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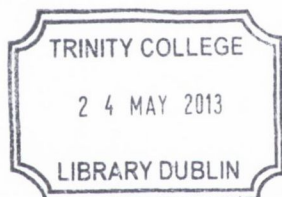
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**The effect of carbohydrate-protein and
carbohydrate beverages on subsequent exercise
capacity and hormonal markers of recovery.**

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Thesis submitted for the award of PhD to the Department of Physiology,
Trinity College Dublin
June 2012



Thesis 9967

Abstract

The effect of carbohydrate-protein and carbohydrate beverages on subsequent exercise capacity and hormonal markers of recovery.

Recovery requires the restoration of physiological and psychological mechanisms and is heavily influenced by the availability and actions of specific hormones and nutrients (Mujika *et al.*, 1996; Kraemer *et al.*, 1998; Filaire *et al.*, 2001). Cortisol (C) and testosterone (T) influence the regeneration phase post-exercise through modulation of anabolic and catabolic processes, and are reported to respond to even slight increases in training load (Coutts *et al.*, 2007). The ingestion of a combined carbohydrate and protein supplement (CHO-PRO) immediately post-exercise has been reported to mediate greater muscle glycogen resynthesis compared with CHO alone (Ivy *et al.*, 2002) as well as significantly improving time to failure in subsequent exercise (Betts *et al.*, 2007). Data suggested that nutritional interventions may suppress the exercise-induced increase in C by providing sufficient exogenous CHO to maintain blood glucose (BGlu) concentration so that muscle glycogen is spared during subsequent exercise, or restored post-exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Gleeson & Bishop, 2000; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a; Lane *et al.*, 2010).

Previous research investigating the effects of CHO-PRO ingestion on subsequent resistance training (RT) performance failed to produce conclusive results (Coyle *et al.*, 2005). Chapter 3 proposed to evaluate if a CHO-PRO supplement, would significantly increase cumulative workload (ΣW ; kg.kg⁻¹ BM) and total work capacity (TWC; kg.kg⁻¹ BM) during a RT regimen performed 240 min after a standard RT protocol in male resistance trained athletes by potentially inducing a greater degree of glycogen restocking compared to iso-carbohydrate (LCHO) or iso-caloric equivalents (HCHO). TWC was significantly greater in the CHO-PRO trial compared with HCHO and LCHO (188 ± 26 vs. 157 ± 21 and 150 ± 16 kg.kg⁻¹ BM, respectively; $P < 0.05$). Ingestion of a CHO-PRO supplement following an initial RT protocol enhanced recovery and resulted in a greater TWC during a subsequent RT protocol when compared with LCHO and HCHO supplements, respectively, possibly due to higher rates of glycogen restoration after the initial RT protocol or an improved availability of BGlu during subsequent exercise.

Considering the necessity to perform functional overreaching investigative studies (Halsen & Jeukendrup, 2004), Chapter 2 proposed to evaluate the hormonal response to in-season training and competition scenarios across three sporting disciplines of varying demands, i.e. soccer, rugby and rowing. Saliva samples were collected pre- and post- in-season training sessions and pre- and post-match/race in male soccer players, rugby players and rowers. Salivary C and T response was significantly amplified in the competition scenario in rugby players. In rowers, C response was significantly greater in the competition scenario, but T concentrations were similar between training and competition scenarios. There was no significant difference in C concentrations between scenarios in soccer players due to a significant interaction effect (pre-training vs. pre-competition; $P < 0.001$) and no significant difference in salivary T responses. Training and competition induced psychophysiological stresses were sufficient to manifest significant changes in the salivary C and T concentrations in rugby players and in salivary C only, in rowers. The psychophysiological stress associated with soccer match-play was sufficient to

induce an increased salivary C concentration over time but did not significantly affect the T response in soccer players.

In light of data reported in Chapter 4, Chapter 5 proposed to evaluate the effect of a CHO-PRO beverage on salivary C and T concentrations compared to a LCHO beverage and a HCHO beverage during standard, in-season soccer training. Limited research has investigated hormonal responses in professional soccer players (Filaire *et al.*, 2001; Ispirlidis *et al.*, 2008) and, to date, no research has investigated the short-term supplementation of sports drinks post-exercise on the salivary C and T response in soccer players. Saliva samples were collected pre-, post- and 1 h post-standardised and monitored in season training sessions on 2 days of the training week, Days 1 and 2 (0 and 24 h, respectively), with an additional sample collected pre-training on Day 4 (72 h). Participants consumed one of 3 test drinks immediately post-training on Day 1 and 2. Mean C concentrations were significantly lower at 72 h relative to 0 after ingestion of both HCHO (6.4 ± 0.8 vs. 12.3 ± 1.7 nmol.L⁻¹, respectively; $P < 0.05$) and CHO-PRO (4.7 ± 0.9 vs. 12.7 ± 2.6 nmol.L⁻¹, respectively; $P < 0.01$). Mean T concentration was significantly lower 1 h post-ingestion of LCHO (0.59 ± 0.07 vs. 0.45 ± 0.04 nmol.L⁻¹, respectively; $P < 0.05$) and CHO-PRO (0.56 ± 0.06 vs. 0.43 ± 0.03 nmol.L⁻¹, respectively; $P < 0.05$) only. However, mean T/C ratio was significantly lower 1 h post-ingestion. Consumption of CHO-PRO and HCHO drinks after in-season soccer training may have partly contributed to a significant decrease in salivary C concentration assessed throughout a standard training week possibly aiding a timely return to an optimal hormonal state.

Considering the findings of previous chapters, Chapter 6 proposed to investigate the effect of different commercially available sports drinks and a placebo (PL) on salivary C and T responses during recovery from two exercise bouts completed in a period of limited nutritional intake and rest. Such a scenario reportedly resulted in increased C concentrations (Lane *et al.*, 2010). Trained cyclists/triathletes performed an intermittent cycle protocol (GD) and a time to failure exercise trial (ExTr) on consecutive evenings and mornings. Area under the curve (AuC) for cumulative BGlu response was significantly greater during HCHO and CHO-PRO vs. PL, respectively (92.0 ± 13.7 and 51.8 ± 5.1 vs. -4.7 ± 5.4 mmol.min.L⁻¹, respectively; $P < 0.001$) and significantly higher for HCHO vs. CHO-PRO ($P < 0.05$). The AuC for cumulative insulin response was significantly greater for both CHO-PRO and HCHO supplementation compared with PL (1161.8 ± 312.1 and 1127.9 ± 296.0 vs. 68.9 ± 21.2 μ IU.min.mL⁻¹, respectively; $P < 0.05$), with no significant difference between CHO-PRO and HCHO. BGlu response varied significantly between CHO-PRO and HCHO and between CHO-PRO and HCHO vs. PL. Salivary C and T concentrations were not significantly different across time, drink trial or pre- to post-ingestion. It appears that in spite of better availability of peripheral glucose and a sustained insulin response, C concentrations and T/C were not different post-ingestion of a CHO-PRO or HCHO sports drink or a PL.

Ingestion of a CHO-PRO supplement had similar effects to ingestion of a HCHO supplement post-exercise. Therefore, considering previous research and the current findings it appears that a CHO-PRO supplement does not induce a significantly improved 'recovery' from either resistance, intermittent or endurance exercise.

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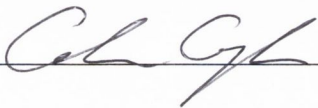
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Declaration

I declare that this thesis is all my own work, with the exception of data presented in Chapter 4 which was collected in collaboration with Mr. Karl Denvir, and has/will not been submitted to this or any other university as part of any other postgraduate program. I grant permission to the Trinity College Dublin librarian to lend or copy this thesis in part on request.

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June 2012

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List of Abbreviations

°C	Degree Celsius
Δ	Change of variable quantity
%HRavg	Mean heart rate as a percentage of heart rate maximum
%HRmax	Percentage of maximum heart rate
ΣW	Cumulative load
$\mu\text{IU.min.mL}^{-1}$	Micro international units per minute per millilitre
$\mu\text{IU.mL}^{-1}$	Micro international units per millilitre
μL	Microlitre
$\mu\text{mol.g}^{-1}$ wet weight	Micromole per gram wet weight
$\mu\text{mol.kg dry muscle}^{-1}.\text{h}^{-1}$	Micromole per kilogram dry muscle per hour
$\mu\text{U.mL}^{-1}$	Micro unit per millilitre
AA	Amino acid
ACTH	Adrenocorticotrophic hormone
Ag	Silver
AgCl	Silver Chloride
Akt	Also known as protein kinase B
ANOVA	Analysis of variance
AuC	Area under the curve
ATP	Adenosine triphosphate
B/B0	Percent bound
B	Mean optical density for the unknowns, standards and controls
B0	Mean optical density for zero wells
beats.min ⁻¹	Beats per minute
BGlu	Blood glucose
BLa	Blood lactate
BM	Body mass
BMI	Body mass index
C	Cortisol
CHO	Carbohydrate
CHO-AA	Carbohydrate amino acid
CHO-FRU	Carbohydrate fructose

CHO-PRO	Carbohydrate-protein
CHO-PRO-Fat	Carbohydrate protein fat
CHO-PRO-LEU	Carbohydrate protein leucine
CK	Creatine kinase
Cl ⁻	Chloride
cm	Centimetre
CNS	Central nervous system
CO ₂	Carbon dioxide
CRH	Corticotropin-releasing hormone
CV%	Percent coefficient of variation
e ⁻	Electron
EDTA	Ethylene diamine tetra acetate
ExTr	Exercise trial
FSH	Follicle stimulating hormone
g	Gram
g.70 kg ⁻¹	Gram per 70 kilogram
g AA.kg ⁻¹	Gram of amino acid per kilogram
g CHO.100mL ⁻¹	Gram of carbohydrate per 100 millilitre
g CHO.kg ⁻¹ .h ⁻¹	Gram of carbohydrate per kilogram per hour
g CHO.kg ⁻¹	Gram of carbohydrate per kilogram
g.dL ⁻¹ :	Gram per decilitre
g PRO.100mL ⁻¹	Gram of protein per 100 millilitre
g PRO.kg BM ⁻¹ .h ⁻¹	Gram of protein per kilogram body mass per hour
g.kg ⁻¹	Grams per kilogram
g.kg ⁻¹ .2 h ⁻¹	Gram per kilogram per two hour
g.kg ⁻¹ .day ⁻¹	Gram per kilogram per day
g.kg ⁻¹ .h ⁻¹	Gram per kilogram per hour
g.min ⁻¹	Gram per minute
GD	Intermittent exercise protocol to induce glycogen depletion
GH	Growth hormone
G1P	Glucose-1-phosphate
G6P	Glucose-6-phosphate
GLUT-1	Glucose transport isoform 1

GLUT-4	Glucose transport isoform 4
h	Hour
H ⁺	Hydrogen ion
H ₂ O	Water
H ₂ O ₂	Hydrogen peroxide
h.day ⁻¹	Hours per day
h.week ⁻¹	Hours per week
Hb	Haemoglobin
HCHO	Iso-caloric to CHO-PRO, high carbohydrate compared to LCHO
Hct	Haematocrit
HR	Heart rate
HRavg	Average heart rate
HRmax	Maximum heart rate
HRmin	Minimum heart rate
Inter-CV%	Inter-assay coefficient of variation
Intra-CV%	Intra-assay coefficient of variation
IRB	International Rugby Board
IRFU	Irish Rugby Football Union
IT	Intensified training
K ⁺	Potassium ion
kCal.kg ⁻¹	Kilocalorie per kilogram
kg	Kilogram
kg.kg ⁻¹ BM	Kilogram lifted per kilogram body mass
kg.m ²	Kilogram per metre squared
km	Kilometre
L	Litre
LCHO	Carbohydrate only equivalent to CHO-PRO
LDH	Lactate dehydrogenase
LH	Luteinising hormone
m	Metre
min	Minute
MJ	Megajoule
mL	Millilitre

mL.70 kg ⁻¹	Millilitre per seventy kilogram
mL.kg ⁻¹ .min ⁻¹	Millilitre per kilogram per minute
mL.kg ⁻¹	Millilitre per kilogram
mm	Millimetre
mmol	Millimole
mmol.h ⁻¹	Millimole per hour
mmol.kg ⁻¹	Millimole per kilogram
mmol.kg ⁻¹ dry mass	Millimole per kilogram of dry mass
mmol.kg ⁻¹ dry muscle	Millimole per kilogram dry muscle
mmol.kg dry muscle ⁻¹ .h ⁻¹	Millimole per kilogram dry muscle per hour
mmol.kg ⁻¹ dw.h ⁻¹	Millimole per kilogram dry weight per hour
mmol.kg ⁻¹ .h ⁻¹	Millimole per kilogram per hour
mmol.L ⁻¹	Millimole per litre
mmol.min.L ⁻¹	Millimole per minute per litre
mmol.min ⁻¹	Millimole per minute
ms	Millisecond
mU.kg ⁻¹ .min ⁻¹	Milliunit per kilogram per minute
NaCl	Sodium chloride
NEFA	Non-esterified fatty acids
NFOR	Non-functional overreaching
nm	Nanometer
NMR	Nuclear magnetic resonance
nmol.240min ⁻¹ .L ⁻¹	Nanomole per 240 minutes per litre
nmol.L ⁻¹	Nanomole per litre
NSB	Non-specific binding well
NT	Normal training
O ₂	Oxygen
OBLA	Onset of blood lactate accumulation
OD	Optical density
OR	Overreaching
OT	Over-training syndrome
<i>P</i>	Statistical probability
PAS	Periodic acid-Schiff
PCr	Phosphocreatine

PL	Placebo
PRO	Protein
PPi	Pyrophosphate
r^2	Pearson's product moment correlation coefficient
RBC	Red blood cell
rev.min ⁻¹	Revolutions per minute
rev.s ⁻¹	Revolutions per second
RM	Repetition maximum
RPE	Rating of perceived exertion
RT	Resistance training
s	Second
SD	Standard deviation
SEM	Standard error of the mean
SGLT-1	Sodium dependant glucose transporter
SHBG	Sex hormone binding globulin
T	Testosterone
T/C	Testosterone to cortisol ratio
<i>t</i>	Time
TCA	Tricarboxylic acid
T _{LAC}	Lactate threshold
TMB	Tetramethylbenzidine
TTF	Test to failure
TWC	Total work capacity
U.5h ⁻¹ .L ⁻¹	Unit per five hour per litre
UDP	Uridine diphosphate
$\dot{V}O_{2max}$	Maximal volume of oxygen consumption
vs.	Versus
w/v	Weight by volume
W	Watt
WBC	White blood cell
Wmax	Peak power output
YoYo IR2	YoYo Intermittent recovery test: level 2
yr	Year

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Chapter 1

General Introduction

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General Introduction

1.1 The role of recovery when training for improved performance

The pursuit of higher levels of performance is unrelenting in both professional and amateur athletes and their coaches. Recently, the idea of improving performance has shifted from the work done in training to maximising recovery between training sessions (Barnett, 2006). Achieving greater and maximal levels of recovery in theory allows the athlete to train at the optimal level when frequent training is required (Barnett, 2006). Athletes and games players typically complete large volumes of training to improve performance. Training is periodised and planned around competition or performance with the highest training load undertaken in the pre-season period and other appropriate periods throughout the competitive season. These periods of training are commonly referred to as functional overreaching (OR). Functional-OR can be deliberately induced in athletes prior to a period of recovery to induce a performance super-compensation effect (Kuipers & Keizer, 1988).

(Deutsch *et al.*, 1998) stated that maximum performance benefits were obtained when the training stimulus mimicked or overloaded the physiological performance conditions. Functional-OR is often utilised by athletes during a typical training cycle, notably during the pre-season period, when applied training loads are sufficient to induce aerobic enhancement through short-term functional-OR in combination with appropriate periods of recovery (Meeusen *et al.*, 2004; Meeusen *et al.*, 2006). By inducing this period of functional-OR prior to a programmed period of recovery, performance 'supercompensation' typically occurs (Kuipers & Keizer, 1988; Meeusen *et al.*, 2006). Acute feelings of fatigue and decreases in performance may occur after such training periods, but can also occur throughout the normal training process (Halsen & Jeukendrup, 2004; Meeusen *et al.*, 2006). However, during periods of functional-OR, greater emphasis is placed on recovery because the success of the training cycle and subsequent performance is dependent on the quality of training (Halsen & Jeukendrup, 2004; Mujika *et al.*, 2004). If recovery is inadequate it can manifest in the athlete as altered mood states and short term performance reduction (Kuipers & Keizer, 1988; Halsen & Jeukendrup, 2004; Mujika *et al.*, 2004; Meeusen *et al.*, 2006; Coutts *et al.*, 2007). If recovery is

inadequate between training sessions throughout periods of intensified training then non-functional overreaching (NFOR) or over-training syndrome (OT) may occur (Kuipers & Keizer, 1988; Jentjens *et al.*, 2006).

Recovery has been defined as the restoration of psychological or physiological mechanisms that allow the athlete to compete or train at a similar level (Mujika *et al.*, 1996). These physiological processes referred to by (Mujika *et al.*, 1996) are heavily influenced by the availability and actions of specific hormones and nutrients (Kraemer *et al.*, 1998). Training allows the athlete to improve their physical, technical and psychological abilities. Recovery allows athletes to train more, and thus improves overall fitness (aerobic, strength and power), technique and efficiency (Kellmann, 2010). For both coach and athlete, achievement of the highest levels of performance on a continual basis during a competitive season is the priority. The training cycle must be precisely controlled to ensure that the athlete can withstand the physiological and psychological demands throughout the season. Ensuring that maximal performance is achieved at key stages during the season is of utmost importance (Mujika *et al.*, 1996). Short-term recovery, between sessions, can potentially prevent NFOR, and long-term recovery within a training cycle can aid in prevention of OT.

Characteristics of OT include persistent fatigue, poor performance in sport despite continued training, changes in mood state and neuroendocrine factors and frequent illness, such as upper respiratory tract infection (MacKinnon, 2000). It has been stated that OT reflects the body's inability to adapt to cumulative fatigue resulting from daily, intense exercise training and performance that is not balanced with appropriate and sufficient rest (MacKinnon, 2000). Effective recovery is essential for the athlete and recovery regimens become more important when considering the period of temporary immunosuppression that occurs following, a bout of prolonged strenuous exercise (Pedersen & Bruunsgaard, 1995; Gleeson & Bishop, 2000; Marcos *et al.*, 2003), or the physical demands of a rugby (Cunniffe *et al.*, 2010) or soccer match (Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a; Ispirlidis *et al.*, 2008). As many researchers have stated (Mujika *et al.*, 1996; Gleeson & Bishop, 2000; MacKinnon, 2000; Betts *et al.*, 2007) effective recovery within a training cycle can reduce the risk of OT, increase the quality of training and enhance performance.

In pre-season and during the regular playing season, the interval between training sessions and sometimes games can be very short. Professional athletes often undertake structured recovery sessions as part of their regular training and post-competition regimen (Barnett, 2006). There is, however, inconclusive evidence of any positive effect of current recovery modalities, such as cold and contrast water immersion, on recovery between stressful training sessions (Barnett, 2006). Despite the wide variety of recovery modalities commonly used, nutritional strategies are critical for optimal physical performance (Coyle, 1992) and can aid an athlete's recovery (Ivy, 1998; Ivy *et al.*, 2002; Betts *et al.*, 2007). Restoration of work capacity through replenishing utilised glycogen and rehydration between training sessions remains the primary physiological facet of recovery (Jentjens & Jeukendrup, 2003; Shirreffs *et al.*, 2004; Maughan *et al.*, 2007).

An important goal of an athlete's everyday diet is to provide the muscle with substrates to fuel the training programme that will achieve optimal adaptation and performance enhancements (Burke *et al.*, 2004). In the absence of adequate carbohydrate (CHO) in the post-exercise period, blood glucose (BGlu) often remains at normal levels although glycogen restoration is severely limited, taking many hours to restore optimum levels (Coyle, 1992). Both competition and training place great demands on the athlete resulting in decreased glycogen content in intra-muscular and hepatic stores with a concomitant increase in cortisol (C) concentration, as well as a significant inflammatory response as C acts to maintain BGlu concentrations via lipolysis and proteolysis. The nutritional strategy implemented by the athlete post-exercise can influence recovery enormously (Coyle, 1992; Burke *et al.*, 2004) and research has demonstrated the effect of CHO ingestion on muscle glycogen restoration post-exercise. However, research by (Ivy *et al.*, 2002) demonstrated that glycogen restoration, the primary facet of physiological recovery, is significantly increased in the post-exercise period when a carbohydrate-protein (CHO-PRO) beverage is ingested. More recent research by Betts *et al.* (2007) demonstrated that ingestion of such a beverage post-exercise resulted in improved exercise capacity in a subsequent exercise bout. Inadequate nutritional intake in combination with recovery of an inadequate duration has been reported to result in impaired immunity (Lane *et al.*, 2010; Mikulski *et al.*, 2010). Low CHO intake has been associated with increased C concentrations, as glucose is a primary fuel source for leukocytes, while

inadequate protein (PRO) intake can result in an impairment of the number of fully differentiated T-cells (Gleeson & Bishop, 2000). Ingestion of CHO drinks during exercise reportedly attenuated the exercise induced increase in C concentration and limited the degree of exercise-induced immunosuppression (Bishop *et al.*, 1999b). The anabolic hormone testosterone (T) has a protective effect against the proteolytic effects mediated by the catabolic hormone C, thus protecting lean tissue (Elloumi *et al.*, 2003). It is therefore, vital for the athlete to have a testosterone to cortisol (T/C) ratio that is reflective of an anabolic state so that muscle mass can be maintained and the catabolic gluconeogenesis pathways mediated by C are limited by the availability of sufficient substrate post-exercise to meet the needs of the athletes recovery. Together, the available data suggested that the employment of such a nutritional intervention may be advantageous in improving quality of training during days of the training cycle that require multiple training sessions, but also may contribute to maximum adaptation of training within the training cycle as stated by Burke *et al.* (2004) and Meeusen *et al.* (2006).

With due consideration for the vast amount of literature investigating the wide ranging intricacies of the physiology of recovery from exercise, this review of literature will attempt to relate relevant secondary source findings to the definition of recovery as stated by Mujika *et al.* (1996) and with due consideration for the development of OT syndrome as defined by MacKinnon (2000). This review of literature will discuss the demands and effects of training for performance on muscle glycogen and hormonal concentrations, and the associated challenges presented to the physiology of the athlete in response to multiple training sessions within a relatively short period of time, or periods of functional-OR. The sport of rugby union was utilised to illustrate this due to the multitude of skills & varying demands of the game that must be addressed in training for maintenance and continual advancement of performance. The necessity and rationale for balance between training and recovery, and the effects of exercise with inadequate recovery on the responses of the hormones C & T will be addressed, along with the potential for use of these variables in determining exercise stress or tolerance of the training load. The relationship between fuel for exercise and manipulations of the post-exercise environment with nutritional interventions will be discussed, in particular, the addition of PRO or amino acids (AA) to CHO or the combined ingestion of CHO-

PRO on muscle glycogen restoration and subsequent performance. The effects of such nutritional interventions, or lack thereof, will also be addressed in order to outline a functional and sport-specific application of commercially available sports drinks in maintaining performance throughout a training cycle and if they may be useful in maintaining an anabolic post-exercise environment with sustained immune function.

1.2 Balancing training demands

Research detailing the volume and varying facets and demands of team sports is limited. Several studies have presented data evaluating the effects of pre-season, high-volume periods and even entire seasons of training and match play across varying field sports (Filaire *et al.*, 2001; Coutts *et al.*, 2007; Cormack *et al.*, 2008). Most recently, Argus *et al.* (2010) outlined the large volume of training; varying in intensity, duration and emphasis undertaken by professional rugby union players during a pre-season training block (Table 1.1). Rugby union is a game that requires an athlete to possess a variety of attributes to fulfil the demands of the varied positions of the game. However, the fundamentals of endurance, strength, speed and power all place great and varying demands on the players throughout a season. However, an important difference lies in the structure of the fixtures calendar between the northern and southern hemispheres. Southern hemisphere players compete in a shorter, concurrent league (Super 15) followed by International test matches before competing in provincial/club leagues and end of season tours. Northern hemispheres players participate in lengthy league seasons punctuated by higher intensity European and International test matches and thus perform one lengthy pre-season training block with varying periods of maintenance. Information from southern hemisphere rugby union reported that short pre-season training phases, varying from 2 to 6 weeks before the beginning of each separate competition, provided conditioning coaches and athletes with limited opportunities to significantly enhance aspects of physical conditioning (Argus *et al.*, 2010). Professional Irish rugby union players participate in a 10 week high-intensity pre-season training period prior to the beginning of their season with later periods of high-intensity training dictated by natural breaks in competition.

The aims of the pre-season training phase are to increase aerobic and anaerobic fitness, running velocity, strength and power, and improve body composition (Argus *et al.*, 2010). However, once competition commences, the volume of conditioning training is reduced while the volume of rugby specific tactical and skill sessions are increased (Argus *et al.*, 2010). Throughout pre-season and regular playing season, the interval between training sessions may be very short. Regardless of their origin, due to the intense nature of the game and the varying skill set required by players in different positions (Deutsch *et al.*, 1998; Duthie *et al.*, 2003; Deutsch *et al.*, 2007; Roberts *et al.*, 2008; Cunniffe *et al.*, 2009) achieving the balance between training and competition stresses and recovery is important in maximising the performance of these athletes (Barnett, 2006).

Training mode	Week 1	Week 2	Week 3	Week 4	Total	Mean RPE
Resistance training	3	5	7	5	20	7.1
Hill sprints	2	1	1	0	4	9
Boxing training	2	2	1	2	7	8.4
Aerobic / conditioning	2	5	5	4	16	7.4
Speed	0	0	2	1	3	6.9
Rugby specific training	0	0	0	3	3	5.6
Total sessions	9	13	16	15	53	7.4 (Mean)

Table 1.1: Training mode, training frequency and rating of perceived exertion (RPE) in elite rugby union players over a 4-week pre-season training phase (Argus *et al.*, 2010).

1.3 Cortisol and testosterone response to training and competition

Halsen and Jeukendrup (2004) in a review of OT and NFOR research stated that there are many concerns associated with investigating these areas. These included a lack of a consensus on a single physiological variable that could be used as a diagnostic tool for either NFOR or OT. The identification of any reduction in performance, and similarly, a negative or positive adaptation to the training are important symptoms for diagnoses of NFOR and OT so there is a need to measure and report performance decrements (Halsen & Jeukendrup, 2004). Responses to intensified training are likely to be individual to the athlete and differences may exist between different sporting activities, however, it is necessary to perform functional-OR studies in order for the physiological adaptations to training to be induced and accurately documented (Halsen & Jeukendrup, 2004). Urhausen *et al.* (1998) stated that the behaviour of C and T provide a physiological indicator of the current physiological stress of training as opposed to an indicator of OT and may be useful indicators for monitoring responses to training and competition (Halsen & Jeukendrup, 2004; Coutts *et al.*, 2007; Cormack *et al.*, 2008).

Coutts *et al.* (2007) identified OR in semi-professional rugby league players with only a relatively small increase in the training load above normal. They investigated the effect of OR by comparing one group of players who performed normal training (NT) for 6 weeks with a second group of players performing intensified training (IT) over the same period. Several biochemical markers, including T and C concentrations in serum were investigated with assessment of relevant physiological measures before, during and after the 6 week training period. Significant performance deficits were observed in the IT group despite no significant differences in T concentration and in the T/C ratio. An athlete's sensitivity to increases in training load should be of concern to the coach and it is this fine balance between training and straining that highlights the importance of not only adequate, but effective recovery, in a training cycle.

Elloumi *et al.* (2003) and Cunniffe *et al.* (2010) have both quantified the hormonal response to a competitive rugby match. Post-match samples were collected immediately, 14 and 38 h (Cunniffe *et al.*, 2010) post-match or immediately, 2 h and twice daily over the following 6 days post-match (Elloumi *et al.*, 2003). Elloumi *et*

al. (2003) reported that post-match salivary C concentrations were 148% higher than pre-match data recorded on a rest day prior to the match. Cunniffe *et al.* (2010) documented that plasma C concentration was significantly elevated immediately and 14 h post-match and that players displayed disturbed immune function (neutrophil degranulation) for up to 38 h post-match. Both studies reported significant increases in C concentration with an accompanying decrease in T concentration immediately post-match, manifesting in a large decrease in the T/C ratio (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010). Elloumi *et al.* (2003) reported significant elevation in the T/C ratio when compared to rest for up to 5 days post-match, suggesting such a post-competitive hormonal response may be required to restore the breakdown of homeostasis induced by the mental and physical strain associated with a rugby match. Post-competitive T/C data documented by Cunniffe *et al.* (2010) remained lower when compared to rest for up to 14 h post-match. However, C concentration decreased below resting data at 38 h post-match with a corresponding increase in T resulting in a significantly elevated T/C ratio. Such a significant increase in the T/C ratio at 38 h post-match when compared with rest may indicate a rebound anabolic stimulus during the recovery period (Cunniffe *et al.*, 2010).

A high-intensity soccer training programme reportedly resulted in a significant decrease in salivary T concentration and a significant increase in salivary C concentration (Filaire *et al.*, 2001). Bishop *et al.* (1999a) observed no significant change in serum C response after 90 min of simulated match play although they reported a decreasing trend from 0 to 90 min of exercise. More recent data published by Moreira *et al.* (2009) identified a trend of increasing salivary C concentration with no significant differences pre- to post-match. During prolonged low-intensity sculling (~75% of OBLA), Jürimäe *et al.* (2001) reported that plasma C and T concentrations were related to the distance covered, but no significant differences in T/C ratios were observed. Mäestu *et al.* (2005) later reported significant reductions in resting free T and T/C ratio after 3 weeks of heavy rowing training. However, these variables returned to resting levels after a 2 week tapering period. Mäestu *et al.* (2005) concluded that hormonal responses recorded after heavy training stress in their study could be interpreted as the first sign of decreased tolerance to training load in athletes and the risk of the appearance of OT.

Hough *et al.* (2011) reported significantly elevated C (saliva and plasma) concentrations after both intermittent and steady state cycling. However, the post-exercise C response was more prolonged after a 30 min cycling protocol alternating between 1 min at 55% and 4 min at 80% of peak power output (W_{max}) when compared with a steady-state (75% W_{max}) cycle to fatigue. Hough *et al.* (2011) also observed a significant T (saliva and plasma) response post-exercise; the greatest increase experienced post-intermittent cycling. The data presented by Hough *et al.* (2011) suggested that intermittent exercise caused more robust elevations of plasma and salivary T and C concentrations compared with steady-state exercise.

Available research to date has attempted to investigate and quantify the short- and long-term hormonal and immune responses to training and competition across varying athletic populations (Table 1.2). There appeared to be a relationship between the physical and psychological stress inherent in competitive situations across the documented sporting arenas (Bishop *et al.*, 1999a; Filaire *et al.*, 2001; Jürimäe *et al.*, 2001; Elloumi *et al.*, 2003; Mäestu *et al.*, 2005; Ispirlidis *et al.*, 2008; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). However, limited data is currently available on the effect of the consumption of CHO sports drinks on hormonal variables, particularly C and T, which are stated to be the more useful variables in assessing an athlete's tolerance of a given training load in real time sporting scenarios (Coutts *et al.*, 2007).

The role of hormones in controlling metabolism and their importance to the athlete's well-being are well documented in the literature and will be discussed further throughout this thesis. Research has also revealed that both C and T are sensitive to everyday factors important in facilitating recovery, namely sleep, nutrition and dietary factors (Volek *et al.*, 1997; Backhaus *et al.*, 2004; Hackney *et al.*, 2005; Lane *et al.*, 2010; Mikulski *et al.*, 2010). However, our knowledge concerning the effect of commercially available recovery drinks, predominantly consisting of CHO with PRO or a variety of AA's, on sport specific and 'real time' sporting scenarios is limited. Previously, Halson and Jeukendrup (2004) stated that there is a necessity to perform functional-OR studies to assess the effects of functional-OR on the physiology of performance, and although the effects of OR on hormonal markers and immune response has been documented (Coutts *et al.*, 2007), the effect of

consumption of a post-exercise recovery drink, a common place practice across all athletic populations, on these hormonal markers in a sport specific scenario warrants assessment to determine the worth of both the beverage and the practice. The available data suggested that the consumption a CHO-PRO drink post-exercise was most advantageous in restoring the physiological and, in some way, the psychological aspects of athletic performance (Ivy *et al.*, 2002; Berardi *et al.*, 2006; Kaastra *et al.*, 2006; Betts *et al.*, 2007), both essential requisites of Mujika's definition of recovery (Mujika *et al.*, 1996).

Therefore, the rationale of this body of research attempted to focus on, firstly, the effects of commercially available CHO versus CHO-PRO drinks on indices of recovery and subsequent performance capacity, and secondly, the effect of similar sports drinks on the salivary concentrations of C and T, as well as the T/C ratio, in a sport specific real time training and competition scenario, and finally a sport-specific scenario with limited recovery duration and nutritional intake between exercise bouts.

Paper	Sport	Stimulus and medium of assessment	Cortisol response	Testosterone response	T/C ratio response
Urhausen <i>et al.</i> (1998)	Cycling	↑ training frequency with similar training volume; serum	No significant difference	No significant difference	No significant difference
Bishop <i>et al.</i> (1999a)	Soccer (simulated)	2 x 45 min soccer specific circuit; serum	No significant difference post-exercise. No effect of CHO ingestion.	Not assessed	Not assessed
Filaire <i>et al.</i> (2001)	Soccer	16 week high-load training bloc; saliva	Significantly ↑ post-exercise	Significantly ↓ post-exercise	Significant ↓ post-exercise
Jürimäe <i>et al.</i> (2001)	Rowing	Single endurance training session: 2 h at 75% OBLA; serum	Unchanged pre- to post- or 2 h post-training	Unchanged pre- to post- or 2 h post-training	No significant difference
Elloumi <i>et al.</i> (2003)	Rugby Union	Rugby union test match; saliva	Significant ↑ post-match	Significant ↓ immediately post-match	Elevated for up to 5 days post-match
Mäestu <i>et al.</i> (2005)	Rowing	2000m (maximal) at rest, after 3 week high-intensity training bloc (HI) and after 2 week taper; serum	3 week HI: Significantly ↓ at rest. No maximal exercise induced response	3 week HI: free T significantly ↓ Significant ↓ post-maximal exercise 2 week taper: Restored to normal, pre-training levels	3 week HI: significantly ↓ 2 week taper: Restored to normal, pre-training levels
Coutts <i>et al.</i> (2007)	Rugby League	6 week intensified training bloc (IT); saliva	No significant difference post-IT	Significant ↓ post-IT	Significant ↓ post-IT

Cormack <i>et al.</i> (2008)	Australian Rules Football	Longitudinal assessment throughout season; saliva	Significant ↓ relative to rest	Varied response	Significant ↑ in 70% of evaluations
Moreira <i>et al.</i> (2009)	Soccer (simulated)	Intra-squad training match; saliva	No significant difference pre- to post-match	Not assessed	Not assessed
Cunniffe <i>et al.</i> (2010)	Rugby Union	Rugby union test match; serum	Significant ↑ immediately and 14 h post-match	Significant ↓ immediately post-match	Suppressed up to 14 h post-match
Hough <i>et al.</i> (2011)	Cycling	Intermittent and steady State protocol; serum and saliva	Significantly ↑ post-exercise. More prolonged significant ↑ post-intermittent protocol	Significantly ↑ post-exercise. More pronounced ↑ post-intermittent protocol	Not assessed

Table 1.2: Summary of hormonal responses to varying stimuli across varying sports and sporting scenarios from previous research.

1.4 Regulation and synthesis of cortisol & testosterone

Endogenous hormones play an essential role in regulative and adaptive mechanisms associated with physical exercise and influence the regeneration phase after exercise through modulation of anabolic and catabolic processes (Urhausen *et al.*, 1998; Jürimäe *et al.*, 2011). Physiological and psychological systems work together to determine energy intake and output (McMurray & Hackney, 2005). Both C and T are secreted in response to high intensity (>60% maximal volume of oxygen consumption; $\dot{V}O_{2max}$) aerobic and anaerobic exercise (Kirschbaum & Hellhammer, 1989; Stone, 1991; Lac & Berthon, 2000; Hill *et al.*, 2008). Investigation of biochemical and hormonal markers of exercise stress, such as C and T, has provided an understanding of the relationship between the volume and intensity of exercise and homeostasis.

Adrenocorticotrophic hormone (ACTH), creatine kinase (CK), ferritin, growth hormone (GH), insulin, lactate dehydrogenase (LDH), uric acid, along with C and T have previously been investigated in response to exercise and some have been mentioned as hormonal and biochemical indicators of exercise stress (Clarkson & Tremblay, 1988; Chandler *et al.*, 1994; Kraemer *et al.*, 1998; Rogol & Kraemer, 2005; Brancaccio *et al.*, 2007). Some biochemical markers, such as CK and LDH, indicate muscle damage and training status, respectively (Clarkson & Tremblay, 1988; Brancaccio *et al.*, 2007) while others are markers of oxidative stress and the regenerative response to exercise. Of particular interest is the post-exercise response of the anabolic and catabolic hormones T and C, respectively. A catabolic hormone of adrenal origin, C has a role as a stress hormone and its presence is suggested as an indicator of the endocrine systems response to exercise stress (Urhausen *et al.*, 1995), while T, an anabolic hormone of testicular origin, is important in the post-exercise recovery response (Urhausen *et al.*, 1995).

Normally C is secreted in response to stress, mediated through ACTH (Bishop *et al.*, 2005). However, when BGlu is low, corticotropin-releasing hormone (CRH) is stimulated by the hypothalamus as a result of elevated circulating catecholamines associated with lipolysis and fatty-acid oxidation, and the sympathetic nervous system (McMurray & Hackney, 2005). CRH results in an increased secretion of

ACTH and ultimately C, which improves glucose conservation and lipid usage for energy production (McMurray & Hackney, 2005). ACTH is responsible for the production and release of glucocorticoids, namely C in the adrenal cortex. ACTH is produced in the anterior pituitary gland by recognition of a threat or challenge and is circulated throughout the body by the systemic blood. The breakdown of PRO to AA in the liver is also stimulated by C and the liberated carbon chains can enter the tricarboxylic acid (TCA) cycle or be used for gluconeogenesis. In most cells of the body a simultaneous suppression of PRO synthesis is mediated by C to save energy, maintain BGlu and conserve the body's glycogen by promoting lipolysis (Rogol & Kraemer, 2005).

The Leydig cells of the testes produce T which stimulates development of male sex characteristics, growth and PRO anabolism (Hackney *et al.*, 2005; Rogol & Kraemer, 2005). The number of Leydig cells is regulated by luteinising hormone (LH) and follicle stimulating hormone (FSH), and the amount of T produced by existing Leydig cells is under the control of LH which regulates the expression of 17- β hydroxysteroid dehydrogenase (Swerdloff *et al.*, 1992; Hackney *et al.*, 2005). Synthesis of T is regulated by the hypothalamic-pituitary-testicular axis and occurs when gonadotropin-releasing hormone is released by the hypothalamus which in turn stimulates the pituitary gland to release FSH and LH. Both FSH and LH stimulate the testes to synthesise T (Swerdloff *et al.*, 1992). A negative feedback loop acting on the hypothalamus and pituitary inhibit the release of gonadotropin-releasing hormone, FSH and LH, respectively, once T levels are at optimum. A small amount (~5%) of T can be produced in the adrenal gland of males (Hackney *et al.*, 2005). Along with T, insulin and GH form part of the regenerative response to exercise with an inverse pattern of response existing between the two anabolic hormones, insulin and T (Chandler *et al.*, 1994; Kraemer *et al.*, 1998). Insulin stimulates glucose absorption and storage of glycogen while GH stimulates PRO synthesis, growth and intermediary metabolism (Rogol & Kraemer, 2005). Anabolic effects of T include stimulation of nitrogen retention and PRO synthesis within the body (Hackney *et al.*, 2005).

Secretion of both C and T are determined by circadian rhythm, which is ACTH-dependent for C. Pulsatile release of LH results in fluctuations of circulating T, with

2 to 4 pulses observed over a 6 to 8 h period (Hackney *et al.*, 2005). Plasma C concentrations are at their highest shortly after waking and then decline throughout the day to reach a nadir in the late evening (Bishop *et al.*, 2005). At 09:00 C has a reference range of between 140 and 690 nmol.L⁻¹ which decreases to <100 nmol.L⁻¹ by midnight (Bishop *et al.*, 2005). Hackney *et al.* (2005) stated that nocturnal elevations of T can be observed in comparison to daytime levels. The peak in T concentration occurs in the afternoon (Krieger & Allen, 1975) with reference concentrations ranging from 9 to 30 nmol.L⁻¹ in male volunteers (Bishop *et al.*, 2005). However, research suggested that T concentration is affected by how stimulated or 'psyched up' an athlete is (Mason *et al.*, 1973; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005). Krieger and Allen (1975) stated that C can be secreted independent of circadian rhythm in response to physical and psychological stress, while T concentration increases post-exercise to induce anabolic effects. In addition, a lack of sleep and dietary factors can affect both C and T concentrations (Volek *et al.*, 1997; Backhaus *et al.*, 2004; Hackney *et al.*, 2005; Lane *et al.*, 2010; Mikulski *et al.*, 2010).

Both C and T are present in the blood bound to their respective carrier proteins, transcortin and sex hormone binding globulin (SHBG), or in their free, unbound form (Bishop *et al.*, 2005; Hackney *et al.*, 2005). Ninety-five percent of C is present in the bound form (Bishop *et al.*, 2005), while Hackney *et al.* (2005) stated that approximately 98% of circulating T is transported in the bound form. Unbound C and T can enter the saliva via intracellular mechanisms. Unbound steroid molecules are described as small, non-polar and lipophilic and can readily diffuse through the phospholipid layer of the cell walls in salivary ducts (Vining & McGinley, 1987). The concentrations of both C and T in their unbound states present in the saliva can vary from 2 upwards to 10% and are unaffected by salivary flow rate (Vining & McGinley, 1987).

The physiological relationship of these two hormones dictates that T decreases as C increases due to inhibition of the gonadotropic axis at a hypothalamic level by the corticotropic axis, either by a direct effect of CRH on gonadotropin-releasing hormone secretion, or via an increase in β -endorphin (Barbarino *et al.*, 1989). A

typical high load training or competition response would therefore result in elevated C with suppressed T in saliva or serum, resulting in a shift towards a catabolic effect (Urhausen *et al.*, 1998; Lac & Berthon, 2000; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Mäestu *et al.*, 2005; Cunniffe *et al.*, 2010). These catabolic effects are reportedly reversed by regenerative measures (Adlercreutz *et al.*, 1986; Urhausen *et al.*, 1998; Tarpenning *et al.*, 2001; Cunniffe *et al.*, 2010). A decreased T/C ratio does not automatically lead to a decrease in performance or a state of OT but instead acts as an indicator of stress or physiological strain due to training (Urhausen *et al.*, 1995; Filaire *et al.*, 2001; Meeusen *et al.*, 2004; Coutts *et al.*, 2007) or a biological marker of a breakdown in homeostasis following competition (Adlercreutz *et al.*, 1986; Elloumi *et al.*, 2003).

1.5 Factors affecting cortisol and testosterone responses

Jürimäe *et al.* (2011) stated that exercise appeared to be a major factor affecting hormonal modulation of energy intake and energy output. Exercise stress increases energy expenditure directly, but also affects a number of hormones and cytokines that control metabolic rate (McMurray & Hackney, 2005). Recent studies have stated that T/C ratio may be a useful variable in monitoring the response to training and competition in elite athletes (Halsen & Jeukendrup, 2004; Coutts *et al.*, 2007; Cormack *et al.*, 2008) due to its nature of increasing with intensity and duration (Kirschbaum & Hellhammer, 1989).

Considering that the physiological drive of the C and T response to exercise remains unchanged, differing hormonal responses reported may be attributable to exercise intensity, exercise type, psychological intensity of the match-play or training situation or the varied populations studied. Differences in hormonal responses between studies and sports may be due to the competitive element or the physical contact induced trauma element of the stimulus. For example, in the protocol utilised by Bishop *et al.* (1999a), participants performed a simulated soccer protocol consisting of a circuit of soccer specific movements that excluded jumping, tackling or competing for ball in the air. Removing such physical aspects of soccer match play limits the degree of trauma relative to that experienced in competitive match-play, and to an extent, the intermittent nature of soccer match-play, reducing energy expenditure, tissue damage and the expected C response. Bishop *et al.* (1999a)

reported no significant neutrophil degranulation which may have limited the C response. Cunliffe *et al.* (2010) reported a significant increase in neutrophil degranulation and a decrease in neutrophil responsiveness 38 h post-match which was possibly mediated by the C concentration. Cunliffe *et al.* (2010) stated that the sustained decreased neutrophil responsiveness pointed to the nature of competitive rugby union. Similarly, the training game protocol employed by Moreira *et al.* (2009) consisted of two 35 min halves with a 10 min intermission. While this training game protocol may have been a truer reflection of soccer match-play compared to the protocol utilised by Bishop *et al.* (1999a), Moreira *et al.* (2009) stated that the training element of the match may have reduced the emotional stress inherent in a competitive soccer environment. Moreira *et al.* (2009) also reported that large intra- and inter-individual C responses may be reflective of the emotional stress individuals place upon such a situation. The athletes' contribution to the game of rugby or soccer, or to a race in rowing or cycling, as well as the respective outcome, can determine the C and T response (Salvador, 2005).

Jensen *et al.* (1991) stated the adrenal-cortisol response is stronger for intermittent anaerobic versus aerobic exercise and this was in agreement with the recent data by Hough *et al.* (2011). During small-sided games, commonly utilised in soccer training, players can be exercising at an average exercise intensity of 80 to 90% of heart rate maximum (HRmax) for 2 to 6 min (Helgerud *et al.*, 2001; Little & Williams, 2006; Little & Williams, 2007). During match-play, a player can cover up to 12 km and performs multiple short sprints, turns, jumps, and low-intensity runs but the overall intensity can be lower (Bangsbo, 2007). A rugby players' contribution to the game is determined by playing position. Cunliffe *et al.* (2009) reported that rugby players covered a mean distance of 6953 m during 83 min of match-play varying from 6680 m by forwards and 7227 m by backs at an average exercise intensity of 172 and 170 beats.min⁻¹ for forward and back line players, respectively.

Eliminating the competitive situation can significantly affect the C and T response. Gonzalez-Bono *et al.* (1999) stated that in a highly competitive situation, changes in T concentration are related to the contribution the individual makes to the outcome and the causes he attributes to it. Both C and T concentrations are affected by

psycho-somatic and arousal phenomena (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005). Suay *et al.* (1999) reported positive correlations of T and C concentrations with number of threats, attacks and fights in Judo fighters, but noted that the response was individual. Mean T concentration in those that responded increased by 15% prior to competition and this was accompanied by a greater motivation to win and higher C concentrations just before the competition (Suay *et al.*, 1999). C has also been stated to increase in an anticipatory manner if a high energetic cost is foreseeable in the near future (Mason *et al.*, 1973) while T concentrations in soccer players can fluctuate according to perception of the opponent, as well as the game venue (Neave & Wolfson, 2003).

It has been stated that there are significant differences in the metabolic and hormonal responses to exercise between athletically trained and un-trained individuals, even when physically fit individuals work at the same percentage of their maximal capacity as unfit individuals (Bloom *et al.*, 1976; Hackney *et al.*, 2005). Competitive sport requires a degree of physical exertion capable of affecting C and T (Cunniffe *et al.*, 2010). Physical effort is dictated by physical fitness (Helgerud *et al.*, 2001), therefore, as technical and tactical performance in a game are determined by physical fitness, physical fitness can in turn determine C and T response. It has been suggested that prolonged exposure to high-intensity training may increase the diurnal secretion of C (Filaire *et al.*, 2001) and decrease T (Hackney *et al.*, 2005). This appears to be due to an adjustment in the regulatory axis of T (Hackney *et al.*, 2005). The participants in these studies vary from collegiate (Bishop *et al.*, 1999a) to professional soccer players (Filaire *et al.*, 2001; Moreira *et al.*, 2009), IRB tier 2 nation rugby players (Elloumi *et al.*, 2003) and IRB tier 1 nation, 6 Nations rugby players (Cunniffe *et al.*, 2010), possibly accounting for differences in hormonal responses. Hough *et al.* (2011) reported salivary and plasma responses in male cyclists training 3 times per week in non-competitive laboratory situations while Jürimäe *et al.* (2001) reported data from national standard male rowers.

Although a link between physical fitness and C and T concentrations has not been confirmed, a recent study by Grandys *et al.* (2009) reported an increase in T concentrations accompanied a 3.7% increase in $\dot{V}O_2\text{max}$ after 5 weeks of moderate-

intensity low volume endurance training in untrained men. Arnason *et al.* (2004) concluded that athletes possessing a lesser degree of aerobic conditioning will endure a greater degree of stress during both training and competition.

1.6 Fatigue

Noakes *et al.* (2004) proposed that exercise performance was regulated by the central nervous system (CNS) to prevent catastrophic physiological failure and that conscious sensations of fatigue do not originate directly from the build-up of metabolites in the muscle but rather from the subconscious parts of the brain. Central fatigue occurs when the CNS no longer adequately activates the motor neurons supplying the working muscles, which causes the person to slow down or terminate exercise even though the muscles themselves are still able to perform. Central fatigue is often psychologically based and may stem from discomfort during strenuous exercise. Well-trained and highly motivated athletes are able to overcome this to a certain extent.

A number of variables can contribute to the onset of peripheral fatigue, which impairs local muscle function and contributes to the eventual onset of central fatigue. Lactate accumulates in the muscle tissue and blood (Juel *et al.*, 2004) as a result of adenosine triphosphate (ATP) production via anaerobic glycolysis during strenuous exercise. Much of the lactate will be released into the circulation during the post-exercise recovery phase but some of this accumulated lactate will be metabolised in the muscle itself (Bangsbo *et al.*, 1991a). This lactate can then be transported via the circulation to the liver as a gluconeogenic precursor (Dubouchaud *et al.*, 2000) and to the heart where it is a major respiratory fuel (Juel & Halestrap, 1999). Lactate accumulation has also been implicated in contributing to the onset of fatigue by disrupting excitation-contraction coupling, and thus, promoting a decline in tension development independent of pH (Hogan *et al.*, 1995; Favero *et al.*, 1997; Spangenburg *et al.*, 1998).

During high-intensity exercise lactic acid is produced by the exercising muscle and this subsequently dissociates in solution at physiological pH to yield lactate and free H^+ (Philp *et al.*, 2005). Within the muscle, a build up of H^+ during high-intensity exercise may limit the ability to generate tension via disruption of excitation-

contraction coupling and regulation of the cross-bridge cycle (Spriet *et al.*, 1985; Metzger & Fitts, 1987; Chin & Allen, 1998). The reduction in muscle pH following exercise is related to the exercise intensity (Street *et al.*, 2001). Movement of lactate is coupled to the movement of H^+ in a 1:1 ratio, so it is reasonable to assume that lactate transport is an important factor in muscle pH regulation (Juel & Halestrap, 1999). After leaving the active muscle cell H^+ accumulates in the blood where it may lead to the onset of metabolic acidosis (Hollidge-Horvat *et al.*, 1999). Much of this H^+ is buffered chemically via reversible reactions such as the carbonic acid/bicarbonate buffer system, which forms carbon dioxide (CO_2) that is readily “blown off” via an elevation in ventilation appropriate to the exercise intensity (Hirakoba *et al.*, 1996).

During high-intensity short duration exercise requiring rapid generation of force, a substantial amount of ATP is derived from the breakdown of phosphocreatine (PCr), which is stored in skeletal muscle (Hill, 1962). Phosphocreatine can be re-synthesized via a reversible reaction catalysed by the enzyme CK, which transfers the phosphate group from ATP back to creatine. However, stores in the active muscle are quickly depleted during intense exercise and this can limit further exercise performance (Naveri *et al.*, 1997).

The accumulation of extracellular K^+ as a result of rapid and repetitive muscle activation during intense exercise, may contribute to muscle fatigue by increasing the action potential threshold and reducing the excitability of the muscle fibres (Cairns *et al.*, 1997). McKenna *et al.* (2006) investigated the link between reactive oxygen (O_2) species, K^+ regulation and skeletal muscle fatigue. Infusion of the antioxidant compound N-acetylcysteine, both prior to and during cycle ergometer exercise, increased time to fatigue compared with control ($23.8 \pm 8.3\%$) and this was attributed, at least in part, to improved K^+ regulation. Mohr *et al.* (2004) investigated 3 one-legged knee extension exercise bouts to exhaustion, separated by 10 min recovery and demonstrated that the rate of K^+ accumulation progressively declined during the repeated bouts, suggesting that accumulation of interstitial K^+ *per se* did not cause fatigue when intense exercise was repeated.

Exhaustion often coincides with muscle glycogen depletion, particularly during endurance exercise of >90 min duration (Hawley *et al.*, 1997), and CHO ingestion during prolonged exercise has been demonstrated to delay fatigue (Coggan & Coyle, 1991). While glycogen availability may be a limiting factor for prolonged endurance exercise below the (T_{LAC}) lactate threshold (Febbraio & Dancsey, 1999), it is not a limiting factor for high-intensity, short-duration exercise. With repeated high-intensity exercise bouts, large decreases in muscle ATP and phosphocreatine, in conjunction with increases in H^+ and muscle and blood lactate (BLa), are more likely to contribute to a reduction in performance than muscle glycogen depletion (Hargreaves *et al.*, 1998). The rate of muscle glycogen resynthesis is higher following short-duration, high-intensity exercise than following prolonged, moderate intensity exercise. Bangsbo *et al.* (1991a) reported a glycogen resynthesis rate of $15.1 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ following 3 min of intense knee extension exercise while Choi *et al.* (1994) reported a rate of $18 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ following three 1 min cycling bouts at $130\% \dot{V}O_{2max}$. In comparison, the resynthesis rate following prolonged exercise is approximately $2 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ (Blom *et al.*, 1986; Ivy *et al.*, 1988). Peak muscle and BLa is higher following high-intensity exercise compared with prolonged exercise and as lactate is a glycolytic intermediate this may contribute to the increased rate of glycogen synthesis.

Stevenson *et al.* (1987) suggested that resynthesis of glycogen from lactate requires a high BLa concentration in order to prevent lactate diffusing from the muscle cells into the circulation. Nordheim and Vollestad (1990) demonstrated that when muscle lactate concentration is elevated, glycogen is resynthesised in inactive Type II fibres, however, it is oxidised in preference to glycogen in Type I fibres. During active recovery following high-intensity exercise, Fairchild *et al.* (2003) reported that glycogen present in Type I fibres was broken down yet glycogen resynthesis in Type II fibres was unaffected by low intensity exercise ($40\% \dot{V}O_{2max}$). These findings support the hypothesis that during the early stages of recovery following high-intensity exercise, much of the lactate produced becomes a substrate for glycogen resynthesis within the Type II muscle fibres.

1.7 The importance of muscle glycogen for performance & recovery

Muscle glycogen is the primary fuel source for moderate to high intensity exercise and when muscle glycogen stores are depleted exercise capacity at these intensities is limited. Any increase in contractile activity induces major changes in the capacity of muscle to metabolise substrates. Previously, Yaspelkis and Ivy (1999) stated that adequate muscle glycogen stores were essential, not only for optimising performance during competition, but also for maintenance of training quality due to stores declining after consecutive days of training. Consecutive, intensive training days are common in many professional and semi-professional sporting set-ups, especially rugby and soccer. Glycogen resynthesis is the most important facet of recovery and this is impaired by exercise induced muscle damage (Costill *et al.*, 1990) and hormonal imbalance (Kraemer *et al.*, 1998; Lane *et al.*, 2010). The most influential factors affecting glycogen resynthesis are type, amount and timing of CHO ingestion (Blom *et al.*, 1986; Blom *et al.*, 1987; Friedman *et al.*, 1991). There appears to be a preferential resynthesis of muscle glycogen reserves following exhaustive exercise, with liver glycogen becoming restocked once muscle glycogen has been fully replenished (Hargreaves, 1992).

Muscle glycogen stores are at their lowest at the end of prolonged heavy exercise and it is essential that they are replenished as quickly as possible because muscle glycogen restocking is at an optimum during the 4 to 5 h immediately post-exercise (Robergs, 1991). Jentjens *et al.* (2001) concluded that it was unlikely that muscle glycogen stores could be completely resynthesised within a few hours, however, restocking could occur within a 24 h period depending on the degree of depletion and provided sufficient CHO was ingested. Nevertheless it was considered beneficial for an athlete to maximise their rate of glycogen restocking in the early hours post-exercise (Williams, 1995). Theoretically, the faster muscle glycogen stores are restocked post-exercise the faster the recovery, and the greater the return of performance capacity. Williams (1995) stated that the rate of glycogen restocking was determined by the amount of CHO available post-exercise, and the general consensus for CHO ingestion was 1.0 to 1.5 g.kg⁻¹.h⁻¹ for optimal glycogen restocking (Ivy *et al.*, 1988).

1.8 Fuelling exercise

The main substrates that fuel the chemical reactions of the body are CHO, PRO and fat and these are obtained from daily food intake or from stores contained within the body in order to produce energy. Of these three substrates, PRO only plays a minor role in energy production, except in extreme conditions (Coyle, 2000). Fat stored as triacylglycerol, mostly in adipocytes around the body (Coyle, 2000) is mobilised through lipolysis to non-esterified fatty acids (NEFA) for transport into the mitochondria. However, Coyle (2000) also stated that all NEFA must be transported across the mitochondrial membrane for oxidation, and this process may be a rate limiting step. CHO is stored in the body as glycogen, a starch comprising of glucose molecules that can be readily hydrolysed. Glycogen is stored directly in the muscle fibre and also in the liver, and is the major source of substrate during most types of exercise (Coyle, 2000). Liver glycogen stores serve to maintain BGlu concentration and are mobilised as exercise intensity increases in response to a decreasing BGlu concentration due to increasing muscle glucose uptake from the circulation. Ahlborg and Felig (1976) stated that BGlu can be elevated from liver glycogenolysis or from entry of ingested glucose into the blood stream. Coyle (2000) stated that during high-intensity (65 to 75% $\dot{V}O_{2\max}$) exercise, as the duration increased the contribution of muscle glycogen declined; stores gradually became depleted and the contribution of BGlu increased. BGlu can last for approximately 1 to 2 h of exercise at these intensities, but eventually declines due to an imbalance between glucose utilisation and glucose production in the fasted state (McConnell *et al.*, 2000). For this reason CHO supplementation has frequently been used as fuel for substrate phosphorylation and mitochondrial oxidation delaying the onset of fatigue during exercise.

During periods of high-intensity training, athletes should consume sufficient CHO to cover about 60% of their energy costs (Hawley *et al.*, 1995). An athlete that trains for more than 2 h.day⁻¹ has a recommended daily intake for CHO of 8 to 10 g.kg⁻¹ body mass (BM). These recommendations are primarily aimed at restoration of intra-muscular and hepatic glycogen stores to ensure adequate CHO availability for exercise on successive training days. However, Gleeson and Bishop (2000) outlined the importance of glucose for immune function due to the very high metabolic rates

of glucose fuelled lymphocytes, neutrophils and macrophages. In addition to maintaining immunocompetence, low CHO availability results in increased release of C (Lane *et al.*, 2010) and inadequate protein intake ($<1.6 \text{ g.kg}^{-1}.\text{day}^{-1}$) impairs immunity with particularly detrimental effects on the T lymphocyte cell system (Chandra, 1997).

Haff *et al.* (2003) postulated that if an athlete could increase the amount of glycogen resynthesis between exercise bouts by way of CHO supplementation, an increase in performance may occur during a second bout of exercise on a given training day. They also stated that inclusion of such a supplement may enhance PRO synthesis or decrease muscle breakdown and thereby enhance the effects of resistance exercise (Roy *et al.*, 1997; Haff *et al.*, 2003) as a result of increased plasma insulin levels. Such a supplemental regimen would be of benefit to those athletes attempting to enhance overall muscle strength (Haff *et al.*, 2003), such as rugby players. Haff *et al.* (1999) previously reported a significantly greater number of sets and repetitions performed after ingestion of a CHO supplement when compared to a placebo (PL). Administration of a CHO supplement before and after a resistance exercise session aided in the maintenance of muscle glycogen and resulted in a better performance in a test to exhaustion after a 4 h recovery period (Haff *et al.*, 1999). Despite the widely cited benefits of pre-ingestion of CHO, a study by Roberts *et al.* (2010) observed no effect of CHO ingestion on time to failure in a rugby specific performance test or in sprint performance. This finding may suggest that muscle glycogen stores were adequate for maintenance of performance in an intermittent type exercise protocol and that where duration increases, so too does the necessity for exogenous CHO.

Recent studies investigating glycogen restocking have focused on the timing of ingestion, the frequency of ingestion, the amount of CHO supplemented and the type of supplement ingested. Many studies have reported that supplementation of CHO with PRO was more effective than CHO alone during a 4 h period immediately post-exercise (Zawadzki *et al.*, 1992; Roy *et al.*, 1997; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000b; Van Loon *et al.*, 2000c; Tipton *et al.*, 2001; Ivy *et al.*, 2002; Williams *et al.*, 2003; Rankin *et al.*, 2004; Berardi *et al.*, 2006; Betts *et al.*, 2007; Rodriguez *et al.*, 2007; Howarth *et al.*, 2009). It has been reported that the addition

of PRO to a CHO supplement significantly increased the rate of post-exercise muscle glycogen resynthesis. Both PRO and certain AA were effective stimulators of insulin secretion and synergistically increased the blood insulin responses when combined with a CHO supplement (Zawadzki *et al.*, 1992; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Van Loon *et al.*, 2000c; Jentjens *et al.*, 2001). This greater rate of glycogen restocking was thought to be the result of a greater plasma insulin response brought about by the addition of PRO to a CHO supplement (Tarnopolsky *et al.*, 1997). It has also been noted that decreased AA availability post-exercise may limit the stimulatory effect of insulin on muscle PRO synthesis after exercise (Biolo *et al.*, 1999).

1.9 Glycogen resynthesis

Glucose uptake in the muscle occurs through facilitated diffusion across the sarcolemma via the GLUT-4 glucose transporter (Jentjens & Jeukendrup, 2003). These transporters are stored in vesicles but can translocate to, and merge with, the cell membrane to allow increased glucose transport into the cell (Jeukendrup & Gleeson, 2004). Once in the sarcolemma, glucose is phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase. G6P is converted to glucose-1-phosphate (G1P) by the enzyme phosphoglucomutase, and subsequently combined with uridine triphosphate to form uridine diphosphate (UDP)-glucose and pyrophosphate (PPi) in a reaction catalyzed by 1-phosphate uridyltransferase (Jeukendrup & Gleeson, 2004). UDP is a carrier of glucose units and takes the glucose molecule to the terminal glucose residue of a pre-existing glycogen molecule (Jeukendrup & Gleeson, 2004). UDP-glucose is catalysed by glycogen synthase to form an α -1,4 glycosidic bond, resulting in one long, straight chain of glucose molecules. However, α -1,6 glycosidic bond branch points are introduced into the glycogen structure by a branching enzyme, and once the length of a chain is about 12 glucose residues long, the branching enzyme detaches a chain about 7 residues long and reattaches it to a neighbouring chain by an α -1,6 glycosidic bond, resulting in a very large but compact glycogen molecule (Jeukendrup & Gleeson, 2004).

Research has demonstrated a biphasic pattern of muscle glycogen restocking after exercise induced glycogen depletion. Price *et al.* (1994) observed the effects of glycogen depletion and insulin concentration on glycogen resynthesis. In their

study, *gastrocnemius* muscle glycogen content was assessed using ^{13}C -nuclear magnetic resonance (NMR) after an exercise period consisting of 1 min of toe raises interspersed with 1 min rest to deplete glycogen stores to approximately 75, 50 or 25% of baseline (protocol I). The insulin dependence of glycogen synthesis was assessed after depletion to 25% of baseline with (protocol II) and without (protocol III) infusion of somatostatin, an endogenous insulin secretion inhibitor. Price *et al.* (1994) stated that glycogen resynthesis rates after depletion to 75 or 50% of baseline were similar but recovery became biphasic when glycogen was depleted to 25% of baseline. When glycogen was depleted to 25% of baseline there was an initial rapid phase of resynthesis during the first 30 min which decreased significantly ($P<0.05$) during the next 30 min period. Price *et al.* (1994) showed a further significant decrease ($P<0.01$) after 60 min to a rate which remained constant thereafter. The glycogen resynthesis rates quoted in this study followed a trend relative to glycogen concentration. When glycogen levels were below 30 mmol, the net resynthesis rate was high ($33 \pm 7 \text{ mmol}\cdot\text{h}^{-1}$), and as post-exercise glycogen levels increased to approximately 30 to 35 mmol the rate of resynthesis decreased significantly ($11 \pm 0.3 \text{ mmol}\cdot\text{h}^{-1}$; $P<0.05$). Price *et al.* (1994) also reported data that show this rate decreased further when concentrations increased again to approximately 35 to 40 mmol ($6 \pm 2 \text{ mmol}\cdot\text{h}^{-1}$; $P<0.05$), where it subsequently remained.

The results reported by Price *et al.* (1994) showed that with inhibition of endogenous insulin secretion following infusion of somatostatin, glycogen resynthesis rates were similar following depletion to 75 and 50% of resting levels, but differed significantly at lower concentrations of glycogen post-exercise. At glycogen levels of <35 mmol, resynthesis rates were observed to be unaffected by somatostatin induced hypoinsulinaemia. However, a halt in resynthesis was observed when the glycogen concentration exceeded 35 mmol during hypoinsulinaemia induced by somatostatin infusion. Price *et al.* (1994) demonstrated that glycogen resynthesis occurred in a biphasic manner, with an initial fast insulin-independent glycogen-dependent phase which occurred at glycogen levels <30 mmol, and a subsequent slow insulin-dependent glycogen resynthesis phase that decreased with a concomitant increase in glycogen concentration. Previously, Richter *et al.* (1984) had stated that the initial rapid phase of glycogen resynthesis was modulated by local contraction induced factors. However, Price *et al.* (1994) concluded that the insulin independent phase

of resynthesis and the minimal changes in blood chemistry induced by the small-muscle exercise protocol utilised in their study suggested that this phase was regulated by local intramuscular factors.

Researchers have observed that the insulin independent, fast phase was possibly mediated by increased glycogen synthase activity immediately following exercise (Blom *et al.*, 1987; Ivy *et al.*, 1988; Jentjens & Jeukendrup, 2003) which lasted for 30 to 60 min (Jentjens *et al.*, 2001). This early post-exercise recovery phase was characterised by an exercise-induced permeability of the muscle cell membrane to glucose. Exercise induced translocation of GLUT-4 skeletal muscle glucose transporters and increased glycogen synthase activity were thought to bring about a rapid rate of glycogen resynthesis (Jentjens *et al.*, 2001). Glycogen synthase has been suggested as the rate limiting enzyme for muscle glycogen resynthesis (Ivy *et al.*, 1988; Jentjens *et al.*, 2001), therefore a rapid rate of glycogen synthesis may depend upon adequate glucose availability within the muscle (Jentjens & Jeukendrup, 2003).

Training status must also be considered as a factor affecting glucose availability when training induced adaptations at the muscular level, namely, increased GLUT-4 content, increased insulin action, and increased capillary density are considered (Jeukendrup & Jentjens, 2000). Exercise has been reported to result in increased insulin sensitivity and a non-insulin dependent increase in glucose transport in active musculature (Ivy *et al.*, 1988). Glycogen resynthesis reportedly occurred at a decreased rate immediately after this fast phase and became insulin dependent (Jeukendrup & Jentjens, 2000). Stimulation of glucose transport by insulin was mediated by translocation of GLUT-4 from intracellular sites to the plasma membrane (Van Hall *et al.*, 2000). Kiens and Richter (1998) stated that the slow glycogen resynthesis phase occurred despite the presence of CHO and high insulin levels and was possibly due to decreased glycogen synthase activity as muscle glycogen concentration increased, leading to a decreased rate of glycogen production via the glycogen synthetic pathway and increased glucose disposal through glucose oxidation (Ivy *et al.*, 1988). It was suggested that the effect of muscle glycogen content on insulin dependent glucose resynthesis activity was fibre type specific

(Vøllestad *et al.*, 1989; Casey *et al.*, 1995), or that other factors may also contributed to the enhanced insulin sensitivity post-exercise.

Laurent *et al.* (2000) stated that glycogen limits its own synthesis through feedback inhibition. Laurent *et al.* (2000) measured muscle glycogen and G6P concentration during a euglycaemic hyperinsulinaemic clamp using $^{13}\text{C}/^{31}\text{P}$ NMR spectroscopy before and after a muscle glycogen loading protocol. Data from Laurent *et al.* (2000) reported that a 1.6 fold increase in muscle glycogen concentration to approximately 130 mmol.L^{-1} resulted in an approximately 30% reduction in the rate of glycogen synthesis. It was thought that this reduction in insulin-stimulated muscle glycogen synthesis could occur through a reduction in glucose transport, hexokinase or glycogen synthase activity (Laurent *et al.*, 2000). In this study, after glycogen loading, an approximate 2-fold increase in G6P intracellular concentration was observed. Laurent *et al.* (2000) suggested that a decrease in glycogen synthase activity was responsible for the lower rate of muscle glycogen synthesis observed. They also reported no significant difference between the rates of insulin-stimulated whole body glucose disposal comparing baseline and post-glycogen loading; implying that under glycogen loaded conditions a significant portion of the glucose infused was being shunted into alternative pathways (Laurent *et al.*, 2000). Higher rates of whole body glucose oxidation and BLA concentration observed after glycogen loading suggested that glucose was diverted from muscle glycogen synthesis into aerobic and anaerobic glycolysis, with some of the glycolytically derived lactate being taken up by the liver for gluconeogenesis and/or lipogenesis and by other tissue for oxidation (Laurent *et al.*, 2000).

1.10 Exercise, hormones and the role of nutrition

Dietary energy and nutrients may influence hormonal concentrations and in turn help to mediate physiological mechanisms related to recovery from heavy resistance exercise (Chandler *et al.*, 1994; Volek *et al.*, 1997; Kraemer *et al.*, 1998). Volek *et al.* (1997) identified the link between the percentage of energy-providing macronutrients in the diet and their importance in determination of T homeostasis in healthy athletic men. Kraemer *et al.* (1998) noted that regulation of hormones by nutrients may become increasingly important during consecutive days of intensive resistance training (RT) in which anabolic/catabolic turnover is accelerated. Results

from a study by Chandler *et al.* (1994) suggested that supplementation of CHO, PRO or CHO-PRO after RT can produce a hormonal environment during recovery that may be favourable for muscle growth by stimulating insulin and GH release. In this study, a significant decrease in post-exercise T concentration was observed post-supplementation despite no associated decrease in the hormone responsible for T production and secretion, namely LH. Chandler *et al.* (1994) postulated that this was most likely due to enhanced T clearance post-supplementation which was later confirmed by Kraemer *et al.* (1998). Concentrations of C are also known to increase post-resistance exercise (Volek *et al.*, 1997; Tarpenning *et al.*, 2001; Kreider *et al.*, 2007). However, ingestion of CHO during progressive RT exercise reportedly attenuated the C response to exercise (Tarpenning *et al.*, 2001).

Bishop *et al.* (1999a) investigated the effect of CHO supplementation versus PL on the immune and plasma C responses to a soccer-specific exercise protocol in collegiate soccer players. Players received either a CHO or PL beverage before, during and after two 90 min soccer-specific exercises consisting of activity patterns simulating the activity patterns of a soccer match (Bangsbo *et al.*, 1991b). They reported significantly lower BGlu concentrations following ingestion of PL when compared to CHO but no significant difference in plasma C concentration, circulating lymphocyte count and saliva immunoglobulin-A secretion between trials. Bishop *et al.* (1999a) conceded that the intensity of the exercise protocol utilised in their study was not adequate to induce a C response despite their heart rate (HR) and BLa data suggesting that the protocol compared well with a match. They concluded that when overall exercise intensity is moderate, and changes in BGlu and stress hormones are minimal, CHO supplementation has a negligible effect. However, Bishop *et al.* (1999b) reported an attenuating effect of CHO ingestion on C response to steady-state cycling (2 h at 70% $\dot{V}O_{2max}$) in comparison to a PL and fluid restriction. Bishop *et al.* (1999b) stated that CHO intake before and during exercise under such conditions attenuated the circulating leukocyte and neutrophil responses to exercise, effects which were possibly mediated by the maintenance of plasma glucose concentrations and the accompanying attenuation of the rise in plasma C concentrations.

Lane *et al.* (2010) investigated the effect of dietary CHO consumption on plasma T/C ratio during a short-term intense micro-cycle of exercise training and reported that plasma T/C ratio decreased significantly from pre- to post-study in their low-CHO (~30% daily intake) group with no significant change detected in the control-CHO (~70% daily intake) group (1.53 ± 1.30 vs. $0.82 \pm 0.71 \times 10^{-3}$, mean $\pm$ SD, respectively). At a metabolic level, it was suggested that the low-CHO diet in combination with the exercise would have prevented glycogen stores from being completely resynthesised between training sessions, thus elevating the fuel-mobilising hormone C, while concomitantly suppressing T secretion (Lane *et al.*, 2010).

As noted previously, CHO feeding during intermittent exercise can increase exercise capacity (Fielding *et al.*, 1985; Nicholas *et al.*, 1995; Jeukendrup, 2004; Jentjens *et al.*, 2004b) as can elevating muscle glycogen stores pre-exercise (Bangsbo *et al.*, 1992). Henson *et al.* (1998) reported that plasma C concentration was significantly higher in their PL trial when compared to their CHO trial following 2.5 h of high-intensity running in trained marathon runners. However, data would appear to suggest that CHO ingestion attenuated the moderate rise in neutrophil function or saliva immunoglobulin-A secretion (Bishop *et al.*, 1999a; Gleeson & Bishop, 2000) and could also attenuate secretion of C (Henson *et al.*, 1998; Lane *et al.*, 2010).

With this in mind, games players and endurance athletes alike must maximise their intramuscular and hepatic glycogen stores by choosing pre-match/training meals with adequate CHO content to maintain BGlu in the latter stages of exercise. The data available would suggest that the practice of strict and functional nutritional practices before, during and after training and competition is essential for games and endurance athletes as a means of not only maintaining quality of training and ensuring adequate preparation for competition, but also as a means of maintaining an optimum immune and hormonal state. Many sports teams in the professional and amateur arenas will perform their high-load training early in the week subsequently decreasing the load towards the end of the week in time for the next competitive outing. The available research would suggest that inadequate nutritional intake in combination with limited recovery duration presented a significant obstacle to recovery from exercise (Lane *et al.*, 2010). However, a recovery strategy involving

consumption of a CHO-PRO sports drink may be a worthwhile intervention if the expected C increase under such conditions was in some way attenuated and a positive T/C ratio was manifest in the athlete throughout the training week and, more importantly, immediately prior to the next competitive outing. Should a nutritional intervention present such a timely return to a positive hormonal state, as reflected in the T/C ratio, employing such a nutritional strategy and recovery intervention would prove invaluable to both athlete and team successes.

1.11 Glycogen resynthesis and carbohydrate ingestion

Jentjens and Jeukendrup (2003) stated that exercise training induced several adaptations at a muscular level that potentially contributed to increased insulin sensitivity including, increased GLUT-4 content, improved insulin signalling, and increased blood flow. Adaptations such as these would favour glucose uptake and glycogen synthesis. This may be the case in trained individuals who may have higher glycogen synthesis rates when compared to sedentary individuals. Muscle fibre type may also contribute to differing rates of glycogen restocking. In humans, whole body glucose uptake after insulin stimulation has been reported to correlate positively with the percentage of Type I fibres and negatively with the percentage of Type IIb fibres (Lillioja *et al.*, 1987). Hickner *et al.* (1997) documented that both the rate of glycogen synthesis and the GLUT-4 content were positively correlated with the percentage of Type I fibres. This higher GLUT-4 expression in Type I fibres may partly explain the higher glycogen synthesis rates recorded in this fibre type. Fairchild *et al.* (2003) stated that the unfavourable hormonal environment associated with active recovery was unlikely to affect glycogen resynthesis in Type II muscle fibres. They observed that plasma and muscle lactate data, along with blood pH, decreased more rapidly during active recovery. However, little effect was observed for BGlu data during the active recovery.

Muscle fibre types also differ in the rate of glycogen resynthesis within each respective fibre type. Vøllestad *et al.* (1989) observed a significant 65% faster rate of glycogen resynthesis in Type IIa and Type IIb fibres when compared to Type I fibres during the first 90 min of a 3 h recovery period with CHO ingestion. However, during the subsequent 90 min period (90 to 180 min) a faster Type I muscle glycogen resynthesis rate was observed when compared with the first 90 min,

and no such difference was observed for the Type II fibres. Initial post-exercise glycogen concentration observations showed a 25% higher Type II fibre glycogen content when compared to Type I fibres. This finding by Vøllestad *et al.* (1989) may also reinforce the statement of Tsintzas *et al.* (2001) that Type II fibres were recruited late in exercise as Type I fibres became fatigued, thus delaying the decrease in glycogen stores. The results of Vøllestad *et al.* (1989) may also reinforce the concept of an insulin independent glycogen resynthesis phase, as insulin concentrations during the initial 90 min were 35% lower than during the subsequent 90 min recovery period.

Tsintzas *et al.* (2001) also reported that CHO ingestion during exercise attenuated the decline in PCr in Type I and Type II fibres, but spared glycogen depletion in Type I fibres only. At the end of exercise, the glycogen concentration in Type I fibres was higher after supplementation with CHO ingestion before (8 mL.kg^{-1}) and during (2 mL.kg^{-1} at 20 min intervals) exercise when compared with a control trial. CHO supplementation in this study resulted in a 56% reduction in glycogen utilisation in Type I fibres when compared with a control. It also appeared that since selective glycogen depletion in Type I fibres was observed at the point of fatigue in the absence of CHO, that CHO ingestion delayed fatigue development during prolonged exercise by better maintaining oxidative ATP resynthesis principally in Type I fibres.

In a study by Casey *et al.* (1995) volunteers performed one-legged cycling exercise to exhaustion. During the initial 2 h of recovery, subjects consumed CHO (3 g.kg^{-1}) and a high CHO diet thereafter. After 3 h of recovery a $25 \pm 8\%$ higher rate of glycogen resynthesis was observed for Type I compared with Type II fibres. From 3 to 10 h of recovery, resynthesis in Type I fibres decreased significantly, however, in Type II fibres resynthesis over the same time period remained unchanged from that observed during the initial 3 h of recovery. From 10 to 24 h of recovery resynthesis declined in both fibre types. Casey *et al.* (1995) also observed that the initial rate of resynthesis across fibre type differed significantly ($P < 0.001$) with Type I greater than Type II. However, the decrease in the rate of glycogen resynthesis observed in Type I fibres during the 3 to 10 h recovery period resulted in a comparatively higher rate of resynthesis for Type II fibres during this time period.

The biochemically analysed results reported by Casey *et al.* (1995) conflicted with the photometrically analysed results of Vøllestad *et al.* (1989). Vøllestad *et al.* (1989) observed a 65% faster rate of glycogen resynthesis in Type II fibres when compared to Type I fibres during the first 1.5 h of recovery. No further differences were observed in this study between Type I and Type II between 1.5 and 3 h of recovery. Vøllestad *et al.* (1989) stated that the different initial rates of resynthesis appeared to be due to the process of transfer of glucose from G6P to glycogen. According to Casey *et al.* (1995), the difference in rates observed in the studies cannot be attributable to the extent of glycogen depletion achieved during exercise or to either the timing or composition of the glucose solutions administered post-exercise. Casey *et al.* (1995) administered CHO at 0, 1 and 2 h of recovery at a rate of 1.4, 0.8 and 0.8 g.kg⁻¹, respectively, whereas Vøllestad *et al.* (1989) supplemented CHO (glucose) immediately, 1 and 2 h after cessation of exercise at a rate of 1.4, 0.7 and 0.7 g.kg⁻¹, respectively. Casey *et al.* (1995) stressed that a poor linear relationship was observed between periodic acid-Schiff (PAS) stain intensity and muscle glycogen concentration in the study by Vøllestad *et al.* (1989) whereas good agreement was observed between mixed fibre muscle glycogen concentration and the mean of the concentrations recorded for Type I and Type II fibres from biopsy samples. These differences may have accounted for the lack of agreement between studies.

Differences observed between fibre type glycogen resynthesis rates quoted by Casey *et al.* (1995) may be due to fibre type differences in glucose transporter isoform numbers and insulin sensitivity, and variations in muscle glucose uptake and regulation of glycogen resynthesis. It has previously been shown that Type I muscle fibres have a greater number of plasma membrane glucose transporter isoforms (Goodyear *et al.*, 1991), and more specifically the highest content of GLUT-4 glucose transporter isoforms. Some differences may also be attributable to the greater capillary to muscle fibre ratio associated with Type I fibres. It must also be suggested that insulin sensitivity between muscle fibre types may be of further importance due to the action of insulin on the activation of glycogen synthase and inhibition of phosphorylase kinase.

Fairchild *et al.* (2003) concluded that active recovery resulted in net glycogen

mobilisation in Type I muscle fibres without affecting glycogen resynthesis in Type II muscle fibres. They observed significant glycogen increases in all muscle fibre types (Type IIa, Type IIb and Type I) during passive recovery with increases similar during active recovery only observed in Type IIa and Type IIb fibres. However, during active recovery histological data indicated that glycogen was being mobilised in these Type I fibres. Significant increases in Type I muscle glycogen concentration were observed during the 30 min of rest after active recovery, whereas glycogen concentration remained stable in Type II muscle fibres over the same time period (Fairchild *et al.*, 2003).

The amounts of CHO supplemented and timing of supplementation have varied widely between studies. This, and many other additional factors, may have contributed to varying results reported and glycogen restocking rates documented. However, what is known is that when no CHO is supplemented after exercise, muscle glycogen restocking rates have been reported to be low (7 to 12 mmol.kg⁻¹ dw.h⁻¹, (Ivy *et al.*, 1988; Tarnopolsky *et al.*, 1997). A study by Blom *et al.* (1987) demonstrated that increasing the CHO consumption rate from 0.18 to 0.35 g.kg⁻¹.h⁻¹ increased the rate of glycogen restocking by more than 150%. However, when the CHO intake was increased further (0.35 to 0.7 g.kg⁻¹.h⁻¹) no additional increase in the glycogen restocking rate was detected. The findings of Ivy *et al.* (1988) agreed with the data reported by Blom *et al.* (1987). Ivy *et al.* (1988) observed no significant difference in muscle glycogen restocking when CHO was supplemented at a dose of 0.75 to 1.5 g.kg⁻¹.h⁻¹. In contrast, Van Loon *et al.* (2000a) observed higher muscle glycogen restocking rates when CHO supplementation was increased from 0.8 to 1.2 g.kg⁻¹.h⁻¹, and CHO supplements were administered every 30 min over a 5 h restocking period. Supplementation in the studies by Blom *et al.* (1987) and Ivy *et al.* (1988) occurred at 2 h intervals, which may partly explain the differences between their results and the data reported by Van Loon *et al.* (2000a).

Together the data of Van Hall *et al.* (2000), Van Loon *et al.* (2000a) and Jentjens *et al.* (2001) suggested that the availability of substrate was the rate-limiting factor for glycogen restocking. Jentjens *et al.* (2001) and Van Loon *et al.* (2000a) suggested that insulin was not the rate-limiting factor for muscle glycogen restocking when total CHO intake was high and provided at 30 min intervals. Jentjens and

Jeukendrup (2003) suggested that maximal glycogen restocking rates occurred at a CHO intake of $\sim 1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and suggested that intestinal absorption of glucose may be a limiting factor for exogenous CHO oxidation (Jentjens *et al.*, 2004a; Jentjens *et al.*, 2004b). Although the doses ingested in each of the cited studies differed significantly, possibly resulting in differing rates of gastric emptying, Jentjens *et al.* (2001) stated that the rate of gastric emptying was not the rate-limiting factor for glycogen restocking, but may be limited by the rate of digestion and absorption of CHO in the intestine and subsequent transport into the blood stream regulated by the liver.

1.12 Glucose transport

Replenishment of muscle and liver glycogen stores requires glucose derived from either dietary sources or resulting from gluconeogenesis (Jentjens & Jeukendrup, 2003). Insulin and exercise are the two most physiologically relevant stimulators of glucose uptake in skeletal muscle (Thorell *et al.*, 1999). Glucose is absorbed from the intestine by a sodium dependent glucose transporter (SGLT-1) and it has previously been suggested that the upper rate of glucose absorption was approximately $1.0 \text{ g} \cdot \text{min}^{-1}$. Jentjens *et al.* (2004b) speculated that SGLT-1 transporters may become saturated at a glucose ingestion rate of 1.0 to $1.2 \text{ g} \cdot \text{min}^{-1}$ and therefore no further increase in exogenous glucose oxidation would be observed when glucose intake was further increased.

Goodyear *et al.* (1991) stated that glucose transport into skeletal muscle occurred by facilitated diffusion via the two isoforms of GLUT transporter expressed in skeletal muscle, GLUT-1 and GLUT-4. GLUT-1 played a role in basal glucose uptake by the muscle, but not insulin or contraction-stimulated glucose uptake (Ren *et al.*, 1994). The stimulation of glucose transport by insulin was mediated by translocation of GLUT-4 from intracellular sites to the plasma membrane (Van Hall *et al.*, 2000). In the un-stimulated state the GLUT-4 isoform was located intracellularly and was translocated to the plasma membrane when insulin was bound to its receptor. Consequently, GLUT-4 translocation in humans was an important mechanism for the increase in glycogen restocking during physical activity (Thorell *et al.*, 1999). Thorell *et al.* (1999) also stated that the mechanism for increased insulin-stimulated post-exercise glucose uptake and GLUT-4

translocation was also due to enhanced insulin receptor signalling. Kuo *et al.* (1999) stated that CHO supplementation exerted a modifying effect as opposed to a main stimulatory effect on GLUT-4 expression. Kuo *et al.* (1999) demonstrated that an acute exercise session potentially initiated an increase in muscle GLUT-4 expression and that a complex interaction existed between exercise with CHO supplementation and GLUT-4 expression in skeletal muscle. Muscle contractions stimulated glucose transport directly and independently from insulin by inducing the GLUT-4 transporter to the cell surface (Kuo *et al.*, 1999; Jentjens & Jeukendrup, 2003). Thorell *et al.* (1999) stated that the larger the resultant plasma insulin concentration the greater the increase in plasma membrane GLUT-4 translocation.

Skeletal muscle translocation of GLUT-4 to the plasma membrane appears also to be facilitated by muscle contraction independent of insulin. Van Hall *et al.* (2000) stated that during the initial phase of recovery from exercise, GLUT-4 still resided in the plasma membrane because of contraction mediated GLUT-4 translocation during the exercise bout, and thus glucose could be transported independently of insulin (Hansen *et al.*, 1998; Van Hall *et al.*, 2000). After the relatively short insulin independent muscle glucose uptake phase, glucose uptake and GLUT-4 translocation to the plasma membrane became insulin dependent. Previously, Price *et al.* (1994) stated that during recovery there was a marked increase in the sensitivity of muscle glucose uptake and glycogen synthesis to insulin. Recently, it has been suggested that the rate of muscle glucose uptake may control the rate of glycogen resynthesis after exercise. Van Hall *et al.* (2000) suggested that this proposed mechanism of glucose uptake fitted with their data. The results of their study showed that glucose uptake was highest during the initial recovery period, and leg glucose uptake decreased with recovery duration. Van Hall *et al.* (2000) proposed that the GLUT-4 transporters that remained in the plasma membrane from the exercise bout may have caused the higher glucose uptake during the initial stage. As recovery proceeded the muscle became more dependent on insulin to maintain GLUT-4 transporters in the plasma membrane and/or to translocate GLUT-4 to the plasma membrane.

Thorell *et al.* (1999) demonstrated that GLUT-4 translocation and Akt phosphorylation were induced by moderate intensity exercise and hyperinsulinaemia ($\sim 56 \mu\text{U}\cdot\text{mL}^{-1}$) and that this insulin concentration caused a significant recruitment of

GLUT-4 to the plasma membrane. In their study participants were studied on two days. Initially, biopsies were obtained before and after a euglycaemic-hyperinsulinaemic clamp ($0.8 \text{ mU.kg}^{-1}.\text{min}^{-1}$). On the second day, participants performed 60 min of cycling at 70% $\dot{V}\text{O}_2\text{max}$, a biopsy was obtained and a similar clamp and biopsy procedure was subsequently performed. Thorell *et al.* (1999) observed a 32% increase in plasma membrane GLUT-4 in response to a physiological hyperinsulinaemia when compared with rest. Exercise for 60 min at 70% $\dot{V}\text{O}_2\text{max}$ also resulted in a significant (34%) increase in plasma membrane GLUT-4 when compared with rest (Thorell *et al.*, 1999). Overall a 44% increase in plasma membrane GLUT-4 was observed for the combination of exercise and insulin compared to rest, which was not significantly different when compared to either exercise or hyperinsulinaemia alone.

Ren *et al.* (1994) stated that GLUT-4 expression rapidly increased in response to exercise that resulted in a sufficiently sustained muscle demand for CHO and appeared to be mediated by pre-translational mechanisms. Ren *et al.* (1994) observed that GLUT-4 expression in skeletal muscle was increased by approximately 50% at 16 h post-exercise and twofold at 48 h post-exercise. Ren *et al.* (1994) stated that the correlation between total GLUT-4 content and stimulated glucose transport rate in muscles implied that the number of glucose transporters translocated into the plasma membrane in response to insulin, exercise or hypoxia was proportional to the GLUT-4 content of the cell when the signalling pathways were intact. Ren *et al.* (1994) also stated that the rapid increase in GLUT-4 expression could provide a survival advantage by potentiating a higher rate of muscle glycogen replenishment when CHO was consumed between exercise bouts.

1.13 Effect of carbohydrate supplementation post-exercise

It is known that the timing of CHO ingestion post-exercise can affect the rate of glycogen repletion (Ivy *et al.*, 1988; Ivy *et al.*, 2002). When the CHO supplement was ingested within 7 to 10 min of exercise cessation there was a more rapid rate of muscle glycogen repletion, in comparison to ingestion of the same CHO supplement 2 h after exercise cessation (Ivy *et al.*, 1988). These findings have implications for athletes in demanding training regimen as the rate of glycogen repletion can be

accentuated by controlling the timing of CHO supplementation post-exercise. Theoretically, this higher restocking rate would allow athletes to physically recover faster thereby providing a faster return of performance capacity.

Ivy *et al.* (1988) observed a 45% lower muscle glycogen restocking rate when CHO ingestion post-exercise was delayed by 2 h compared to when CHO was ingested immediately post-exercise. It had been previously noted that 2 h post-exercise there were fewer glucose transporters available than immediately post-exercise (Goodyear *et al.*, 1991) and this observation may have contributed to the findings of Ivy *et al.* (1988). These data illustrated that the timing of post-exercise supplementation with CHO potentially affected the rate of muscle glycogen restocking. Jentjens and Jeukendrup (2003) concluded that immediate post-exercise supplementation with CHO increased the rate of glycogen restocking, and this may be critical when there was less than 8 h between successive training sessions.

Fallowfield and Williams (1997) concluded that increasing post-exercise CHO supplementation threefold (0.5 to 1.5 g.kg⁻¹.h⁻¹) did not improve endurance capacity after an initial high intensity run and a 4 h recovery supplementation period. Trials consisted of a 90-min run on a level treadmill at 70% $\dot{V}O_{2max}$ (R1) followed by a 4 h recovery-supplementation period and a final exhaustive run at 70% $\dot{V}O_{2max}$ (R2). During recovery, volunteers ingested either two feedings of a 6.9% glucose polymer (low CHO) solution or two feedings of a 19.3% glucose polymer solution (high CHO). Fallowfield and Williams (1997) reported that high CHO (3.0 g.kg⁻¹.2 h⁻¹) was associated with nausea, lethargy and a less positive attitude to subsequent exercise. Despite the absence of performance improvement with increased CHO supplementation, an enhanced BGlu availability response was observed which was associated with elevated serum insulin concentrations. Fallowfield and Williams (1997) stated that the lack of performance enhancement, despite increased BGlu availability, suggested that the limitation to post-exercise recovery in terms of a return to exercise capacity may have been located within the muscle cell.

Zachwieja *et al.* (1991) concluded that the magnitude of the glycogen depletion determined the rate of resynthesis during the early hours post-exercise. Cyclists (n =

6) completed an exercise protocol involving both one- and two-legged cycling to induce either a large or small amount of glycogen depletion. Glycogen depletion was induced by a large amount through the completion of 30 min of single-leg cycling, ten by one-min sprints, and 30 min cycling with both legs. A small amount of glycogen depletion was induced by way of two legged cycling for 30 min. Cyclists rested for 6 h post-exercise and were fed a 24% CHO solution every 20 min in order to achieve a CHO intake of $0.7 \text{ g.kg}^{-1}.\text{h}^{-1}$. Biopsies were obtained from the *vastus lateralis* immediately after exercise and at 2 and 6 h of recovery. Depletion protocols resulted in a glycogen utilisation of 93.9 ± 11.6 and 49.3 ± 5.7 mmol, respectively. Glycogen restocking rate after the large depletion protocol increased during the 6 h recovery/supplementation period (mean rate: $8.8 \pm 2.4 \text{ mmol.kg}^{-1}.\text{h}^{-1}$) and was significantly higher ($3.0 \pm 1.0 \text{ mmol.kg}^{-1}.\text{h}^{-1}$) than the rate observed for the small depletion protocol.

Doyle *et al.* (1993) investigated whether consumption of large amounts of CHO at 15 min intervals ($1.6 \text{ g.kg}^{-1}.\text{h}^{-1}$) during the first 4 h after glycogen depleting exercise would produce similar glycogen content in muscle that had undergone eccentric versus concentric actions 2 and 48 h earlier. Participants initially cycled for 75 min and then undertook interval exercise to deplete glycogen on days 1 and 3. After the cycling exercise on day 1, participants performed 10 sets of 10 repetitions of either concentric or eccentric actions in opposite legs. During the 4 h post-exercise, they consumed $0.4 \text{ g CHO.kg}^{-1}$ every 15 min. Biopsies were obtained immediately before the feedings and 4 h later. Doyle *et al.* (1993) observed similar glycogen resynthesis rates for participants performing either eccentric or concentric actions. However, glycogen resynthesis rates were 25% lower ($P < 0.05$) in muscle that had undergone eccentric actions 48 h earlier than in concentrically exercised muscle, despite the presence of similar circulating concentrations of BGlu and insulin. Doyle *et al.* (1993) stated that the reduced glycogen replenishment rate 2 days after eccentric exercise corresponded to the time course for the development of delayed-onset muscle soreness, muscle damage and inflammation after unaccustomed eccentric exercise. A significant disruption of the muscle cell membrane by eccentric exercise might reduce insulin-stimulated glucose transport or the activation of glycogen synthase (Doyle *et al.*, 1993). Doyle *et al.* (1993) stated that the mechanism for the reduced rate of glycogen restocking may have been related to reduced insulin action

and glucose transport. These two functions are muscle membrane-mediated events that may have been impaired as a result of the increasing muscle damage, membrane disruption and inflammation commonly observed during the days after eccentric exercise (Doyle *et al.*, 1993).

In a study performed by Roy *et al.* (1997) volunteers ($n = 8$) performed 10 repetitions of unilateral knee extensor exercises at approximately 85% of 1 repetition maximum (RM) 8 times and consumed a CHO supplement (1 g.kg^{-1}) or PL immediately and 1 h post-exercise. Roy *et al.* (1997) stated that CHO supplementation (1.0 g.kg^{-1}) immediately and 1 h after resistance exercise could decrease myofibrillar PRO breakdown and urinary urea excretion, resulting in more positive PRO balance. They reported significantly higher plasma insulin and glucose concentrations during the CHO trial when compared to PL. These increases in plasma glucose and insulin levels were associated with less urinary 3-methylhistidine and urea nitrogen excretion, with no significant difference in *vastus lateralis* fractional muscle PRO synthetic rate or rate of whole body PRO degradation detected (Roy *et al.*, 1997). The net effect of post-exercise CHO ingestion was anabolic and would result in a more positive net muscle PRO balance. This study by Roy *et al.* (1997) was the first to report the effect of oral glucose supplementation on post-resistance-exercise PRO metabolism in humans. This study highlighted the effect of elevated insulin on muscle PRO degradation rate as the administration of a CHO supplement led to a significant decrease in urinary 3-methylhistidine excretion over the day of the study (Roy *et al.*, 1997).

1.14 Carbohydrate-protein supplementation

Volek (2004) stated that metabolically active hormones regulated the synthesis and breakdown of PRO, CHO and lipids; insulin is one such hormone. CHO ingestion resulted in elevated insulin concentrations that depended to some extent on the glycemic effect of the food (Volek, 2004). Insulin stimulates both muscle glycogen uptake and activation of glycogen synthase, the rate limiting enzyme for glycogen synthesis. Certain AA and PRO in addition to CHO have been shown to exert a synergistic effect on insulin release (Van Loon *et al.*, 2000a; Jentjens & Jeukendrup, 2003; Volek, 2004), however, according to Jentjens and Jeukendrup (2003) the most important physiological stimulus for pancreatic insulin secretion was an increased

BGlu concentration. Based on this theory many studies have investigated the effect of a combination of CHO and PRO on maximising insulin secretion with the aim of increasing glycogen resynthesis (Zawadzki *et al.*, 1992; Ivy, 1998; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Jentjens *et al.*, 2001; Ivy *et al.*, 2002).

Van Loon *et al.* (2000a) demonstrated that ingestion of a highly insulinotropic PRO hydrolysate AA mixture in combination with a moderate amount of CHO resulted in significantly increased muscle glycogen restocking rates when compared with the ingestion of LCHO ($0.8 \text{ g.kg}^{-1}.\text{h}^{-1}$) only. The increased rate of glycogen restocking in the study by Van Loon *et al.* (2000a) was attributed to the increased uptake of glucose into the muscle as a result of higher insulin levels (15.9 ± 2.2 , 12.3 ± 1.8 and $8.6 \pm 0.9 \text{ U.5 h}^{-1}.\text{L}^{-1}$ for CHO-PRO, HCHO and LCHO, respectively). However, Van Loon *et al.* (2000a) also reported that there were no significant differences between glycogen resynthesis rates when glycogen depleted volunteers were administered CHO-PRO or HCHO during the 5 h recovery/supplementation period. The results of Van Loon *et al.* (2000a) suggested that a CHO-PRO supplement was no more effective than a HCHO supplement when total energy content was maintained constant. The ingestion of CHO ($0.8 \text{ g.kg}^{-1}.\text{h}^{-1}$) with a highly insulinotropic AA and/or PRO mixture ($0.4 \text{ g.kg}^{-1}.\text{h}^{-1}$) in the study by Van Loon *et al.* (2000a) resulted in an attenuated elevation of plasma insulin concentrations which could be of experimental, as well as of practical importance in sports nutrition. Therefore, it would make sense to take advantage of this reaction by attempting to increase post-exercise insulin concentrations to optimise the rate of muscle glycogen restocking (Yaspelkis & Ivy, 1999; Van Hall *et al.*, 2000; Van Loon *et al.*, 2000a; Jentjens *et al.*, 2001).

Van Hall *et al.* (2000) observed no significant difference in post-exercise muscle glycogen storage when comparing a CHO-PRO (CHO and PRO hydrolysate) beverage with a CHO beverage and water. Participants ingested any of the three beverages following glycogen depleting exercise at 15 min intervals for the duration of the 4 h recovery period. Muscle biopsy samples were obtained at 0, 90 and 240 min. Glycogen resynthesis rates during the first 90 min of recovery were $\sim 50 \text{ mmol.kg dry muscle}^{-1}.\text{h}^{-1}$ and decreased to $30 \text{ mmol.kg dry muscle}^{-1}.\text{h}^{-1}$ between 90 and 240 min of recovery. Leg glucose uptake in this study was observed to be

highest ($1.6 \text{ mmol} \cdot \text{min}^{-1}$) at 10 and 20 min following ingestion of the first bolus of both CHO-PRO and CHO drinks. Glucose uptake then decreased to a steady state of $\sim 0.9 \text{ mmol} \cdot \text{min}^{-1}$ after 60 min. Van Hall *et al.* (2000) also noted that between 90 and 240 min of recovery it appeared that more glucose was taken up by the leg than was incorporated into glycogen if it was assumed that 6.5 kg of an 8 kg active leg muscle was depleted. Van Hall *et al.* (2000) concluded that during the first 90 min of recovery an almost 100% match was recorded for the amount of glucose taken up by the leg and the amount of glucose needed for the observed increase in muscle glycogen content. Their results indicated that during the initial phase of recovery, glucose was mainly used for glycogen restocking and less for oxidation (Van Hall *et al.*, 2000).

Despite substantial differences in insulin concentrations between CHO-PRO and CHO trials, a higher rate of glycogen restocking was not evident (Van Hall *et al.*, 2000). This may be explained by the following reasons. Firstly, the rate of glycogen restocking observed by Zawadzki *et al.* (1992) for CHO-PRO was similar to the rates of glycogen restocking observed by Van Hall *et al.* (2000) for both CHO-PRO and CHO, if the average glycogen restocking rate from 0 to 240 min ($39.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was considered. According to Van Hall *et al.* (2000) this rate of glycogen restocking may be the maximum achievable after prolonged dynamic exercise with sufficient and regular CHO loading, despite higher insulin levels. Secondly, the higher insulin concentration in the CHO-PRO trial might not have been effective. Therefore, a relative low insulin level might have elicited the maximal positive achievable effect on glycogen restocking during recovery from intense exercise (Van Hall *et al.*, 2000). Thirdly, Price *et al.* (1994) previously had suggested that the rate of glycogen restocking was affected by the initial concentration, when muscle glycogen content was $<120 \text{ mmol} \cdot \text{kg}^{-1}$ dry muscle, as was the case for muscle glycogen levels immediately post-exercise ($79 \text{ mmol} \cdot \text{kg}^{-1}$ dry muscle) in the study reported by Van Hall *et al.* (2000).

Price *et al.* (1994) also observed a significant increase in NEFA concentration during their somatostatin infusion trial after approximately 1 h (0.6 mmol at rest, 1.9 mmol 1 h post-exercise, 1.6 mmol 2 to 3 h post-exercise; $P < 0.05$). This increase in NEFA concentration as opposed to a direct effect of insulin on muscle may have resulted in

the cessation of glycogen synthesis during the period of hypoinsulinaemia induced by somatostatin infusion. Plasma NEFA during the third protocol employed in this study displayed no significant differences, but rose slightly between rest and 5 h post-exercise possibly due to fasting conditions. In the study by Fallowfield and Williams (1997) the post-recovery concentrations of NEFA and glycerol and the elevated BLa concentrations during their high CHO trial may have reflected depressed lipid mobilisation and metabolism due to increased availability of BGlu. This statement is in agreement with the results of Achten and Jeukendrup (2003) who observed an approximately 28% decreased rate of maximal fat oxidation after CHO supplementation before exercise.

Jentjens *et al.* (2001) investigated the effect of co-ingestion of an insulinotropic AA mixture ($0.4 \text{ g.kg}^{-1}.\text{h}^{-1}$) and a high rate of CHO intake (1.2 g.kg.h^{-1}) provided at 30 min intervals on muscle glycogen restocking in the post-exercise recovery period when compared with a high CHO intake ($1.2 \text{ g.kg}^{-1}.\text{h}^{-1}$) only. The CHO-PRO supplement used in this study had previously been reported to result in high insulin responses and increased rates of glycogen restocking when ingested with moderate amounts of CHO (Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b). Jentjens *et al.* (2001) reported a significantly higher insulin response, expressed as area under the curve (AuC), from 60 to 180 min and 0 to 180 min of recovery after combined PRO/AA and CHO ingestion when compared with CHO alone. However, despite the higher plasma insulin concentration there was no significant increase in glycogen restocking, a finding in contrast to the data reported by Zawadzki *et al.* (1992). Over the 3 h recovery period muscle glycogen increased from post-exercise concentrations to 225 ± 19 and $252 \pm 48 \text{ mmol.kg}^{-1}$ dry mass in the CHO and CHO-PRO trials, respectively. In addition, Jentjens *et al.* (2001) observed no significant differences in muscle glycogen restocking rates between CHO and CHO-PRO trials during the first 60 min or between 60 and 180 min post-exercise. Jentjens *et al.* (2001) did not investigate the effects of an iso-caloric equivalent to CHO-PRO, but stated that it was possible that the elevated glycogen restocking rate was indeed the result of elevated insulin levels caused by the addition of the PRO, as opposed to a difference in energy intake between trials.

Ivy *et al.* (1988) reported muscle glycogen resynthesis rates of 5.2 ± 0.9 and $5.8 \pm 0.7 \mu\text{mol.g}^{-1}$ wet weight during their LCHO and HCHO supplement trials over the first 2 h of recovery post-glycogen depleting exercise, respectively. Supplements were ingested immediately and 2 h after the glycogen depleting exercise protocol. During the second 2 h of recovery glycogen restocking rates for LCHO and HCHO were 24 and 22% slower than during the first 2 h, but these differences were not statistically significant. Similar glycogen restocking rates were observed despite a marked difference in total CHO consumption, 225 g during LCHO treatment and 450 g during HCHO treatment.

Ivy *et al.* (2002) evaluated the potential of a CHO-PRO supplement to enhance muscle glycogen storage after vigorous exercise using ^{13}C NMR spectroscopy to assess glycogen concentrations in the *vastus lateralis* with some small contributions from the *rectus femoris* and *vastus medialis* muscles in glycogen depleted male triathletes. Ivy *et al.* (2002) reported that a CHO-PRO supplement yielded significantly greater muscle glycogen storage in the 240 min immediately after a glycogen depleting exercise protocol when compared with an iso-caloric CHO (HCHO) supplement or an iso-CHO (LCHO) supplement (Figure 1.1). They also reported that ingestion of a CHO-PRO supplement immediately post-exercise enhanced glycogen synthesis during the initial minutes (0 to 40 min) of recovery, and that muscle glycogen recovery was further enhanced when a second CHO-PRO supplement was ingested 120 min into the 240 min recovery period. Ivy *et al.* (2002) observed no significant differences in muscle glycogen concentrations across the three treatments before or immediately after a standard glycogen depleting exercise. Total muscle glycogen after 240 min of recovery was significantly greater during the CHO-PRO trial when compared with the HCHO and LCHO treatments ($P < 0.05$), while there was no significant difference detected between HCHO and LCHO treatments.

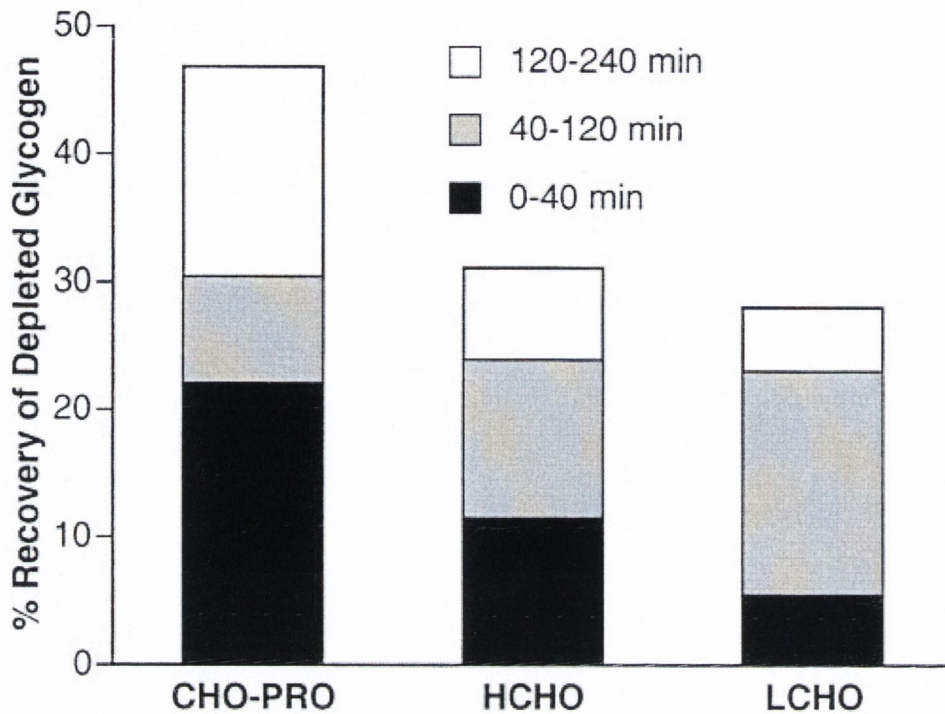


Figure 1.1: Percent recovery of depleted glycogen stores of the *vastus lateralis* during 0 to 40, 40 to 120, and 120 to 240 min of recovery. Pre- and post-exercise muscle glycogen stores were not significantly different across treatments (adapted from Ivy *et al.*, 2002).

During recovery Ivy *et al.* (2002) reported significantly lower plasma lactate data ($P<0.05$) for CHO-PRO when compared with the HCHO and LCHO ingestion at 30, 60, 180 and 240 min. Plasma glucose rose significantly from post-exercise data within 30 min of ingestion of the first supplement, regardless of the supplement ingested, and was significantly greater after HCHO and LCHO than after CHO-PRO ingestion (Ivy *et al.*, 2002). However, Ivy *et al.* (2002) reported that no significant differences were observed in plasma insulin levels at any time across treatments. Zawadzki *et al.* (1992) observed that following CHO-PRO and CHO treatments plasma insulin levels were significantly elevated above PRO treatment at 15 min after cessation of exercise and remained significantly elevated throughout the 4 h recovery period. They also noted that plasma insulin concentrations for their CHO-PRO treatment were generally higher than those for CHO, and were significantly higher at 15, 90, 150 and 180 min post-exercise (Zawadzki *et al.*, 1992; Van Loon *et al.*, 2000a).

The findings that a CHO-PRO treatment enhanced glycogen storage when compared with LCHO were in agreement with data reported by Zawadzki *et al.* (1992) and Van Loon *et al.* (2000a). Zawadzki *et al.* (1992) reported that ingestion of 112 g of CHO or 112 g of CHO and 40.7 g of PRO (CHO-PRO) at 0 and 120 min after a glycogen depleting exercise resulted in a rate of glycogen storage 38% faster following CHO-PRO. In the study by Zawadzki *et al.* (1992) volunteers performed a different glycogen depleting protocol than that implemented by Ivy *et al.* (2002), and muscle glycogen in the *vastus lateralis* was quantified via muscle biopsy technique. The glycogen depletion protocol employed by Zawadzki *et al.* (1992) required volunteers to fast for 12 h prior to each trial and maintain a similar diet and exercise pattern in the days preceding each trial. Van Loon *et al.* (2000a) reported similar average muscle glycogen content 5 h post-exercise between their HCHO and CHO-PRO trials, respectively. It must be noted that Van Loon *et al.* (2000a) administered beverages at 30 min intervals from 0 min to 270 min post-exercise.

Williams *et al.* (2003) noted that ingestion of a CHO-PRO supplement resulted in a 128% greater rate of glycogen storage compared to CHO ($P<0.05$). Participants performed a timed test to failure (TTF) after a 2 h glycogen depletion phase and a 4 h recovery/supplementation phase. Performance time was increased by 55% with CHO-PRO when compared with CHO supplementation during TTF trial ($P<0.05$). Plasma glucose concentrations increased compared to pre-exercise data at 30 min post-ingestion of CHO-PRO and CHO beverages. Plasma glucose concentrations were observed to be significantly higher 60 min after the first CHO-PRO supplement and 30 and 60 min after ingestion of the second CHO-PRO supplement when compared to CHO. This resulted in a 17% higher plasma glucose response for CHO-PRO when compared to CHO. Significantly elevated plasma insulin concentrations were also observed for CHO-PRO when compared to CHO.

In this study by Williams *et al.* (2003), the greater rate of glycogen restocking, greater plasma insulin and glucose responses may have possibly resulted from the discrepancy in the caloric content of the beverages supplemented. Similar results were previously reported by Zawadzki *et al.* (1992) in which investigated drinks were not matched for caloric content. Williams *et al.* (2003) supplemented their participants with 106 g CHO ($\sim 0.40 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and 28 g PRO ($\sim 0.10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)

during the CHO-PRO supplementation phase and only 42 g CHO ($\sim 0.15 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) during the CHO supplementation. Although previous studies have stated that the addition of PRO to a CHO supplement could induce a greater degree of hyperinsulinaemia, a greater plasma glucose response and a greater restocking of glycogen stores, the effect of the increased calorie ingestion resulting from CHO-PRO supplementation cannot be ruled out as a contributing factor to the responses observed by Williams *et al.* (2003).

Betts *et al.* (2007) investigated if the potential benefit of CHO-PRO drinks was a consequence of the PRO fraction or due to the additional energy it provides. Six active male participants performed three trials each involving a 90 min treadmill run at 70% $\dot{V}\text{O}_2\text{max}$ followed by a 4 h recovery period. At 30 min intervals during recovery, participants ingested solutions containing $0.8 \text{ g CHO} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ plus $0.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of whey isolate (CHO-PRO), or 0.8 or 1.1 g $\text{CHO} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (LCHO and HCHO, respectively). The latter two solutions matched the CHO-PRO solution for CHO and for energy, respectively. Following recovery, participants completed a run to exhaustion at 70% $\dot{V}\text{O}_2\text{max}$ (TTF). Betts *et al.* (2007) reported significantly increased serum insulin concentrations at the onset of recovery in all three of their trials, but the largest mean insulinaemic response occurred after CHO-PRO ingestion when compared with HCHO and LCHO (47.0 ± 18.6 vs. 43.9 ± 22.9 and $28.9 \pm 12.4 \text{ nmol} \cdot 240\text{min}^{-1} \cdot \text{L}^{-1}$, respectively). Also, plasma urea rose significantly after ingestion of CHO-PRO when compared with HCHO and LCHO.

Exercise capacity during Betts *et al.* (2007) TTF trial was significantly greater following ingestion of CHO-PRO and HCHO compared to ingestion of LCHO, and no significant difference was detected between the CHO-PRO and HCHO treatments. Betts *et al.* (2007) concluded that increasing the energy content of the recovery solutions extended run time to exhaustion, irrespective of whether the additional energy originated from sucrose or whey isolate. Betts *et al.* (2007) reported that BGlu concentrations were significantly more stable during recovery following CHO-PRO when compared with HCHO and LCHO. This enhanced maintenance of BGlu concentrations following CHO-PRO ingestion may have

contributed to a greater availability of BGlu during the subsequent TTF trial from the gastrointestinal tract or liver, resulting in improved time to fatigue.

Many recent studies have reported no effect of PRO supplementation on muscle glycogen restocking post-exercise (Carrithers *et al.*, 2000; Van Loon *et al.*, 2000a) or when PRO was supplemented with a CHO supplement (Van Hall *et al.*, 2000; Jentjens *et al.*, 2001) that provided $>0.8 \text{ g.kg}^{-1}.\text{h}^{-1}$, the dosage administered by Zawadzki *et al.* (1992). Research by Zawadzki *et al.* (1992), Carrithers *et al.* (2000) and Tarnopolsky *et al.* (1997) observed no significant difference in muscle glycogen stores during the initial hours of recovery with ingestion of LCHO or HCHO. Carrithers *et al.* (2000) investigated the effect of HCHO (iso-caloric to CHO-PRO), CHO-PRO and CHO-AA feedings on muscle glycogen restocking after exhaustive exercise, and the glucose and insulin response at rest via an oral glucose tolerance test. Carrithers *et al.* (2000) reported a significant increase ($P<0.05$) in muscle glycogen concentration after 4 h of recovery (231 ± 37 , 230 ± 29 and $205 \pm 40 \text{ mmol.kg}^{-1}$ for HCHO, CHO-PRO and CHO-AA, respectively), although no differences were apparent among trials. Carrithers *et al.* (2000) also reported a greater concentration of serum glucose during the HCHO trial ($P<0.05$) at 1 h of the recovery period than that observed during their CHO-PRO or CHO-AA trials.

Blom *et al.* (1987) demonstrated that HCHO ingestion between 0.35 and $0.7 \text{ g.kg}^{-1}.\text{h}^{-1}$ after exhaustive exercise provided an adequate stimulus for maximal muscle glycogen restocking over a period of 4 h. However, additional HCHO ingestion ($>0.7 \text{ g.kg}^{-1}.\text{h}^{-1}$) did not appear to provide any enhanced benefit in the restoration of muscle glycogen. The CHO content used by Carrithers *et al.* (2000) differed (1.0 , 0.71 and $0.86 \text{ g.kg}^{-1}.\text{h}^{-1}$ for HCHO, CHO-PRO and CHO-AA, respectively) but muscle glycogen restocking rates were similar, supporting the earlier finding of Blom *et al.* (1987). Therefore, according to the findings of Carrithers *et al.* (2000) muscle glycogen restoration did not appear to be altered with CHO-PRO feeding, provided adequate CHO was supplemented, and the addition of PRO or AA to the HCHO feeding did not elicit a synergistic insulin response. Carrithers *et al.* (2000) concluded that the type of PRO used for the feedings may have contributed to the differences observed among studies in terms of insulin and glycogen concentrations.

Yaspelkis and Ivy (1999) stated that post-exercise supplementation with CHO-AA resulted in significantly reduced rates of CHO oxidation post-exercise and therefore may increase availability of glucose for glycogen restoration. A 35% increase in the rate of glycogen restoration was reported post-exercise with CHO-AA supplementation when compared with CHO supplementation, although this difference did not attain significance (Yaspelkis & Ivy, 1999). Yaspelkis and Ivy (1999) provided 1.0 g.kg⁻¹ of supplements at 0, 1, 2 and 3 h post-exercise. Plasma insulin and glucose concentrations were similar and not significantly different between trials. In comparison to the study by Zawadzki *et al.* (1992), Yaspelkis and Ivy (1999) demonstrated that a single AA in addition to a CHO supplement was not as effective as a CHO-PRO supplement in stimulating insulin secretion and thus elevating glycogen storage. In this study, well-trained cyclists (n = 12) exercised for 2 h on two separate occasions to deplete their muscle glycogen stores. At 0, 1, 2 and 3 h after each exercise bout they ingested either a CHO supplement (1 g CHO.kg⁻¹) or a CHO supplement combined with the AA arginine (1 g CHO.kg⁻¹ and 0.08 g AA.kg⁻¹).

Tarnopolsky *et al.* (1997) compared the rate of glycogen restocking after endurance exercise when the content of both post-exercise supplement (CHO compared with CHO-PRO-Fat) and 24 h energy intake of all trials were maintained constant. Tarnopolsky *et al.* (1997) reported significantly higher muscle glycogen concentrations at 240 compared with 0 min (immediately post-exercise) for CHO-PRO-Fat and CHO trials (CHO-PRO-Fat from 142.2 ± 69 to 245.2 ± 75 mmol.kg⁻¹ dry mass; CHO from 163.4 ± 53.6 to 312.8 ± 56 mmol.kg⁻¹ dry mass). Their results illustrated that the glycogen restocking rates for CHO and CHO-PRO-Fat was similar, suggesting that the disparity in the findings of other investigations may have been affected by the energy intake. It may be argued that gastric emptying may be a limiting factor, and may have been inhibited by the fat content in the supplement administered in this study. However, gastric emptying was considered not to be a contributing factor because the rapid rise in insulin in the CHO-PRO-Fat trial was similar to that observed in the CHO trial and the difference between trials was not statistically significant (Tarnopolsky *et al.*, 1997).

Biolo *et al.* (1999) determined the individual and combined effects of insulin and

prior exercise on leg muscle PRO synthesis and degradation, AA transport, glucose uptake and alanine metabolism. Participants ($n = 5$) were studied 3 h post heavy leg resistance exercise and in a post-absorptive state. Insulin was infused into a femoral artery to increase leg insulin concentration to high physiologic levels without substantially affecting the whole-body level. Biolo *et al.* (1999) demonstrated that the abilities of insulin to stimulate glucose uptake and alanine transport, and to suppress PRO breakdown were increased after resistance exercise, but that stimulation of muscle PRO synthesis and of blood flow were not augmented by prior exercise. The absence of a stimulatory effect of insulin on muscle blood flow observed by Biolo *et al.* (1999) after exercise reduced any effect of insulin on the increased transport of AA from the blood. Biolo *et al.* (1999) concluded that the availability of intracellular AA possibly became limiting for PRO synthesis, as reflected by the decrease in AA concentration, and may be compensated for by the addition of additional AA. Biolo *et al.* (1999) concluded that increased alanine transport as a result of insulin infusion after exercise was met with a similar increase in intracellular alanine turnover. This increased rate of alanine appearance and utilisation was accounted for by increased rates of non-PRO alanine utilisation. This alanine utilisation was exceeded by the rate of synthesis during insulin infusion after exercise and may have contributed to restocking of glycogen stores post-exercise. This was due to the fact that non-PRO alanine utilisation involved the synthesis of pyruvate whose metabolic fate could be met through oxidation and synthesis of glycogen (Biolo *et al.*, 1999).

Data reported by Reed *et al.* (1989), Zawadzki *et al.* (1992) and Van Loon *et al.* (2000a) have suggested that the rate of muscle glycogen restocking was related to the plasma insulin response, thus the rationale for adding PRO to the CHO supplement has been to increase the effectiveness of the supplement in raising the plasma insulin concentration (Zawadzki *et al.*, 1992; Van Loon *et al.*, 2000a). In the study by Ivy *et al.* (2002) the increased rate of glycogen restocking during their CHO-PRO trial could not be attributed to the greater plasma insulin response or to differences in plasma catecholamines and circulating NEFA which could potentially antagonise the action of insulin.

Reed *et al.* (1989) observed glycogen restocking rates of $5.1 \pm 1.0 \mu\text{mol.g}^{-1}$ wet weight for liquid treatment, $5.5 \pm 0.8 \mu\text{mol.g}^{-1}$ wet weight for solid treatment and $5.6 \pm 0.5 \mu\text{mol.g}^{-1}$ wet weight for intravenous treatment. Concentrations of BGlu following intravenous treatment were significantly higher than those for both liquid and solid treatments from 15 min post-exercise until the end of recovery. In the study by Reed *et al.* (1989) glycogen restocking rates were similar across all three treatments, although the mean insulin response for the liquid treatment was significantly higher than the solid treatment, but not intravenous treatment. Reed *et al.* (1989) stated that at plasma insulin concentrations from 150 to $500 \mu\text{U.mL}^{-1}$, the major site of glucose disposal shifted from oxidation to storage with a concomitant increase in the activity of glycogen synthase. The peak insulin concentrations recorded by Reed *et al.* (1989) were well below the specified value (98.4 ± 16.3 , 130.5 ± 14.2 and $104.5 \pm 13.4 \mu\text{U.mL}^{-1}$ during liquid, solid and intravenous treatments, respectively) and therefore, the difference in the insulin responses across the three treatments may not have been of sufficient magnitude to induce significant differences in the rates of glycogen storage. In this study volunteers ($n = 8$) cycled for 2 h on three separate occasions to deplete their muscle glycogen stores. After each exercise bout they received 3 g CHO.kg^{-1} in liquid (50% glucose polymer) or solid (rice/banana cake) or by intravenous infusion (20% sterile glucose). The liquid and solid supplements were divided into two equal doses and administered immediately after and 120 min post-exercise, whereas the intravenous supplement was administered continuously during the first 235 min of the monitored recovery period and blood samples were drawn at regular intervals throughout both exercise and recovery.

Considering the findings of Zawadzki *et al.* (1992), Van Loon *et al.* (2000a) and Ivy *et al.* (2002) it appears that the addition of PRO to a CHO supplement will increase the rate of muscle glycogen restocking during the initial hours after exercise if the supplement only contains a low to moderate amount of CHO. What seems less evident is whether the advantage of a CHO-PRO supplement relative to muscle glycogen restocking is maintained when compared with a HCHO supplement.

In studies that used lower PRO concentrations and supplemented at higher frequencies (Tarnopolsky *et al.*, 1997; Carrithers *et al.*, 2000; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Jentjens *et al.*, 2001) no differences in glycogen restocking between treatments were reported. Ivy *et al.* (2002) provided supplements at 0 min and 120 min of recovery and stated that large doses of CHO provided at regular (15 min) intervals have been reported to promote glycogen storage rates considerably higher than those observed when supplementing at 2 h intervals. Thus, supplementing with smaller doses at higher frequencies may have altered the rates of absorption of CHO and PRO, and may have limited the advantage of the PRO (Van Loon *et al.*, 2000a; Ivy *et al.*, 2002).

Differences in total muscle glycogen restocked between CHO-PRO treatment and HCHO treatment occurred after 40 min and 4 h of recovery (Ivy *et al.*, 2002). However, detection of an outcome effect may also have depended on experimental design differences concerning the recovery protocols. Van Hall *et al.* (2000) reported that glycogen restocking rates were ~20% higher, but failed to infer statistical significance, using supplementation at hourly intervals with either CHO or CHO-PRO over a 3 h period. Their results were similar to those of reported by Ivy *et al.* (2002) and according to Ivy *et al.* (2002) this raised the possibility of a significant difference being detected if the recovery time had been extended by an additional hour. Tsintzas *et al.* (2003) reported that an increase in CHO intake by a small amount could result in increased muscle glycogen restocking during recovery but would not increase the glycogen degradation rate during subsequent exercise.

The data presented in this section regarding the physiological and performance responses to CHO-PRO ingestion, particularly the recent findings by Betts *et al.* (2007), suggested that such a nutritional recovery intervention may provide a means of maintaining training quality and improving the potential outcome of a training cycle.

1.15 Resistance training and glycogen restocking

Volek (2004) stated that RT was an integral part of almost every athletic training regimen and recent studies have focused on enhancing the acute responses of RT, namely PRO synthesis, glycogen resynthesis and breakdown, with the use of specific

nutritional strategies. Exercise has been shown to increase blood flow to skeletal muscle resulting in enhanced nutrient delivery, metabolite removal, and the potential to enhance hormone interactions at target receptors (Volek, 2004). Therefore training status and adaptations to training may influence glycogen resynthesis rates post-exercise. Ren *et al.* (1994) stated that exercise training induced adaptations that have a CHO sparing effect and actually resulted in a slower rate of glucose utilisation by muscle during exercise. Volek (2004) stated that nutrient availability was a critical factor during this time, a factor which had been documented by previous studies (Pascoe & Gladden, 1996; Roy & Tarnopolsky, 1998).

Previous research in applying a protocol such as that utilised by Betts *et al.* (2007) to a RT protocol failed to produce conclusive results (Coyle *et al.*, 2005). Despite a lack of statistical significance, the mean total work capacity (TWC) after ingestion of a CHO-PRO supplement was 16, 10 and 2% higher than after ingestion of LCHO, HCHO and combined CHO and fructose (CHO-FRU) supplements, respectively. It is possible that had glycogen stores been fully depleted, the difference in CHO uptake due to insulinotropic stimuli may have resulted in a significant performance outcome as previously postulated by Ivy *et al.* (2002) and demonstrated by Betts *et al.* (2007). Also, the mode of exercise may itself be a contributing factor (Betts *et al.*, 2007). Research in the area of CHO and CHO-PRO ingestion during recovery from RT has identified the ability of CHO-PRO supplementation to alter the metabolic and hormonal responses (Tarpenning *et al.*, 2001; Williams *et al.*, 2002). A modest effect of CHO ingestion on net leg PRO balance (Børsheim *et al.*, 2004) and similar adaptations to RT have been noted after supplementation with milk or a CHO beverage (Rankin *et al.*, 2004). Also, ingestion of CHO-PRO following RT significantly stimulated insulin and the anabolic process (Miller *et al.*, 2003; Kreider *et al.*, 2007). Other research has determined that ingestion of combined CHO-PRO during recovery from aerobic exercise increased fractional synthetic rate and improved whole body nitrogen balance when compared with CHO alone (Howarth *et al.*, 2009).

1.16 Protein synthesis

Protein turnover is a specific area of concern for individuals who want to increase or maintain lean BM (Thyfault *et al.*, 2004). Chandler *et al.* (1994) demonstrated that

skeletal muscle PRO turnover was increased after short-term bouts of high-intensity resistance exercise. It was thought that the catabolic hormone C increased the rate of skeletal muscle PRO degradation which may have a detrimental effect on muscle after resistance exercise (Thyfault *et al.*, 2004). In the study reported by Thyfault *et al.* (2004) supplementation with CHO did not affect C concentrations post-exercise or effect PRO degradation post-resistance exercise. However, Tipton *et al.* (2001) previously reported that a combination of AA, to increase availability, and CHO to stimulate insulin release could be a potent stimulator of net PRO synthesis. Thyfault *et al.* (2004) stated that the increase in insulin concentrations post-supplementation with CHO improved the anabolic environment following resistance exercise. However, Rasmussen *et al.* (2000) observed an increase in muscle anabolism when a CHO-AA supplement was administered at 1 or 3 h post-exercise. Results from Rasmussen *et al.* (2000) suggested that essential AA were the primary stimulators of muscle PRO synthesis. Rasmussen *et al.* (2000) therefore concluded that the timing of supplementation was of no consequence to their findings. In contrast, Tipton *et al.* (2001) reported that the response of muscle PRO metabolism to a CHO-AA supplement ingested immediately before exercise was greater than the response elicited when the same supplement was consumed post-exercise. Tipton *et al.* (2001) concluded that their more prolonged response was most likely due to a sustained AA delivery.

1.17 Summary of findings

Successful training must involve overload but must avoid the combination of exercise overload and inadequate recovery as this can lead to NFOR or OT (Kuipers & Keizer, 1988; Mujika *et al.*, 1996; MacKinnon, 2000; Halson & Jeukendrup, 2004; Mujika *et al.*, 2004; Meeusen *et al.*, 2006; Betts *et al.*, 2007; Coutts *et al.*, 2007). There is a need to achieve a balance between the stresses inherent in both training and competitive situations with adequate recovery when the effects of such stresses on post-exercise immune function, hormonal responses and intra-muscular glycogen stores are considered (Fukatsu *et al.*, 1996; Bishop *et al.*, 1999b; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Mäestu *et al.*, 2005; Bangsbo *et al.*, 2006; Barnett, 2006; Krstrup *et al.*, 2006a; Cunniffe *et al.*, 2010; Lane *et al.*, 2010; Hough *et al.*, 2011). Described as an indicator of the athletes' ability to tolerate the training load, the T/C ratio is sensitive to changes in training load and competition stresses with

many studies reporting increased C concentration accompanied by decreased T concentration when the training load is increased even slightly and in the aftermath of competitive events, which can persist for up to 38 h under some conditions (Fukatsu *et al.*, 1996; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Coutts *et al.*, 2007; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010; Lane *et al.*, 2010).

Recovery requires the restoration of physiological mechanisms and is heavily influenced by the availability and actions of specific hormones and nutrients (Mujika *et al.*, 1996; Kraemer *et al.*, 1998; Filaire *et al.*, 2001). The combined ingestion of a CHO-PRO supplement immediately post-exercise is of particular benefit to the athlete, as such a nutritional intervention has been reported to mediate greater muscle glycogen resynthesis compared with CHO alone as well as significantly improved time to failure in subsequent exercise (Zawadzki *et al.*, 1992; Roy *et al.*, 1997; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000b; Van Loon *et al.*, 2000c; Tipton *et al.*, 2001; Ivy *et al.*, 2002; Williams *et al.*, 2003; Rankin *et al.*, 2004; Berardi *et al.*, 2006; Betts *et al.*, 2007; Rodriguez *et al.*, 2007; Howarth *et al.*, 2009). Nutritional interventions may also suppress the catabolic effects of strenuous exercise or match-play by providing sufficient exogenous CHO to maintain BGlu concentration so that muscle glycogen is spared during, or restored post-exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Gleeson & Bishop, 2000; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a; Lane *et al.*, 2010).

Restoration of work capacity through replenishing utilised glycogen and rehydration between training sessions remains the primary physiological facet of recovery (Jentjens & Jeukendrup, 2003; Shirreffs *et al.*, 2004; Maughan *et al.*, 2007). Maximising glycogen restoration and minimising disturbances in immunocompetence after training cycles and competitive situations through supplementation with sports drinks appears to reduce the necessity for extended rest periods post-exercise. Nutritional interventions may provide a means of restoring homeostasis via attenuation of catabolic hormonal responses post-exercise possibly enhancing the athletes' ability to tolerate intense training loads and repeated high-intensity, competitive exertions. Quantification of the effects of sports drinks on subsequent performance and the response of T/C ratio may assist in determining

whether these so called “recovery drinks” truly merit such a label and the accompanying prestige in the scientific and sporting arena.

1.18 Aims and hypotheses of the thesis

In view of the conflicting findings in previous research, Chapter 3 proposed to evaluate if a CHO-PRO supplement, by potentially inducing a greater degree of glycogen restocking compared to iso-CHO (LCHO) or iso-caloric equivalents (HCHO), would significantly increase performance during the RT regimen performed 240 min after a standard RT protocol in male resistance trained athletes. Recent research by Betts *et al.* (2007) had established that CHO-PRO ingestion can improve subsequent treadmill running performance. However, no data was available regarding the effects of CHO-PRO ingestion on subsequent RT performance which presented an important sport-specific scenario that warranted investigation considering the prevalence of multi-day training sessions in amateur and professional sporting arenas.

- The hypothesis was that CHO-PRO supplementation would significantly increase time to failure resulting in an increased normalised cumulative workload (ΣW ; kg.kg^{-1} BM) and TWC (kg.kg^{-1} BM) during the RT regimen performed 240 min following a standard RT protocol designed to induce glycogen depletion when compared with supplements of equivalent CHO (LCHO) and caloric content (HCHO).

With consideration for the research outlining the physiological, hormonal and immunological responses to functional-OR training and competition scenarios, and the previously stated necessity to perform functional-OR investigative studies (Halsen & Jeukendrup, 2004), Chapter 4 proposed to evaluate the hormonal responses to in-season training and competition scenarios across three sporting disciplines of varying demands, i.e. soccer, rugby and rowing.

- The primary hypothesis was that the competition scenario would elicit significantly greater hormonal changes compared to the training scenario in all sporting disciplines.
- The secondary hypothesis was that the hormonal changes would be significantly greater in rugby union players than soccer players or rowers as

previous data suggested that the nature of rugby union caused longer term disturbances to host immunity and greater disturbance to both C and T concentrations (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010).

The nature of competitive soccer reportedly places great demands on intra-muscular glycogen stores to fuel performance and resulted in a significant increase in C concentration post-match (Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008). Post-match, glycogen depletion can reportedly persist for up to 48 h despite the presence of a high CHO content diet presenting obvious implications for training intensity (Bangsbo *et al.*, 2006). Ingestion of a CHO-PRO supplement post-exercise presents the most advantageous means of maximising glycogen resynthesis, maintaining subsequent performance (Ivy *et al.*, 2002; Betts *et al.*, 2007) and may decrease the cumulative catabolic response associated with intense exercise and soccer match play (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Ispirlidis *et al.*, 2008; Lane *et al.*, 2010; Mikulski *et al.*, 2010). Chapter 5 proposed to evaluate the effect of a CHO-PRO beverage on salivary C and T concentrations compared to a CHO supplement alone (LCHO) and an iso-caloric equivalent to CHO-PRO supplement (HCHO) during standard, in-season soccer training.

- The hypothesis for Chapter 5 was that a CHO-PRO and HCHO beverage consumed immediately post-training would reduce the exercise induced C response while augmenting the T response, compared to LCHO ingestion, and result in a T/C ratio reflective of an improved ability to tolerate training load.

In periods of functional-OR training, athletes may perform numerous training bouts with limited recovery time between each training session, placing great demands on host immunity (Gleeson & Bishop, 2000; Lane *et al.*, 2010; Mikulski *et al.*, 2010). Greater emphasis is placed on recovery during such training cycles because the success of the training cycle and subsequent performance is dependent on the quality of training (Halsen & Jeukendrup, 2004; Mujika *et al.*, 2004). Ingestion of a CHO-PRO supplement post-exercise presents the most advantageous means of maximising glycogen resynthesis, maintaining subsequent performance (Ivy *et al.*, 2002; Betts *et al.*, 2007) and may decrease the cumulative catabolic response to intense exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Lane *et al.*, 2010; Mikulski *et al.*, 2010).

The aim of Chapter 6 was to investigate the effect of different commercially available sports drinks on salivary C and T responses during recovery from two exercise bouts completed in a period of inadequate rest and nutritional intake.

- The hypothesis of Chapter 6 was that ingestion of a CHO-PRO sports drink after two bouts of high-intensity exercise would result in more effective recovery, when compared with an iso-caloric CHO drink (HCHO) or a PL drink, indicated by a more positive T/C ratio, by means of suppressing the reported exercise induced increase in salivary C concentration as a result of an enhanced insulin response and increased BGlu availability post-ingestion.

Chapter 2

General methods

Chapter 2

General methods

2.1 Anthropometric measurements

For external testing locations, height in metre (m) and BM in kilogram (kg) were measured using an electronic scales (Salter, Tonbridge, UK) and a tape measure (Stanley, Sheffield, UK) secured to a flat surface. The weighing scale was calibrated regularly using weights of known mass (25 kg). For assessment of stature, participants stood upright against the measuring device located on a flat upright surface. Their head was positioned with the ear opening and the lower border of the eye socket in the same horizontal plane (Frankfurt plane). Participants were instructed to take a deep breath and height was measured in centimetre (cm) to the nearest 1.0 cm. For BM assessment each participant stood in the centre of the scales and BM in kg was measured to the nearest 0.1 kg. During the laboratory based study, height and BM were measured using a counter balance scales and stadiometer (Seca, Hamburg, Germany). All participants were weighed barefoot in the clothes they would subsequently exercise in. Body mass index (BMI) was then calculated using the following equation.

$$\text{BMI (kg.m}^2\text{)} = \text{Body mass (kg)}/\text{Height}^2 \text{ (m}^2\text{)}.$$

Percentage body fat was estimated from the sum of skinfold thickness assessed using a Harpenden skinfold caliper (Baty International, West Sussex, UK). Measurements of skinfold thickness were recorded from four sites, namely, tricep, bicep, subscapular and suprailiac, all on the volunteers' dominant side. All measurements were recorded while volunteers were standing on a level surface. A fold of skin and subcutaneous tissue was picked up with the left hand and the plates of the caliper, which were held in the right hand, were allowed to exert full pressure below the position of the left hand, before recording the skinfold thickness to the nearest 0.1 mm. Three measurements were recorded from each site and the average was calculated.

1. Tricep: The fold was picked up at the midpoint of a line connecting the acromion and the olecranon process while the arm was hanging loosely with the elbow extended.
2. Biceps: The skinfold was measured directly above the centre of the cubital fossa at the same level at which the tricep reading was recorded.
3. Subscapular: The subscapular skinfold was picked up just beneath the inferior angle of the scapula in a direction obliquely downwards and outwards at a 45° angle.
4. Supra-iliac: The fold was picked up 5 to 7 cm above the Anterior Superior Iliac Spine (ASIS) at an angle of 45° above the horizontal.

The sum of the four skinfolds was noted and percentage body fat calculated using the equivalent fat content tables (Durnin & Womersley, 1974) grouped by age and gender.

2.2 Haematological analysis

Blood samples were collected under aseptic conditions from the medial cubital veins in the cubital fossa of the arm using the Vacutainer system; this system involved the use of a 21G 1.5 inch needle (Precision Glide, Becton Dickinson, Oxford, UK) and a 4 mL EDTA or lithium heparin tube or a 10 mL Z-Serum tube (Vacutainer, Becton Dickinson, Oxford, UK). A full haematological analysis was performed on necessary blood samples using an automated cell counter (AcT diff, Beckman Coulter., High Wycombe, UK). Participants had blood drawn from alternate arms on each visit. Data for the variables haemoglobin (Hb) in g.dL^{-1} , haematocrit (Hct) in %, red blood cell count ($10^{12}.\text{L}^{-1}$) and white blood cell count ($10^9.\text{L}^{-1}$) were recorded from these blood samples. Where necessary, blood samples were allowed to stand for 20 min to allow clotting before being centrifuged at $5000 \text{ rev.min}^{-1}$ (EBA 20, Hettich, Tuttlingen, Germany). The serum was separated into clean, labelled eppendorf tubes and frozen at -80°C for subsequent batch analysis of specific variables of interest, such as, insulin, C and T.

2.2.1 Capillary blood samples

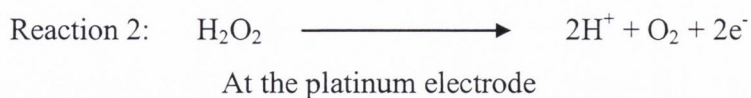
Where necessary, capillary blood samples were drawn from the participant's fingertip for determination of exercise metabolites. Intermittent pressure was applied to the fingertip to aid blood flow. The fingertip was thoroughly cleaned with a 70% w/v isopropyl alcohol swab (Medi-Swab, Smith & Nephew, London, UK) and allowed to dry. The finger was held firm while a sterile standard steel lancet (Solofix, Braun, Melsungen, Germany) was used to puncture the fingertip. The initial drop of blood was cleaned away using absorbent cotton wool. A sweat free sample of capillary blood was collected in a heparinised capillary tube (Marienfeld Laboratory Glassware, Lauda-Königshofen, Germany).

2.3 Blood lactate analysis

A YSI 1500 Sport lactate analyser (YSI, Ohio, USA) was used to measure BLA concentrations during $\dot{V}O_2$ max tests and exercise trials. The measurement of BLA involved a series of chemical reactions within the sensor probe of the analyser. The probe consisted of a silver cathode and platinum anode. A three layered membrane consisting of polycarbonate, immobilised L-Lactate oxidase and cellulose acetate covered the probe. When a whole blood sample was injected in the reaction chamber using the 25 μ L syringe pipette (YSI, Ohio, USA) some of the sample diffused through the outer polycarbonate membrane, this membrane was porous and resisted diffusion of enzymes, but was large enough to allow the passage of O_2 , water (H_2O), hydrogen peroxide (H_2O_2), sodium chloride ($NaCl$) and lactate. The inside membrane of cellulose acetate was permeable to H_2O_2 , but impermeable to ascorbic acid and other substances with a molecular weight greater than 200. When the lactate in the blood sample diffused through the outer polycarbonate membrane and came into contact with the L-Lactate oxidase membrane, lactate was rapidly oxidised producing H_2O_2 and pyruvate in the reaction below



The H_2O_2 produced passed through the inner membrane of cellulose acetate and was oxidised at the platinum anode producing electrons.



The circuit was completed at the silver reference cathode where the following reaction occurred.



The current produced was linearly proportional to the lactate concentration in the sample chamber. The analyser calculated the lactate concentration of the sample by comparing the current produced by the sample with the reference sample of a known lactate concentration (5.0 mmol.L⁻¹).

2.3.1 Calibration

Fresh buffer, consisting of 500 mL water and 6.35 ± 0.35 g YSI 2357 Buffer (YSI, Ohio, USA) and a reference solution consisting of 250 mL buffer solution and 2 mL of 30 mmol.L⁻¹ lactate standard (YSI, Ohio, USA) were placed in the machine prior to each test. To calibrate the analyser 25 µL of a 5.0 mmol.L⁻¹ lactate standard solution was passed into the reaction chamber using the syringe pipette when prompted by the software.

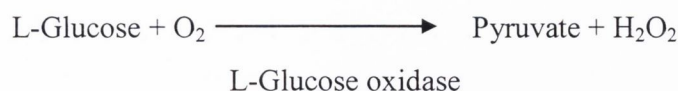
2.3.2 Testing procedures

During testing, blood samples (25 µL) were withdrawn from either 4 mL K⁺ EDTA or lithium heparin vacutainers, or heparinised capillary tube via the syringe pipette to assess BLa concentration. The sample was passed into the reaction chamber when prompted by the software and following measurement the resulting lactate concentration (mmol.L⁻¹) was printed on the LCD display and data noted in the volunteer's data sheet.

2.4 Blood glucose analysis

The BGlu concentration from whole blood samples were assessed using a YSI 2700 combined chemistry analyser (YSI, Ohio, USA). Samples were withdrawn from the vacutainer or eppendorf using the sipper before being instilled into a buffer filled

chamber. Glucose then diffused across a polycarbonate membrane along with O₂ and other molecules. When the glucose was brought into contact with L-glucose oxidase the following reaction occurred.



The H₂O₂ produced diffused across the thin cellulose acetate membrane, which allowed only molecules with a molecular weight of less than 200 to pass through, thereby reducing the risk of contamination. The H₂O₂ produced was then in contact with a platinum anode which catalysed the following reaction



The subsequent electron flow was linearly proportional to the steady state glucose concentration. A buffer solution was used to wash out the chamber after each sample was analysed, and if the baseline current was found to be unstable (comparing initial and final baseline currents) the buffer pump ran a second flush cycle.

2.4.1 Calibration

The buffer and 10 mmol.L⁻¹ glucose standard solution were automatically passed from their reservoir bottles, which were stored within the machine, into the reaction chamber upon entering the run mode. Auto-calibration was carried out under software control every 2 h.

2.4.2 Testing procedures

During trials, blood samples (25 µL) were drawn from either the 4 mL K⁺ EDTA or lithium heparin vacutainers via the sipper to assess BGlu concentration. Where samples were collected in a heparinised capillary tube, the whole blood sample was expelled into a clean eppendorf using a disposable pipette and withdrawn from the eppendorf using the sipper. The sample was passed into the reaction chamber when prompted by the software and, following measurement, the resulting BGlu and BLa

concentrations (mmol.L^{-1}) were printed on the LCD display and data noted in the participant's data sheet.

2.5 Heart rate telemetry

Individual HR (beats.min^{-1}) was measured during training sessions in two ways, real-time by way of the TMPro SW200 software, version 1.6.3.1 (Hosand, Verbania, Italy) or by standard radio-telemetry using the Cardiosport heart rate monitor (Cardiosport, Wrexham, UK). All heart rate monitors used coded technology to prevent crosstalk between units.

2.5.1 Standard radio-telemetry

As per the manufacturer's instructions, the chest strap transmitter was moistened with warm water to allow for more accurate and consistent readings. Participants wore the chest strap tightly and securely across the sternum, as per manufacturer's instructions, and the heart rate monitor was worn on the volunteers' wrist. The heart rate monitor was activated when players were in position to begin the training session and was deactivated immediately on cessation of exercise. Maximum (HR_{max}), minimum (HR_{min}) and average (HR_{avg}) HR (beats.min^{-1}) were recorded for each individual at the end of active high intensity intervals, and also at the end of the session.

2.5.2 Real-time telemetry

The TMPro HR monitoring system provided a remote-controlled graphic representation of the heart rate of twelve players engaged in the monitored training session. The TMPro system transmitted a participant's HR (beats.min^{-1}) to a computer up to 200 m away, and the SW200 software, version 1.6.3.1, allowed data to be graphically displayed and recorded in real-time for each individual.

The TMPro system comprised of a set of transmitters and a receiving antenna. The TX200 transmitter (Hosand, Verbania, Italy) was a small electronic device weighing only 40 g that intercepted the heart rate measured by a chest strap transmitter (Polar WearLink, Polar Electro, Kempele, Finland) and relayed it to the receiving antenna connected to a laptop computer (Hewlett Packard, Leixlip, Ireland). The TX200

transmitter was inserted into the pocket of a specially designed vest (Hosand, Verbania, Italy) and worn without discomfort to the participant.

As per the manufacturer's instructions, the WearLink chest strap was moistened with warm water to allow for more accurate and consistent data collection. During the monitored training session, volunteers wore the chest strap tightly and securely positioned across the sternum. Each TX200 transmitter was numbered 1 through 12 and was, therefore, assigned to an individual participating in the monitored training session. A TX200 transmitter unit was assigned and activated only when the WearLink chest strap was positioned correctly. When uninterrupted signal between chest strap and TX200 transmitter was detected, signified by a red LED flashing rhythmically, the TX200 was placed into the pocket of the specially designed vest. When all volunteers were in position to begin the training session the lead investigator initialised recording of heart rate data with the TMPro software. During the recording period, the lead investigator had access to real-time group or individual data including HRmax and HRavg in $\text{beats}\cdot\text{min}^{-1}$ and the % HRmax training zone that individual participants or the group (mean % HRmax) were exercising in. Training zones were based on participants' individual HR data which were determined through prior maximal testing. The training zones corresponded to the definitions of aerobic low intensity training, aerobic moderate intensity training and aerobic high intensity training with a range of 0 to 60, 70 to 80, 80 to 90 and 90 to 100% of HRmax, respectively (Bangsbo, 2007). The TMPro software was deactivated immediately on cessation of exercise, and recorded data were saved in individual participants' files and a group (mean) file.

2.6 Saliva collection procedure

Saliva samples were collected without exogenous stimulation. All participants were instructed on correct saliva collection technique. Participants were requested to drool passively into an eppendorf tube. The eppendorf was then sealed and frozen at -80°C until time of analyses. Prior to assaying, the saliva sample was allowed to thaw before being centrifuged (Sigma Laboratory Centrifuges, Osterode am Harz, Germany) at $15000\text{ rev}\cdot\text{min}^{-1}$ for 15 min to precipitate mucus. The saliva, less particulate matter, was subsequently decanted to a clean and clearly labelled eppendorf tube using a disposable pipette.

2.7 Salivary cortisol and testosterone ELISA

Saliva samples were assayed for C and T concentrations using highly sensitive enzyme immunoassay kits specifically designed for the quantitative measurement of salivary C and T (Salimetrics, Suffolk, UK). The salivary C assay used 25 μL (for singlet determination) and had a lower limit sensitivity of 0.08 nmol.L^{-1} and an intra- and inter-CV% between 3.4 to 3.7 and 3.8 to 6.4, respectively. The salivary T assay used 25 μL (for singlet determination) and had a lower limit sensitivity of <0.004 nmol.L^{-1} with an intra- and inter-CV% between 2.5 to 6.7 and 5.6 to 14.1 for high and low concentrations, respectively.

All standards were assayed in duplicate, while unknowns and controls were assayed in triplicate. Standard and controls were assayed on all ELISA plates used during data assessment. Ninety-six well microtitre plates coated with monoclonal antibodies to C and T were used. During the assay procedure C and T in standards, controls and saliva samples competed with the C or T linked to horseradish peroxidase for antibody binding sites. After incubation, unbound components were washed away. Bound C and T peroxidase was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB); this reaction produced a blue colour. On completion of the second incubation period, sulphuric acid stop solution was added and the optical density (OD) was measured using a microplate reader (ELx800 Universal Microplate Reader, Biotek, Bedfordshire, UK) at 450 nm. The amount of C and T peroxidase detected was inversely proportional to the amount of C or T present in the sample (Chard, 1990).

2.7.1 Salivary cortisol ELISA procedure

All reagents were brought to room temperature. Known standards of C, controls and saliva samples were added to the microplate. The supplied standards of cortisol were 82.77, 27.59, 9.19, 3.06, 1.02 and 0.33 nmol.L^{-1} . Then 25 μL of assay diluent was transferred into 2 wells for the determination of zero and into each NSB well. A 1:1600 dilution of enzyme conjugate was made by adding 15 μL of enzyme conjugate to the 24 mL of assay diluent prepared previously. The dilute conjugate solution was mixed and 200 μL transferred into each well. The plate was mixed on a plate rotator (Vortex Genie 2, Scientific Industries, New York, USA) for 5 min at 500 rev.min^{-1} to ensure all reagents mixed before the plate was incubated for a

further 55 min at room temperature. On completion of this incubation period, the microplate was washed 4 times with working strength (diluted) wash buffer and blotted thoroughly between washes on absorbent paper towels. On the final wash cycle, the plate was blotted and dried before adding 200 μL of a TMB solution to each well. The plate was mixed on the plate rotator for 5 min at 500 $\text{rev}\cdot\text{min}^{-1}$ and incubated for a further 25 min in a dark room at room temperature. Finally, 50 μL of stop solution was then added to each well. The plate was mixed on a rotator for 3 min at 500 $\text{rev}\cdot\text{min}^{-1}$, wiped dry and OD was measured using a microplate reader (Biotek, Bedfordshire, UK) at 450 nm, within 10 min of adding the stop solution.

2.7.2 Salivary testosterone ELISA procedure

All reagents were brought to room temperature. Five eppendorf tubes were labelled 2 through 6 and 90 μL of assay diluent was transferred into tubes 2 through 6. The supplied 2.08 $\text{nmol}\cdot\text{L}^{-1}$ standard was serially diluted by adding 60 μL of the standard (tube 1) to tube 2 and mixed well. After changing pipette tip, 60 μL dilute standard was transferred from tube 2 to tube 3 and mixed well. This process was repeated for tubes 4, 5 and 6. The final concentrations of standards for tubes 1 through 6 were 2.080, 0.832, 0.333, 0.133, 0.053 and 0.021 $\text{nmol}\cdot\text{L}^{-1}$, respectively. Then 25 μL of standards, controls and unknowns (saliva samples) was transferred into opposite wells. In addition, 25 μL of assay diluent was transferred into 2 wells for the determination of zero and into each NSB well. A 1:1000 of the conjugate was made by adding 18 μL of enzyme conjugate to 18 mL of assay diluent. The dilute conjugate was mixed thoroughly and 150 μL was transferred into each well. The plate was mixed on a plate rotator for 5 min at 500 $\text{rev}\cdot\text{min}^{-1}$ and incubated at room temperature for 55 min. On completion of this incubation period, the microplate was washed 4 times with working strength (diluted) wash buffer and blotted thoroughly between washes on absorbent paper towels. On the final wash cycle, the plate was blotted and dried before adding 200 μL of TMB solution to each well. The plate was mixed on a plate rotator for 5 min at 500 $\text{rev}\cdot\text{min}^{-1}$ and incubated for a further 25 min in a dark room at room temperature. Finally, 50 μL of stop solution was then added to each well, the plate was mixed on a rotator for 3 min at 500 $\text{rev}\cdot\text{min}^{-1}$, wiped dry and OD was measured using a microplate reader (Biotek, Bedfordshire, UK) at 450 nm within 10 min of adding the stop solution.

2.7.3 Calculation of salivary cortisol and testosterone concentrations

For each ELISA plate, the mean OD was calculated for duplicate or triplicate wells. Where all OD values for the sample in triplicate were of similar value, the mean OD of the triplicate was determined. If one OD value in a sample triplicate was significantly out of range of the accompanying two, that sample OD was discarded and the mean of the duplicate OD values was calculated for that sample. The mean OD of the NSB wells was subtracted from the OD of the zero, standards, controls and unknowns. The percent bound (B/B_0) for each standard, where B is the mean OD for the unknowns, standards and controls and B_0 is the mean OD of the zero, was calculated by dividing B by B_0 . The concentration of the controls and their equivalent B/B_0 data were entered in a graphic interpolation package (Cricket Graph version 1.3.1, Cricket Graph Software, California, USA). For each ELISA plate, a graph of log of standard concentrations versus B/B_0 was determined. A third order polynomial equation was then fitted to the data and the line of best fit calculated for each ELISA plate. The coefficients of determination exceeded 0.95 for all plates used during the course of this set of studies. The resultant constants for the cubic equation were entered into a spreadsheet to assess the concentration of C and T of the unknown samples on each plate.

2.8 Pilot Investigation: Serum versus saliva hormone concentrations

As stated previously (Section 1.3), approximately 95 and 98% of C and T respectively is present in their bound forms in the circulation with remaining concentrations present in the unbound active form (Bishop *et al.*, 2005; Hackney *et al.*, 2005). The concentrations of both C and T in their unbound states present in the saliva can vary from 2 upwards to 10% and are unaffected by salivary flow rate (Vining & McGinley, 1987). In order to determine the relationship between the bound and unbound concentrations of C and T, and allow comparison between the previous and current data, an additional, supplementary investigation was performed to investigate the relationship of C and T hormonal concentrations in both serum and saliva. A group of male volunteers provided samples at 4 appointed collection times (09:00, 12:00, 15:00 and 18:00) on 3 consecutive days. Participants provided blood and saliva samples simultaneously at the 4 appointed collection times on day 1 in order to determine the association and ratio of serum to saliva concentrations and if the hormonal concentrations of the two mediums could affect the T/C ratio. This

was a ‘free-living’ investigation, therefore, participants were not required to restrict diet or activity levels over the 3 day sampling period. Mean data and methodology information are presented in Appendix VII. The relationships between serum and salivary C and T concentrations are displayed in Figure 2.1 and 2.2, respectively.

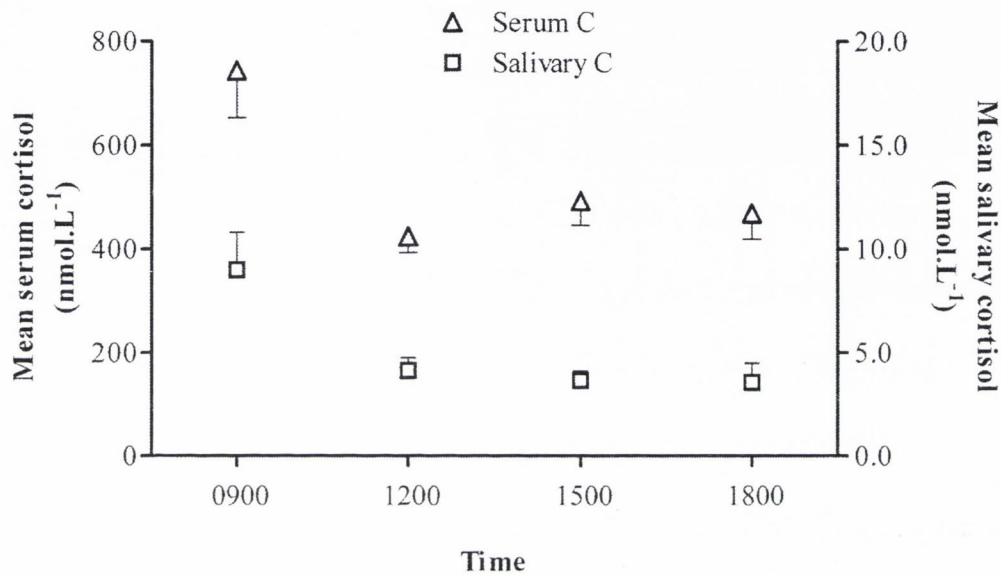


Figure 2.1: Relationship between salivary and serum C concentrations (nmol.L⁻¹) from morning to evening, $\pm$ SEM, n = 10.

Mean salivary C and T concentration was approximately 1 and 6% of mean serum concentrations, respectively, and were within the expected ratios reported in previous research. The concentration of C and T determined in both mediums appeared to follow the previously described diurnal variations in spite of large inter- and intra-subject variability (Vining & McGinley, 1987; Bishop *et al.*, 2005; Hackney *et al.*, 2005).

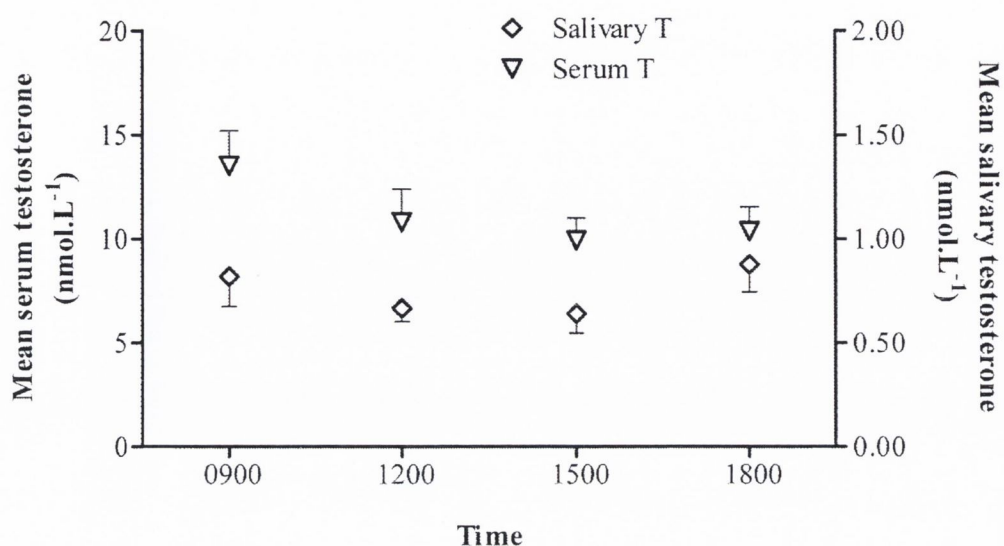


Figure 2.2: Relationship between salivary and serum T concentrations (nmol.L⁻¹) from morning to evening, $\pm$ SEM, $n = 10$.

Pearson product moment correlation coefficients were used to establish possible relationships between variables. A moderate to good correlation was observed between serum and salivary C concentrations ($r^2 = 0.577$), however the relationship between serum and salivary T was poor ($r^2 = 0.058$). The poor correlation observed between salivary and serum T may be indicative of the susceptibility of salivary T measurement to interference effects caused by leakage of blood (plasma) into saliva and storage conditions (Granger *et al.*, 2004). Granger *et al.* (2004) stated that salivary T measurements, more so than other salivary analytes, like C, were keenly sensitive to the manner in which saliva samples were collected and stored and were influenced by the presence of blood, or components of blood, even when the contaminant may not be visible. However, Granger *et al.* (2004) also stated that T behaviour-correlations determined using saliva as a surrogate for serum were likely to substantially underestimate any relationship, citing a 23% underestimation in T behaviour correlation when an enzyme immunoassay technique is used for detection of salivary T.

Chapter 3

The effects of carbohydrate-protein ingestion
post-resistance training on subsequent
exercise capacity in male rugby players.

Chapter 3

3.1 Introduction

3.1.1 The demands of training in Rugby Union

Glycogen is a primary fuel for rugby union performance and, along with maintaining a positive PRO balance, restoration of muscle glycogen is essential for sustained rugby union performance (Tipton *et al.*, 2001; Roberts *et al.*, 2008; Cunniffe *et al.*, 2009). The varying skill set required by rugby players coupled with the intense nature of the game (Deutsch *et al.*, 1998; Duthie *et al.*, 2003; Deutsch *et al.*, 2007; Bevan *et al.*, 2009; Cunniffe *et al.*, 2009; Roberts *et al.*, 2010) present challenges in achieving a balance between training and competition stresses and recovery so that performance can be maximised (Barnett, 2006). The demanding nature of rugby union training has previously been discussed (see section 1.2). RT forms an integral part of rugby players' training (Argus *et al.*, 2010) as it elicits a milieu of hormonal responses critical to acute muscular force and power production, including subsequent tissue growth and remodelling (Kraemer & Ratamess, 2005).

3.1.2 Considerations for recovery from Rugby Union training and game play

An athlete's ability to sustain consistent intensive training and competition without succumbing to chronic fatigue, injury and illness will be influenced, not only by the foods eaten, but by the amount and timing of the food intake (Maughan *et al.*, 2007). Similarly, Barnett (2006) stated that when a training session induced glycogen depletion, the load attainable by the athlete in a subsequent session may be limited by the post-training glycogen synthesis that has occurred. Maximising recovery by rehydration (Shirreffs *et al.*, 2004), liver and muscle glycogen restoration (Jentjens & Jeukendrup, 2003; Maughan *et al.*, 2007) and minimising disturbances to the immune system post-training by sports drinks supplementation (Kreider *et al.*, 2007; Roberts *et al.*, 2010) reduces the necessity for extended rest periods after high load training.

A CHO intake of approximately $1.2 \text{ g.kg}^{-1}.\text{h}^{-1}$ commencing immediately upon completion of strenuous exercise and continuing at regular intervals is recommended in order for utilised glycogen to be replenished (Ivy *et al.*, 2003; Jentjens *et al.*, 2004a). Recommended PRO intake for strength or speed athletes ranges between

1.2 and 1.7 g.kg⁻¹.day⁻¹ (Tipton, 2009). The greatest stimulation of PRO synthesis results from resistance exercise plus AA availability (Rodriguez *et al.*, 2007). Exogenous AA facilitated PRO synthesis and decreased the impact of the post-exercise catabolic environment (Rodriguez *et al.*, 2007).

Evidence suggested that ingestion of CHO-PRO drinks post-exercise would be an advantageous nutritional recovery intervention (Zawadzki *et al.*, 1992; Van Loon *et al.*, 2000b; Tarpenning *et al.*, 2001; Ivy *et al.*, 2002; Williams *et al.*, 2002; Børsheim *et al.*, 2004; Rankin *et al.*, 2004; Berardi *et al.*, 2006; Betts *et al.*, 2007; Howarth *et al.*, 2009). Ingestion of CHO-PRO drinks post-exercise have reportedly resulted in significantly higher muscle glycogen replenishment (Ivy *et al.*, 2002; Berardi *et al.*, 2006) and, furthermore, limited evidence has suggested significantly enhanced subsequent exercise performance (Betts *et al.*, 2007) when compared with iso-caloric and equivalent CHO drinks. These responses have been attributed to an insulinotropic effect (Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b) with better glucose availability potentially responsible for enhanced post-recovery performance (Betts *et al.*, 2007). Certain AA and PRO were effective stimulators of insulin secretion and synergistically increased the blood insulin responses when combined with a CHO supplement (Zawadzki *et al.*, 1992; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Jentjens *et al.*, 2001). This greater rate of glycogen restocking was thought to be the result of a greater plasma insulin response brought about by the addition of PRO to a CHO supplement (Tarnopolsky *et al.*, 1997).

Previous research into an ergogenic advantage of a CHO-PRO beverage on RT performance failed to produce conclusive results (Coyle *et al.*, 2005). However, a possible, but inconclusive, ergogenic benefit of addition of PRO to CHO may exist (Betts *et al.*, 2007) occurring via an interaction of ingested AA with the CNS (Mittleman *et al.*, 1998). An effect of addition of PRO to CHO on the central fatigue hypothesis was previously postulated (Ivy *et al.*, 2003). Research evaluating co-ingestion of PRO with CHO has produced varying results; addition of approximately 0.15 g PRO.kg BM⁻¹.h⁻¹ to a CHO supplement enhanced performance (Shirreffs *et al.*, 2004; Roberts *et al.*, 2008), however, a higher dose (0.26 g PRO.kg BM⁻¹.h⁻¹) did not (Van Essen & Gibala, 2006).

3.1.3 Aims and hypothesis

Recent research by Betts *et al.* (2007) had established that CHO-PRO ingestion can improve subsequent treadmill running performance. However, no data was available regarding the effects of CHO-PRO ingestion on subsequent RT performance which presented an important sport-specific scenario that warranted investigation when the prevalence of multi-day training sessions in amateur and professional sporting arenas was considered. In view of the conflicting findings in previous research, this study proposed to evaluate the effect of a CHO-PRO supplement on recovery from a standard RT session and subsequent RT performance, compared to iso-CHO (LCHO) or iso-caloric equivalents (HCHO).

The hypothesis was that CHO-PRO supplementation would significantly increase time to failure resulting in an increased normalised ΣW (kg.kg^{-1} BM) and TWC (kg.kg^{-1} BM) during the RT regimen performed 240 min following a standard RT protocol designed to induce glycogen depletion when compared with supplements of equivalent CHO (LCHO) and caloric content (HCHO).

Chapter 3

3.2 Methods

3.2.1 Study design

This study involved a single-blinded, repeated measures design protocol requiring three visits to the gymnasium at St. Michael's College, Nutley Lane, Dublin. During each visit volunteers undertook a protocol lasting several hours that included a standard RT phase (duration 2 h 8 min), a recovery-supplementation period (duration 4 h) and a RT TTF protocol. During the 4 h recovery period supplements were ingested in a randomised order in liquid form at two separate, predetermined time intervals. The study design and experimental procedures involved in this study were approved by Faculty Research Ethics Group, Trinity College Dublin.

3.2.2 Volunteers

Eighteen trained male rugby players were initially recruited as volunteers for this study via the Irish Rugby Football Union (IRFU) and all were members of the Leinster Rugby Development Academy. All volunteers had been playing club or schools rugby the previous season and most volunteers had provincial and national underage representative honours. Of the eighteen volunteers initially recruited, only fourteen completed all drinks trials due to injury, attrition and unscheduled IRFU representative commitments ($n = 14$). Exercises performed in this study were similar to those that rugby union players would perform in a standard RT sessions, and all volunteers were well versed in RT technique. All volunteers were provided with a study information sheet and gave informed written consent prior to commencing the study. All testing took place during the pre-season training cycle for the volunteers during July and August.

3.2.3 Inclusion and exclusion criteria

All volunteers were male and aged between 18 and 23 years. They were medically screened prior to their first testing session by a qualified medical practitioner and had previously undergone medical interview and screening through the IRFU. Individuals suffering from diabetes, hypertension, heart defects and metabolic disorders were excluded. Volunteers who were suffering from minor sporting

injuries were allowed to participate and performed exercises within the limits of their injuries.

3.2.4 Anthropometric assessments

Anthropometric assessment of height (m), BM (kg), percentage body fat and BMI (kg.m^2) were performed as outlined in Section 2.1.

3.2.5 Haematological analysis

All blood samples were collected from the medial cubital veins in the cubital fossa using the Vacutainer system and a full haematological assessment was performed, as described in Section 2.2.

3.2.6 Blood lactate assessment

Assessment of BLa concentrations (mmol.L^{-1}) were performed as described in Section 2.3.

3.2.7 Blood glucose assessment

Assessment of BGlu concentrations (mmol.L^{-1}) were performed as described in Section 2.4.

3.2.8 Experimental protocol

Volunteers participated in the RT and TTF protocol on 3 separate occasions with three to four days between successive visits. Each visit lasted approximately 7 h. The order of treatments was randomised to reduce any sequence effects. Volunteers were requested to standardise their fluid intake and training regimen, in agreement with Leinster Academy coaches, in the 24 h period prior to each trial to limit extrinsic effects on results. Volunteers were also requested to standardise their diet in the 24 h preceding each trial and provided a diet diary for the previous 24 h period upon arrival at their first testing session, volunteers replicated this diet prior to subsequent testing sessions.

On the morning of each test, volunteers were provided with a predetermined breakfast providing $10 \text{ g CHO.kg}^{-1} \text{ BM}$ for consumption no less than 60 min prior to

commencing the exercise protocol. Volunteers were asked to present themselves at St Michael's College, Nutley Lane, Dublin, in a rested, well-hydrated state.

Before undertaking the initial RT exercise protocol during session 1, height, BM and skinfold thickness data were assessed, during subsequent test sessions only BM data were re-assessed. A resting blood sample was then collected before volunteers commenced exercising. Volunteers then performed their initial RT exercise protocol and received 1 mL.kg⁻¹ BM of water at 15 min intervals throughout this exercise period. Collected blood samples were immediately analysed on-line to determine relative concentrations of BGlu and BLA.

On cessation of exercise, a blood sample was immediately (t_0) collected. Within 10 min of completing the initial RT session and after the required blood sample had been collected volunteers received their first bolus of assigned supplement. Further blood samples were collected at 2 h of recovery (t_{120}), and at 4 h of recovery (t_{240}) for analysis of BGlu and BLA concentration during recovery. At 120 min into the recovery period after the third blood sample had been collected, volunteers were administered their second individualised bolus of assigned supplement. Volunteers were administered either a commercially available CHO-PRO nutritional supplement, a standard commercially available carbohydrate-electrolyte sports drink (LCHO) or an iso-caloric equivalent to CHO-PRO made from the commercially available carbohydrate-electrolyte sports drink enriched with glucose (HCHO). The volume consumed was individualised for BM equating to 7 mL.kg⁻¹ BM. All volunteers were required to ingest the entire volume within a 10 min time period. Volunteers were required to abstain from drinking water during the 240 min recovery phase. During the 240 min recovery phase volunteers remained resting in a separate room either watching television or reading and were provided with air mattresses to rest on. After blood sample collection following the 240 min recovery period volunteers commenced their TTF protocol, in order to assess the extent, if any, of the recovery induced by each investigated beverage.

3.2.9 Resistance training protocol

All RT exercises were performed after sufficient warm-up supervised by a Leinster Rugby strength and conditioning coach. The warm-up involved a series of dynamic,

exercise specific movements with load, lasting approximately 10 min. Volunteers performed an initial RT session consisting of 8 circuits of 5 discrete exercises, namely arm curls (York, Daventry, UK), squat (York, Daventry, UK; see Figures 3.1a and 3.1b), bench press (T-Rex Multisystem, Beijing, China; see Figure 3.2), leg press (Jimsa, Istanbul, Turkey; see Figure 3.3) and hamstring curls (Techno Gym, Gambettola, Italy; see Figure 3.4). Volunteers RM for each exercise was determined several days preceding the first scheduled drinks trial. This was to ensure that relative exercise stress was to the same degree during each trial, thus potentially reducing variability in glycogen restocking during recovery. The prescribed load for the RT exercises was 10-RM (approximately 75% of individual 1-RM). Volunteers exercised in pairs and each pair had 90 s to complete the required ten repetitions at each exercise station. Exercise duration varied according to the exercise involved. However, volunteers performed approximately 30 s of work during each 90 s interval thus performing approximately 20 min exercising under 10-RM load.

3.2.10 Resistance training test to failure

On completion of the 240 min recovery/supplementation phase, after the fourth blood sample had been collected, volunteers commenced their TTF protocol performing as many circuits of the 5 exercises at 10-RM to failure using the same sequence of exercises employed in the earlier RT exercise phase. This trial was terminated when volunteers failed to complete the required ten repetitions during any three exercises within one circuit. Participants performed a dynamic warm-up supervised by a strength and conditioning coach prior to commencing their TTF protocol. Water was made available for volunteers to drink *ad-libitum* and individual volumes consumed were recorded. A blood sample was also collected immediately upon completion of the TTF protocol. The $\sum W$ for individual exercises and TWC during the TTF protocol under each condition, namely CHO-PRO, LCHO and HCHO were recorded. Both TWC and $\sum W$ were normalised for individual BM and expressed in kg.kg^{-1} BM.

Figure 3.1a: Back Squat (start)



Figure 3.1b: Back Squat (end)



Figure 3.1a-b: Picture illustrating start position (3.1a) end position (3.1b) of back squat resistance training exercise.

Figure 3.2: Bench press



Figure 3.2: Picture illustrating start position for bench press resistance exercise.

Figure 3.3: Leg press



Figure 3.3: Picture illustrating the resistance training exercise leg press.

Figure 3.4: Hamstring curls



Figure 3.4: Picture illustrating the hamstring curls resistance exercise.

3.2.11 Sports drinks

During each single-blinded trial, volunteers ingested one of three sports drinks, CHO-PRO, LCHO or HCHO. Volunteers received 2 individualised volumes of beverage at pre-determined times equivalent to $7 \text{ mL.kg}^{-1} \text{ BM}$. Drinks were consumed within 10 min of cessation of the initial RT and again at 120 min after cessation of the initial RT phase. All drinks were ingested within 10 min, and

despite not being matched for taste were administered in non-transparent vessels. HCHO and LCHO consisted of carbohydrate-electrolyte based sports drink (Club Energise Sport, Britvic, Dublin, Ireland). It was not possible to conduct a double-blinded trial due to the drink flavours and colours. LCHO comprised of the commercially available carbohydrate-electrolyte sports drink and provided 6.2 g CHO.100 mL⁻¹. HCHO consisted of the carbohydrate-electrolyte sports drink with added glucose to provide 8.2 g CHO.100 mL⁻¹. CHO-PRO consisted of a commercially available CHO and PRO based sports drink (Club Energise Sport Recovery 20, Britvic, Dublin, Ireland) and provided 6.2 g CHO.100 mL⁻¹ and 3.1 g PRO.100 mL⁻¹. HCHO and CHO-PRO were volumetrically and iso-calorically equivalent while LCHO and CHO-PRO were equivalent for volume and CHO content.

3.2.12 Statistical analysis

Physical characteristics are presented as mean $\pm$ standard deviation (SD) and all graphical data as mean $\pm$ standard error of the mean (SEM). TWC across trial arm was analysed using a single factor, repeated measures ANOVA with significance quantified using *post-hoc* Student-Neuman-Keuls pair-wise comparisons using InStat (GraphPad Prism, LaJolla, USA). Mean normalised $\sum W$ for individual exercises across trial arm were also analysed using a single factor, repeated measures ANOVA with *post-hoc* Student-Neuman-Keuls comparisons where appropriate. Statistical analysis of BGlu and BLa data across time and intervention (time x drink) was performed using a 2-factor repeated measures ANOVA with *post-hoc* Tukey tests to quantify identified significant differences using SigmaStat (SigmaStat, SanJose, USA). For all statistical analyses, the α level to infer significance was set at $P < 0.05$.

Chapter 3

3.3 Results

3.3.1 Volunteers

Of the 18 volunteers enlisted to commence this study, only 14 completed all 3 drinks trials (n = 14). Four volunteers were forced to withdraw due to injury, attrition and unscheduled IRFU representative commitments. Physical characteristics are displayed in Table 3.1.

Volunteer	Age (yr)	Body Mass (kg)	Height (m)	% Body Fat	Playing Position
1	18	109.1	1.93	22.2	Second Row
2	19	84.9	1.70	19.2	Flyhalf
3	19	91.1	1.88	13.8	Centre/Wing
4	19	87.6	1.80	13.0	Back Row
5	18	96.2	1.85	17.5	Back Row
6	18	89.2	1.75	24.1	Wing
7	21	107.2	1.85	22.5	Prop
8	20	107.1	1.84	19.5	Prop
9	19	96.0	1.91	14.5	Second Row
10	19	91.2	1.84	13.0	Back Row
11	19	90.1	1.75	19.5	Hooker
12	23	85.7	1.74	15.0	Flyhalf/Centre
13	19	84.8	1.77	9.5	Wing
14	18	107.0	1.89	15.5	Prop
Mean	19	94.8	1.82	17.1	
SD	1	9.1	0.07	4.3	

Table 3.1: Mean and individual physical characteristics, along with playing position, for volunteers that completed all three supplementation trials, ± SD, n = 14.

3.3.2 Blood lactate data

Mean BLa data in mmol.L⁻¹ revealed a significant time effect ($P<0.001$), but no significant drink effect ($P>0.05$) and there was no time by drink interaction. Mean BLa data, see Figure 3.5, were as expected significantly lower at resting time points; pre-exercise, mid-recovery phase (t_{120}) and pre-TTF (t_{240}), mean data are presented in Appendix III. There was no significant drink effect manifesting for BLa and no significant time by trial interaction was detected. There was, however, a significant time effect. Mean BLa data across all trials were significantly higher following both exercise phases, post-RT and post-TTF, respectively, compared to all resting time points ($P<0.001$). It was also noted, that BLa data at failure (post-TTF) were significantly higher ($P<0.05$) than data recorded after the initial RT phase (post-RT).

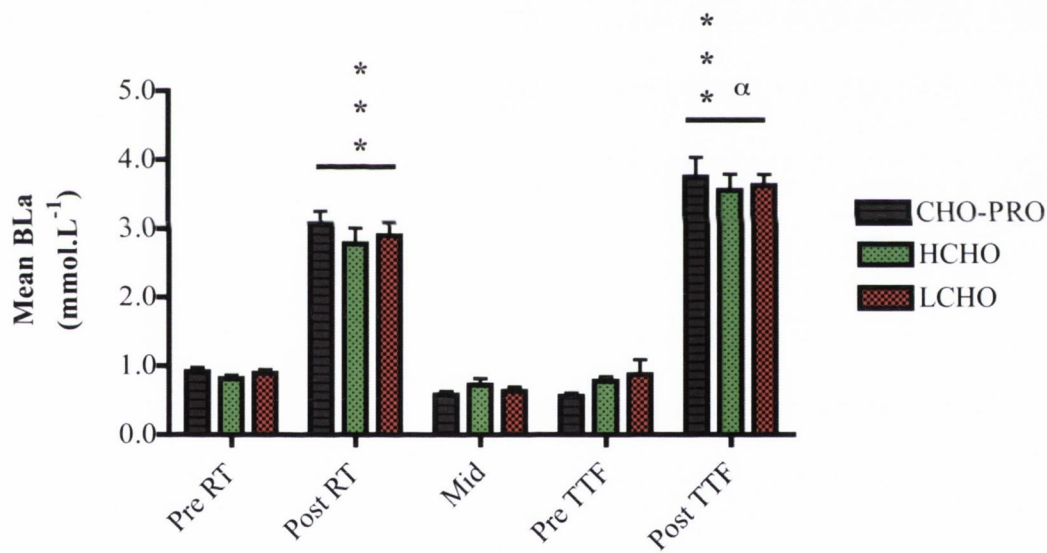


Figure 3.5: Bar graph illustrating changes in mean BLa concentrations (mmol.L⁻¹) across measured time points and drink trials, bars denote SEM, n = 14. α $P<0.05$ vs. post-RT. * * * $P<0.001$ vs. resting time points (Pre RT, Mid & Pre TTF).

3.3.3 Blood glucose data

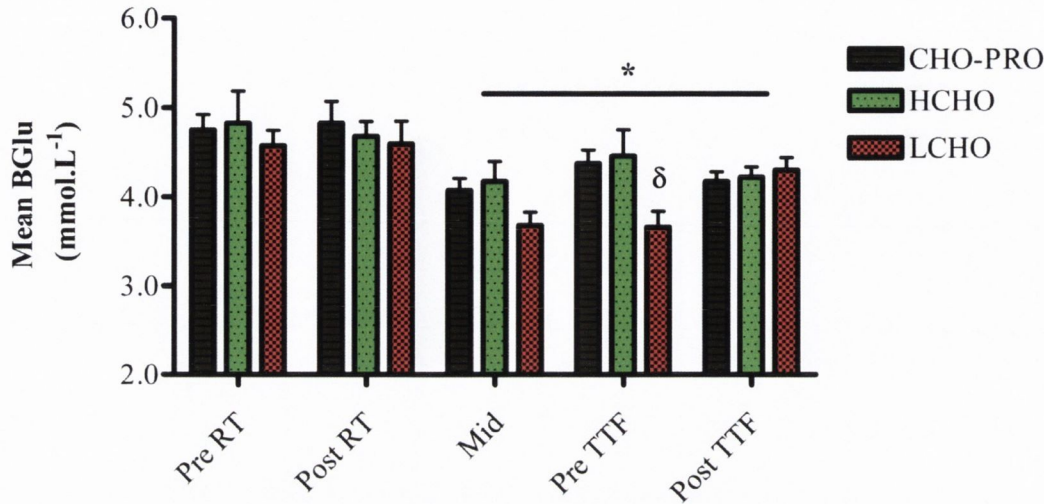


Figure 3.6: Bar graph illustrating changes in mean BGlu concentrations (mmol.L⁻¹) across measured time points and drink trials, bars denote SEM, n = 14. δ $P < 0.05$ vs. HCHO and CHO-PRO at Pre-TTF. * $P < 0.05$, vs. pre- and post-RT across all drinks trials.

Analysis of BGlu data revealed a significant time effect ($P < 0.001$) and a significant drink effect ($P < 0.05$), however, no time by drink interaction was detected. Mean BGlu data were significantly lower at all measurement points following the initial RT phase ($P < 0.05$; Figure 3.6), mean data are presented in Appendix III. Mean BGlu remained steady both before and after the initial RT phase with no significant differences detected between trial days. Mean BGlu at the midpoint (t_{120}) of the 4 h recovery-supplementation phase were not significantly different despite a trend for increased BGlu concentrations for HCHO and CHO-PRO, respectively, compared with the LCHO trial (4.4 ± 0.2 , 4.1 ± 0.1 and 3.8 ± 0.1 mmol.L⁻¹ for HCHO, CHO-PRO and LCHO, respectively). Mean BGlu recorded at the end of the 4 h recovery-supplementation phase (t_{240}), prior to commencing the TTF protocol, were significantly lower during the LCHO trial when compared with HCHO and CHO-PRO trials, respectively (4.1 ± 0.2 vs. 4.6 ± 0.3 and 4.4 ± 0.2 mmol.L⁻¹, respectively; $P < 0.05$). At this time point, HCHO and CHO PRO were not significantly different. On completion of the TTF protocol, BGlu data for all three trials were similar (4.2 ± 0.1 , 4.4 ± 0.1 and 4.2 ± 0.1 mmol.L⁻¹ for CHO-PRO, LCHO and HCHO, respectively).

3.3.4 Total work capacity

Despite large intra-subject variability between trials, TWC, when normalised for body mass ($\text{kg.kg}^{-1} \text{ BM}$), was significantly greater following CHO-PRO ingestion when compared with HCHO and LCHO ($P<0.05$). The greatest mean TWC was recorded post-ingestion of CHO-PRO, with decreasing TWC recorded following HCHO and LCHO supplementation, see Figure 3.7 (188 ± 26 vs. 157 ± 21 and $150 \pm 16 \text{ kg.kg}^{-1} \text{ BM}$, respectively). Mean TWC following ingestion of HCHO and LCHO drinks were not significantly different despite their differing CHO and caloric contents. Mean TWC data are presented in Appendix III.

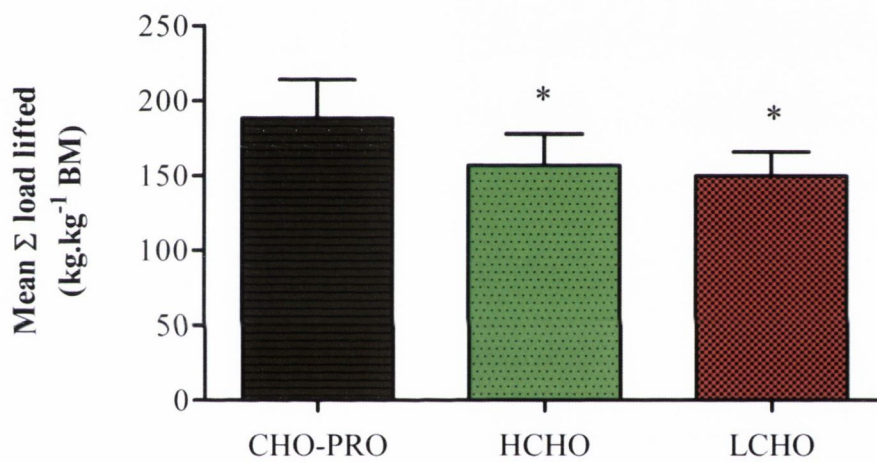


Figure 3.7: Mean TWC ($\text{kg.kg}^{-1} \text{ BM}$) during the TTF protocol across drink trials, bar denote SEM $n = 14$. * $P<0.05$ vs. CHO-PRO.

3.3.5 Individual exercise performance

Individual exercise performance varied greatly from subject-to-subject and also following each drink trial. Mean ΣW data normalised for BM from four out of the five exercises performed demonstrated the trend that the ΣW was greatest following ingestion of CHO-PRO and HCHO when compared with LCHO, respectively (see Table 7, Appendix III). Leg curl was the exception where the ΣW was greater after ingestion of CHO-PRO and decreased for LCHO and HCHO, respectively. Mean data are presented in Appendix III.

3.3.5.1 Squat

Mean ΣW (kg.kg⁻¹ BM) for squat was significantly greater after CHO-PRO ingestion when compared with HCHO and LCHO, respectively (*P*<0.05). The greatest mean ΣW was recorded post-ingestion of CHO-PRO, with a decreasing amount of work performed following HCHO and LCHO supplementation, respectively (56.9 ± 6.1 vs. 47.1 ± 5.3, 45.7 ± 3.9 kg.kg⁻¹ BM , respectively). Figure 3.8 illustrates mean ΣW across drink for squat. Both HCHO and LCHO drink trials manifested no difference in load despite the differing CHO content.

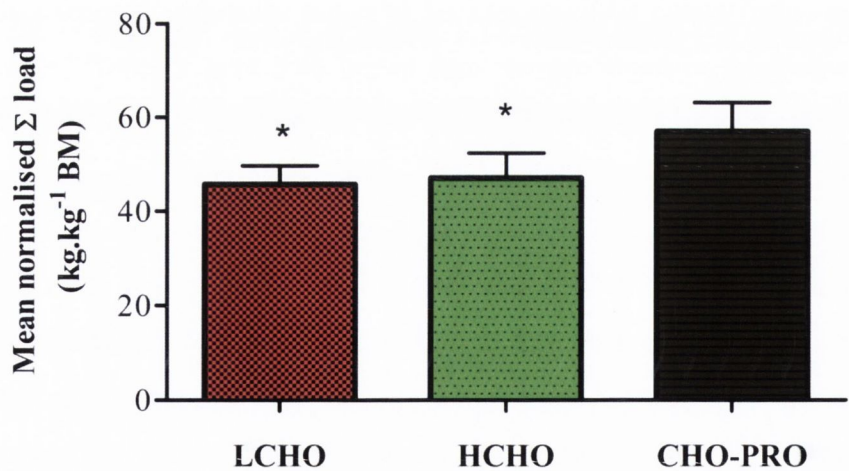


Figure 3.8: Bar graph illustrating mean ΣW for squat (kg.kg⁻¹ BM) across drinks, bars denote SEM, n = 14. * *P* < 0.05 vs. CHO-PRO.

3.3.5.2 Bench Press

The greatest mean ΣW ($\text{kg} \cdot \text{kg}^{-1}$ BM) for bench press was performed post-ingestion of CHO-PRO, with a decreasing amount of work performed following HCHO and LCHO supplementation, respectively (36.0 ± 4.8 vs. 30.8 ± 4.7 and 28.7 ± 3.5 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively). However, there were no significant differences detected across drinks. Figure 3.9 illustrates mean normalised ΣW across drink for bench press.

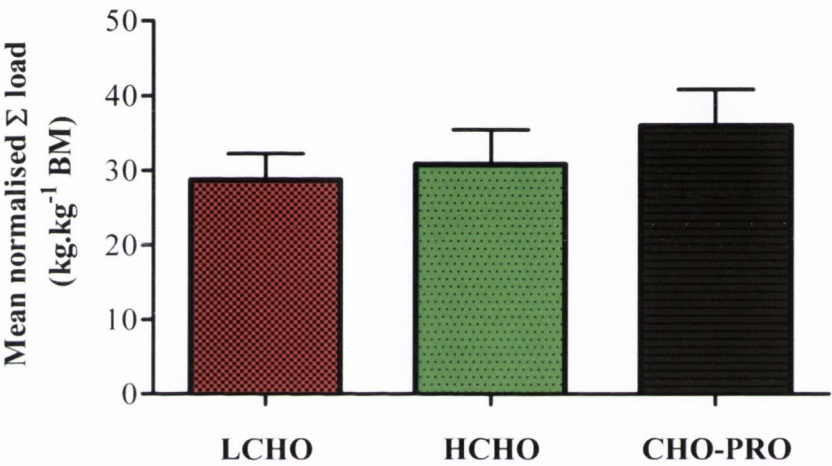


Figure 3.9: Bar graph illustrating mean ΣW for bench press ($\text{kg} \cdot \text{kg}^{-1}$ BM) across drinks, bars denote SEM, $n = 14$.

3.3.5.3 Leg Press

Mean normalised ΣW ($\text{kg} \cdot \text{kg}^{-1}$ BM) for leg press across drinks was not significantly different, despite a strong trend for increased load for CHO-PRO when compared with HCHO and LCHO. The greatest mean ΣW for leg press was performed post-ingestion of CHO-PRO compared with HCHO and LCHO, respectively (57.6 ± 7.3 vs. 48.3 ± 6.5 and 45.2 ± 4.6 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively). Figure 3.10 illustrates mean normalised ΣW across drink for leg press.

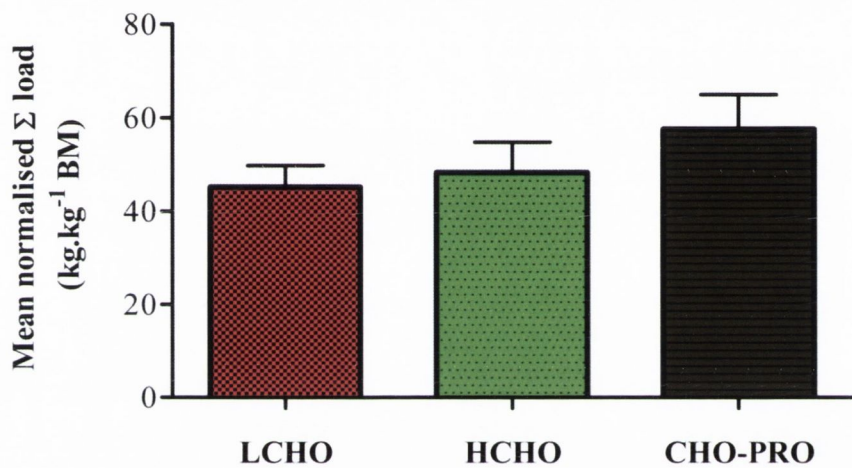


Figure 3.10: Bar graph illustrating mean ΣW for leg press ($\text{kg} \cdot \text{kg}^{-1}$ BM) across drinks, bars denote SEM, $n = 14$.

3.3.5.4 Leg Curl

Mean normalised ΣW ($\text{kg} \cdot \text{kg}^{-1}$ BM) for leg curl was significantly greater for CHO-PRO ingestion when compared with LCHO and HCHO, respectively ($P < 0.05$). Leg curl was the only exercise where the load lifted after LCHO was higher than that after HCHO supplementation (16.9 ± 2.0 vs. 16.4 ± 1.6 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively), although this difference was not statistically significant. The greatest mean ΣW was performed post-ingestion of CHO-PRO, with a decreasing amount of work performed following LCHO and HCHO supplementation, respectively (20.6 ± 2.4 vs. 16.9 ± 2.0 and 16.4 ± 1.6 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively). Figure 3.11 illustrates mean normalised ΣW across drink for leg curl.

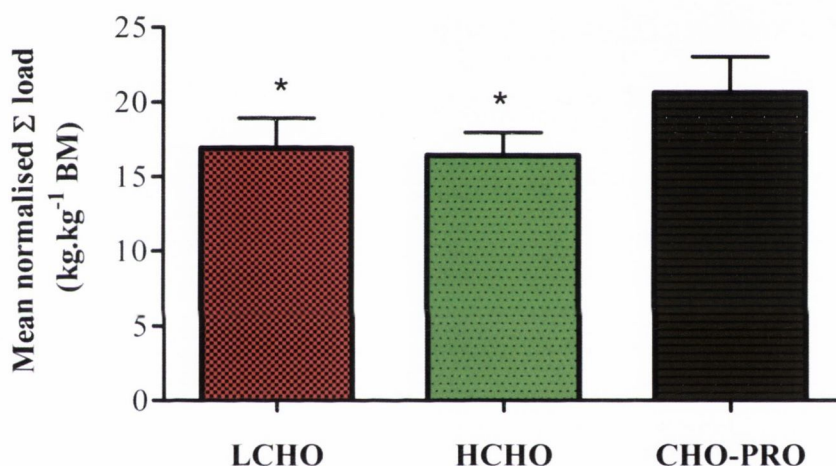


Figure 3.11: Bar graph illustrating mean ΣW for leg curl ($\text{kg} \cdot \text{kg}^{-1}$ BM) across drinks, bars denote SEM, $n = 14$. * $P < 0.05$ vs. CHO-PRO.

3.3.5.5 Bicep Curl

Mean normalised cumulative ΣW ($\text{kg} \cdot \text{kg}^{-1}$ BM) for bicep curl was significantly greater for CHO-PRO ingestion when compared with LCHO (16.8 ± 1.7 vs. 13.1 ± 1.4 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively; $P < 0.05$). There were no significant differences detected between load lifted after ingestion of CHO-PRO and HCHO (16.8 ± 1.7 vs. 14.1 ± 1.5 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively) or HCHO and LCHO (14.1 ± 1.5 vs. 13.1 ± 1.4 $\text{kg} \cdot \text{kg}^{-1}$ BM, respectively). Figure 3.12 illustrates mean normalised ΣW across drink for bicep curl.

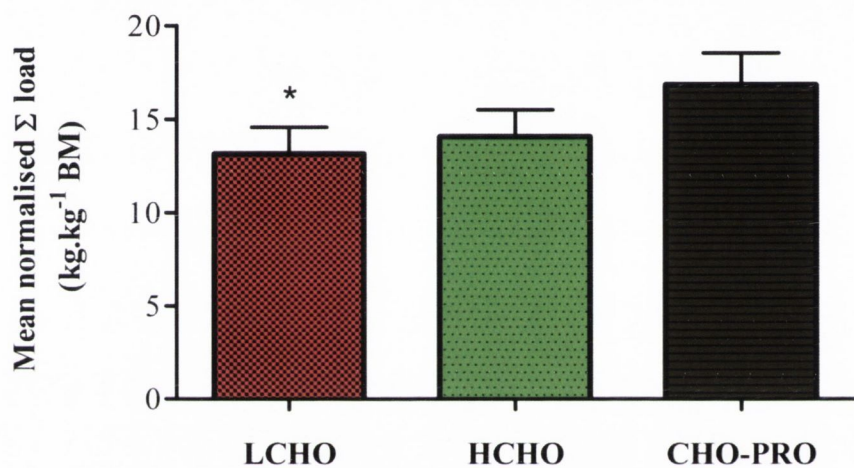


Figure 3.12: Bar graph illustrating mean ΣW for bicep curl ($\text{kg} \cdot \text{kg}^{-1}$ BM) across drinks, bars denote SEM, $n = 14$. * $P < 0.05$ vs. CHO-PRO.

Chapter 3

3.4 Discussion

3.4.1 Summary of findings

In view of the conflicting findings in previous research, this study proposed to evaluate the effect of a CHO-PRO supplement on recovery from a standard RT session and subsequent RT performance, compared to iso-CHO (LCHO) or isocaloric equivalents (HCHO). The hypothesis was that CHO-PRO supplementation would significantly increase time to failure resulting in an increased normalised ΣW (kg.kg^{-1} BM) and TWC (kg.kg^{-1} BM) during the RT regimen performed 240 min following a standard RT protocol designed to induce glycogen depletion when compared with supplements of equivalent CHO (LCHO) and caloric content (HCHO).

Individual performance varied greatly from subject-to-subject and also following each drink trial. Mean ΣW normalised data from 4 out of 5 exercises performed demonstrated the trend that ΣW was greatest following ingestion of CHO-PRO and HCHO, respectively, when compared with LCHO. Despite large intra-subject variability TWC, when normalised for body mass, was significantly greater following CHO-PRO ingestion when compared with HCHO and LCHO (188 ± 26 vs. 157 ± 21 and $150 \pm 16 \text{ kg.kg}^{-1}$ BM, respectively; $P < 0.05$). Also, a trend for improved BGlu was recorded post-ingestion of HCHO and CHO-PRO, respectively, compared with LCHO. However, several factors must be considered regarding exercise to failure, with substrate availability just one component of the intricate physiological response.

3.4.2 Drinks

The benefits of carbohydrate-electrolyte and CHO-PRO sports drinks on performance (Gonzalez-Alonso *et al.*, 1992; Nicholas *et al.*, 1995; Currell *et al.*, 2009; Roberts *et al.*, 2010), restoration of glycogen after intermittent or endurance exercise (Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c; Ivy *et al.*, 2002; Berardi *et al.*, 2006) and on subsequent performance (Betts *et al.*, 2007) have previously been reported. Various supplementation ratios of CHO:PRO have been utilised in the

literature, from 2:1 (Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Berardi *et al.*, 2006), 3:1 (Ivy *et al.*, 2002; Betts *et al.*, 2007) and upwards of 4:1 (Williams *et al.*, 2003) and 6:1 (Van Loon *et al.*, 2000c). A ratio of 2:1 CHO:PRO was administered in the current study. Kerksick *et al.* (2008) recently stated that CHO and PRO ingested at a ratio of 3:1 promoted recovery and replenished muscle glycogen stores, regardless of timing, once ingested regularly. The addition of PRO and AA mixtures to CHO has an insulintropic response (Zawadzki *et al.*, 1992; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b; Van Loon *et al.*, 2000c; Jentjens *et al.*, 2001), with the available glucose being easily absorbed by the tissue during this period of high blood insulin concentrations.

In this study, despite large intra-subject variability between trials, mean TWC (expressed in $\text{kg} \cdot \text{kg}^{-1}$ BM) was significantly greater following CHO-PRO compared with HCHO or LCHO. In addition, differences in mean TWC after ingestion of HCHO or LCHO were not detected despite the differing CHO content, see Figure 3.7. The results of this study are in agreement with previous findings reported by Betts *et al.* (2007). At first glance, the results merit the conclusion that ingestion of the CHO-PRO supplement following exercise enhanced recovery and resulted in greater work capacity when compared with HCHO and LCHO drinks. The lack of significant differences between HCHO and LCHO suggest that CHO and caloric differences were not contributory factors, implying that the PRO component of the CHO-PRO supplement was responsible for the enhanced performance capacity. However, these results are also indicative that CHO-PRO may be more effective for the restoration of exercise capacity than CHO alone and are broadly in line with data previously reported (Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c; Ivy *et al.*, 2002; Williams *et al.*, 2003; Berardi *et al.*, 2006; Betts *et al.*, 2007).

Individual exercise data provided an insight into the similarity between mean ΣW lifted after HCHO and LCHO. Mean ΣW lifted after ingestion of CHO-PRO was significantly higher in 3 of the 5 exercises performed when compared with LCHO (Table 7, Appendix III); no significant differences were detected comparing HCHO and LCHO. One possible explanation for the differing workloads in individual exercises, in particular that observed for leg curl, is the difference in muscle mass of

players due to their playing position specificity. Study participants consisted of 9 forwards and 5 backs (see Table 3.1) with power, strength and endurance in horizontal pushing/wrestling activities reported to be important requisites for forwards and fast running an important requisite for backs (Roberts *et al.*, 2008).

Concentrations of BGlu recorded pre-TTF, after ingestion of drinks at 2 and 4 h previous, were significantly higher during the CHO-PRO and HCHO trial, respectively, when compared with the LCHO trial, see Figure 3.6. Considering there were no significant differences between mean TWC after ingestion of a HCHO and LCHO drink, it is possible that the CHO-PRO drink enhanced recovery through higher rates of glycogen restocking during the recovery/supplementation period and aided subsequent performance via an improved maintenance of BGlu in the post-recovery exercise protocol. Betts *et al.* (2007) stated that the differences in exercise capacity observed between LCHO and HCHO in their study clearly demonstrated that any bias towards a CHO-PRO mixture in terms of CHO content would be expected to prolong exercise capacity irrespective of the additional PRO.

3.4.3 Physiological mechanisms for enhanced subsequent performance

Greater glycogen restoration after ingestion of a combined CHO-PRO supplement has been attributed to the greater plasma insulin response (Zawadzki *et al.*, 1992; Ivy, 1998; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c; Ivy *et al.*, 2002) with the subsequent rise in insulin supposedly accelerating glucose disposal, and a possible increase in glycogen synthase activity accelerating glycogen synthesis (Blom *et al.*, 1987; Ivy, 1998; Jentjens & Jeukendrup, 2003; Kaastra *et al.*, 2006). Many studies report similar insulin responses across supplements with varying BGlu responses (Zawadzki *et al.*, 1992; Tarnopolsky *et al.*, 1997; Ivy *et al.*, 2002; Howarth *et al.*, 2009). Despite reporting similar insulin responses across drinks, Ivy *et al.* (2002) observed significantly lower BGlu concentrations for the first 3 h and at all time points, with the exception of 120 min, after ingestion of HCHO and LCHO drinks during their 4 h recovery period, respectively, when compared with CHO-PRO.

Ivy *et al.* (2002) could not attribute the increased glycogen restocking during the CHO-PRO treatment to a greater plasma insulin response, or differences in

circulating plasma catecholamine concentrations or NEFA. Ivy *et al.* (2002) theorised that the lower plasma glucose and lactate concentrations detected during recovery post CHO-PRO ingestion, possibly indicated increased uptake of plasma glucose and a redistribution of intracellular glucose disposal mediated by the addition of PRO to a CHO supplement. This theory was supported by earlier data from Yaspelkis and Ivy (1999) in which a single AA ingested with a CHO supplement lowered oxidation of glucose despite a tendency for an increasing rate of muscle glycogen storage.

Higher insulin concentrations do not necessarily translate into a higher muscle glycogen resynthesis rate, especially when large volumes of CHO are ingested (Jentjens *et al.*, 2001; Jentjens & Jeukendrup, 2003) and currently not all researchers agree that increasing CHO intake during recovery will facilitate restoration of exercise capacity (Fallowfield & Williams, 1997; Tsintzas *et al.*, 2003). A study by Wong and Williams (2000) reported similar endurance capacity following ingestion of either 0.2 or 0.5 g CHO.kg⁻¹ BM. Howarth *et al.* (2009) also reported no further enhancement of glycogen resynthesis during recovery when PRO or additional CHO was added to a feeding strategy providing 1.2 g CHO.kg⁻¹.h⁻¹. Tsintzas *et al.* (2003) demonstrated that ingestion of a large amount of CHO (175 vs. 50 g) at frequent intervals during recovery from exercise resulted in greater glycogen restoration during recovery but did not affect the rate of muscle glycogen utilisation during subsequent exercise. While supplementation in the present study was infrequent by comparison with Betts *et al.* (2007) and Tsintzas *et al.* (2003), it is possible that this was the mechanism by which HCHO augmented an ergogenic effect over LCHO in the current study and that of Betts *et al.* (2007).

Recent research does not support any effect of CHO-PRO solutions on plasma glucose disposal during recovery (Kaastra *et al.*, 2006). Kaastra *et al.* (2006) investigated the extent to which combined CHO and PRO (casein hydrolysate) ingestion with or without additional free leucine (CHO-PRO-LEU or CHO-PRO, respectively) could increase insulin levels during post-exercise recovery in endurance trained male cyclists. Participants performed 2 h cycling at 55% W_{max}, following which, they ingested 0.8 g.kg⁻¹.h⁻¹ CHO, 0.8, 0.4 and 0.1 g.kg⁻¹.h⁻¹ CHO-PRO-LEU, respectively, or 0.8 and 0.4 g.kg⁻¹.h⁻¹ CHO-PRO, respectively. The

lower plasma glucose response in their CHO-PRO and CHO-PRO-LEU trials can partly be attributed to reduced plasma glucose appearance rates during the early phases of recovery as mean plasma glucose appearance rates were $12 \pm 2\%$ lower when compared with those observed in the CHO trial (Kaastra *et al.*, 2006). This apparent significant reduction in plasma glucose appearance was possibly due to lower gastric emptying and/or intestinal uptake rate of the CHO-PRO and CHO-PRO-LEU mixtures. Kaastra *et al.* (2006) speculated that hepatic glucose output after cessation of exercise was more rapidly reduced in the CHO-PRO and CHO-PRO-LEU trials due to the faster initial rise in plasma insulin concentration.

Kaastra *et al.* (2006) reported that glucose disposal (percent of glucose appearance taken up by the circulation) was identical in all trials, representing $100 \pm 0.03\%$ within 30 min of ingesting the first bolus of test drink in all trials. This implied that the initial rise in plasma glucose concentration was attributed to a less than 30 min delay in glucose disposal before matching the appearance rate of the ingested glucose in the circulation (Kaastra *et al.*, 2006). Kaastra *et al.* (2006) also reported that the effects of the preceding exercise and/or the training status of the subjects resulted in a glucose disposal capacity that exceeded the amount of CHO that was provided and that glucose disposal remained similar during 3.5 h of the post-exercise recovery period.

In the present study, BGlu concentrations were significantly lower during the recovery/supplementation period, mid and pre-TTF, and at failure (post-TTF) compared with pre- and post-RT. Significant differences in BGlu concentrations between drinks were only detected pre-TTF, where BGlu concentration after LCHO ingestion was significantly lower than CHO-PRO and HCHO, respectively, see Figure 3.6. At this time-point volunteers had received their 2 individualised boluses of the drinks at 2 and 4 h previous, yet BGlu concentration after ingestion of LCHO had decreased significantly. This potentially validates the speculation that CHO-PRO ingestion elicited the greatest mean TWC (kg.kg^{-1} BM) through better maintenance of BGlu concentrations, especially when post-TTF BGlu concentrations are considered. During the LCHO trial BGlu concentrations rose after the TTF protocol and were similar to concentrations measured at the same time point in both the HCHO and CHO-PRO trials. Post-TTF BGlu data demonstrated an increase in

circulating glucose during the TTF protocol suggesting that while glucose was readily available for oxidation from the circulation during the CHO-PRO and HCHO trials, the gastrointestinal tract, intra-muscular and possibly even hepatic glycogen stores were providing substrate during the LCHO trial (Betts *et al.*, 2007).

Previously, improved endurance performance during CHO-PRO supplementation was speculated to be due to PRO providing precursors for anaplerotic reactions required to maintain TCA cycle intermediaries in skeletal muscle (Ivy *et al.*, 2002). Elevated concentrations of plasma urea observed during the TTF protocol after CHO-PRO ingestion could indicate increased availability of α -keto acids for gluconeogenesis (Betts *et al.*, 2007). Another possible explanation for the BGlu response after ingestion of CHO-PRO vs. LCHO was that gastric emptying may have been delayed due to the additional energy in the form of PRO (Betts *et al.*, 2007) and/or intestinal uptake rate of CHO-PRO mixtures (Kaastra *et al.*, 2006) leading to exogenous CHO still appearing in the circulation at the start of the TTF protocol (Betts *et al.*, 2007). It is possible that glucose ingested during the HCHO trial may have remained unabsorbed and manifested as high BGlu concentrations throughout the recovery/supplementation period. It must also be noted that BGlu concentration during the LCHO trial measured at 120 min into the recovery/supplementation period were similar to pre-TTF, yet statistical significance was not detected most likely due to the slightly lower BGlu concentration during the CHO-PRO trial at this time-point when compared with the pre-TTF time point.

In comparison to the present study, Van Loon *et al.* (2000a) reported a trend of increasing plasma glucose concentration across all drink trials up to 60 min post-ingestion that experienced a slow decrease thereafter. Van Loon *et al.* (2000a) reported significantly lower plasma glucose responses, expressed as AuC, during their CHO-PRO and CHO-CHO trials compared to CHO alone. Plasma insulin concentrations were significantly elevated up to 2 h post-exercise across trials and reached a plateau thereafter in both the CHO and CHO-CHO trials. During the CHO-PRO trial, however, plasma insulin concentration remained elevated for a further 2 h, and only decreased during the last hour of observation. Van Loon *et al.* (2000a) stated that it was unlikely that the accelerated glycogen synthesis rates observed in the CHO-PRO trials could be attributed to increased gluconeogenic flux

after the ingestion of PRO or AA. This was due to higher insulin concentrations inhibiting gluconeogenesis rather than stimulating it (Van Loon *et al.*, 2000a). Glycogen synthase activity has been described as the rate limiting factor in the glycogen synthetic pathway (Ivy, 1991) therefore, hyperinsulinaemia could accelerate glycogen synthesis by increasing both glucose uptake and glycogen synthase activity in the presence of adequate substrate (Zawadzki *et al.*, 1992). However, Van Loon *et al.* (2000a) reported no significant differences in the percentage of glycogen synthase in its active form at 0 or 5 h of recovery, or between trials. Van Loon *et al.* (2000a) postulated that timing of muscle biopsy sampling may potentially explain this finding because, at 5 h, plasma insulin concentrations in both the CHO-PRO and CHO trials had returned to similar values.

Together the data of Van Hall *et al.* (2000), Van Loon *et al.* (2000a) and Jentjens *et al.* (2001) suggested that the availability of substrate was the rate-limiting factor for glycogen restocking. In this study, mean TWC was not significantly different between CHO-PRO and HCHO, similar to findings reported in endurance running times (Betts *et al.*, 2007). However, the insulinotropic response mediated by the addition of PRO to CHO is not impervious to the upper limit of glucose absorption, approximately 1 g.min⁻¹, through SGLT-1 transporters (Jentjens *et al.*, 2004a). Earlier research by Jentjens *et al.* (2001) observed that when total CHO content was high (1.2 g CHO.kg⁻¹.h⁻¹) the addition of PRO/AA mixture does not result in increased rates of muscle glycogen storage. Later research by Jentjens *et al.* (2004a) speculated that SGLT-1 transporters became saturated at a glucose ingestion rate of 1.0 to 1.2 g.min⁻¹ and therefore, no further increase in exogenous glucose oxidation would be observed when glucose intake was further increased. This could possibly account for our observations between CHO-PRO and HCHO. Drinks in this study were administered according to individual BM, therefore, an individual weighing 100 kg received 700 mL of test drink, providing 0.62 g CHO.kg⁻¹.h⁻¹ for LCHO and CHO-PRO, increasing to 0.93 g CHO.kg⁻¹.h⁻¹ for HCHO.

Another factor of importance relating to glycogen resynthesis rate is the level of depleted glycogen. Berardi *et al.* (2006) stated that during relatively low levels of glycogen depletion (30 to 35 mmol.L⁻¹) CHO feedings alone may be sufficient to stimulate maximal resynthesis rates due to the insulin independence of glycogen

resynthesis and the high physiological drive to resynthesise utilised muscle glycogen (Price *et al.*, 1999) under such conditions. The insulinotropic effects of combined CHO-PRO ingestion may be better suited where glycogen depletion is greater than 30 to 35 mmol.L⁻¹ (Berardi *et al.*, 2006). In the current study, despite the fact that participants were not fasted and, considering the close proximity of their morning meal prior to the beginning of the initial RT exercise phase, glycogen stores may not necessarily have been depleted. However, the ingestion of CHO-PRO in this study clearly presented an advantage for restoration of exercise capacity within this population and exercise modality.

3.4.4 Assessment of recovery and performance effects

Ivy *et al.* (2002) measured BGlu data at more frequent time intervals during their recovery/supplementation period (30 and 60 min) with further BGlu data determinations at 30 min intervals from the 120 to the 240 min time point during their recovery supplementation/phase. Clearly the more frequent measurements allowed for greater assessment of potential physiological response to each sports drink, especially when concomitant measurements of muscle glycogen levels through ¹³C-NMR scans were performed. That said, and considering previous research, a CHO-PRO supplement can increase the rate of muscle glycogen storage during the hours immediately after exercise if the supplement contains a low to moderate amount of CHO (Zawadzki *et al.*, 1992; Van Loon *et al.*, 2000a; Ivy *et al.*, 2002) and also, will restore exercise capacity more completely within 4 h of prior exercise (Betts *et al.*, 2007). Berardi *et al.* (2006) supplemented 1 L boluses at 10, 60 and 120 min during recovery with one solid meal ingested at 240 min. The meal ingested during the CHO trials (7 kCal.kg⁻¹ contained 1.2 g.kg⁻¹ CHO, 0.3 g.kg⁻¹ PRO and 0.1 g.kg⁻¹ fat) provided one third of that ingested during the placebo trials. A more recent study by Betts *et al.* (2007) provided 8 boluses of test drink at 30 min intervals over a 4 h recovery/supplementation period, similar to Van Loon *et al.* (2000a), with blood samples collected at hourly intervals. However, Betts *et al.* (2007) did not determine muscle glycogen data and no blood borne markers of recovery were reported by Berardi *et al.* (2006) despite the use of natural abundance ¹³C-NMR spectroscopy to determine muscle glycogen content.

Exercise intensity varied among studies attempting to assess a performance effect indicative of the enhanced recovery (Berardi *et al.*, 2006; Betts *et al.*, 2007). Participants in the study by Berardi *et al.* (2006) performed a 60 minute ‘best-effort’ cycling bout pre- and post- a 5 h recovery-supplementation period. Betts *et al.* (2007) performed a 90 min run at 70% $\dot{V}O_{2\max}$, a 4 h recovery/supplementation period and a subsequent performance assessment of running time to exhaustion at 70% $\dot{V}O_{2\max}$. Berardi *et al.* (2006) conceded that the glycogen depletion protocol employed in their study was less severe than that utilised in previous studies (Tarnopolsky *et al.*, 1997; Carrithers *et al.*, 2000; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c). This is an important observation as the level of muscle glycogen depletion determines the rate of resynthesis (Zachwieja *et al.*, 1991; Berardi *et al.*, 2006).

3.4.5 Conflicting studies

Research has previously reported no difference between CHO-PRO or CHO ingestion on muscle glycogen restocking following exercise (Carrithers *et al.*, 2000) or from additional CHO supplementation (HCHO) compared to CHO-PRO (Van Hall *et al.*, 2000; Van Loon *et al.*, 2000a; Jentjens *et al.*, 2001). Data from Zawadzki *et al.* (1992), Tarnopolsky *et al.* (1997) and Carrithers *et al.* (2000) reported no significant difference in muscle glycogen stores during the initial hours of recovery with ingestion of LCHO or HCHO. It is important to note that the study by Zawadzki *et al.* (1992) did not use a control beverage and it was argued that the results observed in that study were attributable to the caloric imbalance. Carrithers *et al.* (2000) stated that muscle glycogen restoration did not appear to be altered with CHO-PRO feeding, provided adequate CHO was dispensed, and the addition of PRO or AA to their HCHO feeding did not elicit a synergistic insulin response. Most recently, researchers did not counterbalance drinks for CHO possibly contributing to the 55% greater time to exhaustion after CHO-PRO ingestion (Williams *et al.*, 2003).

There are obvious differences between the present study and the studies of Ivy *et al.* (2002) and Betts *et al.* (2007). This study enlisted trained male academy rugby players as opposed to trained male cyclists or active, endurance trained male runners.

Volunteers in the study of Ivy *et al.* (2002) were fasted and fully glycogen depleted prior to supplementation, as were participants in the studies by Zawadzki *et al.* (1992) and Van Loon *et al.* (2000a). Ivy *et al.* (2002) determined muscle glycogen content using ^{13}C NMR whilst Van Loon *et al.* (2000a) and Zawadzki *et al.* (1992) determined muscle glycogen content using the biopsy technique. Betts *et al.* (2007) assessed a performance outcome of enhanced recovery though CHO-PRO ingestion but did not perform assessment of muscle glycogen restocking. It was not possible to assess muscle glycogen content by either method due to ethical and financial limitations. In addition, blood samples were drawn at different intervals in each study, therefore, data from Ivy *et al.* (2002), Betts *et al.* (2007) and the present study potentially infer differing profiles of glycogen resynthesis and BGlu response for each drink.

Differing study design could also account for the negative findings of Tarnopolsky *et al.* (1997) and Carrithers *et al.* (2000) such as the lower PRO concentrations administered and the frequency of supplementation. Generally, supplements in the studies that reported no significant difference between iso-caloric supplements have administered supplements every 15 to 30 min (Tarnopolsky *et al.*, 1997; Carrithers *et al.*, 2000; Van Loon *et al.*, 2000a; Jentjens *et al.*, 2001). The study designs utilised by Carrithers *et al.* (2000), Van Hall *et al.* (2000) and Jentjens *et al.* (2001) have all used a shorter recovery/supplementation periods (3 h) and increased frequency of supplementation. In particular, Van Hall *et al.* (2000) observed no significant difference between CHO-PRO and HCHO despite ~20% higher glycogen storage rate recorded for CHO-PRO. More frequent administration of supplements with high CHO content may invariably alter the rate of CHO and PRO absorption, possibly limiting the advantage of PRO due to the higher rates of glycogen storage occurring during more frequent feedings (Doyle *et al.*, 1993).

3.4.6 Exercise modality

Betts *et al.* (2007) stated that the mechanism through which CHO-PRO facilitates the restoration of exercise capacity may differ according to the precise mode of exercise that is performed prior to recovery. Betts *et al.* (2007), Berardi *et al.* (2006) and Ivy *et al.* (2002) provided data for exhaustive running and cycling protocols. Data for resistance exercise is limited, and to our knowledge, this study represents the first

evidence of enhanced restoration of exercise capacity in RT. Previous research in this field failed to produce conclusive results in the area of RT (Coyle *et al.*, 2005). Despite a lack of statistical significance, Coyle *et al.* (2005) reported that mean TWC after ingestion of a CHO-PRO supplement was 16, 10 and 2% higher than after ingestion of LCHO, HCHO and CHO-FRU supplements, respectively. It was theorised that had glycogen stores been fully depleted in this earlier study, the difference in CHO uptake due to insulinotropic stimuli and/or substrate uptake pathways may have resulted in significantly enhanced glycogen restoration thus enhancing subsequent performance, as postulated by Ivy *et al.* (2002), Jentjens *et al.* (2004b) and Betts *et al.* (2007).

3.4.7 Fatigue

This discussion so far has focused on factors affecting glycogen restoration, impact of exercise modality on glycogen restoration, differing amounts of CHO and PRO elicit differing post-exercise responses and substrate availability. Clearly an athlete could not continue to exercise at the intensity required for the current experimental protocol for an infinite amount of time. While in the current study exercise duration was relatively low *per se*, exercise intensity was high. The rugby players that participated in this study would typically undertake several RT sessions during their prescribed weekly training schedule, but of a significantly shorter duration (~45 min). During this protocol, it is possible that increased perception of fatigue, possibly originating from the CNS, affected performance and possibly substrate availability. Whether PRO ingestion has an ergogenic affect on the CNS though the Central Fatigue Hypothesis postulated by Ivy *et al.* (2003) is inconclusive, and a relatively unexplored area of research, however, Betts *et al.* (2007) stated that there is a possibility that CHO-PRO ingestion may improve the central drive for exercise in certain individuals. Ivy *et al.* (2003) stated possible reasons for this performance difference between CHO-PRO and CHO including the Central Fatigue Hypothesis and that the PRO provided precursors for the anaplerotic reactions required to maintain TCA intermediaries in skeletal muscle. Previous to the observation of Ivy *et al.* (2003), Mittleman *et al.* (1998) reported a modest performance improvement with supplementation of branched-chain AA.

3.4.8 Recommendations

Further study is warranted in this area to determine the influence of peripheral and central factors of fatigue on the enhanced subsequent performance which was possibly mediated by an enhanced recovery after ingestion of a CHO-PRO drink. Determination of glycogen utilised and glycogen restored would be particularly helpful in quantifying the true glycogen restoration effect of the drinks. These data, with additional analyses of blood borne markers such as insulin, urea, glycerol and NEFA may allow a more complete picture of the peripheral and consequently, central factors limiting performance at high intensities and the fate of the constituents of the drinks at a cellular level. Such data would then allow athletes to conclude whether these so-called beverages truly warrant the name recovery drinks.

3.4.9 Conclusion

Ingestion of a CHO-PRO supplement following resistance exercise enhanced recovery and resulted in a greater work capacity during subsequent resistance exercise when compared with LCHO and HCHO supplements, respectively. This CHO-PRO induced enhancement of recovery was possibly due to higher rates of glycogen restoration after an initial glycogen depleting exercise protocol and better maintenance of BGlu concentrations as outlined by Ivy *et al.* (2002) and, more recently, Betts *et al.* (2007).

Chapter 4

Salivary cortisol and testosterone response to soccer training and competition: Comparison with rowing and rugby union.

Chapter 4

4.1 Introduction

4.1.1 Training and hormonal responses

To improve physical performance, athletes often use periods of heavy physical stress followed by a reduction in stress level to achieve specific adaptations at the cellular level (Jürimäe *et al.*, 2011). At all levels of participation in sport, recovery from training and competition is of paramount importance considering that the onset of OR has been reported when athletes experience even a slight increase in normal training load (Halsen *et al.*, 2002; Coutts *et al.*, 2007). An inability to tolerate training load and the accompanying temporary post-exercise immunosuppression can significantly detract from performances in both training and competition (Meeusen *et al.*, 2004). Previous research has described various neuromuscular and endocrine responses to training and competition (Bloom *et al.*, 1976; Bishop *et al.*, 1999a; Lac & Berthon, 2000; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Maso *et al.*, 2004; Meeusen *et al.*, 2004; Mäestu *et al.*, 2005; Coutts *et al.*, 2007; Ispirlidis *et al.*, 2008; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). Both C and T concentrations have been widely investigated as indicators of the stress response to both training and competition due to their high sensitivity to exercise (Urhausen *et al.*, 1998; Lac & Berthon, 2000; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Maso *et al.*, 2004; Meeusen *et al.*, 2004; Mäestu *et al.*, 2005; Coutts *et al.*, 2007; Cormack *et al.*, 2008; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010). Both C and T support anabolic and catabolic responses to exercise, and in particular, C plays a pivotal role in BGlu homeostasis which in turn impacts upon glycogen restoration.

4.1.2 Cortisol and testosterone response

Significant changes in T/C ratio have been reported after only a slight increase in training load in rugby league players (Coutts *et al.*, 2007) and with pre-season training loads in both soccer players and rowers (Filaire *et al.*, 2001; Mäestu *et al.*, 2005). In soccer players, hormonal responses to simulated and competitive soccer match-play, and, during the high-load pre-season period and reduced load competitive season vary greatly (Bishop *et al.*, 1999a; Filaire *et al.*, 2001; Ispirlidis *et al.*, 2008; Moreira *et al.*, 2009). Data from Filaire *et al.* (2001) demonstrated that 9 months of training and competition had no significant effect on the C response.

However, an acute increase in C concentration, leukocyte count and inflammatory cytokines has been reported post-match (Ispirlidis *et al.*, 2008) along with significant glycogen depletion. Krstrup *et al.* (2006a) reported that approximately 36% of the individual muscle fibres were almost depleted with an additional ~11% completely depleted of glycogen. Such a significant disturbance in homeostasis may result in increased C secretion to stimulate gluconeogenesis for maintenance of BGlu concentrations (Brooks *et al.*, 2005).

Elloumi *et al.* (2003) and Cunniffe *et al.* (2010) both reported significantly elevated C responses with accompanying decreased T responses following a competitive rugby match. Such changes in hormonal markers of stress can remain elevated or suppressed from 2 to 5 days, most likely due to the training status of the athlete (Bloom *et al.*, 1976; Elloumi *et al.*, 2003; Hackney *et al.*, 2005; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). These data suggested that soccer match-play and competitive rugby union may have a significant impact on the player's immunocompetence and ability to train in the days following competition.

Mäestu *et al.* (2005) reported that C concentration was not significantly different at rest after a 3 week high-load training period and 2 week tapering period in rowers when compared with rest. However, the 3 week high-load and 2 week tapering period had a significant negative effect on the free T response and the free T/C ratio when compared to rest (Mäestu *et al.*, 2005). In addition, the bound T response was blunted after the 3 week high-load training period and remained blunted after the 2 week tapering period. A maximal 2000m performance test completed after each training period had no effect on the C response assessed immediately or 30 min post-2000m maximal rowing test. However, the free T/C ratio was significantly increased in the immediate aftermath of the performance test after each training period but was significantly decreased when assessed 30 min later.

Differences have also been reported between the hormonal responses to competition and training (Bishop *et al.*, 1999a; Elloumi *et al.*, 2003; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). Perception of threat and opponent, interactions with other players, playing venue and significance of the event have also been reported as

potential effectors of C and T response in soccer players (Suay *et al.*, 1999; Neave & Wolfson, 2003; Moreira *et al.*, 2009).

4.1.3 Aims and hypothesis

With consideration for the research outlining the physiological, hormonal and immunological responses to functional-OR training and competition scenarios, and the previously stated necessity to perform functional-OR investigative studies (Halsen & Jeukendrup, 2004), this study proposed to evaluate the hormonal response to in-season training and competition scenarios across three sporting disciplines of varying demands, i.e. soccer, rugby and rowing.

The primary hypothesis was that the competition scenario would elicit significantly greater hormonal changes compared to the training scenario in all sporting disciplines. The secondary hypothesis was that the hormonal changes would be significantly greater in rugby union players than soccer players or rowers as previous data suggested that the nature of rugby union caused longer term disturbances to host immunity and greater disturbance to both C and T concentrations (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010).

Chapter 4

4.2 Methods

4.2.1 Study Design

A longitudinal observational study design was used to quantify training and competition induced changes of hormonal markers of stress in soccer players. Saliva samples were collected before and after two measured and replicated in-season soccer training sessions. Training sessions were monitored by on-line HR telemetry. Saliva samples were also collected before and after two scheduled League of Ireland First Division fixtures. The study design and procedures involved in this study were approved by the Faculty Research Ethics Group, Trinity College Dublin.

4.2.2 Participants

Twenty three ($n = 23$) trained first division League of Ireland soccer players were recruited as participants for this observational study. Of the 23 soccer players recruited, 12 completed the required sample collection and monitored training period ($n = 12$). All participants were recruited through contacts made with Sporting Fingal Football Clubs' Director of Football. All participants were contracted with Sporting Fingal Football Club and were members of the playing panel for the 2009 League of Ireland First Division season. All participants had been playing North Dublin Schoolboy League, League of Ireland Premier or First Division, English Football League 1 or, in one case, Barclays Premiership the previous season and some players had national underage representative honours. The training sessions undertaken by the players were their standard squad training sessions conducted and supervised by Sporting Fingal Football Clubs' Head Coach. All participants were familiar with the drills, activities and exercise intensities used in monitored training sessions. Participants were provided with a study information sheet and gave informed written consent 7 days after receiving study information leaflets and, at least 7 days prior to beginning the test. Testing was performed during the mid-season training cycle for the players.

The data presented for rugby players and rowers was collated from a previous collaborative study performed in the Human Performance Laboratory, Trinity College Dublin (Denvir, 2009). The data were collected in an ethically approved

longitudinal observational study assessing training and competition induced changes in hormonal markers of stress and recovery. Fourteen rowers and 16 rugby players were recruited as volunteers. Of these 30 initial volunteers recruited, 21 individuals participated in the required data collection protocol. Participants were novice collegiate members of Dublin University Boat Club ($n = 9$) and 1st team members of Naas RFC U20's squad ($n = 12$). Saliva samples were collected pre- and post-training and competition on a week-on-week-off basis over an eight week period. All testing and data collection took place between March and June 2008.

4.2.2.1 Inclusion and exclusion criteria

All soccer players were male and aged between 18 and 32 years. All soccer players were medically screened prior to the first testing session by a Sporting Fingal Football Club medical practitioner. All rowers and rugby players were male and aged between 18 and 35 years. All rowers and rugby players were medically screened by a medical practitioner prior to the first sample collection training session. Individuals suffering from any musculoskeletal injury that had prevented participation in training within the sample collection period or, deemed unfit to participate by the medical practitioner on completion of a medical examination were excluded from sample collection. Participants with diabetes, hypertension, heart defects, any metabolic disorders, chronic sports injury or any other contra-indicatory symptoms were excluded from sample collection completely. Participants who were suffering from minor sporting injuries were allowed to participate and performed exercises within the limits of their injuries and saliva samples were collected accordingly.

4.2.3 Experimental protocol

Fitness assessment and estimation of $\dot{V}O_2\text{max}$ in soccer players was determined using the YoYo intermittent recovery test: Level 2 (YoYo IR2), Hoff test and 12 min Cooper run prior to the data collection period to determine neuromuscular efficiency, soccer specific $\dot{V}O_2\text{max}$ and predicted $\dot{V}O_2\text{max}$, respectively. Saliva samples were collected pre- and post- standard training sessions and pre- and post- League of Ireland Division 1 fixtures. All fitness assessment and training sessions were

performed at ALSAA, Old Airport Road, Co. Dublin, the appointed training venue of Sporting Fingal Football Club.

4.2.3.1 Monitored training sessions

Soccer players participated in the monitored training sessions on two separate occasions. The Day 1 training session during the standard in-season training week was selected as it was deemed to be the more vigorous, aerobic based session and also had the best scope for reproducibility. Each training session lasted approximately 90 min. Participants were requested to standardise both fluid intake and diet in the preceding 24 h of each monitored training session or match and to replicate this prior to subsequent monitored training sessions and matches. The training regimen was also standardised, in agreement with Sporting Fingal Football Club management, across data collection weeks to limit extrinsic effects on results.

Players assembled on the morning of each monitored training session and provided a saliva sample in a labelled eppendorf tube. Players were provided with a HR monitor, as described in Section 2.5, which was worn for the duration of the training session to allow quantification of the exercise intensities. Participants then participated in the training session as normal. On cessation of exercise, the second post-training saliva sample was collected in a second labelled eppendorf tube. Saliva samples pre- and post-training were collected between 10:00 and 12:30.

The training session was standardised and consisted of ~20 min warm-up, dynamic in nature and involving the ball, ~30 min of skill based 'grids' emphasising ball control and possession, with ~40 min small-sided games (7 vs. 7, 6 vs. 6, 5 vs. 5) played on half pitch for intervals of 6 to 8 min with a variable 1 to 2 min rest period between each game. Both monitored training sessions concluded with a period of low-intensity exercise and static stretching for 5 to 10 min. The HRavg during the standardised training session equated to 81% HRmax.

4.2.3.2 Match day sampling

One home and one away League of Ireland First Division fixture was monitored for salivary hormone changes. Sampling time pre- and post-match varied according to kick-off time, venue of the fixture, and management's request. Samples at the home

fixture were collected between 18:45 and 22:00 in Morton Stadium, Santry, Co. Dublin. Away fixture samples were collected at 17:00 and 22:00 on the team bus. Match day saliva samples were collected within 60 or 180 min pre- and 30 min post-match.

4.2.3.3 Rowers and rugby players

All participants of the rowing and rugby groups were submitted to the same respective training regime throughout the sporting season. Rugby players completed 3-4 hr.week⁻¹ training and participated in one competitive match per week. The rowers completed 6-8 hr.week⁻¹ training and one regatta every 6-8 weeks. Saliva samples were collected on a Tuesday (training session 1) and Thursday (training session 2) between 19:00 and 21:00 for both groups on a week on week off basis over the 8 week collection period. Unlike the current study, the training sessions conducted as part of the study by Denvir, (2009) were neither standardised nor monitored for intensity. For rugby players, training session 1 was longer and more intense than training session 2, which served as team preparation for the impending league fixture. For the rowers, training session 1 consisted of a sub-maximal fitness test (timed 5000 m or incremental test), which then dictated the intensity of training session 2. Rugby players participated in 3 competitive matches during this period, all home fixtures, played between 14:00 and 16:00 and saliva samples were collected after the team concluded their warm-up, 10 minutes prior to kick-off (pre-) and within 30 min post-match. Rowing competition samples were collected during the Dublin University Regatta at approximately 10:00, 14:00 and 16:00. Participants competed in head-to-head 1.5 km races lasting approximately 3 min 40 s and saliva samples were collected pre- and post-race.

4.2.4 Anthropometric assessment

Anthropometric variables, namely; height, body mass, percentage body fat and BMI were assessed as outlined in Section 2.1.

4.2.5 Fitness assessment and estimation of $\dot{V}O_{2\max}$ in soccer players

The YoYo IR2 test, a soccer specific field test, was proven to correlate to countermovement jump peak power and peak treadmill speed (Castagna *et al.*,

2006). These correlations suggested that the YoYo IR2 test was influenced by neuromuscular efficiency (explosive power level) and suggested that the player's level of maximal muscular power, expressed during fast stretch-shortening actions, affected performance during high-intensity intermittent exercise (Castagna *et al.*, 2006).

Grant *et al.* (1995) reported that Cooper run score had a correlation coefficient of 0.92 with a treadmill $\dot{V}O_2\text{max}$ test in young healthy males. However, earlier research had suggested that the Cooper run underestimated $\dot{V}O_2\text{max}$ (McCutcheon *et al.*, 1990). For this reason, and to determine $\dot{V}O_2\text{max}$ with a soccer specific test, participants performed the Hoff test. Hoff *et al.* (2002) stated that the $\dot{V}O_2$ to HRmax correlation in the Hoff test was not significantly different to that of treadmill running ($r = 0.84$). The $\dot{V}O_2\text{max}$ data reported by Hoff *et al.* (2002) cannot be generalised to all soccer players as the data was not representative of a whole team. However, more recent research by Chamari *et al.* (2005) demonstrated a significant correlation between laboratory $\dot{V}O_2\text{max}$ determination and performance in the Hoff test ($r = 0.68$) in elite youth players. As with the Cooper run, the age variation, status and number of subjects, and the homogeneity or otherwise of the group must be considered when comparison with other groups is made (Grant *et al.*, 1995).

The inclusion of these physiological tests were to allow comparison of this population with previously reported data, as well as present information regarding participants physical fitness considering the potential ramifications for tolerance to training load, and consequently, T/C ratio.

4.2.5.1 Cooper run

Soccer players completed a 12 min Cooper run on an outdoor 8 lane, 400m athletic track (ALSAA, Old Airport Road, Co. Dublin) as a means of estimating $\dot{V}O_2\text{max}$. The athletic track was marked at 20m intervals using an odometer (Irion & Vosseler, Schwenningen, Germany) and coloured marker cones. Participants were instructed to cover the greatest distance possible within the 12 min period. All testing took place after a full soccer specific dynamic warm-up was completed. All participants were familiarised with the protocol of the 12 min Cooper run by completing a

familiarisation test during a previous training session. Participants began the 12 min run in one group, a whistle signalled the start of the 12 min period. A vocal warning was given at 11 min and the whistle was blown to signal the end of the 12 min period. On hearing the final whistle, participants remained stationary in order for the distance covered in the final lap, to the nearest 10m, to be determined. The final distance covered (m) was recorded and represented the test score.

Each participant's score was later used to predict their estimated $\dot{V}O_2\text{max}$ using Equation 1 (Cooper, 1968).

$$\text{Equation 1: Predicted } \dot{V}O_2\text{max} = (\text{Distance (m)} - 504.9) / 44.73$$

4.2.5.2 YoYo intermittent recovery test: Level 2

Soccer players performed a YoYo IR2 test to determine neuromuscular efficiency (explosive power level). The YoYo IR2 consisted of a repeated 2 by 20 m shuttle run at a progressively increasing velocity controlled by audio signals from a CD player. Each participant completed a YoYo IR2 test as described by Krstrup *et al.* (2006b). The test was performed on an outdoor 8 lane 400 m running track marked with cones along the 6 middle lanes of the straight, see Figure 4.1. Cones were placed along the lanes of the track at a length of 20 m. An additional cone was placed 5 m behind the starting/finishing line to mark the running distance during the active recovery period. An odometer (Irion & Vosseler, Schwenningen, Germany) and tape measure (Stanley, Sheffield, UK) were used to determine distances between cones. All testing took place after a full soccer specific dynamic warm-up was completed. All participants were familiarised with the procedure of the YoYo IR2 test as they had performed the test during a previous training session.

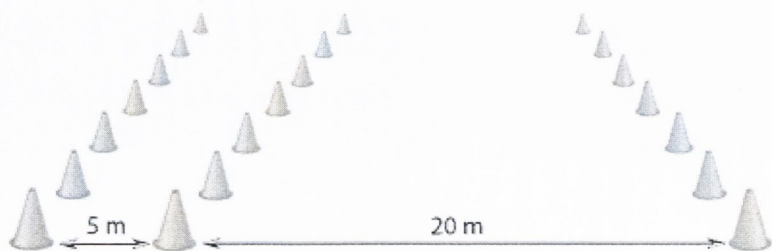


Figure 4.1: YoYo IR2 layout and distances of cones (Bangsbo, 2007).

Participants started the test with their feet on or behind the starting/finishing line and began running 20 m when instructed by the CD. Participants turned and returned to the starting point when signalled by the recorded signal on the CD, covering 40 m per bout. Between each running bout individuals had a 10 s period of active recovery consisting of 2 by 5 m of jogging. When participants had failed to reach a cone on, or, before a signal, they received a verbal warning. When participants had twice failed to reach a cone in time with a signal, the participant was withdrawn from the test. The final distance covered was recorded and represented their test result.

4.2.5.3 Hoff test

The soccer specific Hoff test was performed as described by Chamari *et al.* (2005). The Hoff test was performed on a sand based Astroturf pitch as this allowed for the dimensions of the Hoff track to be accommodated and eliminated differences in under foot conditions. All testing took place after a full soccer specific dynamic warm-up was completed. As proposed by Hoff *et al.* (2002), the participant was required to control a soccer ball around the track (Figure 4.2) by dribbling. The Hoff test track length was 290 m, as modified by Chamari *et al.* (2005). An odometer (Irion & Vosseler, Schwenningen, Germany) and tape measure (Stanley, Sheffield, UK) were used to determine distances between cones or hurdles. Once distances were determined, the point was marked with insulating tape before the cone or hurdle was placed on the surface and secured with duct tape.

The Hoff test consisted of dribbling the football through all the cones and over the 30 cm hurdles. Between cones 8 and 9 participants moved backwards while controlling the football before turning and resuming the remainder of the lap as normal. The backwards dribbling portion of the Hoff test was signified by a 3 m wide gate and different coloured cones. All obstacles were fully secured to the playing surface with duct tape and assistants were on hand to ensure all obstacles were repositioned if necessary. The purpose of the test was to cover the maximum distance possible while maintaining close ball control, akin to match-play, during a fixed 10 min period. Participants were instructed to increase their exercise intensity gradually until they got to their maximum intensity.

Diagram of a 55 m long water tunnel with 9 test sections. The tunnel has a 30 m wide test section and a 5 m wide passage. The total length is 55 m. The test section is divided into 9 sections, each 10 m long, except for the last one which is 15 m long. The sections are labeled 1 through 9. Section 1 is at the entrance, followed by sections 2 through 6, then section 7, then section 8, and finally section 9 at the exit. The sections are separated by 7.0 m, 7.0 m, 7.5 m, 18 m, and 12 m. A 3 m gate is located between sections 7 and 8. A 2 m long section of the tunnel is marked with a wavy line. The flow direction is indicated by arrows.

Individuals started the test at 1 min intervals and were informed of the elapsed time at the halfway point (5 min) and at the final minute of their test (9 min). Thus, the assessor who was timing the test had 4 min in which to start 5 players and then switch to announcing the halfway test signal that occurred in the successive minute for the first participant (5 min). The halfway test signal for the 5th player coincided with the last minute signal for the 1st player (at 9 min) and were signalled

simultaneously to the respective individuals. A second assessor marked laps completed as participants passed the start point. At 10 min, individuals were instructed to stop and the final distance covered (m) was recorded. Participants were familiar with the structure and pacing of the Hoff test as it was part of a battery of pre-season fitness tests used by Sporting Fingal Football Club.

4.2.6 Saliva sample collection

All saliva samples were collected as described in Section 2.6.

4.2.7 Salivary cortisol and testosterone

Salivary C and T concentrations were assessed by ELISA technique and results in nmol.L⁻¹ were computed, as outlined in Section 2.7

4.2.8 Heart rate assessment

During performance testing, HR data (beats.min⁻¹) were monitored and recorded as outlined in Section 2.5.

4.2.9 Statistical analysis

All subject physical characteristics are presented in table format as mean $\pm$ SD and all graphical data as mean $\pm$ SEM. Performance data is presented as mean $\pm$ SEM. Statistical analysis of pooled salivary C and T concentrations within discrete sports across time and scenario (training or competition) were performed using a 2 factor ANOVA, *post-hoc* Bonferroni tests quantified detected significant differences, using Prism (GraphPad Prism, La Jolla, USA). An additional statistical analysis of pooled salivary C and T concentrations across sport and time within scenario (training or competition) was performed using a 2 factor ANOVA with time (pre vs. post) as a repeated measure, *post-hoc* Bonferroni tests quantified detected significant differences, using Prism (GraphPad Prism, La Jolla, USA). For all statistical analyses, significance was set with $P < 0.05$.

Chapter 4

4.3 Results

4.3.1 Physical characteristics

Of the 23 soccer players recruited, at varying times throughout the testing period and monitored training days, some players did not participate due to illness, injury, ‘A’ squad or Under 20’s squad commitments and one player due to mid-season transfer. The transferred players’ data has not been included. Mean physical characteristics are presented in Table 4.1. Mean data for this chapter are presented in Appendix IV.

	Soccer n = 14	Rugby n = 12	Rowing n = 9
Age (yr)	24 ± 3	19 ± 1	19 ± 1
Height (m)	1.81 ± 0.08	1.80 ± 0.05	1.87 ± 0.04
Body mass (kg)	78.4 ± 9.1	86.1 ± 11.7	80.4 ± 6.9
BMI (kg.m ²)	25.1 ± 2.3	26.4 ± 2.9	23.1 ± 2.3
Body fat (%)	12.2 ± 3.0	20.9 ± 3.3	18.7 ± 2.6

Table 4.1: Mean physical characteristics (± SD) for soccer players (n = 14), rugby players (n = 12) and rowers (n = 9).

4.3.2 Performance tests

The mean distance covered during the YoYo IR2 test was 562.9 ± 25.7 m (n = 14), on average soccer players reached a corresponding level of 20.2 ± 0.1 at failure. The mean distance covered during the Hoff test was 1777.5 ± 24.1 m (n = 14) corresponding to a predicted $\dot{V}O_2\text{max}$ of 66.2 ± 1.2 mL.kg⁻¹.min⁻¹. Soccer players covered a mean distance of 3060.7 ± 32.4 m (n = 14) during a 12 min Cooper run corresponding to a predicted $\dot{V}O_2\text{max}$ of 57.1 ± 0.7 mL.kg⁻¹.min⁻¹. Mean squad data are presented in Appendix IV.

4.3.3 Cortisol response

4.3.3.1 Rowing

Cumulative mean C concentration (nmol.L⁻¹) demonstrated a significant scenario effect; competition was significantly higher than training (*P*<0.01). A significant time effect was also noted for cumulative mean C concentrations in rowing; pre- vs. post- (*P*<0.001). However, mean C concentrations pre- and post-training (4.6 ± 0.6 and 10.0 ± 1.7 nmol.L⁻¹, respectively) were not significantly different to pre- and post-competition data (9.1 ± 0.9 and 14.4 ± 1.9 nmol.L⁻¹, respectively; Figure 4.3).

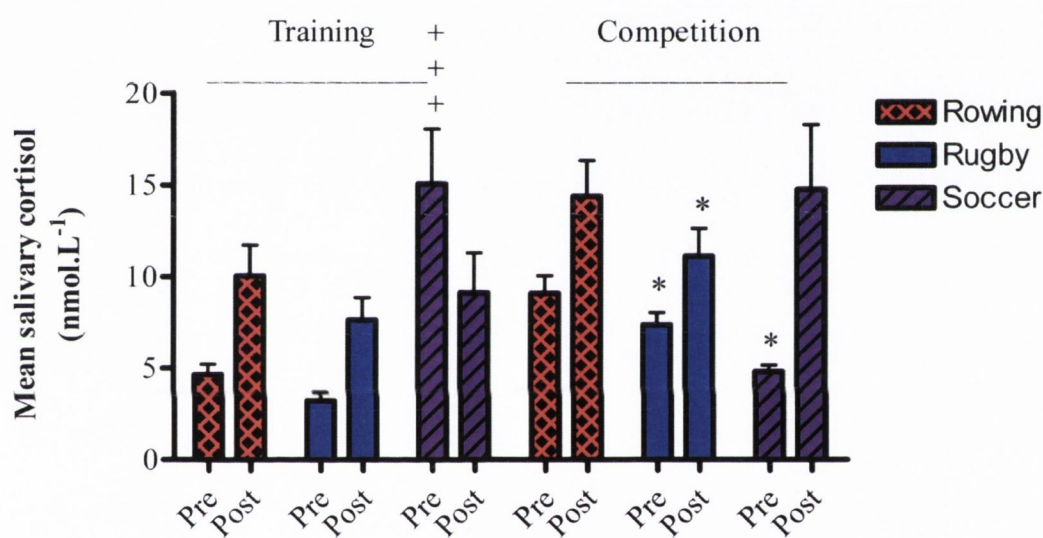


Figure 4.3: Mean pooled salivary C (nmol.L⁻¹) response from pre- to post-exercise during training and competition, bars denote SEM. Rowing, n = 9; rugby, n = 12; soccer, n = 14.

* *P*<0.05 vs. training within sport. +++ *P*<0.001 vs. rowing pre-training within scenario (training).

Δ Cortisol	Rowing	Rugby	Soccer
Training (%)	+115.7	+135.0	-39.3
Competition (%)	+58.2	+50.7	+206.7

Table 4.2: Mean Δ cortisol (%) pre- to post-training and competition across sport.

4.3.3.2 Rugby

A significant scenario effect was evident for cumulative mean salivary C concentration (nmol.L⁻¹) in rugby; competition was significantly higher when compared with training (*P*<0.001). In addition, a significant time effect was also

recorded for mean C concentrations in rugby; pre- vs. post ($P<0.001$). Mean C concentrations pre- and post-match in rugby were significantly higher when compared with pre- and post-training, respectively (3.2 ± 0.4 and 7.4 ± 0.7 vs. 7.6 ± 1.2 and 11.1 ± 1.5 nmol.L⁻¹, respectively; $P<0.05$; Figure 4.3).

4.3.3.3 Soccer

In contrast to rowing and rugby, no significant scenario or time effects were detected in cumulative mean C concentrations (nmol.L⁻¹) in soccer. However, a significant interaction effect was observed ($P<0.01$). The pre-training mean C concentration during soccer was significantly higher when compared with pre-competition (15.0 ± 3.0 vs. 4.8 ± 0.4 nmol.L⁻¹, respectively; $P<0.05$) however, no significant difference was detected between post-training and competition C concentrations (9.1 ± 2.2 vs. 14.8 ± 3.5 nmol.L⁻¹, respectively; Figure 4.3).

4.3.3.4 Cortisol training response

Mean C concentrations (nmol.L⁻¹) within the training scenario across all sports displayed a significant effect due to sport ($P<0.05$) and a significant time by sport interaction ($P<0.001$) however, no significant time effect was detected. Within the training scenario, mean C concentrations pre-training in rowing and rugby were significantly lower than pre-training in soccer players (4.6 ± 0.6 and 3.2 ± 0.4 vs. 15.0 ± 3.0 nmol.L⁻¹, respectively; $P<0.001$). There were no significant differences detected in mean C concentrations recorded for rowing and rugby (pre: 4.6 ± 0.6 vs. 3.2 ± 0.4 , post: 10.0 ± 1.7 vs. 7.6 ± 1.2 nmol.L⁻¹, respectively). In addition, mean C concentrations post-training were not significantly different comparing rowing, rugby and soccer (10.0 ± 1.7 , 7.6 ± 1.2 and 9.1 ± 2.2 nmol.L⁻¹, respectively; Figure 4.3).

4.3.3.5 Cortisol competition response

Mean C concentrations (nmol.L⁻¹) within the competition scenario across all sports displayed a significant time effect ($P<0.001$) with no significant effect due to sport or time by sport interaction. Within the competition scenario, salivary C increased significantly post-competition across all three sports ($P<0.001$). There were no significant differences detected in the competition induced change in C concentration between sports (9.1 ± 0.9 , 7.4 ± 0.7 and 4.8 ± 0.4 vs. 14.4 ± 1.9 , $11.1 \pm$

1.5 and $14.8 \pm 3.5 \text{ nmol.L}^{-1}$, pre- vs. post-competition rowing, rugby and soccer, respectively; Figure 4.3) despite the large ΔC detected in soccer, primarily due to the low pre-competition mean C concentration.

4.3.4 Testosterone response

4.3.4.1 Rowing

No significant scenario or time by sport interaction effects were detected in cumulative mean T concentrations (nmol.L^{-1}) in rowing. However, a significant time effect was detected in cumulative mean T concentrations; pre- vs. post- ($P<0.01$). There were no significant differences between pre-training and pre-competition (0.34 ± 0.03 vs. $0.44 \pm 0.04 \text{ nmol.L}^{-1}$, respectively) or post-training and competition T concentrations (0.54 ± 0.07 vs. $0.56 \pm 0.07 \text{ nmol.L}^{-1}$, respectively; Figure 4.4).

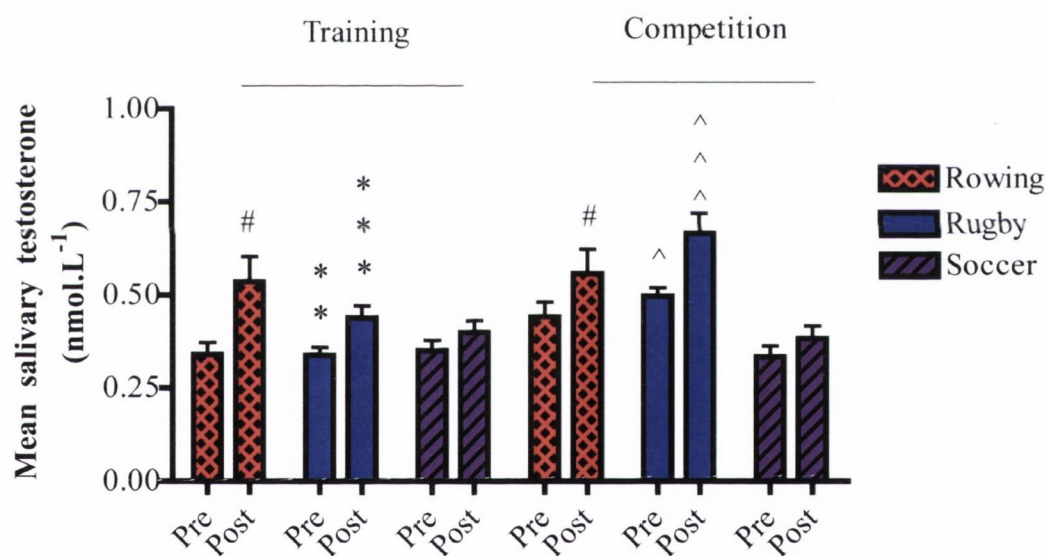


Figure 4.4: Mean pooled salivary T (nmol.L^{-1}) response from pre- to post-exercise during training and competition, bars denote SEM. Rowing, $n = 9$; rugby, $n = 12$; soccer, $n = 14$.
 ** $P<0.01$, *** $P<0.001$ vs. Competition within rugby. # $P<0.05$ vs. post-soccer within scenario (training). ^ $P<0.05$, ^^ $P<0.001$ vs. soccer within scenario.

Δ Testosterone	Rowing	Rugby	Soccer
Training (%)	+57.5	+29.7	+13.9
Competition (%)	+26.5	+34.2	+14.8

Table 4.3: Mean Δ testosterone (%) pre- to post-training and competition across sport.

4.3.4.2 Rugby

Significant scenario and time effects were observed in cumulative mean T concentrations (nmol.L^{-1}) in rugby, but no significant time by scenario interaction was recorded. Cumulative mean T concentrations in rugby were significantly different between scenario (competition vs. training; $P < 0.001$), and were also significantly higher pre- to post- ($P < 0.001$). The T concentration pre-competition was significantly higher than pre-training (0.50 ± 0.02 vs. 0.34 ± 0.02 nmol.L^{-1} , respectively; $P < 0.01$). Similarly, rugby union match play induced a significantly higher T response compared to training (0.44 ± 0.03 vs. 0.67 ± 0.05 nmol.L^{-1} , respectively; $P < 0.001$; Figure 4.4).

4.3.4.3 Soccer

No significant time or scenario effects or time by scenario interactions were recorded in cumulative mean T concentrations (nmol.L^{-1}) for soccer. No significant difference was observed between T concentrations pre-training and pre-competition (0.35 ± 0.03 vs. 0.33 ± 0.03 nmol.L^{-1} , respectively) and were similarly not significant between post-training and competition (0.40 ± 0.03 vs. 0.38 ± 0.03 nmol.L^{-1} , respectively; Figure 4.4).

4.3.4.4 Testosterone training response

Mean T concentrations (nmol.L^{-1}) within the training scenario across sports displayed a significant time effect ($P < 0.001$) and a significant time by sport interaction ($P < 0.01$) but no significant sport effect. Within the training scenario, mean T concentration post-training in rowing was significantly higher than post-training in soccer (0.54 ± 0.07 vs. 0.40 ± 0.03 nmol.L^{-1} , respectively; $P < 0.05$). Mean T concentrations for rowing and rugby training were not significantly different (pre: 0.34 ± 0.03 vs. 0.34 ± 0.02 nmol.L^{-1} , respectively, post: 0.54 ± 0.07 vs. 0.44 ± 0.03 nmol.L^{-1} , respectively; Figure 4.4), nor was there a significant difference between pre-training T concentrations reported for rowing and soccer (0.34 ± 0.03 vs. 0.35 ± 0.03 nmol.L^{-1} , respectively). In addition, mean T concentrations were similar and, therefore, not significant during both rugby (0.34 ± 0.02 and 0.44 ± 0.03 nmol.L^{-1} , pre- and post-, respectively) and soccer training (0.35 ± 0.03 and 0.40 ± 0.03 nmol.L^{-1} , pre- and post-, respectively).

4.3.4.5 Testosterone competition response

Mean T concentrations (nmol.L^{-1}) within the competition scenario across all sports displayed significant time and sport effects ($P < 0.001$) but no significant time by sport interaction. Although pre-competition T concentrations for rowing and soccer were similar, and not significantly different (0.44 ± 0.04 and $0.33 \pm 0.03 \text{ nmol.L}^{-1}$, respectively; Figure 4.4), rugby pre-competition T concentrations were significantly higher than soccer pre-competition (0.50 ± 0.02 vs. $0.33 \pm 0.03 \text{ nmol.L}^{-1}$, respectively; $P < 0.05$). Within the competition scenario, mean T concentrations post- in rugby and rowing were significantly higher than post- in soccer (0.67 ± 0.05 and 0.56 ± 0.07 vs. $0.38 \pm 0.03 \text{ nmol.L}^{-1}$, respectively; $P < 0.001$). Mean T concentrations were not significantly different between rugby union and rowing.

Chapter 4

4.4 Discussion

4.4.1 Summary of Findings

Halson and Jeukendrup (2004) stated that there was a necessity to perform functional-OR investigative studies in order to gain insight into the incidence, markers and possible causes of OT. This study proposed to evaluate the hormonal response to in-season training and competition scenarios across three sporting disciplines of varying demands, i.e. soccer, rugby and rowing. The primary hypothesis was that the competition scenario would elicit significantly greater hormonal changes compared to the training scenario in all sporting disciplines. The secondary hypothesis was that the hormonal changes would be significantly greater in rugby union players than soccer players or rowers as previous data suggested that the nature of rugby union caused longer term disturbances to host immunity and greater disturbance to both C and T concentrations (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010).

The salivary T responses to soccer training and match-play were not significantly different. In soccer, mean salivary C concentration decreased in response to training and, although not significant, a trend of increasing C was observed post-match. Similar hormonal responses to rugby union training and competition were recorded; both C and T concentrations were increased post-training and competition. However, the response of both hormones was significantly amplified in the competition scenario. In rowing, the mean salivary C response to the competition scenario was greater than that recorded in the training scenario. Within rowing, mean salivary C response over time was greater post- compared to pre-. However, mean C concentrations were similar and not significantly different between pre-training and competition, and between post-training and competition. The salivary T response to training and competition in rowing was similar and, therefore, not significantly different.

4.4.2 Soccer

The hormonal response to in-season soccer training and competitive match-play varied. Salivary T concentrations were not significantly different comparing training

and competition (see Figure 4.4). Salivary C concentration decreased in response to soccer training (pre- to post-training, see Figure 4.3) but this difference failed to attain statistical significance most likely due to time of sampling and wide variability between players. However, mean salivary C concentration displayed a tendency of increasing concentration post-match, possibly indicating the potential impact of competitive match-play (Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008) on host homeostasis as previously reported in rugby union players (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010).

Data presented by Krustrup *et al.* (2006a) illustrated the effect of soccer match-play on muscle glycogen stores. An increased C concentration, as observed in the present study, may be indicative of a drive to restore lost glycogen or maintain BGlu in the immediate post-match period and is similar to that reported by Ispirlidis *et al.* (2008). Krustrup *et al.* (2006a) reported no significant decrease in BGlu concentration during match-play, which is a key factor in the immune and C response to exercise (Bishop *et al.*, 1999a). Previously reported data suggested a link between inflammatory markers and plasma C concentrations (Fukatsu *et al.*, 1996; Bishop *et al.*, 1999a; Cunniffe *et al.*, 2010). Bishop *et al.* (1999a) reported no significant effect of simulated match-play on neutrophil degranulation which may have accounted for their lack of an increase in C concentration. However, the reported acute increase in post-match C concentration in the study by Ispirlidis *et al.* (2008) was accompanied by a significant inflammatory response. It is possible that the elimination of several high-intensity activities (jumping, tackling, heading the ball) from the protocol utilised by Bishop *et al.* (1999a) may have significantly reduced any trauma related inflammatory responses that typically occur during competition, as reported by Ispirlidis *et al.* (2008), thus augmenting the catecholamine and hormonal response. Also, match-play is proven to place greater demands on intra-muscular glycogen stores whilst BGlu concentration is maintained (Krustrup *et al.*, 2006a), therefore, any potential effects of match-play on C concentrations may be mediated by a combination of inflammatory factors and the drive to maintain BGlu or recover utilised glycogen (Fukatsu *et al.*, 1996; Bishop *et al.*, 1999a; Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010).

4.4.3 Rugby Union

The hormonal response to rugby union training and competition was similar. Both C and T increased post-training and competition and was also significantly increased significantly in the competition scenario relative to training. This significant acute post-match elevation in salivary C is in agreement with previous research (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010). The salivary T response to rugby union match-play in the current study, however, differed from previous studies. Elloumi *et al.* (2003) reported a slight, but non-significant, decrease in post-match salivary T concentration while the more recent data presented by Cunniffe *et al.* (2010) reported a significantly decreased serum T response immediately post-match which returned to resting levels by 14 h post-match. Such a decrease in T concentration post-match may limit the protective effects of T against the catabolic actions mediated by C for gluconeogenesis, such as proteolysis and lipolysis which are accompanied by a sparing effect of glycogen stores (Elloumi *et al.*, 2003). However, in the circumstances of the current study, the increased T concentrations in rugby players post-match may have been elicited in order to induce these protective effects. Perhaps such a T response may be an adaptation, or habitual response, to long-term rugby union training, similar to that observed in C responses in professional soccer players (Filaire *et al.*, 2001), so that homeostasis is maintained or restored.

Differences in the C responses between training and match-play may be due to the intense tissue damaging nature of rugby (Cunniffe *et al.*, 2010). Cunniffe *et al.* (2010) reported increases in both serum C and blood neutrophils after a competitive rugby union game which is similar to that reported in endurance athletes and professional soccer players (Fukatsu *et al.*, 1996; Bishop *et al.*, 1999b; Ispirlidis *et al.*, 2008). Such a prolonged inflammatory response may be essential in further stimulation of necessary growth and repair processes following intense tissue damaging exercise like rugby (Cunniffe *et al.*, 2010). It is important to note that Elloumi *et al.* (2003) did not collect samples immediately pre-match, as both Cunniffe *et al.* (2010) and the current study did, which may account for differences in the T responses between studies.

4.4.4 Rowing

A greater salivary C response was observed in response to the competition scenario and from pre- to post-. However, mean C concentrations were similar and not significantly different between pre-training and competition as well as between post-training and competition. The salivary T response to training and competition in rowing was similar and, therefore, not significantly different. Research has reported an increase in C concentration following steady-state exercise (Fukatsu *et al.*, 1996; Henson *et al.*, 1998). Mäestu *et al.* (2005) reported similar elevations in C and T concentrations immediately following maximal exercise in rested rowers. Periods of overload training have reportedly resulted in decreased resting free T and free T/C ratio, however, both variables returned to baseline after the training load was reduced (Mäestu *et al.*, 2005). The salivary hormonal response presented here may suggest that the rowers in the current study were in a period of the season where training load was tolerable considering that the free T response to a 2000m ergometer test was reportedly blunted after a 3 week overload period and remained decreased after a further 2 weeks of tapering (Mäestu *et al.*, 2005).

4.4.5 Competition versus training hormonal responses.

The response of C has been linked to exercise intensity and duration (Stone, 1991; Lac & Berthon, 2000; Hill *et al.*, 2008). However, recent research has suggested that the hormonal response to exercise is highly individual (Suay *et al.*, 1999; Moreira *et al.*, 2009), varies according to exercise type (Jensen *et al.*, 1991; Hough *et al.*, 2011) and is related to physical fitness (Grandys *et al.*, 2009). The observation that competition hormonal responses were significantly higher is most likely due to the intensity and the duration of exertions in actual match-play (Lac & Berthon, 2000). The environmental conditions inherent in a competitive arena would have posed a high degree of psychophysiological strain (Cunniffe *et al.*, 2010). The enhanced emotional stress of competition, along with interactions with, and perception of opponents and their perceived threat are reported to reinforce this C response (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009). Previous research into simulated match-play in soccer, involving both collegiate and professional soccer players, has reported no significant increase in C post-exercise (Bishop *et al.*, 1999a; Moreira *et al.*, 2009). However, Ispirlidis *et al.* (2008) reported significantly elevated C in

response to competitive soccer match-play. It is possible that intrinsic motivational factors, in combination with a lesser energetic cost, were a factor in the C responses reported and may account for differences reported between simulated or training situations and competitive match-play. However, competition between squad members for places in a starting line-up is common place in professional and club institutions, and is often encouraged, and could possibly account for individual, if not mean differences, in salivary C and T concentrations (Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009).

The response of both C and T are reportedly highly individual (Suay *et al.*, 1999; Neave & Wolfson, 2003; Moreira *et al.*, 2009). Variation in C and T responses between individuals may be due to situational factors or individual aspects, such as, interactions with opposition players and importance of the game, yet they appeared to be independent of match outcome (Gonzalez-Bono *et al.*, 1999; Suay *et al.*, 1999; Moreira *et al.*, 2009). These factors may have attributed to the significant salivary C response within the competition scenario compared to training for soccer and may also account for the large variability observed between individual players. The 'home advantage' phenomenon may account for the non-significant salivary T response according to Neave and Wolfson (2003) who reported significantly higher salivary T concentrations before a home game than an away game or at a neutral training session. Neave and Wolfson (2003) also reported significantly higher T concentrations before playing an 'extreme' rather than a 'moderate' rival and that strikers exhibited differing hormonal responses across venues while the goalkeepers had the highest T concentration against the 'extreme' rival.

The rugby data presented here was collected from 3 home games, all resulting in defeat and, although C response was similar to that reported by Cunniffe *et al.* (2010) who presented data from a home defeat in an international rugby game, the T responses differed. Likewise, the first monitored soccer match was an away game, and was the first competitive fixture after a week-long mid-season break. Both venue of the game and periods of rest are reported to affect C and T concentrations (Filaire *et al.*, 2001; Neave & Wolfson, 2003) and may account for large variability in the soccer data. The rowing competition samples were collected at a regatta where participants raced against a rival university team, although this does not

appear to have affected post-competition T concentrations in the rowers. However, a distinct intra-squad competition was noted in the rowing training sessions during which, participants performed a sub-maximal time-trial which pushed them to the brink of collapse. This was in contrast to competition rowing events where the same levels of exertion were not evident. Therefore, it seems possible that the competitive and strenuous nature of the time-trial based rowing training session could possibly have provided sufficient physiological and psychological stress or arousal phenomena to result in increased global C and T concentrations from pre- to post-training and competition (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Salvador, 2005).

The adrenal-cortisol response is stronger for intermittent anaerobic versus aerobic exercise (Jensen *et al.*, 1991; Hough *et al.*, 2011). Soccer and rugby training sessions were of a very high-intensity and were intermittent and anaerobically based. Although it was not possible to measure intensity of the soccer league fixtures, research suggests that match play is of a highly intense intermittent nature during which players regularly experienced periods of temporary fatigue which could possibly last for up to 5 min (Mohr *et al.*, 2003; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a; Bangsbo, 2007; Di Mascio & Bradley, 2011). Like soccer, rugby union is also an intermittent sport but has a more physical and traumatic nature (Duthie *et al.*, 2005; Roberts *et al.*, 2008; Cunniffe *et al.*, 2010). Cunniffe *et al.* (2009) stated that rugby players covered an average distance of 6953 m at an average intensity of ~88 %HRmax during match-play. Although the back line players performed a greater number of sprints during the game (34 vs. 19), the forwards expended more energy during match-play than the backs (8.2 vs. 6.9 MJ, respectively). Rowing is relatively steady-state high intensity exercise, yet the C concentration reportedly increases after such exercise (Fukatsu *et al.*, 1996; Henson *et al.*, 1998; Mäestu *et al.*, 2005). Mäestu *et al.* (2005) reported that mean C concentration were significantly elevated immediately and 30 min following a maximal rowing test when compared to rest following a period of normal, overload and tapered training. It appears that the hormonal response to rowing reported in the current study was similar to that observed by Mäestu *et al.* (2005) and may point to a psychophysiological elevation of hormones occurring during competition. Both the rugby and soccer training session were selected for hormonal evaluation were the

more physically demanding training session and, despite the relative intensity, psychological and arousal factors may explain the differing C and T responses between training and competition.

Although a link between physical fitness and concentrations of C and T have not been confirmed, a recent study by Grandys *et al.* (2009) reported that a 16% increase in T concentration accompanied a 3.7% increase in $\dot{V}O_2\text{max}$ after 5 weeks of moderate-intensity and low volume endurance training in untrained male volunteers. The fact remains that athletes possessing a lesser degree of aerobic conditioning will endure a greater degree of stress during training and competition (Arnason *et al.*, 2004; Caldwell & Peters, 2009) which may manifest as mental and physical fatigue during the latter stages of the season. Such mental and physical fatigue is potentially associated with a high catabolic status (Kraemer *et al.*, 2004). In this study it would be anticipated that the professional soccer players, due to their full-time status, would possess higher $\dot{V}O_2\text{max}$ data than collegiate rowers and club level rugby players, respectively. The rowers that participated in this study had a higher training to competition ratio than the rugby players completing ~ 6 to $8 \text{ h}\cdot\text{week}^{-1}$ of training, totalling 48 to 64 h over the duration of the study period, and competing in 4 races, each lasting approximately 3 min 40 s within a single day. Participating club rugby players performed ~ 3 to $4 \text{ h}\cdot\text{week}^{-1}$, totalling 24 to 32 h and competed in 4 matches each lasting ~ 80 min. In contrast, the soccer players participating in this study were full-time professional League of Ireland soccer players performing 5 training sessions per week of approximately 90 min in duration, totalling $7.5 \text{ h}\cdot\text{week}^{-1}$. The soccer players also participated in one competitive league fixture per week lasting ~ 90 min.

4.4.6 Diurnal variation

Timing of sample collection was the most evident difference between data sets presented in this study. Saliva samples in soccer players were collected pre-training between 10:00 and 10:30 and post-training between 12:30 and 13:00. However, soccer competition samples were collected in the evening and varied according to fixture kick-off times. The pre-match samples were collected between 17:00 and 19:00 and the post-match samples were collected between 21:45 and 22:30. For

rugby players and rowers, pre-training saliva samples were collected in the late evening and post-training samples were collected in the early night time, both dictated by respective training schedules. Rowing competition samples were collected at 10:00 and 14:00 and rugby match-day samples were collected in mid-afternoon.

Augmented diurnal changes could possibly account for pre-competition mean C concentrations for soccer. Filaire *et al.* (2001) stated that after a period of high-intensity training circadian rhythm could possibly be modified. Wittert *et al.* (1996) observed that the expected early morning diurnal change in C and ACTH concentrations occurred earlier in athletes than in controls. Filaire *et al.* (2001) stated that intense training could induce a prolonged increase in the diurnal secretion of C, as reflected by the significant elevation of C at 11:30 in their study. The salivary C response to training and match-play in this study was also similar to Ispirlidis *et al.* (2008) but differed from that presented for simulated match-play reported by Bishop *et al.* (1999a) and Moreira *et al.* (2009) possibly due to reasons outlined previously. The differing C and T responses observed in soccer training when compared with rowing and rugby union may be due to timing of sample or an habitual adaptation to exercise (Wittert *et al.*, 1996; Filaire *et al.*, 2001). However, some adaptation to required weekly league participation may have resulted in professional soccer players being more relaxed than their collegiate rowing or club rugby counterparts.

It is evident that collection times within each sport occurred at differing times within the respective diurnal cycles of both measured hormones. However, Cunniffe *et al.* (2010) argues that as concentrations are known to decrease throughout the day, changes in these biomarkers immediately post-match would be indicative of a real exercise induced effect. Differences in the C response may be explained by the degree of psychophysiological stimulation, habitual adaptations and the varying timings of sample collection between studies (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Elloumi *et al.*, 2003; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). Although every effort was made to control time of sampling, this was a free-living observational study and dietary

and lifestyle influences cannot be ruled out (Backhaus *et al.*, 2004; Lane *et al.*, 2010; Mikulski *et al.*, 2010; Paton *et al.*, 2010).

4.4.7 Conclusions

When the inflammatory and immunosuppressive responses to exercise are considered, available data suggests that both C and T play pivotal roles in the post-match environment for maintenance of homeostasis (Fukatsu *et al.*, 1996; Henson *et al.*, 1998; Elloumi *et al.*, 2003; Mäestu *et al.*, 2005; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010). It appears that the psychologically engaging, traumatic and high-intensity, intermittent nature of match-play in rugby and, to a lesser extent, soccer are central to the hormonal response, particularly that of C (Bishop *et al.*, 1999a; Elloumi *et al.*, 2003; Ispirlidis *et al.*, 2008; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). Rugby is a high-intensity collision sport where players experience repeated trauma (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010), therefore, it was hypothesised that rugby training and match-play would induce a significantly greater disturbance in hormonal response than either rowing or soccer training and competition data. However, this effect was not observed. Competition is known to impose greater psychophysiological demands on the athlete which can lead to a greater hormonal response although not all individuals will respond in the same manner (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010).

The hormonal responses observed in the current study demonstrated training and competition induced psychophysiological stresses were sufficient to manifest significant changes in the salivary C and T response for club rugby and collegiate rowers. The psychophysiological stress associated with soccer match-play in a League of Ireland Division 1 fixture was sufficient to induce an increased salivary C concentration but did not significantly affect the T response in professional Irish soccer players. Soccer match-play places great demands on intra-muscular glycogen stores (Krustrup *et al.*, 2006a) and the reported increase in C in the acute post-match period (Ispirlidis *et al.*, 2008) may be indicative of the role C plays in the mobilisation of substrate through catabolic mechanisms to maintain BGlu concentration in the latter stages of exercise and in the post-exercise glycogen depleted state. Considering that reported disturbances to immunity, hormonal

concentrations and intra-muscular glycogen stores can last for lengthy periods post-exercise, an altered hormonal response may be a necessary means of restoring homeostasis (Elloumi *et al.*, 2003; Mäestu *et al.*, 2005; Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010). However, with these reported hormonal responses to training and competition scenarios, the question remains, whether nutritional interventions can significantly augment these responses and provide a greater, more complete recovery.

Chapter 5

The effect of carbohydrate versus carbohydrate-protein ingestion on salivary cortisol and testosterone response in professional soccer players.

Chapter 5

5.1 Introduction

5.1.1 Soccer

Soccer is an intermittent sport in which the aerobic and anaerobic energy systems are highly taxed as game play is punctuated with changes in activity every 3 to 5 s varying from jumping and tackling, to changing direction, turning and high intensity sprinting (Mohr *et al.*, 2003; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006b). The anaerobic energy system provides energy for periods of acceleration, tackling, turning and jumping (Mohr *et al.*, 2003). However, a players' aerobic capacity plays a significant role in modern soccer, influencing both technical performance and tactical decision making (Helgerud *et al.*, 2001; Chamari *et al.*, 2005). Helgerud *et al.* (2001) demonstrated that increases in both $\dot{V}O_{2\max}$ and running economy, by a magnitude of approximately $5 \text{ mL.kg}^{-1}.\text{min}^{-1}$ and 7% respectively, substantially influenced the player's technical and tactical performance during match-play. Such increases in performance indices resulted in players covering 20% greater distance, completing double the number of sprints and a 24% increase in interactions with the ball during a game (Helgerud *et al.*, 2001).

Krstrup *et al.* (2006a) reported that HRavg and HRmax during match-play varied between 85 and 98 %HRmax respectively, and that the average exercise intensity for top-class players was approximately 70% $\dot{V}O_{2\max}$ despite research identifying that a majority of the distance covered in a game is by walking or low-intensity running (Bangsbo, 1994; Reilly *et al.*, 2000; Mohr *et al.*, 2003; Esposito *et al.*, 2004; Bangsbo *et al.*, 2006). Maintaining this intensity for the duration of a game (90 min) places high demands on the O_2 transport systems and the endurance capacity of skeletal muscles.

5.1.2 Considerations for recovery in soccer players

Research has reported that elite soccer players experienced temporary fatigue after intense periods of the game which limited the amount of high-intensity running and persisted for up to 5 min (Mohr *et al.*, 2003; Di Mascio & Bradley, 2011). Elevating muscle glycogen stores pre-exercise and CHO ingestion during intermittent exercise

has resulted in increased exercise capacity and presents obvious benefits to the soccer player so that performance can be maintained (Fielding *et al.*, 1985; Bangsbo *et al.*, 1992; Nicholas *et al.*, 1995; Jeukendrup, 2004; Foskett *et al.*, 2008)

However, sprint performance was significantly reduced immediately post-match possibly due to significant depletion of muscle glycogen in half of the individual players' muscle fibres of both types (Krustrup *et al.*, 2006a). This evidence suggested that in the latter stages of a soccer match, a players' immunocompetence may be compromised (Pedersen & Bruunsgaard, 1995; Gleeson & Bishop, 2000; Marcos *et al.*, 2003) as low availability of muscle glycogen will result in increased secretion of C so that BGlu concentrations can be maintained (Bishop *et al.*, 1999a; Marcos *et al.*, 2003; Brooks *et al.*, 2005; Lane *et al.*, 2010). Data presented in Chapter 4 reported increased salivary C concentration post-match but no significant change in salivary T concentration. Similarly, Ispirlidis *et al.* (2008) reported an acute increase in C concentration post-match in professional soccer players.

Often, soccer players are required to participate in competitive matches within short-periods and this can result in an accumulation of fatigue and an increased risk of injury (Dupont *et al.*, 2010). Bangsbo *et al.* (2006) reported that even though players consumed a high CHO diet post-match, muscle glycogen was only slightly higher when compared to players consuming a normal diet. Bangsbo *et al.* (2006) stated that these decreased glycogen stores (compared with resting levels) persisted for up to 48 h post-match and had obvious implications for subsequent training intensity. Considering the physiological demands of soccer (Helgerud *et al.*, 2001; Mohr *et al.*, 2003; Chamari *et al.*, 2005; Bangsbo *et al.*, 2006; Krustrup *et al.*, 2006a; Krustrup *et al.*, 2006b), along with these naturally occurring threats to immunocompetence (Pedersen & Bruunsgaard, 1995; Gleeson & Bishop, 2000; Marcos *et al.*, 2003; Bangsbo *et al.*, 2006; Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008) and potential implications for development of injury and OT (Dupont *et al.*, 2010), it appears further nutritional interventions may prove essential for maintenance of homeostasis and as a means of increasing the players' ability to tolerate training and competitive loads throughout the season.

5.1.3 Aims and hypothesis

The nature of competitive soccer reportedly places great demands on intra-muscular glycogen stores to fuel performance and resulted in a significant increase in C concentration post-match (Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008). Sustained post-match glycogen depletion can present obvious implications for subsequent training intensity (Bangsbo *et al.*, 2006). Ingestion of a CHO-PRO supplement post-exercise presents the most advantageous means of maximising glycogen resynthesis, maintaining subsequent performance (Ivy *et al.*, 2002; Betts *et al.*, 2007) and may decrease the cumulative catabolic response associated with intense exercise and soccer match play (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Ispirlidis *et al.*, 2008; Lane *et al.*, 2010; Mikulski *et al.*, 2010). Limited research has investigated post-match hormonal responses in soccer players (Ispirlidis *et al.*, 2008) and, to date, no research has investigated the short-term supplementation of sports drinks on hormonal markers of recovery in soccer players.

This study proposed to evaluate the effect of a CHO-PRO beverage on salivary C and T concentrations compared to a CHO supplement alone (LCHO) and an isocaloric equivalent to CHO-PRO supplement (HCHO) during standard, in-season soccer training. The hypothesis was that a CHO-PRO and HCHO beverage consumed immediately post-training would reduce the exercise induced C response while augmenting the T response, compared to LCHO ingestion, and result in a T/C ratio reflective of an improved ability to tolerate training load.

Chapter 5

5.2 Methods

5.2.1 Experimental design

The protocol involved a single-blinded, repeated measures design to assess the acute impact of sports drinks of varying composition on hormonal markers of stress within 1 h post-training/ingestion and between pre-training samples collected at rest on days 1, 2 and 4 of the training week. Data was collected on the first 2 days of the training week (Day 1 and 2), with an additional saliva sample collected at rest on Day 4. Participants were provided with a study information sheet and provided written informed consent at least 7 days prior to the commencement of testing. Participants were then required to undertake HR monitored training sessions twice weekly and provide saliva samples at pre-determined times over 3 in-season training weeks. At the conclusion of each monitored training session, participants received one of the 3 sports drinks under investigation. All testing took place during the mid-season (May-July) training cycle for the participants. The study design and experimental procedures involved in this study were approved by Faculty Research Ethics Committee, Trinity College Dublin.

5.2.2 Participants

Twenty three trained first division, League of Ireland soccer players were recruited as volunteers for the study. Of the 23 soccer players recruited, only 12 ($n = 12$) completed all supplementation and monitored training weeks, some of whom had previously participated in the data collection for Chapter 4. All volunteers were recruited through Sporting Fingal Football Clubs' Director of Football. All volunteers were contracted players of Sporting Fingal Football Club and competed in the League of Ireland First Division during the 2009 season. All volunteers had been playing North Dublin Schoolboy League, League of Ireland Premier or First Division, English Football League 1 or, in one case, Barclays Premiership the previous season and some participants had national underage representative honours.

5.2.2.1 Inclusion and exclusion criteria

All participants were medically screened prior to the first testing session by the Sporting Fingal Football Club physician. Individuals suffering from any

musculoskeletal injury that had prevented participation in training within the sample collection period or, deemed unfit to participate by the physician were excluded from the monitored training and recovery intervention protocol. Volunteers with diabetes, hypertension, heart defects, any metabolic disorders, chronic sports injury or any other contra-indicatory symptoms were excluded from sample collection completely. Volunteers who were suffering from minor sporting injuries were allowed to participate and performed exercises within the limits of their injuries.

5.2.3 Study design

Prior to beginning the in-season monitored training and recovery intervention protocol, participants performed a series of soccer specific physiological assessments to determine $\dot{V}O_2\text{max}$, namely a 12 min Cooper run, a YoYo IR2 test and a Hoff test as described in Section 4.2.5. During the monitored training and recovery intervention protocol, participants presented themselves at ALSAA, Old Airport Road, Co. Dublin, in a rested, well-hydrated state at appointed training times. Participants were requested to standardise pre-training fluid intake and diet to limit extrinsic effects on trial outcomes. Players assembled on the morning of each monitored training session and were provided with a HR monitor for real-time wireless monitoring of HR, as described in Section 2.5.2. Volunteers then participated in standard squad training sessions conducted and supervised by Sporting Fingal Football Clubs' Head Coach. The HR monitor was worn by participants for the duration of the training session. All players were familiar with the drills, activities and intensities in the training sessions. At the end of the monitored training session participants ingested one of the three sports drinks under investigation.

5.2.4 Saliva sample collection

Saliva samples for determination of C and T concentrations were collected before, immediately post- and 1 h post-training sessions (pre-, post- and +60 min, respectively) on Day 1 and Day 2. An additional saliva sample was also collected at rest (pre-training) on Day 4; 72 h after the first saliva sample (Day 1 Pre). All samples were collected as described in Section 2.6 between 10:00 and 13:30. Post-exercise drink effect was analysed by comparing C and T data immediately post-

training to 1 h post-training (post- vs. +60 min). Differences across pre-training concentrations were analysed by comparing resting samples across Day 1, 2 and 4 (0, 24 and 72 h post-ingestion, respectively).

5.2.5 Salivary cortisol and testosterone

Determination of salivary cortisol and testosterone concentrations (nmol.L^{-1}) were performed using ELISA technique and results were calculated as outlined in Section 2.7.

5.2.6 Sports drinks

Participants ingested one of three 10 mL.kg^{-1} BM bolus of sports drink at cessation of the training session and all drinks were consumed within 10 min. The sports drinks used in this study are commercially available and were delivered in liquid form. HCHO and LCHO consisted of a carbohydrate-electrolyte based sports drink (Club Energise Sport, Britvic, Dublin, Ireland). LCHO comprised of the commercially available carbohydrate-electrolyte sports drink and provided $6.2 \text{ g CHO.100 mL}^{-1}$. HCHO consisted of the carbohydrate-electrolyte sports drink with added glucose to provide $9.3 \text{ g CHO.100 mL}^{-1}$. CHO-PRO consisted of a commercially available CHO-PRO sports drink (Club Energise Sport Recovery 20, Britvic, Dublin, Ireland) and provided $6.2 \text{ g CHO.100 mL}^{-1}$ and $3.1 \text{ g PRO.100 mL}^{-1}$. HCHO and CHO-PRO were volumetrically and iso-calorically equivalent while LCHO and CHO-PRO were equivalent for volume and CHO intake.

5.2.7 Statistical Analysis

All subject physical characteristics are presented in tabular format as mean $\pm$ SD and all graphical data are presented as mean $\pm$ SEM. Statistical analyses of salivary C, T and T/C ratio data across time and intervention (time by drink) were performed using a 2-factor repeated measures ANOVA with *post-hoc* Tukey tests to quantify identified significant differences. Recorded HR data for each training session, namely %HRmax, HRavg and time in zone were also analysed using a 2 factor repeated measure ANOVA with *post-hoc* Tukey tests to quantify identified significant differences. All statistical testing was performed using SigmaStat

(SigmaStat Ltd., SanJose, USA). For all statistical analyses, significance was set with $P < 0.05$.

Chapter 5

5.3 Results

5.3.1 Participants

Of the 23 soccer players recruited, only 12 (n = 12) completed all supplementation and monitored training weeks either due to illness or injury, other squad commitments and one player due to mid-season transfer. The transferred players' data was excluded. Where necessary, sample number is provided with data. Physical characteristics for the participants that completed all three trials are presented as mean ± SD while all salivary and performance data are presented as mean ± SEM. Mean data for this chapter are presented in Appendix V. Participants' physical characteristics are presented in Table 5.1

Participant	Mass (kg)	Height (m)	BMI (kg.m ²)	% Body Fat
Mean	78.0	1.79	24.9	11.6
SD	9.3	0.07	1.9	2.3

Table 5.1: Mean physical characteristics for participants that completed all three supplementation trials, ± SD, n = 12.

Physiological test scores are presented in Table 5.2.

	YoYo IR2	Cooper Run	Hoff Test
Mean score (m)	553.3 ± 32.6	3055.0 ± 37.5	1772.4 ± 22.5
Mean predicted $\dot{V}O_2$ max (mL.kg ⁻¹ .min ⁻¹)		57.0 ± 0.8	68.1 ± 2.0

Table 5.2: Mean physiological test data for participants that completed all three supplementation trials, ± SEM, n = 12.

5.3.2 Heart rate data

Mean HR data for soccer players measured during the monitored training sessions were not significantly different across training sessions and drinks (Table 5.3). Mean %HRmax was significantly lower during the training session performed on Day 2 of the LCHO trial when data were compared with CHO-PRO (97.4 vs. 91.0 %HRmax; $P<0.05$). No significant differences in mean %HRmax were detected between any other sessions across drinks. Mean %HRavg were not significantly different between training sessions. All training sessions consisted of ~20 min warm-up, dynamic in nature and involving the ball, ~30 min of skill based ‘grids’ emphasising ball control and possession, with ~40 min small-sided games (7 vs. 7, 6 vs. 6 or 5 vs. 5) played on half pitch for intervals of 6 to 8 min with a variable 1 to 2 min rest period between each game. Both monitored training sessions concluded with a period of low-intensity exercise and static stretching for 5 to 10 min which was not recorded.

Week	Day	<60 (%HRmax)	60-70 (%HRmax)	70-80 (%HRmax)	80-90 (%HRmax)	90-100 (%HRmax)
1	1	19.2	12.8	14.6	17.6	9.1
		8.3	6.4	6.3	8.8	6.0
1	2	20.7	10.7	11.2	16.7	8.0
		11.3	5.0	5.7	4.8	11.0
2	1	17.5	12.3	13.1	18.4	7.5
		14.4	4.8	4.4	7.9	8.3
2	2	22.1	14.8	13.2	14.2	7.2
		9.8	4.9	3.4	6.6	6.3
3	1	20.6	11.9	13.9	17.1	10.1
		9.8	6.5	3.7	5.9	2.7
3	2	15.9	13.2	15.8	17.3	9.1
		11.9	4.3	5.4	7.6	7.0

Table 5.3: Mean duration (min) spent at specific exercise intensities (%HRmax) during standard in-season soccer training sessions, ± SD, n = 12.

5.3.3 Effect of supplementation on pre-training hormonal response

Globally, the mean salivary C concentrations were significantly lower at 72 h relative to 0 and 24 h ($P<0.01$ and $P<0.05$, respectively; Figure 5.1a). *Post-hoc* analysis revealed that mean C concentrations were significantly lower at 72 h relative to 0 after ingestion of both HCHO (6.4 ± 0.8 vs. 12.3 ± 1.7 nmol.L⁻¹, respectively; $P<0.05$) and CHO-PRO (4.7 ± 0.9 vs. 12.7 ± 2.6 nmol.L⁻¹, respectively; $P<0.01$). There was no significant difference in C response between time points. A similar response in terms of the global difference over the time-frame of the training week was not evident in mean T or T/C response (Figure 5.1b and 5.1c, respectively). No significant differences within drinks were observed for T and T/C ratio data. Mean data are presented in Appendix V.

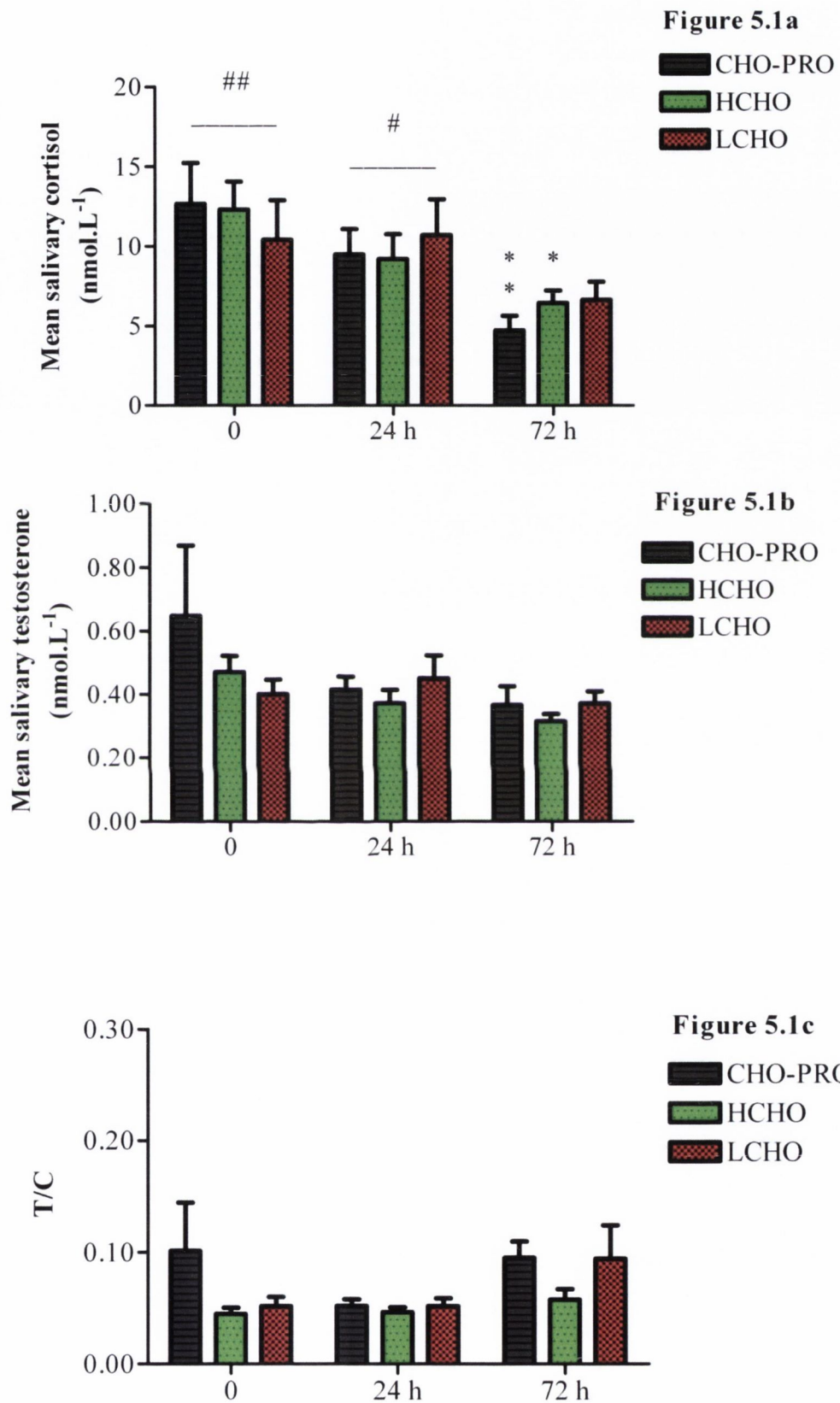


Figure 5.1: Hormonal response between training sessions (0, 24 and 72 h) across drink trials (mean $\pm$ SEM, $n = 12$). **(A)** Salivary C (nmol.L⁻¹) **(B)** Salivary T (nmol.L⁻¹) **(C)** Salivary T/C ratio. # $P < 0.05$ vs. 72 h; ## $P < 0.01$ vs. 72 h; * $P < 0.05$ vs. 0 within specific drink trial; ** $P < 0.01$ vs. 0 within specific drink trial.

5.3.4 Hormonal response pre- to post-ingestion

Globally, the mean salivary C concentrations pre- to post-drink were not significantly different (Figure 5.2a). However, a global time effect was reported for both mean T ($P<0.01$; Figure 5.2b) and T/C ratio ($P<0.05$; Figure 5.2c), demonstrating that both were significantly lower post-ingestion. *Post-hoc* analysis revealed that mean T concentration was significantly lower post-ingestion of LCHO (0.59 ± 0.07 vs. 0.45 ± 0.04 nmol.L⁻¹, respectively; $P<0.05$) and CHO-PRO (0.56 ± 0.06 vs. 0.43 ± 0.03 nmol.L⁻¹, respectively; $P<0.05$) drink trials but not for HCHO. *Post-hoc* analysis of T/C ratio data revealed that mean T/C ratio was significantly lower post-ingestion of LCHO (0.22 ± 0.05 vs. 0.14 ± 0.02 , respectively; $P<0.05$). Mean data are presented in Appendix V.

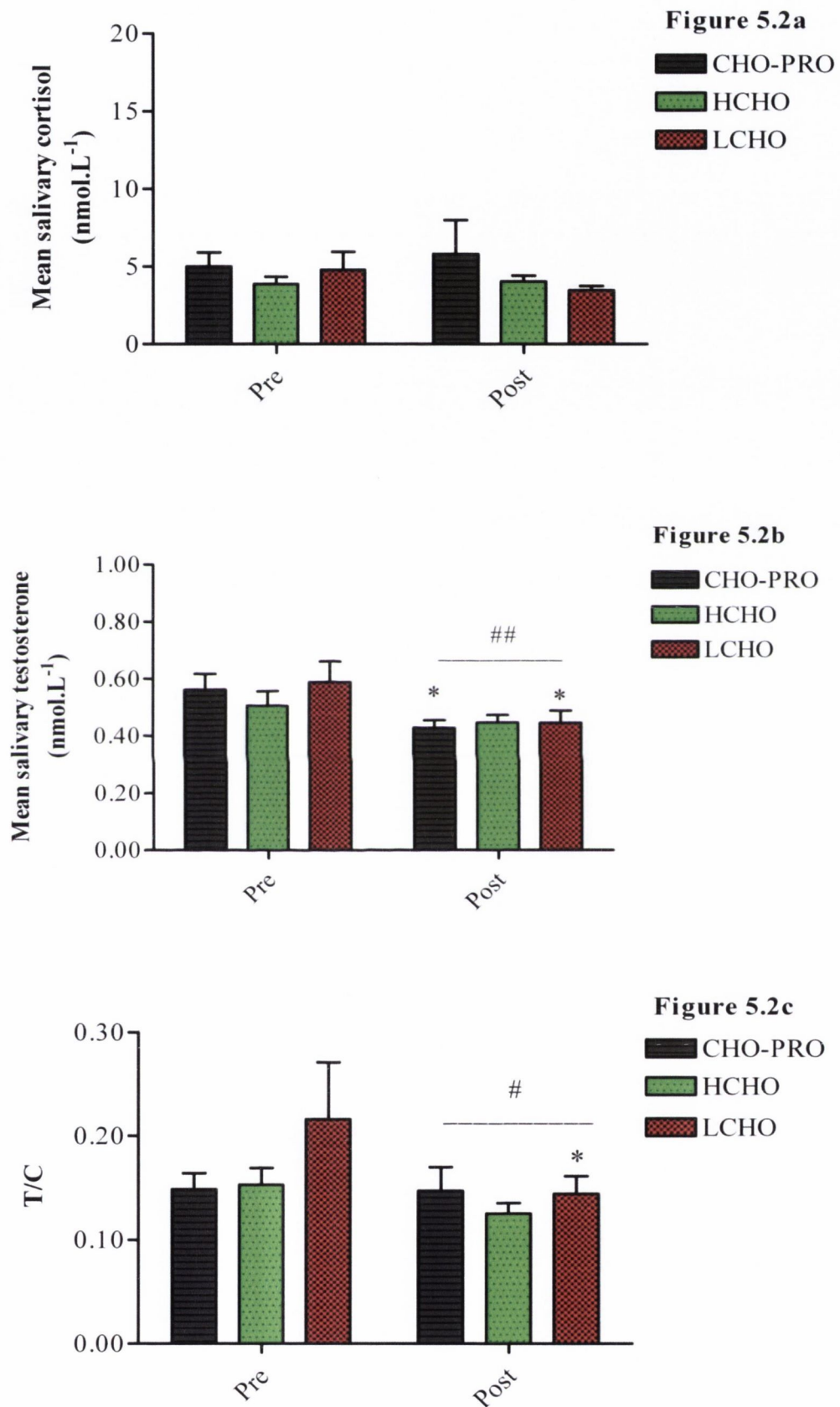


Figure 5.2: Pooled hormonal response pre- to post-ingestion of sports drinks (mean $\pm$ SEM, $n = 12 \times 2$). (A) Salivary C (nmol.L⁻¹) (B) Salivary T (nmol.L⁻¹) (C) Salivary T/C ratio. # $P < 0.05$ vs. Pre; ## $P < 0.01$ vs. Pre; * $P < 0.05$ vs. Pre within specific drink trial.

Chapter 5

5.4 Discussion

5.4.1 Summary of findings

Limited research has investigated post-match hormonal responses in soccer players (Ispirlidis *et al.*, 2008) and, to date, no research has investigated the short-term supplementation of sports drinks on hormonal markers of recovery in soccer players. This study proposed to evaluate the effect of a CHO-PRO beverage on salivary C and T concentrations compared to a CHO supplement alone (LCHO) and an iso-caloric equivalent to CHO-PRO supplement (HCHO) during standard, in-season soccer training.

Despite wide variability in hormonal concentrations from subject to subject, globally, the mean salivary C concentrations were significantly lower at rest on Day 4 (72 h) relative to Day 1 (0) and Day 2 (24 h) resting time points ($P<0.01$ and $P<0.05$, respectively; Figure 5.1a). Mean C concentrations were significantly lower at 72 h relative to 0 after ingestion of both HCHO (6.4 ± 0.8 vs. 12.3 ± 1.7 nmol.L⁻¹, respectively; $P<0.05$) and CHO-PRO (4.7 ± 0.9 vs. 12.7 ± 2.6 nmol.L⁻¹, respectively; $P<0.01$). One hour post-ingestion, mean T concentration was significantly lower during the LCHO (0.59 ± 0.07 vs. 0.45 ± 0.04 nmol.L⁻¹, respectively; $P<0.05$) and CHO-PRO (0.56 ± 0.06 vs. 0.43 ± 0.03 nmol.L⁻¹, respectively; $P<0.05$) drink trials only. The T/C ratio was significantly lower 1 h post-ingestion of LCHO (0.22 ± 0.05 vs. 0.14 ± 0.02 , respectively; $P<0.05$).

5.4.2 Effect of sports drinks on pre-training cortisol and testosterone

Availability of CHO in the immediate and short-term can affect the post-exercise response of C and can have a potentially positive impact on the athletes' ability to tolerate training load (Henson *et al.*, 1998; Bishop *et al.*, 1999a; Brooks *et al.*, 2005; Coutts *et al.*, 2007; Lane *et al.*, 2010). It appears that the consumption of CHO-PRO and HCHO drinks after in-season soccer training may have contributed to a significant decrease in salivary C concentration assessed throughout a standard training week. At the 72 h time point, the mean C response was significantly lower than rest and 24 h, possibly indicating a cumulative effect of the drinks in terms of modulating the C response to the training load. It must be noted that, the day

preceding the 72 h time-point, Day 3 of each training week, was a scheduled rest day and therefore, may have partly contributed to such a decreased C response. These results are in agreement with Henson *et al.* (1998) who reported a significant effect of CHO ingestion on the C response to endurance exercise.

The dietary intake and activity levels for participants in this study were not controlled in the intervening period between ingestion of the drink and collection of the 72 h sampling time point and may have contributed to the significantly decreased salivary C concentrations. This study was designed to be a 'free-living' and sport-specific study. However, research has reported C concentrations remained significantly increased compared to rest, on completion and 24 h after completion of a 3 day high-intensity training micro-cycle when participants consumed a low CHO content diet (Lane *et al.*, 2010). Significantly, Lane *et al.* (2010) reported no significant changes in resting C concentrations in participants on a CHO control diet. With due consideration for the findings of Lane *et al.* (2010), there appeared to be merit in the claim that ingestion of either CHO-PRO or HCHO drinks post-training, during an in-season training week can contribute to a significant reduction of the potential catabolic effects mediated by inadequate recovery via nutritional means and/or duration.

Within the CHO-PRO and HCHO trials, independent drink effects were observed at 72 h relative to rest (0), this was not evident for LCHO, possibly indicating that caloric intake, total CHO availability or the addition of PRO were sufficient to attenuate the expected post-exercise C increase. When CHO availability post-exercise is inadequate for restoration of glycogen stores and maintenance of BGlu, the C concentration increases (Brooks *et al.*, 2005; Lane *et al.*, 2010). In such an instance, muscle proteolysis and AA oxidation is stimulated by C so that BGlu concentrations can be maintained (Bishop *et al.*, 1999a; Brooks *et al.*, 2005; Mikulski *et al.*, 2010). In the current study, mean C concentrations had decreased significantly by 72 h relative to rest, see Figure 5.1a. A possible explanation for the suppressed C secretion after ingestion of the CHO-PRO and HCHO drinks post-exercise could be that adequate CHO availability suppressed the catabolic pathways for substrate liberation such as, NEFA and muscle proteolysis.

However, the mean T response was not significantly different across time. It could be argued that the T response was blunted post-exercise as a result of adequate CHO availability resulting in a suppressed post-exercise C response. Elloumi *et al.* (2003) previously stated that T has a protective effect against the catabolic effects of C. The availability of an exogenous source of CHO, and particularly PRO, in the immediate aftermath of training possibly limited the extent to which the actions of both hormones were required post-training as BGlu would have been sufficiently maintained. In addition, the fact that training load was tolerable may also have been a factor in the observed T response. Filaire *et al.* (2001) reported changes to C and T concentrations only after a high-intensity pre-season training period. In-season training was of a distinctly decreased load and during such a period, C concentration reportedly returned to resting levels (Filaire *et al.*, 2001). The mean T/C response did not attain statistical significance most likely due to the large variability in T and C concentrations at rest (Figure 5.1) and may be indicative of the players' ability to tolerate in-season training load (Coutts *et al.*, 2007). Filaire *et al.* (2001) reported significantly decreased in-season T concentrations when compared with resting levels but in similarity with the current study, no changes in their T/C ratio were reported in-season.

5.4.3 Acute response

Overall a cumulative effect of the drinks in terms of modulating the acute T response to soccer training was observed. Mean salivary T concentration was significantly lower post-ingestion of drinks potentially due to increased T clearance post-exercise (Chandler *et al.*, 1994; Kraemer *et al.*, 1998; Mikulski *et al.*, 2010). In addition, within both CHO-PRO and HCHO trials the mean salivary T response was reduced post-exercise. This is similar to Mikulski *et al.* (2010) reporting a significant decrease in T after ingestion of a high or low CHO content meal. Mikulski *et al.* (2010) stated that this clearance of T was most likely due to increased post-ingestion hepatic blood flow resulting in degradation of T by hepatocytes. Similarly, the acute T/C ratio response was lower post-ingestion of drinks in the current study. However, this was possibly due to large variability between participants' C responses and the observed decreases in T concentrations.

These results are similar to those published by Bishop *et al.* (1999a) and Moreira *et al.* (2009) reporting no effect of CHO ingestion on the C response to simulated match-play and wide inter- and intra-individual variability in C responses to a training situation. Bishop *et al.* (1999a) stated that when exercise intensity was moderate and changes in stress hormones and immune variables were minimal, CHO supplementation had a negligible effect. Moreira *et al.* (2009) suggested that differences in player's C responses to a training situation were due to the reduced emotional stress inherent within the competitive soccer match-play environment, and that stressors such as interactions with opponents, the match situation and importance of the game were lacking. It is, therefore, difficult to ascertain whether ingestion of sport drinks had an acute effect on players' ability to tolerate training load, as the C response to training would appear to be significantly affected by the lack of psychophysiological stimuli inherent in a competitive match-play situation (Cunniffe *et al.*, 2010).

5.4.4 Regulation of cortisol and testosterone

Urhausen *et al.* (1995) previously suggested that the T/C ratio in men is driven by the action of free-T. Recently, Lane *et al.* (2010) suggested that T responses were affected by SHBG concentrations and that the low CHO diet exacerbated the impact on PRO synthesis and glycogen resynthesis. Research has suggested that, should exercise continue in a situation where insufficient CHO availability affected circulating free-T, then accelerated and augmented endocrine responses in lipolytic regulatory hormones would result, potentially promoting lipolysis and providing available substrate (Hackney, 1996; Hackney, 2006). Evidently, C plays a key regulatory role in lipolysis and, along with a reduction in circulating free-T, could perpetuate the cycle of further suppressed T concentration through continued elevations in C concentration, adding to a growing disparity with each training session. Lane *et al.* (2010) suggested other potential reasons for their observed reduction in T/C ratio pre- to post-training with a low CHO diet, including reduction in LH and FSH as a result of the training sessions (Galbo *et al.*, 1979) as well as suppression by the stress hormone prolactin.

It is difficult to identify from individual players' T and C concentrations whether a typical T and C response was occurring. However, a circadian variation for C was

observed when evaluating pre-training data which, according to Filaire *et al.* (2001), coincided with the daily morning flux in natural C concentrations. Filaire *et al.* (2001) suggested that the circadian rhythm of C was potentially modified after a high-intensity training period so that C reached a peak later in the morning. Filaire *et al.* (2001) also reported significantly decreased salivary T concentration after the same period. Samples in the current study were collected at approximately 10:00, 12:00 and 13:00. This pre-training elevation of C concentration was also noticeable in data presented in Chapter 4. However, the elevated mean C concentrations observed at rest in this study and in Chapter 4 may be indicative of the match-play induced glycogen depletion that can last for up to 48 h post-match (Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a). It is possible, that recovery of muscle glycogen was limited in the post-match period due to training structure, as well as lifestyle factors, resulting in players presenting for training with less than optimal recovery. A typical training week for Sporting Fingal players consisted of consecutive early week training sessions with low-intensity training on Thursday preceding a Friday night league fixture. Wednesday and Sunday were typically rest days, and players typically performed a recovery session on Saturday. There was no significant difference detected in mean C concentration from 0 to 24 h, suggesting that players may still have been glycogen depleted in this period. Research has suggested that both inadequate time and low CHO availability between training sessions can result in elevated C concentrations (Lane *et al.*, 2010). In such a scenario, the C response may be faster and more robust in order to mobilise fuel (Galbo *et al.*, 1979) and last for up to 24 h post-exercise (Hackney, 2006). However, such an elevation in resting C concentrations may be indicative of a breakdown in homeostasis due to the strenuous and severe glycogen depleting nature of match-play and the lag in glycogen restoration (Elloumi *et al.*, 2003; Mohr *et al.*, 2003; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a).

5.4.5 Conclusions

In the current study, the acute T/C response was significantly different post ingestion of drinks but no cumulative difference was observed throughout the training week. However, mean salivary C was significantly reduced after ingestion of CHO-PRO and HCHO when compared with LCHO throughout a training week. Many external factors may have contributed to this result, including the preceding rest day prior to

the 72 h sample point and the significantly elevated C at rest, potentially indicating limited recovery from the previous league match. With due consideration to research available it appears that adequate availability of CHO, caloric intake, total CHO availability or the addition of PRO was sufficient to attenuate the expected post-exercise C increase throughout a training week. Considering previous research, it appears that such suppression of C concentrations may have been a cumulative effect of both the duration between training sessions and nutritional intervention. Clearly, a comparison of salivary hormone response during a training week with no nutritional intervention would allow for clearer determination of such a cumulative effect. However, although it appears that training load was tolerable during the in-season period in professional Irish soccer players; nutritional strategies may facilitate optimal recovery between matches when combined with adequate rest.

Chapter 6

The effect of carbohydrate versus carbohydrate-protein ingestion on hormonal markers of recovery in cycling.

Chapter 6

6.1 Introduction

6.1.1 Maintaining homeostasis in the athlete

Exercise of sufficient duration and physiological stress can result in immunosuppression (Fukatsu *et al.*, 1996; Gleeson & Bishop, 2000; Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010). Immunosuppression following exercise can also occur due to inadequate nutritional recovery with potential negative consequences for performances in both training and competition (Gleeson & Bishop, 2000; Meeusen *et al.*, 2004). Indeed, evidence suggests that inadequate CHO availability has negative implications for the athlete (Marcos *et al.*, 2003; Brooks *et al.*, 2005; Lane *et al.*, 2010). The optimum time for athletes to replenish their glycogen stores is immediately after exercise (Blom *et al.*, 1987; Ivy *et al.*, 1988; Price *et al.*, 1994; Jentjens *et al.*, 2001; Jentjens & Jeukendrup, 2003). Therefore, the correct nutritional recovery strategy can yield benefits for host immunity as well as subsequent performance. Ingestion of sports drinks before, during or after high-intensity exercise not only delayed fatigue and improved glycogen restocking between sessions (Fielding *et al.*, 1985; Nicholas *et al.*, 1995; Ivy *et al.*, 2002; Saunders *et al.*, 2004; Betts *et al.*, 2007; Sawka *et al.*, 2007), but also offset post-exercise disturbances in immune response possibly due to the role glucose plays in fuelling leucocytes; key elements in the response mechanism to tissue damage (Gleeson & Bishop, 2000). Such drinks can also play a role in post-exercise recovery by influencing the hormonal response to exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Tarpenning *et al.*, 2001).

During high-intensity training blocks or periods of functional OR, exercise intensities are sufficient to induce substantial stress on the physiological systems of the body (Meeusen *et al.*, 2006). It has been suggested that the hormonal and catecholamine response to exercise differs according to the training status of participants and the intensity, duration and nature of the exercise (Jensen *et al.*, 1991; Stone, 1991; Lac & Berthon, 2000; Arnason *et al.*, 2004; Mikulski *et al.*, 2008; Caldwell & Peters, 2009; Grandys *et al.*, 2009; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). However, a slight increase in training load can result in significant changes to an individuals' T/C ratio, which is an indicator of their tolerance to the

current training load (Halsen & Jeukendrup, 2004; Coutts *et al.*, 2007; Cormack *et al.*, 2008).

6.1.2 Substrate availability and hormonal response

Research by Mikulski *et al.* (2008) reported that low CHO availability, as a result of exercise and fasting, resulted in an elevated concentration of catecholamines in untrained individuals, which was diminished upon ingestion of a CHO meal. When insufficient exogenous CHO was available to replenish muscle glycogen or maintain BGlu concentrations, C was secreted to stimulate proteolysis and AA oxidation to provide substrate (Hackney, 1996; Brooks *et al.*, 2005; Hackney, 2006). Both C and T play particularly important roles in the post-exercise physiology of recovery (Krieger & Allen, 1975; Urhausen *et al.*, 1995; Bishop *et al.*, 1999a) and, in some instances, can remain altered for significant periods post-competition (Elloumi *et al.*, 2003; Cunniffe *et al.*, 2010). C acts in tandem with GH to enhance leptin synthesis so that energy balance is maintained (Mikulski *et al.*, 2010). T reportedly has a protective effect against the proteolytic effect of C (Elloumi *et al.*, 2003). Research investigating hormonal responses to glycogen depletion and limited CHO availability has reported varying responses. Mikulski *et al.* (2010) reported no effect of glycogen depletion on the serum C or T response in trained individuals, however, insulin was significantly decreased. According to Mikulski *et al.* (2010), decreased insulin secretion results in enhanced lipolysis, proteolysis and gluconeogenesis and an attenuation of peripheral glucose uptake. Recent research by Lane *et al.* (2010) illustrated the effect of insufficient CHO intake between exercise bouts, reporting a decreased T/C response after a 3 day micro-cycle of high-intensity training with low dietary CHO intake.

Depletion and subsequent restoration of body CHO stores is one of the key elements of the training process (Mikulski *et al.*, 2010). Low muscle glycogen levels have been reported to enhance transcriptional activation of some metabolic genes in response to exercise, raising the possibility that signalling mechanisms sensitive to glycogen content or free fatty acid availability may be linked with the transcriptional control of exercise-responsive genes (Pilegaard *et al.*, 2002). Ivy *et al.* (2002) reported significantly decreased muscle glycogen content following a 2 h cycling protocol which was more significantly restored post-ingestion of a CHO-PRO

supplement compared with HCHO and LCHO supplements. Ingestion of CHO-PRO has also been reported to improve BGlu availability in subsequent exercise sessions thus resulting in greater performance capacity compared with CHO alone (Betts *et al.*, 2007). The additional PRO may also improve performance by improving the central drive for exercise in individuals (Ivy *et al.*, 2002; Betts *et al.*, 2007). CHO-PRO ingestion also reduced muscle damage in trained cyclists compared to CHO alone (Saunders *et al.*, 2004) while Henson *et al.* (1998) reported a significantly higher C response after ingestion of a placebo compared with CHO following 2.5 h of high-intensity running.

6.1.3 Aims and hypothesis

In periods of functional-OR training, athletes may perform numerous training bouts with limited recovery time between each training session, placing great demands on host immunity (Gleeson & Bishop, 2000; Lane *et al.*, 2010; Mikulski *et al.*, 2010). Greater emphasis is placed on recovery during such training cycles because the success of the training cycle and subsequent performance is dependent on the quality of training (Halson & Jeukendrup, 2004; Mujika *et al.*, 2004). Both intermittent and steady-state cycling resulted in significantly increased secretion of C and when inadequate CHO availability is combined with inadequate rest intervals post-exercise, a catabolic hormonal response can occur even during relatively short, high-intensity training cycles (Lane *et al.*, 2010; Hough *et al.*, 2011). Nutritional interventions should be encouraged during such periods of overload, or where intervals between competition and training are short in order to minimise the effects of reduced glycogen stores on performance and homeostasis, so that quality of performance in training is maintained (Saunders *et al.*, 2004; Brooks *et al.*, 2005; Krstrup *et al.*, 2006a; Betts *et al.*, 2007; Mikulski *et al.*, 2008; Lane *et al.*, 2010; Mikulski *et al.*, 2010).

Recent research has investigated the effect of CHO ingestion alone in a similar protocol (Lane *et al.*, 2010). Ingestion of a CHO-PRO supplement post-exercise presents the most advantageous means of maximising glycogen resynthesis, maintaining subsequent performance (Ivy *et al.*, 2002; Betts *et al.*, 2007) and may decrease the cumulative catabolic response to intense exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Lane *et al.*, 2010; Mikulski *et al.*, 2010). The aim of this study

was to investigate the effect of different commercially available sports drinks on salivary C and T responses during recovery from two exercise bouts completed in a period of limited nutritional intake and rest. The hypothesis was that ingestion of a CHO-PRO sports drink after two bouts of high-intensity exercise would result in more effective recovery, when compared with an iso-caloric CHO drink (HCHO) or a PL drink, indicated by a more positive T/C ratio, by means of suppressing the reported exercise induced increase in salivary C concentration as a result of an enhanced insulin response and increased BGlu availability post-ingestion.

Chapter 6

6.2 Methods

6.2.1 Experimental design

The protocol involved a single-blinded, repeated measures design. After signing informed consent, participants visited the Human Performance Laboratory on 4 occasions. After an initial assessment of $\dot{V}O_2\text{max}$, participants completed an exercise trial that included an intermittent cycling protocol (GD) intended to induce glycogen depletion and a time to failure exercise trial (ExTr) on consecutive evenings and mornings. On completion of the GD protocol participants received a standardised meal, after which, they fasted overnight. The following morning, participants presented at the laboratory to begin the ExTr. At the end of the ExTr participants ingested one of three drinks under investigation and remained resting for a 60 min monitored recovery period. The study design and experimental procedures involved in this study were approved by Faculty Research Ethics Group, Trinity College Dublin.

6.2.2 Participants

Eleven cyclists and triathletes volunteered for this exercise study. However, only 7 completed all three exercise trials ($n = 7$). Participants had been training and competing in either triathlon or cycling for the previous 2 seasons. Each participant was informed of all procedures and any potential risks associated with the study before written consent was provided prior to testing. All testing took place between January and August in the Human Performance Laboratory, Trinity College Dublin.

6.2.3 Inclusion and exclusion criteria

Subject to passing the prerequisite medical assessment, individuals were deemed fit to complete the $\dot{V}O_2\text{max}$ test and subsequent exercise trials. Individuals suffering from serious musculoskeletal injuries or deemed unfit to participate on completion of the medical examination were excluded. Volunteers with diabetes, hypertension, heart defects, any metabolic disorders, chronic sports injury or any other contra-indicatory symptoms were excluded from the study.

6.2.4 Experimental protocol

Each individual's $\dot{V}O_2\text{max}$ was determined several days prior to the first scheduled exercise trial so that individualised workloads could be calculated. Participants presented themselves at the Human Performance Laboratory in a rested, well-hydrated state on evenings of exercise trials. Pre-test fluid intake, diet and training were standardised in the 24 h prior to each trial to limit extrinsic effects on trial outcomes. The exercise trials consisted of the GD protocol, previously employed by Vøllestad *et al.* (1992). The following morning, after an overnight fast (~14 h), participants presented themselves at the Human Performance Laboratory to perform the steady-state ExTr protocol, similar to that utilised by Pedersen *et al.* (2008). All exercise trials were performed on an electronically loaded, Excalibur Sport cycle ergometer (Lode, Groningen, The Netherlands). Participants ingested a CHO controlled meal after the GD phase on each visit. Participants were permitted to drink water *ad libitum* throughout both exercise periods, the volume of which was recorded. On completion of the ExTr, participants received a bolus of assigned drink and remained resting during the 60 min monitored recovery period. Participants voided prior to, and after exercise trials and the recovery period so that urine volume could be recorded. All drinks trials were separated by a minimum of 7 and a maximum of 14 days.

6.2.5 Maximal oxygen uptake test

Each participant underwent an incremental $\dot{V}O_2\text{max}$ test to volitional exhaustion to determine loads at equivalent percentage $\dot{V}O_2\text{max}$ (70 and 120%) for their subsequent exercise trials. On arrival at the laboratory each participant completed a detailed medical questionnaire (Appendix II) and underwent a detailed medical examination to rule out any contraindications to maximal exercise. Cardiovascular and respiratory variables were assessed; in addition, the attending physician examined their throat and lymph glands. A pulmonary function test was performed using a spirometer (Microlab ML3500S, Micro Medical, Kent, UK). Anthropometric assessment of height (m), BM (kg), percentage body fat and BMI (kg.m^2) were performed as outlined in Section 2.1. A blood sample was collected from the cubital vein using the Vacutainer system and a full haematological assessment was performed, as described in Section 2.2. The electronically loaded

Excalibur Sport cycle ergometer (Lode, Groningen, The Netherlands) was adjusted according to the participants' needs before beginning a 12 min warm-up at a nominal workload of 120 W.

The incremental test began with 3 min at rest during which time the participant was in his cycling position. On completion of the 3 min rest period, the participant began cycling at a load of 120 W; the load subsequently increased by 40 W at 3 min intervals until volitional exhaustion. The participant wore a HR monitor (Cardiosport, Wrexham, UK), as described in Section 2.5, and HR ($\text{beats}\cdot\text{min}^{-1}$) was recorded at 30 s intervals in the final minute of each 3 min increment. To allow gas exchange variable to be recorded during all stages of the incremental test, the participant wore a V2 facemask (Cosmed, Rome, Italy) connected to a Quark B² (Cosmed, Rome, Italy) breath-by-breath pulmonary gas exchange analyser. This system consisted of a flowmeter, a Zirconia O₂ analyser and an infra-red CO₂ analyser. The flowmeter used a bi-directional digital turbine with air passing through helical conveyors, causing the rotation of the turbine rotor. The rotating blade interrupted an infrared light beam emitted by the three diodes of the optoelectronic reader. Every interruption represented a 16.6% turn of the rotor; this allowed for accurate measurement of the number of rotations in a fixed time ($\text{rev}\cdot\text{s}^{-1}$). There was a constant ratio between the air passing through the turbine and the number of turbine rotations thus allowing for the accurate measurement of volume and flow rate. The Zirconia-oxygen analyser had a range of 1 to 100% O₂, a response time of <120 ms and an accuracy of <0.05% O₂. The fast CO₂ analyser measured CO₂ concentration by infra-red radiation absorption (response time <150 ms). The CO₂ that passed through the sensor cell absorbed a certain amount of radiation; the absorption was proportional to the quantity of CO₂ in the sample line.

Upon reaching volitional exhaustion, participants performed a cool-down over a 10 min period at 120 W. Exhaustion was defined as the participant being unable to maintain sufficient cadence ($<60 \text{ rev}\cdot\text{min}^{-1}$) for more than 10 s. The peak value of individual $\dot{V}\text{O}_2\text{max}$ ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), as calculated by the software, was subsequently recorded. Post-exercise lung function data were performed to rule out exercise-induced broncho-constriction. Individual T_{LAC} was determined from the BLa data

collected from capillary blood samples during the last minute of each test increment completed, as outlined in section 2.2.1. T_{LAC} was derived from graphical interpolation using the V-slope method (Beaver *et al.*, 1986).

6.2.6 Intermittent exercise protocol

After completing a 10 min warm-up at 120 W, participants began the GD protocol. The GD trial was an intermittent protocol consisting of 30 min at a workload of 70% $\dot{V}O_{2max}$, three sprints ($\sim 120\%$ $\dot{V}O_{2max}$) of 1 min duration with a 2 min recovery interval at 180 W before completing a further 45 min at 70% $\dot{V}O_{2max}$. If participants failed to complete the duration of the GD trial, time to exhaustion was recorded with exhaustion being defined as the athlete being unable to maintain the required workload for 10 s. On completion of this GD trial, and after providing a saliva sample, participants consumed a pre-determined meal consisting of tuna in sunflower oil (200 g.70 kg⁻¹ BM), egg fried rice (250 g.70 kg⁻¹ BM), fortified whole milk (250 mL.70 kg⁻¹ BM) and cheese cake (125 g.70 kg⁻¹ BM). Participants were instructed to consume only water overnight after this low CHO meal (46% CHO, 29% PRO and 25% fat).

The post-GD meal provided participants with 3.6 g.kg⁻¹ BM of egg-fried rice, 2.9 g.kg⁻¹ BM of tuna in sunflower oil, 1.8 g.kg⁻¹ BM of cheese cake and 3.6 mL.kg⁻¹ BM of fortified, whole-milk. This meal provided 5.8, 5.4, 8.9 and 2.3 kCal.kg⁻¹ BM for egg-fried rice, tuna in sunflower oil, cheese cake and fortified, whole-milk, respectively. All participants consumed this post-GD meal in its entirety.

6.2.7 Exercise trial

The following morning participants arrived at the Human Performance Laboratory in a fasted state. Prior to exercise, an intravenous catheter was inserted into a forearm vein by a medical practitioner. After performing a 10 min warm-up at 120 W, the ExTr began. The ExTr consisted of a cycle to volitional exhaustion at a workload equivalent to 70% $\dot{V}O_{2max}$. Exhaustion was defined as the participant being unable to maintain the required workload for more than 10 s. During the ExTr, $\dot{V}O_2$ was measured during the final 3 min of every 15 min interval completed. On completion of the ExTr participants consumed an assigned drink and rested passively for 60 min.

6.2.8 Insertion of the IV catheter

To ease access of blood sampling and to reduce the invasive nature of repeated blood sampling during the ExTr, an indwelling catheter was inserted into a forearm vein. The peripheral venous catheter (20GA 1.1 x 30mm, Insite Autoguard, Becton Dickinson, Oxford, UK) was inserted by a medical practitioner. The area of insertion was thoroughly wiped with a 70% w/v isopropyl alcohol swab (Medi-Swab, Smith & Nephew, London, UK). Having inserted the catheter into the forearm vein, a 6 inch Luer-Lok extension set (Posiflow Luer-Lok, Becton Dickinson, Oxford, UK) was attached. A yellow 'bung' with injection membrane (Vygon, Swindon, UK) was attached to the end of the Luer-Lok extension set for use during sampling. The catheter and extension set were secured in place using Alupore hypoallergenic tape (Smith & Nephew, London, UK). A blood sample was drawn immediately using the previously described Vacutainer system (Section 2.2) and the catheter was kept patent by flushing with 0.9% w/v BP NaCl solution (Antigen International, Roscrea, Ireland).

6.2.9 Heart rate data

Participants HR (beats.min⁻¹) data was recorded by radio-telemetry (Section 2.5) using a Cardiosport heart rate monitor (Cardiosport, Wrexham, UK) at 10 min intervals throughout both the GD and ExTr protocols. Ultrasound transmission gel (Aquasound 100, Parker Laboratories, New Jersey, USA) was applied to the transmitter electrodes on the chest strap to assist with constant readings.

6.2.10 Blood and saliva sample collection

Saliva samples were collected, as outlined previously (Section 2.6), pre- and post-GD protocol to determine salivary concentrations of C and T. Participants provided saliva samples before beginning the ExTr (Pre), on completion of the ExTr (Post) and at the end of the 60 min monitored recovery period (+60 min). Capillary blood samples were collected, as described in Section 2.2.1, before and after the GD protocol for determination of BGlu and BLa concentrations. During the ExTr blood samples were drawn through the indwelling IV catheter inserted in the participants arm prior to beginning the warm-up. Samples were collected before ExTr (Pre), on completion of exercise (Post) and at the end of the 60 min monitored recovery period (+60 min) to correspond with saliva sample collection. Additional blood samples

were collected at 10 min intervals during the ExTr for determination of BL_a and BGlu, and at 15 min intervals during the 60 min monitored recovery period to determine BGlu, BL_a and plasma insulin responses in the post-exercise period.

6.2.11 Blood lactate and glucose analysis

Measurements of BL_a and BGlu concentrations (mmol.L⁻¹) were performed as described in Sections 2.3 and 2.4, respectively.

6.2.12 Sports drinks

Participants received 10 mL.kg⁻¹ BM bolus of sports drink or placebo on completion of the ExTr protocol and all drinks were consumed within 10 min. HCHO consisted of a commercially available carbohydrate-electrolyte sports drink (Club Energise Sport, Britvic, Ireland) with added glucose providing 8.37 g CHO.100 mL⁻¹. CHO-PRO consisted of a commercially available CHO-PRO sports drink (Powerbar Recovery, Nestle, Vevey, Switzerland) providing 6.2 g CHO.100 mL⁻¹ and 2.17 g PRO.100 mL⁻¹. Placebo was a sugar free cordial diluted at a ratio of 10 parts water to 1 part cordial (MiWadi, Britvic, Dublin, Ireland). Both HCHO and CHO-PRO were volumetrically and iso-calorically equivalent.

6.2.13 Salivary cortisol and testosterone

Salivary C and T concentrations were assessed by ELISA technique and results in nmol.L⁻¹ were computed as outlined in Section 2.7.

6.2.14 Serum insulin

Serum samples were assayed for insulin using an ultrasensitive sandwich type immunoassay ELISA kit (Insulin Ultrasensitive ELISA, ALPCO Diagnostics, Salem, USA). The kit operates on the principle of competition between insulin in the sample and the enzyme-labelled antibodies for a limited number of antibody binding sites on the microwell plate. The ELISA kit had a sensitivity of 0.398 µIU.mL⁻¹ with a standard range of 3 to 200 µIU.mL⁻¹.

A 96-well microplate coated with a monoclonal antibody specific for insulin was used. Known standards of insulin, diabetes controls and serum samples were added

to the microplate wells simultaneously with the detection antibody (Horseradish peroxidase enzyme-labelled monoclonal antibody). The microplate was incubated on an orbital plate shaker at 700 to 900 rev.min⁻¹. After incubation, the wells were washed with wash buffer and blotted dry. TMB substrate was added and the microplate was incubated a second time, again on an orbital plate shaker at 700 to 900 rev.min⁻¹. On completion of the second incubation period, stop solution was added and the OD was measured by a micro plate reader (Biotek, Bedfordshire, UK) between 450 and 630 nm. The intensity of the colour generated was directly proportionate to the amount of insulin in the sample.

6.2.14.1 Assay procedure

The 96-well microplate along with reagents and samples were brought to room temperature. Serum samples were centrifuged (Sigma Laboratory Centrifuges, Osterode am Harz, Germany) for 10 min at 15000 rev.min⁻¹, while all reagents and controls were vortexed prior to use, as per manufacturer guidelines. A standard curve was included with each assay plate and all standards, controls and samples were run in duplicate. The standard curve included known insulin concentrations; zero, 3, 10, 30, 100 and 200 μ IU.mL⁻¹. Diabetes controls of high and low insulin concentrations were also included. 25 μ L of each standard, control and sample were transferred into their respective wells and 100 μ L of detection antibody was then added to each well using a multi-channel pipette. The plate was covered with a plate sealer and incubated at room temperature for 1 h on an orbital plate shaker at 700 to 900 rev.min⁻¹.

On completion of this incubation period, the microplate was washed 6 times with working strength wash buffer and blotted thoroughly between washes on absorbent paper towels. Using the multi-channel pipette, 100 μ L of TMB substrate was then added to each well. The plate was immediately covered with a plate sealer and incubated for 30 min at room temperature on an orbital plate shaker set to 700 to 900 rev.min⁻¹. On completion of the second incubation period, 100 μ L of stop solution was added to each well using a multi-channel pipette and the plate was gently shaken to ensure the stop solution mixed completely. The OD was measured using a platereader (Biotek, Bedfordshire, UK) between 450 and 630 nm within 30 min of the addition of the stop solution.

The insulin ELISA was a ligand binding assay, with response exhibiting a sigmoidal relationship to the analyte concentration. A standard curve was constructed by plotting mean OD against concentration. A logarithmic curve fit yielded correlation coefficient data. Following manipulation, the equations were applied to determine the concentration of insulin in each sample.

6.2.15 Statistical Analysis

Physical characteristics are presented as mean $\pm$ SD and all graphical data as mean $\pm$ SEM. The AuC for cumulative BGlu (mmol.min.L⁻¹) and cumulative insulin (μ IU.min.mL⁻¹) responses, respectively, were computed by calculating area relative to baseline (Post-ExTr concentration) across time using the trapezoidal method (Graphpad Prism, La Jolla, USA). Statistical analyses of plasma insulin, salivary C and T concentrations, BGlu and BLa data across time and intervention (time by drink) were performed using a 2-factor repeated measures ANOVA with *post-hoc* Tukey tests to quantify identified significant differences. Statistical analysis of performance indices were analysed using a single factor repeated measures ANOVA with *post-hoc* Tukey tests to quantify identified significant differences. Statistical analysis was performed using SigmaStat (SigmaStat, SanJose, USA) and the α level to infer significance was set with $P < 0.05$.

Chapter 6

6.3 Results

6.3.1 Participants

Of the 11 participants that volunteered for this study, only 7 completed all three exercise trials and their performance data were included in analysis. Four volunteers completed $\dot{V}O_2\text{max}$ tests but were unable to participate in the exercise trials due to training commitments and scheduling difficulties. Physical characteristics for the remaining study participants ($n = 7$) that completed all three trials are presented as mean $\pm$ SD while all metabolic and performance data are presented as mean $\pm$ standard error of the mean $\pm$ SEM. Participants' physical characteristics and performance indices are presented in Table 6.1 and 6.2, respectively. Mean data for this chapter are presented in Appendix VI.

Participant	Age (yr)	Body Mass (kg)	Height (m)	Body Fat %	BMI (kg.m ²)
Mean	30	82.1	1.84	13.3	24.5
SD	7	6.4	0.04	4.4	2.2

Table 6.1: Mean physical characteristics for participants that completed all three supplementation trials, $\pm$ SD, $n = 7$.

Participant	$\dot{V}O_2\text{max}$ (mL.kg ⁻¹ .min ⁻¹)	Load at T _{LAC} (W)	70% $\dot{V}O_2\text{max}$ (W)	Sprint (W)
Mean	58.7	283	253	432
SEM	1.6	13	8	13

Table 6.2: Mean performance indices for participants that completed all three supplementation trials, $\pm$ SEM, $n = 7$.

6.3.2 Blood lactate data

Mean BLa concentrations (mmol.L⁻¹) were significantly higher post-GD compared with rest across all trials (Post-GD: 3.6 ± 0.9, 3.0 ± 0.5 and 3.1 ± 0.8 mmol.L⁻¹ for CHO-PRO, HCHO and PL, respectively: *P*<0.05, Figure 6.1). Also, mean BLa response was significantly higher post-exercise during the ExTr than all other time points, across all drinks (3.0 ± 0.4, 2.3 ± 0.3 and 2.4 ± 0.3 mmol.L⁻¹, for CHO-PRO, HCHO and PL, respectively; *P*<0.001, Figure 6.2).

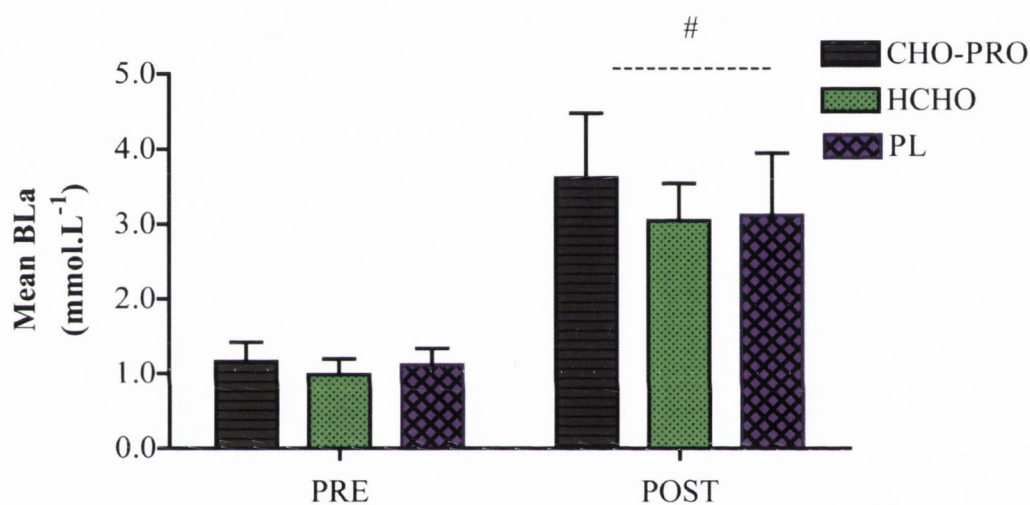


Figure 6.1: Mean BLa (mmol.L⁻¹) response from pre- to post-exercise and across drink trials during the GD protocol, bars denote SEM, n = 7. # *P*<0.05 vs. PRE.

Mean BLa concentration was not significantly different across drink trials at any other time point during the 60 min monitored recovery period. Mean BLa recorded post-exercise in the CHO-PRO trial was significantly higher compared with corresponding data for HCHO and PL trials (3.0 ± 0.4 vs. 2.3 ± 0.3 and 2.4 ± 0.3 mmol.L⁻¹, respectively; $P<0.05$, Figure 6.2). Also, mean BLa during the CHO-PRO trial was significantly lower at rest when compared to all other measured time-points ($P<0.05$). During the HCHO trial, mean resting BLa was significantly lower compared to +15 and +60 min of the monitored recovery period ($P<0.05$). Mean resting BLa data during the PL trial were significantly lower than post-exercise across all time-points ($P<0.001$).

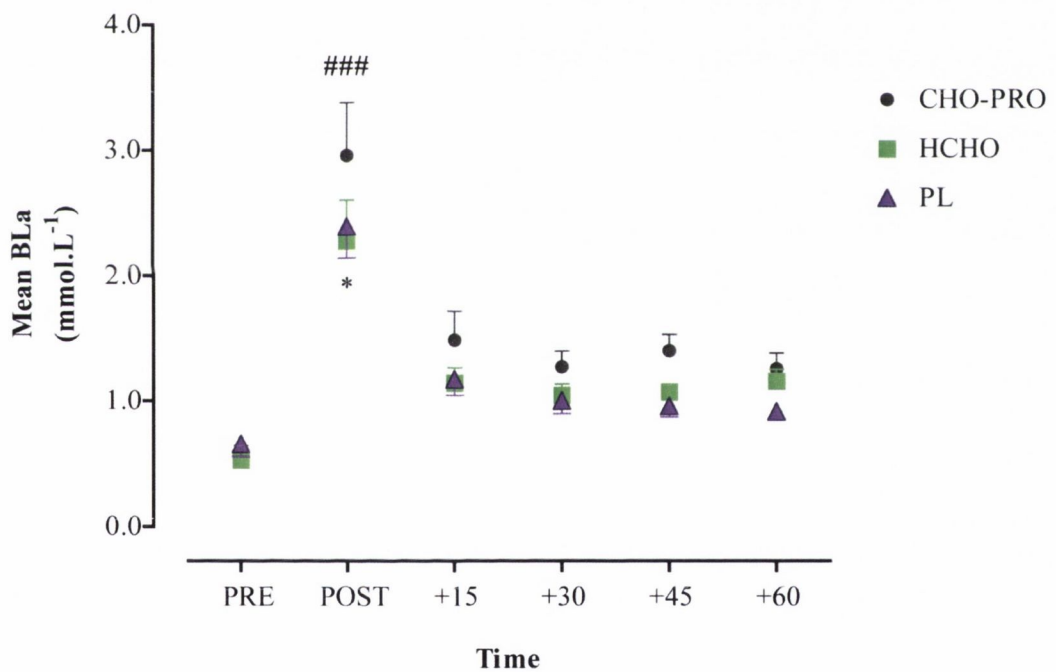


Figure 6.2: Mean BLa (mmol.L⁻¹) response pre- to post-ExTr and during the 60 min monitored recovery period across measured time points and drink trials, bars denote SEM, n = 7. Significantly lower than CHO-PRO, * $P<0.05$. Significantly higher than PRE, ### $P<0.001$.

6.3.3 Blood glucose data

Mean BGlu concentrations (mmol.L⁻¹) recorded pre- to post-GD were not significantly different across drink or time (Figure 6.3).

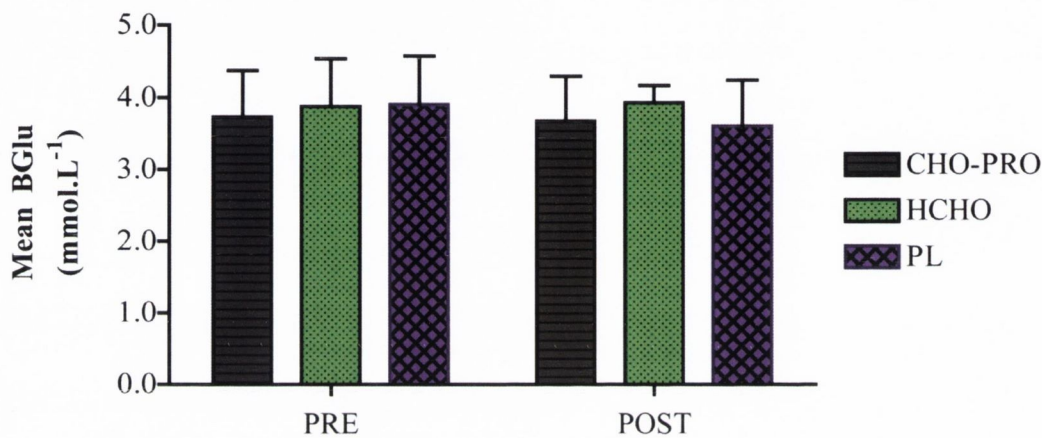


Figure 6.3: Mean BGlu (mmol.L⁻¹) response during the GD protocol from pre- to post-exercise and across drink trials, bars denote SEM, n = 7.

Mean BGlu concentrations revealed differing responses to each of the test drinks during the 60 min monitored recovery period (Figure 6.4). Ingestion of HCHO and CHO-PRO induced significantly higher mean BGlu concentrations by +15 min compared with PL (5.6 ± 0.2 and 5.3 ± 0.4 vs. 4.1 ± 0.1 mmol.L⁻¹, $P<0.001$ and $P<0.05$, respectively; Figure 6.4). Mean BGlu had further increased at +30 min post-ingestion of HCHO and CHO-PRO and remained significantly higher than PL (6.4 ± 0.4 and 6.1 ± 0.4 vs. 4.0 ± 0.1 mmol.L⁻¹, respectively; $P<0.001$). By +45 min, mean BGlu concentration in the CHO-PRO trial began to decrease and was significantly lower than that observed post-ingestion of HCHO (5.0 ± 0.5 vs. 6.2 ± 0.5 mmol.L⁻¹, respectively; $P<0.05$), yet BGlu concentrations in both the HCHO and CHO-PRO trials remained significantly higher than those observed in the PL trial (6.2 ± 0.5 and 5.0 ± 0.5 vs. 3.9 ± 0.1 mmol.L⁻¹; $P<0.05$ and $P<0.001$, respectively). By the end of the 60 min monitored recovery period, mean BGlu concentrations post-ingestion of CHO-PRO and HCHO had further decreased. Mean BGlu concentration observed at +60 min in the HCHO trial remained significantly elevated compared to BGlu concentrations observed in the CHO-PRO and PL trials

(5.4 ± 0.4 vs. 4.2 ± 0.2 and 3.9 ± 0.1 mmol.L⁻¹, $P<0.05$ and $P<0.001$, respectively). However, mean BGlu concentration 60 min post-ingestion of CHO-PRO had decreased to a concentration similar to that recorded post-ingestion of PL and, therefore, concentrations were not significantly different between drinks at +60 min.

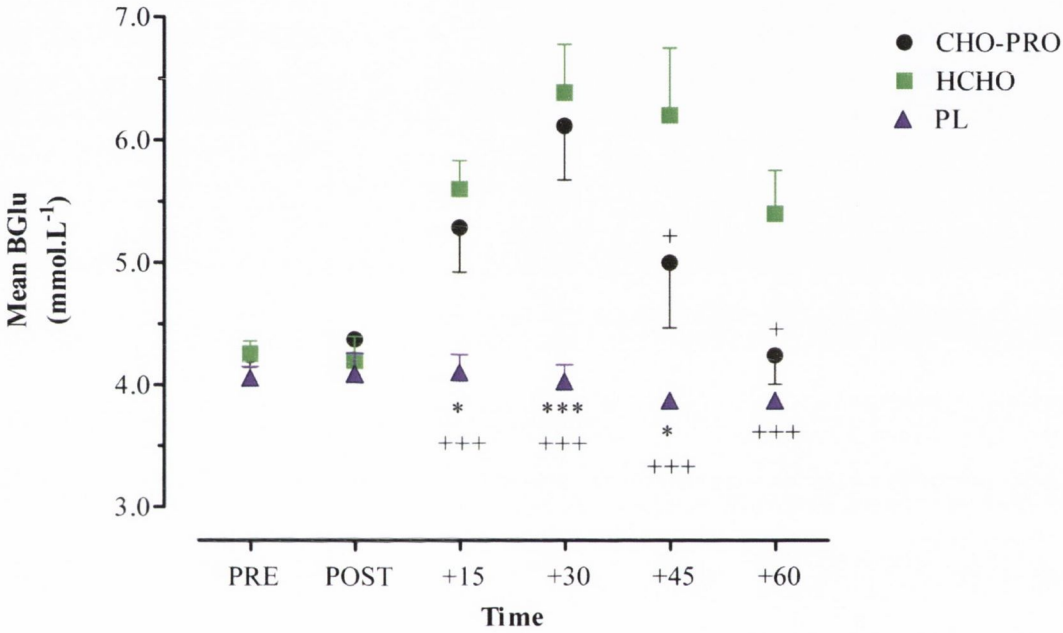


Figure 6.4: Mean BGlu (mmol.L⁻¹) response pre- to post-ExTr and through the 60 min monitored recovery period across measured time points and drink trials, bars denote SEM, n = 7.

* $P<0.05$, *** $P<0.001$ vs. CHO-PRO. + $P<0.05$, +++ $P<0.001$ vs. HCHO.

Mean BGlu concentrations within the CHO-PRO trial were significantly higher 30 min post-ingestion when compared with rest, post-exercise, 60 min post-ingestion ($P<0.001$) and 45 min post-ingestion ($P<0.05$). Mean BGlu 15 min post-ingestion of CHO-PRO was also significantly higher than pre- and post-exercise, as well as the +60 min time-point ($P<0.05$). During the HCHO trial, mean BGlu concentration was significantly higher at all time points post-ingestion (+15, +30, +45 min; $P<0.001$, and +60 min; $P<0.05$) while mean BGlu concentrations 60 min post-ingestion were significantly lower than 30 min post-ingestion ($P<0.05$). There were no significant differences in mean BGlu observed across time points during the PL trials.

6.3.4 Insulin

Mean serum insulin ($\mu\text{IU.mL}^{-1}$) response post-ingestion of drinks revealed significant differences between the HCHO, CHO-PRO and PL trials during the 60 min monitored recovery period (Figure 6.5). At 15 min post-ingestion, mean serum insulin in the HCHO trial was significantly higher than that observed in the PL trial (21.0 ± 8.5 vs. $4.5 \pm 0.7 \mu\text{IU.mL}^{-1}$, respectively; $P < 0.05$) but was not significantly different from the concentration recorded at the same time point in the CHO-PRO trial ($17.2 \pm 7.6 \mu\text{IU.mL}^{-1}$). Mean serum insulin concentrations 30 min post-ingestion of CHO-PRO and HCHO were significantly higher than PL (34.7 ± 7.5 and 24.5 ± 6.8 vs. $2.6 \pm 0.7 \mu\text{IU.mL}^{-1}$, $P < 0.001$ and $P < 0.05$, respectively) but not significantly different when compared to each other. Similarly, mean serum insulin concentrations recorded at 45 min post-ingestion of HCHO and CHO-PRO remained significantly elevated compared to PL (23.2 ± 5.2 and 21.5 ± 7.2 vs. $1.7 \pm 0.6 \mu\text{IU.mL}^{-1}$, respectively; $P < 0.05$). Serum insulin in the CHO-PRO trial had decreased by the end of the 60 min monitored recovery period ($13.3 \pm 4.9 \mu\text{IU.mL}^{-1}$) and was no longer significantly different when compared to PL ($1.7 \pm 0.6 \mu\text{IU.mL}^{-1}$). However, mean serum insulin at the end of the 60 min monitored recovery period in the HCHO trial remained significantly higher than that observed in the PL trial (18.2 ± 3.8 vs. $1.7 \pm 0.6 \mu\text{IU.mL}^{-1}$, respectively; $P < 0.05$).

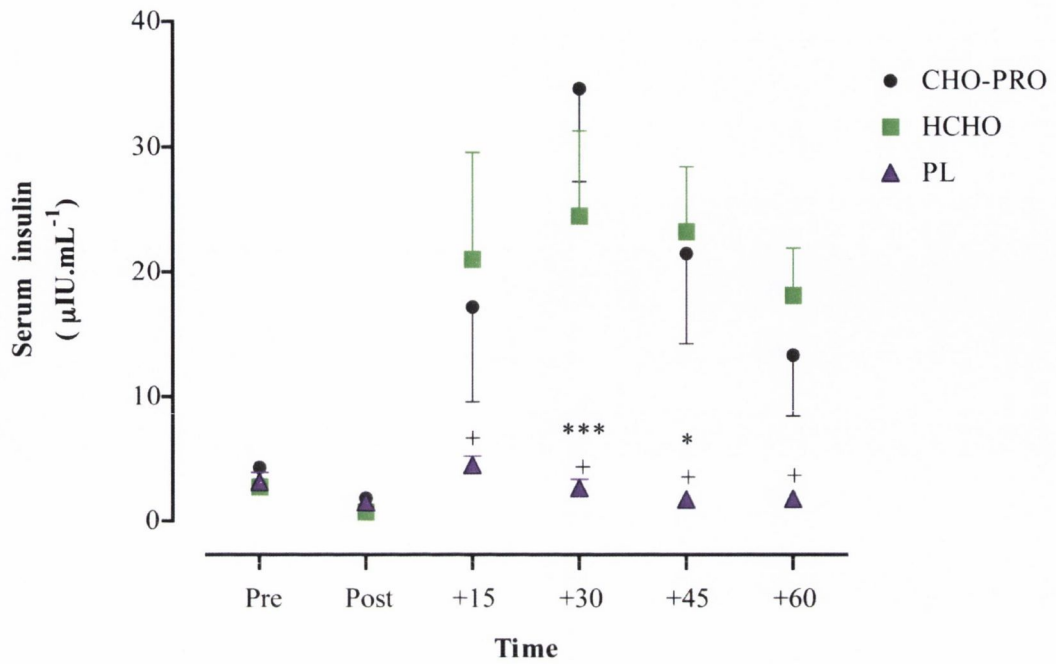


Figure 6.5: Mean serum insulin ($\mu\text{IU}\cdot\text{mL}^{-1}$) response pre- to post-ExTr and through the 60 min monitored recovery period across measured time points and drink trials, bars denote SEM, $n = 7$. * $P < 0.05$, *** $P < 0.001$ vs. CHO-PRO. + $P < 0.05$ vs. HCHO.

Mean serum insulin 30 min post-ingestion of CHO-PRO was significantly higher than rest and post-exercise ($P < 0.001$) and 15 and 60 min post-exercise ($P < 0.05$). Mean serum insulin recorded 45 min post-ingestion of CHO-PRO was significantly higher than rest and post-exercise ($P < 0.05$) while data at 15 min post-exercise were significantly higher than post-exercise ($P < 0.05$). Mean serum insulin concentrations at all time points post-ingestion were significantly higher than at rest and post-exercise during the HCHO trial (pre- and post- vs. +15 and +60 min; $P < 0.05$, pre- and post- vs. +30 min; $P < 0.001$, rest vs. +45 min; $P < 0.05$, post- vs. +45 min; $P < 0.001$). Mean serum insulin concentrations during the PL trials were not significantly difference across time.

6.3.5 Area under the curve

The AuC for cumulative BGlu (mmol.min.L⁻¹) response was significantly greater during HCHO and CHO-PRO trials compared to PL, respectively (92.0 ± 13.7 and 51.8 ± 5.1 vs. -4.7 ± 5.4 mmol.min.L⁻¹, respectively; *P*<0.001). In addition, the AuC observed for cumulative BGlu response was significantly higher for HCHO when compared to CHO-PRO (*P*<0.05). The AuC for cumulative insulin (μIU.min.mL⁻¹) response was significantly greater for both CHO-PRO and HCHO supplementation compared with PL (1161.8 ± 312.1 and 1127.9 ± 296.0 vs. 68.9 ± 21.2 μIU.min.mL⁻¹, respectively; *P*<0.05). Insulin AuC was not significantly different comparing CHO-PRO and HCHO trials.

6.3.6 Cortisol response

Mean salivary C (nmol.L⁻¹) responses were similar and not significantly different across drink and time, with the exception of the post-ExTr time-point (Figure 6.6).

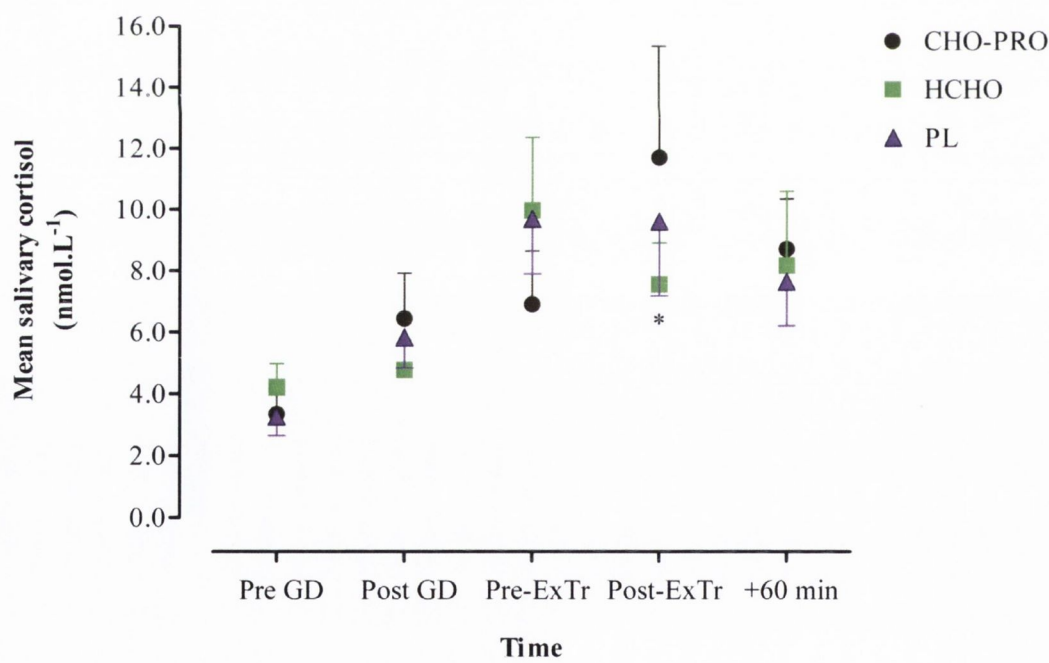


Figure 6.6: Mean salivary cortisol (nmol.L⁻¹) response across measured time points and drink trials, bars denote SEM, n = 7. * *P*<0.05, HCHO vs. CHO-PRO.

Mean salivary C concentration at the post-ExTr time-point was significantly lower during the HCHO trial than the CHO-PRO trial (7.6 ± 1.3 vs. 11.7 ± 3.6 nmol.L⁻¹, respectively; *P*<0.05). Both CHO-PRO and HCHO were not significantly different

to PL ($9.6 \pm 2.4 \text{ nmol.L}^{-1}$) at this time-point. Notably, there were no significant differences detected in mean salivary C response from pre- to post-ingestion of drinks (Post-ExTr and +60 min, respectively; Table 6.3).

Cortisol (nmol.L ⁻¹)	CHO-PRO	HCHO	PL
Pre-Drink	11.7 ± 3.6	7.6 ± 1.3	9.6 ± 2.4
Post-Drink	8.7 ± 1.6	8.1 ± 2.4	7.7 ± 1.4

Table 6.3: Mean salivary cortisol (nmol.L⁻¹) concentrations pre- and 60 min post-ingestion of drinks, ± SEM, n = 7.

6.3.7 Testosterone response

Mean salivary testosterone (nmol.L⁻¹) response was not significantly different between drinks (Figure 6.7). Furthermore, mean salivary T recorded pre- (Post-ExTr) and 60 min post-ingestion of drinks (+60 min) were not significantly different (Table 6.4).

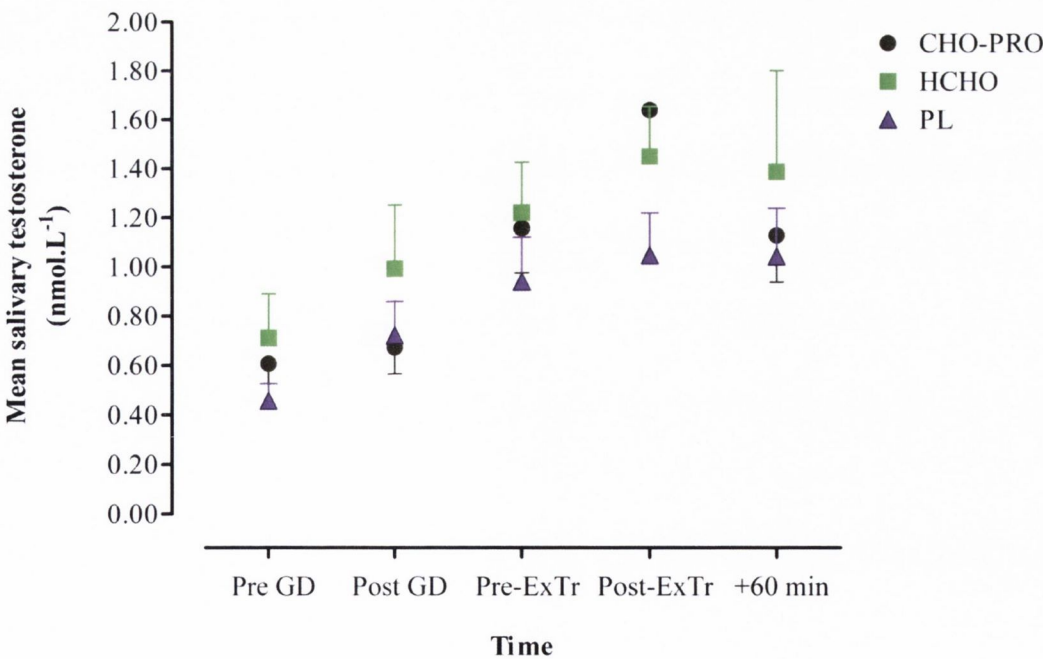


Figure 6.7: Mean salivary testosterone (nmol.L⁻¹) response across measured time points and drink trials, bars denote SEM, n = 7.

During the CHO-PRO trials, mean salivary T concentrations were significantly lower at both pre- and post-GD time-points when compared with the Post-ExTr time-point ($P<0.05$). Concentrations did not differ across measured time-points during HCHO and PL trials.

Testosterone (nmol.L ⁻¹)	CHO-PRO	HCHO	PL
Pre-Drink	1.64 ± 0.17	1.45 ± 0.20	1.05 ± 0.17
Post-Drink	1.13 ± 0.19	1.39 ± 0.41	1.04 ± 0.20

Table 6.4: Mean salivary testosterone (nmol.L⁻¹) concentrations pre- and 60 min post-ingestion of drinks, ± SEM, n = 7.

6.3.8 Testosterone to cortisol ratio

Mean T/C ratio was not significantly different across time and drink (Figure 6.8) and there were no significant differences detected within drink.

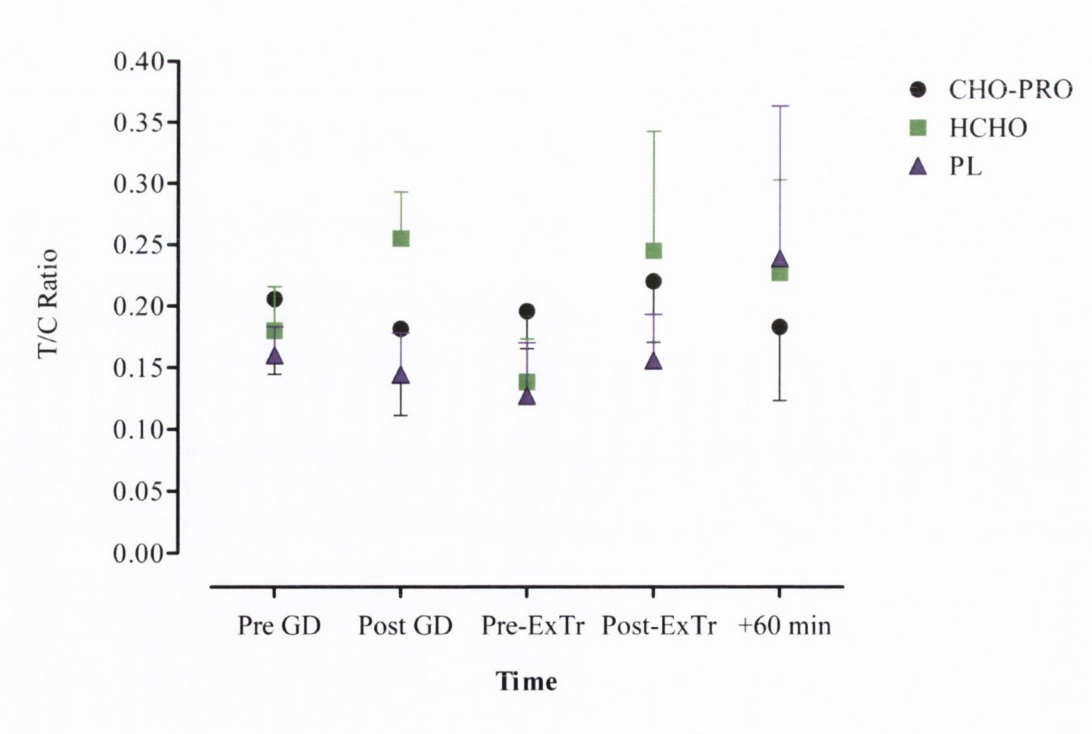


Figure 6.8: Mean salivary T/C ratio across measured time points and drink trials, bars denote SEM, n = 7.

Considering mean C and mean T response, there were no significant differences observed in mean T/C ratio pre- to post-ingestion of drinks (0.22 ± 0.05 , 0.23 ± 0.10 and 0.16 ± 0.04 vs. 0.18 ± 0.06 , 0.23 ± 0.08 and 0.24 ± 0.12 for CHO-PRO, HCHO and PL, Post-ExTr to +60 min, respectively; Table 6.5).

T/C Ratio	CHO-PRO	HCHO	PL
Pre-Drink	0.22 ± 0.05	0.23 ± 0.10	0.16 ± 0.04
Post-Drink	0.18 ± 0.06	0.23 ± 0.08	0.24 ± 0.12

Table 6.5: Mean salivary T/C ratio pre- and 60 min post-ingestion of drinks, $\pm$ SEM, n = 7.

6.3.9 Time to Failure

Time to exhaustion during both exercise protocols, GD and ExTr, were not significantly different across drinks. Mean exercise time during the GD protocol was 77.9 ± 7.9 , 72.9 ± 7.9 and 79.1 ± 7.5 min for CHO-PRO, HCHO and PL, respectively (Figure 6.9).

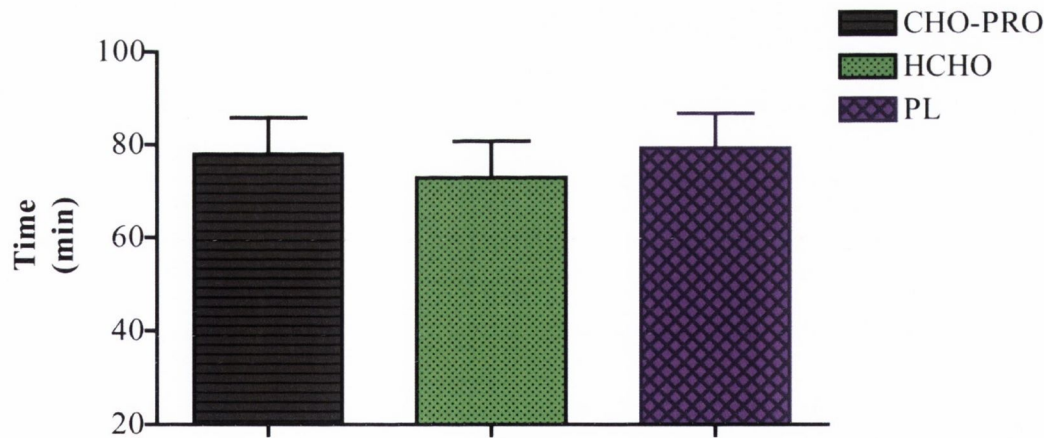


Figure 6.9: Mean exercise duration (min) completed during the GD protocol across drink trials, bars denote SEM, n = 7.

Only 3 individuals completed the GD protocol in its entirety during their 3 glycogen depletion rides, while 1 individual completed the duration of the GD protocol on two out of their 3 visits.

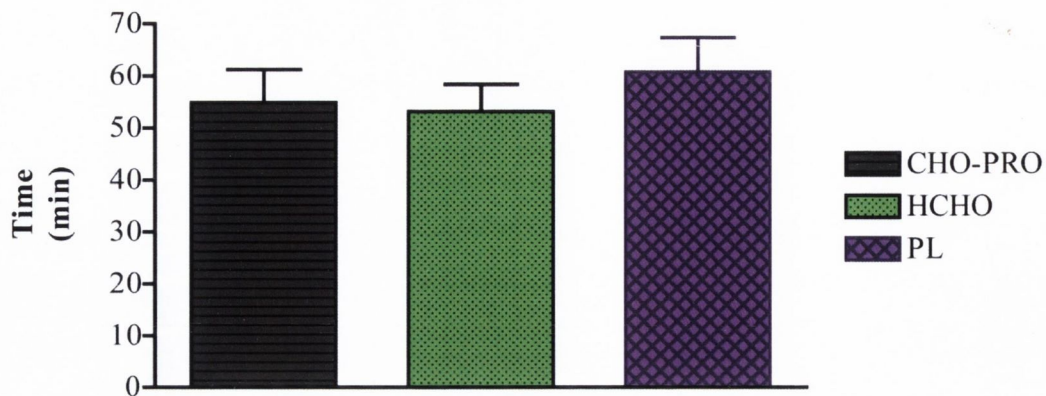


Figure 6.10: Bar graph illustrating exercise duration (min) of the ExTr protocol across drink trials, bars denote SEM, n = 7.

Mean exercise duration during the ExTr was 54.9 ± 6.3 , 53.1 ± 5.2 and 60.7 ± 6.7 min for CHO-PRO, HCHO and PL, respectively (Figure 6.10). Mean heart rate data expressed as %HRmax was not significantly different between GD trials or ExTr (Table 6.6).

%HRmax	CHO-PRO	HCHO	PL
GD Protocol	85.7 ± 1.8	83.4 ± 1.5	83.1 ± 2.2
ExTr Protocol	83.1 ± 2.2	80.6 ± 1.6	81.4 ± 2.0

Table 6.6: Mean %HRmax for GD and ExTr protocols, $\pm$ SEM, n = 7.

Chapter 6

6.4 Discussion

6.4.1 Summary of Findings

The aim of this study was to investigate the effect of different commercially available sports drinks on salivary C and T responses during recovery from two exercise bouts completed in a period of limited nutritional intake and rest. The protocol replicated the scenario of two fatiguing training sessions separated by an overnight period with restricted CHO intake; a scenario which reportedly resulted in increased C secretion (Lane *et al.*, 2010; Hough *et al.*, 2011). The hypothesis was that ingestion of a CHO-PRO sports drink after two bouts of high-intensity exercise would result in more effective recovery, when compared with an iso-caloric CHO drink (HCHO) or a PL drink, indicated by a more positive T/C ratio, by means of suppressing the reported exercise induced increase in salivary C concentration as a result of an enhanced insulin response and increased BGlu availability post-ingestion.

The current study recorded differing BGlu and serum insulin responses between each of the drink trials during the 60 min monitored recovery period. Ingestion of HCHO and CHO-PRO induced similar and significantly higher mean BGlu concentrations at 15 and 30 min post-ingestion compared with PL. A significantly faster decline in BGlu concentration was evident during the last 30 min post-ingestion of CHO-PRO compared to HCHO. Mean BGlu concentration observed at 60 min post-ingestion in the HCHO trial remained significantly elevated compared to CHO-PRO and PL trials, however, concentrations 60 min post-ingestion of CHO-PRO and PL were similar. The AuC for cumulative BGlu ($\text{mmol} \cdot \text{min} \cdot \text{L}^{-1}$) response was significantly greater during HCHO and CHO-PRO trials compared to PL, respectively. Also, the AuC observed for cumulative BGlu response was significantly higher for HCHO when compared to CHO-PRO.

Post-ingestion serum insulin responses were similar for both CHO-PRO and HCHO. However, post-ingestion serum insulin concentrations in the HCHO trial were significantly higher than those observed in the PL trial for the duration of the 60 min monitored period. Serum insulin concentrations recorded during the CHO-PRO trial

only attained significance relative to the PL trial at 30 and 45 min post-ingestion. The AuC for cumulative insulin ($\mu\text{IU}\cdot\text{min}\cdot\text{mL}^{-1}$) response was significantly greater for both CHO-PRO and HCHO supplementation compared with PL, respectively. Insulin AuC was not significantly different comparing CHO-PRO and HCHO trials. There was no significant difference in salivary C and T concentrations, or T/C ratio as a result of exercise or post-ingestion of test drinks.

6.4.2 Insulin and blood glucose response

The hormones investigated in this study, play varying roles during recovery from exercise. Specifically, C acts to maintain BGlu concentrations through catabolic pathways, while T and insulin have roles in glycogen resynthesis, post-exercise PRO anabolism, and the uptake and disposal of glucose (Adlercreutz *et al.*, 1986; Urhausen *et al.*, 1995; Kraemer *et al.*, 1998; Bishop *et al.*, 1999a; Brooks *et al.*, 2005; Rogol & Kraemer, 2005; Kaastra *et al.*, 2006; Betts *et al.*, 2007; Lane *et al.*, 2010; Mikulski *et al.*, 2010). The T/C ratio was stated to be an indicator of an athlete's ability to tolerate the current training load and a decrease in this ratio can be indicative of a breakdown in homeostasis (Adlercreutz *et al.*, 1986; Urhausen *et al.*, 1995; Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Meeusen *et al.*, 2004; Coutts *et al.*, 2007). Both intermittent and steady-state cycling have resulted in significantly increased secretion of C and when inadequate CHO availability is combined with inadequate rest intervals post-exercise, a catabolic hormonal response has been reported even during relatively short, high-intensity training cycles (Lane *et al.*, 2010; Hough *et al.*, 2011). Considering the effect of a low CHO diet on C concentration immediately and 24 h after a 3 day high-intensity training micro cycle, investigating supplementation of CHO-PRO or HCHO immediately after two high-intensity fatiguing training sessions separated by an overnight period with limited CHO intake presented an opportunity to investigate the effect of these beverages, or their component nutrition, on facilitating an acute post-exercise anabolic environment during a period when stimuli for glycogen synthesis are optimal.

Greater glycogen restoration after ingestion of a combined CHO-PRO supplement has been attributed to the greater plasma insulin response (Zawadzki *et al.*, 1992; Ivy, 1998; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c; Ivy *et al.*, 2002) and some research suggested that CHO ingestion can attenuate the C and catecholamine

response to exercise (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Mikulski *et al.*, 2008). Ingestion of a CHO-PRO supplement reportedly resulted in improved performance in subsequent exercise bouts, an effect mediated by an enhanced maintenance of BGlu concentrations (Betts *et al.*, 2007).

Enhanced lipolysis, proteolysis and gluconeogenesis along with an attenuation of peripheral glucose uptake, all of which are mediated by C, occur when insulin secretion is decreased (Mikulski *et al.*, 2010). The presence of high concentrations of insulin and BGlu post-exercise may negate the necessity for C mediated BGlu maintenance through these catabolic pathways. However, despite many studies reporting similar insulin responses and varying BGlu responses with various nutritional interventions (Zawadzki *et al.*, 1992; Tarnopolsky *et al.*, 1997; Ivy *et al.*, 2002; Howarth *et al.*, 2009), few studies have investigated the effects of ingestion of such beverages on hormonal markers of recovery in combination with the reported insulinotropic response.

In the current study, mean serum insulin concentrations were significantly higher post-ingestion of HCHO and CHO-PRO supplements in comparison to a PL throughout the 60 min monitored recovery period (Figure 6.5). The AuC cumulative for insulin in the current study was not significantly different between CHO-PRO and HCHO trials throughout the recovery period. Nonetheless, BGlu response appeared blunted at +45 and +60 min post-ingestion of CHO-PRO in comparison to the HCHO trial. Similarly, Betts *et al.* (2007) reported that BGlu concentration was significantly lower 60 min into their CHO-PRO trial compared to a HCHO trial during a 4 h monitored recovery period. However, BGlu concentrations in their HCHO trials had decreased to a concentration similar to that reported in their CHO-PRO and CHO trials 60 min later (Betts *et al.*, 2007). The earlier research by Ivy *et al.* (2002) reported significantly higher BGlu responses during both HCHO and LCHO trials compared with CHO-PRO which lasted for up to 120 min post-exercise. However, previous research has reported no significant differences in insulin response post-ingestion of any of the drinks supplemented in respective trials (Ivy *et al.*, 2002; Kaastra *et al.*, 2006; Betts *et al.*, 2007). Betts *et al.* (2007) did however report a significantly higher AuC cumulative for insulin in their CHO-PRO trial compared to their CHO trial.

The AuC cumulative for BGlu was significantly higher in response to the HCHO trial compared with the CHO-PRO trial suggesting that there was greater peripheral glucose availability in the circulation for the duration of the 60 min monitored recovery period. Despite the similarities in insulin responses in both the HCHO and CHO-PRO trials, peripheral uptake of glucose appeared significantly enhanced during the latter stages of the CHO-PRO trial (Figure 6.4). During the recovery period, peripheral glucose uptake by skeletal muscle or hepatic cells may have occurred at a greater rate in the CHO-PRO trial via an insulin mediated increased availability of glycogen synthase and GLUT-4 transporters (Blom *et al.*, 1987; Ivy, 1998; Ivy *et al.*, 2002; Jentjens & Jeukendrup, 2003; Kaastra *et al.*, 2006). It is reported that muscular level adaptations to exercise, such as increased GLUT-4 content, increased insulin action, and an increased capillary density (Jeukendrup & Jentjens, 2000) resulted in this increased glucose transport in the musculature. However, it could be speculated that the elevated insulin leads to a more tissue specific increase in insulin-stimulated glucose uptake (Kaastra *et al.*, 2006). Therefore, differences in insulin sensitivity may have contributed to large variations in insulin response between subjects (Kaastra *et al.*, 2006; Mikulski *et al.*, 2010).

The current data suggested that additional energy in the form of PRO, or that combined ingestion of CHO-PRO, resulted in a significantly enhanced rate of BGlu uptake compared to additional calories in the form of CHO. However, this observed difference in the rate at which BGlu increased and decreased, or appearance and disappearance, in the 60 min monitored recovery period may have been limited by intestinal transport of glucose via SGLT transporters (Jentjens *et al.*, 2004b). Therefore, during the HCHO trial, the additional glucose in the HCHO drink may have been responsible for the sustained BGlu concentration in the latter stages of the 60 min recovery period as the rate of absorption of this additional glucose was possibly limited by the availability of SGLT-1 transporters. This scenario is a more likely explanation of the results in the current study considering the similarity in AuC cumulative for insulin between both HCHO and CHO-PRO and the findings of Kaastra *et al.* (2006) who reported no significant difference in glucose disposal after ingestion of CHO, CHO-PRO or CHO-PRO-LEU.

Kaastra *et al.* (2006) reported a lower plasma glucose response in their CHO-PRO and CHO-PRO-LEU trials in comparison to CHO despite a significantly lower insulin response in that trial. The results presented by Kaastra *et al.* (2006) demonstrated that circulating insulin levels are not limiting to whole-body plasma glucose disposal when endurance-trained athletes ingest $0.8 \text{ g CHO.kg}^{-1}$. It therefore appears that the stimulatory effect of PRO and/or AA co-ingestion with CHO on post-exercise muscle glycogen synthesis is more likely attributed to the insulin stimulated activation of glycogen synthase (Kaastra *et al.*, 2006).

6.4.3 Cortisol and testosterone responses

Plasma glucose concentration may be a key factor in determining immune and hormonal responses to exercise and previous studies have reported that CHO ingestion has suppressed the exercise induced C response in endurance running and cycling (Henson *et al.*, 1998; Bishop *et al.*, 1999b). However, in the current study, T/C was not significantly different across time or between drink trials (Figure 6.8). Mean salivary C concentration was significantly higher post-ExTr in the CHO-PRO trial compared with the HCHO trial; however this difference may have been due to motivational factors or variations in time of sampling in individual exercise trials (Krieger & Allen, 1975; Bishop *et al.*, 2005; Moreira *et al.*, 2009). Despite this, mean salivary C response was not significantly different between drink trials or across time (Figure 6.6). Similarly, no differences were observed in mean salivary T response between drink trials or across time (Figure 6.7).

Furthermore, ingestion of sports drinks appeared to have no effect on the responses of T/C ratio. Considering the reported effects of CHO ingestion on exercise stimulated C secretion (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Lane *et al.*, 2010), it was anticipated that the C response during the PL trial would be significantly different to the HCHO and CHO-PRO trials. It seems plausible on consideration of the evidence, that the C response would be amplified in the PL trials as a lack of CHO to maintain BGlu concentration, restore utilised glycogen or increase insulin concentration would increase the drive for C secretion as a means of maintaining BGlu via catabolic substrate liberation (Brooks *et al.*, 2005; Lane *et al.*, 2010; Mikulski *et al.*, 2010). It has been reported that increased C concentration occurred in the presence of decreased insulin concentration after a 50-mile walking race

(Fukatsu *et al.*, 1996) and that decreased insulin secretion resulted in decreased peripheral glucose uptake and enhanced lipolysis; one of the catabolic substrate liberation actions of C (Mikulski *et al.*, 2010).

However, mean C concentrations remained at a similar level from pre- to post-GD and were not significantly different from pre- to post-ExTr. In the current study mean T concentrations appeared to increase slightly from the pre-GD to +60 min but were not significantly different. It is possible that the diurnal variability of these hormones contributed to these slight shifts in salivary concentration. The observed non-significant T/C ratio response pre- to post-exercise or across the 15 h period when trials were completed is in disagreement with Mäestü *et al.* (2005) who reported significant changes in T/C post-maximal performance testing.

Considering a C response is stimulated at a threshold of 60% $\dot{V}O_2\text{max}$ (Kirschbaum & Hellhammer, 1989), both workload and HR data (Table 6.2 & 6.6) support the fact that exercise intensity in the current study was sufficient to induce a C response. Most participants performed a similar duration of exercise in their respective GD and ExTr protocols and HR data suggested no difference in efforts across trials. Mean BLa concentrations were also significantly higher post-exercise (Figure 6.1 & 6.2). However, the lack of a C response to the exercise may have been due to insufficient duration despite participants exercising at an intensity greater than 80% HRmax in a fasted state with limited nutritional intake and rest: a scenario which was reported to result in increased C concentration (Kirschbaum & Hellhammer, 1989; Stone, 1991; Lac & Berthon, 2000; Hill *et al.*, 2008; Lane *et al.*, 2010; Mikulski *et al.*, 2010). It is important to note that during this protocol, saliva sampling times varied according to the timings of both the GD protocol (late evening) and the ExTr (early morning) and may account for variability across time points. Mean C concentrations were lowest prior to the GD phase which coincided with evening time (17:00 to 18:00), a known diurnal low phase (Krieger & Allen, 1975; Bishop *et al.*, 2005). However, some of the participants performed protocols earlier in the evening or morning of a given trial day to accommodate work commitments.

As previously discussed, both C and T vary naturally in accordance with circadian rhythm (Krieger & Allen, 1975) and can be affected by lifestyle factors, such as lack of sleep (Backhaus *et al.*, 2004), which may have masked any drink effect on hormonal markers of stress. Training load has also been reported to modify the circadian rhythm of C, which was modified after periods of heavy training. In such an instance, C was increased relative to resting values and reached a peak later in the morning while T concentration was suppressed relative to resting concentration (Wittert *et al.*, 1996; Filaire *et al.*, 2001; Coutts *et al.*, 2007). The majority of testing in the current study took place in the competitive racing and triathlon season although two participants were training in preparation for a Trans-Alpine cycle marathon. These individuals participated in the drink trials as a means of part preparation for their cycling event in combination with their normal training which was of a relatively high load and therefore, may have affected these participants' individual hormonal responses. In the current study, both mean T and C response, and subsequent T/C ratio, varied greatly within participants and across both time and drink trial. Previous research has pointed to a highly individualised response of such hormones to exercise (Suay *et al.*, 1999; Moreira *et al.*, 2009). Data published by Suay *et al.* (1999) and Moreira *et al.* (2009) demonstrated that individual response to a given situation varied and it has been suggested that there was a need to individually analyse the results.

Moreira *et al.* (2009) suggested that individuals will respond favourably or marginally to a training program depending on the type of athlete and their ability to cope with training demands and non-training stress factors. Data presented by Bishop *et al.* (1999a) and Moreira *et al.* (2009) are from simulated and training match play, respectively, scenarios that would not elicit the same psychophysiological impact inherent in competitive situations (Mason *et al.*, 1973; Aubets & Segura, 1995; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). The data presented by Cunniffe *et al.* (2010) and Elloumi *et al.* (2003) are competition data and it is known that such conditions would impose a degree of psychophysiological strain sufficient to augment the secretion of C and T (Mason *et al.*, 1973; Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005). Moreira *et al.* (2009) reported that salivary C response to a training match was blunted highlighting potential factors such as

individuals placing less emphasis on the game and thus, reducing the perceived emotional stress associated with a competitive situation. This potentially offers explanation for the significantly lower C concentration at post-ExTr between HCHO and CHO-PRO trials.

Research has presented data from both professional and amateur athletic populations which may also contribute to the differing T/C response (Bishop *et al.*, 1999a; Elloumi *et al.*, 2003; Moreira *et al.*, 2009; Cunniffe *et al.*, 2010). Variations in physical fitness can account for individual hormonal responses (Grandys *et al.*, 2009). Mikulski *et al.* (2010) highlighted that differing insulin responses to glycogen depleting exercise may have been due to the greater exercise capacity of the trained individuals and that a 70% HRmax workload was approximately twice higher for untrained individuals. Every effort was made to exert control on potentially influential external factors, however, factors such as amount and quality of sleep and intrinsic motivational factors may have affected both C and T responses in the current study (Mason *et al.*, 1973; Suay *et al.*, 1999; Neave & Wolfson, 2003; Backhaus *et al.*, 2004; Salvador, 2005).

6.4.4 Conclusion

In Chapter 3, it was observed that a CHO-PRO supplement following resistance exercise enhanced recovery and resulted in greater work capacity in a subsequent exercise session when compared with LCHO and HCHO. Previous research suggested that the insulinitropic effects of the addition of PRO to CHO enhanced recovery through higher rates of BGlu disposal during the recovery period (Ivy *et al.*, 2002). Ingestion of CHO-PRO also facilitated improved subsequent work capacity due to improved maintenance of BGlu after recovery compared with CHO alone (Betts *et al.*, 2007). Greater glycogen restoration after ingestion of a combined CHO-PRO supplement has been attributed to the greater plasma insulin response (Zawadzki *et al.*, 1992; Ivy, 1998; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000c; Ivy *et al.*, 2002; Kaastra *et al.*, 2006). Therefore, in the current study the acute elevation in BGlu and serum insulin concentrations post-ingestion of HCHO may suggest that the SGLT-1 and GLUT-4 transporters were saturated and that the insulinitropic response to CHO-PRO presented more glycogen synthase activity for subsequent BGlu disposal, potentially accelerating glycogen synthesis in the latter

stages of recovery (Blom *et al.*, 1987; Ivy, 1998; Jentjens & Jeukendrup, 2003; Kaastra *et al.*, 2006). Recent research suggested that repeated strenuous exercise performed with inadequate rest and CHO availability resulted in an increased catabolic state (Lane *et al.*, 2010). It appears that in spite of better availability of peripheral glucose and a sustained insulin response, C concentrations and T/C were not different between CHO-PRO, HCHO and PL trials. It may be necessary to exert further control on factors affecting hormonal responses, particularly timing of sample collection, if T/C is to be used as a diagnostic or informative measure of training status. It may also be necessary to determine a diurnal cycle of T/C ratio in individuals due to the individual response reported in the current study and previous research.

Chapter 7

General Discussion

Chapter 7

General Discussion

7.1 Summary of findings

Chapter 3 proposed to evaluate if a CHO-PRO supplement would significantly increase time to failure resulting in greater mean ΣW and TWC during a subsequent TTF protocol performed 4 h after a standard RT protocol in male resistance trained academy rugby players. Despite large intra-subject variability TWC (kg.kg^{-1} BM) was significantly greater following CHO-PRO ingestion when compared with HCHO and LCHO (188 ± 26 vs. 157 ± 21 and 150 ± 16 kg.kg^{-1} BM, respectively; $P < 0.05$). Also, a trend for improved BGlu was recorded post-ingestion of HCHO and CHO-PRO, respectively, compared with LCHO.

Chapter 4 proposed to evaluate the hormonal response to in-season training and competition scenarios across three sporting disciplines of varying demands, i.e. soccer, rugby and rowing. The competition scenario induced a significantly greater salivary C response than the training scenario in rowing and rugby, with no effect of scenario evident in soccer. In addition, the competition scenario in rugby induced a significantly greater mean salivary T response compared to training, an effect that was not evident in either rowing or soccer. A trend of increasing C concentration was observed from pre- to post-match in soccer.

Chapter 5 proposed to evaluate the effect of a CHO-PRO beverage on salivary C and T concentrations compared to a CHO supplement alone (LCHO) and an iso-caloric equivalent supplement (HCHO) during standard, in-season soccer training. Despite wide variability in hormonal concentrations from subject-to-subject, globally, the mean salivary C concentrations were significantly lower at rest on Day 4 (72 h) relative to Day 1 (0) and Day 2 (24 h) resting time points. Mean C concentrations were significantly lower at 72 h relative to 0 after ingestion of both HCHO and CHO-PRO drinks only.

Chapter 6 proposed to investigate the effect of different commercially available sports drinks on salivary C and T responses during recovery from two exercise bouts

completed in a period of inadequate rest and nutritional intake. In spite of improved peripheral glucose availability and a sustained insulin response post-ingestion of CHO-PRO and HCHO drinks relative to PL, concentrations of C and T, and the T/C ratio were not significantly different between trials.

7.2 Ingestion of combined carbohydrate-protein post-exercise

Competition and training can significantly deplete muscle glycogen stores and produce a significant inflammatory response which included a significant increase in C concentration (Elloumi *et al.*, 2003; Bangsbo *et al.*, 2006; Krstrup *et al.*, 2006a; Coutts *et al.*, 2007; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010). Continued training or competition with inadequate recovery can potentially result in the development of a persistent catabolic state synonymous with the manifestation of OT syndrome and highlights the necessity to balance training and competition stresses with adequate recovery. Achieving a balance between recovery and overload throughout a competitive season can facilitate maximum adaptation to a training cycle and improve performance (Meeusen *et al.*, 2006). Nutritional aids are reported to complement post-exercise recovery by providing abundant sources of CHO, PRO and electrolytes to replenish those lost through exercise and can manipulate the physiological post-exercise recovery mechanisms, such as PRO turnover and hormonal response, so that adaptations to training can be maximised and the disturbances provoked by high-intensity training or competition performance can theoretically be reduced. However, despite performing structured recovery sessions which may include a variety of recovery modalities, restoration of utilised muscle glycogen remains the primary physiological facet of recovery (Jentjens & Jeukendrup, 2003; Shirreffs *et al.*, 2004; Barnett, 2006; Maughan *et al.*, 2007). One of the principal objectives of this research was to investigate the efficacy of post-exercise CHO-PRO ingestion on the restoration of exercise capacity, and to determine whether such a nutritional intervention was more effective at suppressing the catabolic response associated with strenuous exercise. A CHO-PRO beverage was investigated due to the vast body of research indicating an enhanced recovery rate of utilised glycogen in the post-exercise period and an improved maintenance of BGlu concentration in subsequent exercise bouts when compared with a caloric equivalent sports drink and a sports drink matched for CHO content (Roy *et al.*, 1997; Tarnopolsky *et al.*, 1997; Van Loon *et al.*, 2000a; Van Loon *et al.*, 2000b;

Jentjens *et al.*, 2001; Tipton *et al.*, 2001; Ivy *et al.*, 2002; Williams *et al.*, 2003; Rankin *et al.*, 2004; Berardi *et al.*, 2006; Betts *et al.*, 2007; Rodriguez *et al.*, 2007; Howarth *et al.*, 2009).

The data presented in Chapter 3 reported that CHO and caloric differences were not contributory factors to the enhanced subsequent resistance exercise performance as there were no significant differences in performance capacity detected between the HCHO and LCHO supplements. This would suggest that the PRO component of the CHO-PRO supplement was responsible for the enhanced performance capacity. Ivy *et al.* (2002) reported that ingestion of a CHO-PRO supplement immediately post-exercise enhanced glycogen resynthesis during the initial recovery period (0 to 40 min), and that muscle glycogen restoration was further enhanced when a second CHO-PRO supplement was ingested 120 min into the 240 min recovery period (Figure 1.1). The total muscle glycogen recovery reported by Ivy *et al.* (2002) at the end of a 240 min monitored recovery period was significantly greater during the CHO-PRO trial when compared with the HCHO and LCHO treatments ($P < 0.05$) and, similar to the current study, there was no significant difference detected between HCHO and LCHO treatments. Betts *et al.* (2007) demonstrated that ingestion of CHO-PRO and HCHO at 30 min intervals during a 240 min recovery period significantly increased exercise capacity during a TTF protocol when compared with ingestion of LCHO. However, unlike the data presented in this thesis, Betts *et al.* (2007) reported no significant difference in time-to-failure in the CHO-PRO and HCHO treatments during their subsequent running performance. The CHO-PRO drink ingested in the study presented in Chapter 3 may have contributed to a higher rate of glycogen restoration after the initial glycogen depleting exercise protocol whilst also providing a means for an improved availability of BGlu concentration during the subsequent exercise bout.

Betts *et al.* (2007) concluded that irrespective of whether the additional energy originated from sucrose or PRO in whey isolate form, increasing the energy content of the recovery solution extended run time to exhaustion. However, Betts *et al.* (2007) reported a more stable BGlu concentration during recovery following CHO-PRO ingestion when compared with HCHO and LCHO ingestion which may have contributed to a greater availability of BGlu during the subsequent TTF protocol

from the gastrointestinal tract or liver. The results of Chapter 3 are in partial agreement with Betts *et al.* (2007), as the significant improvement in subsequent exercise after CHO-PRO ingestion may have been mediated by enhanced maintenance of BGlu. In Chapter 3 mean BGlu concentrations were more consistent during both CHO-PRO and HCHO trials compared to LCHO (Figure 3.6) and this may have contributed to the greater availability of BGlu during the subsequent RT session as postulated by Betts *et al.* (2007).

The psychophysiological stresses inherent in competitive match play reportedly induced significantly elevated C concentrations in rugby union players and soccer players (Elloumi *et al.*, 2003; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010). While the disturbance was significantly acute in soccer players, a longer term significant disturbance was reported post-match in rugby players. The results of Chapter 4 are in agreement with those reported by Elloumi *et al.* (2003) and Cunniffe *et al.* (2010) as competitive rugby union match-play induced significant increases in C concentration pre- and post-match compared to training. A trend of increased C concentration was recorded post-match in soccer players (Table 4.2). However, T concentrations in soccer training and competition were similar and not significantly different.

Salivary T concentration was significantly higher pre- to post- in both scenarios in rugby players, although the magnitude of the response was slightly larger in the competition scenario. The reported responses to rugby union match-play differed in available research. Cunniffe *et al.* (2010) reported a significantly decreased serum T response immediately post-match which only returned to baseline by 14 h post-match. Elloumi *et al.* (2003) reported a slight decrease in post-match salivary T concentration, which was not significant when compared to pre-match. In Chapter 4, the increased T concentrations recorded post-match in rugby players may have been elicited in order to induce the protective effects of T against the catabolic effects of C (Elloumi *et al.*, 2003). Perhaps the observed T response to match-play may be an adaptation, or habitual response, to long-term rugby union training, similar to that observed in C responses in professional soccer players (Filaire *et al.*, 2001) to facilitate a return to homeostasis (Elloumi *et al.*, 2003). The hormonal responses to rowing training and competition documented in Chapter 4 were similar to those

reported by Mäestu *et al.* (2005). Mäestu *et al.* (2005) reported that C and T concentrations were significantly elevated immediately following maximal exercise in rested rowers. However, a 3 week overload period significantly blunted free-T concentration before and after a 2000m ergometer maximal test (Mäestu *et al.*, 2005). Following a further 2 weeks of tapered training, free-T concentration returned to baseline concentration pre-maximal exercise despite the free-T response post-maximal exercise remaining significantly blunted. The salivary T response reported in Chapter 4 may suggest that the rowers were in a phase of their season where training load was tolerable.

Both C and T vary according to their respective diurnal rhythms and are affected by various intrinsic and external factors which may have influenced the responses observed in Chapter 4. Nevertheless the differing hormonal responses in the professional footballers may be due to an adaptation to constant training and an improved ability to cope with the imposed weekly stress of competitive fixtures when compared to the amateur club rugby players and collegiate rowers. Moreira *et al.* (2009) stated that top-class professional soccer players training systematically and regularly are very well adapted to this type of training and therefore respond differently. Filaire *et al.* (2001) stated that a period of high-intensity training may modify circadian rhythm manifesting in a late morning peak in C secretion which may lend explanation to the significantly higher C concentration recorded pre-training in Chapters 4 and 5. Filaire *et al.* (2001) stated that circadian rhythms observed in the soccer players in their study reflected those documented in the literature, that is, decreasing from morning to evening, and are similar to the variations observed in C concentrations in Chapters 4 and 5. Variations in C and T responses between and within individuals may be due to situational factors or individual aspects, such as, interactions with opposition players and the importance of the game, yet they appeared to be independent of the result (Gonzalez-Bono *et al.*, 1999; Suay *et al.*, 1999; Moreira *et al.*, 2009). Also, psychological stresses inherent in competitive situations in combination with the high energetic cost associated with competitive exertions may induce a significant elevation in C concentration and cannot be recreated in training (Suay *et al.*, 1999; Neave & Wolfson, 2003; Salvador, 2005; Moreira *et al.*, 2009).

Data suggested that soccer match-play not only resulted in increased C concentration but induced a significant and severe depletion in intra-muscular glycogen stores (Krustrup *et al.*, 2006a; Ispirlidis *et al.*, 2008). Bangsbo *et al.* (2006) stated that match-play induced glycogen depletion was still evident 48 h post-match, despite high dietary CHO intake, and this placed significant limitations on the player in subsequent training sessions. In Chapter 5, ingestion of CHO-PRO and HCHO drinks immediately post-training did not affect C concentration. Bishop *et al.* (1999a) reported that ingestion of CHO had no effect on post-exercise C response after simulated soccer performance when compared with a PL. It is important to note that the soccer protocol employed in the study by Bishop *et al.* (1999a) excluded jumping, tackling or competing for the ball in the air which may have limited the trauma element of match-play, and to an extent, the intermittent nature of soccer match-play, thereby reducing energy expenditure, tissue damage and an expected C response. Bishop *et al.* (1999a) also reported no significant occurrence of neutrophil degranulation which may have limited the C response and is in contrast to data presented by Cunniffe *et al.* (2010). Cunniffe *et al.* (2010) reported a significant increase in neutrophil degranulation and a decrease in neutrophil responsiveness 38 h post-match; both of these responses were possibly mediated by their significantly increased C concentration. However, in Chapter 5 ingestion of sports drinks appeared to contribute to a significantly decreased C concentration in the latter stages of the training week (Figure 5.1). Mean salivary C concentration was significantly decreased at rest on Day 4 (72 h) of the training week when CHO-PRO and HCHO drinks were consumed immediately post-training on Days 1 and 2. As both training sessions were similar in nature and relative intensity, it would seem plausible to suggest that ingestion of sports drinks contributed, in some way, to the observed decrease in C concentration.

Another possible contributory factor to the decreased C concentration observed at 72 h (vs. 0 and 24 h) was that training Day 4 was preceded by a day of rest for all players. The responses to C and T are reported to be altered after periods of training, competition and rest therefore, it may not be sufficient to only provide a means of substrate recovery. With regards to these two hormones, specifically C, it may be necessary to combine a nutritional intervention with a period of rest during which muscle damage and psychological fatigue can be reduced which may be indicated by

a decreased concentration of C. In Chapter 5, this decreased C concentration on Day 4 (72 h) relative to pre-training on Day 1 (0) may indicate a timely return to an optimal state of recovery considering a competitive fixture was pending in the next 36 h and highlight the benefit of nutritional intervention in maintaining a level of optimal performance within professional athletes training and competing on a weekly basis.

Research has suggested that CHO ingestion not only delays fatigue (Fielding *et al.*, 1985; Nicholas *et al.*, 1995; Saunders *et al.*, 2004; Sawka *et al.*, 2007) but suppresses the post-exercise C response (Henson *et al.*, 1998; Bishop *et al.*, 1999b; Lane *et al.*, 2010; Mikulski *et al.*, 2010). Considering the reported insulinotropic response mediated by the ingestion of a CHO-PRO supplement, it was anticipated that an enhanced anabolic response would be evident post-ingestion of the CHO-PRO supplement when compared with HCHO or a PL in Chapter 6. Hough *et al.* (2011) reported that intermittent and steady-state exercise stimulated a significant increase in C concentration that was not significantly different between exercise types. However, a protocol consisting of steady-state cycling at 70% $\dot{V}O_2\text{max}$ with 3 sprints at 120% $\dot{V}O_2\text{max}$ did not induce a significant hormonal response, despite previous research highlighting the efficacy of such a protocol on glycogen depletion (Vøllestad *et al.*, 1992; Pedersen *et al.*, 2008). Also, a subsequent steady-state cycle at 70% $\dot{V}O_2\text{max}$ under restricted dietary conditions did not affect hormone concentration. However, it is possible that performing 2 bouts of strenuous exercise in a limited time frame (~14 h) may be a tolerable load for trained individuals, such that a C response is not elicited. Lane *et al.* (2010) reported that C concentrations increased significantly after a 3 day high-intensity training period where rest was limited and CHO intake was restricted, and that C concentrations after the first and second bouts of exercise were not significantly higher when compared with rest. There appeared to be no beneficial effect of ingestion of CHO-PRO or HCHO detected on acute C and T concentrations post-exercise in Chapter 6. However, Bishop *et al.* (1999b) reported that CHO ingestion attenuated C response to steady-state cycling (2 h at 70% $\dot{V}O_2\text{max}$) when compared to a PL trial and fluid restriction trial. Bishop *et al.* (1999b) stated that CHO intake before and during exercise under such conditions attenuated the circulating leukocyte and neutrophil responses to

exercise; effects which were possibly mediated by the maintenance of plasma glucose concentrations and the accompanying suppression in plasma C concentrations. Mikulski *et al.* (2010) reported that C decreased from post-exercise concentrations after ingestion of a CHO meal and that the response was similar despite differing CHO content (low vs. high). However, the results presented in Chapter 6 are inconsistent with the findings of Mikulski *et al.* (2010) as CHO ingestion did not affect the acute hormonal response to the exercise.

In Chapter 6, mean serum insulin concentrations were significantly higher post-ingestion of HCHO and CHO-PRO supplements when compared to a PL throughout the 60 min monitored recovery period (Figure 6.5). However, cumulative AuC for insulin ($\mu\text{IU}\cdot\text{min}\cdot\text{mL}^{-1}$) was not significantly different between CHO-PRO and HCHO trials, and conflicts with the findings of Betts *et al.* (2007). Betts *et al.* (2007) reported that cumulative AuC for insulin was significantly higher in their CHO-PRO trial when compared to their CHO trial with no difference reported between CHO-PRO and HCHO. Kaastra *et al.* (2006) reported no significant difference in glucose disposal after ingestion of CHO, CHO-PRO or CHO-PRO-LEU. They also reported a lower plasma glucose response in their CHO-PRO and CHO-PRO-LEU trials relative to the CHO trial despite a significantly lower insulin response in that trial. The mean BGlu response observed in Chapter 6 appeared blunted at +45 and +60 min post-ingestion of CHO-PRO in comparison to the HCHO trial and was similar to Betts *et al.* (2007) who reported that BGlu concentration was significantly lower 60 min after ingestion of CHO-PRO when compared with HCHO. However, BGlu concentrations in their HCHO trials had decreased to a concentration similar to that reported in their CHO-PRO and CHO trials 60 min later (Betts *et al.*, 2007) which is possibly indicative of the delayed absorption associated with higher volumes of CHO at an intestinal level. Earlier research by Ivy *et al.* (2002) reported significantly higher BGlu responses during both HCHO and LCHO trials when compared with CHO-PRO which lasted for up to 120 min post-exercise. However, previously research has reported no significant differences in insulin responses post-ingestion of any of the drinks supplemented in their respective trials (Ivy *et al.*, 2002; Kaastra *et al.*, 2006; Betts *et al.*, 2007).

The results presented by Kaastra *et al.* (2006) demonstrated that circulating insulin was not limiting to whole-body plasma glucose disposal when endurance-trained athletes ingested 0.8 g CHO.kg⁻¹. Entrance of glucose into the systemic circulation is the rate limiting factor for exogenous glucose oxidation rather than intramuscular factors (Jeukendrup, 2004). It therefore appears that the stimulatory effect of PRO and/or AA co-ingestion with CHO on post-exercise muscle glycogen synthesis is more likely attributed to the insulin stimulated activation of glycogen synthase (Kaastra *et al.*, 2006).

It has been suggested that a synergistic link exists between C and BGlu concentration. When CHO availability is low, C is reportedly secreted to induce gluconeogenesis via lipolysis and proteolysis (Brooks *et al.*, 2005; Lane *et al.*, 2010; Mikulski *et al.*, 2010) and may be a possible explanation for increased C concentration after periods of high-intensity exercise (Filaire *et al.*, 2001; Mäestu *et al.*, 2005; Coutts *et al.*, 2007). The acute hormonal responses observed in Chapter 6 were not significantly different post-ingestion of CHO-PRO, HCHO or PL. However, there was no significant difference in hormonal concentrations observed during an exercise protocol where nutritional intake and rest interval between consecutive exercise trials was limited. The serum insulin response post-ingestion of CHO-PRO and HCHO were not significantly different but were significantly higher when compared to PL and, although BGlu concentration was significantly lower towards the latter stages of a 60 min monitored recovery period, there appeared to be no difference of additional PRO or CHO ingestion on the responses of C and T in the acute phase of recovery. With due consideration for the data presented, it is apparent insufficient evidence is available to suggest that recovery was enhanced post-ingestion of a CHO-PRO supplement, and considering certain monitored variables, the CHO-PRO supplement was no more effective than ingestion of a PL immediately post-exercise.

7.3 Saliva versus serum hormone concentrations

Researchers have quantified the response of C and T in various populations and across differing exercise scenarios with hormonal concentrations determined from both serum and saliva samples (Fukatsu *et al.*, 1996; Henson *et al.*, 1998; Bishop *et al.*, 1999a; Bishop *et al.*, 1999b; Lac & Berthon, 2000; Filaire *et al.*, 2001; Elloumi

et al., 2003; Mäestu *et al.*, 2005; Coutts *et al.*, 2007; Cormack *et al.*, 2008; Ispirlidis *et al.*, 2008; Cunniffe *et al.*, 2010; Lane *et al.*, 2010; Mikulski *et al.*, 2010; Hough *et al.*, 2011). The varying exercise modes and field-based data collection documented in this dissertation were necessary to determine a real-life and sport-specific hormonal response, as well as evaluating any relationship that may have existed between C, T and post-exercise nutritional recovery interventions in a variety of athletic populations. However, the field based data collection and the dynamic nature of the exercise modes in the respective protocols presented obvious limitations with regard to blood sampling; most-pertinent were concerns regarding hygiene and well-being of the participants. Performing RT exercise or soccer training with an indwelling cannula in place may not be practical for a participant undertaking such exercise due to the high risk of trauma or infection, or both, that could possibly have occurred due to the nature of the exercise or the non-sterile environment in which training/data collection took place. Therefore, saliva samples provided an easily accessible medium through which concentrations of both C and T could be reliably quantified in sport-specific environments while minimising the risk to the individual. High correlations have been reported between serum and salivary concentrations of both C and T indicating that salivary concentration reliably estimated serum concentration in both hormones (Vining & McGinley, 1987; Aardal & Holm, 1995; Shirtcliff *et al.*, 2002; Daniel *et al.*, 2006; Dorn *et al.*, 2009).

A pilot investigation, outlined in section 2.8, investigated the relationship between the bound and unbound concentrations of C and T. A moderate to good correlation was observed between serum and salivary C concentrations ($r^2 = 0.577$), however the relationship between serum and salivary T was poor ($r^2 = 0.058$). The poor correlation observed between salivary and serum T was possibly due to the higher susceptibility of salivary T, more so than other salivary analytes like C, to the manner in which saliva samples were collected and stored, as well as contaminants like blood or components of blood, even when the contaminant may not be visible (Granger *et al.*, 2004). Previous research has also reported that behaviour correlations using saliva as a surrogate for serum were likely to substantially underestimate any relationship (Granger *et al.*, 2004).

These data therefore, validate comparisons of salivary C with the available research and also add to the body of research investigating the effects of training on the C response to exercise and post-exercise nutritional interventions. However, salivary T concentrations may have limitations when assessing an intervention effect and may have affected the T/C ratio reported in this thesis due to the sensitivity of T in saliva to external effectors and potential contaminants.

7.4 Limitations and recommendations

Both C and T respond to increased training load, nutritional supplementation and psychological stress (Filaire *et al.*, 2001; Elloumi *et al.*, 2003; Brooks *et al.*, 2005; Meeusen *et al.*, 2006; Coutts *et al.*, 2007; Cunniffe *et al.*, 2010). However, C and T are reported to return to near baseline concentration when the subsequent training load is reduced or, in the case of C, upon post-exercise CHO ingestion (Filaire *et al.*, 2001; Mikulski *et al.*, 2010). Considering the diurnal responses and potential effectors, it may be necessary to determine baseline concentrations of the hormones in question, so that quantification of any ergogenic drink effect can be measured in comparison to these baseline concentrations. In Chapter 5, the inclusion of a control training week or a PL trial arm may have allowed for determination of the aforementioned ergogenic effect of CHO-PRO ingestion vs. HCHO and LCHO considering that research has suggested that C concentration was similarly attenuated after ingestion of a low and high-CHO meal. Large inter- and intra-individual hormonal responses have been reported in this thesis and are similar to data reported by Suay *et al.* (1999) and Moreira *et al.* (2009). Moreira *et al.* (2009) stated that large intra- and inter-subject variability was possibly due to athletes responding either favourably or marginally to a training program depending on their ability to cope with training demands and psychological stresses. These data suggest that hormonal responses are highly individual and, therefore, it may be necessary to individually analyse the results, particularly within team sports, and compare changes within the individual to their pre-determined baseline to determine the effect of an intervention.

The data presented in Chapters 3 and 5 may have benefited from the inclusion of an additional PL or control trial arm. The inclusion of a PL trial would have been advantageous in determining the reasons for the enhanced recovery and improved

subsequent performance in Chapter 3 and may have allowed positive identification of an effect of CHO or CHO-PRO supplementation on C and T concentrations in Chapter 5. Betts *et al.* (2007) stated that their enhanced subsequent performance may have been due to ingestion of their final bolus of drink 30 min prior to beginning the TTF protocol providing a source of abundant glucose to maintain BGlu concentration, thus improving performance. Considering this observation, varying the timing and frequency of supplementation may enhance the subsequent performance capacity and potentially may have further implications for hormonal concentrations at the latter stages of the training week. In Chapter 6, a LCHO supplement may have accounted for the difference caused by additional energy in the form of PRO or CHO, possibly contributing to the findings of Betts *et al.* (2007), and may have proved an interesting comparison considering the insulin responses reported by Ivy *et al.* (2002), Kaastra *et al.* (2006) and Betts *et al.* (2007). The data presented in Chapters 5 and 6 suggest that a longer term intervention may be required to determine if there is a significant benefit associated with CHO or CHO-PRO ingestion on assisting the athlete's ability to tolerate a training load and may point to a necessity to collect data in the high-load pre-season phase.

Further study is warranted in this area to determine the influence of peripheral and central factors of fatigue on the recorded enhanced performance outcomes as a result of an enhanced recovery with a CHO-PRO drink. Determination of glycogen utilised and glycogen restored would be particularly helpful in quantifying the true glycogen restoration effect of supplemented drinks. These data, with additional analyses of urea, glycerol and NEFA may allow a more complete picture of the peripheral and consequently, central factors limiting performance at high intensities and the fate of the constituents of the drinks at a cellular level. Measurement of ACTH and SHBG may also provide useful information relating to C and T secretion, respectively. Such data would then allow athletes to conclude whether these so-called beverages truly warrant the name 'recovery' drinks.

7.5 Conclusions

The ingestion of a CHO-PRO supplement post-exercise is reported to facilitate greater recovery through an insulin mediated increase in glucose uptake and glycogen synthesis and a possible ergogenic effect of the interactions of AA with the

CNS resulting in reduced feelings of lethargy (Ivy *et al.*, 2002; Kaastra *et al.*, 2006). The additional PRO certainly provides a source of di- and tri-peptides which, in combination with the abundant availability of CHO, may be necessary for restoration of cell and immune function and repair of tissue damage in the post-exercise recovery phase (Bishop *et al.*, 1999b; Gleeson & Bishop, 2000; Tipton & Wolfe, 2004; Cunniffe *et al.*, 2010; Mikulski *et al.*, 2010). In addition, the ingestion of CHO post-exercise reportedly attenuated the post-exercise catecholamine and C response (Fukatsu *et al.*, 1996; Cunniffe *et al.*, 2010; Mikulski *et al.*, 2010).

Any differences between a CHO supplement with additional energy in the form of PRO or CHO marginally favour the addition of PRO, as evidence suggested that the lower glucose load in conjunction with the insulinotropic effects of the additional PRO result in faster and more enhanced subsequent glycogen resynthesis (Jentjens *et al.*, 2001; Ivy *et al.*, 2002). A CHO-PRO supplement ingested post-exercise can significantly increase serum insulin concentration, however, the insulin response was similar post-ingestion of an iso-caloric HCHO drink. However, BGlu concentrations were only significantly different between CHO-PRO and HCHO trials towards the end of a 60 min recovery period, most likely due to intestinal transport limiting exogenous CHO uptake (Jeukendrup, 2004). There is an insufficient body of evidence to justify using the term 'recovery' in relation to CHO-PRO drinks as the hormonal responses were similar post-ingestion of HCHO, and with the exception of serum insulin concentrations, remained unaffected. Mean BGlu responses post-ingestion of CHO-PRO and HCHO appear to be markedly better than a LCHO drink and a PL supporting the fact that glucose availability is improved in the post-exercise period after consumption of a CHO-PRO and HCHO sports drink. Whether there is an advantage to be gained from longer term supplementation as opposed to immediate short-term usage is worthy of further investigation, due to the tendency of a significant outcome in terms of improved early morning C concentration throughout a training week in soccer players (Chapter 5). Possibly there would be a benefit in looking at early morning C concentrations in a squad of players undertaking training over a much longer time frame to determine the beneficial effects, if any, of additional PRO in reducing this C peak when combined with additional CHO vs. CHO alone.

Ingestion of a CHO-PRO supplement had similar effects to ingestion of a HCHO supplement post-exercise. Therefore, considering previous research and the current findings it appears that a CHO-PRO supplement does not induce a significantly improved 'recovery' from either resistance, intermittent or endurance exercise.

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Appendix I

Appendix I

Publications

Coyle C, Donne B, Hennessy L & Mahony N. (2005). Short-term effects of carbohydrate versus carbohydrate-protein ingestion on subsequent resistance training performance in male rugby players. In *Proceedings of the 10th Annual Congress of the European College of Sports Science*, Belgrade. pp. 220.

Coyle C, Donne B & Mahony N. (2012). Effects of carbohydrate-protein ingestion post-resistance training in male rugby players. *Int J Exerc Sci* **5**, 39 – 49.

Appendix II

Appendix II

Participant consent form & medical questionnaire

INFORMED CONSENT FORM

Project: The effect of carbohydrate and carbohydrate-protein sports drinks on post-exercise salivary cortisol and testosterone response.

Principal Investigators: Mr. Colin Coyle and Mr. Bernard Donne

If any adverse events occur during testing, or medical abnormalities are discovered during medical screening, I consent for my GP to be advised of these events / abnormalities.

DECLARATION:

This study and this consent form have been explained to me. The investigator(s) has/have answered all my questions to my satisfaction. I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

I understand I may withdraw from the study at any time. I understand that any data obtained as a result of my participating in this study will be held in a secure location for a period of 5 years and not subsequently used for any further studies without my due consent. I also understand that should any data from this study be published, my anonymity and confidentiality shall remain intact.

PARTICIPANT'S NAME:
CONTACT DETAILS
PARTICIPANT'S SIGNATURE:
Date:

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE:
Date:

(Keep the original of this form in the investigator's file, give one copy to the participant, and send one copy to the sponsor (if there is a sponsor).

Medical Questionnaire and Participant Examination Form Date / / 20

Name:Discipline

Age:No. of years in Sport:

Date of Birth:Contact Tel No.:

Occupation:Email:

Address:G.P's name:

.....G.P's address:

.....

.....

Training indicators

AM Resting HR:beats.min⁻¹

Sleep:hr.day⁻¹

Mood: 1 2 3 4 5 6 7 8 9 10

Fatigue: 1 2 3 4 5 6 7 8 9 10

Training sessions per week

AerobicInterval

SpeedResistance

Last training Session (time/details)

.....

.....

Personal & Family History illnesses

	Circle			Circle	
Smoking	Y	N	Heart Disease	Y	N
Alcohol	Y	N	Strokes	Y	N
Tea	Y	N	Diabetes	Y	N
Coffee	Y	N	Asthma	Y	N
Vegetarian	Y	N	Epilepsy	Y	N

State details & any other family

Drug History

MedicationY N

Over The Counter MedsY N

AllergiesY N

VitaminsY N

State Details

Current medical problems in the last 3 months

Recent Illnesses:

Recent Injuries:

<i>Last 7 days</i>	<i>Circle</i>		<i>At any time...</i>	<i>Circle</i>		<i>Details</i>
Flu Symptoms	Y	N	Hospital admissions	Y	N	
Sore Throat	Y	N	Operations	Y	N	
Cough	Y	N	Fractures	Y	N	
Wheeze	Y	N	Sports Injuries	Y	N	
Chest Pains	Y	N	Diabetes	Y	N	
Palpitations	Y	N	Asthma	Y	N	
Nausea	Y	N	Epilepsy	Y	N	
Vomiting	Y	N	Jaundice	Y	N	
Diarrhoea	Y	N	Kidney problem	Y	N	
Headaches	Y	N	Heart Murmur	Y	N	
Fits or Faints	Y	N	Eye Problems	Y	N	
Others	Y	N	Other Illness	Y	N	

General Examination Doctors Use Only

Pulsebeats.min⁻¹ Reg. / Irreg.

BP/.....mmHg

Head: Nose	Throat	FBC Result:
Neck: Nodes	Thyroid	
CVS: Apex beat	Heart Sounds	PFT Result:
RS: Exp ⁿ	Perc. / Ausc.	

Medical Summary

Fit for pre-season training study Y N Signature:

Appendix III

Appendix III
Chapter 3

Table 1: Mean BLa (mmol.L⁻¹) data for HCHO trial, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	0.8	2.8	0.7	0.8	3.6
SEM	0.1	0.2	0.1	0.1	0.2

Table 2: Mean BLa (mmol.L⁻¹) data for LCHO trial, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	0.9	2.9	0.6	0.9	3.6
SEM	0.1	0.2	0.1	0.2	0.2

Table 3: Mean BLa (mmol.L⁻¹) data for CHO-PRO trial, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	0.9	3.1	0.6	0.6	3.7
SEM	0.1	0.2	0.0	0.0	0.3

Table 4: Mean BGlu (mmol.L⁻¹) data for HCHO trial, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	4.8	4.7	4.2	4.5	4.2
SEM	0.4	0.2	0.2	0.3	0.1

Table 5: Mean BGlu (mmol.L⁻¹) data for LCHO trial, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	4.6	4.6	3.7	3.7	4.4
SEM	0.2	0.3	0.1	0.2	0.1

Table 6: Mean BGlu (mmol.L⁻¹) data for CHO-PRO, n = 14

	Pre-RT	Post-RT	Mid	Pre-TTF	Post-TTF
Mean	4.8	4.8	4.1	4.4	4.2
SEM	0.2	0.3	0.1	0.2	0.1

Table 7: Mean ΣW (kg.kg⁻¹ BM) for exercises across drinks trials, $\pm$ SEM, n = 14.

* $P<0.05$ vs. HCHO and LCHO; # $P<0.05$ vs. LCHO only.

Exercise	CHO-PRO	HCHO	LCHO
Back Squat	56.9 $\pm$ 6.1*	47.1 $\pm$ 5.3	45.7 $\pm$ 3.9
Bench press	36.0 $\pm$ 4.8	30.8 $\pm$ 4.7	28.7 $\pm$ 3.5
Leg press	57.6 $\pm$ 7.3	48.3 $\pm$ 6.5	45.2 $\pm$ 4.6
Leg curl	20.6 $\pm$ 2.4*	16.4 $\pm$ 1.6	16.9 $\pm$ 2.0
Bicep curl	16.8 $\pm$ 1.7 #	14.1 $\pm$ 1.5	13.1 $\pm$ 1.4

Table 8: Mean TWC (kg.kg⁻¹ BM) across trials, n = 14

	LCHO	HCHO	CHO-PRO
Mean	149.6	156.6	188.0
SEM	16.0	21.0	26.2

Appendix IV

Appendix IV

Chapter 4

Table 1: Mean 12 min Cooper run score (m) and predicted $\dot{V}O_2\text{max}$ (mL.kg⁻¹.min⁻¹); soccer players, n =17

	Cooper Test Score (m)	$\dot{V}O_2\text{max}$ (mL.kg ⁻¹ .min ⁻¹)
Mean	3095.3	57.9
SD	146.6	3.3
SEM	35.6	0.8

Table 2: Mean YoYo IR2 score (m) and corresponding level completed; soccer players n = 17

	Distance (m)	YoYo IR2 level
Mean	588.2	20.3
SD	119.0	0.5
SEM	28.9	0.1

Table 3: Mean Hoff test score (m) with predicted $\dot{V}O_2\text{max}$ (mL.kg⁻¹.min⁻¹); soccer players n = 16

	Hoff Test (m)	$\dot{V}O_2\text{max}$ (mL.kg ⁻¹ .min ⁻¹)
Mean	1784.8	68.1
SD	82.0	6.0
SEM	20.5	1.5

Table 4: Mean pooled cortisol (nmol.L⁻¹) concentration pre- and post-training across rugby (n = 12), rowing (n = 9) and soccer (n = 14).

	Rowing Pre	Rowing Post	Rugby Pre	Rugby Post	Soccer Pre	Soccer Post
Mean	4.6	10.0	3.2	7.6	15.0	9.1
SEM	0.6	1.7	0.4	1.2	3.0	2.2

Table 5: Mean pooled cortisol (nmol.L⁻¹) concentration pre- and post-competition across rugby (n = 12), rowing (n = 9) and soccer (n = 14).

	Rowing Pre	Rowing Post	Rugby Pre	Rugby Post	Soccer Pre	Soccer Post
Mean	9.1	14.4	7.4	11.1	4.8	14.8
SEM	0.9	1.9	0.7	1.5	0.4	3.5

Table 6: Mean pooled testosterone (nmol.L⁻¹) concentrations pre- and post-training across rugby (n = 12), rowing (n = 9) and soccer (n = 14).

	Rowing Pre	Rowing Post	Rugby Pre	Rugby Post	Soccer Pre	Soccer Post
Mean	0.34	0.54	0.34	0.44	0.35	0.40
SEM	0.03	0.07	0.02	0.03	0.03	0.03

Table 7: Mean pooled testosterone (nmol.L⁻¹) concentrations pre- and post-competition across rugby (n = 12), rowing (n = 9) and soccer (n = 14).

	Rowing Pre	Rowing Post	Rugby Pre	Rugby Post	Soccer Pre	Soccer Post
Mean	0.44	0.56	0.50	0.67	0.33	0.38
SEM	0.04	0.07	0.02	0.05	0.03	0.03

Appendix V

Appendix V

Chapter 5

Table 1: Mean 12 min Cooper run score (m) with predicted $\dot{V}O_2\text{max}$ (mL.kg⁻¹.min⁻¹), n = 12.

	Cooper Run (m)	Predicted $\dot{V}O_2\text{max}$ (mL.kg ⁻¹ .min ⁻¹)
Mean	3055	57.0
SD	129.9	2.9
SEM	37.5	0.8

Table 2: Mean Hoff test score (m) with predicted $\dot{V}O_2\text{max}$ (mL.kg⁻¹.min⁻¹), n = 12

	Hoff Test (m)	Predicted $\dot{V}O_2\text{max}$ (mL.kg ⁻¹ .min ⁻¹)
Mean	1772.4	68.1
SD	77.9	6.9
SEM	22.5	2.0

Table 3: Mean YoYo IR2 score (m) and corresponding level completed, n = 12

	Distance (m)	YoYo IR2 level
Mean	553.3	20.2
SD	112.9	0.5
SEM	32.6	0.1

Table 4: Mean salivary cortisol (nmol.L⁻¹) during LCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	10.4	10.7	6.7
SEM	2.5	2.2	1.1

Table 5: Mean salivary cortisol (nmol.L⁻¹) during HCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	12.3	9.2	6.4
SEM	1.7	1.6	0.8

Table 6: Mean salivary cortisol (nmol.L⁻¹) during CHO-PRO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	12.7	9.5	4.7
SEM	2.6	1.6	0.9

Table 7: Mean salivary testosterone (nmol.L⁻¹) during LCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.40	0.45	0.37
SEM	0.05	0.07	0.04

Table 8: Mean salivary testosterone (nmol.L⁻¹) during HCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.47	0.37	0.32
SEM	0.05	0.04	0.02

Table 9: Mean salivary testosterone (nmol.L⁻¹) during CHO-PRO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.65	0.41	0.37
SEM	0.22	0.04	0.06

Table 10: Mean salivary T/C ratio during LCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.052	0.052	0.094
SEM	0.008	0.007	0.030

Table 11: Mean salivary T/C ratio during HCHO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.045	0.046	0.057
SEM	0.006	0.004	0.010

Table 12: Mean salivary T/C ratio during CHO-PRO trials on Day 1, 2 and 4 pre-training, n = 12.

	Day 1 Pre	Day 2 Pre	Day 4 Pre
Mean	0.101	0.052	0.095
SEM	0.043	0.006	0.015

Table 13: Mean pooled (x2) salivary cortisol (nmol.L⁻¹) during CHO-PRO, HCHO and LCHO trials pre- to post-ingestion on Days 1 and 2, n = 12.

	LCHO Pre	LCHO Post	HCHO Pre	HCHO Post	CHO-PRO Pre	CHO-PRO Post
Mean	4.8	3.5	3.9	4.0	5.0	5.8
SEM	1.2	0.3	0.5	0.4	0.9	2.2

Table 14: Mean pooled (x2) salivary testosterone (nmol.L⁻¹) during CHO-PRO, HCHO and LCHO trials pre- to post-ingestion on Days 1 and 2, n = 12.

	LCHO Pre	LCHO Post	HCHO Pre	HCHO Post	CHO-PRO Pre	CHO-PRO Post
Mean	0.59	0.45	0.50	0.45	0.56	0.43
SEM	0.07	0.04	0.05	0.03	0.06	0.03

Table 15: Mean pooled (x2) salivary T/C ratio during CHO-PRO, HCHO and LCHO trials pre- to post-ingestion on Days 1 and 2, n = 12.

	LCHO Pre	LCHO Post	HCHO Pre	HCHO Post	CHO-PRO Pre	CHO-PRO Post
Mean	0.216	0.144	0.153	0.125	0.148	0.147
SEM	0.055	0.017	0.016	0.010	0.016	0.023

Appendix VI

Appendix VI

Chapter 6

Table 1: Mean BLa concentration (mmol.L⁻¹) pre- to post GD protocol, ± SEM, n = 7.

	CHO-PRO	HCHO	PL
Pre-GD Protocol	1.2 ± 0.3	1.0 ± 0.2	1.1 ± 0.2
Post-GD Protocol	3.6 ± 0.9	3.0 ± 0.5	3.1 ± 0.8

Table 2: Mean BLa concentration (mmol.L⁻¹) pre- to post-ExTr and throughout the 60 min monitored recovery period, n =7

CHO PRO	Mean	SEM
Pre-ExTr	0.6	0.1
Post-ExTr	3.0	0.4
+15	1.5	0.2
+30	1.3	0.1
+45	1.4	0.1
+60	1.3	0.1

HCHO	Mean	SEM
Pre-ExTr	0.5	0.0
Post-ExTr	2.3	0.3
+15	1.1	0.1
+30	1.0	0.1
+45	1.1	0.1
+60	1.2	0.1

PL	Mean	SEM
Pre-ExTr	0.7	0.1
Post-ExTr	2.4	0.3
+15	1.2	0.1
+30	1.0	0.1
+45	1.0	0.1
+60	0.9	0.1

Table 3: Mean BGlu concentration (mmol.L⁻¹) pre- to post GD protocol, $\pm$ SEM, n = 7.

	CHO-PRO	HCHO	PL
Pre-GD Protocol	3.7 $\pm$ 0.6	3.9 $\pm$ 0.7	3.9 $\pm$ 0.7
Post-GD Protocol	3.7 $\pm$ 0.6	3.9 $\pm$ 0.2	3.6 $\pm$ 0.6

Table 4: Mean BGlu response (mmol.L⁻¹) pre- to post-ExTr and throughout the 60 min monitored recovery period, n = 7.

CHO PRO	Mean	SEM
Pre-ExTr	4.2	0.1
Post-ExTr	4.4	0.3
+15	5.3	0.4
+30	6.1	0.4
+45	5.0	0.5
+60	4.2	0.2

HCHO	Mean	SEM
Pre-ExTr	4.3	0.1
Post-ExTr	4.2	0.2
+15	5.6	0.2
+30	6.4	0.4
+45	6.2	0.5
+60	5.4	0.4

PL	Mean	SEM
Pre-ExTr	4.1	0.1
Post-ExTr	4.1	0.2
+15	4.1	0.1
+30	4.0	0.1
+45	3.9	0.1
+60	3.9	0.1

Table 5: Mean serum insulin response ($\mu\text{IU.mL}^{-1}$) pre- to post-ExTr and throughout the 60 min monitored recovery period, n = 7.

CHO PRO	Mean	SEM
Pre-ExTr	4.3	1.2
Post-ExTr	1.9	1.3
+15	17.2	7.6
+30	34.7	7.5
+45	21.5	7.2
+60	13.3	4.9

HCHO	Mean	SEM
Pre-ExTr	2.8	0.6
Post-ExTr	0.7	0.5
+15	21.0	8.5
+30	24.5	6.8
+45	23.2	5.2
+60	18.1	3.8

PL	Mean	SEM
Pre-ExTr	3.1	0.8
Post-ExTr	1.5	0.6
+15	4.5	0.7
+30	2.6	0.7
+45	1.7	0.6
+60	1.8	0.6

Table 6: Mean area under the curve cumulative for BGlu (mmol.min.L⁻¹) and insulin (μIU.min.mL⁻¹) response throughout the 60 min monitored recovery period, n = 7.

CHO-PRO	BGlu (mmol.min.L ⁻¹)	Insulin (μIU.min.mL ⁻¹)
Mean	51.8	1161.8
SEM	5.1	312.1

HCHO	BGlu (mmol.min.L ⁻¹)	Insulin (μIU.min.mL ⁻¹)
Mean	92.0	1127.9
SEM	13.7	296.0

PL	BGlu (mmol.min.L ⁻¹)	Insulin (μIU.min.mL ⁻¹)
Mean	-4.7	68.9
SEM	5.4	21.2

Table 7: Mean salivary cortisol concentration (nmol.L⁻¹) across time, ± SEM, n = 7.

	Pre-GD	Post-GD	Pre-ExTr	Post-ExTr	+60 min
CHO-PRO	3.3 ± 0.6	6.4 ± 1.5	6.9 ± 1.7	11.7 ± 3.6	8.7 ± 1.6
HCHO	4.2 ± 0.8	4.8 ± 0.9	10.0 ± 2.4	7.6 ± 1.3	8.2 ± 2.4
PL	3.3 ± 0.6	5.8 ± 1.0	9.7 ± 1.8	9.6 ± 2.4	7.7 ± 1.4

Table 8: Mean salivary testosterone concentration (nmol.L⁻¹) across time, ± SEM, n = 7.

	Pre-GD	Post-GD	Pre-ExTr	Post-ExTr	+60 min
CHO-PRO	0.61 ± 0.17	0.67 ± 0.11	1.16 ± 0.18	1.64 ± 0.17	1.13 ± 0.19
HCHO	0.71 ± 0.18	0.99 ± 0.26	1.22 ± 0.21	1.45 ± 0.20	1.39 ± 0.41
PL	0.46 ± 0.07	0.72 ± 0.14	0.94 ± 0.18	1.05 ± 0.17	1.04 ± 0.20

Appendix VII

Pilot investigation: Serum versus saliva hormone concentrations

Serum cortisol and testosterone ELISA procedure

Serum samples were assayed for C and T using a sandwich type immunoassay ELISA kit (ALPCO Diagnostics, Salem, USA). The kits operated on the principle of competition between C and T in the serum sample and the enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting steps removed unbound materials. After washing, the enzyme substrate was added and the plate left to incubate at room temperature. The enzymatic reaction was then terminated by adding stop solution and absorbance measured on a plate reader (Biotek, Bedfordshire, UK) at 450 nm. The intensity of the colour change was inversely proportional to the concentration of cortisol in the sample.

Standard and controls were assayed on all plates. All standards, controls and serum samples were run in duplicate. The serum C ELISA used 20 μL (for singlet determination) and had a lower limit sensitivity of 1.10 nmol.L^{-1} and an intra- and inter-CV% between 2.9 to 9.4 and 3.8 to 8.1, respectively. The serum T ELISA used 50 μL (for singlet determination) and had a lower limit sensitivity of 0.76 nmol.L^{-1} with an intra- and inter-CV% between 6.6 to 9.6 and 6.1 to 8.5, respectively.

Serum testosterone and cortisol ELISA procedure

The 96-well microplate along with reagents, standards, controls and samples were brought to room temperature. Serum samples were centrifuged (Sigma Laboratory Centrifuges, Osterode am Harz, Germany) for 10 min at $15000 \text{ rev.min}^{-1}$, while all reagents, standards and controls were vortexed (Scientific Industries, New York, USA) prior to use, as per manufacturer guidelines. The standard curve for T included known concentrations; zero, 0.28, 1.46, 5.79, 17.34 and $57.91 \text{ nmol.L}^{-1}$. Controls of high ($17.34 \text{ nmol.L}^{-1}$) and low (2.6 nmol.L^{-1}) T concentrations were also included. 50 μL of each standard, control and sample were transferred into their respective wells and 100 μL of detection enzyme conjugate was then added to each well using a mutli-channel pipette. The plate was covered with a plate sealer and

incubated at room temperature for 60 min on an orbital plate shaker (Scientific Industries, New York, USA) set to 200 rev.min⁻¹.

The C standard curve included known concentrations; zero, 13.8, 55.2, 138.0, 276.0, 828.00 and 1656 nmol.L⁻¹. Controls of high (828.0 to 1380 nmol.L⁻¹) and low (289.8 to 759.0 nmol.L⁻¹) C concentrations were also included. 20 µL of each standard, control and sample were transferred into their respective wells. 100 µL of detection enzyme conjugate was then added to each well using a multi-channel pipette. The plate was covered with a plate sealer and incubated at room temperature for 45 min on an orbital plate shaker (Scientific Industries, New York, USA) set to 200 rev.min⁻¹.

On completion of this incubation period, the microplate was washed 3 times with working strength (diluted) wash buffer and blotted thoroughly between washes on absorbent paper towels. Using the multi-channel pipette, 150 µL of TMB substrate was then added to each well. The plate was immediately covered with a plate sealer and incubated for 15 min at room temperature on an orbital plate shaker set to 200 rev.min⁻¹. On completion of the second incubation period, 50 µL of stop solution was added to each well using a multi-channel pipette and the plate was gently shaken to ensure the stop solution mixed completely. The microplate was then placed on a reader at an OD of 450 nm within 20 min of the addition of the stop solution.

Calculation of serum cortisol and testosterone concentrations

For each ELISA plate, the mean OD was calculated for the duplicate wells. The concentration of the controls and mean OD of unknowns were entered in a graphic interpolation package (Cricket Graph version 1.3.1, Cricket Graph Software, California, USA). For each ELISA plate, a graph of log of control concentration versus mean OD was determined. A third order polynomial equation was fitted to the data and the line of best fit calculated for each ELISA plate. The coefficients of determination exceeded 0.95 for all plates. The resultant constants for the cubic equation were entered into a spreadsheet to assess the concentration of C and T of the unknown samples on each plate.

Salivary cortisol and testosterone

Salivary cortisol and testosterone concentrations were assessed by ELISA technique as outlined in Section 2.7 and results in nmol.L⁻¹ were computed as outlined in Section 2.7.3

Table 1: Mean salivary cortisol concentrations (nmol.L⁻¹) across time, ± SEM.

	0900	1200	1500	1800
Day 1	8.4 ± 1.9 n = 10	4.2 ± 0.6 n = 9	3.7 ± 0.4 n = 10	3.6 ± 0.9 n = 10
Day 2	6.5 ± 0.7 n = 12	3.6 ± 0.4 n = 12	4.7 ± 1.2 n = 12	3.4 ± 0.5 n = 12
Day 3	9.2 ± 2.0 n = 12	8.9 ± 4.0 n = 12	5.7 ± 2.5 n = 12	6.5 ± 3.3 n = 12

Table 2: Mean salivary testosterone concentrations (nmol.L⁻¹) across time, ± SEM.

	0900	1200	1500	1800
Day 1	0.82 ± 0.14 n = 10	0.66 ± 0.06 n = 9	0.64 ± 0.09 n = 10	0.87 ± 0.13 n = 9
Day 2	0.76 ± 0.13 n = 12	0.82 ± 0.24 n = 12	0.88 ± 0.24 n = 12	0.85 ± 0.25 n = 12
Day 3	0.59 ± 0.09 n = 12	0.57 ± 0.15 n = 12	0.69 ± 0.12 n = 12	0.69 ± 0.15 n = 12

Figure 1: Mean salivary cortisol concentration (nmol.L^{-1}) recorded at fixed intervals across 3 consecutive days in free-living male volunteers, bars denote SEM.

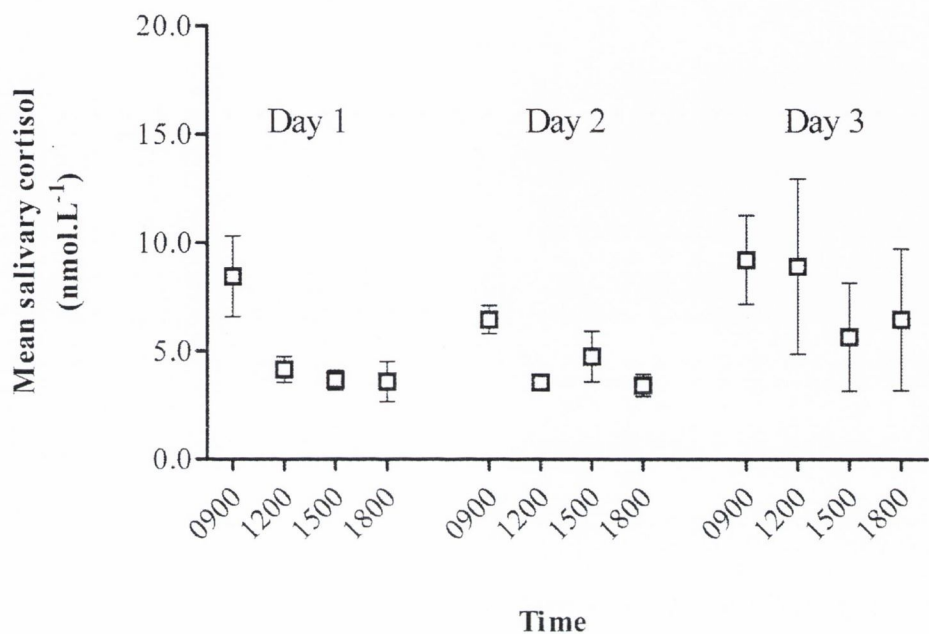


Figure 2: Mean serum testosterone concentration (nmol.L^{-1}) recorded at fixed intervals across 3 consecutive days in free-living volunteers, bars denote SEM.

