h (Price *et al.*, 2000).

With this phase depending mainly on the presence of raised insulin levels, the athlete must consume some form of carbohydrate to elicit such a response, in addition to providing a steady supply of glucose for incorporation into the replenishing muscle glycogen stores.

One way that the nutritional demands of both the fast and slow phases of muscle glycogen synthesis are met (i.e. a rapid supply of glucose and a high insulin response) is through the consumption of high glycaemic index carbohydrates. It is clearly evident that high muscle glycogen synthesis rates occur during the initial hours after exercise when a high glycaemic index carbohydrate is ingested (Jentjens & Jeukendrup, 2003). Consuming these high glycaemic index carbohydrates in liquid form, especially in the early period following cessation of exercise, may prove more effective than solid foods due to the faster gastric emptying and easier digestion of carbohydrate supplied in liquid form. The additional benefit of supplying carbohydrates in liquid form is that this will also aid in the athlete's rehydration following exercise due to the osmotic effect of carbohydrates increasing water absorption.

In order to maximise the rate of glycogen resynthesis immediately following exercise, the timing of consumption of carbohydrate is very important. In a study by Ivy *et al.,* (1988, cited in Levenhagen, *et al*, 2001) it was found that consumption of a 25% glucose polymer immediately following exercise dramatically increased the rate of glycogenesis. It was also found that the rate of glycogenesis was markedly decreased (by 45%) if ingestion of the glucose polymer was delayed by 2 hours. This finding is supported in a study by Levenhagen *et al.,* (2001), where it was shown that peak stimulation of whole body glucose utilisation and leg glucose uptake occurred within the first hour after exercise.

Research into the optimal amount of carbohydrate required to maximise muscle glycogen synthesis rates has produced conflicting results. In a recent review by Jentjens & Jeukendrup (2003) examining the amount of carbohydrate consumed and the resulting muscle glycogen synthesis rate (Figure 1.6), carbohydrate intakes of between 1.0-1.83 g/kg body weight (BWT)/h have led to very high glycogen synthesis rates when consumed at 15-60 minute intervals. From the literature it is reasonable to conclude that maximal glycogen synthesis rates occur at a carbohydrate intake of approximately 1.2 g/kg BWT/hour. In support of this conclusion, Van Loon *et al.,* (2000b) demonstrated that increasing the carbohydrate intake during recovery from 0.8g/kg BWT/hour to 1.2 g/kg BWT/hour resulted in a significantly greater plasma insulin response (46 ±18%) and significantly higher muscle glycogen synthesis (44.8 ±6.8 μmol glycosol units/g dry muscle wt/h compared with 16.6 ±7.8 μmol glycosol units/g dry muscle wt/h). It should be noted, however, that in this study no difference was found between consumption of 1.2 g/kg BWT/hour of carbohydrate and consumption of a mixture of 0.8 g/kg BWT/hour of carbohydrate and 0.4 g/kg BWT/hour of protein. In this latter study by Van Loon *et al.,* (2000b), carbohydrate supplements were provided at 30-minute intervals. Studies that have supplemented at longer (2 hour) intervals may not find the same association between muscle glycogen synthesis rates with

increasing carbohydrate intake because they may not adequately increase and maintain blood glucose and insulin levels over the entire period (Jentjens & Jeukendrup, 2003). The choice of study design is important to ensure that study conditions closely mimic current post-exercise nutritional strategies used by athletes. The use of more frequent ingestion of carbohydrate is one way of achieving this and will therefore increase the applications of the research in terms of presenting practical recommendations.

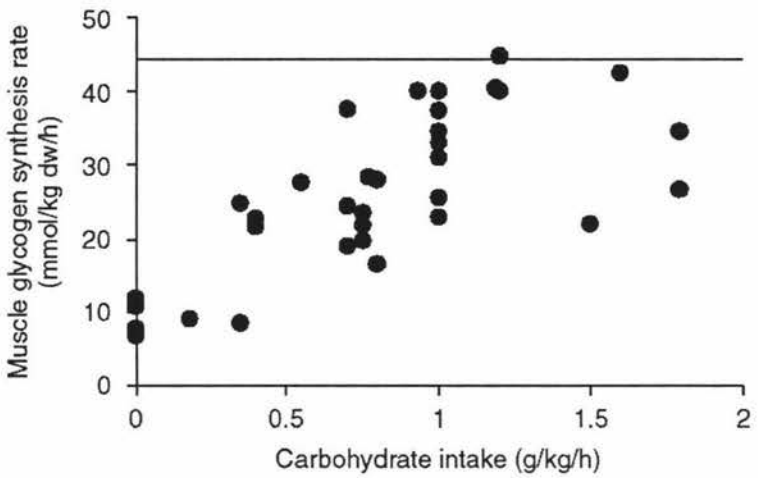

Figure 1.6. Muscle glycogen synthesis rates are depicted against the rate of carbohydrate ingestion. The horizontal line depicts the absolute maximum for muscle glycogen synthesis (Jentjens & Jeukendrup, 2003).

Carbohydrate intake following exercise may be of increased importance in cases where significant muscle damage has occurred during exercise. Muscle damage typically impairs the rate of post-exercise glycogen synthesis, especially following endurance exercise, where it has been proposed that this may be the result of decreased GLUT4 content in the muscle. However, in a study by Asp *et al.* (1997), it was found that following a marathon the GLUT4

content of muscle was unaltered. Further research on muscle damage and glucose uptake mechanisms by Aguila, *et al.* (2000) found that muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase and Akt-kinase which leads to decreased insulin-mediated glucose uptake. These results indicate that it is the signalling of GLUT4 translocation into the cell membrane that causes impaired muscle glycogen synthesis as a result of muscle damage. This decreased rate of glucose uptake by the muscle may be partially overcome, however, by increased carbohydrate intake following exercise (Burke, 2000). Therefore, it is important for athletes to use nutritional strategies to maximise both the fast and slow phases of muscle glycogen synthesis.

Protein is now also being included in exercise recovery beverages. It has been shown that the availability of amino acids to the muscle stimulates protein recovery (Tipton & Wolfe, 2001). Therefore, inclusion of protein in a recovery beverage may improve the athlete's recovery through the protein's synergistic effect on carbohydrate metabolism and a stimulatory effect on muscle recovery.

1.3.3. PROTEINS

Protein is now being included as part of an athlete's recovery for two main reasons: firstly, protein seems to have a stimulatory effect on insulin levels, thus improving uptake of glucose into the glycogen depleted muscle, and secondly, dietary protein may also have an anabolic effect on muscle by providing the essential substrate amino acids needed to support muscle protein synthesis.

1.3.3.1.PROTEIN AND THE INSULIN RESPONSE

The ingestion of protein, in addition to carbohydrate, following exercise has been suggested to accelerate muscle glycogen synthesis. The proposed mechanism is through an insulinotropic effect, eliciting an insulin response greater than that normally associated with ingestion of carbohydrate alone. There appears to be a link between insulin and protein metabolism which was highlighted in a study by Levenhagen *et al.,* (2001) where the rate of protein synthesis was increased during the same period that insulin responsiveness to glucose metabolism appeared to be increased. Another suggested mechanism for protein enhancing muscle glycogen recovery is that following exercise protein may provide the carbon skeletons necessary to facilitate a gluconeogenic supply of glucose, thereby enhancing muscle glycogen synthesis (Zawadzki *et al.,* 1992).

A number of studies have shown the insulinotropic effect of protein to be very significant, with one study by Van Loon *et al.,* (2000a), demonstrating an insulin response (from the intake of protein, in addition to carbohydrate) 100% greater than that found from ingestion of carbohydrate alone. This increased insulin response may be a potential mechanism for promoting increased glycogen synthesis rates in the fatigued muscle. This was demonstrated in another study by Van Loon *et al.,* (2000b) where glycogen synthesis rates were more than 113% higher than those found from the carbohydrate-only control group during a five hour post-exercise period. This finding also supported the earlier findings of Zawadzki *et al.*, (1992) which described an increased rate of glycogen synthesis following consumption of protein, in addition to carbohydrate, during the exercise recovery period. The study by Zawadzki *et al.,* (1992) showed that supplementation of protein alone was not enough to stimulate muscle glycogen synthesis, and that in this case the results did not differ from the response when no supplement was given. This finding aids in refuting the

argument that the higher glycogen synthesis rates observed when protein was consumed in addition to carbohydrate were the result of an increased gluconeogenic flux. Additionally, the higher concentration of insulin found in these studies would have acted to inhibit gluconeogenesis rather than stimulate it. The need for a recovery beverage containing carbohydrate and protein, as opposed to currently available sports drinks (containing carbohydrate only), was confirmed in a study by Williams *et al.,* (2003). In this study it was found that a beverage containing carbohydrate and protein (supplying ~0.8 g/kg of carbohydrate) resulted in a 17% greater plasma glucose response, a 92% greater insulin response and a 128% greater storage of muscle glycogen when compared with a 6% carbohydrate-electrolyte sports beverage (supplying ~0.3g/kg carbohydrate). It is common practice for athletes to use sports drinks (actually designed for consumption during exercise) following exercise, whereas the use of a recovery beverage would appear to be more beneficial.

Despite physiology providing logical support for the carbohydrate and protein mix in a post-exercise beverage, research on the effects of the co-ingestion of carbohydrate and protein on muscle glycogen synthesis have produced conflicting results (Table 1.2). This is highlighted by differences in the methodology of these studies, in terms of the type of protein used, the inclusion of additional amino acids and the timing of beverage intake, which may influence the resultant muscle glycogen synthesis rates. Despite this, one strong theory emerged suggesting that the increase in muscle glycogen synthesis rates associated with protein intake, in combination with carbohydrate, may have been due to maximal glycogen synthesis rates not being reached with a moderate (~0.8 g/kg/h) carbohydrate intake (Jentjens & Jeukendrup, 2003). One study, already mentioned, by Van Loon *et al.,* (2000b) appeared to support this suggestion, when an increase in the rate of carbohydrate intake from 0.8 - 1.2 g/kg BWT/h resulted in higher muscle glycogen synthesis rates.

When the effects of this higher intake of carbohydrate (1.2 g/kg/h) was examined, however, it was found that the addition of a protein-amino acid mixture did not increase muscle glycogen synthesis rates, despite a much higher insulin response (Jentjens *et al.*, 2001). This result supported the earlier research by Van Hall *et al.,* (2000) which concluded that when carbohydrate was ingested in sufficient quantities, the co-ingestion of carbohydrate and protein provided no additional increase in glycogen resynthesis rates. These findings appeared to suggest that insulin is not the limiting factor for muscle glycogen synthesis when total carbohydrate intake is high (1.0-1.2 g/kg/h) and that the availability of carbohydrate post-exercise plays a more important role (Van Loon *et al.*, 2000b).

More recently, however, a study by Ivy *et al.,* (2002) found that a carbohydrate and protein supplement resulted in higher muscle glycogen levels after four hours of exercise recovery compared with consumption of beverages containing either a high carbohydrate content (of equal energy value to the carbohydrate-protein beverage) or equal carbohydrate content without the protein. While no significant difference was found in the plasma insulin responses between treatments, the plasma glucose concentrations tended to be significantly lower in the carbohydrate-protein treatment. Although the amount of carbohydrate supplied in this study was low, it may suggest that protein has a role in the uptake of glucose into the muscle, above that seen from the consumption of carbohydrate alone.

Table 1.2. Summary of research examining the effect of the consumption of protein in addition to carbohydrate on insulin response and muscle glycogen resynthesis.

Reference	n	Carbohydrate g/kg/h	Protein g/kg/h	Protein Types Used	Time of Intake	Findings
Zawadzki *et al.*, 1992.	9	0.77 0.77	---- 0.28	---- Whey protein isolate	0, 120	▪ Plasma glucose concentration significantly higher for Carb only trial compared to Carb + Protein trial. ▪ Plasma insulin concentration significantly higher for Carb + Protein trial than Carb only trial. ▪ Rate of muscle glycogen storage significantly faster in Carb + Protein trial compared to Carb only trial.
Carrithers *et al.*, 2000	8	1.0 0.71 0.86	 0.20 0.14	---- Whey protein/Casein mix (included 0.09g/kg/h milk fat) Essential amino acids	0, 30, 60, 90, 120, 150, 180, 210	▪ No significant differences in muscle glycogen concentration, serum glucose or serum insulin between three trials.
Van Hall *et al.*, 2000a	5	1.25 1.25	---- 0.38	---- Whey protein hydrolysate	0 (Bolus 600m), Then 150ml/15min for 4h	▪ No significant difference in muscle glycogen resynthesis rate. ▪ Arterial insulin levels significantly higher in the Carb + Protein trial compared to the Carb only trial.
Van Hall *et al.*, 2000b	8	0.8 0.8 0.8	---- 0.3 0.3	---- Wheat protein hydrolysate Whey protein hydrolysate	0, 60, 120	▪ No significant difference in rate of muscle glycogen resynthesis. Rates were higher after ingestion of whey and wheat hydrolysate trials compared to control but not significant. ▪ Plasma insulin concentration significantly higher following ingestion of the whey and wheat hydrolysate trials compared to the control.

Table 1.2. *(continued)*)

Reference	n	Carbohydrate g/kg/h	Protein g/kg/h	Protein Types Used	Time of Intake	Findings
Van Loon *et al.*, 2000a	8	0.8 0.8 0.8 0.8 0.8	---- 0.4 0.4 0.4 0.4	---- Whey protein hydrolysate Wheat protein hydrolysate Pea protein hydrolysate Casein protein *(combinations of wheat hydrolysate and/or free amino acids also included)*	0, 30, 60, 90	▪ Insulin responses positively correlated with plasma leucine, phenylalanine and tyrosine concentrations. ▪ No significant difference between whey and wheat protein hydrolysates on insulin concentration. ▪ Intact protein ingestion resulted in lower plasma amino acid levels and an insulin response that was not significantly different from the control. ▪ Carb + Wheat protein hydrolysate with added free leucine + phenylalanine resulted in highest insulin concentration.
Van Loon *et al.*, 2000b	8	0.8 1.2 0.8	---- ---- 0.4	---- ---- Wheat hydrolysate	0, 30, 60, 90, 120, 150, 180, 210, 240, 270	▪ Plasma insulin levels and muscle glycogen synthesis rates were significantly higher in Carb + Protein and Carb (1.2g/kg/h) trials than Carb (0.8g/kg/h).. ▪ No significant differences between Carb+Protein and Carb (1.2g/kg/h) trials.
Van Loon *et al.*, 2000c	8	1.2 1.2 1.2	---- 0.2 0.4	---- Wheat protein hydrolysate Wheat protein hydrolysate *(combinations of wheat hydrolysate and free amino acids also included)*	0, 30, 60, 90, 120, 150	▪ Insulin responses positively correlated with plasma leucine, phenylalanine and tyrosine concentrations. ▪ Insulin response higher than Carb only and Carb + protein hydrolysate following ingestion of protein hydrolysate + added free leucine and phenylalanine (0.4g/kg/h protein = higher insulin response than 0.2g/kg/h)
Jentjens et al., 2001	8	1.2 1.2	---- 0.4	---- Wheat protein hydrolysate	0, 30, 60, 90, 120, 150	▪ Plasma insulin: Significantly higher in Carb + Protein than Carb trial. ▪ No significant differences for plasma glucose or muscle glycogen synthesis.

Table 1.2. (*continued*))

Reference	n	Carbohydrate g/kg/h	Protein g/kg/h	Protein Types Used	Time of Intake	Findings
Ivy et al., 2002	7	0.54 0.54 0.73	---- 0.19 ----	---- not stated ----	0, 120	▪ Muscle glycogen significantly higher in Carb + Protein trial compared to other two trials. ▪ No significant differences in plasma insulin levels. ▪ Plasma glucose levels lower following Carb + Protein trial compared to other two trials.

One possible explanation for the protein-carbohydrate synergistic effect may be that with muscle damage decreasing glucose absorption into muscle, the stimulatory effect of protein on muscle recovery following exercise may facilitate muscle cell repair and more specifically insulin-mediated glucose uptake mechanisms early during recovery, which will in turn promote greater glucose uptake and muscle glycogen synthesis.

A number of different types of protein have been studied to determine whether their insulinotropic effects are similar. It has been found that the percentage of amino acids that stimulate secretion of insulin is the same for both whey and casein proteins (Fruhbeck, 1998). However, the higher proportion of branched chain amino acids in whey protein may lead to a greater synergistic effect with insulin on protein metabolism compared to casein protein. In a study by Van Loon *et al.,* (2000a) the insulinotropic effects of protein hydrolysates (whey, wheat and pea) and intact protein (casein) were compared when provided in addition to carbohydrate in a recovery beverage, the composition of which is given in Table 1.3. The result for the intact protein (casein) was an insulin response that was not significantly different from that found with the control diet (carbohydrate only) and tended to be less than the responses observed after ingestion of the whey and wheat protein hydrolysates. After ingestion of intact protein, plasma amino acid responses over the two hour period tended to be lower than that observed following ingestion of the protein hydrolysates. The conclusion from this study was that the use of protein hydrolysates (whey or wheat) was preferred to stimulate insulin secretion because this results in a more rapid increase in plasma amino acid levels during a two hour period following exercise compared to that occurring after ingestion of the intact protein. Another finding of this study by Van Loon *et al.,* (2000a) was that insulin responses were positively correlated with plasma leucine, phenylalanine and tyrosine concentrations. Comparing the composition of the wheat and whey protein hydrolysates used in the study by Van Loon *et*

al., (2000a) (Table 1.3) highlights that the whey protein consists of a higher percentage of leucine, while wheat protein contains a higher proportion of phenylalanine, with the tyrosine levels being similar. The resultant insulin response of these two proteins may be similar, should these protein compositions be reflected in the plasma following consumption.

Table 1.3. Amino acid composition of hydrolysates of whey, pea and wheat protein, and intact casein protein (van Loon *et al.*, 2000a).

Amino acid	Whey	Pea	Wheat	Casein
		% by wt		
L-Alanine	4.7	3.8	1.8	3.1
L-Cysteine	1.2	0.4	0.9	0.4
L-Aspartate	5.4	4.4	0.2	3.7
L-Glutamate	9.1	7.4	3.2	11.2
L-Phenylalanine	2.4	3.2	4.8	5.4
L-Glycine	1.6	2.8	2.8	1.9
L-Histidine	1.6	1.7	1.6	3.2
L-Isoleucine	5.1	2.4	2.6	5.8
L-Lysine	8.4	5.9	—	8.3
L-Leucine	8.7	5.1	5.6	10.1
L-Methionine	1.3	0.6	1.1	3.0
L-Asparagine	4.4	3.8	1.9	3.7
L-Proline	5.9	2.8	12.3	10.5
L-Glutamine	7.4	6.6	29.0	11.2
L-Arginine	2.0	6.9	2.2	3.8
L-Serine	5.1	4.0	4.4	6.3
L-Threonine	6.6	2.8	2.0	4.6
L-Valine	4.5	2.7	3.0	7.4
L-Tryptophan	1.2	—	—	1.4
L-Tyrosine	2.3	2.6	2.5	5.8

It should be noted that the role of protein in an athlete's recovery from exercise is not limited to the effects of insulin on restoration of carbohydrate stores depleted through exercise. Insulin has also been identified as an important factor in protein metabolism (Kimball *et al.,* 2002). Acute

physiologic elevations in plasma insulin levels, especially during conditions of hyperaminoacidemia, result in an additional increase in net muscle protein anabolism *in vivo* in humans (van Loon *et al.*, 2000c). However, insulin should not be regarded as the primary regulator because in the absence of elevated amino acid concentrations, insulin only exerts a modest effect on muscle protein synthesis (van Loon *et al.*, 2000c).

1.3.3.2.PROTEIN AND PROTEIN SYNTHESIS / DEGRADATION

If an athlete can maximise protein synthesis and minimise protein degradation in the first few hours following exercise then this would provide a situation promoting muscle remodelling and potentially hypertrophy. With protein degradation elevated during exercise, which continues into the post-exercise phase, protein intake following exercise is essential in the stimulation of muscle protein recovery.

The metabolic basis for protein accretion from net muscle protein synthesis is a situation where protein synthesis exceeds protein breakdown over the given period in question (Tipton & Wolfe, 2004). It has been demonstrated that increased amino acid availability from exogenous amino acids elevates muscle protein synthesis and results in the maintenance of a positive net muscle protein balance following exercise (Tipton & Wolfe, 1998; Tipton & Wolfe, 2001). In the absence of food intake, the net response of protein metabolism to an acute bout of exercise remains negative (Rennie & Tipton, 2000). Supplying amino acids during the early post-exercise period, at a time when blood flow to the muscle is increased, appears to promote greater amino acid availability and to maximise the stimulation of protein synthesis (Sawka *et al.*, 2000).

The importance of amino acid availability on protein metabolism was also highlighted by Levenhagen *et al.*, (2001) where the effect of providing a supplement (containing a combination of protein (casein), carbohydrate and lipid) either immediately following exercise (EARLY) or three hours post-exercise (LATE) was examined. Supplementation immediately following exercise resulted in an increased rate of protein synthesis compared with when the supplement was supplied three hours later. The rate of leg protein synthesis was three times greater for EARLY than that found for LATE, while the whole body rate of protein synthesis was 12% greater with the supplement for EARLY compared with that found for LATE (Figure 1.7). There was no difference in the measurement for leg protein breakdown or whole body proteolysis between the two conditions (EARLY verses LATE). This study illustrates the importance of supplying amino acids immediately following exercise to stimulate protein recovery, with amino acid availability dictating the rate of protein synthesis during the recovery period. It also illustrates that protein synthesis is not only stimulated during the early stages of the recovery period, but protein synthesis also increased when protein was supplied three hours following exercise. Therefore, in order to maximise recovery following exercise, it is important to supply the athlete with protein during the early stages of recovery and to ensure a continued protein supply over at least the next three hours. This may potentially be achieved through the consumption of a combination of both protein hydrolysates and whole protein, because both these forms of protein would have varying rates of amino acid release during digestion.

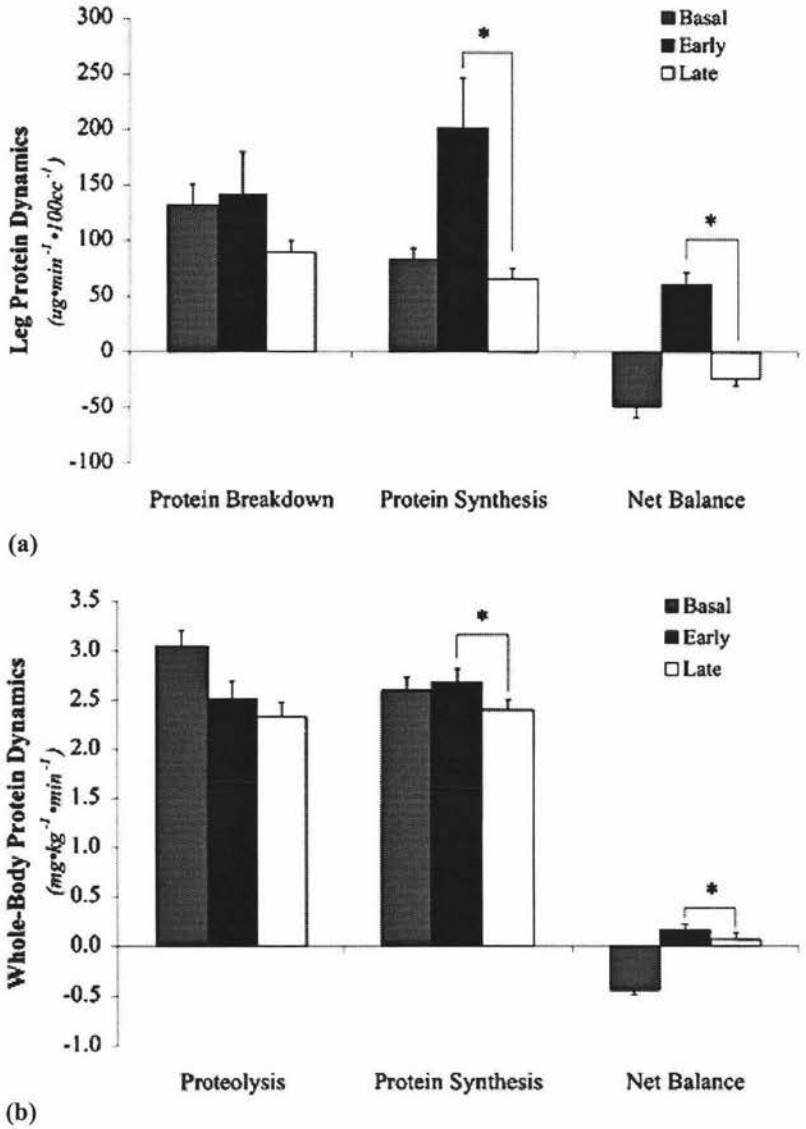

Figure 1.7. Rates of leg protein dynamics (a) and whole body protein dynamics (b) for 10 subjects given an oral nutrient supplementation either immediately after exercise (EARLY) or 3 h postexercise (LATE). * Significantly different ($p<0.05$), EARLY vs LATE (Levenhagen *et al.*, 2001).

Hyperaminoacidemia has been shown to enhance the rate of muscle protein synthesis both at rest and following exercise (Rennie & Tipton, 2000). However, in a study by Biolo *et al.,* (1997) it was found that infusion of an amino acid mixture after exercise enhanced the rate of muscle protein synthesis compared with the infusion of mixed amino acids at rest. This

finding, also supported in recent research by Tipton *et al.,* (2003), suggests an additive effect between the availability of amino acids and the effects of exercise on the muscle and that this early post-exercise effect (in response to consumption of essential amino acids) reflects the 24-hour post-exercise response. The study by Biolo *et al.*, (1997) also showed that during the period of hyperaminoacidemia after exercise the rate of muscle protein breakdown, which is normally elevated, did not rise. While this study examined the effect of the intravenous infusion of amino acids, it has also been shown that oral amino acid ingestion is effective in ensuring amino acid availability to the muscle following exercise (Rennie & Tipton, 2000). In a further study by Biolo *et al.,* (1999) the normal post-exercise stimulation of protein breakdown was ameliorated by local insulin infusion after exercise. Therefore, consumption of amino acids in addition to carbohydrates (insulin release stimulatory) may promote an optimal protein balance in the muscle following exercise.

The ability of dietary protein to have an effect on protein synthesis and degradation was highlighted in a study by Boirie *et al.,* (1997), which examined the effects of whey and casein on amino acid absorption and the resulting effects on protein metabolism. When subjects were given whey protein, it was found that the plasma appearance of dietary amino acids was fast, elevated and transient, a profile that was associated with an increase in protein synthesis with no change in protein degradation. When athletes were given the casein protein, it was found that the plasma appearance of dietary amino acids was slower, less elevated and more prolonged compared to that seen for the whey protein. This pattern was associated with a slight increase in protein synthesis, and a marked decrease in protein degradation. In this study by Boirie *et al.,* (1997) it was proposed that the differences were mainly the result of differences in the gastric emptying of these two proteins. This proposed difference in the gastric emptying, and therefore supply of amino acids, from different proteins, has lead to more focus on the

use of whey protein, often in the form of protein hydrolysates, as opposed to intact protein, as the preferred form of dietary protein for exercise recovery. As protein hydrolysates contain smaller amino acid chains (depending on the degree of hydrolysis), the absorption of amino acids from the protein may be faster as less digestion is required compared to the release of amino acids from intact protein. However, the effect of whole protein or hydrolysates of whole protein on protein synthesis and degradation remains unclear.

In nutrition research related to exercise recovery, the protein hydrolysates used are mainly derived from two major sources: whey protein and wheat protein. With whey protein containing the higher proportion of branched chain amino acids, the milk protein may have an advantage over wheat protein. Table 1.4 highlights the leucine and other branched chain amino acid contents of different proteins. While the availability of amino acids is a limiting factor for protein synthesis, it appears that the branched chain amino acid leucine, in particular, is involved in stimulating protein synthesis (Kimball *et al.*, 2002; Layman & Baum, 2004). The regulatory role of leucine on protein synthesis appears to depend on its intracellular concentration, and it is now known to involve regulation of the insulin signalling pathway. The site of leucine action is on the kinase mTOR (mammalian target of rapamycin) (Figure 1.8), which acts to stimulate rates of protein synthesis via two pathways: phosphorylation control of eIF4 translational initiating complex and from activation of S6 ribosomal protein (Layman & Baum, 2004). Parallel to the effects on protein synthesis, mTOR has been shown to potentially alter the insulin receptor signal via a stimulation of upstream phosphorylation of IRS-1 (insulin receptor substrate) (Layman & Baum, 2004). At rest, this down-regulation of the insulin signal did not affect glucose transport, potentially due to either (1) basal levels of GLUT4 transporters being adequate to maintain glucose transport or (2) that levels of GLUT1 (non-insulin dependent glucose

transporters) may play an important role maintaining baseline levels of glucose transport (Layman & Baum, 2004). It is unknown what effect this down-regulation of the insulin signal will have on glucose transport during exercise recovery.

Table 1.4.

Leucine and Branched Chain Amino Acid (BCAA) content of foods[1] (Layman & Baum, 2004).

	Leucine	BCAA
Whey protein isolate	14%	26%
Milk protein	10%	21%
Egg protein	8.5%	20%
Muscle protein	8%	18%
Soy protein	8%	18%
Wheat protein	7%	15%

[1]Values reflect g of amino acids/100g of proteion Source: USDA Food Composition Tables.

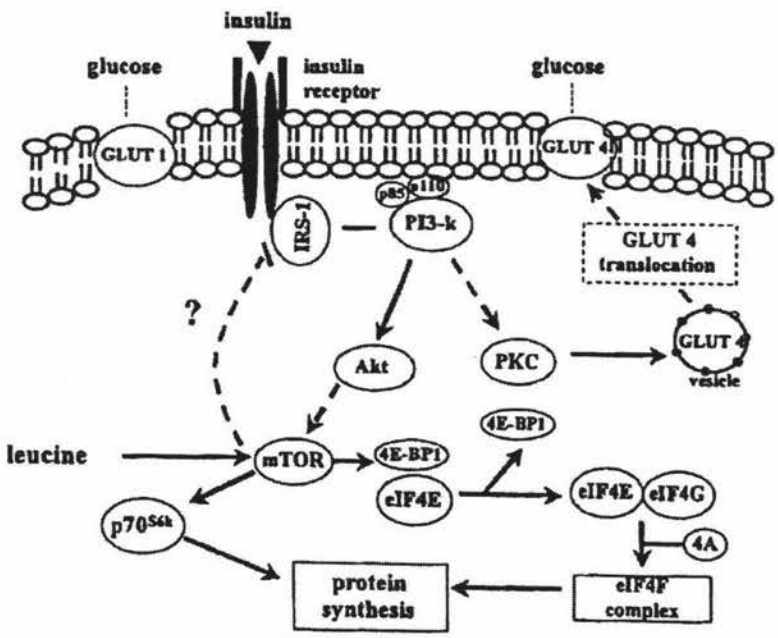

Figure 1.8. Insulin signalling cascade. GLUT1, insulin independent glucose transporter; PKC, protein kinase C; eIF, translational initiation factors. (Layman & Baum, 2004).

In order to gain the greatest response on muscle protein balance from the consumption of amino acids and carbohydrates, staggering the ingestion of these beverages may be beneficial, so that the responses are superimposed and therefore maximised (Tipton & Wolfe, 2004). Another potential benefit of whey protein over wheat protein relates to its respective essential amino acid composition. Net muscle protein synthesis appears to result from ingestion of essential amino acids only, which may act to stimulate muscle protein synthesis in two ways: (1) by supplying substrate for muscle protein synthesis and (2) acting as a regulatory factor (Tipton & Wolfe, 2004). From the composition of whey and wheat protein (Table 1.3) it can be seen that whey protein contains all of the essential amino acids, whereas wheat protein is limiting in the essential amino acids lysine and tryptophan. Because of this whey protein is classed as a 'complete protein' in that it contains all of the essential amino acids in sufficient amounts to match mans' protein requirements (FAO/WHO/UNU, 1991). Whey protein contains higher levels of all of the essential amino acids except for phenylalanine, which is greater in the wheat protein. Therefore, while the insulin stimulatory effects of these two proteins may be similar, support for protein synthesis may be greater with whey protein than with wheat because of the essential amino acid composition. The amounts of essential amino acid necessary to stimulate protein synthesis appears to be relatively small, with there also being a limit to the stimulatory effect of essential amino acids. Tipton *et al.,* (1999), demonstrated that net protein synthesis was similar when ~20g and 40g of essential amino acids were ingested after exercise.

Therefore, promoting the availability of amino acids to the muscle, thus ensuring maximal amino acid uptake into muscle following exercise, through the consumption of a protein in a form that is quickly digested and absorbed should promote a positive muscle protein balance (where the rate of protein synthesis exceeds protein degradation). Because of this, protein

hydrolysates are favoured for use in research to determine the effects of protein, when consumed together with carbohydrate, on muscle protein metabolism. The increased composition of essential amino acids in whey protein may give it an advantage over wheat protein as a recovery protein source due to the stimulatory effects of essential amino acids on muscle protein synthesis.

1.4 SUMMARY / HYPOTHESIS

Exercise results in the depletion of muscle glycogen stores as well as net protein breakdown of muscle protein. The consumption of a fluid beverage containing a combination of carbohydrate and protein during the early stages of recovery appears to be more effective in maximising both carbohydrate and protein recovery compared with the recovery achieved following consumption of a beverage containing only carbohydrate. Protein has been shown to enhance both carbohydrate and protein recovery through its additional insulintropic effects, while protein recovery is also enhanced by an increased amino acid availability to the exercised muscle. While research has focused on the use of protein hydrolysates during recovery, there may also be potential benefits arising from the consumption of whole protein. In addition the effect of a combination of whole protein and hydrolysed protein on protein metabolism following exercise remains unclear.

Therefore, the present study aims to determine (1) whether there is an insulinotropic effect of milk proteins, when consumed in addition to carbohydrate, which is assumed to enhance muscle glycogen resynthesis and (2) whether a blend of hydrolysate and intact protein, when consumed in addition to carbohydrate, will enhance the athlete's recovery from exercise.

Chapter 2

METHODS

2.1.Subjects

Twelve trained top level cyclists volunteered and signed consent forms for this study examining the effects of dietary protein, in addition to carbohydrate, on metabolic recovery from exercise. Both the Massey University Ethics Committee (PN Protocol – 02/77) and the Manawatu-Whanganui Ethics Committee (Ethics Register: 19/02) approved this study. Subjects were recruited based on the following exclusion criteria:

(1) Not currently taking any performance enhancing substances,
(2) No known allergy or intolerance to milk, and
(3) No heart disease, diabetes or uncontrolled asthma.

The subjects also need to be in a competitive phase of training to meet a desired fitness level.

2.1.1. Screening of Subjects

All cyclists were pre-screened by a medical doctor involved in the trial to ensure they did not meet any of the exclusion criteria. This also involved measurement of resting blood pressure and a discussion of the subject's health history. Prior to inclusion in the trial, subjects were handed a list of potential performance enhancing substances (Table 2.1) that may effect their carbohydrate or protein metabolism and asked to confirm that they were not currently taking any of these substances prior to inclusion in this trial.

Table 2.1.

List of proposed performance enhancing substances that have the potential to influence a subject's carbohydrate and / or protein metabolism if used prior to or during a post-exercise recovery study.

Anabolic Steroids	Vanadyl Sulphate
Growth Hormone	Beta-2 agonists
Erythropoietin (EPO) or an equivalent drug	Androstenedione
Testosterone precursors or derivatives	Nandrolone
Beta-Blockers	Glucocorticosteroids
Ephedrine / Pseudoephedrine	IGF-1
Cholesterol Lowering Drugs	Narcotics
DHEA	Diuretics
Insulin	Tribulus Terrestris

2.2. Experimental Design

Cyclists were exercised to exhaustion to elicit a state of assumed glycogen depletion, then given a recovery beverage at timed intervals, with parameters of recovery monitored over an eight-hour period following exercise. Four test dietary beverages were investigated in this double-blind, cross-over designed study. Each subject repeated a seven-day experimental period during four consecutive weeks, with a different beverage tested in each experimental period. Details of the four dietary beverages are given in a later section.

2.2.1. Pre-Experimental Period

2.2.1.1. Determination of Peak Oxygen Uptake and Maximum Power Output

In the week prior to the commencement of the study subjects reported to the laboratory for their pre-experimental trial fitness testing. This involved determination of Peak Oxygen Uptake (VO_{2peak}) and Maximum Power Output (W_{max}). VO_{2peak} was measured via indirect calorimetry using the Metamax® 3B protable gas analyser (Cortex, Germany) and an incremental exercise

programme performed on the Kingcycle Trainer (Kingcycle, Buckinghamshire, UK) with the use of the subjects' own cycles.

Each subject's weight was measured prior to the maximal exercise test. The weight recorded at this time was used to determine the beverage and water quantities to be consumed on each of the four experimental trial days.

Following calibration of the subject's own cycle on the Kingcycle Trainer, subjects stretched, then warmed up for 10 minutes at 100 Watts. The starting power output was set at 200 Watts with a 33 Watts per minute continuous increase. Criteria for attainment of VO_{2peak} and W_{max} were:

(1) Attainment of maximum heart rate (estimated at 220 – age);
(2) Respiratory Exchange Ratio (RER) ≥ 1.10;
(3) An increase in VO_2 less than 0.2 L/min over one minute; and
(4) Volitional fatigue.

Following completion of the exercise test, subjects warmed down for 10 minutes at 100 Watts. VO_{2peak} was calculated as the greatest mean VO_2 over a five-second interval. The five-second interval was chosen, as opposed to one of longer duration, due to the continuous incremental nature of the test. W_{max} was calculated on the Kingcycle Trainer software (v10.0, 2002, Kingcycle, Buckinghamshire, UK) as the maximum power output attained during the test.

2.2.1.2. Determination of Body Composition

Each subject's body composition was measured in the third week of the trial and followed the standardised ISAK (International Society for the Advancement of Kinanthropometry) Level 1 Proforma. Measurements were taken on the right side of the body with a combination of skinfolds, girths, and bone breadths (Table 2.2). All skinfolds were taken by the same investigator using Harpenden Skinfold Calipers (Quality Measurement Ltd, West Sussex, United Kingdom) and a Lufkin® W606 Executive Diameter Steel Tape (CooperTools, Apex, NC).

Table 2.2.

Body composition assessment sites[#] used in a post-exercise recovery study.

Skinfolds	Girths	Bone Breadths
Biceps	Upper arm (Relaxed)	Humeral
Triceps	Upper Arm (Flexed)	Femoral
Subscapular	Waist	
Suprailiac	Hip	
Supraspinale	Calf	
Abdominal		
Thigh		
Calf		

[#] This measurement procedure follows the standardised ISAK Level 1 Proforma.

2.2.1.3. Sum of Skinfolds

Two sets of Sum of Skinfolds (units = mm) measurements were calculated according to the formula stated below:

Sum 6 Skinfolds

= Σ[Subscapular, Triceps, Supraspinale, Abdominal, Front Thigh, Medial Calf]

Sum 8 Skinfolds

= Σ[Subscapular, Biceps, Triceps, Iliac Crest, Supraspinale, Abdominal, Front Thigh,
Medial Calf]

2.2.1.4. Percentage Body Fat

Percentage body fat was calculated using the Yuhasz (1974) equation for males:

Yuhasz Equation (1974):

Percentage Body Fat

= (1.1051 x sum of triceps, subscapular, supraspinale, abdominal, thigh, calf) + 2.585

2.2.1.5. Body Mass Index (BMI)

BMI was calculated using the following equation:

$$BMI = \frac{\text{Weight (kg)}}{[\text{Height(m)}]^2}$$

2.2.1.6. Waist : Hip Ratio

Waist : Hip Ratio was calculated using the following equation:

$$\text{Waist : Hip Ratio} = \frac{\text{Waist Circumference (mm)}}{\text{Hip Circumference (mm)}}$$

Definitions:

Waist Circumference	=	minimum circumference around waist
Hip Circumference	=	maximum circumference around hips

2.2.1.7. Somatotype

Somatotype was calculated using the Heath-Carter Somatotype Method (Carter, 1980). Somatotype is a method of quantification of the shape and composition of the human body. It is generally expressed in a three-number rating system representing endomorph, mesomorph, and ectomorph components, respectively.

Endomorph: Relates to the relative fatness of the subject;

Mesomorph: Relates to the relative musculoskeletal robustness of the subject; and

Ectomorph: Relates to the relative linearity or slenderness of the subject.

2.2.2. Experimental Design

2.2.2.1. Dietary Beverage Composition

The four dietary beverages tested were:

a. Carbohydrate Only (Carb))
b. Carbohydrate + Whey Hydrolysate Protein (Carb + H
c. Carbohydrate + Whey Intact Protein (Carb + I)
d. Carbohydrate + 50% Whey Protein Hydrolysate + 50% Whey Intact Protein (Carb + M)

Each dietary beverage was designed to deliver a set amount of carbohydrate and protein relative to body weight per hour during the initial stages of recovery. Table 2.3 gives the ingredient composition of the four dietary beverages (per 1000g of powder), while Table 2.4 details the amount of each beverage that needed to be consumed relative to body weight to ensure the correct supply of both nutrients and water. The volume of water consumed with the Carb beverage is higher than that for the protein-containing beverages as a result of the water content of the protein powders being accounted for. The protein percentage of the intact protein and protein hydrolysate powders also differed (whey intact protein: 94.2%DM, 93.5% protein (N x 6.25); whey protein hydrolysate: 95.3%DM, 87.3% protein (N x 6.25)).

Table 2.3.

Ingredient composition[#] of the four dietary beverages powders used in a post-exercise recovery study.

	Dietary Beverages			
	Carb	Carb + I	Carb + H	Carb + M
Protein Hydrolysate[1] (g)	-----	-----	272.15	136.42
Intact Protein[1] (g)	-----	253.37	-----	127.37
Glucose[2] (g)	489.64	359.15	356.38	357.27
Maltodextrin[3] (g)	489.64	359.15	356.38	357.27
Sodium Saccharin[4] (g)	1.11	0.81	0.81	0.81
Citric Acid[5] (g)	13.06	9.58	9.50	9.53
Orange Flavour[6] (g)	6.56	4.78	4.78	4.78
Sodium Bicarbonate[7] (g)	-----	13.17	-----	6.55
Total Powder (g)	1000	1000	1000	1000

[#] g/1000g air dry weight
Carb = Carbohydrate only
Carb + I = Carbohydrate + Intact Protein
Carb + H = Carbohydrate + Protein Hydrolysate
Carb + M = Carbohydrate + Protein Mix
[1] Fonterra Co-operative Group Ltd, Palmerston North, New Zealand
[2] Corn Products International, USA, lot 700 1057 002J
[3] Maltrin®, Salkat New Zealand Ltd, Auckland, New Zealand
[4] Lot 00498, 450FC, ex Bronson & Jacobs Pty Ltd, Auckland, New Zealand
[5] ex Jungbunzlauer, FG000 Lot 7681/02.01, Interchem Angencies Ltd, Auckland, New Zealand
[6] Natural SD, 9/033581 1/11/2002, Dragoco Australia Pty Ltd, Australia
[7] $NaHCO_3$

Table 2.4.

Expected intake rates for both of the dietary beverage powder (grams of dry matter (DM)) and water, expressed per kg of body weight, used in a post-exercise recovery study.

	Dietary Beverages			
	Carb	Carb + I	Carb + H	Carb + M
gDM/kg BWT	1.225	1.671	1.684	1.679
gWater/kg BWT	8.775	8.329	8.316	8.321

Carb = Carbohydrate only
Carb + I = Carbohydrate + Intact Protein
Carb + H = Carbohydrate + Protein Hydrolysate
Carb + M = Carbohydrate + Protein Mix

2.2.2.1.1. Carbohydrate Content of Dietary Beverages

The carbohydrate component of the dietary beverages was formulated to supply 1.2 grams of carbohydrate / kg BWT / hour during the first three hours of the recovery period. The carbohydrate content comprised of 50% glucose (sourced from Corn Products International, USA, lot 700 1057 002J) and 50% maltodextrins (MALTRIN®, sourced from Salkat New Zealand Ltd, Auckland, New Zealand) and supplied at a carbohydrate concentration of 12% (w/v) in the treatment beverages.

2.2.2.1.2. Protein Content of Treatment Beverages

The total protein component of the drink was formulated to supply 0.4 grams of protein / kg BWT / hour for the first three hours of the recovery period. The protein fraction consisted of a milk whey protein isolate (ALACEN™ 895 Whey Protein Isolate, Fonterra Co-operative Group Ltd, Palmerston North, New Zealand) and its "same batch" hydrolysate (Degree of hydrolysis of 14.1%). The typical amino acid composition of ALACEN™ 895, and the mineral comparisons of the protein isolate and hydrolysate can been seen in Table 2.5 and Table 2.6, respectively.

Table 2.5.

Typical amino acid # composition of ALACEN™ 895 Whey Protein Isolate* used in a post-exercise recovery study.

	Typical Amino Acid Amount (g amino acid per 100g of protein)
ESSENTIAL AMINO ACIDS	
Isoleucine	6.3
Leucine	14.3
Lysine	11.2
Methionine	2.4
Phenylalanine	3.8
Threonine	5.3
Tryptophan	2.4
Valine	5.6
NON ESSENTIAL AMINO ACIDS	
Histidine	2.0
Alanine	5.7
Arginine	3.0
Aspartic Acid	12.5
Cysteine/Cystine	4.0
Glutamic Acid	17.6
Glycine	1.8
Proline	4.5
Serine	4.5
Tyrosine	4.2

g unbound amino acid per 100g protein (e.g. amino acid weight includes the molecular weight of the water of hydrolysis)

* data provided by Fonterra Co-operative Group Ltd, Palmerston North, New Zealand.

Table 2.6.

Mineral composition* of the whey protein isolate and whey protein hydrolysate used in a post-exercise recovery study.

	Whey Protein Isolate (Intact Protein)	Whey Protein Hydrolysate
Sodium (mg/100g)	544	2330
Chloride (mg/100g)	28	723
Potassium (mg/100g)	89	30
Calcium (mg/100g)	75.5	62
Magnesium (mg/100g)	9.3	7.5
Protein (% w/w)	93.5	87.3

* data provided by Fonterra Co-operative Group Ltd, Palmerston North, New Zealand.

2.2.2.1.3. Anion : Cation Balance

The anion : cation balance of the three protein solutions was calculated and then corrected to ensure that they all had the same Dietary Electrolyte Balance (DEB) value. This balance has the potential to affect kidney function and the excretion of urinary NH_4^+ (Kellum, 2000), therefore it is important that the anion : cation balance is the same across all beverages. The initial and final DEB values for each protein beverages is shown in Table 2.7. DEB was calculated using the following equation:

$$DEB = mEq\ (Na^+ + K^+ - Cl^-) / 100gDM$$

Table 2.7.

Initial and Final Dietary Electrolyte Balance (DEB)$^{+}$ for the three protein containing beverages tested in a post-exercise recovery study.

Protein Beverage	Initial DEB	Final DEB
Whey Hydrolysate Protein	227.14	227.14
Whey Intact Protein	70.06	227.14
50% Whey Hydrolysate Protein + 50% Whey Intact Protein	148.60	227.14

$^{+}$DEB = mEq ($Na^{+} + K^{+} - Cl^{-}$)/100g Dry Matter of Diet

In order to correct for this difference, Sodium Bicarbonate ($NaHCO_3$) was added at 13.19 g / kg to the Whey Intact Protein beverage powder and 6.60 g / kg to the 50% Whey Hydrolysate Protein + 50% Whey Intact Protein beverage powder. Following this correction, the DEB values of all protein-containing beverages were equal to that of the whey hydrolysate protein beverage which was 227.14 mEq/100 DM.

2.2.2.1.4. Double-Blinding of Treatments and Administration of the Beverages

The dry material for each of the different beverages was formulated by Fonterra Co-operative Group Ltd (Palmerston North, New Zealand). Each powder contained the relevant amounts of carbohydrate and protein ingredients, together with Dragoco orange flavouring (Natural SD, 9/033581 1/11/2002, Dragoco Australia Pty Ltd, Australia), citric acid anhydrous (ex Jungbunzlauer, FG000 Lot 7681/02.01, Interchem Angencies Ltd, Auckland, New Zealand) and Sodium Saccharide (Lot 00498, 450FC, ex Bronson & Jacobs Pty Ltd) in order to mask the different tastes of the four beverages and to make the drinks

more palatable. The amount of each of these ingredients in each beverage is shown in Table 2.3.

Each of the four beverages was given a randomly assigned a three-digit code. An independent technician prepared the required amount (on a BWT basis) of both the dry material and water that needed to be consumed by each subject for the given experimental day. These were then refrigerated and only mixed by the experimenters immediately prior to consumption by the subject. The experimenters and the subjects had no knowledge of which treatment beverage was being consumed at any time.

The dietary beverages were consumed by the subjects in bolus form at 30 minute intervals over the first two hours (Time 0, 30, 60, 90 and 120 minutes) of the recovery period on each experimental day. The actual time of consumption was recorded.

2.2.2.2. Experimental Period

The seven-day experimental period was divided into two phases (Figure 2.1). The first phase was defined as the true experimental period and lasted four days (which includes the experimental day, Figure 2.2), while the second phase consisted of three days of recovery. As described above, this seven-day period was repeated over four consecutive weeks. The drink order was randomised (Table 2.8) so that the beverages being tested did not routinely follow the same combination for each subject.

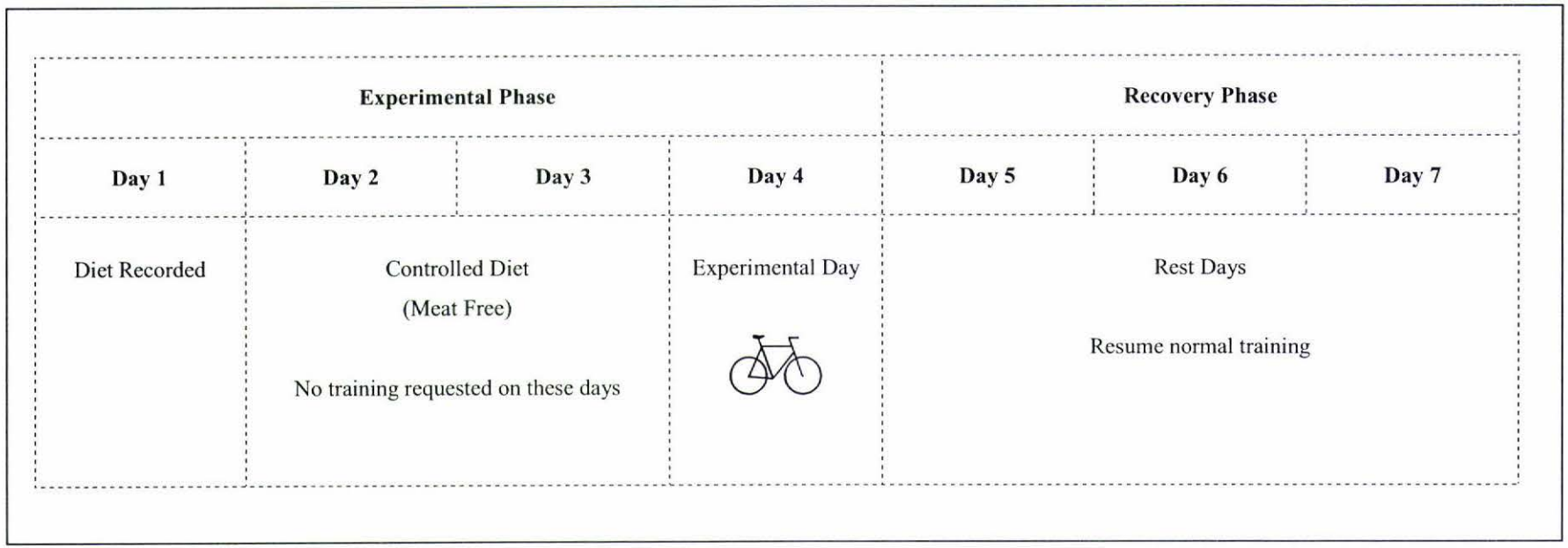

Figure 2.1. Overview of a seven day experimental period highlighting division of the two phases for a post-exercise recovery study.

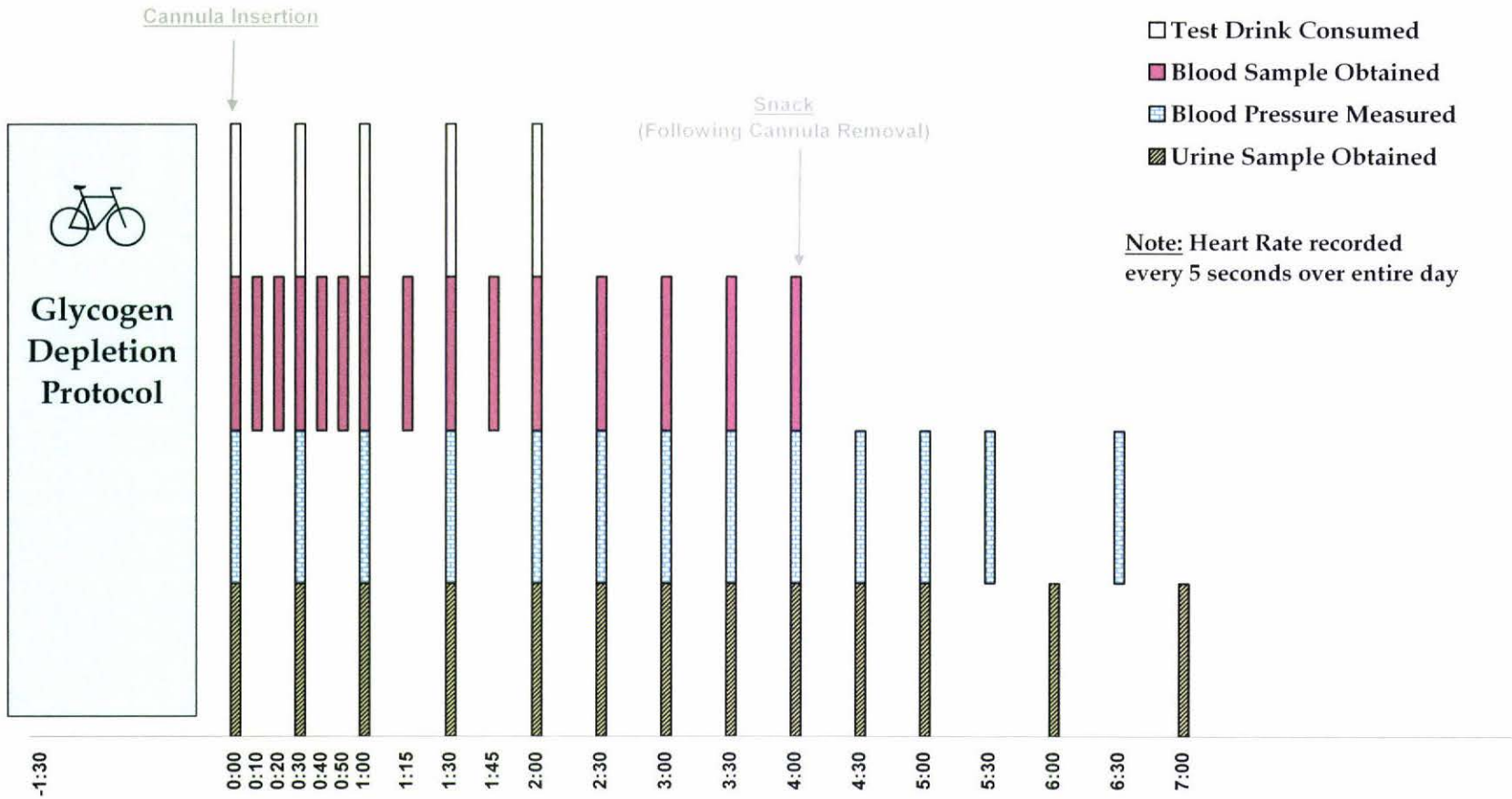

Figure 2.2. Schematic overview of the Day 4 (Experimental Day) for a post-exercise recovery study. Note: Time 0 is set as the time the first beverage was consumed.

Table 2.8.

Randomised allocation of the four beverages tested used in a post-exercise recovery study designed so that each beverage does not routinely follow the same combination in each subject.

Carb = Carbohydrate Only
Carb + H = Carbohydrate / Protein Hydrolysate
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate

	Test Subject											
Week	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
1	Carb	Carb + H	Carb + I	Carb + M	Carb + H	Carb + I	Carb + M	Carb	Carb + I	Carb + M	Carb	Carb + H
2	Carb + M	Carb	Carb + H	Carb + I	Carb	Carb + H	Carb + I	Carb + M	Carb + H	Carb + I	Carb + M	Carb
3	Carb + H	Carb + I	Carb + M	Carb	Carb + I	Carb + M	Carb	Carb + h	Carb + M	Carb	Carb + H	Carb + I
4	Carb + I	Carb + M	Carb	Carb + H	Carb + M	Carb	Carb + H	Carb + I	Carb	Carb + H	Carb + I	Carb + M

2.2.2.2.1. Day 1 (Diet Record)

Subjects recorded the foods they consumed, including type and quantities, so as to determine whether their diet changed significantly over the four week trial period. Diets were analysed using Diet Cruncher (v1.6.0, 2002, Way Down South, Dunedin, New Zealand) nutritional software linked into the 2003 New Zealand Food Composition database (Crop and Food Research, Palmerston North, New Zealand). The results of this analysis, although not presented in this thesis, are being prepared for a separate scientific manuscript.

2.2.2.2.2. Day 2 and Day 3 (Controlled Diet)

Subjects were supplied with a controlled diet for these two days. The diet over these two days was meat-free to allow for accurate measurement of plasma and urinary 3-methylhistidine. The macro-nutrient composition of the supplied diet is listed below (Table 2.9).

Table 2.9.

Diet composition analysis of the controlled diet consumed the two days prior to each experimental day in a post-exercise recovery study.

	Energy[#] kJ (kCal)	Percentage of Total Energy	Grams / kg of body weight*
Total Energy	15347 (3368)		
Carbohydrate	9653 (2307)	62.9	7.90
Fat	3808 (910)	24.8	1.40
Protein	1887 (451)	12.3	1.54

* Based on average weight of cyclists of 73.21kg
[#] Based on Carbohydrate (16.7kJ/g), Fat (37.7kJ/g) and Protein (16.7kJ/g)
Note: Energy Conversion Value => 4.184 kJ = 1 kCal

The controlled diet was designed based on current dietary recommendations for competitive cyclists and to provide sufficient energy to meet the anticipated needs of each subject. Subjects recorded all foods consumed over this two-day period to confirm they had adhered to the controlled diet. Subjects were also requested to limit exercise in the two days prior to each experimental day and to maintain a well-hydrated state during this period so as to achieve a similar hydration state on each day of the experiment.

2.2.2.2.3. Day 4 (Experimental Day)

The experimental day consisted of an initial glycogen depletion exercise protocol followed by a seven-hour recovery period where ongoing sampling of blood, urine, blood pressure, and heart rate occurred (Figure 2.2). At the beginning of each experimental day, subjects reported to the Human Performance Laboratory at Massey University by 8:30am in a fasted state, having consumed 7 ml water/kg BWT in the preceding hour.

2.2.2.2.3.1. Glycogen Depletion Protocol

The procedure for muscle glycogen depletion was performed on the Kingcycle Trainer (Kingcycle, Buckinghamshire, UK) using the subject's own cycle. Prior to beginning the glycogen depletion protocol, each subject was fitted with a Polar S610 Heart Rate Monitor (Polar Electro Oy, Finland) which remained on for the duration of the experimental session.

During the glycogen depletion protocol, subjects consumed water at a rate of 10 ml/kg BWT/hour in order to minimise the level of dehydration. A measured quantity of water was provided at 30-minute intervals with subjects instructed to consume this water within this interval. The total volume of water consumed by each subject was recorded.

Warm Up:	Subject performs a 10 minute warm up at 100W.
Stage 1:	The subject exercises in two-minute intervals alternating between 90% and 50% of W_{max}. Completion of this stage occurs when the power output for the 90% intervals falls by greater than 50 Watts.
Stage 2:	The subject exercises in two-minute intervals alternating between 80% and 50% of W_{max}. Completion of this stage occurs when the power output for the 80% intervals falls by greater than 50 Watts.
Stage 3:	The subject exercises in two-minute intervals alternating between 70% and 50% of W_{max}. Completion of this stage occurs when the power output for the 70% intervals falls by greater than 50 Watts.
Warm Down:	10 minutes at 100 Watts.

Figure 2.3. Glycogen depletion protocol used in a post-exercise recovery.

The method of muscle glycogen depletion used in this study, outlined in Figure 2.3, has been used in previous studies examining both the effects of carbohydrate and/or protein intake on exercise recovery (van Loon *et al.*, 2000b, 2000c; Jentjens *et al.*, 2001; van Hall *et al.*, 2000a, 2000b). However, these previous studies used the criteria for stage completion as a decrease in pedalling frequency below 60 rpm. In order to keep the protocol for this current study as sport-specific as possible, through the use of the subjects' own cycles and the Kingcycle Trainer (Kingcycle, Buckinghamshire, UK), subjects were permitted to change gears, meaning that pedalling frequency could be maintained. Therefore, the criteria used for stage completion was set at a decrease in power output by greater than 50 Watts during the high intensity phase (i.e. 90, 80 or 70% of Wmax) rather than a decrease in pedalling frequency. This indicated that the subject was no longer able to reach and maintain the required power output.

With each exercise session, the subjects' start time for warm up and Stage 1, the time at completion of Stage 1, Stage 2, and Stage 3, and the time when warm-down was completed, were all recorded. Total test time, estimated calories used, distance covered and average power for each subject was also recorded. Upon completion of the warm down, subjects were permitted to have a brief shower (2-3 minutes) prior to taking a three minute walk to the Human Nutrition Studies Laboratory (within the Institute of Food, Nutrition and Human Health, Massey University) where they remained for the rest of the day.

Following the glycogen depletion protocol, fluid was supplied at a rate of 10 ml/kg BWT/hour during the first two hours of recovery as part of the dietary beverages and then at a rate of 7 ml/kg BWT/hour for the following two hours until the end of the blood sampling period, after which time fluid consumption was voluntary. Table 2.10 shows planned rates of fluid intake over the entire experimental day.

Table 2.10.

Desired water consumption throughout the experimental day in a post-exercise recovery study.

Time (minutes)	Period	Desired Water Intake
-150 to -90	Pre-Exercise	7 ml/kg
-90 to 0	During Exercise	10 ml/kg/h
0 to 180	Beverage	10 ml/kg/h
180 to 240	Consumption*	7 ml/kg/h
240 to 420	Post Beverage 1*	Voluntary
	Post Beverage 2#	

* Blood sampling still occurring during this time

No blood sampling occurring during this time

2.2.2.2.4. In-Dwelling Cannulae and Blood Sampling

2.2.2.2.4.1. In-Dwelling Cannulae:

A venous cannula (BD Insyte™ 16 GA 1.7x30mm, 220ml/sec Catheter; Becton Dickson Infusion Therapy Inc., Utah, USA) was inserted by a medical practitioner in the forearm vein of each subject under aseptic conditions to enable frequent blood sampling. The cannulae site was secured with an adherent cover (Teraderm™ I.V. 7cm x 8.5 cm, 3M Health Care, St Paul, MN, USA). Blood samples were withdrawn from the cannula using a blunt plastic cannula BD (Becton Dickson Infusion Therapy Inc., Utah, USA) via a Baxter Interlink® system (Baxter Healthcare Corporation, Deerfield, IL, USA) following cleaning of the sampling port with a Medi-Swab™ pre-injection swab (Smith & Nephew Pty, Ltd, Auckland, New Zealand).

The time from completion of the exercise warm down until catheter insertion was recorded for each subject. The site of cannulation was alternated weekly to ensure adequate healing of the site of prior cannulation. Cannulae were removed at completion of the sampling period under aseptic conditions following which local pressure was applied by the subject until haemostasis was achieved.

2.2.2.2.4.2. Blood Sampling

In-dwelling cannulae were flushed with 3ml of saline (Sodium Chloride Injection BP 0.9%, Astra Zeneca Ltd, Auckland, NZ) with the first 3ml of blood withdrawn from the sampling port discarded so as to avoid inadvertent dilution with residual saline. An 11ml sample was then taken in a fresh syringe and transferred into a 10ml EDTA tube (BD Vacutainer Systems, Plymouth, UK).

The 10ml EDTA tubes containing each blood sample were spun in a Heraeus Megafuge 1.0R Refridgerated Bench Centrifuge (Heraeus Instruments GmbH, Labortechark, Hanau). Following this, the blood plasma was separated using a pipette (Jencons 200-1000 SealPette, Bedfordshire, England) and transferred into 2ml Cryos Cellstar® tubes (Greiner bio-one, RayLab, Auckland, New Zealand) that were subsequently frozen at –17°C (Frigidaire Freezer, Auckland, New Zealand).

The remaining 1ml of blood was used to fill a heparinised capillary tube (Chase Scientific Glass Inc., Rockwood, TN) to determine haematocrit. Each filled capillary tube was subsequently plugged at one end using Critoseal (Sherwood Services, The Kendall Company, Massachusetts, USA) and spun in a Jouan Hemi C micro-haematocrit centrifuge (Jouan Inc., Winchester, Virginia, USA) with the haematocrit recorded using a micro-haematocrit reader (Hawksley, England)

A total of 15 blood samples were collected at the following time points; 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210 and 240 minutes, with time 0 being from the time of commencement of consumption of the first recovery beverage. Baseline blood samples were taken immediately prior to consumption of the first dietary beverage.

2.2.2.2.5.Urine Sampling:

Each subject was asked to empty his bladder and collect the evacuated urine in a plastic container at time 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, and 420 minutes. Baseline urine samples were taken immediately prior to consumption of the first dietary beverage. The urinary pH of each sample was lowered to below pH 5 using 0.1% HCl solution to prevent ongoing bacterial degradation of any metabolites that may have been present in the urine. The quantity of urine produced by each subject for each individual sample was determined through weighing. From each urine sample, two 10ml samples were transferred into 15ml PP-Test tubes (Greiner bio-one, RayLab, Auckland, New Zealand) and one 2ml sample was placed in a Cryos Cellstar® tube (Greiner bio-one, RayLab, Auckland, New Zealand), respectively. The two 10ml samples were transferred using a 10ml graduated pipette (TD-Ex 20 Serological, Greiner bio-one, RayLab, Auckland, New Zealand) connected to a Powerpette Plus (Jencons, Bedfordshire, England). The 2ml sample was transferred using a Samco transfer pipette (BioLab Ltd, Auckland, New Zealand). The urine samples were frozen (Frigidaire Freezer, Auckland, New Zealand) at $-17^{o}C$ and the remaining urine discarded.

2.2.2.2.6. Heart Rate Recording

Heart rate data was collected by telemetry using Polar S610 Heart Rate Monitors (Polar Electro Oy, Finland). Recorded heart rate data was downloaded from the Polar S610 Heart Rate monitors to the Polar Precision Performance software (v3.0, 2001, Polar Electro Oy, Finland) via an infrared connection. Heart rate was automatically recorded every five seconds during the entire experimental period (from the time the subjects began their warm-up for the glycogen depletion protocol, until following the collection of the final urine sample of the day). This equated to approximately 8.5 hours of heart rate data on each occasion. Data was then transferred to a Microsoft Excel (v10.0, Microsoft Corporation, USA) spreadsheet at the completion of each experimental day. Mean values of one-minute recordings were used in subsequent statistical analysis. Data used for heart rate recovery was taken from 30 minutes following completion of the final completed workload. The basis behind this was to account for the heart rate effects of the 10-minute warm down, a brief shower, and insertion of the in-dwelling cannulae, which allowed the focus to remain on the effect of beverage consumption on heart rate recovery.

2.2.2.2.7. Blood Pressure Measurement

All blood pressure recording was determined by the same researcher throughout the period of the study so as to eliminate any operator effect. All blood pressure measurements were duplicated at each time interval, with the two measurements taken immediately following each other. Both measures for blood pressure were included in the statistical analysis. The blood pressure measurements were taken on the opposite arm to the one inserted with the in-dwelling cannula for safety reasons, and were measured using a mercury sphygmometer (Riester, Birmingham, UK) with a 14.5cm cuff and

a stethoscope (Riester, Birmingham, UK). Systolic blood pressure was measured as the onset of sounds and diastolic blood pressure as the change (muffling) of sounds. As the cannulae were inserted into alternate arms each week of the trial, the arm that the blood pressure measurement was taken on was also alternated each week. Blood pressure measurements were taken at time points 0, 30, 60, 90, 120, 150, 180, 210, 270, 300, 330, and 390 minutes. The blood pressure measurement at 240 minutes was not used in subsequent calculations as this was the time of cannulae removal, making measurement of blood pressure difficult.

2.2.2.2.8.Snack

Following completion of the blood sample collection period, and removal of the in-dwelling cannula, subjects were provided with a non-protein carbohydrate snack. Until this time of the day subjects had not consumed any solid foods, with their only energy coming from the dietary beverages. The snack provided was Heards Barley Sugar lollies (Nestle New Zealand Ltd, Auckland, New Zealand) and was only supplied following completion of the blood sampling period which was the end of assessment of carbohydrate metabolism. Only the measurement of protein status in urine was assessed following this time point, in addition to monitoring of heart rate and blood pressure.

2.3.Chemical Analysis

All analyses of blood and urine samples, with the exception of determination of amino acid levels, were performed using diagnostic kits (Table 2.11). All chemical analyses were performed at Massey University (Palmerston North) in the Institute of Food, Nutrition and Human Health's IANZ Accredited Nutrition Laboratory.

Table 2.11.

Diagnostic kits used for chemical analysis performed in a post-exercise recovery study.

Analysis Variable	Diagnostic Kit Used
Insulin	RIA kit, Linco Research, St Charles, Missouri, USA.
Glucose Albumin Urea Creatinine	Roche Diagnostics kit, Hoffmann-LaRoche, Basel, Switzerland. Analyses were performed on a Roche Cobas Fara II, Hoffmann-LaRoche, Basel, Switzerland.
Ammonia	Sigma Diagnostic kit, Sigma-Aldrich, St Louis, Missouri, USA. Analyses were performed on a Roche Cobas Fara II, Hoffmann-LaRoche, Basel, Switzerland.

Duplicate samples of the dry powder of each test beverage were analysed for amino acid content following acid hydrolysis in 6M HCl for 24 hours. The amino acids were separated by ion-exchange chromatography and detected following reaction with nin-hydrin.

The amino acid analysis was performed on a Waters 790E HPLC system (Waters Corporation, Milford, Massachusetts, USA). This system includes the Waters Ion Exchange Column, W715 autosampler, W510 pump, Waters column heater and W490E UV-Vis detector (Waters Corporation, Milford, Massachusetts, USA). The gradient program for each run was slowed to approximately three hours to allow adequate separation of 3-methylhistidine from histidine to enable quantification.

2.4.Statistical Analysis

Data was explored mainly through a combination of repeated measures ANOVA and Multivariate (Discriminant) Analysis Statistical Analysis was performed using SYSTAT® (v6.0 for windows®, 1996, SPSS Incorporated, Chicago, Illinois).

Pairwise repeated measures ANOVA was mainly used to determine the presence of a significant effect of the four treatment beverages as well as the presence of a significant difference in the pattern of variation over time for the variables. Statistical analysis of cummulative urine output also involved the use of a one-way ANOVA, with a *post-hoc* Bonferroni test, to determine the presence of significant pairwise comparisons based on there being multiple comparisons across beverages trialled. It is acknowledged that statistical analysis by pairwise comparisons, due to the limitations of the statistical software used, has limitations and as such only highly significant results ($p<0.05$) are reported.

Multivariate (Discriminant) Analysis determined the presence of any pairwise comparisons between beverages with standardised Canonical values used to determine the basis of any significance.

Statistical analysis of pulse rate recovery involved the use of an ANCOVA, to determine the overall effect of beverages in terms of their slope and intersect. In addition, a Student's t-test (equation shown below) of the linear regression slope coefficients was used to determine whether the slopes of pulse rate recovery between the different beverages were significantly different.

$$t_{obs} = \frac{b_1 - b_2}{\sqrt{s^2_{b_1} + s^2_{b_2}}} \text{ and is distributed with } n_1 + n_1 - 4 \text{ df}$$

where b is the slope and s_b is the standard error for the slope for samples 1 and 2.

Chapter 3

RESULTS

3.1 Experimental Trial Overview:

Eleven of the twelve subjects successfully completed all four weeks of the trial. One subject had to be withdrawn after the second week of the trial due to a tendency to develop migraines during the procedure. Examination of food diaries confirmed that subjects had adhered to the diet supplied (meat-free) in the two days prior to each experimental day. One further subject's results were excluded from subsequent analysis as his plasma insulin and plasma glucose measurements showed impaired glucose tolerance and decreased insulin sensitivity.

3.1.1 Exercise Protocol:

Mean overall duration of cycling in the depletion protocol was 55 minutes. The mean duration of the 90% stage was 16.0 (± 8.0) minutes, the 80% stage was 14.0 (± 8.0) minutes, and the 70% stage was 24 (± 13) minutes. All athletes reported a high level of fatigue, with some subjects struggling to maintain the 50% W_{max} recovery intensity. The mean power output of athletes during this period was 260 (± 41) Watts with an estimated energy expenditure, calculated by the Kingcycle software, of 1095 (± 289) Calories.

3.1.2 Trial Beverage Consumption:

Beverages were consumed within two minutes by all the subjects, with no spillage and no episodes of nausea or vomiting following consumption.

3.1.3 Blood & Urine Sampling:

The mean time period between cessation of exercise and insertion of cannulae was 14 minutes, 55 seconds (± 4 minutes, 38 seconds). There were no complications from the cannulation procedure, except that a small number of timed blood samples were missed because of delays due to slow rates of blood flow early in the procedure.

Some subjects had difficulty producing a urine sample at specific times during the early stages of the recovery period. Towards the end of the procedure several subjects experienced discomfort from an overly distended bladder when the interval between sampling was increased to 60 minutes.

3.2 Anthropometric and Exercise Test (VO_{2peak} and W_{max}) Results:

The mean percentage body fat of the athletes (Table 3.1) was calculated using Yuhasz equation for males (Yuhasz, 1970) and the mean (±SD) value was 8.15% (±1.94%). Similarly, mean (±SD) Body Mass Index was 22.82 kgm^2 (±1.77 kgm^2) and Waist: Hip Ratio was 0.80 (±0.04). Somatotype was calculated using the Heath-Carter Somatotype Method (Carter, 1980). Results for somatotype (Table 3.1) indicate a dominant mesomorph body shape rating for the majority of subjects.

The pre-trial fitness tests (Table 3.2), indicated that the subjects were very highly trained. Their mean VO_{2peak} was 5.00 L/min (range: 4.10-5.81 L/min) or 67.24 ml/kg/min (range: 55.84-77.80 ml/kg/min). An example graph of VO_2 output from the pre-experimental trial fitness test is given in Figure 3.1. The mean (±SD) W_{max} of the athletes was 445.45 W (± 66.80 W). The mean values for VO_{2peak} and W_{max} did not include values from the subject with insulin resistance.

Table 3.1.

Summary of the data gained from anthropometric assessment[#] of the ten male subjects that were involved in a post-exercise recovery study.

Subject	Age (years)	Height (cm)	Weight (kg)	SKINFOLDS			BMI	Waist : Hip Ratio	SOMATOTYPE****		
				% Body Fat *	Sum 6 ** Skinfolds (mm)	Sum 8 *** Skinfolds (mm)			Endomorph	Mesomorph	Ectomorph
A	29	175.4	78	12.4	93.2	121.2	25.3	0.81	3.8	0.1	1.6
B	36	187.9	74.7	6.8	39.9	48.2	21.2	0.78	1.4	4.7	4.1
C	23	177.6	66.7	7.7	48.7	61.4	21.1	0.80	1.8	3.8	3.5
D	20	179.4	72.9	7.6	47.4	61.5	22.7	0.85	2.1	5.5	2.9
E	23	181.0	74.8	6.8	40.4	53.3	22.8	0.83	1.9	4.9	2.9
F	26	180.0	85.9	9.4	64.7	81.5	26.5	0.81	2.9	6.3	1.3
G	31	170.4	62.7	6.9	41.3	50.8	21.6	0.81	1.7	4.8	2.9
H	29	183.2	76.1	7.1	42.9	58.0	22.7	0.84	1.7	4.6	3.1
J	18	176.2	70.3	10.3	73.6	100.1	22.6	0.77	3.4	5.4	2.7
L	24	177.8	70	6.5	37.7	48.4	21.7	0.73	1.7	4.7	3.3
MEAN	25.90	178.89	73.21	8.15	52.98	68.44	22.82	0.80	2.24	4.45	2.83
STANDARD DEVIATION	5.43	4.71	6.40	1.94	18.35	24.75	1.77	0.04	0.82	1.76	0.83

[#] According to ISAK procedure (International Society for the Advancement of Kinanthropometry. (2001). International standards for anthropometric assessment.. ISAK, Australia)

* % Body Fat calculated using Yuhasz equation for males [$\%BF_{Yuhasz\ Equation}$ = (0.1051 x sum of Triceps, Subscapular, Supraspinale, Abdominal, Thigh, Calf) + 2.585] (Yuhasz, 1970)

** Sum of 6 Skinfolds includes Subscapular, Triceps, Supraspinale, Abdominal, Front Thigh, and Medial Calf skinfold sites

*** Sum of 8 Skinfolds includes Subscapular, Biceps, Triceps, Iliac Crest, Supraspinale, Abdominal, Front Thigh, and Medial Calf skinfold sites

**** Somatotype categories: 0.5 to 2.5 - low; 2.6 to 5.4 - moderate, 5.5 to 7 - high; 7 plus: extremely high (Carter, (1980).

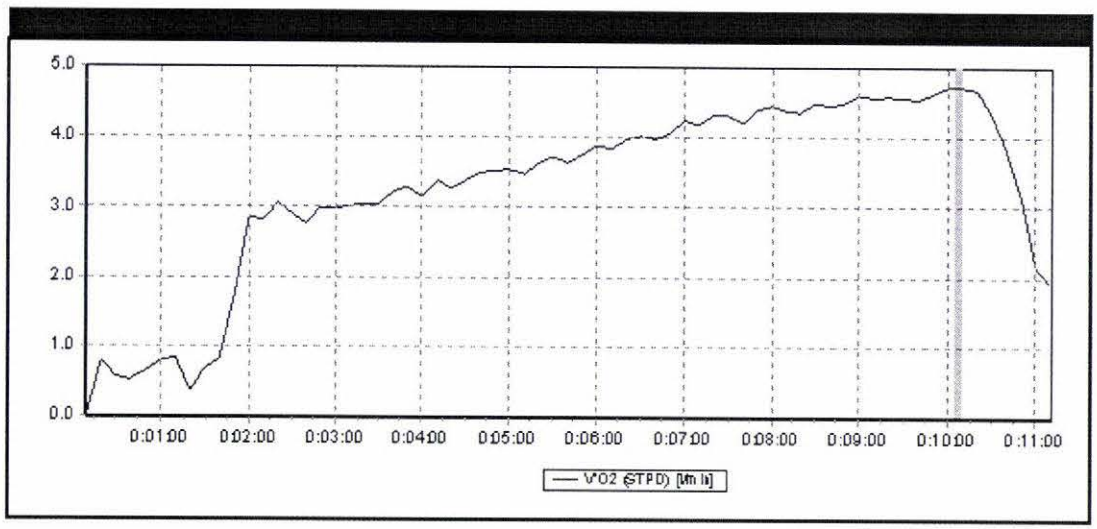

Figure 3.1. Example graph of VO_2 output from a pre-experimental trial fitness test. The five-second interval for determining VO_{2peak} is highlighted on the right hand side of the graph. The result for this athlete was recorded at 4.73 L/min or 71.77 ml/kg/min.

Table 3.2.

Data from the pre-trial fitness assessment for the 11 males scheduled to be involved in a post-exercise recovery study.

Subject	VO_{2peak} (L/min)	VO_{2peak} (ml/kg/min)	W_{max} (Watts)	Glycogen Depletion Workloads			
				90% W_{max}	80% W_{max}	70% W_{max}	50% W_{max}
A	4.60	58.97	371	334	297	260	186
B	4.74	55.84	359	323	287	251	180
C	4.73	71.77	465	419	372	326	233
D	5.60	74.70	516	464	413	361	258
E	5.48	72.06	528	475	422	370	264
F	5.81	65.33	511	460	409	358	256
G	4.10	66.16	346	311	277	242	173
H	5.75	77.80	508	457	406	356	254
I	4.84	62.08	420	378	336	294	210
J	4.44	65.30	421	379	337	295	211
L	4.88	69.68	455	410	364	319	228
MEAN	5.00	67.24	445.45	400.91	356.36	311.82	222.73
STANDARD DEVIATION	0.57	6.71	66.80	60.12	53.44	46.76	33.40

3.3 Variability Associated with Chemical Analyses

All analyses, with the exception of plasma and urinary amino acids, were performed in duplicate. The interassay coefficient of variation (CV%) stated below (Table 3.3) are those of the nutritional laboratory at the Institute of Food, Nutrition and Human Health (College of Sciences, Massey University, Palmerston North, New Zealand) where all analyses were conducted.

Table 3.3.

Variability (CV%) associated with various chemical analyses conducted on samples collected during a post-exercise recovery study.

Diet Sample	CV%
Insulin	<5%
Glucose	3.9%
Creatinine	5.5%
Urea	3.2%
Albumin	3.1%
Ammonia	5.0%

Owing to the time requirement of each analysis (as stated in the Methods section) the analyses of plasma amino acids were not performed in duplicate. However the reliability of the assay was subject to an internal standard with a recovery of 95% (±5%) and was also run with an external control sample with a recovery of 95% (±5%). To determine the amino acid composition of the diet samples, however, duplicate analyses were performed with the CV% recorded for the three protein-containing beverages (Table 3.4).

Table 3.4.

Variability (CV%) associated with the determination of amino acids in the beverages used in a post-exercise recovery study.

Diet Sample	CV%
Carb + I	5%
Carb + H	6%
Carb + M	4%

Carb + I = Carbohydrate / Intact Protein
Carb + H = Carbohydrate / Protein Hydrolysate
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate

3.4 *Amino Acid Composition of Diets*

The amino acid compositions of the four trial beverage powders are shown in Table 3.5. Of the amino acids determined, the Carb + H beverage had slightly higher levels overall compared to the Carb + I beverage. The amino acid levels of the Carb + M beverage were slightly higher than those of both the Carb + H beverage and the Carb + I beverage. Overall, the amino acid compositions of the three dietary beverages containing protein were similar.

Table 3.5.

Determined amino acid composition of the four dietary beverages, expressed as g/kg BWT delivered, in a post-exercise recovery study.

Amino Acids	Carb	Carb + H	Carb + M	Carb + I
ASPARTIC ACID	nd	0.119	0.140	0.112
THREONINE	nd	0.046	0.056	0.043
SERINE	nd	0.031	0.035	0.029
GLUTAMIC ACID	nd	0.157	0.184	0.150
PROLINE	nd	0.043	0.050	0.041
GLYCINE	nd	0.017	0.019	0.015
ALANINE	nd	0.056	0.061	0.049
VALINE	nd	0.054	0.058	0.047
ISOLEUCINE	nd	0.053	0.056	0.047
LEUCINE	nd	0.133	0.150	0.119
TYROSINE	nd	0.030	0.031	0.025
PHENYLALANINE	nd	0.035	0.037	0.031
HISTIDINE	nd	0.019	0.020	0.017
LYSINE	nd	0.109	0.118	0.098
ARGININE	nd	0.024	0.026	0.020
CYSTEINE*	nd	0.031	0.033	0.042
METHIONINE*	nd	0.023	0.024	0.030

Carb = Carbohydrate only
Carb + H = Carbohydrate / Protein Hydrolysate
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + I = Carbohydrate / Intact Protein
nd = not detected
* = detected in performic acid

3.5 Metabolic & Physiological Responses:

The characteristics of metabolic and physiological responses to the four beverages that were consumed by each athlete during recovery are reported in the following order:

- Rehydration
- Cardiovascular Recovery
- Carbohydrate Metabolism
- Protein Metabolism

3.5.1 Rehydration

The rate at which the athletes rehydrated was reflected in changes in their haematocrit, plasma albumin and urine volume during recovery.

3.5.1.1 Haematocrit

The haematocrit decreased more or less monotonically through time in all athletes on all beverages (Figure 3.2, Table 3.6). The fall in haematocrit was greatest over the first 20 minutes and was similar for all beverages. The overall fall in haematocrit during the entire period of monitoring (240 minutes) appeared to be greatest when either the Carb + H beverage or the Carb + M beverage were consumed. However, there was considerable variation between subjects. Thus, statistical analysis showed no significant difference between any pairwise comparisons of beverages on repeated measures ANOVA or on multivariate (discriminant) analysis.

Table 3.6.

The mean and standard error for haematocrit (%) of all athletes for the four beverages during the time period from cessation of exercise to 240 minutes post-exercise during a post-exercise recovery study.

	Carb		Carb + I		Carb + M		Carb + H	
Time	Mean *n=10*	SE	Mean *n=11*	SE	Mean *n=11*	SE	Mean *n=10*	SE
0	47.1	1.37	46.0	0.61	45.5	0.96	45.6	1.18
10	44.2	1.10	43.6	0.91	43.3	0.96	43.0	1.17
20	43.4	1.25	43.4	0.65	42.7	0.73	42.6	1.10
30	44.6	1.36	43.7	0.79	42.9	0.72	42.4	1.14
40	43.4	1.56	43.5	1.01	42.5	0.83	42.6	1.07
50	43.7	1.43	42.8	1.07	42.4	0.85	41.6	1.12
60	43.6	1.43	43.3	0.99	42.6	0.86	41.6	1.04
75	43.0	1.59	43.4	1.04	41.8	0.84	42.8	1.19
90	44.8	1.31	43.5	0.89	42.6	0.86	42.1	1.06
105	43.4	1.46	43.3	0.96	41.5	0.79	42.1	0.87
120	43.9	1.31	43.7	0.80	42.2	0.81	41.9	0.97
150	43.1	1.23	43.5	0.86	41.8	0.96	41.8	0.91
180	43.6	1.48	42.6	0.98	40.8	0.84	40.7	1.13
210	43.5	1.40	42.3	1.07	40.3	0.78	41.0	0.87
240	43.7	1.41	42.4	0.97	40.8	0.81	40.6	0.94

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

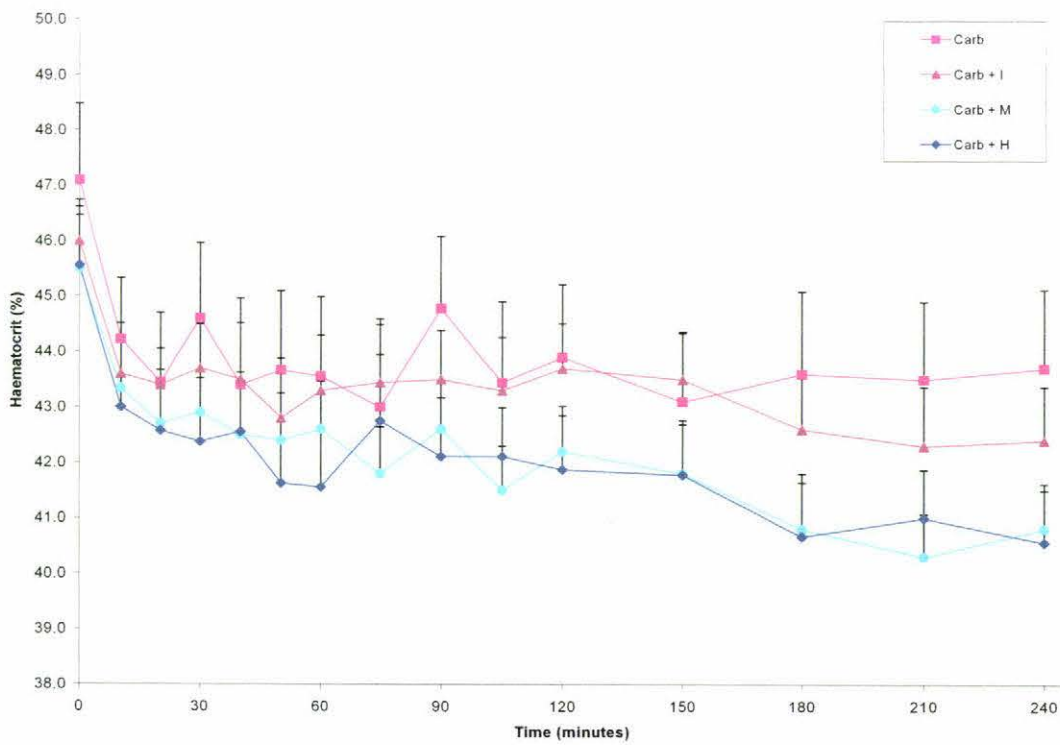

FIGURE 3.2. Mean (± SEM) haematocrit (%) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.

3.5.1.2 Plasma Albumin

The plasma albumin concentration also decreased more or less monotonically with all athletes consuming all beverages (Figure 3.3, Table 3.7). However, the plasma albumin concentration appeared to fall to a lower level when any of the beverages containing protein were being consumed compared with when the beverage containing Carb only was being consumed.

The mean plasma albumin concentrations were found to be significantly higher, on pairwise repeated measures ANOVA, when the beverage containing Carb was being consumed than that when the beverage containing Carb + H was being consumed (df 1,8, $F = 23.34$, $p<0.01$). Similarly, mean plasma albumin concentrations were also found to be significantly higher, on pairwise repeated measures ANOVA, when the Carb beverage was being consumed compared to that when the Carb + M beverage was being consumed (df 1,8, , $F = 7.44$, $p<0.05$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Thus, plasma albumin levels were significantly higher when the Carb beverage was being consumed than when either of the two beverages containing protein hydrolysate were being consumed (i.e. Carb + H and Carb + M).

Table 3.7.

The mean and standard error of plasma albumin concentration (g/L) for the response to the four beverages during the time period from cessation of exercise to 240 minutes post-exercise in a post-exercise recovery study

	Carb		Carb + I		Carb + M		Carb + H	
Time (min)	Mean *n=10*	SEM	Mean *n=11*	SEM	Mean *n=11*	SEM	Mean *n=10*	SEM
0	43.3	0.63	43.0	0.69	42.7	0.71	42.9	0.78
30	40.8	0.74	40.3	0.61	40.3	0.68	40.6	0.71
60	41.0	0.59	40.4	0.63	40.5	0.59	40.3	0.75
120	41.7	0.54	40.6	0.56	40.0	0.52	39.9	0.81
180	40.9	0.83	40.4	0.62	39.2	0.55	39.8	0.30
240	40.6	0.50	39.4	0.60	39.2	0.60	39.4	0.48

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

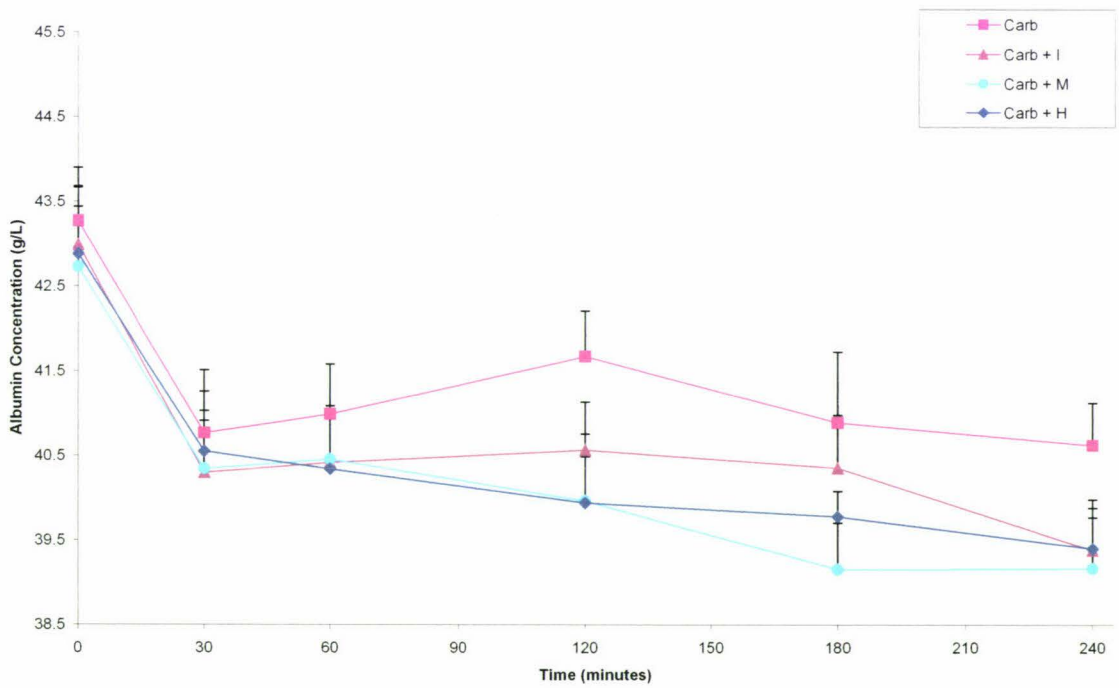

FIGURE 3.3. Mean (± SEM) plasma albumin concentrations (g/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.

3.5.1.3 Urine Production

3.5.1.3.1 Analysis of Cumulative Urine Output Over Initial 240 Minutes:

The total urine output over the first 240 minutes following exercise (Figure 3.4) varied significantly, on one-way ANOVA, depending on the beverage that was being consumed (df 3,38, $F = 4.03$, $p<0.05$). Comparisons of these differences between dietary beverages, using *post-hoc* Bonferroni adjustment, showed that the urine output was significantly higher when the Carb beverage was being consumed compared to when either of the two intact protein-containing beverages were being consumed [Carb verses Carb + I ($p<0.05$); and Carb verses Carb + M ($p<0.05$)].

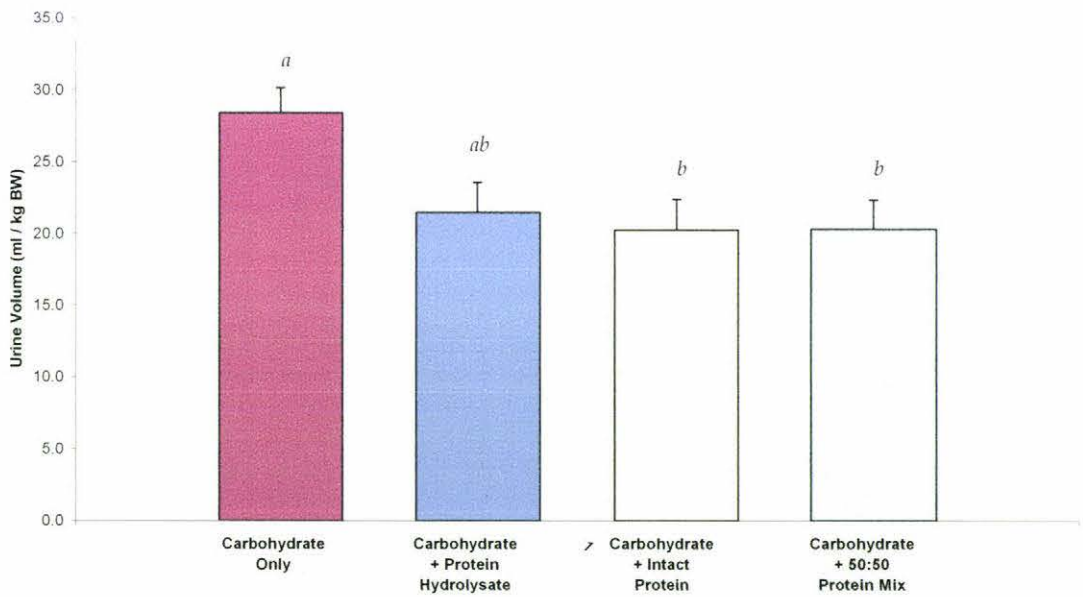

FIGURE 3.4. Mean (± SEM) total urine volumes expressed as ml/kg BWT for the first 240 minutes (fluid intake controlled during this period) in a post-exercise recovery study. *Similar letter designations implies non-significance; non-similar implies significance ($p<0.05$).*

3.5.1.3.2 Temporal Pattern of Urine Output

The urine output decreased in all athletes over the first 30 minutes after exercise (Figure 3.5, Table 3.8). This was followed by a period of increased urine output, during the time when the athletes were continuing to consume the test beverages, and peaked around 120 minutes at the time of consumption of the last drink. Urine output then fell until around 180 minutes when it again increased with all dietary beverages. After 240 minutes and expiry of the period of controlled fluid consumption, the urine output tended to again fall monotonically as water intake was self-regulated. As shown in Table 3.9, the desired levels of water consumption were achieved.

Table 3.8.

The mean and standard error of urine output results (g/30minutes) for the response to the four beverages during the time period from cessation of exercise to 420 minutes post-exercise in a post-exercise recovery study.

	Carb		Carb + I		Carb + M		Carb + H	
Time	Mean *n=10*	SE	Mean *n=11*	SE	Mean *n=11*	SE	Mean *n=10*	SE
0	174.0	23.79	188.1	31.50	189.6	47.35	201.0	29.31
30	74.2	15.64	69.4	12.63	65.2	14.26	66.8	8.35
60	170.0	43.59	158.3	28.68	130.7	22.91	141.3	23.60
90	256.9	32.25	210.6	32.04	173.6	29.89	176.3	30.35
120	325.2	22.43	180.0	25.04	161.7	28.66	175.4	32.18
150	297.3	18.21	178.0	38.06	168.6	36.99	213.9	46.95
180	218.3	15.56	171.4	16.90	166.4	21.35	156.0	35.03
210	233.2	22.04	162.3	34.03	193.7	30.62	166.5	17.07
240	326.1	29.30	210.8	40.49	295.3	21.74	307.3	22.95
300	241.3	24.10	231.8	23.64	262.7	24.36	258.0	33.63
360	166.8	16.05	223.2	21.13	242.9	29.54	218.5	15.45
420	135.6	24.58	146.4	16.13	184.7	24.03	148.7	30.96

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

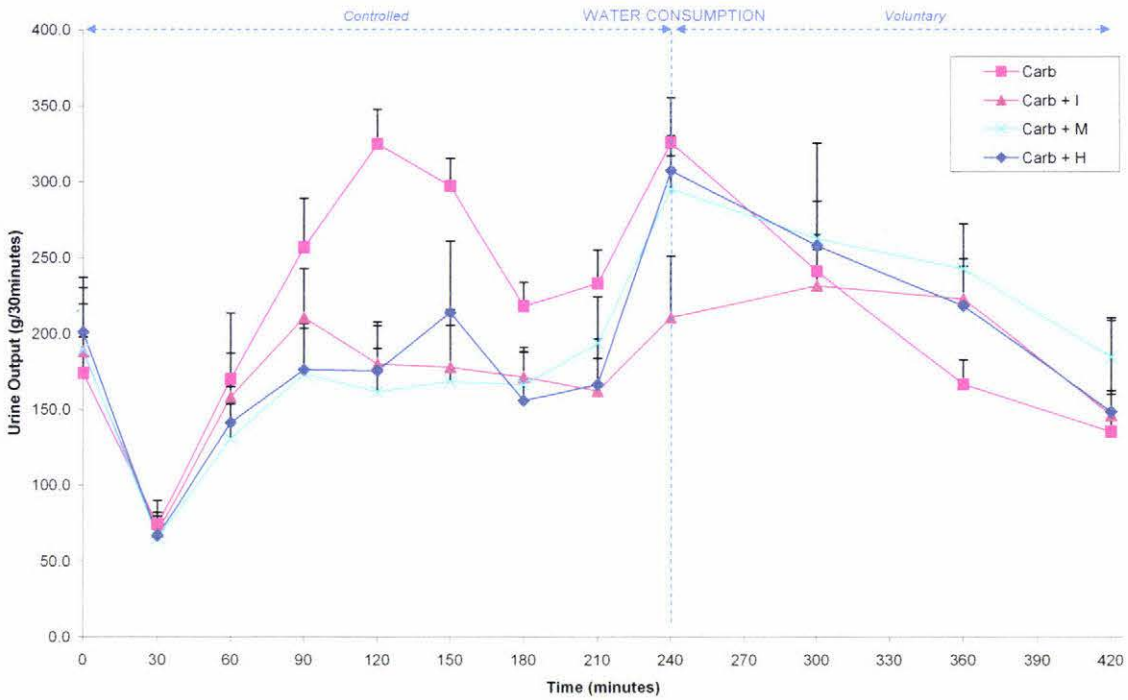

FIGURE 3.5. Mean (± SEM) urine outputs (g/30minutes) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

Table 3.9.

Desired and actual water consumption achieved throughout the experimental day in a post-exercise recovery study.

Time (minutes)	Period	Desired	Actual
-150 to -90	Pre-Exercise	7 ml/kg BWT	7 ml/kg BWT
-90 to 0	During Exercise	10 ml/kg BWT/h	10 ml/kg BWT/h
0 to 180	Beverage Consumption*	10 ml/kg BWT/h	10 ml/kg BWT/h
180 to 240	Post Beverage 1*	7 ml/kg BWT/h	7 ml/kg BWT/h
240 to 420	Post Beverage 2#	---------- Voluntary Consumption ----------	

* Blood sampling occurring during this time
\# No blood sampling occurring during this time

3.5.1.3.3 Temporal Pattern of Urine Output Over Initial 240 Minutes:

The half-hourly mean urine outputs (Table 3.8, Figure 3.5) over the initial 240 minutes following consumption of the beverage containing Carb appears to be greater than that following consumption of either of the three protein-containing beverages. These difference were shown to be significant on pairwise repeated measures ANOVA (Carb verses Carb + I beverage (df 1,7, $F = 27.49$, $p<0.01$); Carb verses Carb + H beverage (df 1,8, $F = 7.19$, $p<0.05$); and Carb verses Carb + M beverage (df 1,8, $F = 10.58$, $p<0.05$)). The same analysis also showed a significantly different profile of urine outputs over time between when the Carb beverage was being consumed and when any of the three protein-containing beverages were being consumed (Carb verses Carb + I (df 8,56, $F = 3.43$, $p<0.01$); Carb verses Carb + H (df 8,64, $F = 2.42$, $p<0.05$); and Carb verses Carb + M (df 8,64, $F = 3.44$, $p<0.01$)). Similarly, there was a significant difference in the profile of urine outputs over time when the Carb + H beverage was consumed compared with when the Carb + I beverage was consumed (df 8,56, $F = 2.42$, $p<0.05$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

3.5.1.3.4 Temporal Pattern of Urine Output Over Full 420 Minutes:

When the beverage containing Carb was being consumed the means of successive urine volumes were significantly higher, on pairwise repeated measures ANOVA, compared to that when the beverage containing Carb + I was being consumed (df 1,7, $F = 8.07$, $p<0.05$). The pattern of urine volumes over time was similarly shown to be significantly different when the Carb beverage was being consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + I beverage (df 11,77, $F = 5.93$, $p<0.01$); Carb verses Carb + H beverage (df 11,88 $F = 2.05$, $p<0.01$,); and Carb verses Carb + M beverage (df 11,88, $F = 4.6$, $p<0.01$)). During the full 420 minute

recovery period, fluid consumption was only controlled during the first 240 minutes and became voluntary following this time (Figure 3.4). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Thus, when the athlete consumed the Carb beverage they had a significantly greater urine output than when they consumed any of the three protein-containing beverages. During the period of controlled fluid consumption (initial 240 minutes), this difference was significant for total urine output, mean half-hourly urine outputs, and the pattern of mean urine outputs over time. In terms of Cumulative Urine Output over the first 240 minutes the urine output was significantly greater following consumption of the Carb beverage compared to following consumption of either the Carb + I or Carb + M beverages.

3.5.2 Carbohydrate Metabolism

Plasma insulin (Figure 3.6 and Table 3.10) and plasma glucose (Figure 3.7 and Table 3.11) showed coincident peaks at 20 and 50 minutes for all athletes and on all beverages. Following these peaks, there was a period of more sustained increase in both plasma insulin and plasma glucose that was maintained until around 180 minutes post-exercise, when both plasma insulin and plasma glucose started to fall. The pattern was similar with all beverages, with consumption of either of the three protein-containing beverages appearing to result in higher mean insulin levels and lower mean glucose levels than that observed following consumption of the beverage containing Carb only.

The pattern and magnitude of plasma insulin and plasma glucose varied significantly between subjects. This variation obscured any distinctions between dietary beverages based on plasma insulin or plasma glucose alone. However, multivariate (discriminant) analysis of the variation of plasma insulin and plasma glucose combined showed a significant level of discrimination according to dietary beverages (df 63,18, $F = 2.44$, $p<0.05$). A significant pairwise distinction was achieved between that when the Carb

beverage was being consumed compared with that observed when the Carb + M beverage was being consumed (df 21,6, $F = 4.66$, $p<0.05$). Similarly, significant pairwise distinction was also achieved between that when the Carb + M beverage was being consumed compared with that observed when the Carb + H beverage was being consumed (df 21,6, $F = 7.73$, $p<0.01$). These distinctions were both on the basis firstly, of a contrast between plasma insulin and plasma glucose during the peaks that occur around 20 and 50 minutes, and secondly on the basis of the magnitude of the increase and subsequent decrease in both plasma insulin and plasma glucose around 120 and 180 minutes. (Note that results for time zero were removed in this multivariate analysis on the basis that at this point it is extremely unlikely that beverage consumption would have influenced any physiological or biological measures under surveillance).

A subsequent analysis was performed on the same data for plasma insulin and plasma glucose that had been corrected for haematocrit using the same statistical methodology in order to determine whether the effects of the dietary beverages described above were independent of the athlete's hydration status. This analysis again showed a significant overall level of discrimination (df 63,12, $F = 2.77$, $p<0.05$) between dietary beverages and a significant distinction between when the Carb beverage was consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 21,4, $F = 9.36$, $p<0.05$); Carb verses Carb + I (df 21,4, $F = 9.79$, $p<0.05$); Carb verses Carb + M (df 21,4, $F = 12.67$, $p<0.05$)). These distinctions were again on the basis of the contrast between plasma insulin and plasma glucose for the peaks that occurred around 20 and 50 minutes and the decrease in concentration following this, as well as a contrast of the peak around 120 and 150 minutes and the magnitude of the decrease following this.

Thus, there was a significant difference in the relationship between plasma insulin and plasma glucose levels when the Carb + M beverage was being consumed compared with the levels recorded when either the beverage containing Carb or the beverage containing Carb + H were being consumed. When data was corrected for haematocrit to account for the hydration status of the athlete, consumption of the beverage containing Carb resulted in significantly higher plasma glucose and lower plasma insulin levels compared to that observed when any of the three protein-containing beverages were being consumed.

Table 3.10.

The mean and standard error results of plasma insulin concentration (mmol/L) for the response to the four beverages during the time period from cessation of exercise to 240 minutes post-exercise in a post-exercise recovery study.

	Carb		Carb + I		Carb + M		Carb + H	
Time	Mean *n=10*	SE	Mean *n=11*	SE	Mean *n=11*	SE	Mean *n=10*	SE
0	8.0	1.097	6.7	1.327	9.3	2.917	7.6	0.993
10	29.3	6.407	35.0	7.875	29.1	3.679	33.5	5.006
20	43.7	9.639	59.8	15.425	52.0	9.150	67.0	12.726
30	30.4	5.078	42.9	8.413	37.1	5.149	34.8	4.786
40	30.8	6.824	40.3	6.707	45.3	5.652	45.6	11.763
50	42.5	11.192	70.1	17.371	64.0	14.949	73.4	24.607
60	27.3	4.299	45.5	7.319	47.8	9.047	43.9	8.028
90	38.6	6.346	64.7	14.691	49.5	10.836	68.5	18.964
120	41.4	6.551	59.4	15.875	57.4	13.132	55.6	12.207
150	42.0	8.249	61.0	14.301	74.2	19.381	66.3	16.966
180	27.9	5.756	70.1	17.098	51.0	14.683	57.9	11.873
240	16.4	3.754	34.9	9.244	21.3	3.231	29.1	11.682

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

Table 3.11.

The mean and standard error results of plasma glucose concentration (mmol/L) for the response to the four beverages during the time period from cessation of exercise to 240 minutes post-exercise in a post-exercise recovery study.

	Carb		Carb + I		Carb + M		Carb + H	
Time	Mean *n=10*	SE	Mean *n=11*	SE	Mean *n=11*	SE	Mean *n=10*	SE
0	4.5	0.24	4.3	0.19	4.4	0.16	4.4	0.27
10	5.7	0.15	5.4	0.17	5.3	0.13	5.5	0.19
20	6.6	0.17	6.1	0.14	5.9	0.24	6.1	0.28
30	5.4	0.37	5.2	0.16	5.2	0.16	5.0	0.23
40	5.2	0.26	4.7	0.12	4.8	0.16	4.6	0.17
50	5.3	0.27	5.1	0.21	5.0	0.17	5.1	0.23
60	5.3	0.19	4.8	0.13	4.6	0.19	4.6	0.16
90	5.2	0.22	4.7	0.17	4.9	0.20	5.0	0.20
120	5.4	0.15	4.9	0.18	5.0	0.12	5.2	0.16
150	5.4	0.20	5.1	0.15	5.4	0.13	5.2	0.19
180	5.5	0.20	5.4	0.15	5.1	0.15	5.3	0.21
240	4.0	0.28	4.5	0.22	4.3	0.21	4.5	0.26

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

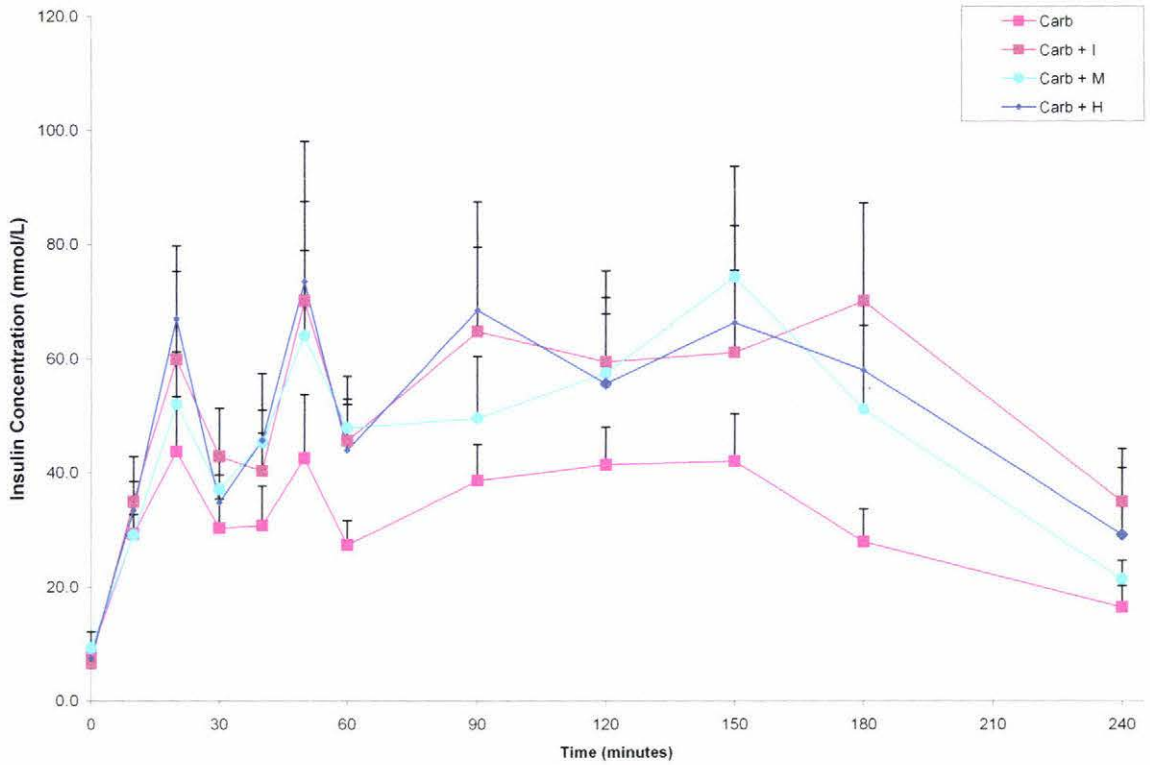

FIGURE 3.6. Mean (± SEM) plasma insulin concentrations (mmol/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.

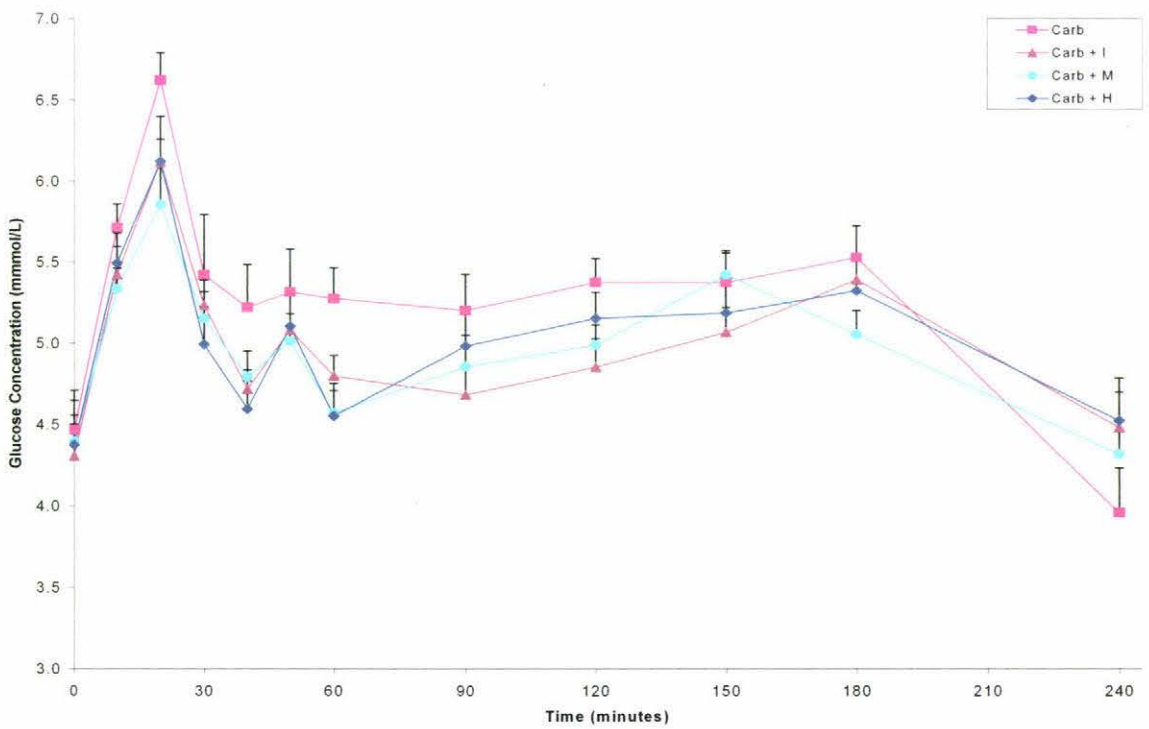

FIGURE 3.7. Mean (± SEM) plasma glucose concentrations (mmol/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 240 minute period following exercise and recovery beverage consumption.

3.5.3 Cardiovascular Recovery

3.5.3.1 Heart Rate

The results for heart rate recovery are an accumulation of 4900 data points for each dietary beverage, which represents a mean heart rate recording across the subjects every five seconds throughout the recovery period.

The fall in mean heart rates (pulse decrement) was more or less linear in all cases (Figure 3.8 and 3.9). Thus pulse decrement could be fitted by linear regression with high levels of correlation (Table 3.12). The slopes of these regression lines were visibly different between diet treatments. The overall significance of these differences in the slopes of pulse decrement was confirmed by the presence of a significant interaction term (df 3,60, $F = 43.66$, $p<0.001$) and a significant difference in slope intersect term (df 3,60, $F = 36.99$, $p<0.001$) on ANCOVA of the effect of the four beverages on the relativity of pulse decrement with time.

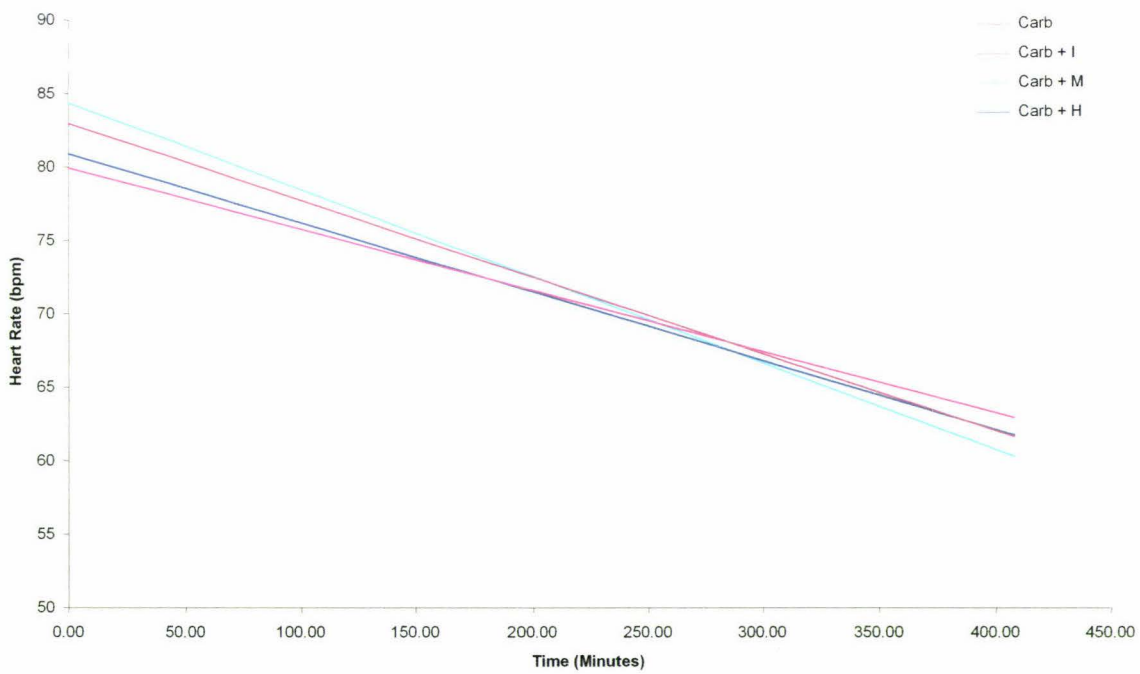

FIGURE 3.8. Linear regression analysis results of mean post-exercise pulse rate recovery (bpm) (n=4900) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H), intact whey protein (Carb + I), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M) in a 240 minute period following exercise and recovery beverage consumption.

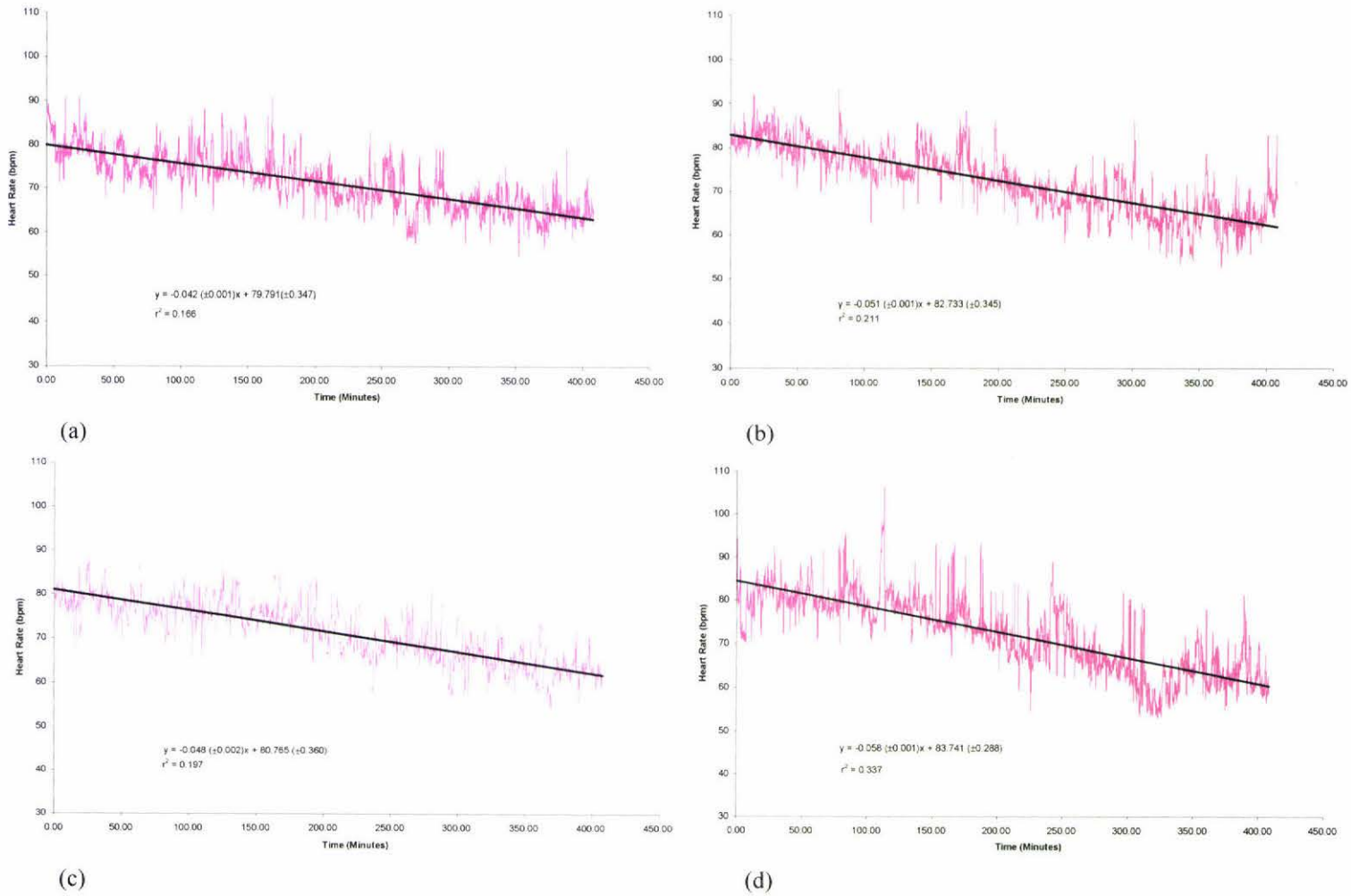

Figure 3.9. Linear regression analysis results of mean post-exercise pulse rate recovery (bpm) following consumption of each of the four dietary beverages (n=4900): (a) Carbohydrate Only; (b) Carbohydrate and Intact Protein; (c) Carbohydrate / Protein Hydrolysate; (d) Carbohydrate and 50% whey protein hydrolysate and 50% intact whey protein, in a 240 minute period following exercise and recovery beverage consumption.

Table 3.12.

Linear Regression results for heart rate during the post-exercise recovery period in a post-exercise recovery study.

Dietary Beverage	Slope	Slope SE	r^2	Intercept	Intercept SE
Carb	-0.042	0.001	0.166	79.791	0.347
Carb + H	-0.048	0.002	0.197	80.765	0.360
Carb + I	-0.051	0.001	0.211	82.733	0.345
Carb + M	-0.058	0.001	0.337	83.741	0.288

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

The slope coefficients of the regressions of pulse rate over time during the period when each of the four beverages were being consumed were also compared directly in a pairwise fashion by student's *t*-test (Table 3.13). These comparisons showed that the rates of fall in pulse rate when the three protein-containing beverages were being consumed were all significantly greater than that when the Carb beverage was consumed. The rate of the fall of pulse rate during the period when the Carb + M beverage was being consumed was also significantly greater compared to when either of the other two other protein-containing beverages were being consumed. The rate of fall of pulse during the period when the Carb + H was being consumed and that when the Carb + I beverages was being consumed was found not to be significantly different on any pairwise comparisons.

Table 3.13.

Analysis results[+] of slope coefficients for post-exercise heart rate recovery for the response to four dietary beverages during a post-exercise recovery study.

	Carb		Carb + H		Carb + I		Carb + M	
	t value	p	t value	p	t value	p	t value	p
Carb	----	----						
Carb + H	2.96	$p<0.01$	----	----				
Carb + I	4.26	$p<0.001$	1.23	NS	----	----		
Carb + M	8.60	$p<0.001$	5.21	$p<0.001$	3.943	$p<0.001$	----	----

[+] Students *t*-test, significance assumed at $p<0.05$
Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

Thus, the rate of decrease in pulse rate during recovery was significantly greater when any of the three protein-containing beverages were being consumed compared to consumption of the beverage containing Carb only. Of these, consumption of the Carb + M beverage had the most significant effect in decreasing pulse rate during recovery. There were no significant differences in the rate of decrease of pulse during the period when the Carb + I and that when the Carb + H beverages were being consumed.

3.5.3.2 Systolic Blood Pressure

The systolic blood pressure tended to increase when all athletes consumed all beverages until 120 minutes after the cessation of exercise (Figure 3.10). Following this period, once all beverages had been consumed, systolic blood pressure tended to decrease in a more or less monotonic fashion until recordings ceased. There was wide variation across treatments over the recording period, so as to cause mean values on different beverages to have no clear relationship to each other.

Analysis of systolic blood pressure by pairwise repeated measures ANOVA showed no significant difference in mean systolic blood pressure during recovery on the different dietary treatments. However, a significant difference in the variation of systolic blood pressure recordings over time was found between when the Carb beverage was being consumed and when the Carb + H beverage (df 11,55, $F = 3.74$, $p<0.005$) was being consumed. Similarly, there was a significant difference between systolic blood pressure change when the Carb + H beverage was being consumed compared to when the Carb + I beverage (df 11,55, $F = 2.34$, $p<0.05$) was being consumed. Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Comparisons of systolic blood pressure over time by multivariate (discriminant) analysis showed a significant overall discrimination (df 36, 98, $F = 2.04$, $p = 0.005$) between beverages. There was a significant distinction in the pattern of response when the Carb + H beverage was being consumed compared to that observed when the Carb beverage (df 12,33, $F = 2.81$, $p<0.01$) was being consumed. Similarly, there was a significant distinction in the pattern of responses when the Carb + H beverage was being consumed from that when the Carb + M (df 12,33, $F = 2.74$, $p<0.05$) was being consumed, and a difference from when the Carb + I beverage was being consumed and that when Carb + M beverage was being consumed (df 12,33, $F = 2.13$, $p<0.05$). These distinctions were all on the basis of the contrast between the early and late systolic blood

pressure values, chiefly on the basis of the values at time 0 and 120 minutes being contrasted with the values at 180 and 300 minutes.

Thus, no significant overall differences were found in mean systolic blood pressure during recovery from exercise between the different dietary beverages. However, significant differences were found in the pattern of variation in mean systolic blood pressures over time, mainly between when the Carb + H beverage was being consumed and that when any of the other three beverages were being consumed. However, no clear relationship between pairwise comparisons could be determined.

3.5.3.3 Diastolic Blood Pressure

The variation of diastolic blood pressure over time (Figure 3.11) shows an opposite trend to that of the systolic blood pressure. Thus, the diastolic blood pressure decreased monotonically for around 180 minutes, and then tended to increase until the end of the recording period on all dietary beverages. As was the case with systolic blood pressure, there was wide variation across beverages over the recording period, so as to cause mean values on individual dietary beverages to have no clear relationship to each other.

The mean diastolic blood pressures following consumption of the Carb + M beverage were shown to be significantly higher, on pairwise repeated measures ANOVA, than that following consumption of the Carb + H beverage (df 1,9, $F = 8.63$, $p<0.05$). There were no significant differences between mean diastolic blood pressures with any other pairwise comparisons of beverages. The pattern of variation of diastolic blood pressure over time was significantly different when the Carb + M beverage was being consumed, from that when any of the three other beverages were being consumed (Carb + M verses Carb (df 11, 55, $F = 3.84$, $p<0.001$); Carb + M verses Carb + I (df 11,77, $F = 2.33$, $p<0.05$); and Carb + M verses Carb + H (df 11,99, $F = 3.95$, $p<0.001$)). Similarly, there was a significant difference in the pattern of variation of diastolic blood pressure over

time when the Carb beverage was consumed compared to when the Carb + H beverage (df 11,55, $F = 2.76$, $p<0.01$) was being consumed. Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

The analysis of the variation of diastolic blood pressure with time by multivariate (discriminant) analysis showed a significant overall discrimination between dietary beverages (df 36,98, $F = 1.68$, $p<0.05$). There was a significant distinction found in the pairwise comparison of the Carb and Carb + H beverages (df 12,41, $F = 2.46$, $p<0.05$) on the basis of a contrast between the early and late diastolic blood pressure values, chiefly on the basis of the blood pressure values at time 0 and 180 minutes being contrasted with those at 270 and 300 minutes.

Thus, consumption of the beverage containing Carb + M resulted in higher mean diastolic blood pressures than that observed following consumption of the Carb + H beverage. In terms of the pattern of variation of diastolic blood pressure over time, consumption of the Carb + M beverage resulted in a significantly different blood pressure response when compared to that following consumption of any of the other three dietary beverages. Similarly, a significant difference was also shown for diastolic blood pressure pattern following consumption of the Carb beverage compared with that observed following consumption of the Carb + H beverage. Due to the large amount of variation in diastolic blood pressures over time, the relationship of these significant differences is unclear.

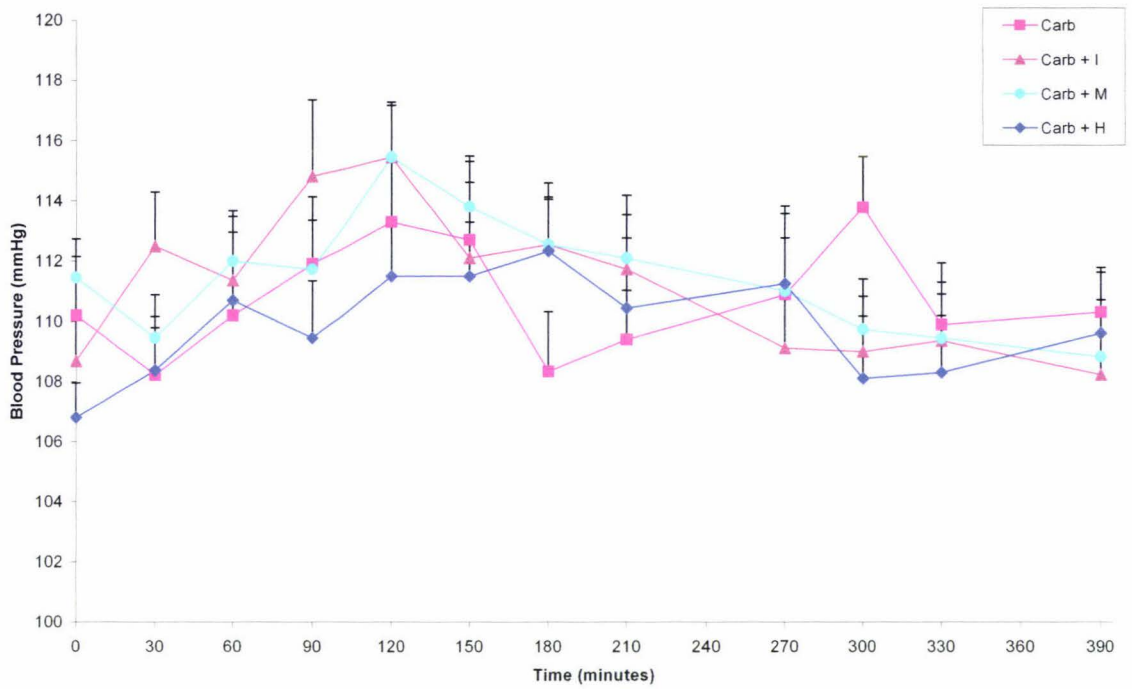

FIGURE 3.10. Mean (± SEM) systolic blood pressures (mmHg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

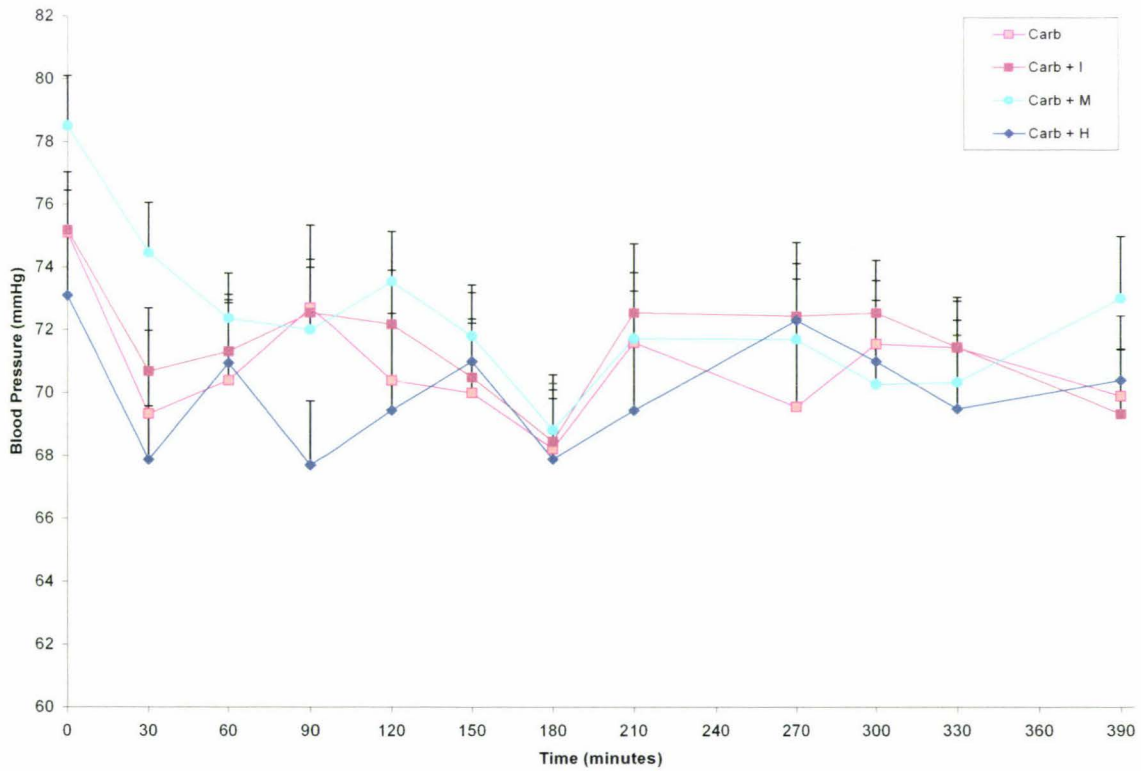

FIGURE 3.11. Mean (± SEM) diastolic blood pressures (mmHg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

3.5.3.4 Pulse Pressure

Pulse pressure, defined as the difference between systolic and diastolic blood pressure, showed a general monotonic increase across all dietary beverages over time until around 150 minutes after cessation of exercise, after which there was a general monotonic decrease that was maintained until the end of the recording period (Figure 3.12).

Analysis of the variation in pulse pressure by pairwise repeated measures ANOVA showed that mean pulse pressure was significantly different when the Carb + I beverages was being consumed compared to when the Carb + M beverage was consumed (df 1,7, $F = 15.74$, $p<0.05$). There was also a significant difference in the pattern of variation of pulse pressure over time when the Carb beverage was being consumed and that when Carb + H beverage was being consumed (df 11,55, $F = 5.13$, $p<0.001$). Similarly, there was a significant difference in the pattern of variation of pulse pressure over time between that reported following the consumption of the Carb and that when the Carb + M beverage was consumed (df 11,55, $F = 2.73$, $p<0.01$), as well as between that following the consumption of the Carb + H and that when the Carb + M beverage was consumed (df 11,99, $F = 2.26$, $p<0.05$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

There was a significant overall discrimination between beverages in the pattern of the variation of pulse pressure over time on multivariate (discriminant) analysis (df 36,98, $F =2.16$, $p<0.005$). There was a significant distinction in the pattern of pulse pressure following consumption of the Carb beverage and that observed when each of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 12,33, $F = 2.32$, $p<0.05$); Carb verses Carb + I (df 12,33, $F = 2.19$, $p<0.05$); and Carb verses Carb + M (df 12,33, $F = 3.19$, $p<0.01$)). There was also a distinction found in the pattern of variation in pulse over time when the Carb + M beverage was being consumed compared with when either of the other two protein-containing beverages were being consumed (Carb + M verses Carb + H (df 12,33, $F = 2.24$, $p<0.05$); Carb + M verses Carb

+ I (df 12,33, F = 2.89, $p<0.01$). These distinctions were primarily on the basis of the contrast between the rise in pulse pressure values, between 30 and 120 minutes, and the fall in pulse pressure following this, between 150 to 210 minutes.

Thus, while a number of significant differences were highlighted on both pairwise repeated measures ANOVA and multivariate (discriminant) analysis, there was no clear difference in the pattern of response following consumption of each of the four dietary beverages

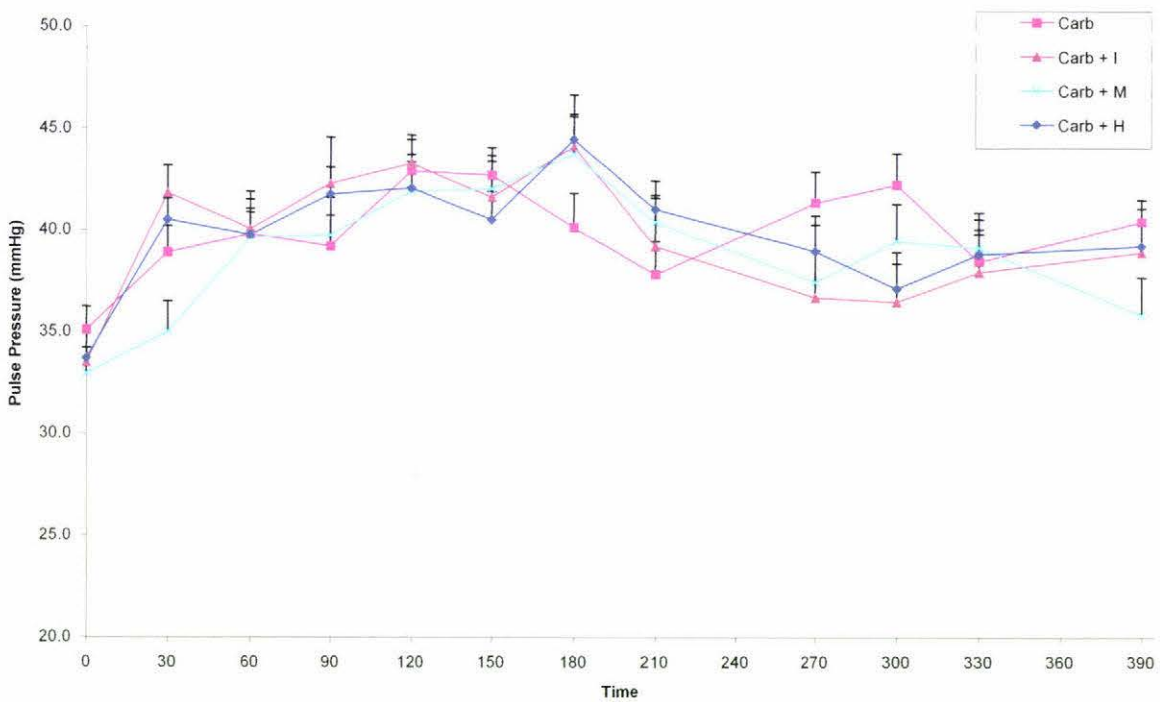

FIGURE 3.12. Mean (± SEM) pulse pressure values (mmHg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

3.5.4 Protein Recovery

3.5.4.1 Urinary 3-methylhistidine

After the initial period of 120 minutes following exercise during which time urinary 3-methylhistidine concentration varied widely, the urinary 3-methylhistidine level excreted tended to decrease monotonically on all beverages (Figure 3.13). The mean amount of urinary 3-methylhistidine excreted during this period appeared to be lower when the Carb beverage was being consumed than that observed when any of the three protein-containing beverages were being consumed.

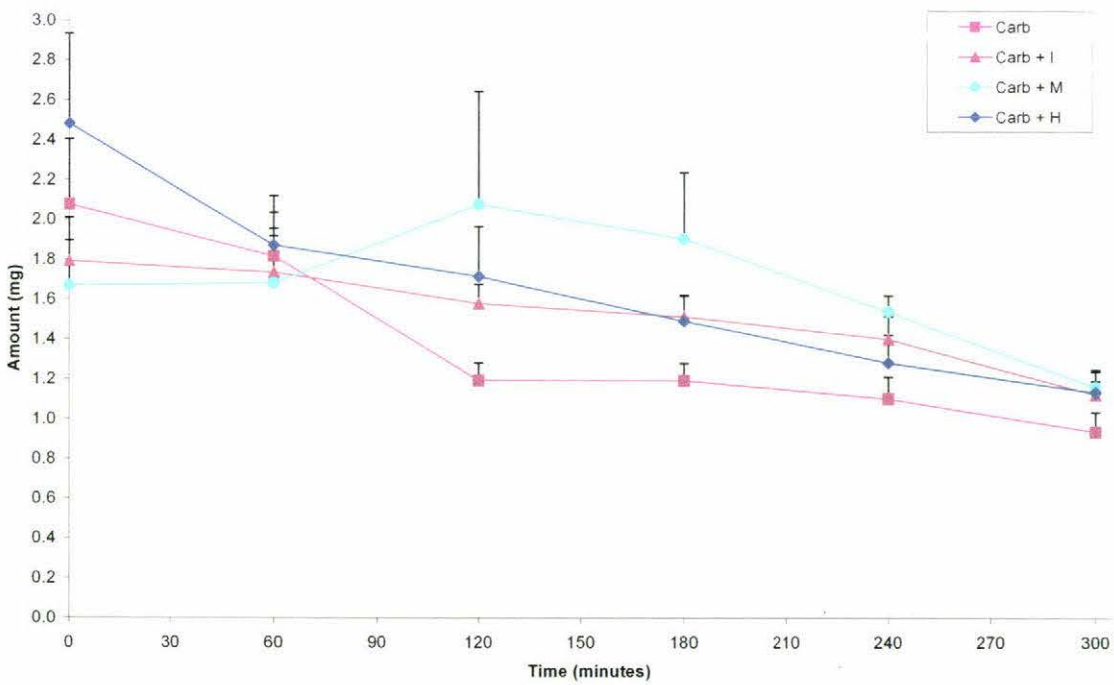

FIGURE 3.13. Mean (± SEM) urinary 3-methylhistidine excretion (mg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

This was confirmed by pairwise repeated measures ANOVA which showed significantly lower mean levels of urinary 3-methylhistidine when the Carb beverage was being consumed than that when the Carb + H beverage was being consumed (df 1,8, $F = 24.62$, $p<0.01$). There was a significant difference in the pattern of variation of urinary 3-methylhistidine concentration with time when the Carb beverage was being consumed compared with when either of the two intact protein containing beverages were being consumed (Carb verse Carb + I (df 5,40, $F = 2.67$, $p<0.05$); and Carb verses Carb + M (df 5,30, $F = 4.01$, $p<0.05$)). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Thus, mean levels of urinary 3-methylhistidine concentration were found to be significantly lower when the Carb beverage was being consumed than that observed when the Carb + H beverage were being consumed. Significant differences were also present in the variation of 3-methylhistine levels over time between that when the Carb beverage was being consumed and that when either of the two intact protein-containing beverages were being consumed.

3.5.4.2 Urinary Urea

Urinary urea nitrogen values fell over the first 30 minutes after the cessation of exercise, and subsequently rose, peaking at approximately 60 minutes when the Carb beverage was being consumed and at approximately 180-210 minutes when the three protein-containing beverages were being consumed (Figure 3.14). The mean urinary urea nitrogen values appeared to be lower after consumption of the beverage containing Carb than after consumption of any of the three protein-containing beverages.

This was confirmed on pairwise repeated measures ANOVA which showed that urinary urea nitrogen levels when the Carb beverage was being consumed were significantly lower than that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 1,8, $F = 21.38$, $p<0.005$); Carb verses Carb + I (df 1,7, $F = 62.20$, $p<0.001$); and Carb verses Carb + M (df 1,8, $F = 51.37$, $p<0.001$)). Similarly, significantly lower mean urinary urea nitrogen levels were also found after consumption of the Carb + H beverage compared to when the Carb + I beverage was being consumed (df 1,7, $F = 8.89$, $p<0.05$). Significant differences in the pattern of variation of urinary urea nitrogen were also found when the beverage containing Carb beverage was being consumed from that when each of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 9,72, $F = 5.30$, $p<0.001$); Carb verses Carb + I (df 9,63, $F = 13.03$, $p<0.001$); and Carb verses Carb + M (df 6,72, $F = 13.29$, $p<0.001$)). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

There was a significant difference in the rate of change of urinary urea nitrogen content over time between the four beverages on multivariate (discriminant) analysis (df 30,65, $F = 3.75$, $p<0.0001$). There was successful discrimination between the pattern of excretion when the Carb beverage was being consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 10,22, $F = 16.00$, $p<0.001$); Carb verses Carb + I (df 10,22, $F = 20.08$, $p<0.001$); and Carb verses Carb + M (df 10,22, $F = 17.90$, $p<0.001$)). These distinctions were all on the basis of the contrast

between the early and late values of urinary urea nitrogen excreted, chiefly on the basis of a contrast between the values at 30 and 60 minutes contrasted with those from 180 minutes onwards.

Thus there was a significantly lower urinary urea nitrogen excretion when the Carb beverage was being consumed than when any of the three protein containing beverages were being consumed. Similarly, a significantly lower urinary urea nitrogen excretion was found following consumption of the Carb + H beverage than that found following consumption of the Carb + I beverage. The pattern of excretion of urinary urea nitrogen when the Carb beverage was being consumed was significantly different than when any of the three protein-containing beverages were being consumed.

3.5.4.3 Plasma Urea

Plasma urea concentrations (Figure 3.15) during the recovery period showed a general tendency to increase over the first 240 minutes during the period when each of the three protein containing beverages were being consumed. However, when the Carb beverage was being consumed, the plasma urea concentrations increased only during the first 30 minutes, and subsequently decreased in a more or less linear fashion during the remainder of the experimental period.

The mean plasma urea concentration was found to be significantly lower, on pairwise repeated measures ANOVA, when the Carb + H beverage was being consumed compared to when the Carb + M beverage was being consumed (df 1,5, $F = 5.91$, $p<0.05$). Furthermore, there was a significant difference in the pattern of variation of plasma urea concentration over time, on pairwise repeated measures ANOVA, between the period when the Carb beverage was being consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 9,45, $F = 96.73$, $p<0.001$); Carb verses Carb + I (df 9,54, $F = 24.27$, $p<0.001$); and Carb verses Carb + M (df 9,54, $F = 5.43$, $p<0.001$)). Similarly, there was a significant difference in the pattern of variation of plasma urea concentration over time between the period when the Carb + H beverage and that when the Carb + I beverage were being consumed (df 9,54, $F = 3.66$, $p<0.005$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

There was significant discrimination on multivariate (discriminant) analysis between the pattern of variation of plasma urea responses over the periods when the four beverages were being consumed (df 30,65, $F = 2.72$, $p=0.0004$). Significant distinctions were found between the period when the Carb beverage was being consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 10,22, $F = 7.08$, $p<0.01$); Carb verses Carb + I (df 10,22, $F = 9.41$, $p<0.01$); and Carb verses Carb + M (df 10,22, $F = 10.41$, $p<0.01$)). These distinctions were on the basis of

a contrast in the magnitude of the plasma urea concentration at time points 50, 60 and 120 minutes with those at time points 20, 90 and 150 to 180 minutes.

Thus, consumption of the Carb beverage resulted in significantly lower plasma urea concentrations in terms of the pattern of variation over time than that following consumption of any of the three protein-containing beverages. The mean plasma urea concentration was significantly lower following consumption of the Carb + H beverage compared with that observed following consumption of the Carb + M beverage.

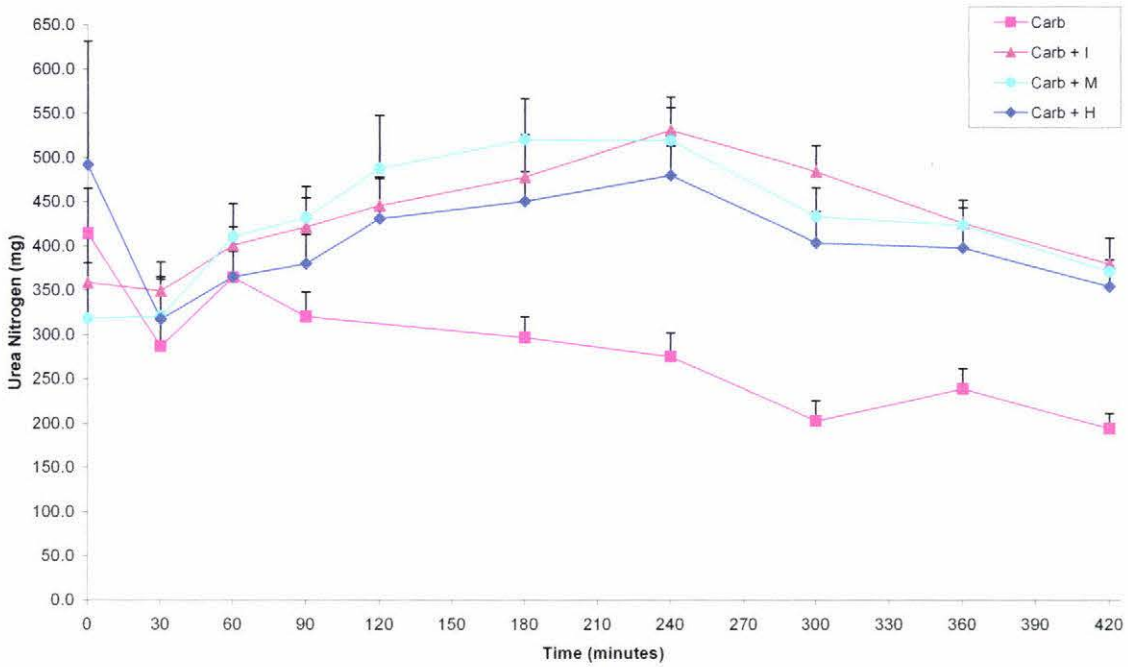

FIGURE 3.14. Mean (± SEM) urinary urea nitrogen excretion (mg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

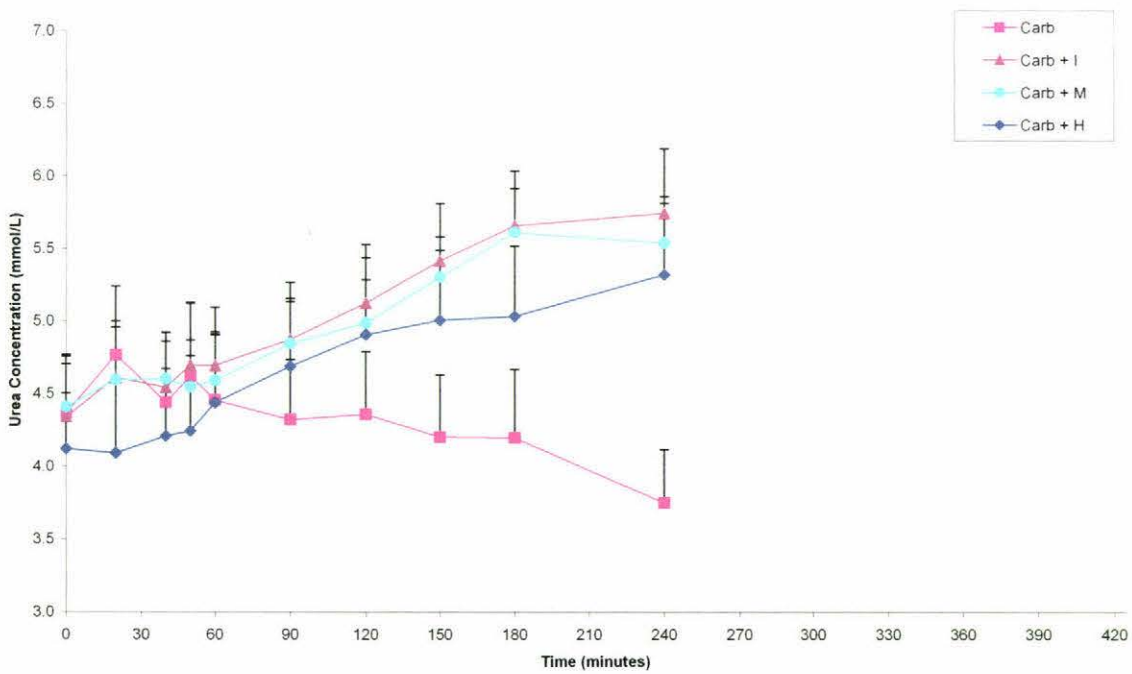

FIGURE 3.15. Mean (± SEM) plasma urea concentration (mmol/L) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

3.5.4.4 Urinary Ammonia

The pattern of urinary ammonia nitrogen concentrations (Figure 3.16) during recovery was broadly similar to that of urinary urea nitrogen excretion. There was a similar decrease over the first 30 minutes with all drinks. Again the urinary ammonia nitrogen concentration when the Carb beverage was being consumed remained more or less constant but peaked around 180-210 minutes when each of the three protein-containing beverages were being consumed.

Consumption of the Carb beverage resulted in significantly lower mean urinary ammonia nitrogen levels, on pairwise repeated measures ANOVA, than that observed following consumption of any of the three protein-containing beverages (Carb verses Carb + H (df 1,8, $F = 8.12$, $p<0.05$); Carb verses Carb + I (df 1,7, $F = 19.17$, $p<0.01$); and Carb verses Carb + M (df 1,8, $F = 20.74$, $p<0.01$)). Similarly, pairwise comparisons of the three protein-containing beverages showed that consumption of the Carb + H beverage resulted in significant lower mean urinary ammonia nitrogen levels than that observed following consumption of either of the other two protein-containing beverages (Carb + H verses Carb + I (df 1,7, $F = 24.24$, $p<0.01$); and Carb + H verses Carb + M (df 1,8, $F = 7.64$, $p<0.05$)). The same analysis showed significant differences in the patterns of variation of urinary ammonia nitrogen levels over time when the Carb beverage was being consumed compared to those when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 9,72, $F = 2.82$, $p<0.01$); Carb verses Carb + I (df 9,63, $F = 6.47$, $p<0.001$); and Carb verses Carb + M (df 9,72, $F = 7.96$, $p<0.001$)). A significant difference in the pattern of variation in urinary ammonia levels over time was also found between consumption of the Carb + H beverage compared to when the Carb + M beverage was being consumed (df 9,72, $F = 3.16$, $p<0.005$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

There was similarly a significant difference on multivariate (discriminant) analysis in the pattern of variation of urinary ammonia nitrogen over time when the four beverages were being consumed (df 30,65, $F = 2.37$, $p=0.0018$). A

significant distinction was found between when the Carb beverage was being consumed and that when any of the three protein-containing beverages were being consumed (Carb verses Carb + H (df 10,22, F = 4.14, $p<0.01$); Carb verses Carb + I (df 10,22, F = 6.77, $p<0.001$); and Carb verses Carb + M (df 10,22, F = 5.83, $p<0.001$)). This distinction was chiefly on the basis of the contrast between the value of urinary ammonia nitrogen at 60 minutes with those at 90 and 180 minutes.

Thus, consumption of the beverage containing Carb resulted in significantly lower urinary ammonia nitrogen levels than consumption of any of the three protein-containing beverages. Of the three protein-containing beverages, consumption the Carb + H beverages resulted in significantly lower urinary ammonia nitrogen levels than that following consumption of either of the other two protein-containing beverages (Carb + I or Carb + M).

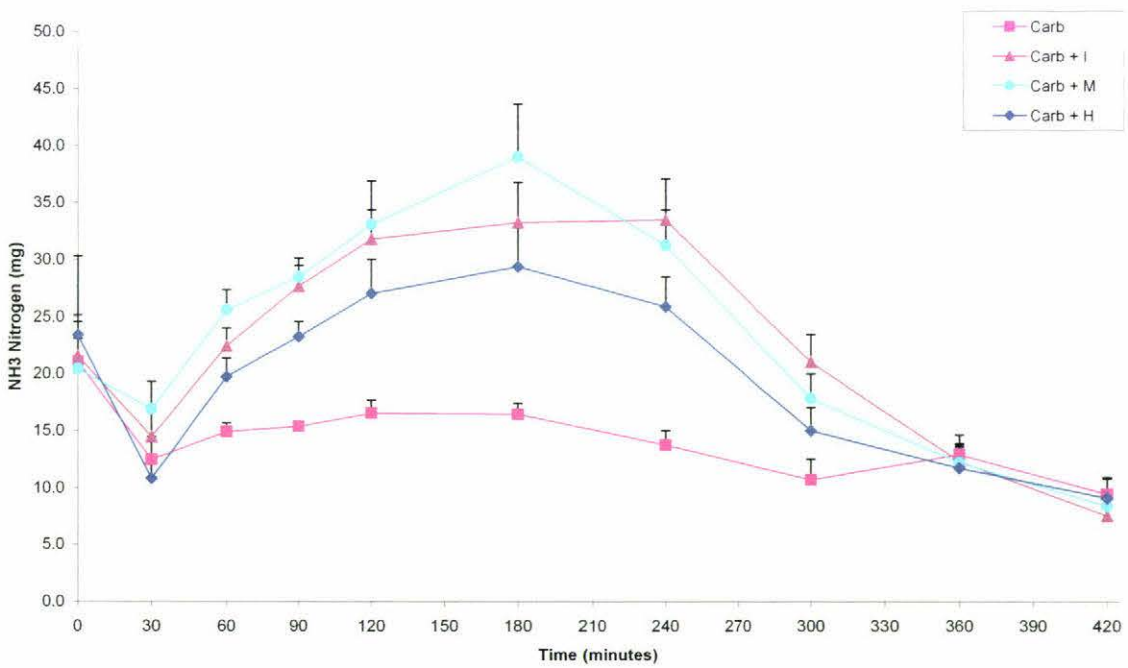

FIGURE 3.16. Mean (± SEM) urinary ammonia nitrogen excretion (mg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

3.5.4.5 Urinary Creatinine

Consumption of all four beverages during recovery gave similar temporal profiles of total urinary creatinine nitrogen excretion (Figure 3.15). This was characterised by a monotonic decrease in urinary creatinine nitrogen over the first 300 minutes. After 300 minutes, all four dietary beverages showed a steady level of urinary creatinine nitrogen excretion. No significant differences, either in the pattern or the quantity of urinary creatinine nitrogen excretion, were found between beverages either by pairwise repeated measures ANOVA or multivariate (discriminant) analysis.

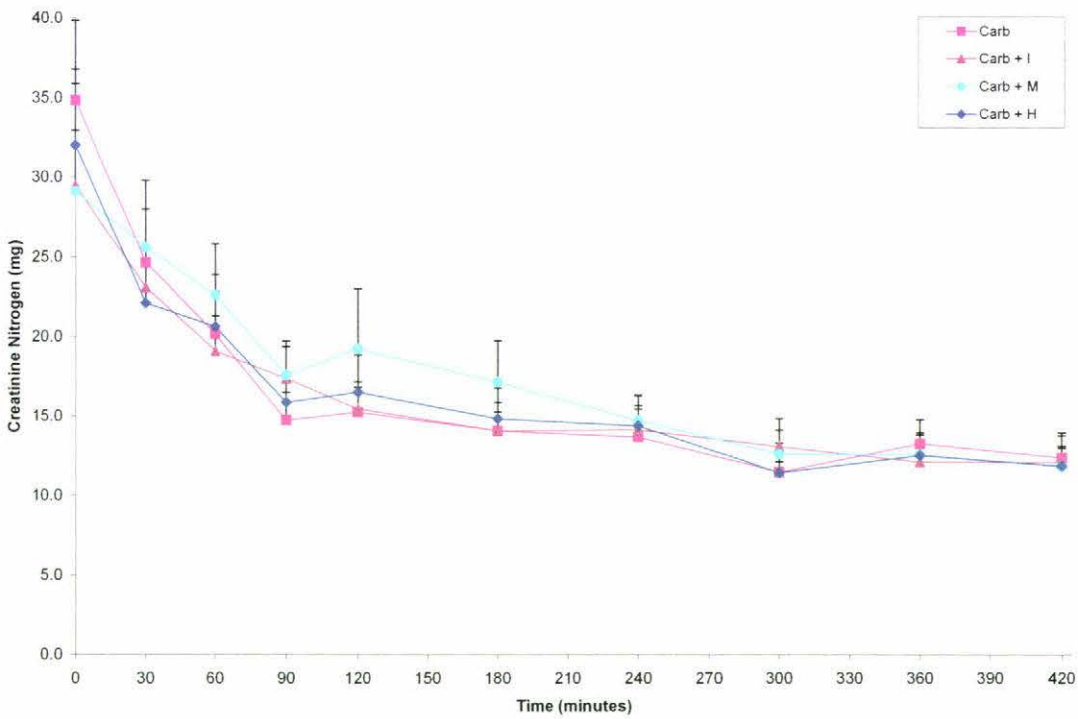

FIGURE 3.17. Mean (± SEM) total urinary creatinine nitrogen excretion (mg) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

3.5.4.6 Plasma Essential Amino Acids

The pattern of variation of the essential amino acids (Valine, Methionine, Leucine, Isoleucine, Phenylalanine, Tryptophan and Lysine) in plasma over time (Figures 17-24) are broadly similar. Consumption of the three protein-containing beverages resulted in a monotonic increase in the levels of all amino acids over the first 180 minutes following cessation of exercise, followed by a decrease towards the end of the recording period. Conversely, the plasma level of essential amino acids showed a monotonic fall in amino acid levels, which was maintained through the recovery period, when the Carb beverage was consumed.

There was significant differences in the mean levels of various essential amino acids on pairwise repeated measures ANOVA following consumption of the Carb beverage compared with those observed following consumption of each of the three protein-containing beverages (Table 3.14). Consumption of the Carb beverage resulted in significantly lower plasma amino acid levels than consumption of any of the three protein-containing beverages. The same analyses showed that consumption of the beverage containing Carb + I resulted in significantly higher plasma amino acid levels than did consumption of the beverage containing Carb + H for the essential amino acids Valine, Leucine, Isoleucine, Phenylalanine, and Tryptophan (Table 3.14). Similarly, consumption of the beverage containing Carb + I resulted in significantly higher amino acid levels than that observed following consumption of the beverage containing Carb + M for the essential amino acids Valine, Leucine and Isoleucine (Table 3.14). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant. When plasma essential amino acid levels are corrected for haematocrit, to account for the athletes' hydration state, the resulting plasma amino acid concentrations following consumption of four beverages remained in the same relative order.

Thus, consumption of the beverage containing Carb resulted in significantly lower mean plasma essential amino acid levels than that observed after consumption of any of the three protein-containing beverages. Consumption of

the beverage containing intact protein resulted in significantly higher plasma essential amino acid levels of valine, leucine, isoleucine, phenylalanine and tryptophan than those following consumption of the beverage containing Carb + H. Similarly, consumption of the Carb + I also resulted in significant higher plasma amino acid levels of valine, leucine and isoleucine than those following consumption of the Carb + M beverage.

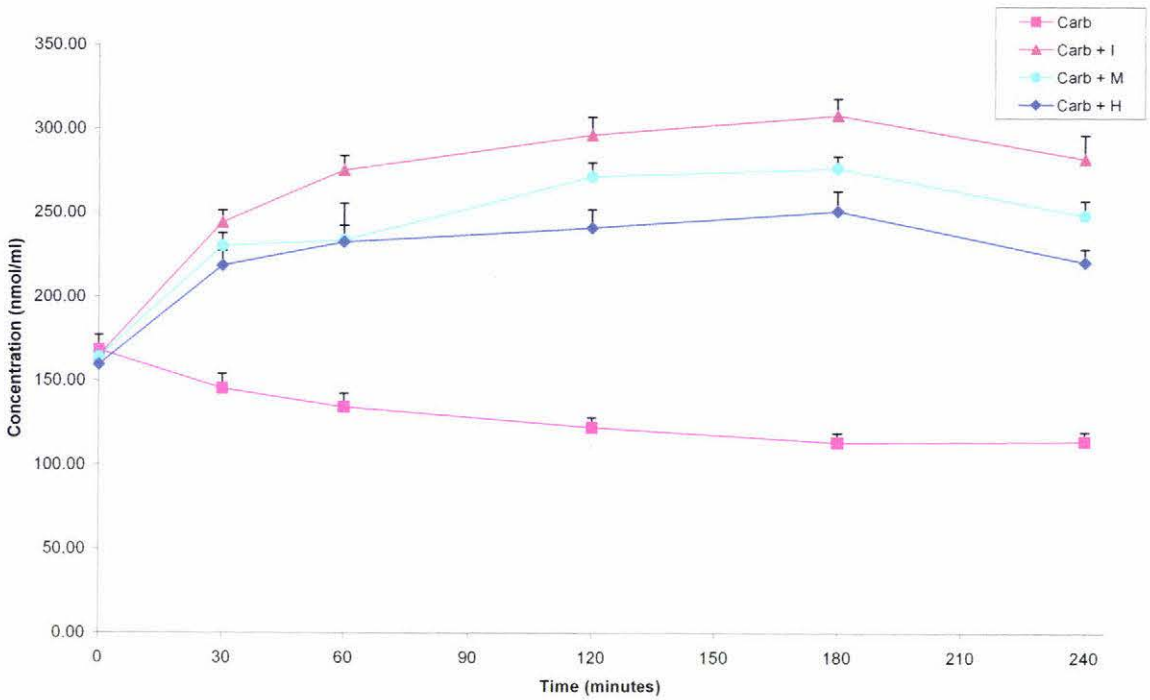

FIGURE 3.18. Mean (± SEM) plasma Valine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

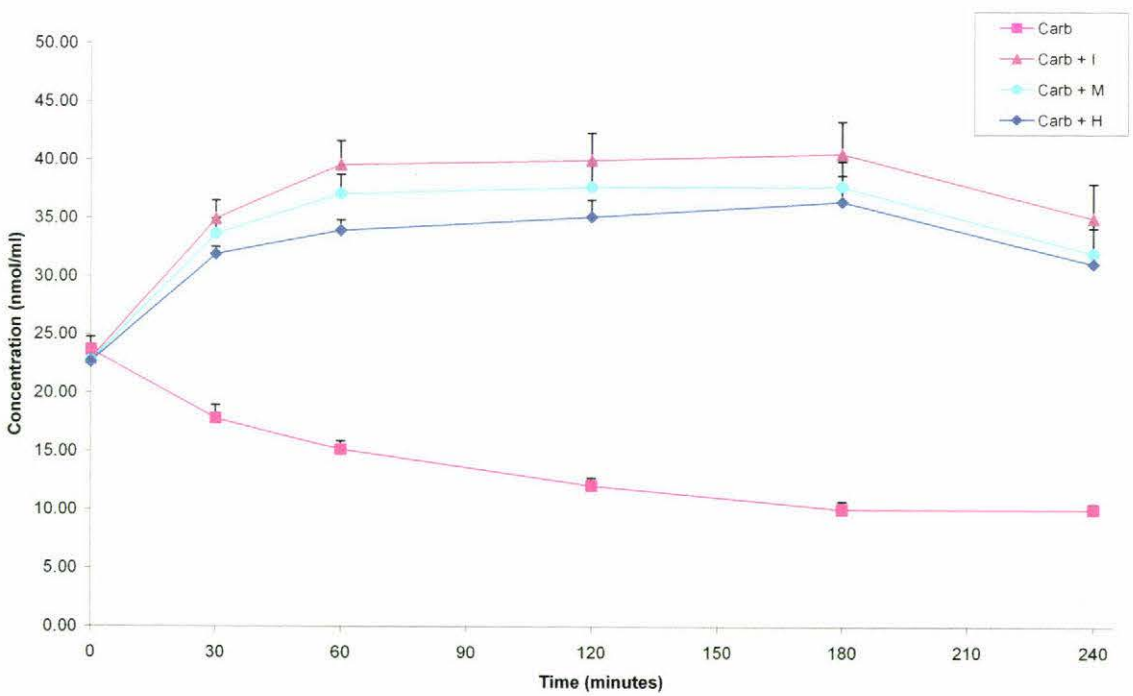

FIGURE 3.19. Mean (± SEM) plasma Methionine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

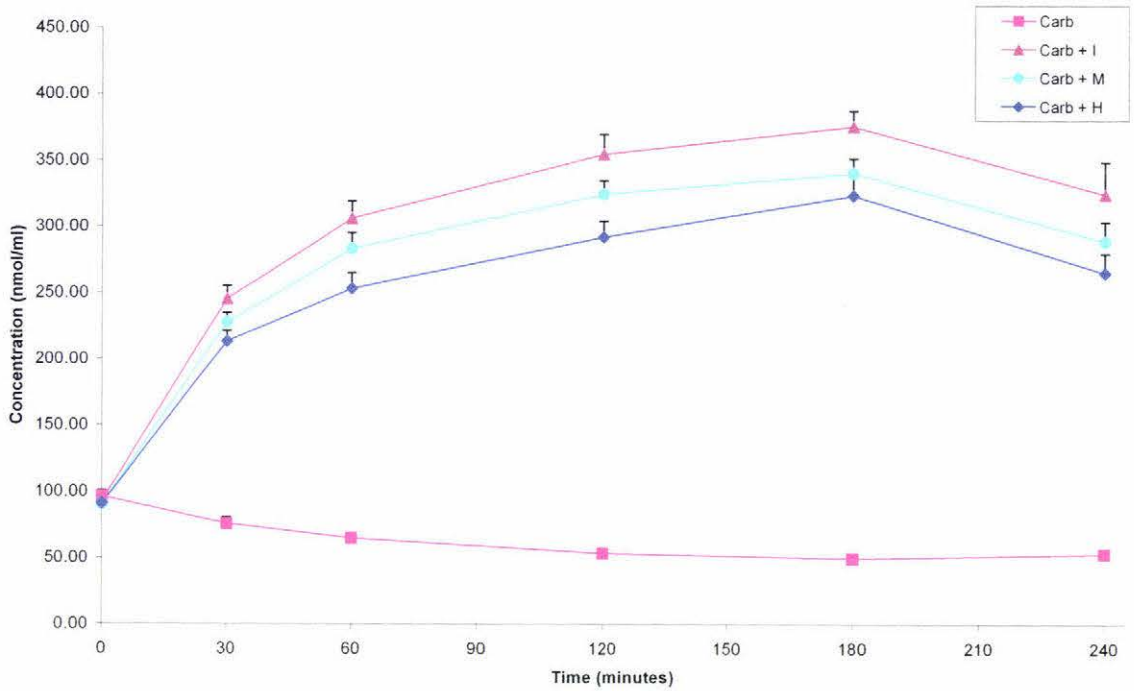

FIGURE 3.20. Mean (± SEM) plasma Leucine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

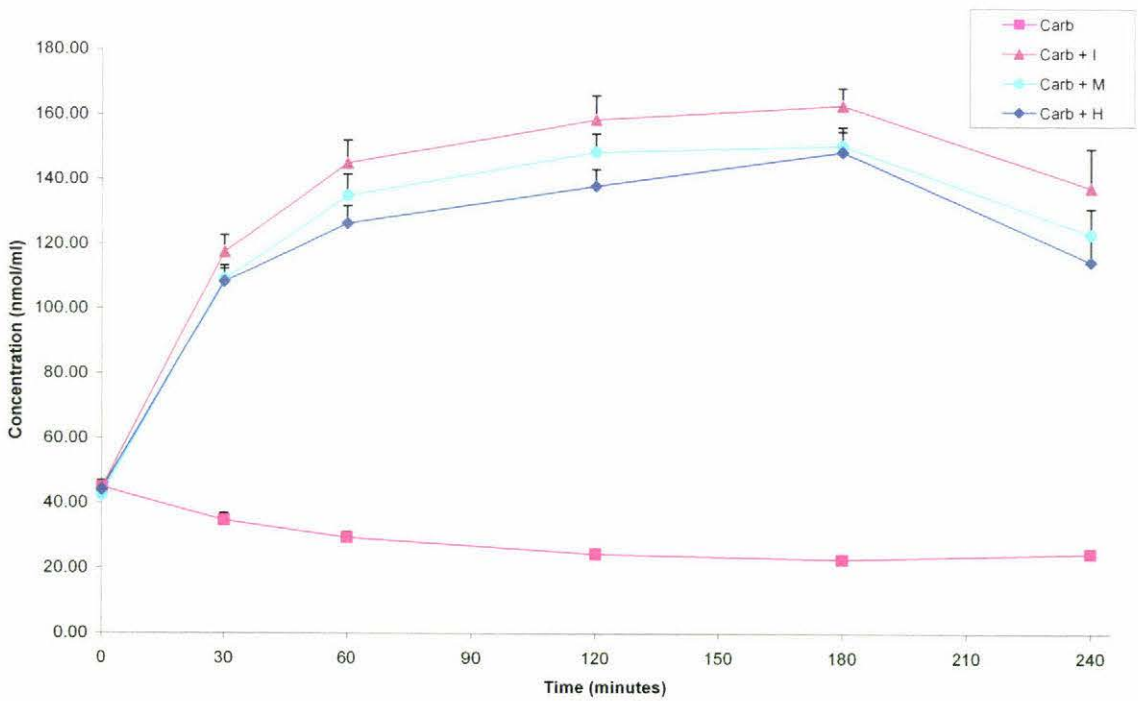

FIGURE 3.21. Mean (± SEM) plasma Isoleucine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

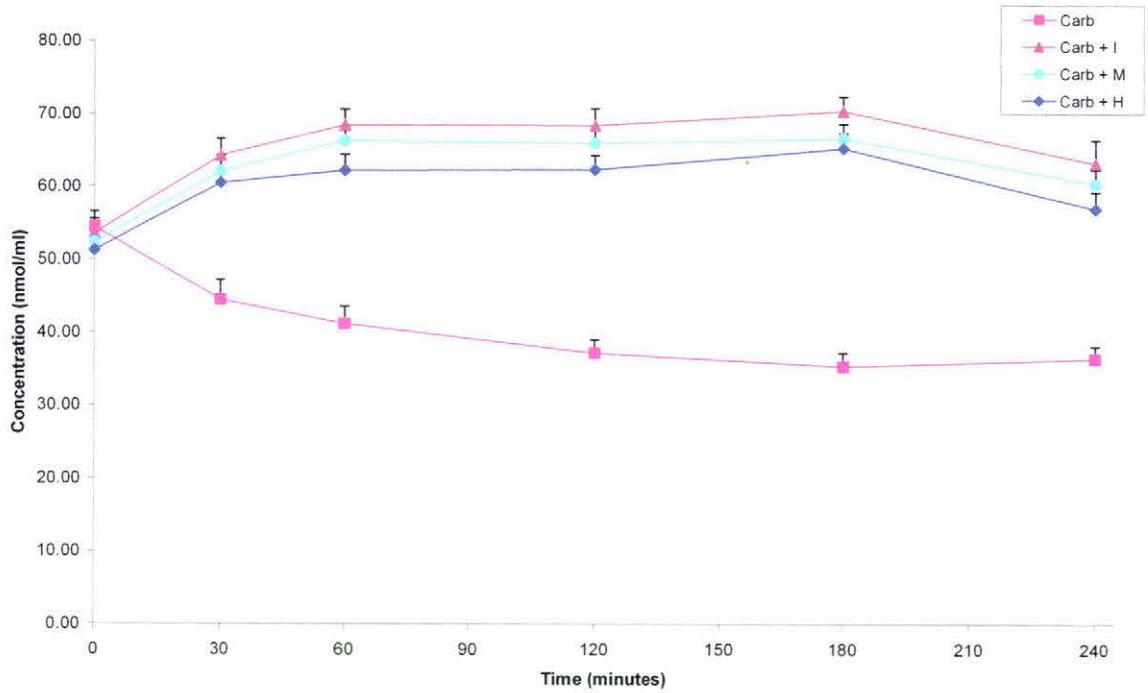

FIGURE 3.22. Mean (± SEM) plasma Phenylalanine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

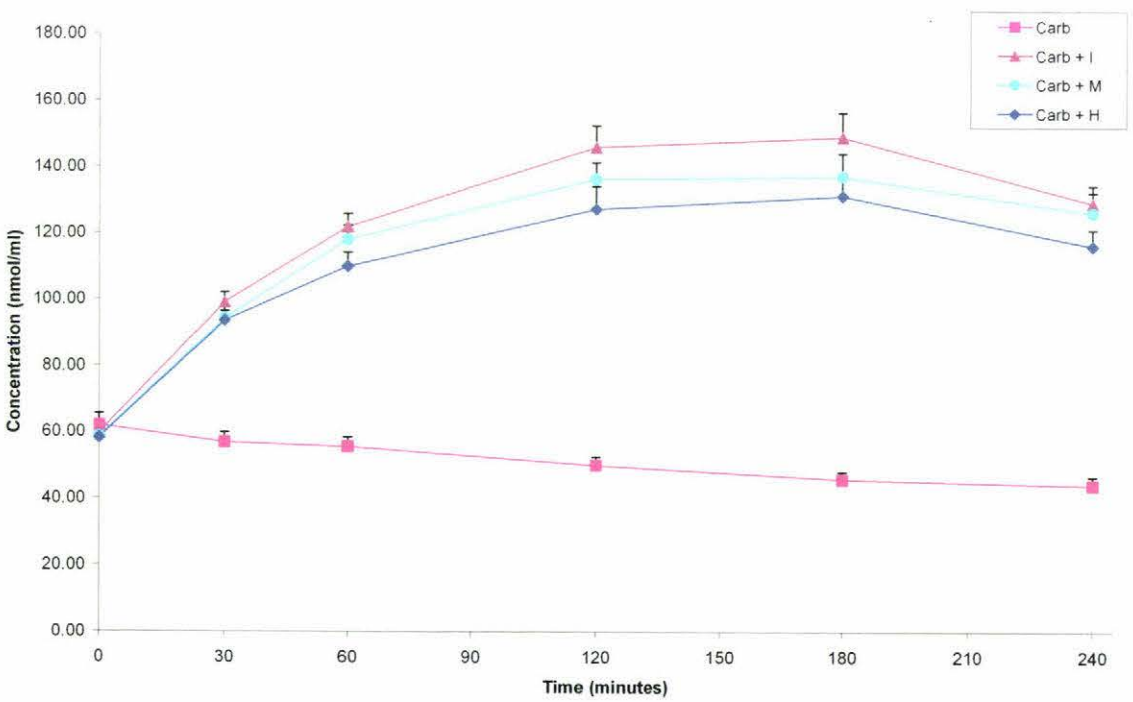

FIGURE 3.23. Mean (± SEM) plasma Tryptophan concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

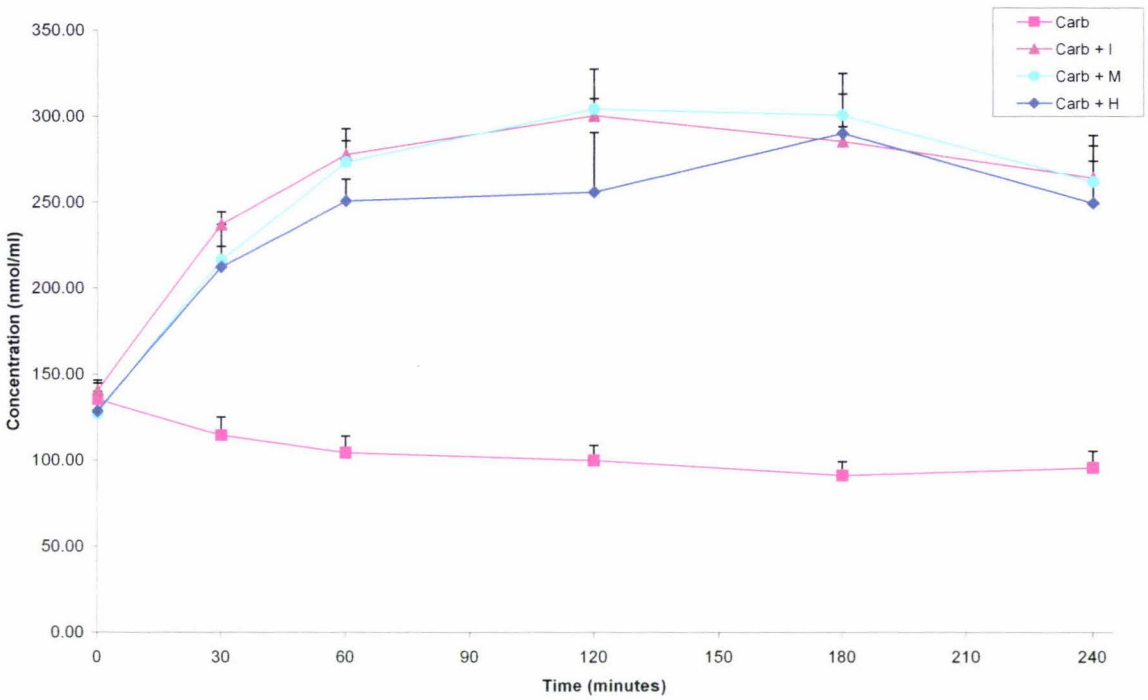

FIGURE 3.24. Mean (± SEM) plasma Lysine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

Table 3.14.
Statistical analysis of the effect of dietary beverages on individual mean plasma essential amino acid levels, by pairwise repeated measures ANOVA, during the time period from cessation of exercise to 240 minutes post-exercise during a post-exercise recovery study.

	Carb verses Carb + H		Carb verses Carb + I		Carb verses Carb + M		Carb + H verses Carb + M		Carb + I verses Carb + M		Carb + I verses Carb + H	
	df	**p**	**df**	**p**	**df**	**p**	**df**	**p**	**df**	**p**	**df**	**p**
Valine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		1,10	P<0.05	1,8	p<0.005
Methionine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		NS		NS	
Leucine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		1,10	P<0.05	1,8	p<0.005
Isoleucine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		1,10	P<0.05	1,8	P<0.05
Phenylalanine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		NS		1,8	p<0.005
Tryptophan	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		NS		1,8	P<0.01
Lysine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.005	NS		NS		NS	

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

3.5.4.7 Plasma Non-Essential Amino Acids

Similar differences in the temporal patterns of plasma amino acids were found with the non-essential amino acids Arginine (Figure 3.24) and Tyrosine (Figure 3.25). Thus, the plasma amino acid concentrations of the two non-essential amino acids tested both decreased over the first 60-120 minutes when the three protein-containing beverages were being consumed, then decreased monotonically until the end of the monitoring period. When the beverage containing Carb was being consumed, the plasma amino acids showed a general monotonic decrease that was maintained throughout the recording period.

Differences in the non-essential plasma amino acid concentrations when the protein-containing beverages were being consumed to that when the Carb beverage was being consumed were confirmed on pairwise repeated measures ANOVA ($p<0.001$, Table 3.15). The same analysis also showed a significant higher amino acid concentration for Tyrosine when the Carb + I beverage was being consumed to that when the Carb + H beverage was being consumed ($p<0.05$, Table 3.15). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Thus, consumption of any of the three protein containing beverages gave higher plasma amino acid levels of the two non-essential amino acids tested (Arginine, Tyrosine) compared with when the beverage containing Carb was consumed. For Tyrosine, consumption of the beverage containing Carb + I gave significantly higher plasma amino acid levels than that when the Carb + H beverage was consumed. These effects were shown to be independent of the athlete's hydration status.

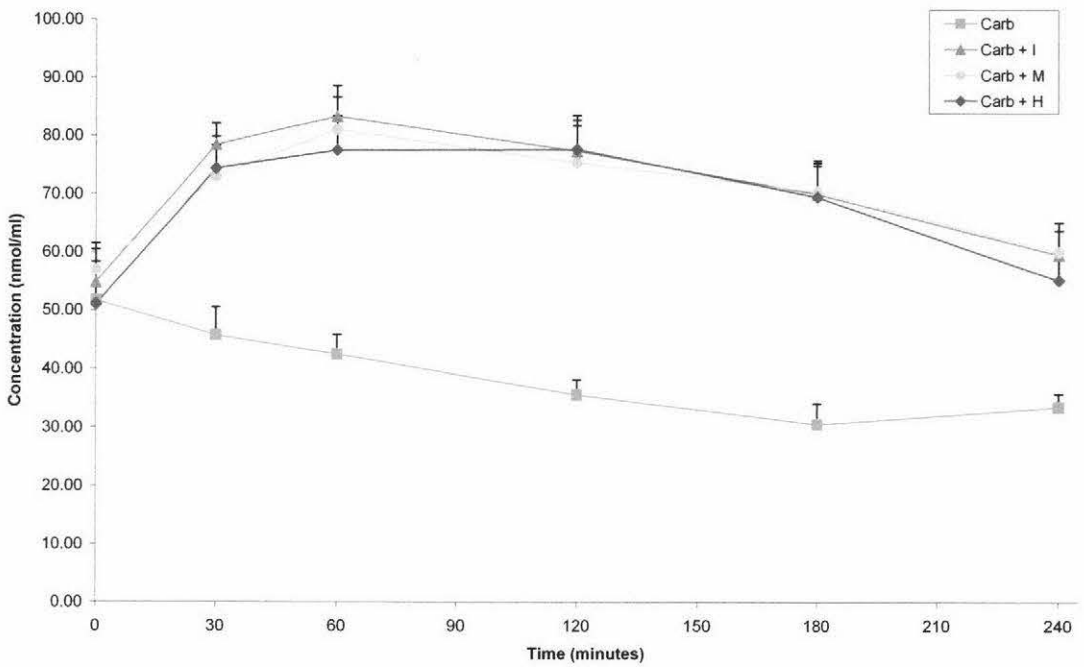

FIGURE 3.25. Mean (± SEM) plasma Arginine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

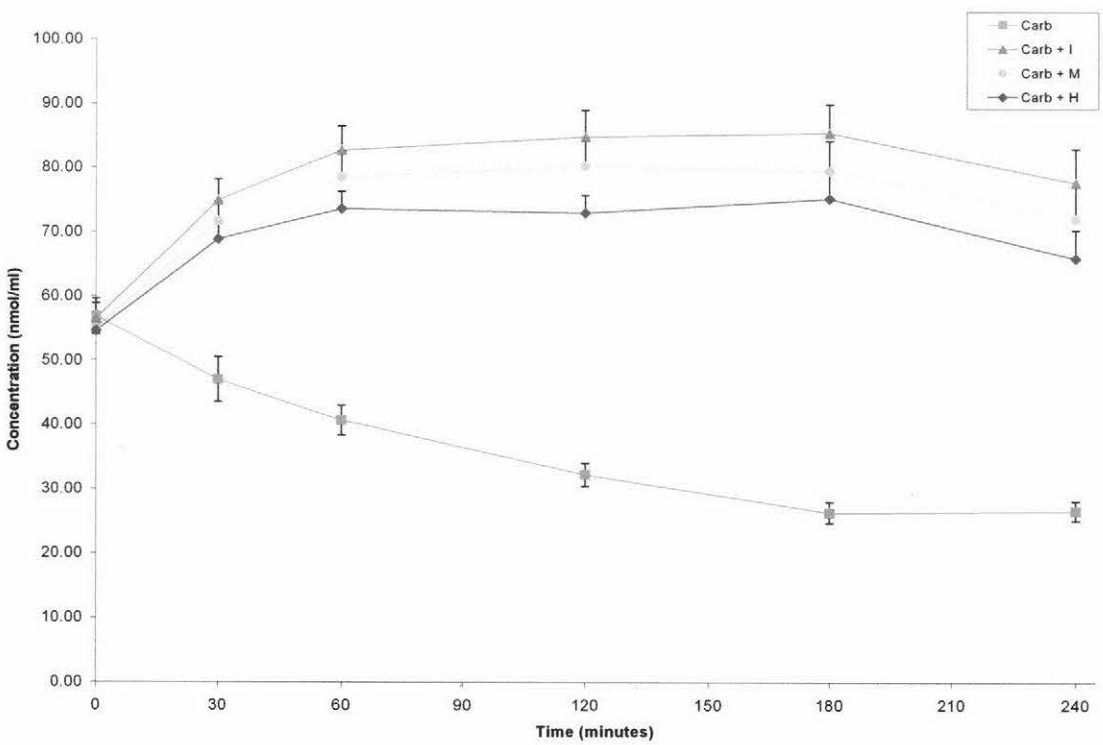

FIGURE 3.26. Mean (± SEM) plasma Tyrosine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

Table 3.15.

Effect of dietary beverages on individual mean plasma non-essential amino acid levels, by pairwise repeated measures ANOVA, during the time period from cessation of exercise to 240 minutes post-exercise during a post-exercise recovery study.

	Carb verses Carb + H		Carb verses Carb + I		Carb verses Carb + M		Carb + H verses Carb + M		Carb + I verses Carb + M		Carb + I verses Carb + H	
	df	**p**	**df**	**p**	**df**	**p**	**df**	**p**	**df**	**p**	**df**	**p**
Arginine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		NS		NS	
Tyrosine	1,7	p<0.001	1,8	p<0.001	1,8	p<0.001	NS		NS		1,8	p<0.05

Carb = Carbohydrate Only
Carb + I = Carbohydrate / Intact Protein
Carb + M = Carbohydrate / Intact Protein / Protein Hydrolysate
Carb + H = Carbohydrate / Protein Hydrolysate

3.5.4.8 Plasma Amino Acids Not Supplied in Beverages

Plasma Ornithine levels (Figure 3.26) decreased more or less monotonically during the measurement period after consumption of all of the beverages except for the beverage containing Carb + M, which resulted in an increase in plasma Ornithine levels that was maintained for 120 minutes, and then decreased until the end of the measurement period.

Whilst no significant difference in mean plasma Ornithine concentrations between the four beverages were found on pairwise repeated measures ANOVA, a significant difference in the pattern of variation of Ornithine concentration over time was found with consumption of the Carb beverage resulting in significantly lower plasma Ornithine concentrations compared to when the Carb + M beverage was being consumed (df 5,40, $F = 2.69$, $p<0.05$). Statistical analyses of remaining pairwise comparisons, on repeated measures ANOVA, were not found to be significant.

Plasma concentrations of 3-methylhistidine increased during the first 30-60 minutes of recovery, then decreased over the remainder of the sampling period for all four dietary beverages. Statistical analysis of levels of plasma 3-methylhistidine results showed no significant difference in the mean levels of 3-methylhistidine, or in the pattern of variation, between the dietary beverages.

Thus, the pattern of variation over time of plasma Ornithine concentration was significantly higher following consumption of the beverage containing Carb + M than that following consumption of the beverage containing Carb. There were no other significant differences in plasma Ornithine levels found in any other pairwise comparisons. No significant differences in plasma 3-methylhistidine concentrations were found between any pairwise comparisons of the four dietary beverages.

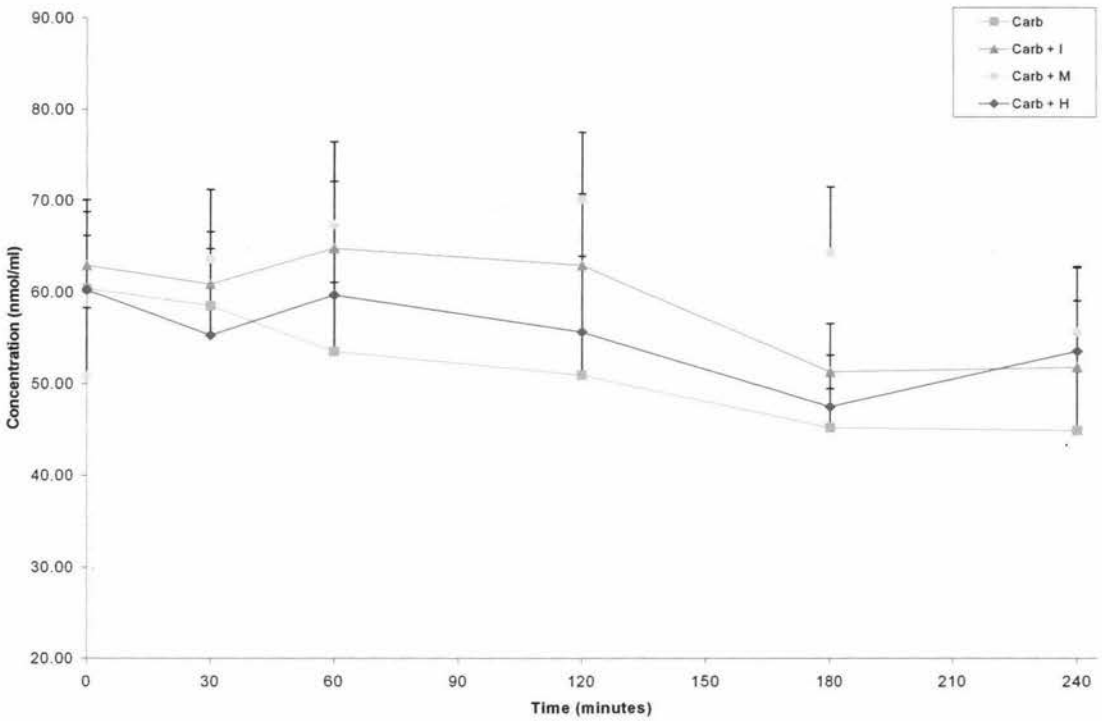

FIGURE 3.27. Mean (± SEM) plasma ornithine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

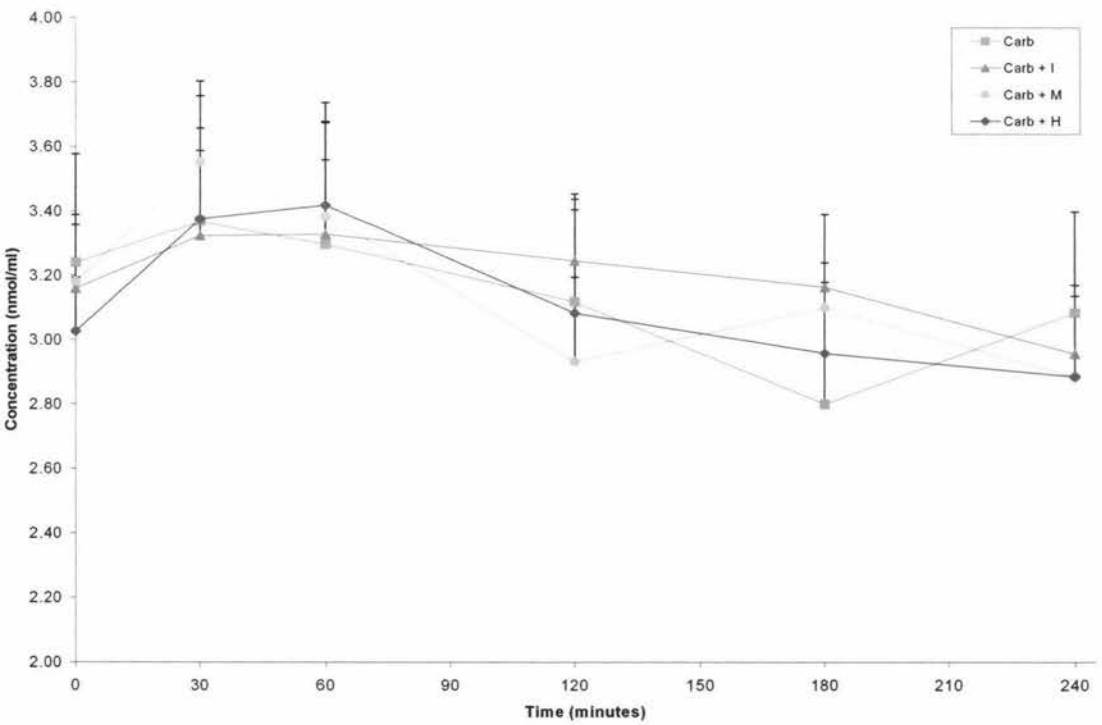

FIGURE 3.28. Mean (± SEM) plasma 3-methylhistidine concentration (nmol/ml) after ingestion of a control drink containing carbohydrate only (Carb; n=10) and drinks containing carbohydrate plus either: whey protein hydrolysate (Carb + H; n=10), intact whey protein (Carb + I; n=11), or 50% whey protein hydrolysate and 50% intact whey protein (Carb + M; *n=11*) in a 420 minute period following exercise and recovery beverage consumption.

Chapter 4

DISCUSSION

In the present study, four trial dietary beverages were tested to determine their effects on an athlete's recovery from glycogen-depleting exercise. The aims of this study were to determine (1) whether there is an insulinotropic effect of milk proteins, when consumed in addition to carbohydrate, which acts to enhance muscle glycogen resynthesis, and (2) whether a blend of hydrolysate and intact protein, when consumed in addition to carbohydrate, will enhance the athlete's recovery from exercise. However, the major finding of this study related to the effect of the dietary beverages on the athletes' rehydration following exercise. Therefore, the results related to rehydration will be discussed prior to those for both carbohydrate and protein metabolism.

4.1.Rehydration

An athlete's degree of rehydration during recovery from exercise is reflected in the status of plasma albumin levels, haematocrit and urine volume. In this study, the greatest rate of rehydration, as reflected in the rate of change of these factors, can be seen to occur within the first 30 minutes following cessation of exercise and consumption of the first aliquot of dietary beverage. During this period, ingested fluid will have appeared in the blood at a time when fluid loss is being limited, as evidenced by a large decrease in urine output over the first 30 minutes (Figure 3.5).

4.1.1. Plasma Albumin

One of the major driving forces for rehydration to occur relates to plasma albumin and its contribution to plasma colloid osmotic pressure. In the present study, plasma albumin concentration was significantly higher following consumption of the Carb beverage than that when either of the two beverages containing protein hydrolysate were being consumed (Carb + H, $p<0.01$; Carb + M, $p<0.05$). A study by Hayes, Lucas & Shi (2000) found that during recovery following intensive exercise, restoration of plasma volume was associated with a decrease in both plasma

albumin concentration and the concentration of total solutes. Lower concentrations of plasma albumin following consumption of the hydrolysate beverages in the current study would appear to suggest, therefore, an association between the consumption of protein hydrolysates and restoration of plasma volume.

Protein hydrolysates have been extensively used in exercise-recovery research due to their apparent more rapid absorption which ensures a ready supply of amino acids to the recovering muscle, which in turn is assumed to stimulate both insulin release and protein synthesis (Van Loon et al., 2000a). This rapid absorption of amino acids from protein hydrolysates may act to increase the total plasma solute concentration through the appearance of both absorbed amino acids and dietary sodium associated with co-transport with amino acids during absorption. These mechanisms will act to increase the osmotic drive for restoration of plasma volume. Sodium is also absorbed via co-transport with glucose in addition to movement via passive transport (Calbet & MacLean, 2002). Therefore, the rate of sodium absorption will be greatly enhanced through the inclusion of both carbohydrate and amino acids in a sodium-containing beverage. Further discussion of the mechanisms associated with the effect sodium has on plasma albumin and rehydration will appear later.

The hydrolysed protein used in the present study contained a significant quantity of sodium (2.3g sodium/100g protein) as a result of the manufacturing process. This initially caused the sodium content of the four dietary beverages to be different. In order to prevent an anion : cation effect on excretion of urinary nitrogen metabolites, the present study design included balancing for this additional sodium in the hydrolysed protein by adding sodium bicarbonate to the other two protein-containing beverages. This resulted in the three protein-containing beverages containing a similar sodium content. However, the beverage containing carbohydrate only had no additional sodium added.

While the sodium contained within the protein-containing beverages probably accounts for most of the dilutional effect on plasma albumin concentration seen in the present study, other factors may have the potential to directly influence albumin content. Haynes, Lucas & Shi (2000) highlighted that a post-exercise hypotension-

induced gain in intravascular protein (primarily attributed to an increased albumin content) provided the major driving force for restoration of plasma volume. This finding was independent of rehydration and was based on the increased albumin content enabling fluid to remain within the intravascular space thus diluting the exercise-induced increases in plasma solutes and sodium concentration. It is also believed that following overperfusion of the skin during exercise, the redistribution of blood during recovery may increase albumin levels via two mechanisms: (1) enhanced lymph return which is rich in albumin, and (2) increased blood flow to the liver which stimulates albumin synthesis (Haynes, Lucas & Shi, 2000). Both of these mechanisms would act to increase colloid osmotic pressure and therefore promote the restoration of plasma volume. In a study by Yang, *et al.* (1998), however, it was found that the increased rate of protein synthesis following exercise was not sufficient to account for the increase in plasma albumin content. While these other factors can directly affect the amount of albumin found within the plasma it is likely that the findings in the present study are mainly as a result of the dilutional effect associated with restoration of plasma volume.

4.1.2. Haematocrit

Haematocrit is a good indication of fluid status following exercise and the study results for haematocrit followed the same trend as those of plasma albumin but failed to reach significance. In a study by Neumayr, *et al.* (2002), examining changes in haematocrit following an endurance cycling event, a significant correlation between change in haematocrit and total body water loss immediately following the race was observed. These findings indicate the presence of hemoconcentration following exercise as a result of dehydration. In the Neumayr, *et al.* (2002) study, haematocrit was also measured one day following the race where it was found to be significantly lower, which was assumed to indicate extended post-exercise plasma volume expansion.

The results for plasma albumin and haematocrit indicate that the enhanced rehydration resulted from extracellular rehydration, as opposed to intracellular rehydration. As we did not make direct measurements of intracellular rehydration, speculation on mechanisms of rehydration will be limited to extracellular rehydration.

4.1.3. Urine Output

A major finding of the present study is the effect consumption of protein-containing beverages had on urine output. When athletes consumed the Carb beverage they had significantly greater mean urine outputs over time than when they consumed any of the three protein-containing beverages (Carb + I, $p<0.01$; Carb + H, $p<0.05$; Carb + M, $p<0.05$). This was most significant in the first 240 minutes of recovery, during which time the athletes' fluid intake was controlled. In terms of cumulative urine output over this period, consumption of the Carb beverage resulted in a significantly higher urine output that either of the two beverages containing intact protein (Carb + I, $p<0.05$; Carb + M, $p<0.05$).

This reduction of fluid loss through urine will aid in rehydration of the athlete by facilitating the restoration of plasma volume. In addition, it highlights the ability of the kidneys to support fluid retention. A number of studies have shown that the primary obstacle to rapid rehydration is the loss of large amounts of fluid in the form of urine (Mitchell, *et al.,* 2000). Schmidt *et al.* (1998) demonstrated that increased levels of plasma aldosterone and plasma renin activity during exercise results in increased renal sodium retention during the first few hours after exercise, which would affect urine output, with these hormones returning to control levels within 3-6 hours after exercise along with sodium excretion. Nagashima, *et al.* (2001) highlighted the importance of plasma sodium levels on restoration of plasma volume by showing that following saline infusion the reflex reductions in fluid regulatory hormones (plasma renin activity and arginine vasopressin) were both significantly delayed and decreased following exercise (Nagashima, *et al.* 2001). The increase in sodium and water reabsorption in the kidneys, either through the effects of hormones or through exercise-induced reabsorption of sodium in the proximal tubule will both act to reduce urine volume.

Therefore, while the results of the present study highlight a potential protein effect in limiting urine output, it is more likely that this is mainly the result of the very high sodium content of these beverages when compared to the sodium-free Carb beverage.

4.1.4. Sodium and Rehydration

In a study by Maughan & Leiper (1995), the urine output of dehydrated subjects, who consumed a volume equal to 150% of body mass loss during exercise, was found to be inversely proportional to the sodium content of the ingested fluid. Only when the sodium content exceeded 50mmol/L did subjects remain in a positive fluid balance throughout the recovery period. The main conclusion from the Maughan & Leiper (1995) study was that providing a sufficient volume of water is consumed, sodium replacement is the most important factor in the restoration of fluid balance. This finding was supported in a further study by Shirreffs & Maughan (1998), where fluid ingestion equivalent to 150% of volume depleted by exercise was consumed. Subjects in that study retained 71% of the ingested volume when the beverage had a high (102 mmol/L) sodium concentration but only 37% when virtually no sodium was present. Consumption of sufficient sodium and water ensures that plasma sodium concentration and osmolality do not markedly decline which would be likely with consumption of plain water only. As a result, plasma levels of arginine vasopressin and aldosterone would be maintained and, therefore, avoid a situation that would result in a negative fluid balance developing (Shirreffs & Maughan, 1998). This shows that consumption of a beverage containing sodium acts to increase plasma osmolality and causes a resulting increase in plasma volume to maintain sodium concentration. Maintenance of high plasma sodium levels may remove any osmotic drive for water to move from the plasma into cells, which in turn is likely to result in an increase in circulating fluid volume but not intracellular fluid volume.

In the present study, consumption of the three protein-containing beverages, containing elevated levels of sodium, would have acted to maintain the sodium concentration of plasma and cause an increase in plasma volume; an effect that was not seen following consumption of the sodium-free Carb beverage. Consumption of the Carb beverage is likely to have caused inhibition of arginine vasopressin release, due to a dilutional effect decreasing plasma osmolality. Removal of the effects of arginine vasopressin will cause decreased water reabsorption within the kidneys and a resulting increase in urine output. This dilutional effect on plasma osmolality may also exacerbate the risk of hyponatremia in the recovering athlete, as it will result in

increased cellular absorption of fluid from plasma in order to counter the fall in osmolality.

Therefore, sodium definitely plays a key role in the rehydration process, through its osmotic effects, and may be a major factor in the observed differences in rehydration following consumption of the Carb beverage and that of any of the three protein-containing beverages. The very high sodium content of the protein-containing beverages was initially the result of the manufacturing process of the protein hydrolysate and then addition of sodium to the beverages containing intact protein in order to remove differences in the anion : cation balance. This, however, lead a large difference between the sodium content of the protein-containing beverages and that of the beverage containing carbohydrate only. In hindsight, it may have been beneficial to add additional sodium to the Carb beverage. However, the study design was based on internationally published designs from other groups where the carbohydrate only beverage was provided without additional sodium.

The optimal concentration of sodium in a recovery beverage is still to be determined through further research. Mitchell, *et al.* (2000) demonstrated that increasing the sodium concentration of a recovery beverage from 25 mM to 50 mM, did not alter urine or hormonal dynamics. When determining the optimal sodium concentration, the fact that excessive sodium intake may stimulate natriuresis needs to be considered, because of the potential for increased urine flow and the loss of large amount of potassium in the urine (Shirreffs & Maughan, 1998). In the present study, the high sodium concentration of the three protein-containing beverages actually acted to enhance rehydration of the athletes. This was unexpected, and may indicate that higher sodium levels in a recovery beverage can be tolerated (i.e. may not cause intestinal reverse osmosis) in the presence of both carbohydrate and amino acids, due to the involvement of sodium in co-transport of these nutrients during absorption in the intestines.

4.1.5. Role of Potassium in Supporting Rehydration

In addition to the sodium content being greater in the three protein-containing beverages than found in the Carb only beverage, the protein-containing beverages also contained potassium (Table 2.6). Potassium is the major ion in intracellular fluid and increases in intracellular potassium may, therefore, support the rehydration of intracellular fluid space following exercise-induced dehydration (Shirreffs, 2001). The potassium lost in sweat during exercise also needs to be replaced, although sodium losses are much higher, highlighting that water loss associated with sweating is more likely to be from the extracellular space (Shirreffs, 2001). During high intensity exercise, it has been found that there is a net loss of potassium from contracting skeletal muscle, at a rate proportional to contraction intensity (Lindinger & Grudzien, 2003). This flux of potassium out of the muscle is mainly due to the activity of voltage-dependent potassium channels associated with action potential.

However, ATP-dependent and calcium-dependent potassium channels may also be involved in the potassium flux (Juel *et al.,* 1999). During exercise, potassium may also be taken up by red blood cells which could play an important role in supporting the clearance of potassium from plasma (Lindinger & Grudzien, 2003). This efflux of potassium during exercise is rapidly reversed upon cessation of exercise via a substantial increase in the activity of the muscle sodium-potassium pump (McKenna, 1999). This reuptake of potassium and the subsequent removal of sodium from the cell into plasma may aid in restoration of plasma volume following exercise, a mechanism which may be further stimulated due to the appearance of dietary potassium in the plasma.

Insulin also plays an important role in the re-uptake of potassium following exercise, and has been found to cause a rapid (within seconds) increase in transport of glucose, amino acids and potassium into insulin-sensitive cells (Ganong, 2001, cited in Manninen, 2004). Therefore, insulin acts to increase the uptake of potassium into the recovering muscle, and as a result increase the movement of sodium out of the cell and into plasma. This will further support the recovery of plasma volume via improved plasma osmolality. Continued release of arginine vasopressin following exercise as a result of increasing plasma osmolality, would

limit urine production and enable the athlete to retain fluids. In addition, any effect that a dietary beverage may have on insulin response (such as the proposed insulin stimulating effects of dietary protein) may also indirectly influence the rate of rehydration following exercise-induced dehydration. Therefore, the potassium content may have influenced the observed result of improved rehydration following consumption of the protein-containing beverages above that seen for the beverage containing carbohydrate only.

4.1.6. Comparison of the Protein-Containing Beverages on Rehydration

Although no significant differences between the three protein-containing beverages were found in the indices of rehydration discussed, when comparing individual responses relative to the Carb only beverage, the beverages containing protein hydrolysate had a significant effect on plasma albumin concentrations, while the beverages containing intact protein containing beverages had a significant effect on the cummulative urine output. The finding that consumption of beverages containing protein hydrolysate (Carb + H or Carb + M) increased plasma volume levels may provide a possible mechanism as to why the intake of intact protein resulted in a lower overall urine output.

One of the potential benefits associated with protein hydrolysates is that they are absorbed more rapidly than either intact protein or free amino acids (Manninen, 2004). This faster absorption rate for protein hydrolysates may help to explain the improved plasma volume restoration (as indicated by plasma albumin and haematocrit results) found in the present study. This increased rate of amino acid absorption will also cause an increased rate of sodium absorption due to the co-transport of these nutrients. The resulting increase in plasma osmolality may provide the driving force for the increased plasma volume. The effect of this would then be to reduce urine output through continued release of arginine vasopressin. However, cumulative urine output was found to be significantly different only in the two intact protein-containing beverages. This latter finding could be the result of stimulation of atrial natriuretic peptide (ANP) in response to consumption of the hydrolysate-containing beverages. ANP has the ability to cause significant alterations in fluid balance due to its natriuretic and diuretic effects. ANP secretion is stimulated via

either mechanical stretch (as a result of increased intravascular volume, blood pressure and any change in body posture that could increase central venous pressure) or as an antagonist to several neurohormones (specifically angiotensin II, endothelin and norepinephrine) (Kim & Piano, 2000; Bentzen, *et al.,* 2002). Therefore, ANP has the potential to increase both water and sodium excretion via its actions on the kidneys, specifically from an increase in glomerular capillary pressure and thus increased glomerular filtration rate (Kim & Piano, 2000). The greater increase in plasma volume following consumption of protein hydrolysate beverages may have acted to increase mechanical stretch and thus stimulate the release of ANP to a greater extent compared with what may have occurred following consumption of the Carb + I beverage. It is interesting that consumption of the Carb + M beverage, containing a mixture of both intact protein and protein hydrolysate, seemed to have resulted in significantly lower plasma albumin concentrations and cumulative urine volume. This may indicate that the presence of both forms of protein acted to maximise the individual benefits of each protein form. This result may be further reflected in the greater cardiovascular recovery associated with consumption of the Carb + M beverage (to be discussed next).

4.2. Cardiovascular Recovery

Another major finding of this study was the observation that consuming protein, in addition to carbohydrate, in a recovery beverage resulted in improved heart recovery following exercise. The rate of decrease in pulse rate during recovery was significantly greater when any of the three protein-containing beverages were being consumed compared to that observed following consumption of the beverage containing Carb only. Of these, consumption of the Carb + M beverage had the greatest effect in decreasing pulse rate during recovery, which was significantly greater than the response from the other two protein-containing beverages. There were no significant differences in the rate of decrease of pulse rate when the Carb + I and Carb + H beverage responses were compared.

These findings can be largely explained by improved rehydration of the athletes following consumption of any of the three protein-containing beverages when compared with consumption of the Carb beverage. Dehydration in humans has

several adverse effects on cardiovascular function via reducing circulatory fluid volume, including a decrease in orthostatic tolerance and an increased heart rate at rest and during exercise (Charkoudian, *et al.,* 2003). In general, dehydration due to exercise is associated with smaller decreases in plasma volume because of post-exercise fluid shifts, which tend to restore intravascular volume (Hayes, *et al.,* 2000; Jimenez, *et al.,* 1999). As discussed previously, absorption of sodium, glucose and amino acids from the protein-containing beverages in the present study would have acted to maintain the plasma osmolality and therefore caused a resulting increase in plasma volume. This, in turn, would have resulted in an increase in circulating fluid volume following exercise, as supported by the finding of decreased urine output (assumed to be the result of maintained arginine vasopressin release stimulated by maintenance of plasma osmolality).

Charkoudian, *et al.* (2003) demonstrated that exercise-induced dehydration caused both an increase in resting heart rate and a decrease in the sensitivity of baroreflex control of heart rate. This observed increase in resting heart rate seen with exercise-induced dehydration was not found to be responsive to a volume infusion (saline) during exercise, even though this acted to increase central venous pressure and returned arterial blood pressure to pre-exercise levels. This finding suggests that baroreflex control of heart rate is not the only mechanism affecting post-exercise heart rate. The present study differs with that of Charkoudian, *et al.* (2003), in that plasma volume expansion did occur following cessation of exercise resulting in improved pulse rate recovery, and appears to indicate that baroreflex control of heart rate following exercise may be an important mechanism controlling post-exercise heart rate recovery.

Inactive recovery from exercise is associated with removal of the primary exercise stimulus from the brain which contributes to post-exercise reduction in cardiac output and arterial pressure (Carter, *et al.,* 1999). The resulting decrease in heart rate is likely to reflect both sympathetic withdrawal and parasympathetic reactivation following cessation of exercise (Kannankeril & Goldberger, 2002). Other factors contributing to heart rate recovery following exercise are thought to be related to slower changes in the stimulation of both metaboreceptors and baroreceptors

accompanying the elimination of body heat and catacholamines (Javorka, *et al.*, 2002).

Stimulation of baroreceptors, via an increase in circulating fluid volume (as observed in the present study following consumption of any of the protein-containing beverages), will further increase the parasympathetic reactivation following exercise and further promote a reduction in heart rate following exercise. The decline in stroke volume during exercise, as a result of dehydration, has been found to be specifically due to reduced blood volume, which probably reduces ventricular filling (González-Alonso, *et al.*, 1997). Correction of dehydration following exercise recovery via the restoration of plasma volume and, as a result, blood volume and stroke volume, may act to improve cardiovascular recovery following exercise. This is supported by the results of the present study which produced evidence that rapid rehydration significantly increased heart rate recovery, and most notably so following consumption of the Carb + M beverage.

4.3. Carbohydrate Recovery

The present study found no significant differences between any of the dietary treatments for either plasma insulin or plasma glucose concentrations. This finding for plasma insulin concentration supported the previous findings of Van Loon (2000b) where they concluded that if sufficient carbohydrate was supplied, the addition of protein to the recovery beverage caused no additional insulin response. However, it should be noted that a large amount of variation in both plasma insulin and plasma glucose concentrations between subjects was evident, which may have masked any significant effects. While no significant differences were found for these variables, the graphs of the response of plasma insulin and plasma glucose concentrations (Figure 3.6 & 3.7) following consumption of the beverages highlights the link between them, with coincident concentration peaks occurring at 20 and 50 minutes for all athletes on all beverages.

There was, however, a significant difference, using uncorrected data, in the ratio between plasma glucose and plasma insulin concentrations following consumption of the Carb + M beverage compared to that following consumption of either the

Carb + H ($p<0.01$) or Carb ($p<0.05$) beverages. When data was corrected for hemoconcentration to account for differences in rehydration during recovery, no significant differences were found in the analysis of either plasma glucose concentrations or plasma insulin concentrations. However, when the ratio between plasma glucose and plasma insulin concentrations was examined, a significant difference was found between the consumption of the Carb beverage and the consumption of any of the three protein-containing beverages. In this case, consumption of Carb beverage resulted in a lower ratio of plasma insulin to glucose concentrations compared to consumption of any the three protein-containing beverages.

Apart from physiological considerations of outcome, the difference in the results of corrected verses uncorrected values suggests that in exercise studies of this type correction of blood analysis results using haematocrit should be routine based on this study's findings related to the sodium and protein effect in expanding plasma volume and enhancing the process of rehydration. Previous studies examining the effects of protein hydrolysates on plasma component concentrations, such as insulin and glucose, may have had their results influenced by the sodium content of the hydrolysates (an artifact of the manufacturing process of hydrolysates) and the subsequent rate of sodium absorption due to co-transport with amino acids. The net effect of this would be enhanced rehydration following consumption of the protein-containing beverage when compared to the carbohydrate only beverage, mainly due to the dilution effect of consuming a sodium-free carbohydrate beverage on plasma osmolality and the associated increase in urine output.

A number of previous studies examining the insulinotropic effects of protein have focused on comparing different types of proteins (i.e. whey verses casein). The present study appears to be the first to compare the effects of an intact whey protein and its 'same batch' hydrolysate. In comparing the athletes' metabolic response to consumption of the three protein-containing beverages used in this study, after taking into account the hydration state of the athletes, no differences in the ratio between plasma insulin and plasma glucose were found. This result may indicate that the different effects of proteins in stimulating insulin release and glucose uptake in previous studies could have been due to the different types of proteins used and

the corresponding differences in their digestion rates. The rate of amino acid appearance in the blood is further slowed by the decrease in intestinal blood flow. The magnitude of this reduction in intestinal blood flow has been found to be directly related to relative exercise intensity, as a percentage of VO_{2max} (McAllister, 1998). In this situation, consumption of an easily digested form of protein, such as the protein hydrolysate, may be beneficial in maximising amino acid absorption.

As discussed previously (Chapter 1, p21), the pattern of muscle glycogen synthesis following glycogen depleting exercise appear to occur in a biphasic manner, consisting of both rapid and slow phases (Jentjens & Jeukendrip, 2003). Both of these phases need to be considered as they have to potential to influence the ratio of plasma insulin to plasma glucose concentrations, however, this influence of this was not measured in the present study. Within the rapid phase, glycogen synthase is mainly stimulated by low muscle glycogen levels. Ensuring a high rate of glucose supply to the recovering muscle will maximise this phase of recovery. In relation to this, sodium may act to enhance muscle recovery via two potential mechanisms. The first is enhanced carbohydrate absorption in the intestine, via the co-transport of sodium and glucose (Loo, *et al.,* 1996). The second is maintenance of plasma osmolality, causing an increase in plasma volume and circulatory fluid volume (Shirreffs & Maughan, 1998), which will enhance the transport of glucose in the blood to the recovering muscle through improved cardiovascular function. Insulin will aid in the uptake of glucose into the muscle during this rapid phase in addition to directly stimulating glycogen synthase activity during the slow phase. Therefore, maximising the insulin response will play an important role in the recovery of muscle glycogen stores following exercise.

The stimulation of glucose uptake into muscle following exercise occurs in response to both muscular contraction and insulin and is mediated by translocation of GLUT4 from intracellular sites into the cell membrane (Kuo *et al.,* 1999). In addition, exercise further increases insulin responsiveness through inducing an increase in GLUT-4 translocation in muscle (Fischer, *et al.,* 2002). Therefore, maximising the insulin release following exercise will have an increased effect on glucose uptake into the muscle due to improved insulin sensitivity. The present study provides evidence of this improved insulin response and the corresponding increase in

glucose uptake into the muscle, in that when the hydration state of the athlete was taken into account, a significant relationship between plasma insulin and plasma glucose was found. This was characterised by the protein-containing beverages inducing significantly higher ($p<0.05$) plasma insulin and lower plasma glucose concentrations during recovery, which indicates increased insulin release and increased glucose uptake into muscles. This situation would act to maximise the recovery of muscle glycogen stores following exercise.

While amino acids act to increase the ratio of plasma insulin to plasma glucose levels, the amino acid leucine may play an additional role within the muscle by affecting the insulin signalling cascade and the translocation of GLUT4 into the cell membrane. The site of leucine action within the muscle is on the kinase mTOR, which mainly acts to stimulate protein synthesis within the muscle (Layman & Baum, 2004; Lynch, *et al.,* 2003). However, mTOR has been shown to potentially alter the insulin receptor signal via stimulation of IRS-1 causing the down-regulation of this signal (Layman & Baum, 2004). This down-regulation may act to limit the movement of glucose into the muscle due to removal of the stimulus for translocation of GLUT4 into the cell membrane. If this mechanism decreases muscle glucose uptake, then stimulation of further insulin release may occur in response to the resulting elevated blood glucose levels. The leucine component of the beverages used in the present study are between 13-14% of total protein content, which is of a similar composition to the diets used in the study of Layman & Baum (2004) who compared the leucine levels of different types of proteins. As the main effect of mTOR stimulation is promotion of protein synthesis, it is still to be determined why this down-regulation occurs as it may hinder other muscle recovery processes, such as the restoration of muscle glycogen stores.

4.4.Protein Recovery

4.4.1. Plasma Amino Acids

In the present study, as expected, amino acid levels were found to be significantly greater following consumption of any of the three protein-containing beverages compared to that observed following consumption of the Carb beverage, for all

amino acids tested. By using the amino acid response following consumption of the Carb beverage as a baseline, it can be assumed that the appearance of amino acids in the plasma above this baseline is predominantly amino acids from the dietary beverages. Therefore, consumption of the beverage containing intact protein resulted in significantly higher plasma essential amino acid levels of valine, leucine, isoleucine, phenylalanine, tryptophan and tyrosine than those observed following consumption of the beverage containing Carb + H. Similarly, consumption of the Carb + I also resulted in significant higher plasma amino acid levels of valine, leucine and isoleucine than those noted following consumption of the Carb + M beverage. In addition, changes in plasma branched chain amino acid levels may also reflect the rate of intestinal absorption of amino acids due to it being well recognised that these amino acids escape metabolism by the liver (Calbet & MacLean, 2002).

The finding that consumption of the Carb + I beverage resulted in significantly higher plasma amino acid concentrations for a number of the amino acids measured was unexpected and conflicts with that found in previous research. Due to the proposed stimulatory effect of plasma amino acids on protein synthesis and insulin secretion during exercise recovery, protein hydrolysates have been targeted as the preferred protein due to their rapid digestion and absorption of resultant amino acids. Biorie, *et al.* (1997) examined the digestion rate of different proteins and found the plasma appearance of dietary amino acids following consumption of whey protein was significantly faster than that following consumption of casein protein. This finding was supported by Van Loon *et al* (2000a) who demonstrated that following ingestion of intact protein (casein), plasma amino acid responses over a two hour period tended to be lower than that observed following ingestion of protein hydrolysates. However, two key factors differentiate the present study from previous studies in examining the different appearance rates of free amino acids in the blood. Firstly, the anion : cation balance of the proteins was corrected to ensure it was balanced. The anion : cation balance of a protein mixture has been shown to affect the absorption rate of amino acids (Haydon & West, 1990), which is most likely due to the influence sodium has in the cellular transport process. And secondly, the proteins used in the present study included intact whey protein and its 'same batch' hydrolysate, as opposed to comparing whey and casein proteins, or as

has been reported in some studies (Van Loon *et al.,* 2000a), intact casein verses wheat or pea protein hydrolysates. As stated previously, the high sodium content in the protein-containing beverages had a significant effect on the rehydration of the athlete. Previous studies where the anion : cation balance was not accounted for may have had their results influenced by the sodium content of the protein hydrolysates used, via its effect on the restoration of plasma volume.

4.4.2. Plasma Urea Concentration and Urinary Excretion of Urea and Ammonia

In the present study, as expected, consumption of the three protein-containing beverages resulted in significantly higher concentrations of plasma urea and significantly higher urinary urea and ammonia nitrogen excretion than that following consumption of the Carb beverage. In comparing the three protein-containing beverages, consumption of the Carb + H beverage resulted in significantly lower concentrations of plasma urea and significantly lower urinary urea nitrogen excretion than that following consumption of the Carb + I beverage. Similarly, consumption of the Carb + H beverage also resulted in significantly lower urinary ammonia nitrogen excretion than that following consumption of either the Carb + I or Carb + M beverages. These findings mirror those for plasma amino acids and may be the direct result of the effect of augmentation of protein synthesis and a resulting reduction in deamination of amino acids in response to consumption of the Carb + H beverage compared to the Carb + I beverage.

The ammonia substrate for the synthesis of urea is predominately derived from the catabolism of excess amino acids (Lardner & O'Donovan, 1998). Urea is metabolically inert, which allows it to be excreted without affecting the acid-base balance of the body, thus avoiding potentially toxic levels of ammonia (Maughan, *et al.,* 1997; Lardner & O'Donovan, 1998). As stated previously, only six amino acids can be oxidised by muscle (leucine, isoleucine, valine, glutamate, aspartate, and arginine). The deamination of these amino acids allows for oxidation of the carbon-skeletons to occur with the resulting nitrogen-containing amino group needing to be removed from the muscle. The two key mechanisms for the removal of this are via either alanine or glutamine (Wagenmakers, 2000). Each of these two amino acids provides a non-toxic carrier of ammonia from the muscle to the liver where it is

eventually excreted as urinary urea. Young, *et al.* (2000) demonstrated that a significant relationship was found between leucine oxidation and urea nitrogen excretion across a wide range of nitrogen and amino acid intakes. This finding may illustrate the fate of surplus amino acids and aid in explaining the greater plasma urea concentration and urinary nitrogen excretion observed following consumption of the Carb + I beverage compared to the Carb + H beverage.

4.4.3. Proposed Mechanism for Difference Between Intact Protein and Protein Hydrolysate Beverages on Protein Recovery

It is appreciated that there are limitations associated with the measurement of amino acids in peripheral blood. This arises from amino acids having already been processed through the liver and that only a few amino acids are processed within muscle. In addition, this study only looked at the branched chain amino acids and the amino acids positioned close to 3-methylhistidine during analysis. However, it would not be expected to see such a uniform difference across all amino acids, as seen in the present study. Possible explanations for this are discussed below.

A possible mechanism for the higher plasma amino acid concentrations observed for the Carb + I beverage compared to the Carb + H beverage may be that the assumed more rapid absorption rate of the protein hydrolysate in the dietary beverages may also have resulted in a relatively more rapid uptake of these amino acids from the blood into muscle tissue. Protein hydrolysates have been found to be absorbed faster across the gut than other types of protein as highlighted by studies showing that oligopeptides are absorbed across the gut epithelium at a faster rate than either whole proteins or free amino acids (Calbet & MacLean, 2002). If these rapidly absorbed amino acids were not being efficiently used by the muscles then we would expect to see greater excretion of nitrogen as urea and ammonia in the blood. However, this was not the case in the present study with nitrogen excretion in the form of urea and ammonia being significantly higher following consumption of the Carb + I beverage compared with the Carb + H beverage. Therefore, a final assumption could be that it would appear as though the protein hydrolysate beverages were not only being absorbed quicker across the gut than the Carb + I

beverage but were also being utilised well by the muscle cells (for protein synthesis rather than deamination) with minimal wastage.

One limitation of the present study, however, is that accurate assessment of the rapid appearance of amino acids in the plasma following consumption of Carb + H beverage was not possible as plasma amino acid analyses were only performed on the blood samples collected every 30-60 minutes during the recovery period. Therefore, there is the possibility that the protein hydrolysate did result in a more rapid appearance of amino acids in the plasma, but that their appearance was not captured in the study's sampling regime. Furthermore, as analyses were performed using venous blood, amino acids may have already been taken up into muscles with a resultant decrease in plasma amino acid levels following consumption of protein hydrolysates.

4.4.4. 3-Methylhistidine

In the assessment of protein breakdown in response to exercise, measurement of 3-methylhistidine has been used as it is an indirect marker of myofibrillar (actin and myosin) protein breakdown (Tipton & Wolfe, 1998). Implicit in this assessment, however, is the assumption that all 3-methylhistidine determined is muscle-derived as opposed to being of dietary origin. To ensure this assumption was valid, the current study design required athletes to adhere to a strict dietary regimen where meat (a primary source of dietary 3-methylhistidine) was excluded from the diet for two days prior to blood sampling. In the current study, urinary 3-methylhistidine excretion was found to be higher following consumption of the Carb + H beverage than following consumption of the Carb beverage. In addition, there was a significant difference in the pattern of variation in 3-methylhistidine levels over time between consumption of the Carb beverage and either the Carb + I or Carb + M beverages. This author speculates that this may indicate that over the initial recovery period, consumption of the protein-containing recovery beverages may facilitate the clearance 3-methylhistidine from the muscle and therefore, may enable faster initiation of the protein recovery process. As yet, no research has examined the acute changes in 3-methylhistidine clearance from the muscle and the effect that diet has on this process. In a study by Goodman (1987), a potential link between

sodium influx into the muscle and 3-methylhistidine release was identified through the use of inhibitors of sodium uptake into muscle cells. The finding of this study found that when the activity of the Na^+-K^+ pump was inhibited the 3-methylhistidine release by the muscle decreased by 21-35%, while inhibitors of either the Na^+/H^+ antiport system or protein kinase C also resulted in decreased 3-methylhistidine release (Goodman, 1987). These findings may indicate a potential for dietary factors to influence the release of 3-methylhistidine from the muscle. In the present study, the significantly higher 3-methylhistidine excretion observed following consumption of the Carb + H beverage may also be linked to the results for urea and ammonia nitrogen excretion. If the excretion of 3-methylhistidine reflects the initiation of the protein recovery process, this would result in the incorporation of amino acids into the muscle, rather than excess amino acids going through deamination resulting in urea and ammonia production. This may therefore indirectly support the more efficient use of amino acids from the Carb + H beverage. Most previous studies have used the full 24 hour urine excretion of 3-methylhistidine as a measure of whole body protein breakdown, rather than measurement of 3-methylhistidine levels during the early stages of recovery. In one such study, the effect of consuming either a carbohydrate only beverage or a milk-based carbohydrate-protein beverage on muscle protein breakdown was examined (Wojcik, *et al.,* 2001). Twenty-four hour urine samples were used for analysis of urinary 3-methylhistidine, with the findings showing a significant increase in the excretion of 3-methylhistidine as a result of eccentric exercise. However, no significant effect of consumption of the different beverages on 3-methylhistidine excretion was found. More research is needed to determine whether there are any acute effect of protein-containing beverages on the release of 3-methylhistidine from the muscle following exercise and the initiation of protein recovery. In the present study, the findings for urinary 3-methylhistidine excretion did not mimic plasma 3-methylhistidine levels, where no significant differences between treatments in plasma 3-methylhistidine concentrations were found.

Chapter 5

CONCLUSIONS AND GENERAL DISCUSSION

In the current sporting environment, extreme demands are placed on an athlete to train and compete at the highest level on multiple occasions throughout a competitive season. Athletes are constantly pushing themselves to the limit in order to gain that elusive edge over their competitors. Nutrition is one factor that has the potential to give the athlete that edge. The focus of nutritional research is no longer just on strategies for before and during exercise, but also on how to maximise an athlete's recovery from exercise so that the effects of one exercise bout will not limit performance in subsequent exercise bouts. The results of the present study highlight the profound influence that nutritional strategies can have on an athlete's recovery from exercise. Traditional post-exercise nutritional strategies have been focused around the intake of beverages, typically designed for during exercise, which supply the body with both carbohydrates and fluids. However, the present study provides evidence that protein is important, not only for the recovery of muscle protein and to facilitate repletion of depleted muscle glycogen stores, but also plays an important role in rehydration of the athlete.

The most significant finding of the present study is the observed improvement in rehydration of the athletes following consumption of any of the three protein-containing beverages. This effect was evidenced by improved restoration of plasma volume and by significantly reduced urine outputs when compared to consumption of the control beverage (carbohydrate only). These two mechanisms combined to cause significantly faster heart rate recovery following consumption of any of the three protein-containing beverages. This finding has major implications for the recovering athlete. The decreased heart rate following exercise may act to decrease the post-exercise metabolic cost of that exercise session, allowing the focus to be on the replacement of depleted energy stores. In addition, the major performance-limiting effects of dehydration are based around a decrease in circulation fluid volume and hemoconcentration. Restoration of circulating fluid volume limits this effect of dehydration, as well as improving the delivery of circulating glucose

and amino acids to the recovering muscle. The results for heart rate recovery in the present study also highlight the value to the athlete in consuming a recovery beverage containing both intact protein and protein hydrolysates. Consumption of the Carb + M beverage provided the best heart rate recovery parameters. Interestingly, this is the first research to look at the effect of using a combination of intact whey protein and its 'same batch' hydrolysate. These two forms of the same batch of whey protein appear to induce slightly different effects on recovery metabolism. The combination of these two, however, as used in the present study as a 50:50 intact : hydrolysate protein mix, shows a potential additive effect from the combination of these in a recovery beverage. This is possibly linked to differences in intestinal absorption rate between these two forms of protein. However, this remains to be determined from further research.

The majority of the differences in rehydration are probably due to the effect of the sodium content of the three protein-containing beverages compared to that in the Carb beverage. The difference in sodium content between the protein-containing beverages and the Carb beverage is a result of the protein hydrolysate manufacturing process and, for the Carb + I and Carb + M beverages, equalising the anion : cation balance across all three protein-containing beverages. A high dietary sodium intake may hinder the rehydration process as the sodium acts to draw water into the intestines via reverse intestinal osmosis. In this study, however, restoration of plasma volume following exercise occurred despite the protein-containing beverages containing very high sodium levels. The presence of protein may, therefore, have prevented reverse intestinal osmosis from occurring, potentially due to the linked absorption mechanism of sodium and certain amino acids. Being able to both consume and absorb large amounts of sodium from a recovery beverage affords the athlete a more rapid correction of exercise-induced dehydration. This would be ideally suited to athletes who compete on multiple occasions over the course of a day or week. Restoration of circulating volume will act to increase stroke volume during recovery and, as a result, cause a reduction in heart rate required for maintenance of cardiac output. The athlete will therefore be in a better physiological state to cope with the demands of subsequent exercise bouts by ensuring a greater nutrient and oxygen supply to the working muscles. Enhancing the restoration of plasma volume may take on increased significance with exercise in hot

conditions, such as the 2004 Athens Olympic Games. In this situation, athletes often perform on multiple occasions over a short period of time with dehydration potentially being a major limitation on athletic performance. Where recovery time is limited, nutritional strategies aimed at maximising hydration status and replacing used energy stores take on increased importance. In most cases the difference between winning and losing may be as small as a matter of seconds. Therefore, ensuring that an athlete is fully recovery from a previous exercise bout may mean the difference becoming finishing first, or last.

This significant rehydration effect following consumption of the protein-containing beverages influenced the results for plasma concentrations for other metabolic parameters of recovery. This is highlighted in the results related to plasma insulin and plasma glucose, where the distinction between the three protein-containing beverages and the control beverage (carbohydrate only) was found to be significant when the rehydration status of the athletes was corrected for. It is likely that the results of previous studies on protein hydrolysates and exercise recovery would have been similarly influenced by the presence of protein and, in addition, the high sodium content of the protein hydrolysates used (an artifact of the manufacturing process). As shown in the present study, consumption of the protein hydrolysate beverages resulted in greater restoration of plasma volume than the Carb beverage which may be a confounding variable in determining the resulting effect of protein consumption of stimulation of insulin release and muscle glucose uptake. Further research is needed to determine the magnitude of this effect on resulting plasma concentrations of metabolic parameters of recovery.

A major benefit of the inclusion of protein into an exercise recovery beverage is that it will invariably have a stimulatory effect on protein recovery following exercise, which cannot be achieved via consumption of a beverage containing carbohydrate alone. Milk proteins are ideally suited for use in beverages focused on exercise recovery. The fact that they contain all of the essential amino acids required by the athlete means that milk proteins have the potential to stimulate muscle protein synthesis above that possible from consumption of lysine-deficient wheat protein. In addition, the higher branched chain amino acid content of milk protein is a further advantage over wheat protein due to the role

these amino acids have in muscle metabolism. The results of the present study also allude to the role of different protein forms on exercise recovery. Based on the results for plasma urea and urinary nitrogen excretion (urea and ammonia), there appears to be a more efficient use of amino acids following consumption of protein hydrolysates compared to intact protein, possibly resulting in greater incorporation of amino acids into muscle protein. The past preference for the use of protein hydrolysates is based around the more rapid absorption rate compared to intact protein. However, intact proteins may also have something to offer the recovering athlete. This is also shown in the results for heart rate recovery, potentially resulting from differences in both the restoration of plasma volume and urine output, indicating that the effects of proteins on muscle recovery are not separate from those of rehydration.

The results of the present study definitely illustrate the importance of dietary protein, when consumed as part of carbohydrate containing beverage, in maximising the recovery of the athlete following endurance exercise. It may also allude to a potential benefit of protein hydrolysates over intact protein on protein recovery. However, the study also shows that there are potential benefits to the recovering athlete associated with both forms of whey protein. As a result, the findings of the present study may support the development of a specifically formulated recovery sports beverage that contains, in addition to carbohydrate, a mixture of both intact and hydrolysed whey protein. This would provide a complete recovery beverage by maximising all three of the metabolic requirements of recovery, namely: restoration of plasma volume; recovery of depleted carbohydrate stores; and recovery of muscle protein.

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