

**The effects of a high molecular weight glucose  
polymer on muscle metabolism and exercise  
performance in humans**

Frances Gunner, BSc, MSc.

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## **Thesis Abstract**

The work in this thesis has investigated the potential for a unique HMW glucose polymer (Vitargo, Swecarb AB, Sweden; MW of 500-700 g.mol<sup>-1</sup>) derived from barley starch to augment short-term post exercise muscle glycogen resynthesis above that of an isoenergetic LMW glucose polymer (Maxijul, SHS International, UK; MW of 900 g.mol<sup>-1</sup>). The HMW glucose polymer has been previously investigated in comparison to a LMW glucose solution with studies reporting a 70% greater muscle glycogen content after 2 hr recovery from glycogen-depleting exercise (Piehl-Aulin et al., 2000) and an enhanced gastric emptying at rest (Leiper et al., 2000). More recently an improved work output (10%) in a maximal exercise test performed 2 hr after exhaustive exercise was demonstrated after ingestion of the HMW glucose polymer compared to an isoenergetic LMW glucose polymer (Stephens et al., 2008). Key observations in this study were a greater rate of rise in blood glucose and serum insulin concentration during recovery with ingestion of the HMW compared to the LMW glucose polymer. Thus it was suggested that the improvement in performance in the secondary exercise bout could potentially be attributed to greater muscle glycogen availability present at the onset of the test.

This hypothesis was subsequently tested initially in this thesis with the quantification of muscle glycogen content after cycling to exhaustion and ingestion of the same HMW and LMW glucose polymers. However, despite undertaking an identical exercise protocol, in contrast with the study by Stephens et al (2008), no differences in the rate of rise in blood glucose or

serum insulin were observed. Accordingly muscle glycogen resynthesis measured 2 hrs after exhaustive exercise was similar following ingestion of the HMW and LMW glucose polymers (118 vs. 123 mmol.kg<sup>-1</sup>). Thus exercise performance in a secondary bout was near identical between both polymers (173 vs. 175 kJ). It was concluded that the LMW and HMW glucose polymers elicited similar post exercise muscle glycogen resynthesis however, since the sampling interval in this study using muscle biopsies was large (2 hr), it may have negated to highlight any early differences in muscle glycogen content. Therefore further investigation was undertaken that focused on more subtle sequential fluctuations in muscle glycogen by using ultra-high field <sup>13</sup>C MRS following feeding of the same HMW and LMW glucose polymers. Marginal increases in muscle glycogen during 1 hr of recovery from prolonged exercise were reported after ingestion of the HMW and LMW glucose polymers (6 and 4% respectively). Additionally, increases in muscle glycogen after ingestion of both glucose polymers above that of a zero-energy control were not seen after 1 hr of recovery when a greater magnitude of resynthesis would be expected with the former. It was thus postulated that irrespective of the improved sensitivity of ultra-high field <sup>13</sup>C MRS, the technique may not be suited to post exercise muscle glycogen resynthesis determination due to the methodological issue of subject positioning inhibiting typical gastric emptying patterns.

When considering the implications of these studies it appears that the HMW glucose polymer does not augment post exercise muscle glycogen resynthesis above that of an isoenergetic glucose polymer with a much lower molecular weight. Nonetheless given that the blood glucose and serum insulin profiles

over a 2 hr recovery in the first study of this thesis and the study by Stephens et al (2008) were notably different with the same test solutions, it was considered that there may be a disparity with the HMW glucose polymers utilised. Importantly the production of the HMW glucose has altered such that the manufacturing process has deviated from granulation to agglomeration with the native starch evolving from potato to corn and more recently barley. It was suggested that the most recent HMW glucose polymer used presently had deviated away from its initial characteristics leading to the blood glucose and serum insulin responses observed in the first study of this thesis. Indeed by then comparing post exercise ingestion of a previous granulated version of the HMW glucose polymer with a more soluble agglomerated version in the same experimental protocol as the first study, an initial greater rise in serum insulin was observed in the first 55 min of post exercise recovery. Thus alterations in manufacturing from granulation to agglomeration do appear to have affected properties related to postprandial insulin secretion. However this effect on insulin was not seen overall over the 2 hr recovery period and no differences in blood glucose or exercise performance in a secondary bout were observed suggesting other factors such as the native starch may be influential.

It can thus be concluded that the difference in postprandial glucose and insulin responses seen between previous work and the present investigation may be due to altered physical characteristics of the HMW glucose polymer. No differences in intrinsic viscosity, rheology or molecular weight were noted between the agglomerated and granulated versions of the HMW glucose polymer thus the alterations in the origin material may account for more

influence on digestibility *in vivo*. Further investigation would be warranted into effects on post exercise muscle glycogen resynthesis and exercise performance provided that the HMW glucose polymer could be returned to its original formulation.

## **Declaration**

This thesis is a presentation of my own original experimental data completed with the assistance of the academic and technical staff in the School of Biomedical Sciences, University of Nottingham. I declare that this thesis contains a record of my own work and in no way has the information been previously submitted for any other degree.

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## List of Abbreviations

|       |   |
|-------|---|
| AICAR | Aminoimidazole carboxamide ribonucleotide |
| AKT   | Serine threonine kinase/ protein kinase B |
| AMPK  | AMP-activated kinase                      |
| ADP   | Adenosine diphosphate                     |
| ATP   | Adenosine triphosphate                    |
| BMI   | Body mass index                           |
| CAMK  | Calmodulin-dependent Protein kinase       |
| Co-A  | Co-enzyme A                               |
| CSS   | Composite satiety score                   |
| DE    | Dextrose equivalent                       |
| DW    | Dry weight                                |
| ECG   | Electrocardiogram                         |
| G6P   | Glucose-6-phosphate                       |
| G6PDH | Glucose-6-phosphate dehydrogenase         |
| GI    | Glycaemic Index                           |
| GLU   | Glucose                                   |
| GLUT  | Glucose transporter                       |
| GS    | Glycogen synthase                         |
| GP    | Glycogen phosphorylase                    |
| GSK3  | Glycogen synthase kinase 3                |
| HCL   | Hydrochloric acid                         |
| HK    | Hexokinase                                |
| HMW   | High molecular weight                     |
| IRS   | Insulin receptor substrate                |
| kDa   | Dalton                                    |
| LMW   | Low molecular weight                      |
| MG    | Macroglycogen (acid-soluble)              |
| MRS   | Magnetic resonance spectroscopy           |
| NADH  | Nicotinamide adenine dinucleotide         |
| NEFA  | Non-esterified fatty acids                |
| PG    | Proglycogen (non-acid soluble)            |
| PIK3  | Phosphatidyl 3-kinase                     |
| PKC   | Protein kinase C                          |
| RVA   | Rapid Visco-Analyser                      |
| SGLT  | Sodium-dependent glucose transporter      |
| T     | Tesla                                     |
| UTP   | Uridine triphosphate                      |
| WW    | Wet weight                                |

**In all cases throughout this thesis, values for muscle glycogen content refer to dry weight (dw) unless otherwise stated as wet weight (ww).**

# **Chapter 1**

## General Introduction

## 1. Introduction

Prolonged, moderate to high intensity exercise is characterised by a reliance on carbohydrate and fat as substrates for ATP resynthesis and energy production in contracting skeletal muscle. Although both carbohydrate and fat will contribute to energy metabolism, as exercise intensity increases there is a greater dependency on muscle glycogen and blood glucose paralleled by a decline in whole-body fat oxidation (Romijn et al., 1993). A reliance on carbohydrate sources has been demonstrated when exercise intensity during cycling was increased from 55% to 75%  $\dot{V}O_2$  max, resulting in the total energy expenditure attributed to muscle glycogen and blood glucose rising from 51% to 78% (van Loon et al., 2001). Endogenous glycogen stores are present in skeletal muscle (~250-400g) and the liver (~80-110g) and in the absence of exogenous sources, provide the predominant source of energy metabolism during moderate to high intensity exercise (>70%  $\dot{V}O_2$  max). The rate of ATP required to sustain prolonged moderate to high intensity effort is ~2.5 mmol.kg.dm<sup>-1</sup>.s<sup>-1</sup> which can be met by carbohydrate oxidation (~2-2.8 mmol.kg.dm<sup>-1</sup>.s<sup>-1</sup>), however muscle glycogen reserves are finite and thus limit energy production to approximately 80-90 min of sustained effort. An integrated response to exercise occurs via oxidation of muscle glycogen, blood borne glucose (derived from the liver or gastrointestinal tract if fed), NEFA and triglycerides to address metabolic ATP demand (Romijn et al., 1993). Due to the limited nature of endogenous carbohydrate reserves, only catabolism of fat through beta-oxidation can allow the continuation of exercise albeit at a moderate intensity ( $\leq$ 70%  $\dot{V}O_2$  max) as total fat oxidation is suppressed at

higher exercise intensities (Horowitz and Klein, 2000). Notably when glycogen content falls and reliance on fat oxidation increases, the ATP production rates are much lower ( $\sim 1 \text{ mmol.kg.dm}^{-1}.\text{s}^{-1}$ ) and are therefore insufficient to meet requirements for higher intensity exercise. Thus ATP homeostasis cannot be maintained and activity must decrease in intensity or cease. In addition the availability of fatty acyl groups in the mitochondria is suggested to be rate-limiting for fat oxidation at moderate to high exercise intensities. This is potentially due to the inhibition of enzyme transport across the muscle membrane via increasing muscle malonyl-CoA content or a low free carnitine availability (Stephens et al., 2007). It follows that for continued ATP production and continuation of physical effort at intensities of  $\sim 70\% \dot{V}O_2 \text{ max}$  and above, the most viable energy source is muscle glycogen.

### **1.1 The effects of prolonged sub maximal exercise on glycogen depletion**

Prolonged exercise can be defined as lasting between 30 and 180 min at an intensity between 60 and 85%  $\dot{V}O_2 \text{ max}$ . This type of exercise is recognised to be limited by muscle glycogen availability, a concept that has been accepted since a body of work originating in Sweden by Bergstrom and Hultman in the 1960's. They reported a gradual decline in muscle glycogen concentration of the Quadriceps Femoris during cycling at 80%  $\dot{V}O_2 \text{ max}$  and observed the rate of muscle glycogen degradation to be fastest in the first 15 min after the onset of the activity (Bergstrom and Hultman 1967). Notably, the development of volitional fatigue in this study coincided with near-total depletion of the muscle glycogen store. The importance of muscle glycogen for optimal performance

was illustrated in another experiment that manipulated dietary intake following an exhausting cycling bout to reduce muscle glycogen. This exercise was followed by a 3 day intake of a normal mixed diet (2800 kcal), a low carbohydrate diet (1500 kcal protein + 1300 kcal fat) or a high carbohydrate diet (2300 kcal carbohydrate + 500 kcal protein) preceding a second exhaustive exercise bout (Bergstrom et al., 1967). Glycogen content after 3 days on the isocaloric diets was 118, 42 and 227 mmol.kg<sup>-1</sup>.ww for the mixed, low and high carbohydrate diets respectively which corresponded to 126, 59 and 189 min endurance cycling times in the second exercise session. Thus increasing pre-exercise muscle glycogen levels can extend cycling times to exhaustion. This study demonstrated a positive relationship between pre-exercise glycogen content of the *Quadriceps Femoris* and exercise capacity, a finding that has been consistently replicated in subsequent research. In resting conditions the level of muscle glycogen is fairly resistant to change irrespective of dietary intake, indeed fasting for 24 hr does not appear to affect resting levels (Loy et al., 1986). For example total starvation for 4 days with no exercise reduced muscle glycogen content by 40% and 1 week of a carbohydrate poor diet caused a reduction of 30% (Hultman and Bergstrom, 1967). Reintroducing a high carbohydrate diet increased the muscle glycogen store moderately when no exercise was performed. However in combination with prolonged exercise a concentration can be accrued above normal, a phenomenon that would be beneficial for performance. This effect of supranormal resynthesis was shown in a study that employed one-legged exhaustive cycling in 2 subjects followed by 3 days of an almost exclusive carbohydrate diet (Bergstrom and Hultman 1966). Muscle biopsies were taken from the *Quadriceps Femoris* in both legs

after exhaustion and daily during 3 days of a 2200-2600 kcal.day<sup>-1</sup> almost total carbohydrate diet and a dramatic increase in muscle glycogen was observed localised to the exercised leg. After 1 day muscle glycogen in the exercised leg had risen above the level of the resting leg, in which glycogen remained largely unchanged, and continued to rise for the following 2 days where it reached almost twice the content compared to the non-exercised leg (~4g per 100g muscle in exercised vs. ~2g per 100g muscle in non-exercised) after 3 days. Notably the consumption of a very high carbohydrate diet did not much alter the muscle glycogen content in the non-exercised leg (increase of <1g per 100g of muscle) in both subjects implying prior exercise is essential for stimulating muscle glycogen resynthesis above normal levels. It was suggested from this study that the process of glycogen depletion in muscle stimulates localised resynthesis and this effect of 'supercompensation' is the basis for carbohydrate loading regimens employed by athletes to maximise pre-exercise muscle glycogen concentrations. Optimising muscle glycogen levels using a combination of prior depleting exercise and high carbohydrate diets (>60% total intake) is a common practice preceding periods of competition. Supercompensated muscle glycogen levels have been shown to persist for at least 3 days after the carbohydrate loading period (Goforth et al., 1997). The carbohydrate loading period in this study involved 3 days of a low carbohydrate diet (10% of total intake) with 115 min of cycling at 75%  $\dot{V}O_2$  peak on day 1 after which a further 2 days required 40 min cycling at the same intensity. This was followed by 3 days of rest and consumption of a high (85% of total intake) carbohydrate diet. The protocol was successful in increasing baseline muscle glycogen by 1.79 times and was resistant to change in the

subsequent 3 days post-loading. Subsequently it was shown that muscle glycogen as measured by MRS was increased (1.45 times above baseline) following 120 min cycling at 65%  $\dot{V}O_2$  peak followed by 1 min sprints to exhaustion and a high carbohydrate diet. This level decreased by 10 mmol.l<sup>-1</sup> when exercise (20 min at 65%  $\dot{V}O_2$  peak) was performed during the post-loading phase but was replenished to the pre-exercise level 24 hr later (Goforth et al., 2003). Thus supercompensated muscle glycogen content persists even when light exercise is undertaken. It has also been shown that after an exhaustive exercise bout and 3 days of feeding an 85% carbohydrate diet to supercompensate muscle glycogen, the reduction in muscle glycogen content thereafter was not significant at 3 and 5 days after the loading phase. However it was significantly lowered after 7 days (decreased by 46%), suggesting that supercompensated muscle glycogen can be maintained up to 5 days if the high carbohydrate intake is sustained following the initial loading phase in trained individuals (Arnall et al., 2007).

### *1.1.1 Muscle fibre types*

During intense exercise (30 sec maximal treadmill sprinting) the rate of glycogenolysis has been reported to be 64% higher in Type II fibres than Type I fibres (Greenhaff et al., 1994). In contrast, glycogen depletion has been shown to occur primarily in type I muscle fibres during prolonged exercise at moderate intensities (Gollnick et al., 1974; Vollestad and Blom 1985). Additionally Ball-Burnett et al (1991) showed that during one-legged cycling at 61%  $\dot{V}O_2$  max until 2 hr or fatigue, a more pronounced glycogen depletion was present in Type I compared to Type II fibres ( $82 \pm 45$  vs.  $175 \pm 62$  mmol.kg<sup>-1</sup>) suggesting that they are preferentially recruited. A study by

Tsintzas et al (1995) demonstrated a 28% reduction in mixed muscle glycogen degradation during a 60 min run at 70%  $\dot{V}O_2$  max with ingestion of a 5.5% carbohydrate-electrolyte solution compared to water. This effect was observed solely in Type 1 fibres suggesting the consumption of carbohydrate during exhaustive sub maximal exercise can offset glycogen depletion in Type I fibres and also indicating they are predominantly recruited during this type of exercise. As exercise progresses, higher glycogen degradation is present in Type II fibres suggesting that they are gradually recruited as Type I fibres become depleted (Gollnick et al., 1973; Vollestad et al., 1984). As a result of prolonged exercise that preferentially depletes glycogen in Type I fibres, some Type II fibres will not be wholly depleted, thus sampling of mixed muscle such as the *Vastus Lateralis* may detect a low level of glycogen content still present at exhaustion.

### 1.1.2 Liver glycogen and exercise

The liver has a significant role in the homeostasis of blood glucose concentration from either catabolism of its glycogen stores or conversion to glucose of lactate, pyruvate, glycerol and amino acids through gluconeogenesis (Wahren and Ekberg 2007). Although decrements in prolonged sub-maximal exercise performance are likely due to muscle depletion, they may also result from the development of hypoglycaemia (Loy et al., 1986). Liver glycogen content has been reported to be  $\sim 270 \text{ mmol.kg}^{-1}\text{ww}$  after a normal mixed diet (Nilsson 1973), however this can be depleted with starvation or a low carbohydrate diet to  $\sim 24\text{-}55 \text{ mmol.kg}^{-1}\text{ww}$  (Nilsson and Hultman 1973). Restoration of liver glycogen above normal resting levels ( $\sim 500 \text{ mmol.kg}^{-1}$ ) can occur within 1 day provided a high carbohydrate diet is consumed. Basal

glucose output at rest is approximated to be  $0.8 \text{ mmol}\cdot\text{min}^{-1}$  (Wahren et al., 1971) and is accomplished via an approximate 50% contribution from glycogenolysis and gluconeogenesis respectively (Wahren and Ekberg 2007). This occurs even during the early postprandial period when hepatic glycogen availability is high (Petersen et al., 1996). It was demonstrated using  $^{13}\text{C}$  MRS that the rate of glycogenolysis was relatively constant in healthy males during an initial 22 hr of fasting at which time gluconeogenesis contributed to 36% of total glucose production (Rothman et al., 1991). As fasting continued and liver glycogen became reduced, the contribution of gluconeogenesis concurrently increased up to 96% of total glucose production after a further 18 hr.

Blood glucose concentration is maintained during light exercise indicating a close matching of hepatic output to extraction by skeletal muscle (Wahren et al., 1971). An increased glucose production with increasing exercise intensity is accounted for primarily by hepatic glycogenolysis with contribution from gluconeogenesis remaining constant (Petersen et al., 2004). As exercise progresses the proportion of liver glucose output derived from glycogenolysis decreases as liver glycogen stores become depleted and gluconeogenesis is accelerated to maintain hepatic glucose output (Ahlborg et al., 1974). During exercise the reciprocal fluctuations in insulin and glucagon are responsible for the increase in hepatic output by augmenting glycogenolysis and increasing the extraction of gluconeogenic precursors for conversion to glucose (Wolfe et al., 1986; Wasserman et al., 1989). A decline in insulin concentration and a concurrent rise in glucagon levels is required during exercise to maintain plasma glucose homeostasis (Wolfe et al., 1986). However during more

strenuous exercise, glucose utilisation may exceed production resulting in the eventual development of hypoglycaemia (Trimmer et al., 2002).

## **1.2 Post-exercise muscle glycogen resynthesis**

Muscle glycogen resynthesis following exercise is characterised by a protracted increase in permeability of the muscle membrane to glucose and an increased activity of glycogen synthase (GS), both of which will be discussed here. The pattern of muscle glycogen resynthesis has been shown to proceed in two distinct phases related to the influence of insulin. Immediately after exercise a more rapid phase dominates that is independent of insulin and has been demonstrated in rodent studies (Maehlum et al., 1977; Garetto et al., 1984; Richter et al., 1984) and importantly in humans (Price et al., 1994). This last study depleted calf muscle glycogen to 25% of resting levels and noted that infusion of somatostatin to inhibit insulin secretion did not affect muscle glycogen formation with a resynthesis rate of  $27 \text{ mmol.l}^{-1}.\text{h}^{-1}$  in the first 30 min post exercise that slowed to 1 tenth of the rate from 30 to 60 min. When muscle glycogen reached  $> 35 \text{ mmol.l}^{-1}.\text{h}^{-1}$ , further increases in muscle glycogen did not occur. This led the authors to suggest that early post exercise resynthesis is unaffected by insulin thus implying local control by intramuscular factors. This initial insulin-independent phase of muscle glycogen resynthesis occurs in the first 30-60 min post-exercise (Jentjens and Jeukendrup 2003) and is thought to only occur when muscle glycogen concentrations are lower than 128-150  $\text{mmol.kg}^{-1}$  (Maehlum et al., 1977; Price et al., 1994). In the later phase in the study by Price et al (1994) the rate of muscle glycogen resynthesis slowed to  $2.9 \pm 0.8 \text{ mmol.l}^{-1}.\text{h}^{-1}$  and was unchanged up to 6 hr into recovery. Following the rapid initial phase after glycogen depleting exercise muscle glycogen

resynthesis proceeds at a slower rate requiring both carbohydrate availability and elevated insulin concentrations (Ivy et al., 1998).

### *1.2.1 Activation of Glycogen Synthase*

Similar to other enzymes, Glycogen Synthase (GS) exists in 2 interconvertible forms, an inactive non-phosphorylated (D) and an active phosphorylated (I) form, the conversion of which is mediated by a glycogen-synthase phosphatase-I enzyme (Danforth 1965). GS catalyses the incorporation of UDP-glucose into glycogen and is regulated allosterically by G6P in addition to reversible phosphorylation and dephosphorylation leading to inactivation and activation respectively (Nielsen and Wojtaszewski 2004). Phosphorylation decreases the activity of glycogen synthase by causing an increase in the  $K_m$  for UDP-glucose (Roach et al., 1976). GS is phosphorylated at nine or more sites and the extent of activation depends on the specific site phosphorylated and on the G6P concentration (Villar-Palasi and Guinovart 1997; Roach 2002). It is well known that GS activity is closely associated with glycogen depletion in that the activity of the I form is inversely related to muscle glycogen content (Ivy and Kuo 1998). Conversion between GS forms is mediated by a phosphorylation-dephosphorylation mechanism with activation of the enzyme caused by protein-phosphatase-1. As the glycogen concentration decreases, glycogen synthase is released and de-phosphorylated to its active form. Additionally the release of insulin after exercise acts on protein-phosphatase 1 to stimulate conversion of GS to its active form. Along with muscle glucose uptake, the activity of GS is thought to be rate-limiting for muscle glycogen synthesis.

### *1.2.2 Muscle glucose uptake*

Glucose availability and glucose transport across the muscle cell are considered rate-limiting factors in glucose utilisation for storage. The delivery of glucose to the muscle is subject to physiological barriers relating to digestion and absorption. Subsequently research has investigated strategies to optimise glucose availability via manipulating the quantity, type and frequency of carbohydrate feedings. These efforts are discussed further in section 1.4. Glucose from the circulation crosses the muscle membrane via the process of facilitated diffusion mediated by GLUT4 transporters which migrate from intracellular storage compartments and fuse to the muscle membrane. Insulin stimulation and physical exercise are the two pathways that are physiologically relevant for increased GLUT4 translocation. The increase in muscle glucose uptake that occurs following glycogen-depleting exercise is due to this increased GLUT4 translocation but has been suggested to involve increased intrinsic activity (Furtado et al., 2003). GLUT4 has also been associated with translocation to the t-tubules to facilitate glucose transport throughout the muscle. It has been shown using a confocal imaging technique in living muscle fibres of anaesthetised mice that insulin diffuses into the t-tubule system to cause interaction with local insulin receptors triggering local insulin signalling and GLUT4 translocation (Lauritzen, 2009). In addition it was demonstrated in this study that most GLUT4 vesicles are distributed locally within the t-tubule system and sarcolemma rather than migrating long distances thus aiding glucose transport throughout the muscle cells. Glucose transport has been proposed as rate-limiting to muscle glycogen synthesis (Fisher et al., 2002) leading to further investigation into the stimulation of increased muscle glucose

uptake in the post-exercise period. The GLUT4 transporters are sensitive to both muscle contractions that can stimulate increased glucose uptake during exercise and insulin release post-exercise (Thorell et al., 1999). As exercise intensity increases and muscle glycogen is degraded, muscle glucose uptake is increased, and glucose enters the muscle cell where it is immediately phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase. The effects of insulin and exercise have been shown to be additive suggesting that two separate pools of GLUT4 transporters may exist that respond to these different signalling mechanisms i.e. exercise and insulin (Coderre et al., 1995; Goodyear and Kahn 1998; Ploug et al., 1998). The proposed mechanisms regulating both contraction and insulin stimulated muscle glucose uptake are not yet fully understood but are discussed here and set out in Figure 1.1.

#### *1.2.2.1 Contraction-induced effects*

The effects of muscular contractions on glucose uptake are thought to be regulated on two levels. One level refers to the increased intracellular calcium concentration from muscular contractions which act as a feed-forward mechanism to initiate increased muscle glucose uptake during exercise. The calcium-sensitive Protein Kinase C (PKC) conventional isoforms  $\alpha$ ,  $\beta$  and  $\gamma$  have been implicated in this process although further research is required into this area (Richter et al., 2003). Another calcium regulated protein which may be involved in this pathway is the family calmodulin-dependent protein kinase (CAMK) which may also be a stimulator of insulin-induced muscle glucose transport (Jessen and Goodyear 2005). Aside from calcium-sensitive signalling, there is a possible second level of regulation stimulated by changes in energy charge and thus the metabolic status of the muscle creating a feedback pathway

for muscle glucose transport. AMP-activated kinase (AMPK) is regarded as a fuel sensor within the muscle that is activated during exercise and therefore has been implicated as a regulator of muscle glucose transport since it has been shown to be activated in the rat in response to exercise *in vivo* (Rasmussen and Winder 1997; Rasmussen et al., 1998). AMPK is activated by an increase in the AMP:ATP ratio to switch off ATP-consuming pathways and switch on alternative pathways for ATP generation (Fujii et al., 2004). The relationship between muscle glucose transport and AMPK has been investigated using AICAR which is metabolised to mimic the effects of AMP on AMPK and has been reported to increase muscle glucose transport in the rat (Fujii et al., 2006). As it was initially shown that AICAR increased glucose uptake and AMPK activity (Merrill., et al 1997) it was then suggested that AMPK is the dominant signalling pathway regulating exercise induced muscle glucose transport. The potential for AMPK to mediate contraction-induced glucose transport was then suggested by a study using transgenic mice that demonstrated a reduction in AMPK activity which subsequently blunted AICAR-stimulated glucose uptake (Mu et al., 2001). In humans AMPK activity is higher when muscle glycogen content is low (Wojtaszewski et al., 2003) as is muscle glucose transport and interest has been shown in recent years in the role of AMPK in contraction-induced glucose transport and also with reference to obesity-related conditions (Hardie et al., 2006). Activation of AMPK by AICAR or hypoxia increases the plasma membrane content of GLUT4 (Koistinen et al., 2003) suggesting it may be at least part involved in contraction-stimulated muscle glucose uptake (Mu et al., 2001). However, due to observations in studies that have used  $\alpha$  1 or  $\alpha$  2 AMPK knockout mouse models and have either observed decreased (Mu et al.,

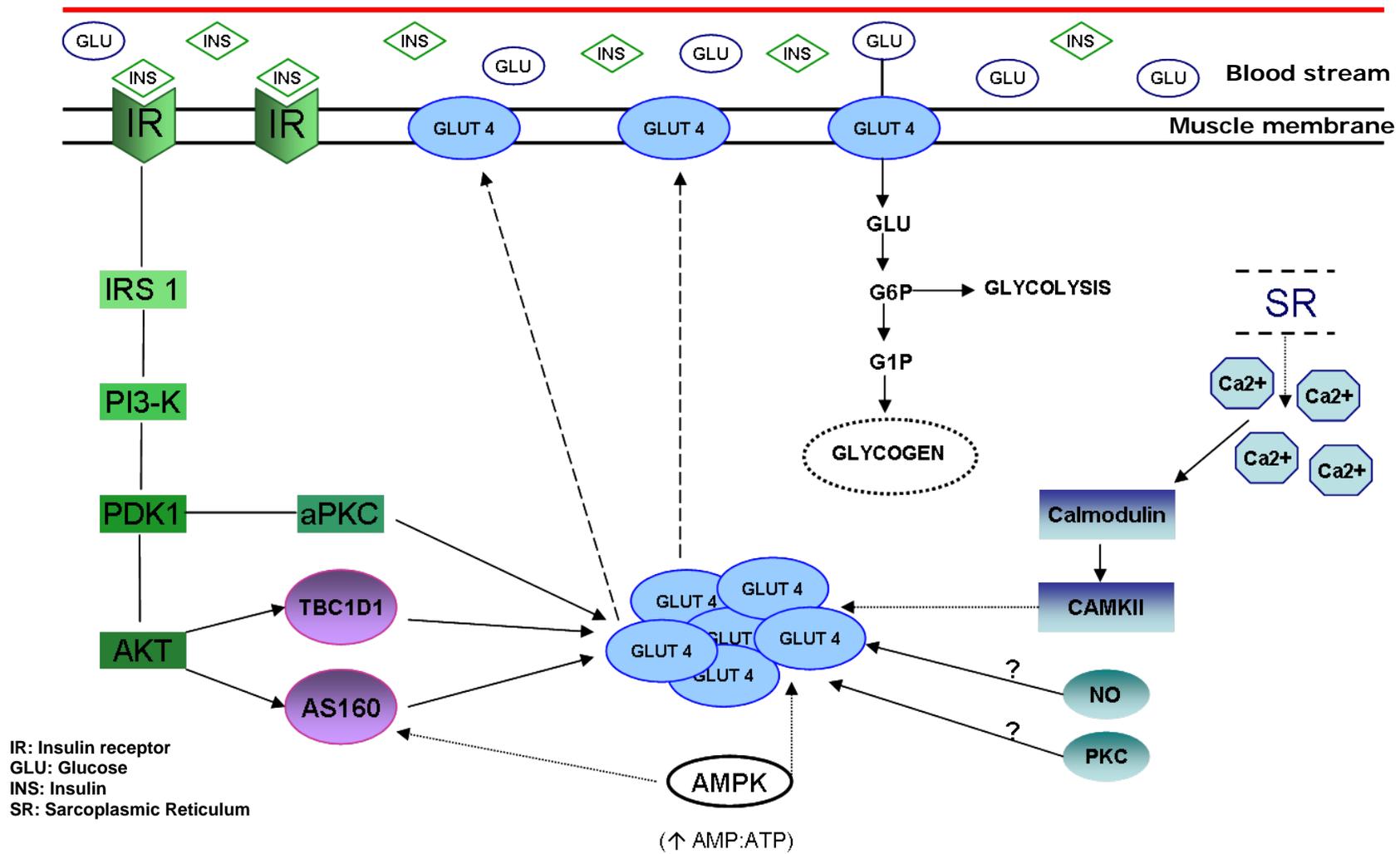
2001) or normal (Jørgensen et al., 2004) glucose transport *ex-vivo* with normal force production, it is suggested that contraction-induced muscle glucose uptake is maintained if one of the  $\alpha$  1 or  $\alpha$  2 AMPK isoforms is expressed normally (Jørgensen et al., 2006). Despite previous studies implicating AMPK in contraction-stimulated transport, it appears that AMPK does not solely regulate contraction-mediated muscle glucose uptake and it seems likely that a combination of signalling proteins may be involved (Jessen and Goodyear 2005). Further research is warranted into the signalling molecules linked to the activation of muscle glucose transport (Richter et al., 2003; Fujii et al., 2004). Recently it was shown that the Akt substrate of 160 kDa (AS160) is phosphorylated by AMPK in response by both AICAR and contraction in skeletal muscle (Kramer et al., 2006). Furthermore, mutation of the calmodulin-binding domain on AS160 inhibits contraction but not insulin-stimulated glucose uptake (Kramer et al., 2007). Therefore it has been suggested that AS160 may represent a convergence or divergence point for both insulin and contraction dependent signalling in the regulation of muscle glucose uptake (Rockl et al., 2008). Increased phosphorylation of AS160 in response to exercise or insulin indicates a potential convergence. However further evidence is needed to identify site-specific phosphorylation and sub cellular localisation to elucidate the influence of AS160 on both contraction and insulin mediated muscle glucose uptake after exercise (Cartee and Funai 2009).

#### *1.2.2.2 Insulin signalling and effects*

Insulin is released from the  $\beta$  cells of the Islets of Langerhans situated in the pancreas in response to an increase in blood glucose concentration. Exercise

contractions stimulate an increase in the permeability of the muscle membrane to glucose, an effect which is maintained for several hours post-exercise by a marked increase in the sensitivity of the muscle to insulin (Hansen et al., 1998). This effect has been shown in rodent studies to persist for long periods when carbohydrate is restricted and is reduced as muscle glycogen returns to normal levels (Cartee et al., 1989). Insulin also facilitates glycogen synthesis by decreasing phosphorylation and subsequently activating glycogen synthase. A single bout of exercise is sufficient to increase insulin sensitivity in the exercised muscles (Wojtaszewski et al., 2002) and the degree of glycogen depletion in the exercised muscle is associated with an enhanced metabolic action of insulin in the recovery period (Richter et al., 2001). The insulin-mediated effect allows muscle glucose transport to continue after the reversal of contraction-induced effects and this enhanced effect is likely responsible for the slower phase muscle glycogen resynthesis. Insulin and exercise are now thought to activate the common effect of GLUT4 translocation by separate signalling mechanisms. The role of the insulin signalling pathway in facilitating glucose utilisation with exercise, which is initiated by the binding of insulin to the  $\alpha$  subunit of the insulin receptor leading to tyrosine phosphorylation of IRS-1/2 and activation of phosphatidylinositol 3-kinase (PI3K), is still debatable (Goodyear and Kahn 1998; Ryder et al., 2001; Holloszy 2005). The insulin signalling cascade has been shown to be similar in exercised versus non-exercised muscle in humans (Wojtaszewski et al., 2000) and also to be unaffected when the metabolic action of insulin is decreased (Thong and Graham 2002). Thus far, it does not appear that enhanced insulin signalling is responsible for increased insulin sensitivity, however a possibility

is that the insulin pathway has only been established up to the Akt/GSK3 stage and signalling further along this pathway may be involved (Wojtaszewski et al., 2002). The contribution of Akt and protein kinase C (PKC) to stimulation of insulin-dependent muscle glucose transport has also yet to be established. However, a study using a hyperinsulinemic-euglycemic clamp to elicit a high plasma insulin concentration found that after an hour of moderate cycling exercise, IRS-2-associated PI3K activity was enhanced compared with insulin alone (Howlett et al., 2006). Further investigation into this effect and an association with the increase in insulin-stimulated glycogen synthase activity has been proposed by the authors. Another avenue of research has focused on the role of AMPK in regulating insulin signalling as activation of AMPK by AICAR has been shown to increase insulin sensitivity (Fisher et al., 2002). Further work is needed to clarify any effect of AMPK on increasing insulin sensitivity following exercise (Fisher 2006; Fujii et al., 2006). Recent investigation has implicated the insulin signalling molecule TBC1D4 via inclusion in the signal transduction pathway leading to AMPK-stimulated GLUT4 translocation (Chavez et al., 2008). In this way it has been indicated to be involved in increased insulin sensitivity and the increased permeability of the muscle membrane to glucose may be accompanied by an elevated insulin stimulated microvascular perfusion after an acute bout of exercise (Maarbjerg et al., 2011). Thus our understanding of the mechanisms involved is far from complete and requires further research into the distal insulin signalling network and the role of other molecules (Rowland et al., 2011).



**Figure 1.1** Potential mechanisms mediating contraction-induced and insulin dependent skeletal muscle glucose uptake into the muscle cell

### 1.2.3 Glycogen synthesis

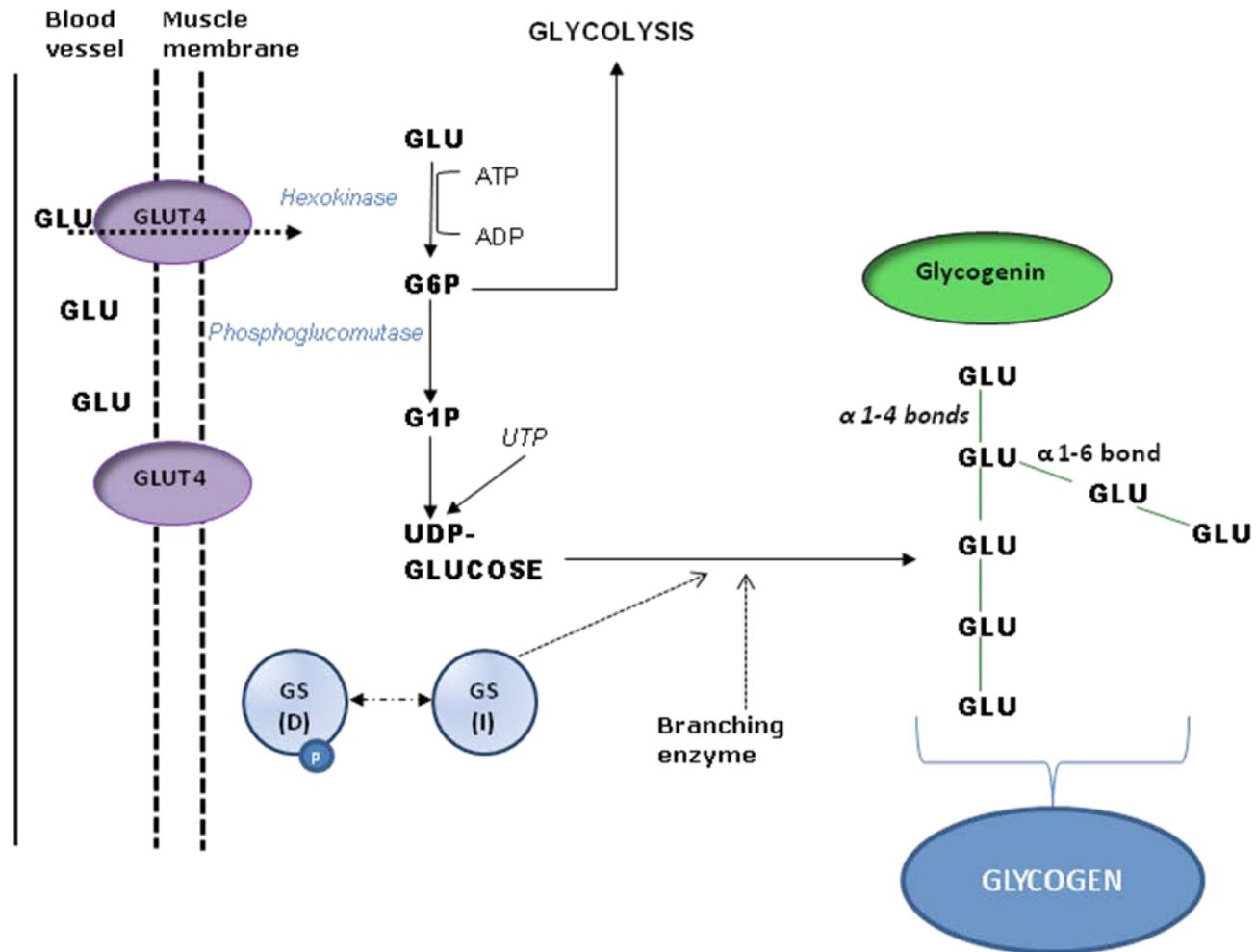
The formation of glycogen molecules within the muscle is initiated with the uptake of blood glucose across the sarcolemma via the transporter protein GLUT4 that migrate from a central location in the cell to the muscle membrane in response to either contraction-stimulated processes or the presence of insulin. Upon entering the muscle cell, glucose is rapidly converted to Glucose-6-phosphate (G6P) (by the action of the enzyme Hexokinase (HK)) which is in turn converted to Glucose-1-phosphate (G1P) via Phosphoglucomutase (Figure 1.2). The combination of Uridine Triphosphate (UTP) and G1P forms UDP-glucose which serves as a glucose carrier. The glucose residue of the UDP glucose is attached by an  $\alpha$ -1,4 glycosidic bond to the free end of an existing glucose chain, an action catalysed by GS. The extension of the glycosyl chains of the glycogen molecule is achieved via the sequential attachment of more glucose residues, each aligned with an  $\alpha$ -1,4 glycosidic bond, up to about 12 residues. The compact, branched structure of the glycogen molecule develops with the transfer of approximately 7 residues from one chain to another pre-existing chain creating a branch point using an  $\alpha$ -1,6 linkage. This structure allows numerous free-ends for rapid degradation by GP or incorporation of glucose residue for synthesis by GS. At the centre of the glycogen molecule is the autoglycosylating protein glycogenin which initiates glycogen granule formation. During prolonged exercise a decrease in activity of the glycogenin protein has been shown to occur (Shearer et al., 2005) and low glycogen levels to correspond to higher glycogenin activity (Shearer et al., 2000). In contrast during recovery from exercise, levels of glycogenin activity increase, indicating a capacity for rapid glycogen formation (Shearer et al., 2005). A

more recent study demonstrated no change in glycogenin concentration in the muscle despite lowering of glycogen content through exhaustive exercise suggesting conservation of glycogenin in small glycogen granules for rapid resynthesis in the post exercise period (Wilson et al., 2007). The synthesis of glycogen has been supposed to create 2 different granular forms termed proglycogen (PG) and macroglycogen (MG) (Marchand et al., 2002) that were interpreted as differing in granule size (MG=  $10^7$  Da vs. PG= 400 kDa). Early glycogen resynthesis in the first 4 hr post-exercise has been shown to relate to a large increase in the number of glycogen particles followed by an increase in the size of individual particles albeit to an intermediate size (Marchand et al., 2007). By measuring the proportion of PG and MG resynthesis following exhaustive exercise and feeding a high carbohydrate diet, Adamo et al (1998) demonstrated that in the first 4 hr of recovery PG resynthesis was dominant whereas between 24 and 48 hr the increase in muscle glycogen was attributed to increases in MG. The metabolic limitation that limits granule size to an average of 20-30 nm instead of a maximum of 42 nm is not understood but presumably there is a metabolic advantage in glycogen degradation of multiple numbers as opposed to fewer large particles (Graham et al., 2010).

However it has been noted that studies of the different fractions of glycogen which have used a homogenization free acid extraction have underestimated the proportion of acid-soluble glycogen (James et al., 2008). As markedly different rates of acid-soluble glycogen (MG) post-exercise resynthesis (content of 11% vs. 77% reported as MG) can be derived from comparing a homogenization free with a homogenization dependent protocol (Barnes et al.,

2009), interpretation of the literature should take account of glycogen-extraction methodological procedures.

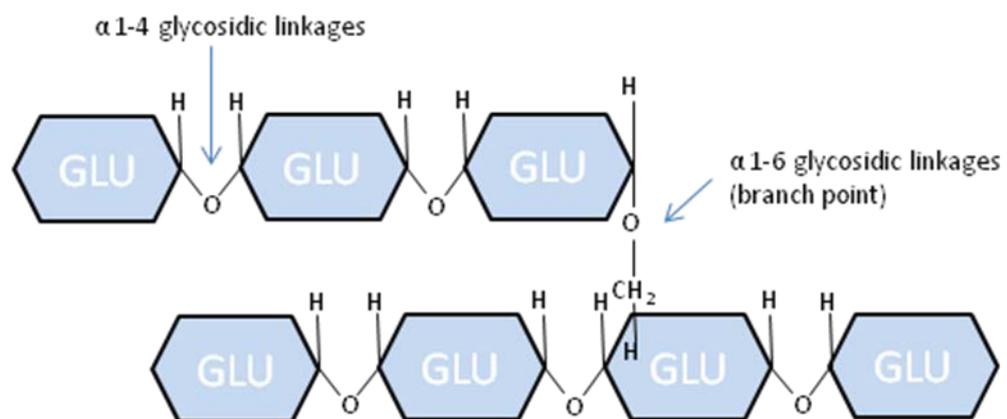
Glycogen particles stored within the muscle cell have been reported to group in 3 separate regions, the subsarcolemmal, intermyofibrillar and intramyofibrillar (Marchand et al., 2002). It is thought that these subcellular glycogen pools are preferentially engaged for specific cellular functions with the intracellular glycogen particles primarily metabolised during exhaustive exercise (Marchand et al., 2007; Prats et al., 2011). It is known that GS is phosphorylated at 9 or more sites differing in their level of activation (Roach 2002). Phosphorylation at specific sites could direct glycogen synthesis to different metabolic pools. Phosphorylation at site 1b is associated with intramyofibrillar glycogen (Prats et al., 2009) further supporting the hypothesis that the muscle cell can direct GS action towards the depleted pool (Prats et al., 2011). By using electrical stimulation of skinned fibres it was shown that glycogen in the intramyofibrillar space was related to fatigue resistance capacity (Nielsen et al., 2009). As such this population of muscle glycogen may play an essential role in fatigue development by interfering with excitation-contraction coupling. However the regulatory mechanisms governing the distribution of glycogen particles in their subsequent subcellular location as yet remains to be fully elucidated (Prats et al., 2011). Glycogen has been suggested to associate with the key enzymes and proteins coupled with its regulation such as GS, Glycogen phosphorylase, glycogen phosphorylase kinase, debranching enzyme, protein phosphatase 1 and AMPK (Graham 2009; Graham et al., 2010). However understanding in the co-ordination of these proteins in the regulation of glycogen metabolism remains elusive.



**Figure 1.2** Glycogen synthesis within the muscle cell

### 1.3 Factors affecting short-term glycogen resynthesis

Effective post-exercise muscle glycogen resynthesis is reliant on the provision of carbohydrate to elicit elevated circulating levels of glucose and insulin, conditions that are favourable for increased muscle glucose uptake (Blom et al., 1987; Ivy et al., 1988; Zawadski et al., 1992; van loon et al., 2001). Starch is the main form of dietary carbohydrate and is composed of varying fractions of amylose (15-30%) and amylopectin (70-85%) (Jenkins and Donald 1995; Fredriksson et al., 1998; Jane et al., 1999). Both amylose and amylopectin are connected by  $\alpha$  1-4 linkages however the amylopectin molecules contain branches that are achieved with  $\alpha$  1-6 linkages (Figure 1.3), thus starch has a highly branched structure similar to glycogen. The physicochemical properties of starch vary markedly between starches of different fractions (Swinkels et al., 1985) thus affecting characteristics related to viscosity and enzymatic degradation (Gallant et al., 1992).



**Figure 1.3** Structure of starch

Digestible starch must be hydrolysed to its monosaccharide components via enzymatic action before it can be absorbed in the small intestine, therefore the rate of digestion and absorption potentially limits the glycaemic response to an oral glucose load (Wolever and Bolognesi 1996). As such the availability of ingested carbohydrate is dependent on the rate of gastric emptying, absorption in the small intestine and subsequent release from the liver (Jentjens and Jeukendrup 2003).

### *1.3.1 Rate of gastric emptying*

It has been reported that higher levels of muscle glycogen resynthesis ( $85\text{-}130\text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) can occur when large amounts of glucose (up to  $2.1\text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) are infused following prolonged sub-maximal glycogen-reducing exercise, when compared to oral ingestion (Bergstrom and Hultman 1967; Roch-Norlund et al., 1972). Blood glucose concentrations in these studies reached supra-physiological concentrations of  $\sim 20\text{ mmol}\cdot\text{l}^{-1}$ , a level not achieved by oral ingestion, suggesting glucose availability appears to be at least in part limited by the digestion and absorption of carbohydrate. The stomach acts as a reservoir for ingested nutrients and thus dictates the release of these nutrients into the circulation (Hellstrom et al., 2006), hence in conjunction with absorption of nutrients across the small intestinal mucosa, it represents a possible rate-limiting process in glycogen synthesis.

There are a number of factors that regulate the rate of gastric emptying with perhaps the most influential being the volume of the ingested fluid (Mitchell and Voss 1991) and the energy content (Calbet and MacLean 1997). Other factors include the osmolality, temperature and pH of the ingested bolus

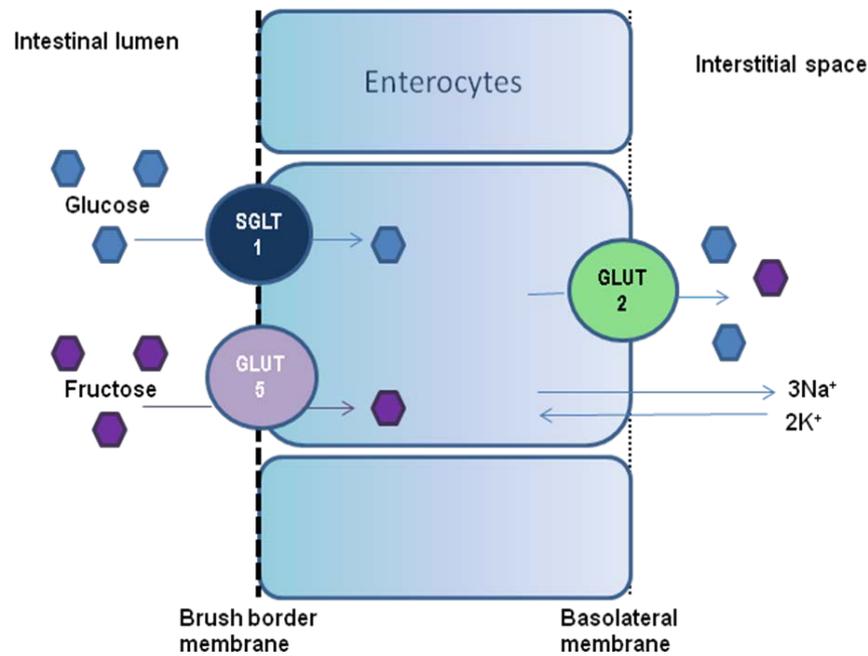
although these are thought to have a less pronounced effect on the rate of gastric emptying. During exercise, increasing the carbohydrate concentration of ingested fluids will reduce the rate of gastric emptying (Costill and Saltin 1974; Vist and Maughan 1994) but will increase the delivery of carbohydrate to the small intestine (Rehrer et al., 1994). Gastric emptying of liquids is regulated by the influence of gastric volume and feedback inhibition from the small intestine. It is this balance that is predominantly responsible for the inverse relationship between carbohydrate content and gastric emptying rate.

The time taken for carbohydrate to empty from the stomach at rest will be mediated primarily by volume and energy density, however when these are constant, other factors may exert an influence. Half-emptying time for a dilute glucose solution (40 g/l) has been shown to be 17 min compared to 130 min for a high concentration glucose solution (188 g/l). These times were reduced to 14 and 64 min respectively when these glucose solutions were replaced by isoenergetic polymers (Vist and Maughan 1995). Lieper et al., (2000) demonstrated half emptying times of 17 and 32 min for a glucose polymer or monomeric glucose consisting of 75g carbohydrate in 550 ml (13.5% solution). It has been reported that, possibly due to a lower osmolality, polymerised glucose empties faster than isoenergetic monomeric glucose solutions (Sole and Noakes 1989), and delivers a greater amount of carbohydrate to the small intestine (Vist and Maughan 1995). The authors of the latter study suggest that incomplete hydrolysis of a concentrated polymer in the small intestine would alter the osmolality before reaching receptors and could affect the feedback process to gastric emptying. As the stomach regulates the delivery of gastric contents to the small intestine, it is plausible that augmenting the rate of gastric

emptying could increase delivery of an oral glucose load to the small intestine and thus affect the bioavailability of carbohydrate for glycogen resynthesis.

### *1.3.2 Intestinal absorption of glucose*

The rate of absorption within the specific intestinal region where sugars are absorbed will partly affect the time course of glucose appearance in the circulation and availability to hepatic tissue (Ferraris 2001). Absorption of monosaccharides across the small intestine occurs via different transporters, SGLT 1 is the major facilitator of glucose transport across the brush-border membrane in conjunction with Na<sup>+</sup> whereas fructose enters by means of GLUT 5, although both share a common exit pathway of GLUT 2 across the basolateral membrane (Gould and Bell 1990) (Figure 1.4). The differing pathways of uptake have led to investigation into the ingestion of a combination of carbohydrates to enhance carbohydrate oxidation via an increased substrate absorption (Jeukendrup 2010). A maximal rate of 1 g.min<sup>-1</sup> of carbohydrate oxidation had been previously suggested, potentially due to saturation of the intestinal transporters. Recent work has reported 65% higher exogenous carbohydrate oxidation with ingestion of glucose and fructose and peak oxidation to be higher than previously reported at 1.7 g.min<sup>-1</sup> (Jentjens and Jeukendrup 2005). In this way, intestinal transport may present a barrier to exogenous carbohydrate oxidation during exercise. It remains to be determined whether post-exercise digestion of multiple transportable carbohydrates exceeds the same limitation.



**Figure 1.4** Intestinal absorption of monosaccharides

### 1.3.3 Role of hepatic glucose uptake and glycogen synthesis

The rate at which glucose appears in the systemic circulation after an oral load depends on the proportion of exogenous glucose that escapes splanchnic extraction (Felig et al., 1975). The GLUT 2 transporter present in liver cells allows a high rate of glucose transport into the liver due to its high affinity for glucose. Thus as the glucose concentration rises, glucose transport into the liver and subsequent phosphorylation increases. This increase in hepatic glucose uptake is achieved by augmented splanchnic fractional extraction of glucose after ingestion (Ferrannini et al., 1980). During the post-exercise period, the proportion of an ingested glucose load reaching the circulation is increased, not only by means of an increased intestinal absorption but by a larger fraction of the load escaping hepatic retention (Maehlum et al., 1978; Hamilton et al., 1996). Despite this, prior exercise in dogs has been shown to

increase hepatic glucose uptake independently of hormonal influence (Galassetti et al., 1999) suggesting exercise-induced changes in insulin and glucagon concentration are not required for increased hepatic glucose uptake (Pencek et al., 2004).

Determination of changes in liver glycogen concentration is complicated by a lack of non-invasive techniques. However, the recent advent of  $^{13}\text{C}$  magnetic resonance spectroscopy has allowed post-exercise measurements to be made (Roden et al., 2001). A consistent decline (~55-60%) in liver glycogen after 83 min of cycling at 70%  $\dot{V}O_2$  max has been demonstrated (Casey et al., 2000). This study reported that ingestion of glucose and sucrose were equally effective in increasing liver glycogen during a 4 hr recovery period. Furthermore a modest relationship was reported between the change in liver glycogen content and exercise capacity in a subsequent bout of exercise, potentially due to increased blood glucose availability from hepatic sources. Recent investigation has shown an increased hepatic glycogen resynthesis with combined ingestion of fructose or galactose compared to glucose in a 15% maltodextrin solution fed after exhaustive exercise (Decombaz et al., 2011). This effect was attributed to a difference in intestinal absorption with multiple transportable carbohydrates. The contribution of hepatic glycogen synthesis to disposal of a 98g glucose load has been previously estimated to be 17% (Petersen et al., 2001). Thus when considering post-exercise glycogen resynthesis, the role of liver glycogen resynthesis should be taken into account.

## **1.4 Strategies to optimise post-exercise glycogen resynthesis**

Carbohydrate feeding to stimulate muscle glycogen resynthesis is widely advocated since the biopsy studies performed by Bergstrom and colleagues in the 1960's established a relationship between muscle glycogen and exercise performance. Since only a small increase in post-exercise muscle glycogen can occur without the ingestion of carbohydrate (Bergstrom and Hultman 1966; Bergstrom and Hultman 1967; Ivy et al., 1988), most likely due to inadequate circulating levels of blood glucose and insulin, rapid provision of carbohydrate is advised. Without adequate substrate, muscle glycogen resynthesis can only proceed at a low rate from gluconeogenic sources at a rate of  $\sim 2 \text{ mmol.kg}^{-1}.\text{h}^{-1}$  (Maehlum and Hermansen 1978; Ivy et al., 1988). In accordance, a wealth of research has focused on manipulating the type, timing and amount of carbohydrate provided post-exercise in an attempt to optimise the rate of muscle glycogen resynthesis. It is important to consider that modern sport often requires repeated training sessions or multiple competitive events with a short time for recovery. In this way it is essential to maximally facilitate the recovery processes in the time available, primarily the restoration of diminished muscle glycogen stores (Beelen et al., 2010; Betts and Williams 2010).

### *1.4.1 Timing of carbohydrate administration*

The time elapsed between the end of exercise and provision of carbohydrate has been demonstrated to impact on the rate of muscle glycogen resynthesis. By delaying ingestion of a 25% glucose polymer solution ( $2 \text{ g.kg}^{-1}$  body weight (bw)) by 2 hr following glycogen-depleting exercise Ivy et al (1988) found that the rate of muscle glycogen resynthesis was reduced by  $\sim 50\%$  when compared to the same supplement provided immediately after exercise. Conversely the

effect of delaying carbohydrate ingestion by 2 hr does not appear to affect muscle glycogen content after 8 and 24 hr (Burke et al., 1993; Parkin et al., 1997) thus timing of carbohydrate administration may have more influence on muscle glycogen resynthesis in the immediate post-exercise period. The slower rate after 2 hr in the study by Ivy (1988) occurred despite elevated blood glucose and insulin concentrations, suggesting the development of a degree of insulin resistance in the muscle. Thus the immediate post exercise period presents a greater metabolic capacity for glycogen synthesis. Prompt ingestion of carbohydrate following exercise is therefore beneficial not only to provide an immediate source of substrate but to take advantage of the period of increased permeability of the muscle to glucose (Goodyear et al., 1990) and transient insulin sensitivity (Richter et al., 1984; Cartee et al., 1989) that occurs after exercise to initiate rapid muscle glycogen resynthesis.

The frequency of the post exercise feeding schedule has been suggested to affect muscle glycogen resynthesis. More frequent ingestion of carbohydrate offsets the decline in blood glucose and insulin typically observed with a single feeding (Ivy et al., 1988). Studies that have administered carbohydrate in a more frequent manner (15-30 min intervals) have observed high levels of post-exercise muscle glycogen resynthesis ( $27-50 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ ) when the amount of carbohydrate ingested was  $1-1.2 \text{ g.kg}^{-1}\text{bw.h}^{-1}$  (Doyle et al., 1993; Piehl Aulin et al., 2000; van Hall et al., 2000; van Loon et al., 2000; Jentjens et al., 2001). However, studies feeding similarly high levels of carbohydrate immediately post exercise and at 1-2 hr intervals have shown comparable rates of muscle glycogen resynthesis (Ivy et al., 1988; Blom 1989; Casey et al., 1995). In addition, Blom et al (1987) reported that elevated blood glucose levels could be

maintained when supplementing either 0.7 or 1.2 g.kg<sup>-1</sup> bw of glucose at 2 hr intervals during recovery from exhaustive cycling exercise. Notably, studies that have measured muscle glycogen content 24 hr after glycogen-depleting exercise have shown no difference when a high carbohydrate diet was provided at frequent intervals or in fewer meals (Costill et al., 1981; Burke et al., 1996). Direct investigation into the frequency of post-exercise carbohydrate feeding on rates of muscle glycogen resynthesis in a short recovery period is thus warranted.

#### *1.4.2 Amount of carbohydrate*

Whilst it is accepted that supplementing with carbohydrate of any considerable quantity is more effective at stimulating increases in muscle glycogen content compared to no carbohydrate (Ivy et al., 1988), the optimal amount of carbohydrate required for maximal muscle glycogen resynthesis is still in contention. The study by Blom et al (1987) suggested that a critical carbohydrate amount exists that corresponds to a threshold level of muscle glycogen resynthesis and increasing the amount of carbohydrate above this point does not further enhance glycogen resynthesis. This study demonstrated that increasing the amount of glucose ingested at 0, 2 and 4 hr post-exercise from absolute amounts of 0.35 to 0.7 g.kg<sup>-1</sup> bw increased the rate of muscle glycogen resynthesis from 2.1 to 5.8 mmol.kg<sup>-1</sup>ww.h<sup>-1</sup>. However, no further increase was observed when increasing the total ingested amount to 1.4 g.kg<sup>-1</sup> bw (5.7 mmol.kg<sup>-1</sup>ww.h<sup>-1</sup>). Similarly Ivy et al (1988) aimed to define the most effective amount of carbohydrate ingestion for post exercise muscle glycogen resynthesis and observed no difference between ingestion of 1.5 or 3 g.kg<sup>-1</sup> bw of a concentrated (50%) glucose polymer in the first 2 hr of recovery from 2 hr

of cycling exercise (5.2 vs. 5.8 mmol.kg<sup>-1</sup>.h<sup>-1</sup> ww respectively). Increasing the amount of carbohydrate fed will increase the rate of muscle glycogen resynthesis however there appears to be a 'cap' to the rate that had been formerly suggested to exist at ~1.2 g.kg<sup>-1</sup> bw (van Loon et al., 2000). From reviews of the relevant literature it can be ascertained that a dose-response relationship exists between the carbohydrate ingestion rate and the muscle glycogen synthetic rate following exercise (Jentjens and Jeukendrup 2003). Comparison of post exercise muscle glycogen resynthesis rates is complicated by the variety of exercise protocols used and the type of carbohydrate provided, nonetheless a recent meta-analysis of human studies following glycogen-depleting exercise has demonstrated a significant positive correlation between the two variables (amount of CHO and rate of muscle glycogen resynthesis,  $r=0.6$ ,  $p<0.01$ ) (Betts and Williams 2010). Notably the studies considered in the analysis exhibited a broad range of post exercise muscle glycogen concentrations at the onset of recovery, thus given that low glycogen content is itself an instigator of resynthesis (Zachwieja et al., 1991; Price et al., 1994), differences in rates of muscle glycogen resynthesis may derive from variations in the extent of muscle glycogen depletion (Betts and Williams 2010). Taking the analysis of the studies, it appears that the highest rates of glycogen resynthesis over a short (2-4 hr) recovery period have been reported in the range of 40-50 mmol.kg<sup>-1</sup>.h<sup>-1</sup> with glucose ingestion of >1 g.kg<sup>-1</sup>bw.h<sup>-1</sup> (Casey et al., 1995; Piehl Aulin et al., 2000; van Loon et al., 2000; Jentjens et al., 2001; Shearer et al., 2005), however higher rates of 58 mmol.kg<sup>-1</sup>.h<sup>-1</sup> have been reported with a high carbohydrate intake in combination with high levels of caffeine (Pedersen et al., 2008). The 'optimal' amount of carbohydrate to be

consumed after exercise to restore muscle glycogen has not yet been fully elucidated, however it seems apparent that at least  $1 \text{ g.kg}^{-1} \text{ bw.h}^{-1}$  is required to attain reasonable levels of resynthesis (Betts and Williams 2010).

#### *1.4.3 Type and form of carbohydrate*

The type of carbohydrate supplement provided can influence muscle glycogen resynthesis as was suggested in a study repeatedly feeding either  $0.7 \text{ g.kg}^{-1} \text{ bw}$  of glucose, fructose or sucrose during a 6 hr recovery (Blom et al., 1987). The rates of muscle glycogen resynthesis were similar between glucose and sucrose ( $5.8$  and  $6.2 \text{ mmol.kg}^{-1} \text{ .h}^{-1}$ ) and were higher than the rate achieved with fructose ingestion ( $3.2 \text{ mmol.kg}^{-1} \text{ .h}^{-1}$ ). Given that fructose is preferentially removed from the bloodstream and metabolised by the liver for glucose production (Nilsson and Hultman 1974; Schaefer et al., 2009), this difference in muscle glycogen synthesis was attributed to the differing metabolic response from fructose and glucose in that the insulin response from glucose is markedly higher compared to fructose (Levine et al., 1983). It was notable that sucrose elicited similar muscle glycogen resynthesis to glucose considering that sucrose is composed of equimolar amounts of glucose and fructose. It was hypothesised that the fructose molecules were taken up in the liver thus allowing more glucose to bypass hepatic uptake and be available for the muscle (Blom et al., 1987), however the combination of fructose and glucose may be preferable for muscle glycogen resynthesis. Fructose and glucose are absorbed across the intestinal mucosa via different transporters (Levin 1994) thus it was postulated that feeding a combination of carbohydrates could enhance total carbohydrate absorption above that of a single carbohydrate type (Shi et al., 1995). In accordance, very high rates of carbohydrate oxidation ( $1.75 \text{ g.min}^{-1}$ )

during exercise have been reported with a high intake ( $2.4 \text{ g}\cdot\text{min}^{-1}$ ) of a combination of glucose and fructose (Jentjens and Jeukendrup 2005). Although this suggests a limitation present in intestinal transport during exercise, further investigation demonstrated no enhancement of post exhaustive exercise muscle glycogen resynthesis with equal amounts of glucose and fructose compared to glucose alone (Wallis et al., 2008). Considering that there were comparable plasma glucose and serum insulin responses in the study by Wallis and colleagues, it follows that total muscle glycogen after ingestion of 90g of glucose or 60g of glucose combined with 30g of fructose over 4 hr was not different ( $176 \text{ vs. } 155 \text{ mmol}\cdot\text{kg}^{-1}$ ).

Whether the form of the carbohydrate supplement provided post exercise is liquid or solid does not appear to influence muscle glycogen resynthesis. Given that gastric emptying is more rapid with a liquid in comparison to a solid (Rehrer et al., 1994), it would be expected that a liquid supplement would enhance carbohydrate delivery above that from a solid meal. Investigation into the form of carbohydrate is limited, nonetheless studies have shown no difference in muscle glycogen resynthesis between a liquid or solid supplement. Although similar rates of muscle glycogen resynthesis ( $24.8$  and  $24.6 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) over 5 hr recovery from exhaustive exercise were reported in a study that fed a liquid or solid carbohydrate meal (Keizer et al., 1987). Absolute amounts of carbohydrate were different between the trials and thus cannot be compared directly. The administration of  $3 \text{ g}\cdot\text{kg}^{-1}$  bw of a 50% glucose polymer or a solid carbohydrate meal immediately after exercise and 2 hr later again showed similar rates of post exercise muscle glycogen

resynthesis (21.8 and 23 mmol.kg<sup>-1</sup>.h<sup>-1</sup> respectively) despite a greater insulin response observed with the liquid supplement (Reed et al., 1989).

Insulin has a fundamental role in facilitating post exercise muscle glycogen storage. An elevated insulin response following meals consisting of carbohydrate classified as high glycaemic index (GI) has been reported in association with augmented muscle glycogen resynthesis over 24 hr post glycogen-depleting exercise compared to low GI meals (Burke et al., 1993). The accumulated glycaemic responses over the 24 hr measurement period were not different between high and low GI conditions in this study. Thus the immediate glycaemic and insulinaemic response following feeding observed with high GI foods and altered absorption of carbohydrate with the low GI foods are likely responsible for the enhancement in muscle glycogen content.

#### *1.4.4 Addition of other nutrients*

The co-ingestion of protein or amino acids augments postprandial insulin release above that of carbohydrate alone (Spiller et al., 1987; van Hall et al., 2000; Jentjens et al., 2001) and this synergistic effect of insulin release with a combination of carbohydrate and protein has been the focus of research aiming to augment short term muscle glycogen resynthesis. Insulin release from pancreatic beta cells in response to infusion of different amino acids in combination with carbohydrate has been previously determined by sampling portal and peripheral blood (Floyd et al., 1970). Later investigation into combinations of amino acids to evoke this response suggested that the levels of leucine, phenylalanine and tyrosine strongly determine the magnitude of insulin secretion (van Loon et al., 2000c). The proposed mechanism by which amino acids stimulate insulin secretion varies according to the individual

amino acid. Briefly, amino acids are suggested to influence mitochondrial metabolism in pancreas beta cells via allosteric activation, membrane depolarisation or a combination of both (Newsholme et al., 2006). The insulinotropic effect of amino acid ingestion may not be restricted to direct effects on secretion by beta cells. The incretin hormone Glucagon-like peptide-1 (GLP-1) is known to be released from enteroendocrine L-cells of the small intestine mucosa in response to meal ingestion, thereby inducing insulin secretion resulting from oral as opposed to intravenous nutrient ingestion. A dose-response relationship between GLP-1 and insulin secretion has been demonstrated via both intravenous (Kjems et al., 2003) and meal ingestion (Ahrén et al., 2003). GLP-1 also stimulates secretion of insulin via  $\beta$ -cell membrane depolarisation and increases in mitochondrial ATP synthesis leading to insulin storage granule exocytosis (Baggio and Drucker 2007). In this way the effect of nutrient ingestion itself regardless of it being a glucose load or mixed meal can augment insulin release via release of gastrointestinal hormones.

The effect of elevated insulin levels on increasing muscle glucose uptake and activation of glycogen synthase is well documented (Goodyear and Kahn 1998; Richter et al., 2001; Wojtaszewski et al., 2002; Maarbjerg et al., 2011). In view of this and the role of insulin in enhancing muscle glycogen resynthesis, it follows that a higher insulin response achieved with protein and carbohydrate may further increase muscle glycogen resynthesis above that which can be achieved with carbohydrate alone. Subsequently it was demonstrated that co-ingestion of carbohydrate, protein hydrolysate, leucine and phenylalanine can elevate plasma insulin levels and stimulate muscle glycogen synthesis (van

Loon et al., 2000c). Although a dose-response relationship between protein intake and insulin release was not found previously, van Loon et al (2000b) reported a greater insulinaemic response when increasing the amount of protein in a carbohydrate-protein combination from 0.2 to 0.4 g.kg<sup>-1</sup>bw. Notably this study showed an enhanced muscle glycogen resynthesis over a 3 hr recovery period with feeding of 0.8 g.kg<sup>-1</sup>bw.h<sup>-1</sup> carbohydrate plus 0.4 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of protein hydrolysate compared to 0.8 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of carbohydrate alone. However this effect was negated when carbohydrate was increased to 1.2 g.kg<sup>-1</sup>bw.h<sup>-1</sup>. A protein intake of 0.3-0.5 g.kg<sup>-1</sup>bw.h<sup>-1</sup> has tended to elicit higher insulin concentrations than carbohydrate alone (van Hall et al., 2000a; van Hall et al., 2000b; van Loon et al., 2000a; van Loon et al., 2000b; Kaastra et al., 2006; Betts et al., 2008) whereas the studies that have not found a difference in insulin response above carbohydrate ingestion have fed lower amounts of protein of ~0.1 g.kg<sup>-1</sup>bw.h<sup>-1</sup> (Carrithers et al., 2000; Ivy et al., 2002; Ivy et al., 2003). Blood glucose concentrations tended to be attenuated in the above mentioned studies with ingestion of carbohydrate-protein mixtures, however it is not clear whether this is due to an increased glucose disposal or a reduced appearance in circulation from delayed gastric emptying (Calbet and MacLean 1997). A reduced rate of glucose appearance and disappearance has been observed with ingestion of 0.8 g.kg<sup>-1</sup>.h<sup>-1</sup> of carbohydrate compared to the same amount of carbohydrate combined with 0.4 g.kg<sup>-1</sup>.h<sup>-1</sup> of protein during 3 hr of recovery from 2 hr of exercise at 55%  $\dot{V}O_2$  max (Kaastra et al., 2006). In addition leg glucose uptake was shown to remain constant at a rate of 0.9 mmol.min<sup>-1</sup> during 4 hr of recovery from exhaustive intermittent exercise when a 600 ml solution containing 1.67 g.kg<sup>-1</sup>bw of sucrose or the same amount with

0.5 g.kg<sup>-1</sup>bw of whey protein hydrolysate added was ingested at 15 min intervals. Given these observations it appears more likely that the reduced glycaemic responses reported with combined protein and carbohydrate ingestion are due to reduced glucose appearance in the circulation rather than an increased rate of glucose disposal.

The research investigating the effect of combining carbohydrate with protein on muscle glycogen resynthesis is conflicting. It was first investigated by feeding 112g carbohydrate with 40.7g protein immediately and 2 hr after glycogen-depleting exercise. This resulted in a 38% greater rate of muscle glycogen resynthesis over a 4 hr recovery compared to 112g of carbohydrate. However as these solutions were markedly different in energy content it is not clear whether the enhanced glycogen synthesis results from the increased response or a greater total energy available in the carbohydrate-protein trial (Roy and Tarnopolsky 1998). Subsequently, further research has compared carbohydrate-protein mixtures with isoenergetic carbohydrate solutions. Ingestion of 0.8 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of carbohydrate with 0.4 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of protein has been found to elicit comparable rates of muscle glycogen resynthesis with 1.2 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of carbohydrate during recovery from exhaustive cycling (van Loon et al., 2000b). This has been confirmed in other investigations (van Hall et al., 2000a; Jentjens et al., 2001; Howarth et al., 2009), suggesting a maximal rate of muscle glycogen resynthesis in post exercise recovery can be attained with a sufficiently high carbohydrate intake and protein cannot enhance this level further. It appears that adding protein rather than additional carbohydrate to elicit a proposed 'maximal' 1.2 g.kg<sup>-1</sup>bw.h<sup>-1</sup> of carbohydrate is no more effective in augmenting muscle glycogen despite a rise in circulating insulin

concentrations (Jentjens et al., 2001; Howarth et al., 2009). Using dual-tracer techniques and positron emission tomography, insulin has been shown to facilitate glucose utilisation by skeletal muscle in a dose-dependent manner (Bonadonna et al., 1993; Williams et al., 2001; Pencek et al., 2006) however the rate-limiting factor is considered to be the proximal stages of glycogenesis including glucose delivery, muscle glucose uptake and phosphorylation in the muscle cell (Pencek et al., 2004). The absence of an accumulation of free glucose during graded insulin release suggests both glucose appearance in circulation and muscle glucose transport are rate-limiting (Katz et al., 1988). Thus despite a further increase in insulin with combined protein and carbohydrate ingestion, subsequent glucose availability may be limiting such that resynthesis of muscle glycogen is sub-optimal unless sufficient amounts ( $\sim 1.2 \text{ g}\cdot\text{kg}^{-1} \text{ bw}$ ) of carbohydrate are ingested (Beelen et al., 2010).

Nonetheless, recent investigation has reported a 66% (58 vs. 38  $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) increased rate of muscle glycogen resynthesis over a 4 hr recovery period from glycogen-reducing exercise that was attained with co-ingestion of caffeine (2  $\text{mg}\cdot\text{kg}^{-1}\cdot\text{bw}\cdot\text{h}^{-1}$ ) with carbohydrate (1  $\text{g}\cdot\text{kg}^{-1}\cdot\text{bw}\cdot\text{h}^{-1}$ ) compared to same amount of carbohydrate alone (Pedersen et al., 2008). Although no benefit on post-exercise muscle glycogen resynthesis was previously seen with caffeine co-ingestion (Battram et al., 2004), the caffeine supplements in this study were fed during exercise. Thus the caffeine-mediated effect might only be present when caffeine is co-ingested with carbohydrate during recovery. The mechanism behind the effect on muscle glycogen resynthesis is not clear, although it could be related to an increased intestinal absorption of glucose with caffeine ingestion (Yeo et al., 2005), however this remains to be determined. A notable

finding of the study by Pedersen et al (2008) is that a higher rate of muscle glycogen resynthesis ( $58 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ ) than previously described was observed despite a typical intake of carbohydrate during recovery ( $\sim 1 \text{ g.kg}^{-1} \text{ bw.h}^{-1}$ ). However the high amount of caffeine consumed during recovery is unfeasible for regular consumption and it remains to be determined whether lower doses of caffeine elicit the same effect.

### **1.5 A unique, high molecular weight (HMW) glucose polymer**

Manipulation of the type, timing and amount of carbohydrate provided post exercise has been extensively investigated in an attempt to optimise the restoration of depleted muscle glycogen content. As previously noted, the delivery of glucose to the muscle is a limiting factor and key determinant of recovery time. Most post exercise supplements contain carbohydrate in the form of maltodextrins due to the compact nature of the glucose molecules allowing the inclusion of more carbohydrate whilst a low osmolality can be maintained. The sweetness and high osmolality of glucose impacts on tolerability and rate of gastric emptying (Vist and Maughan 1995), thus most commercial sports drinks contain glucose polymers to maximise delivery and absorption of both carbohydrate and fluid.

A unique, very HMW glucose polymer ( $500,000\text{-}700,000 \text{ g.mol}^{-1}$ ) has been reported to aid muscle glycogen resynthesis during conditions of short recovery time. This was described by Piehl Aulin et al., (2000) when comparing this glucose polymer with a mixture of monomeric and short chain oligomeric glucose with a much lower molecular weight ( $500 \text{ g.mol}^{-1}$ ). The isoenergetic test solutions were markedly different in osmolality ( $60\text{-}84$  vs.  $\sim 300 \text{ mosmol.kg}^{-1}$  for the high and low molecular weight solutions respectively).

Prolonged exercise depleted muscle glycogen content to similar levels of 53 and 58 mmol.kg<sup>-1</sup>.h<sup>-1</sup> in the high and low molecular weight trials, however, provision of 75g at 30 min intervals of the high compared to the low molecular weight solution resulted in higher muscle glycogen synthesis rates in the first 2 hr post-exercise (50.2 vs. 29.9 mmol.kg<sup>-1</sup>.h<sup>-1</sup> for high versus low molecular weight solutions). Notably blood glucose and insulin concentrations did not differ between the test solutions however differences in either could have been obscured by the sampling of venous blood or the repeated feeding schedule. The main finding from this study that a HMW glucose polymer enhances muscle glycogen resynthesis in the first 2 hr post-exercise compared to an energy equivalent composition of monomers was ascribed to a potential difference in gastric emptying of the solutions. This theory was subsequently investigated by Lieper et al., (2000) by examining the effect on gastric emptying of the same solutions in non-exercised subjects at rest. The HMW glucose polymer was noted to resemble a thick paste when added to water whereupon with mechanical mixing it would form a homogenous gel that would be expected to retard gastric emptying. Unexpectedly it was demonstrated that the volume remaining in the stomach after ingestion of the HMW glucose polymer was lower between 20 and 50 min compared to the LMW solution. The exponential pattern of emptying observed with the HMW glucose polymer meant that the rate of gastric emptying was faster than the LMW solution which followed a more linear pattern. The time to empty half of the test solutions was considerably faster at 17 min compared to 33 min for the high and low molecular weight solutions respectively. Furthermore, carbohydrate delivery to the small intestine was significantly faster in the first

10 min post-ingestion with the HMW glucose polymer thus cumulative delivery of carbohydrate was greater after an hour. From the results of these studies it was suggested that recovery from glycogen-depleting exercise when only a short rest period was available would be enhanced and could therefore improve exercise performance in a subsequent bout of exercise. This was investigated in a study that compared the ingestion of the HMW glucose polymer to an isoenergetic glucose polymer with a LMW ( $\sim 900 \text{ g}\cdot\text{mol}^{-1}$ ) and markedly different osmolality ( $34 \text{ vs. } 124 \text{ mosmol}\cdot\text{kg}^{-1}$  HMW vs. LMW). Subjects completed a bout of exhaustive cycling exercise to deplete muscle glycogen followed by ingestion of the test solution and a 2 hr rest period (Stephens et al., 2008). After the recovery subjects performed a 15 min cycling performance test designed to measure the amount of physical work completed. As expected both carbohydrate solutions elicited higher total work output compared to a flavoured water control but a significant difference of a 10% increase in work output was reported after consumption of the HMW polymer compared to the LMW polymer (164.1 kJ vs. 149.4 kJ). Blood glucose concentration reached a higher peak of  $8.1 \text{ mmol}\cdot\text{l}^{-1}$  at 30 min for HMW compared to  $7.3 \text{ mmol}\cdot\text{l}^{-1}$  at 50 min for LMW, a pattern that was paralleled in insulin concentration with a more rapid peak of  $80.6 \text{ mU}\cdot\text{l}^{-1}$  (HMW) compared to  $68.7 \text{ mU}\cdot\text{l}^{-1}$  (LMW) at 40 and 70 min respectively. Due to this enhanced rate of rise in both blood glucose and serum insulin concentration, the performance effect with HMW ingestion was attributed to a greater resynthesis of skeletal muscle glycogen and thus a higher pre-exercise glycogen content at the onset of the second exercise bout. However, this is only speculative as no measures of muscle or liver glycogen content were obtained. Clearly there are potentially

advantageous characteristics of the HMW glucose polymer in enhancing recovery from prolonged exercise when recovery time is limited although further investigation is warranted to directly determine muscle glycogen resynthesis rates as an effect of ingestion post-exercise. It does not appear that oxidation of the high molecular weight polymer is increased during exercise compared to a standard maltodextrin (Rowlands et al., 2005). It is likely that as the oxidation of carbohydrate is not thought to be limited by gastric emptying and glucose delivery during exercise (Rehrer et al., 1992), the potential benefit of enhanced glucose delivery with the HMW glucose polymer previously described is more related to recovery of reduced muscle glycogen stores.

## **2. Thesis structure and aims**

This thesis aims to quantify muscle glycogen resynthesis as a result of ingestion of a unique HMW glucose polymer and to investigate the potential of the polymer to enhance maximal exercise work output above that of a comparable glucose polymer with a markedly lower molecular weight.

**In the course of this thesis the following research questions will be addressed;**

- Does consumption of a unique, HMW glucose polymer elicit enhanced muscle glycogen resynthesis after a short time period as a result of an augmented glucose and insulin response in comparison to an isoenergetic LMW glucose polymer?
- Are early phase temporal changes in post-exercise muscle glycogen resynthesis different when consuming the high or low molecular weight glucose polymers?
- Is a subsequent bout of maximal effort exercise following a short recovery from prior glycogen-depleting exercise improved after consumption of the HMW glucose polymer above that of the LMW glucose polymer?

## **Chapter 2**

### General Methods

This chapter describes the experimental methods utilised during the course of research studies in this thesis. It comprises details of procedures that are common to the studies including volunteer recruitment, dietary analysis, beverage supplementation, preliminary exercise testing, sample collection and statistical analysis.

## **2.1 Recruitment of study volunteers**

All the studies in this thesis were subject to the same inclusion criteria and recruited healthy, male volunteers, who were required to be aged between 18 and 35 years, non-smokers, non-vegetarians and currently engaging in some form of regular physical activity. All study protocols were submitted to The University of Nottingham Medical School Research Ethics Committee for approval. To recruit appropriate volunteers, poster advertisements were displayed at visible places throughout the University of Nottingham including fitness-based facilities. Additionally, advertisements were placed in gymnasiums and sports-related centres in the city of Nottingham and brief study details were placed on the internet in suitable online classified pages. All methods of recruitment were previously approved by the Ethics Committee. After initial enquiries, detailed written information concerning the study was distributed and volunteers were invited to visit the laboratory for a discussion. During this initial meeting, volunteers were fully informed of the study protocol and written consent was obtained. Volunteers were able to withdraw from the studies at any time.

## **2.2 Medical screening of healthy volunteers**

Volunteers completed a general health questionnaire concerning their dietary and physical activity habits. Any previous history of medical issues was disclosed before volunteers underwent the medical screening. The volunteer's height in centimetres and weight in kilograms was recorded and subsequently used to calculate a value for body mass index (BMI). Blood pressure was then monitored (Dinamap Pro 1000, GE Healthcare, Little Chalfont, UK) in both a standing and supine position. A 12-lead electrocardiogram (Schiller AT-10 Plus, Baar, Switzerland) was then carried out to detect any undiagnosed cardiac abnormalities and shown to a medical practitioner for approval. A sample of venous blood (~5ml) was drawn from the antecubital fossa and analysed for urea and electrolytes and routine blood chemistry (full blood count) at the Department of Clinical Pathology, Queen's Medical Centre, Nottingham, UK. Volunteers were excluded at this point if they were currently on medication, blood pressure was chronically high (>140/90 mmHg) when measured on separate occasions or if blood chemistry was abnormal.

## **2.3 Estimation of maximal oxygen consumption ( $\dot{V}O_2$ max)**

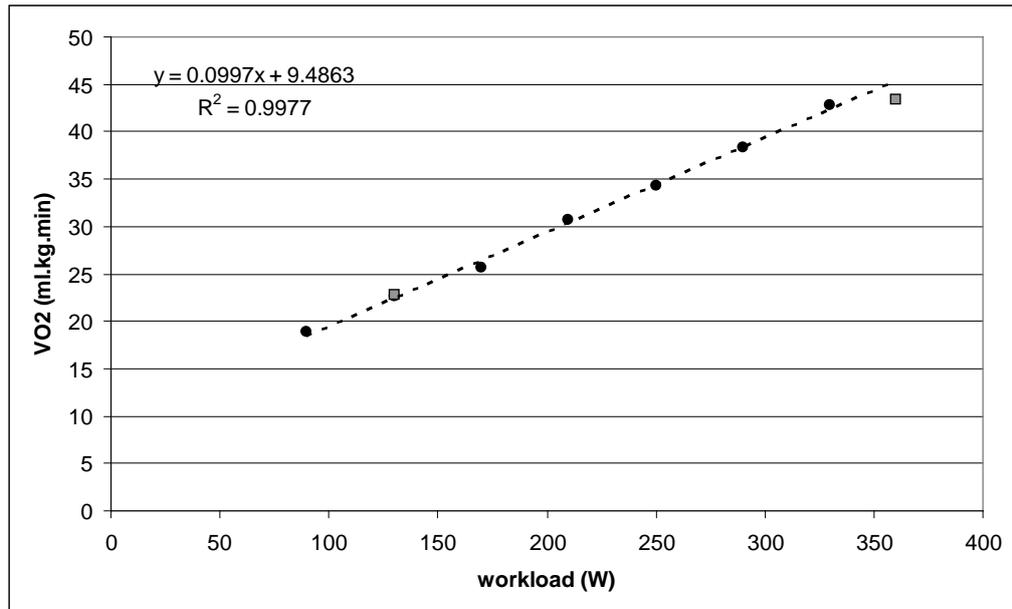
All the experimental protocols required volunteers to exercise at specific percentages of their maximal oxygen uptake. It was therefore necessary to first establish maximal oxygen uptake using a discontinuous incremental exercise test on a cycle ergometer (Lode B.V, Groningen, The Netherlands). Volunteers arrived at the laboratory in the morning following an overnight fast and adjusted the ergometer set-up for their comfort. This set-up was noted and maintained for the duration of the study. A 3-lead ECG was fitted to monitor

heart rate throughout the test. Whilst exercising, volunteers wore a nose clip and a valved mouthpiece that was supported by a fitted head brace for stability and comfort. The mouthpiece was connected to lightweight plastic tubing that carried expired air to a mixing chamber linked to an online analysis system (Vmax 29, SensorMedics, Yorba Linda, CA, USA). The expired air was analysed for oxygen and carbon dioxide content and used to calculate oxygen uptake ( $\text{VO}_2$ ) and respiratory exchange ratio (RER) every 20 sec using an analyser module in the online system. This system was calibrated before use with a syringe pump for air flow and gases of known concentrations (Viasys Healthcare, Yorba Linda, CA, USA). The test comprised a series of short (3-4 min) stages of cycling that increased incrementally in workload until the volunteer fatigued and could not complete a stage. The initial stage was set at a workload of 1 watt per kg body mass. Thereafter the workload for each stage was increased by 30-40 watts depending on the volunteer's level of fitness until the volunteer experienced fatigue and was unable to continue. The first 3 stages of the test were 4 min in duration and later stages were 3 min in duration to allow volunteers to reach a steady state of exercise. After each stage the volunteer stopped cycling and was allowed a 4 min rest break prior to the next stage to avoid peripheral fatigue before maximal oxygen uptake was achieved. Due to the low effort level of the first 3 stages, these were usually performed continually without a rest break as it was deemed unnecessary. Verbal encouragement was provided throughout the test to ensure the volunteer reached their maximal effort during the last completed stage. The test ceased when a volunteer could not continue cycling for the whole 3 min stage. At this point the workload was lowered immediately and the volunteer was

encouraged to continue cycling at a low intensity to recover. The oxygen uptake measurements during the final minute from every completed stage were averaged and then the relationship between  $\dot{V}O_2$  and workload was plotted (Figure 2.1). The  $\dot{V}O_2$  max test should produce a linear relationship between workload and oxygen uptake. Maximal oxygen uptake is defined as the highest value maintained despite a further increase in workload and should be visible as a plateau in oxygen uptake as exercise intensity increases.

#### **2.4 Confirmation of maximal oxygen consumption**

To confirm that the true  $\dot{V}O_2$  max value had been achieved, the volunteers performed a  $\dot{V}O_2$  max confirmation visit on a different occasion separated by a minimum of 1 day. The volunteers reported to the laboratory after an overnight fast and were fitted with the mouthpiece, nose clip and 3 lead ECG. They then completed a 4 min cycling stage at the second stage from the initial  $\dot{V}O_2$  max test to demonstrate consistency and to act as a low-intensity warm-up. At the end of this stage they were given a 4 min rest. They then completed a 3 min stage at a higher workload than the last fully completed stage on the initial  $\dot{V}O_2$  max test. If able, they then attempted a higher workload after another 4 min rest. Oxygen uptake was measured during each cycling stage. A value for  $\dot{V}O_2$  max was established when an increase of  $<2\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  was observed despite an increase in workload. This test should demonstrate a plateau in oxygen uptake even when the workload continued to increase (Figure 2.1).



**Figure 2.1** Results from a  $\dot{V}O_2$  max test (filled circle) and confirmation visit (grey squares) showing the relationship between  $VO_2$  and workload

## 2.5 Familiarisation to study procedures

The  $\dot{V}O_2$  max test and confirmation visit produce a linear relationship between oxygen uptake and workload. Initially 75% of the  $\dot{V}O_2$  max value was calculated and the y-intercept of the linear relationship was used to match this 75% value with a corresponding workload. It is necessary to confirm that this workload did equal this exercise intensity and to familiarise the volunteer with procedures that would be used during an experimental visit. On an occasion before an experimental visit, each volunteer arrived at the laboratory after an overnight fast and performed a bout of cycling on the cycle ergometer at their pre-determined workload for a minimum of 30 and a maximum of 60 min. For the last 3 min of every 10 min period, the mouthpiece and nose clip were fitted to the volunteer whilst they continued to cycle and oxygen uptake was analysed. If required, the workload was adjusted accordingly to attain the

required oxygen uptake. The percentage of  $\dot{V}O_2$  max demonstrated at the 20 min time point was used to calculate the exercise intensity performed by each volunteer in subsequent tests. After a rest period of 20-30 min, the volunteers were required to complete a performance test of 15 min (Chapter 3) or 20 min (Chapter 5) duration that would be performed during the main experimental trials. During this test volunteers were instructed to maintain the highest revolutions per minute (rpm) as they were able and to produce a maximal effort during the cycling bout. The ergometer was programmed to a pedalling-dependent (linear) mode so as to vary work load in accordance with cadence. The computer attached to the ergometer monitored work output (kJ) every 20 sec and the total cumulative work output at the termination of the time period was taken as a measure of exercise performance. Volunteers received verbal encouragement throughout the test and were able to view the time elapsed.

## **2.6 Exhaustive exercise protocol**

In Chapters 3 and 5, the experimental protocol required volunteers to perform cycling exercise on a cycle ergometer until the point of exhaustion. This exercise protocol is designed to reduce endogenous skeletal muscle glycogen reserves to an extremely low level. Volunteers in a fasted state commenced cycling at a previously established workload corresponding to  $\sim 75\%$   $\dot{V}O_2$  max and continued pedalling at  $>70$  rpm until they could not maintain the exercise. The ergometer was programmed to a hyperbolic mode such that if the pedalling rate altered the workload was maintained at the same level. Once the volunteers' fatigued and ceased cycling they were allowed a 5 min rest break before re-starting cycling at the same intensity. This exercise-rest pattern was continued until the volunteer could no longer sustain cycling above 70 rpm for

more than 2 min and the exercise bout was terminated. Throughout the exhaustive exercise protocol the volunteer was verbally encouraged to maintain their present physical effort. On the first experimental visit, water was provided *ab libitum* during the cycling period and the amount consumed was recorded to be replicated during subsequent experimental visits. To compensate for any training effect as a result of subsequent experimental trials, on each occasion volunteers performed the exhaustive exercise protocol to volitional fatigue and were not time-matched to previous visits. As such this would ensure muscle glycogen content was reduced to the lowest level. Previous work that has employed this exhaustive exercise protocol has demonstrated that the method is successful in lowering skeletal muscle glycogen stores to  $\sim 25 \text{ mmol.kg}^{-1}$  (Casey et al., 1995).

## **2.7 Preparation of test solutions**

All the studies contained in this thesis involve post exercise supplementation of volunteers with test solutions. These beverages are a high molecular weight (HMW) glucose polymer (Vitargo, Swecarb AB, Kalmar, Sweden), a standard glucose polymer with a much lower molecular weight (LMW) (Maxijul, SHS International, Liverpool, UK) and a flavoured water placebo. The characteristics of the solutions used in all studies are shown in Table 2.1 (derived from nutritional labelling). All beverages with the exception of the HMW glucose polymer in Chapter 3 were flavoured with a commercial sugar-free orange flavoured squash drink to ensure sufficient taste matching. The HMW glucose polymer used in chapter 3 was pre-flavoured with orange flavouring whereas this same product in subsequent chapters was unflavoured and thus required the addition of sugar-free orange-flavoured squash. The

LMW glucose polymer was also pre-flavoured however this product required additional flavouring to adequately mimic the taste of the HMW glucose polymer. This method of flavouring also ensured good palatability for rapid consumption by volunteers.

The test solutions were formed by dissolving the powdered product into water and mixing thoroughly with a mechanical hand whisk. All test beverages were prepared on the morning of an experimental visit and were contained in a non-transparent drinks bottle to obscure appearance to volunteers thus to avoid indication of which solution was being tested. Test solutions were prepared and consumed at room temperature.

| <b>CHAPTER 3.</b>          |  |                          |                                     |                              |                          |                               |
|----------------------------|--|--------------------------|-------------------------------------|------------------------------|--------------------------|-------------------------------|
| <b>BEVERAGE</b>            | <b>Molecular Weight (g.mol<sup>-1</sup>)</b> | <b>Powder amount (g)</b> | <b>Solutions added</b>              | <b>Total CHO content (g)</b> | <b>Final Volume (ml)</b> | <b>Calorie content (kcal)</b> |
| <b>HMW</b>                 | 500,000-700,000                              | 107                      | 920ml water                         | 100                          | 1000                     | 394                           |
| <b>LMW</b>                 | ~900   | 105.3                    | 100ml flavoured squash + 820ml      | 100                          | 1000                     | 400                           |
| <b>CON</b>                 |  | 0                        | 150ml flavoured squash + 850ml      | 0.6                          | 1000                     | 4                             |
| <b>CHAPTER 4.</b>          |  |                          |                                     |                              |                          |                               |
| <b>BEVERAGE</b>            | <b>Molecular Weight (g.mol<sup>-1</sup>)</b> | <b>Powder amount (g)</b> | <b>Solutions added</b>              | <b>Total CHO content (g)</b> | <b>Final Volume (ml)</b> | <b>Calorie content (kcal)</b> |
| <b>HMW</b>                 | 500,000-700,000                              | 107                      | 100ml flavoured squash +820ml water | 100                          | 1000                     | 394                           |
| <b>LMW</b>                 | ~900   | 105.3                    | 100ml flavoured squash + 820ml      | 100                          | 1000                     | 400                           |
| <b>CON</b>                 |  | 0                        | 150ml flavoured squash + 850ml      | 0.6                          | 1000                     | 4                             |
| <b>CHAPTERS 5 &amp; 6.</b> |  |                          |                                     |                              |                          |                               |
| <b>BEVERAGE</b>            | <b>Molecular Weight (g.mol<sup>-1</sup>)</b> | <b>Powder amount (g)</b> | <b>Solutions added</b>              | <b>Total CHO content (g)</b> | <b>Final Volume (ml)</b> | <b>Calorie content (kcal)</b> |
| <b>AGG</b>                 | 500,000-700,000                              | 107                      | 100ml flavoured squash +820ml water | 100                          | 1000                     | 394                           |
| <b>GRAN</b>                | 500,000-700,000                              | 107                      | 100ml flavoured squash +820ml water | 100                          | 1000                     | 394                           |
| <b>CON</b>                 |  | 0                        | 150ml flavoured squash + 850ml      | 0.6                          | 1000                     | 4                             |

**Table 2.1** Nutritional composition of test solutions

## **2.8 Dietary manipulation and analysis**

Before attending any experimental study visits, volunteers were required to abstain from alcohol, caffeine and strenuous exercise for 24 hr to eliminate any effects from confounding lifestyle factors. For the study in Chapter 3, volunteers completed a habitual food diary for 2 weekdays and 1 weekend day that was then analysed using computer software for typical macronutrient and energy intake (Microdiet, Downlee Systems, Ltd, High Peak, UK). A prescribed diet for each volunteer was constructed from these data comprising four days of average energy intake ( $\pm 100$  kcal) with consumption of carbohydrate not more than 55% of total intake on each day. Volunteers were required to follow this prescribed diet on the day before attending the laboratory for an experimental trial and for 3 days thereafter. On the day of an experimental visit, volunteers were provided with the same food and drink at the completion of the experimental procedure and were instructed to record their food and drink intake for the remainder of the day. For the studies in Chapters 4 and 5, diet diaries were provided and volunteers were instructed to match types and amounts of food intake on the days preceding a study and food that was provided after each experimental visit was identical. Total food intake was recorded for 3 days after an experimental visit using a food diary, and analysed for total energy and carbohydrate intake to ensure that overconsumption of either carbohydrate or total energy had not occurred.

## **2.9 Blood sampling and analyses**

The studies described in Chapters 3 and 5 involved sampling of arterialised-venous blood at regular intervals. In these studies, on arrival to the laboratory, an 18-gauge intravenous cannula (BD, New Jersey, USA) was inserted into a dorsal hand vein in a retrograde direction. This was kept patent throughout the study by the constant infusion of 0.9% sodium chloride solution (Baxter Healthcare, Northampton, UK). During the period of blood sampling the hand was kept inside a warm air box set at 55°C to arterialise the blood thus allowing a close approximation of arterial concentrations of glucose and lactate from venous sampling (Gallen and Macdonald 1990). In the study described by Chapter 3, approximately 8 ml of blood was drawn at 10 min intervals during a 2 hr period. This blood was immediately analysed for glucose and lactate concentration before being distributed into collection tubes for further analysis. Approximately 4 ml was transferred into a gel separation tube (BD Medical, Franklin Lakes, NJ, USA) and left standing for a minimum of 30 min to clot before being spun in a centrifuge at 3000 rpm for 10 min. The resulting serum was extracted and then stored at -80°C for later determination of serum insulin concentration. The remaining blood was transferred into a Lithium Heparin tube (BD Medical, Franklin Lakes, NJ, USA) containing 75 µl ethylene glycol tetraacetic acid (EGTA) and kept on ice until the end of the experimental trial. The tubes were then spun in a centrifuge at 3000 rpm for 10 min before the supernatant was extracted and stored at -80°C for later determination of plasma non-esterified fatty acid (NEFA) concentration. In the study described in Chapter 5, approximately 3 ml of arterialised-venous blood was drawn at 5 min intervals for 60 min and at 10 min intervals for a further 60 min. These samples

were immediately analysed for glucose concentration and the remaining sample was collected for insulin determination as previously described.

#### *2.9.1 Blood glucose and lactate concentration*

Blood glucose (Chapters 3 and 5) and lactate (Chapter 3) concentrations were determined immediately after withdrawal from the cannula using a glucose/lactate analyser (YSI 2300 STATplus, Yellow Springs Instruments, Ohio, USA). The analyser was routinely calibrated every 60 min during the studies against glucose and lactate standards of known concentrations (Yellow Springs Instruments, Ohio, USA).

#### *2.9.2 Serum insulin concentration*

Serum insulin was determined using a radioimmunoassay kit (Coat-a-Count Insulin, Diagnostics Products Corporation, Los Angeles, CA, USA). The assay involves combining samples with <sup>125</sup>Iodine labelled insulin which competes for insulin-specific antibody sites with the insulin contained within a sample. After decanting the tube a gamma counter was used to measure the levels of radioactivity which was then converted via a calibration curve into an insulin concentration for each sample. The full analytical method is described in Appendix 1. In the study described in Chapter 3 the samples were separated into batches of ~200 and 3 separate assays were run. The CV for the method was measured using samples of known concentration (100 and 8 mIu.l<sup>-1</sup>) that were placed at the beginning and end of the sample run. The variation in these samples was used to calculate the CV of the method. The intra assay CV's for the 3 assays performed in Chapter 3 were 3.2, 4.5 and 0.8 %. In the study

described in Chapter 5 another 3 essays were performed with ~200 samples each and the CV's were 0.9, 3.4 and 4.4 %.

### *2.9.3 Non-esterified fatty acids (NEFA)*

The measurement of plasma NEFA concentration was conducted using an automated analyser (ABX Pentra 400, Horiba Medical, Montpellier, France) and chemical reagent kit (WAKO Chemicals GmbH, Neuss, Germany). The concentration of NEFA is determined by conversion to Acyl-CoA which is subsequently oxidised to yield hydrogen peroxide. The hydrogen peroxide forms a purple pigment in the presence of peroxidase and the absorbance of the colour is used to determine NEFA concentration. The assay was run once on all samples and the CV as determined from 3 separate measures of 1 sample during the assay was 0.36%.

## **2.10 Muscle sampling procedure and analyses**

### *2.10.1 Muscle biopsy procedure*

Muscle biopsies were obtained in the study described in Chapter 3 for the determination of muscle glycogen content. A small sample of muscle tissue from the *Vastus Lateralis* (50-200mg wet weight) was removed using the percutaneous needle biopsy technique (Bergstrom 1975). Three muscle biopsies were taken during each experimental trial, 2 were conducted with the volunteer resting semi-supine on a bed and the third was performed with the volunteer sat on the cycle ergometer after completing a 15 min cycling time trial. Briefly, the biopsy area was first measured and shaved (if needed) before the biopsy sites were marked on the skin (~2-3cm apart). For each biopsy, the leg was cleaned with iodine solution before a local anaesthetic (Lignocaine)

was injected using a syringe firstly under the skin and then into the subcutaneous tissue before a small (<1cm) incision was made in the skin and fascia lata using a scalpel blade. Thereafter, a 5mm gauge Bergstrom biopsy needle was inserted into the muscle and a small sample was removed assisted by suction applied to the end of the needle. The muscle biopsy sampling took approximately 10 sec and the sample was then frozen in liquid nitrogen within 2-3 sec of extraction. The wound was then cleaned and dressed by an experienced medical professional. As 3 muscle biopsies were taken on the same leg per visit for each volunteer, the samples were taken in a distal-proximal- medial order with spacing of approximately 2.5 cm between sites to minimise any minor trauma effects.

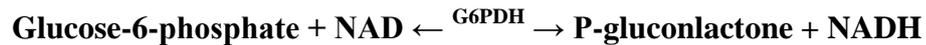
#### *2.10.2 Extraction of muscle glycogen*

The excised muscle samples were firstly cut in liquid nitrogen to produce samples of ~100 mg which were then freeze-dried to remove all water. The dried muscle pellets were examined and all visible blood and connective tissue was removed before the samples were pulverised using a pestle and mortar (20 min per sample), until a fine powder was achieved. The extraction of muscle glycogen proceeded by dissolving the powder in aliquots with 100-160  $\mu$ l of 0.1M sodium hydroxide and incubating at 80°C for 10 min in conjunction with repeated vortexing. The aliquots were then neutralised with 400-640  $\mu$ l of a buffer of 0.2M citric acid and 0.1M HCL after which 15-20  $\mu$ l of the enzyme Amyloglucosidase (AGDase) was added and the samples were left to stand for 60 min. The aliquots were then spun in a centrifuge for 2 min at 14000 rpm and the supernatant was stored at -80°C for further analysis of glycogen concentration. An extraction factor was calculated from the formula below;

**EF** = NaOH + Buffer + AGDase ( $\mu$ l) / muscle powder weight (mg)

### 2.10.3 Assay for muscle glycogen determination

Muscle glycogen content was determined using a spectrophotometric method (Harris et al., 1974) with NADH linked reactions as follows;



A reagent mix containing Triethanolamine, DTT, NAD, ATP and H<sub>2</sub>O was added to each sample in a 96 well plate and the absorbance at a wavelength of 366 nm of the samples (Abs1) and blanks (B11) containing the reagent was measured. Thereafter an automatic pipette was used to add 2  $\mu$ l of a mix of hexokinase and glucose-6-phosphate dehydrogenase to each sample and left to react for 20 min. A second measure of the absorbance of samples (Abs2) and blanks (B12) was taken and the changes in absorbance were used to calculate glycogen concentrations from the formula below;

$$\text{Glycogen (mmol.kg}^{-1} \text{ dw)} = \left[ \frac{((\text{Vol2} * (\text{Abs2}-\text{B12})) - (\text{Vol1} * (\text{Abs1}-\text{B11})))}{(3.4 * \text{Svol})} \right] * \text{EF}$$

Vol 1 = volume in well before enzyme

Vol 2 = volume in well after enzyme added

3.4 = molar extinction co-efficient for NAD at 366 nm wavelength

Svol = sample volume (25  $\mu$ l)

The CV for the muscle glycogen assay as determined from the same sample measured 10 times was 2.5%.

## 2.11 Magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an emerging analytical method to measure the concentration of metabolites *in vivo* without the need for invasive procedures such as biopsies. With the advent of more sensitive, higher strength magnets, MRS can allow continuous monitoring of fluctuations in metabolite levels thus it is a potentially viable investigative tool for glycogen metabolism. The MRS technique is based on the magnetic properties possessed by nuclei with an odd atomic number known as magnetic moments or 'spin' which are randomly orientated under normal conditions. The nuclei which are most commonly investigated using MRS are  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{31}\text{P}$  which all possess spin equal to  $1/2$ . The application of a magnetic field causes the nuclei to line up parallel and precess about the magnetic field, either aligned with or opposed to the magnetic field. The precessional frequency of the nucleus, about the static field, is termed the Larmor frequency and is dependent on the magnetic field experienced by the nucleus. It is this precession that is measured in NMR. The total magnetic field experienced by a given nucleus depends on the circulating electrons which generate a local electrical field that opposes the externally applied field and can vary between the same types of nucleus ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) due to the molecular bonding geometry. Thus, variations in the local magnetic field of the same type of nucleus due to electron distribution lead to differences in the precessional frequency, known as the chemical shift which can provide specific structural information about a molecule. The chemical shift is determined relative to a reference frequency (Tetramethylsilane for  $^{13}\text{C}$ ) which is assigned a chemical shift of zero parts per million (ppm). The

different molecules in a sample can be distinguished in a sample from their characteristic chemical shift.

The principle of MRS is to measure the signal from specific nuclei as they return back to their equilibrium position following application of high frequency radio waves. Briefly, when atomic nuclei are present in a stationary magnetic field, they precess at their characteristic frequencies aligned parallel with the magnetic field. Spin states are not equal such that those orientated parallel with the externally applied field are in a low energy state, whereas those that oppose the externally applied field are in a higher energy state. At equilibrium, the number of spins aligned with the static magnetic field (low energy state) is higher than the number of spins opposed to the static magnetic field (high energy state) giving rise to an overall magnetization (bulk magnetization) of the sample which is proportional to the strength of the static magnetic field. The separation of energy states allows transitions between various spin states by applying an oscillating magnetic field with energy equal to the energy difference between the different states. Applying an electromagnetic pulse at a specific frequency induces changes from a low to high energy state, changing the direction of the bulk magnetization of the sample. For a simple NMR experiment, the electromagnetic pulse is applied which moves the bulk magnetization from the equilibrium position (aligned with the magnetic field) through  $90^\circ$  (perpendicular to the magnetic field). The spins then precess perpendicular to the static field. The precession of this magnetization about the static magnetic field is detected using a radiofrequency (RF) coil which fits over the region of interest ensuring a homogenous sample of desired tissue. Over time the measured signal decreases due to interaction

between the spins (leading to a loss of phase coherence perpendicular to the applied magnetic field) and loss of energy as spins return to the lower energy state. The signal measured from the tissue over time is converted to a series of peaks at specific frequencies to the compounds using Fourier analysis, and the area under the peak corresponds to the concentration of each metabolite.

Increasing the field strength improves the signal to noise ratio (SNR), and thus the sensitivity of the method. Most *in vivo* metabolic studies have been conducted using magnets of strength ranging from 1.5 to 3.0 tesla (tesla refers to the SI unit of magnetic flux density), however recent advances in technology have more than doubled the field strength to 7.0 tesla although these are not as readily available. The natural abundance of nuclei also affects the sensitivity in that the highest sensitivity is observed for close to 100% such as  $^1\text{H}$  and  $^{31}\text{P}$  spectroscopy. In contrast,  $^{13}\text{C}$  has a low natural abundance of 1.1% and therefore a relatively low sensitivity meaning only higher strength magnets (7.0 tesla) that can enhance the visibility of compounds such as skeletal glycogen are suitable.

NMR spectroscopy has been previously used as a method to measure carbohydrate metabolism in skeletal muscle and hepatic tissue in both healthy (Jue et al., 1989; Casey et al., 2000; Krssak et al., 2000) and diabetic subjects (Shulman et al., 1990; Price et al., 1996; Shulman 1996). The advantages of the technique lie in the ability to perform time-course investigations with multiple measurements with minimum discomfort to volunteers.

### *2.11.1 Volunteer screening and safety procedures*

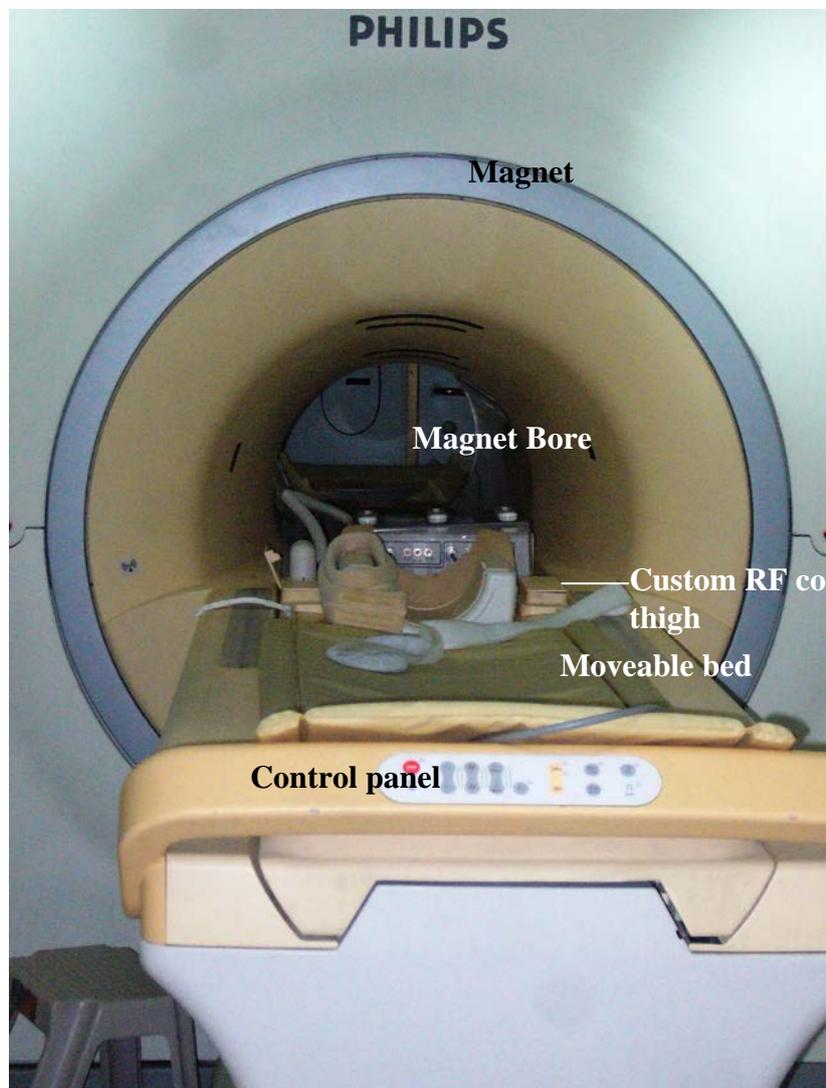
In Chapter 4 volunteers underwent a medical screening and completed a general health questionnaire as described previously in section 2.2. In addition they completed a safety questionnaire to assess their suitability for the scanning procedures (Appendix 2). Before beginning the study, all volunteers were informed about the scanning procedures and were familiarised to being contained within the scanner. Due to the high magnetic field, some volunteers experienced minor dizziness when being moved in or out of the scanner that would dissipate after a short time. Volunteers were informed of this and were transported in and out of the scanner on a preliminary visit to assess their comfort before any study procedures were initiated. During the scanning volunteers were required to wear ear plugs and ear defenders and had possession of an alarm button to alert investigators of any problems.

### *2.11.2 $^{13}\text{C}$ MRS for glycogen determination in skeletal muscle*

The determination of skeletal muscle glycogen concentration described in Chapter 4 was achieved using  $^{13}\text{C}$  NMR spectroscopy, acquired with a 7.0 tesla (T) whole body magnetic resonance scanner with a 1-m diameter bore (Philips Healthcare, The Netherlands, Figure 2.2). A  $^{13}\text{C}$  quadrature coil (Philips Healthcare, The Netherlands) was used for transmission and reception of  $^{13}\text{C}$  frequency RF signals with an inbuilt  $^1\text{H}$  quadrature coil used for transmission and reception of  $^1\text{H}$  frequency signals. Volunteers were placed on the moveable bed in a prone position with their mid section resting on a foam pad and head resting on a pillow for comfort. The right leg was positioned with the upper thigh contained within the RF coil such that the middle of the coil was in line with the mid-thigh and homogenous filling of the coil with the quadriceps

muscle could be achieved. The position of the leg in the coil was marked on the skin to ensure accurate re-positioning of the volunteer between scans.

For the studies described in Chapter 4 all spectra were collected using the 7.0 T magnetic resonance scanner and acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling. A total of 8 spectra with 80 averages each were acquired for each measurement point (temporal resolution of 11 min).



**Figure 2.2** Magnetic resonance scanner (7.0 T) and accompanying hardware

### *2.11.3 Quantification of muscle glycogen*

Once the data was acquired it was analysed by averaging the spectra (to improve SNR ratio) and 50 Hz Lorentzian line broadening was completed. A phase correction was applied using the Magnetic Resonance User Interface (jMRUI). The signal of interest (C1 position of glycogen) arises at 100.5 ppm (TMS =0) and the integral area under the peak was determined using in-house software built in Matlab compared to an external reference peak from a small vial of urea placed within the coil at ~168 ppm. Absolute concentrations were calculated by measuring the volume of glycogen containing tissue within the sensitive region of the coil (measured in each subject using an initial MR image acquired using the <sup>1</sup>H quadrature coil). The variation of the method was calculated from the standard deviation of the baseline spectra obtained in Chapter 4. A variation of 6 mmol/l was measured across all subjects.

### **2.12 Statistical analysis**

All statistical analysis was performed using GraphPad Prism 5.03 (GraphPad Software, Inc, California, USA) and data are expressed as mean ± standard error of the mean (SEM), unless otherwise stated. Two-way analysis of variance with repeated measures (ANOVA) was used to determine differences in Chapters 3 and 5 and further post-hoc analysis was performed using Bonferroni multiple comparisons. One-way ANOVA was used to analyse changes in single measure variables i.e. muscle glycogen concentration and total exercise performance. Significance was accepted at a P level of less than 0.05.

## **Chapter 3**

The effect of post-exercise ingestion of a high molecular weight glucose polymer on skeletal muscle glycogen resynthesis and exercise performance in healthy male volunteers

### 3.1 Introduction

With increasing exercise intensity the relative contribution of carbohydrate oxidation increases with a concurrent decline in fat oxidation such that muscle and liver glycogen becomes the primary fuel for oxidative metabolism (van Loon et al., 2001). As a result skeletal muscle glycogen content largely declines during prolonged to moderate intensity exercise such that fatigue has been shown to coincide with near-total muscle glycogen depletion (Ahlborg et al., 1967; Bergstrom and Hultman 1967; Hermansen et al., 1967). Given that the time to exhaustion for prolonged exercise has been demonstrated to be proportional to the pre-exercise muscle glycogen content (Bergstrom et al., 1967) and provision of exogenous carbohydrate during exercise can offset fatigue and prolong exercise performance (Coyle et al., 1986; Tsintzas et al., 1996; Coggan and Coyle 1987), the importance of muscle glycogen for sustained exercise performance is well established. The availability of muscle glycogen is thus a limiting factor during exercise with fatigue likely occurring due to an inability of glycogen depleted muscle to maintain the required rate of ATP resynthesis (Sahlin et al., 1990). Therefore optimisation of pre-exercise muscle and liver glycogen content is desirable at the onset of exercise.

Following depletion of muscle and liver glycogen stores there is a metabolic priority for glycogenesis and it has been reported that complete recovery of muscle glycogen content can be achieved within 24 hr following provision of 8-10 g.kg<sup>-1</sup> bw of carbohydrate in meal form (Costill et al., 1981; Burke et al., 1995). Subsequent exercise performance at a similar intensity when recovery time is limited may rely on the extent of muscle glycogen reserves, thus much interest has targeted strategies to maximise post exercise muscle glycogen

resynthesis via increasing the amount of fed carbohydrate or combining it with protein to raise insulin concentrations (Beelen et al., 2010). Effective post exercise muscle glycogen resynthesis cannot proceed if muscle glucose availability is limiting and dietary carbohydrate represents the primary source of glucose following muscle and liver glycogen depletion. Only negligible muscle glycogen resynthesis ( $\sim 2 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ ) occurs after glycogen-depleting exercise without exogenous carbohydrate (Bergstrom and Hultman 1966; Bergstrom and Hultman 1967; Ivy et al., 1988) and delaying ingestion of carbohydrate has shown sub-optimal rates (50% lower) of muscle glycogen resynthesis when compared to a supplement provided immediately after glycogen-depleting exercise (Ivy et al., 1988).

Post-exercise muscle glycogen resynthesis is achieved via the increased permeability of the muscle membrane to blood glucose due to translocation of GLUT 4 vesicles and the activation of glycogen synthase. This process has been shown to occur in two distinct phases in humans (Price et al., 1994). The initial phase occurs in the 60 min following exercise and occurs independently of the insulin concentration thus muscle glucose uptake is mediated by local factors. This is followed by a longer secondary phase that can persist up to 48 hours and is characterised by an increase in insulin sensitivity (Maarbjerg et al., 2011; Richter et al., 2001; Goodyear and Kahn, 1998). As such, muscle glycogen resynthesis may be limited by the rate of glucose delivery in the initial phase considering that abstaining from carbohydrate intake confers little increase in muscle glycogen following exercise (Ivy et al., 1998). Additionally studies that have used intravenous infusion of glucose have elicited higher rates of muscle glycogen resynthesis compared to oral carbohydrate ingestion, which

was attributed to high blood glucose concentrations (Bergstrom and Hultman 1967; Roch-Norlund et al., 1972). In the absence of carbohydrate intake the contraction-induced effects on muscle glucose uptake reverses rapidly (Goodyear et al., 1990), therefore the secondary effect of insulin secretion (as a response to increasing blood glucose) is required to initiate a signalling cascade that maintains GLUT 4 translocation and activation of glycogen synthase. Muscle glycogen resynthesis following depleting exercise is thus limited firstly by blood glucose concentration providing a substrate for glycogenesis and furthermore by a secondary effect on insulin secretion to maintain muscle glucose uptake and the activity of glycogen synthase. Post-exercise carbohydrate feeding serves to increase circulating arterial glucose concentration thereby providing a substrate and subsequently elevating insulin concentrations to increase muscle glucose transport.

Glucose flux into muscle is mediated through processes that control glucose delivery, membrane transport and phosphorylation in the muscle (Wasserman et al., 2011). However, the liver, stomach and digestive tract are important determinants of muscle glucose uptake by maintaining blood glucose concentrations. From ingestion to arrival at the muscle membrane, delivery of ingested carbohydrate is most likely to be limited by a combination of factors including the rate of gastric emptying, the rate of intestinal absorption, uptake and output by the liver (Jentjens and Jeukendrup 2003). Strategies to elevate blood glucose and insulin concentrations for rapid muscle glycogen resynthesis have included the addition of protein (van Hall et al., 2000; van Hall et al., 2000; van Loon et al., 2000; van Loon et al., 2000; Kaastra et al., 2006),

caffeine (Pedersen et al., 2008) and feeding high GI carbohydrate (Burke et al., 1993; Wee et al., 2005).

Previous work using a 13.5% solution containing a unique glucose polymer with a very high molecular weight (HMW) (500,000-700,000 g.mol<sup>-1</sup>) and a corresponding low osmolality (84 mosmol.kg<sup>-1</sup>) has indicated a capacity to aid recovery from prolonged exercise as evidenced by studies comparing it to a mixture of glucose and oligosaccharides with a much lower molecular weight (LMW) (500 g.mol<sup>-1</sup>) and higher osmolality (350 mosmol.kg<sup>-1</sup>) in healthy males (Piehl-Aulin et al., 2000; Leiper et al., 2000). Following glycogen-reducing exercise, an augmented muscle glycogen resynthesis rate (50 vs. 30 mmol.kg<sup>-1</sup>.h<sup>-1</sup>) was reported by Piehl-Aulin et al (2000) at 2 hr of resting recovery with ingestion the HMW glucose polymer compared to the LMW glucose solution. This finding was supported by a related study using the same solutions in non-exercised, resting volunteers. This study reported faster half-emptying time of 75g of the HMW glucose polymer in 500ml of water (17 vs. 32 min) coupled with a greater carbohydrate delivery to the small intestine in the first 10 min after ingestion when compared to the same volume of the LMW solution (Leiper et al., 2000). These results appear to indicate more rapid gastric emptying, potentially as a result of the difference in osmolality (Vist and Maughan 1995). These observations led to the supposition that recovery from glycogen-depleting exercise could be enhanced during a short time period with ingestion of the HMW glucose polymer thus performance in a subsequent exercise bout could be improved. In view of this a later study reported an increased work output during a 15 min 'all-out' exercise trial performed 2 hr after exhaustive cycling exercise and ingestion of 100g of the HMW glucose

polymer (Stephens et al., 2008). Notably this study compared the glucose polymer to an isoenergetic glucose polymer, but with a lower molecular weight ( $900 \text{ g}\cdot\text{mol}^{-1}$ ) and higher osmolality ( $124 \text{ mosmol}\cdot\text{kg}^{-1}$ ), that was considered more representative of a typical commercial sports drink. An overall 10% improvement in work output was observed with the high compared to the low molecular weight glucose polymer, and importantly this improved performance effect was demonstrated in all 8 participants. This observation was attributed to greater muscle glycogen availability due to an observed greater rate of rise in blood glucose and plasma insulin concentration over the initial 40 min following ingestion of the HMW compared to the LMW glucose polymer suggesting enhanced muscle glycogen resynthesis. However, no measurements of muscle glycogen were made in this study but in light of the improvement in exercise performance, it can be postulated that muscle glycogen content was higher at the onset of the second exercise bout after ingestion of the HMW glucose polymer. The present study was conducted to quantify muscle glycogen content as a result of glycogen-depleting exercise and ingestion of either a HMW or LMW glucose polymer.

### **3.2 Aims and hypotheses**

The main aim of the study was to quantify changes in muscle glycogen during a short recovery from glycogen-depleting exercise after supplementation with glucose polymer solutions differing in molecular weight and osmolality.

A secondary aim was to record exercise performance in a further high intensity exercise bout and document any improvements in relation to differences in muscle glycogen content.

It was hypothesised that ingestion of the HMW glucose polymer post exercise would augment blood glucose and insulin concentrations above that of an isoenergetic LMW glucose polymer and would subsequently enhance muscle glycogen resynthesis over a 2 hr resting recovery period from glycogen-depleting exercise.

### **3.3 Methods**

#### *3.3.1 Subjects*

Seven healthy, non-smoking, non-vegetarian males (Mean  $\pm$  SD; Age:  $26 \pm 4.7$  yrs; BMI:  $22.6 \pm 2.9$  kg.m<sup>-2</sup>) were recruited for the purposes of the study. The volunteers were all currently engaged in some form of regular physical activity and were familiar with cycling exercise. All the volunteers were informed of the study procedures and associated risks of participation before providing written consent. Once consented into the study, volunteers attended the laboratory on 3 separate occasions for preliminary testing using an online system (Vmax 29, SensorMedics, Yorba Linda, CA, USA), to establish maximal rate of oxygen consumption ( $\dot{V}O_2$  max) and workload corresponding to 75% of  $\dot{V}O_2$  max as described in Chapter 2. Mean  $\dot{V}O_2$  max was  $48.3 \pm 3.2$  ml.kg<sup>-1</sup>min<sup>-1</sup>.

### 3.3.2 Study design and protocol

Volunteers visited the laboratory in the morning following an overnight fast on three separate occasions separated by a minimum of 2 weeks. For 24 hr previously they had abstained from consuming alcohol or caffeine or participating in strenuous physical activity. To minimise the possibility of glycogen supercompensation between visits, volunteers were instructed to adhere to a prescribed food plan comprising no more than 55% of total energy as carbohydrate for 3 days following a study visit (Table 3.1). Volunteers were instructed to follow the same diet for the day preceding each study visit.

|                   | <b>Diet day</b> |             |             |
|-------------------|-----------------|-------------|-------------|
|                   | <b>1</b>        | <b>2</b>    | <b>3</b>    |
| <b>Total kcal</b> | 2510 ± 975      | 2495 ± 1051 | 2461 ± 1012 |
| <b>CHO (%)</b>    | 54 ± 1          | 54 ± 1      | 54 ± 1      |
| <b>FAT (%)</b>    | 32 ± 4          | 30 ± 3      | 30 ± 3      |
| <b>PRO (%)</b>    | 15 ± 3          | 15 ± 4      | 16 ± 4      |

**Table 3.1** Dietary intake pre and post experimental visits

An illustration of the study visit procedure is shown in Figure 3.1. Once at the laboratory, volunteers rested semi-supine on a bed whilst a cannula was inserted retrograde into a superficial vein on the back of the hand for subsequent blood sampling. The cannula was attached to a 0.9% saline drip (Baxter Healthcare, Northampton, UK) to be kept patent throughout the duration of the study. Once the cannula was inserted and secured, the volunteers mounted the cycle ergometer (Lode Excalibur, Lode, Groningen, The Netherlands) and began the exhaustive cycling protocol described in detail in Chapter 2. Once the volunteer was deemed to be at the point of exhaustion,

as indicated by an inability to maintain more than 2 min cycling effort, exercise ceased and a blood sample was taken immediately. Thereafter the volunteer rested semi-supine on a bed and a muscle biopsy was taken. After the biopsy incision was treated, the volunteer was provided with a non-transparent sports bottle containing 1 of 3 test solutions. These 1 litre solutions contained either 100g of 2 different carbohydrate powders (HMW=Vitargo, Swecarb AB, Kalmer, Sweden or LMW=Maxijul, SHS International, Liverpool, UK) mixed with water, or a flavoured water placebo (full details are provided in Chapter 2). All volunteers were able to rapidly consume the test solution within 2-3 min and thereafter they remained semi-supine on the bed for a period of 120 min.

Once placed on the bed, the cannulated hand was immediately placed in a hand-warming unit (temperature of 55°C) for arterialised-venous blood sampling. During the 2 hr resting recovery period, blood samples were drawn from the cannula at 10 min intervals. At 2 hr following drink ingestion, a second muscle biopsy was obtained and the site for the third biopsy was prepared. This third site was then bandaged before the volunteers re-mounted the cycle ergometer. They then commenced the 15 min cycling performance test to determine maximal work output that is detailed in Chapter 2 and is considered a more sensitive measure of endurance exercise performance than cycling to exhaustion (Jeukendrup et al., 1996). Volunteers were instructed to complete the test at the highest effort they could sustain. Verbal encouragement was provided at set time points and standardised between participants. At the completion of the test the volunteers immediately lay supine on a bed positioned adjacent to the ergometer. A third muscle biopsy was then taken in this position. Following treatment of the muscle biopsy sites, volunteers were

fed and left the laboratory. The experimental protocol was completed on 3 occasions for each volunteer in a randomised order to prevent any order effect of the solutions.

### *3.3.3 Sample collection and analysis*

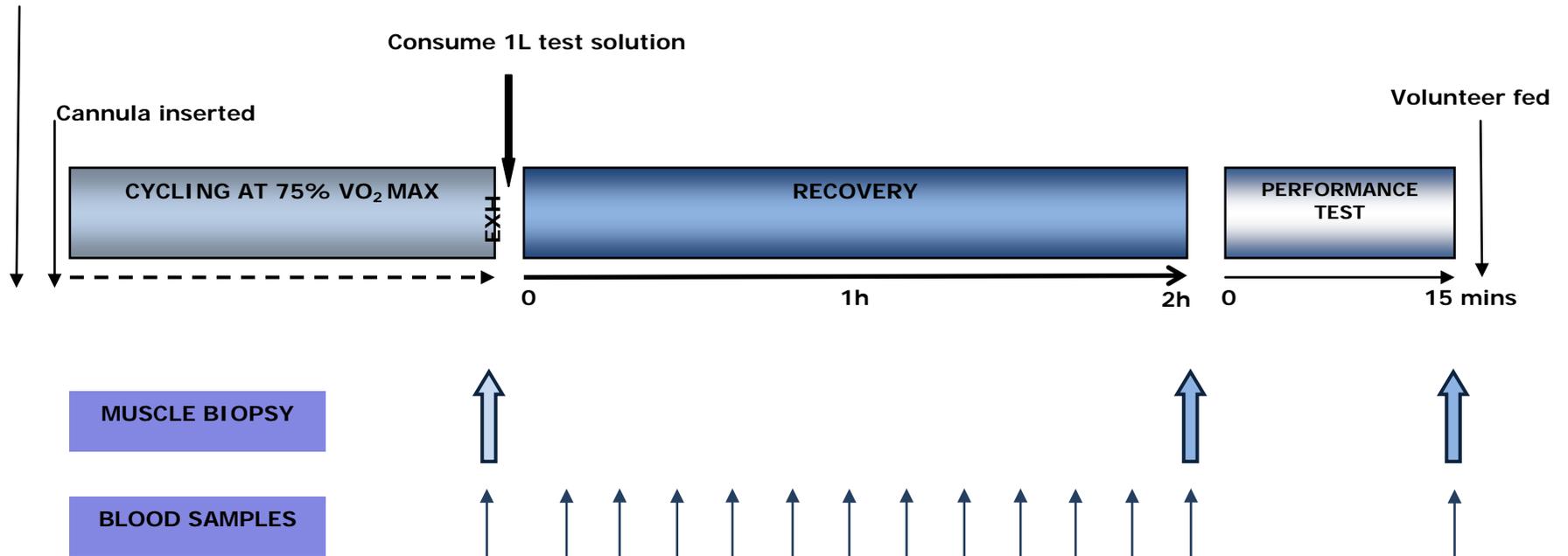
Muscle biopsy samples were obtained from the *vastus lateralis* at 3 time points on each study visit using the percutaneous needle biopsy technique (Bergstrom 1975). Once removed, the muscle samples were snap frozen in liquid nitrogen within 5 seconds to preserve the metabolic state of the tissue. These samples were subsequently stored immersed in liquid nitrogen before analysis. The muscle samples were freeze-dried, powdered and analysed for glycogen concentration as described in Chapter 2. Approximately 1 ml of each arterialised-venous blood sample withdrawn from the cannula at 10 min intervals during the recovery period was analysed for glucose and lactate concentration (YSI 2300 STATplus, Yellow Springs Instruments, OH, USA). The remainder of each blood sample was separated equally into a lithium heparin container and a serum tube (BD vacutainer, Franklin Lake, NJ, USA) and either kept on ice (lithium heparin) or left to clot (serum tube), after which they were centrifuged at 3000 rpm and the plasma stored at -80°C for subsequent determination of free fatty acid (NEFA) and insulin concentrations as described in Chapter 2.

### *3.3.4 Statistical analysis*

Blood metabolite concentrations and muscle glycogen content were analysed using two-way analysis of variance (time and solution effects; GraphPad Prism 5.03, GraphPad Software, Inc, California, USA) to detect differences in

responses after exercise, over the 2 hr recovery period and after the performance test. When a main effect was identified, further post-hoc analysis was performed using Bonferroni multiple comparisons to compare all time points. A one-way analysis of variance was performed to assess any difference in rate of muscle glycogen resynthesis between groups. Further *post hoc* analysis with Tukey's multiple comparison test was completed when a significant main effect was seen. Significance was accepted when P values were less than 0.05 and all values unless stated otherwise are expressed as mean  $\pm$  standard error of the mean (SEM).

Arrive at Laboratory  
(fasted overnight)



**Figure 3.1** Schematic representation of experimental study visit

### 3.4 Results

#### 3.4.1 Exercise time to exhaustion

Volunteers cycled to exhaustion at a workload of  $189 \pm 40$  watts which corresponded to an exercise intensity of  $77.1 \pm 1.5\% \dot{V}O_2$  max. The total exercise time completed during the glycogen-depleting protocol was similar between all 3 conditions. Mean exercise time to reach exhaustion for the high molecular weight glucose polymer trial (HMW), the low molecular weight glucose polymer trial (LMW) and the control trial (CON) was  $106 \pm 6$  min,  $105 \pm 6$  min and  $103 \pm 6$  min respectively.

#### 3.4.2 Blood Metabolites

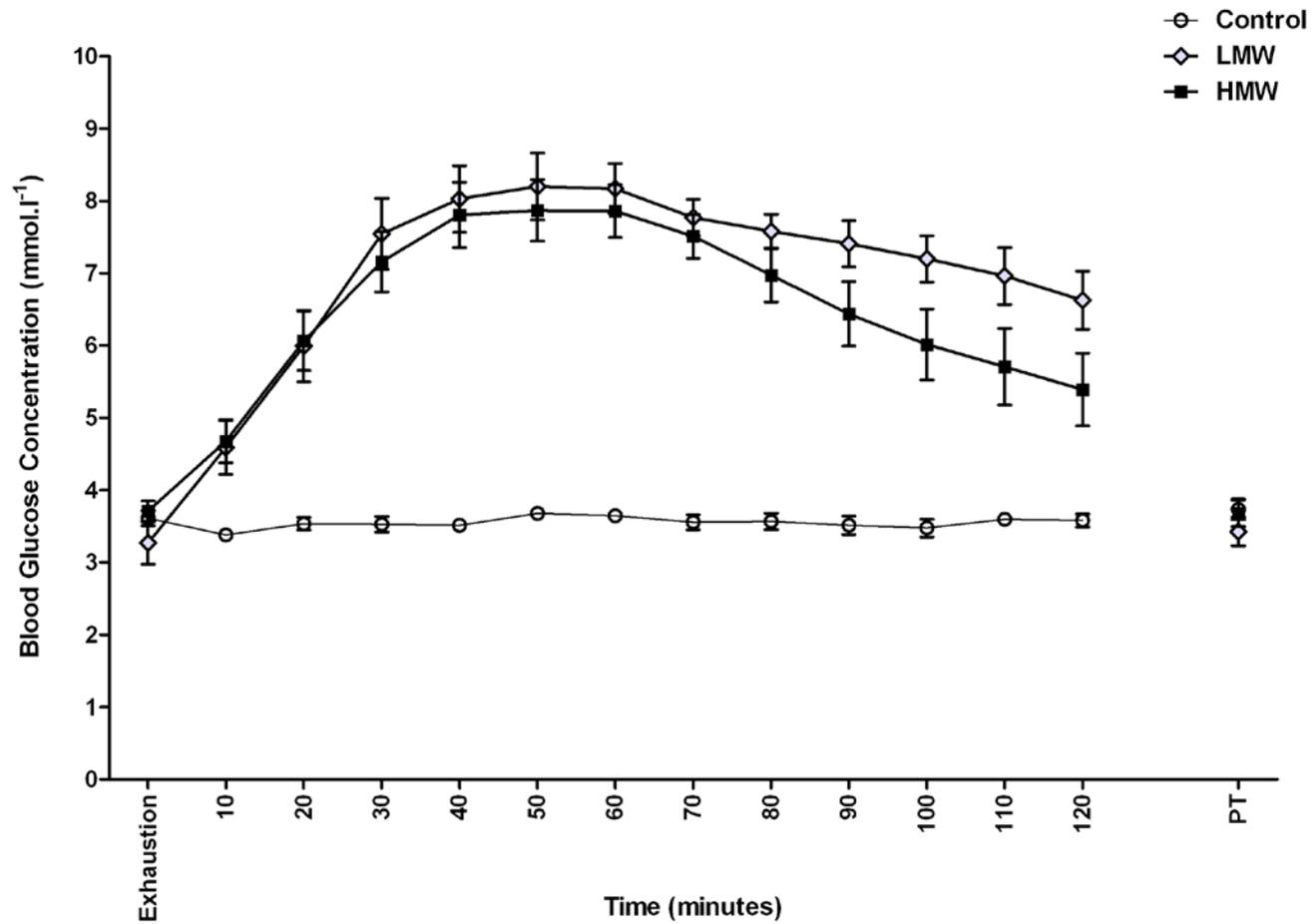
##### 3.4.2.1 Blood glucose concentration

Blood glucose concentration was measured immediately post-exercise and throughout the resting recovery period (Figure 3.2). At the cessation of the initial bout of exhaustive exercise, blood glucose concentration was similar between CON ( $3.6 \pm 0.1 \text{ mmol.l}^{-1}$ ), LMW ( $3.2 \pm 0.3 \text{ mmol.l}^{-1}$ ) and HMW ( $3.7 \pm 0.1 \text{ mmol.l}^{-1}$ ). During the 2 hr resting recovery period following consumption of CON, blood glucose concentration remained between 3.5 and 4  $\text{mmol.l}^{-1}$ . After consumption of both HMW and LMW, blood glucose concentrations were significantly greater compared to CON ( $P < 0.001$ ) from 20-120 min. Both polymer solutions elicited a peak in blood glucose concentration at 50 min of  $8.2 \pm 0.5$  and  $7.9 \pm 0.4 \text{ mmol.l}^{-1}$  for LMW and HMW respectively. Thereafter, the blood glucose concentration in both conditions declined during the second hour of recovery and returned to a level similar to baseline values following the

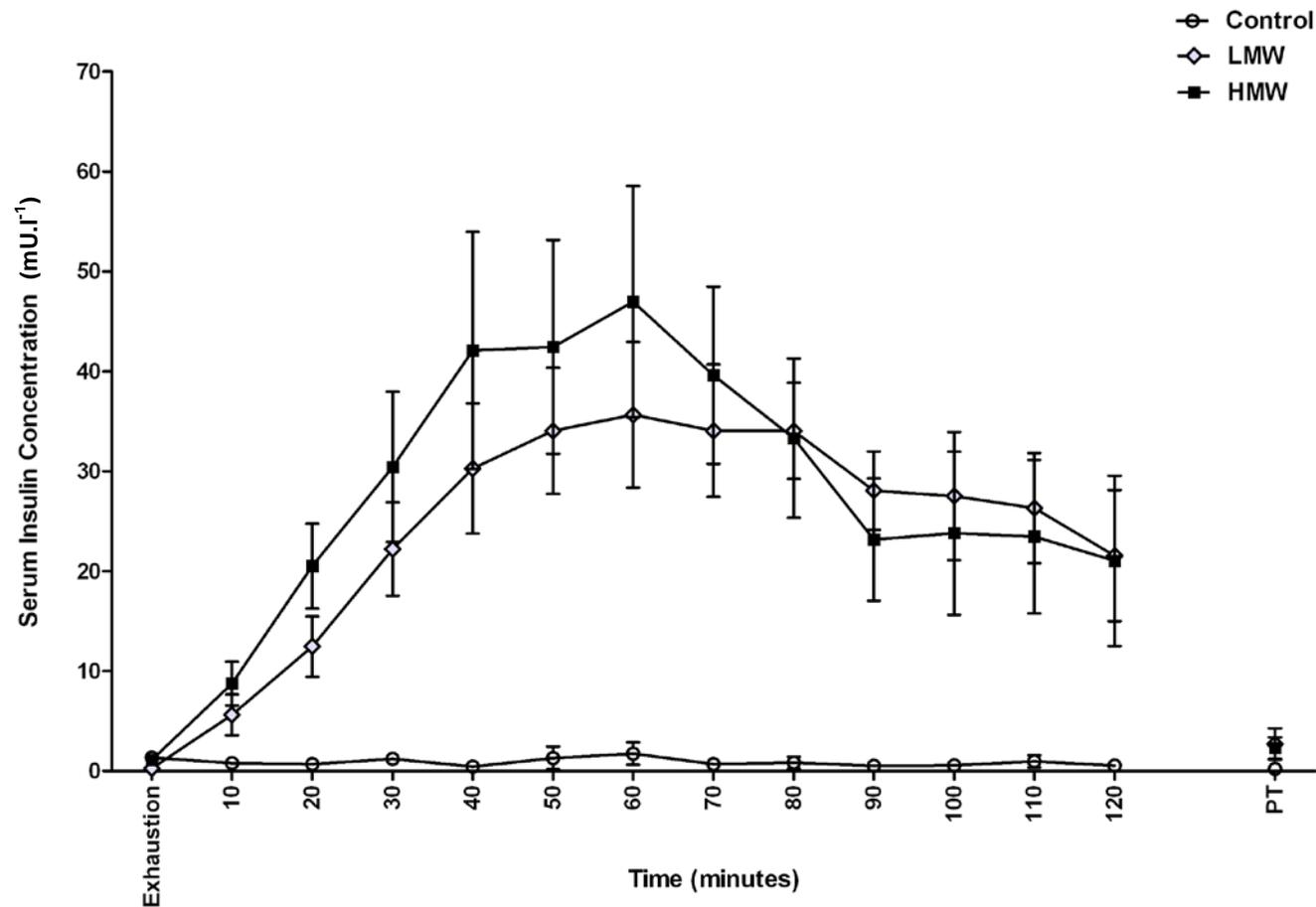
performance test. No significant differences were observed between the glucose polymer trials at any time points.

#### *3.4.2.2 Serum insulin concentration*

Serum insulin concentration is shown in Figure 3.3. At the termination of exhaustive exercise serum insulin was not different at  $1.4 \pm 0.5$  mU.l<sup>-1</sup> (CON),  $0.3 \pm 0.2$  mU.l<sup>-1</sup> (LMW) and  $1.1 \pm 0.4$  mU.l<sup>-1</sup> (HMW). During the 2 hr rest period serum insulin remained at a similar concentration in CON. After consumption of the LMW and HMW polymers, serum insulin increased to a peak of  $35.7 \pm 7.2$  mU.l<sup>-1</sup> and  $47 \pm 11.4$  mU.l<sup>-1</sup> respectively, both at 60 min post ingestion. Serum insulin was greater than control after 30 (HMW) and 40 (LMW) min into the rest period ( $P < 0.01$ ), but was not different between the glucose polymer trials at any time. The serum insulin concentration was at a similar level of  $2.7 \pm 1.5$  mU.l<sup>-1</sup> (LMW) and  $2.7 \pm 1.2$  mU.l<sup>-1</sup> (HMW) at the completion of the performance test.



**Figure 3.2** Blood glucose concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. Values are mean  $\pm$  sem, n=7. 80



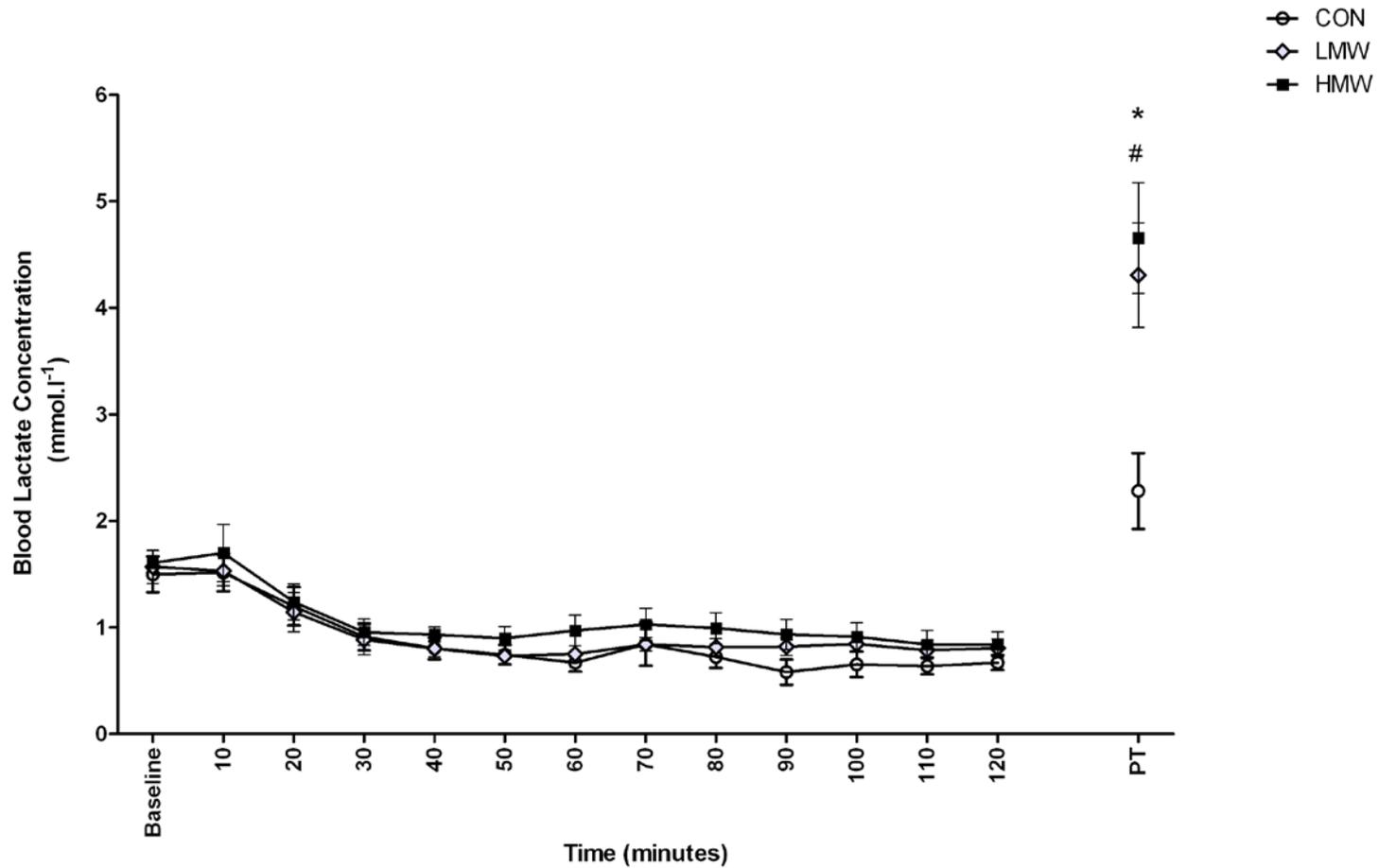
**Figure 3.3** Serum insulin concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. Values are mean  $\pm$  sem, n=7.

#### 3.4.2.3 Blood lactate concentration

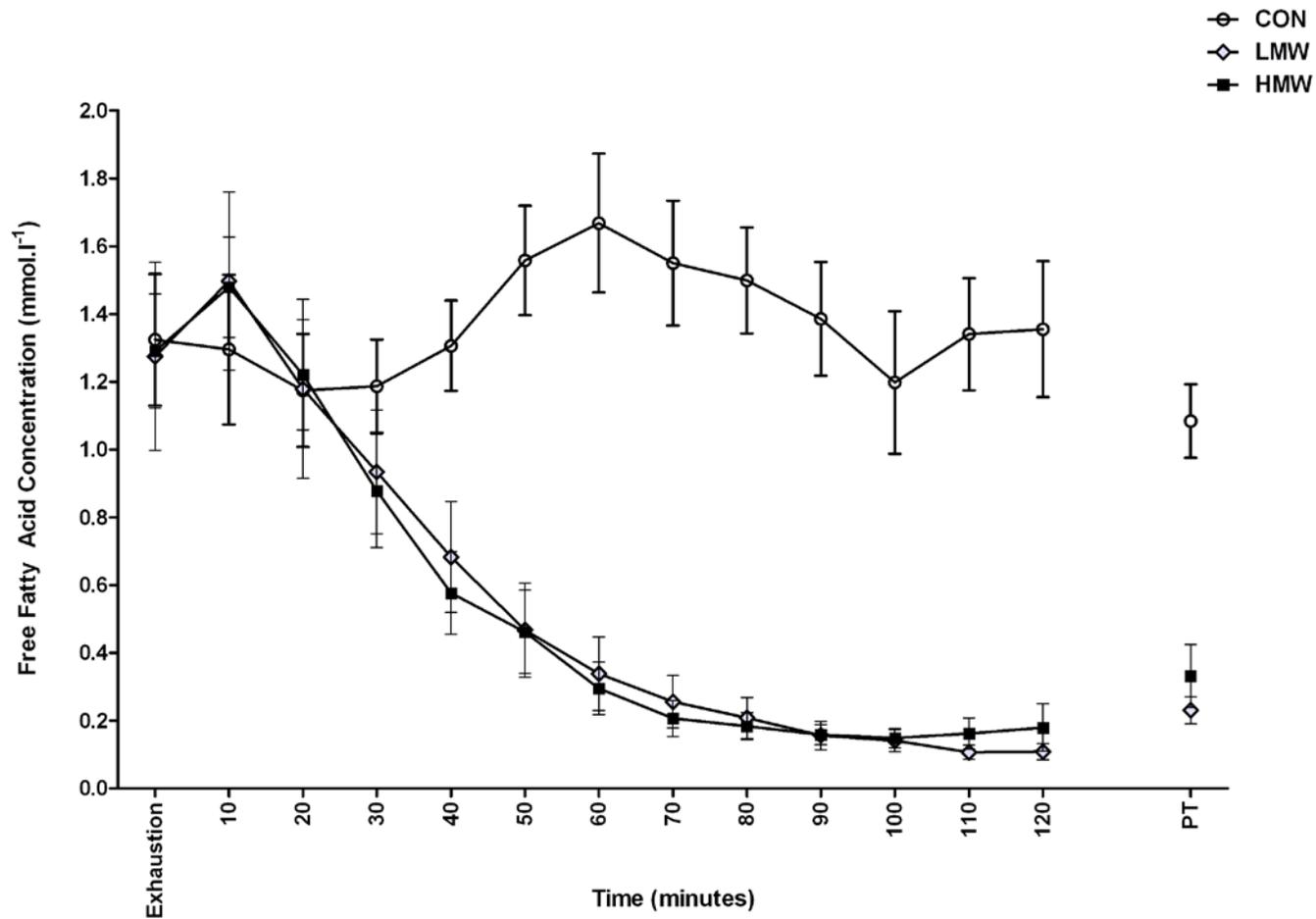
Exhaustive exercise resulted in a similar blood lactate concentration across all 3 conditions;  $1.5 \pm 0.2 \text{ mmol.l}^{-1}$  (CON),  $1.6 \pm 0.2 \text{ mmol.l}^{-1}$  (LMW) and  $1.6 \pm 0.1 \text{ mmol.l}^{-1}$  (HMW). Two hours of rest did not alter blood lactate concentration as it remained at or below  $1 \text{ mmol.l}^{-1}$  throughout this period in all conditions (Figure 3.4). The completion of the performance test increased blood lactate concentration in the glucose polymer trials to  $4.3 \pm 0.5 \text{ mmol.l}^{-1}$  (LMW) and  $4.7 \pm 0.5 \text{ mmol.l}^{-1}$  (HMW), which was greater than in the control condition  $2.3 \pm 0.4 \text{ mmol.l}^{-1}$  ( $P < 0.01$ ).

#### 3.4.2.4 Free fatty acid concentration (NEFA)

Plasma NEFA concentration was similar across all 3 trials after the exhaustive exercise period at  $1.1 \pm 0.2$  (CON),  $1.2 \pm 0.3$  (LMW) and  $1.4 \pm 0.2 \text{ mmol.l}^{-1}$ . Thereafter, FFA initially increased in both glucose polymer trials until 10 min post-ingestion where it then declined at a similar rate over the resting recovery such that no significant differences were detected at any time point (Figure 3.5). Consumption of CON elicited a NEFA concentration that increased in the first 60 min of resting recovery and was consistently greater from 40 min to the end of recovery than both LMW and HMW ( $P < 0.001$ ).



**Figure 3.4** Blood lactate concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. # P<0.01 HMW vs. CON \*P<0.01 LMW vs. CON. Values are mean  $\pm$  sem, n=7.

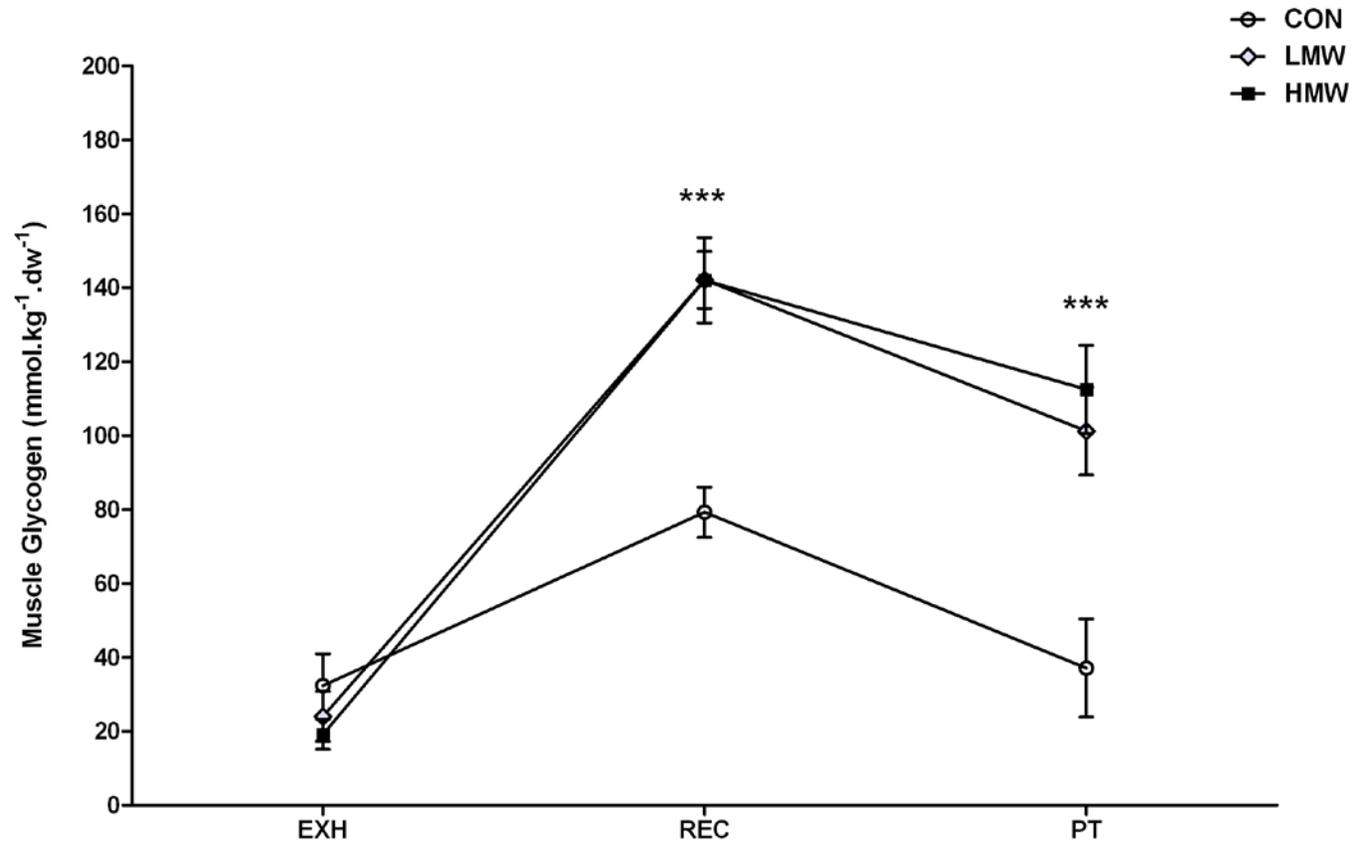


**Figure 3.5** NEFA concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. Values are mean  $\pm$  sem, n=7.

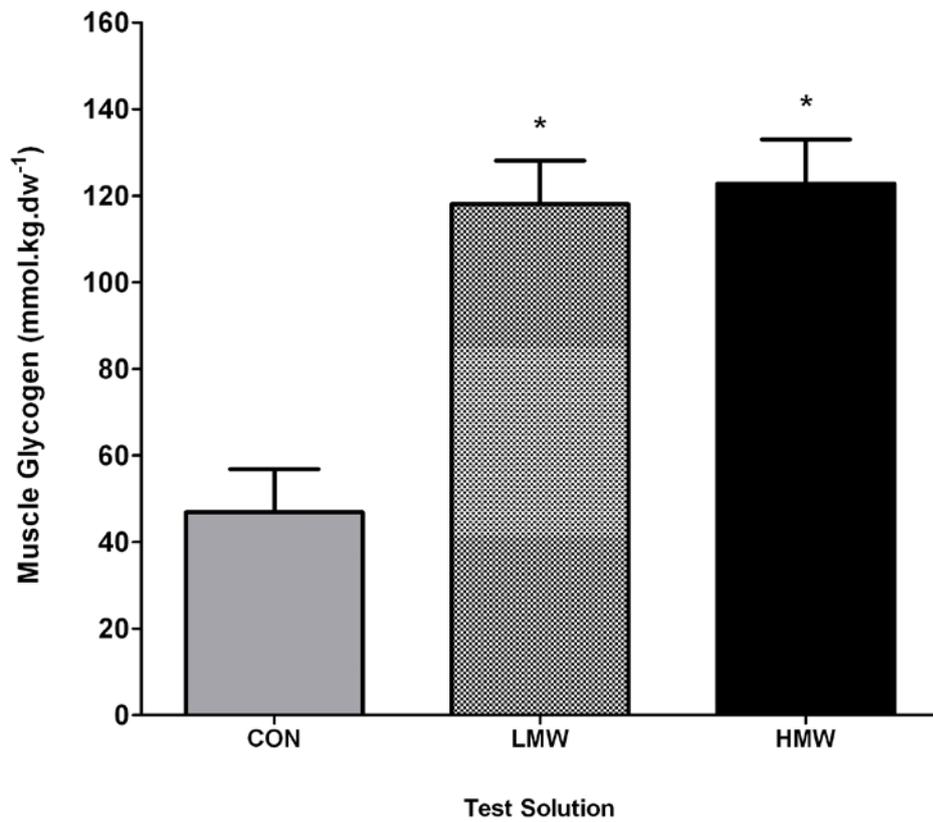
### 3.4.3 Muscle Glycogen

The exhaustive exercise protocol was successful at depleting muscle glycogen content (Figure 3.6). Muscle glycogen content was lowered at exhaustion to similar values in all trials at  $32 \pm 9 \text{ mmol.kg}^{-1}$  in CON,  $24 \pm 7 \text{ mmol.kg}^{-1}$  in LMW and  $19 \pm 4 \text{ mmol.kg}^{-1}$  in HMW condition. At the end of the 2 hr resting recovery period muscle glycogen in CON was  $79 \pm 7 \text{ mmol.kg}^{-1}$ . Supplementation with the glucose polymer solutions increased muscle glycogen content over the 2 hr recovery period to  $142 \pm 8$  and  $142 \pm 12 \text{ mmol.kg}^{-1}$  for the LMW and HMW conditions respectively (Figure 3.7). The magnitude of muscle glycogen resynthesis over the resting recovery was therefore almost identical between the carbohydrate polymers being  $118 \pm 10 \text{ mmol.kg}^{-1}$  (LMW) and  $123 \pm 10 \text{ mmol.kg}^{-1}$  (HMW) which was markedly greater than the control condition ( $P < 0.001$ ).

The work output test performed after the 2 hr rest period reduced muscle glycogen concentration in all conditions to  $41 \pm 15 \text{ mmol.kg}^{-1}$  (CON),  $101 \pm 12 \text{ mmol.kg}^{-1}$  (LMW) and  $113 \pm 12 \text{ mmol.kg}^{-1}$  (HMW). Although muscle glycogen content was higher in both polymer conditions after the performance test ( $P < 0.001$ ) when compared to the control, total muscle glycogen usage during the test was not different between the 3 trials ( $P > 0.05$ ).



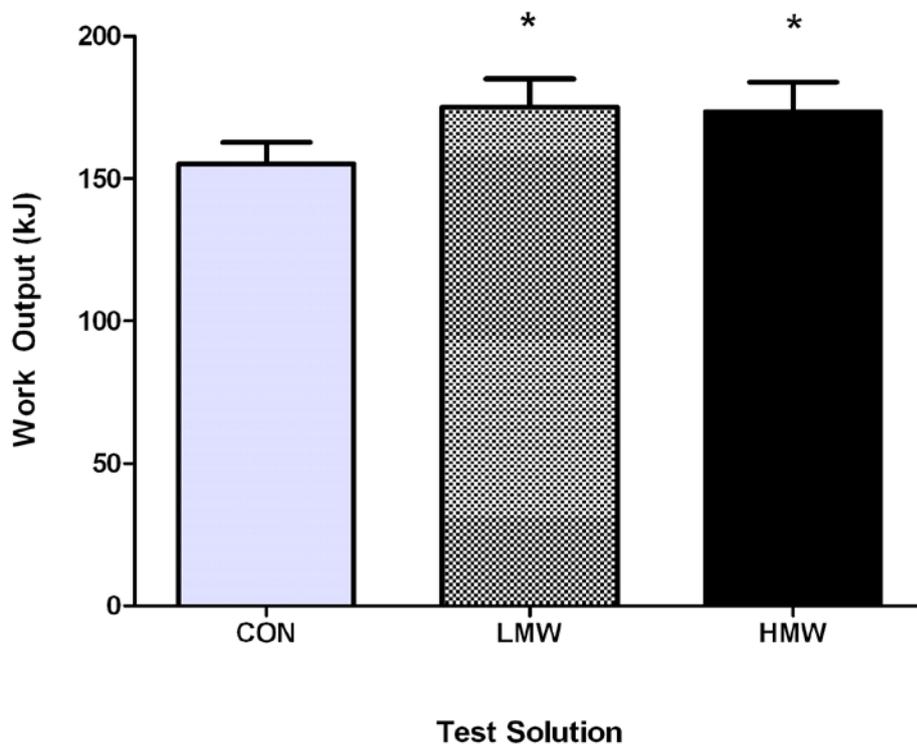
**Figure 3.6** Changes in muscle glycogen concentration from exhaustion, after supplementation with a 1 litre solution containing either 100g of low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control and a 2 hr rest period and after a 15 min cycling performance test. \*  $P < 0.001$  LMW and HMW vs. CON. Values are mean  $\pm$  sem,  $n=7$ .



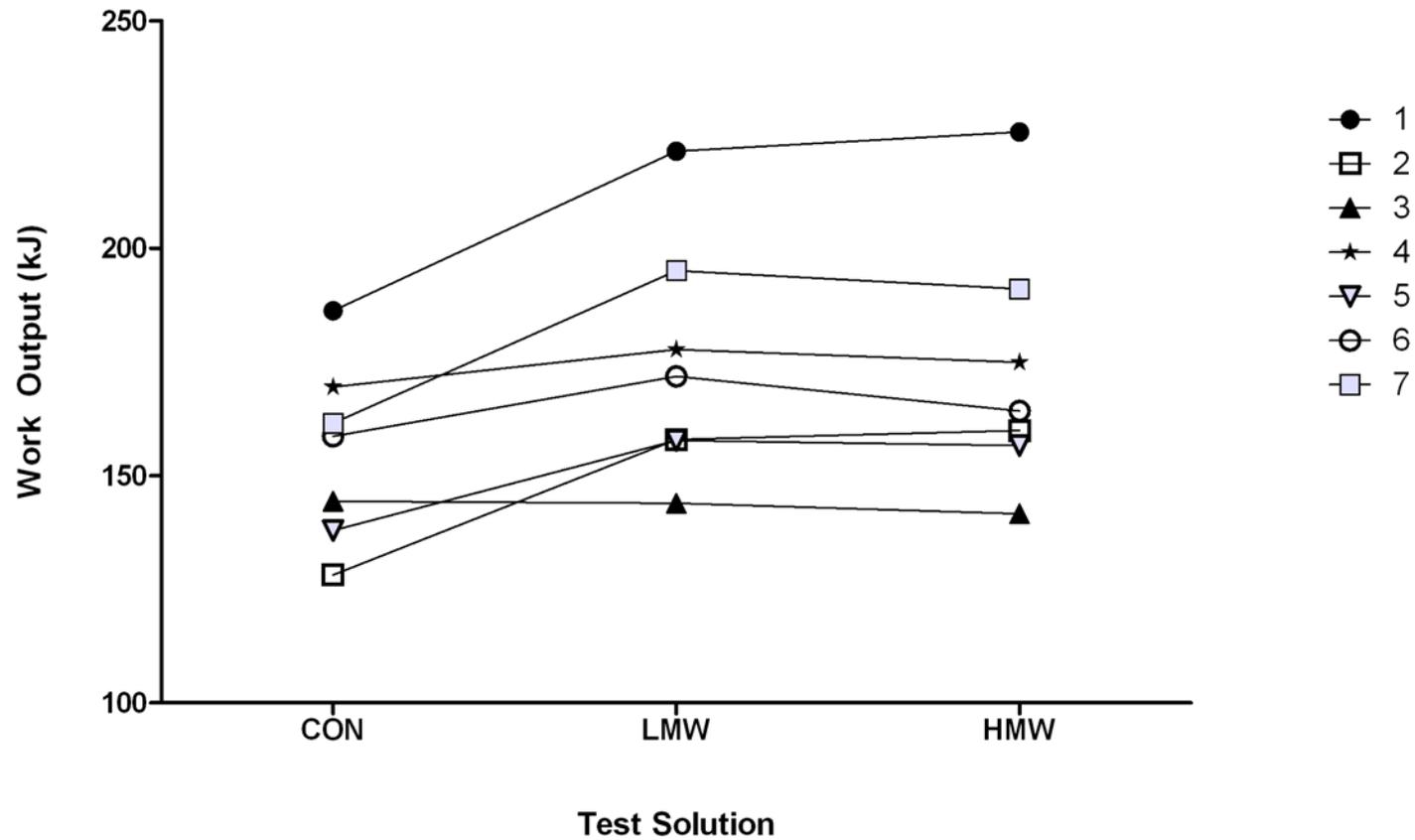
**Figure 3.7** Total muscle glycogen resynthesis during a 2 hr recovery following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of a low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. \*  $P < 0.01$  LMW and HMW vs. CON. Values are mean  $\pm$  sem,  $n=7$ .

### 3.4.4 Exercise performance

Work output during the 15 min performance test in CON ( $155 \pm 8$  kJ) was less than both HMW ( $173.4 \pm 11$  kJ,  $P < 0.01$ ) and LMW ( $175 \pm 10$  kJ,  $P < 0.01$ ). Work output was no different between the LMW and HMW trials (Figure 3.8). Individual work output for all participants is shown in Figure 3.9. Heart rate measured on the tenth minute of exercise was similar between trials at  $172 \pm 4$  bpm (CON),  $172 \pm 4$  bpm (LMW) and  $176 \pm 4$  bpm (HMW).



**Figure 3.8** Total work output produced during a 15 min cycling performance test performed 2 hr after an initial exhaustive exercise bout and supplementation with a 1 litre solution containing either 100g of a low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control before 2 hr rest. \*  $P < 0.01$  LMW and HMW vs. CON. Values are mean  $\pm$  sem,  $n=7$ .



**Figure 3.9** Individual exercise performance during a 15 min cycling performance test following an initial exhaustive exercise bout and supplementation with a 1 litre solution containing either 100g of a low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control before 2 hr resting recovery.

### 3.5 Discussion

The main finding from this study was that ingestion of high and low molecular weight glucose polymers resulted in an almost identical magnitude of muscle glycogen resynthesis over 2 hr recovery following glycogen-depleting exercise. In contrast to expectations, there were no observable differences in muscle glycogen content, blood glucose concentration, or serum insulin concentration after ingestion of both glucose polymers, however all were markedly different compared to the carbohydrate-free control solution as would be expected. Post-exercise muscle glycogen resynthesis appears to have occurred at a similar rate ( $\sim 60 \text{ mmol.kg}^{-1}.\text{h}^{-1}$ ) in both HMW and LMW and as a result similar muscle glycogen content was present at the onset of the second exercise bout ( $142 \text{ mmol.kg}^{-1}$ ) in both trials. It therefore follows that cumulative work output achieved in this test was near-identical in HMW and LMW (173 vs. 175 kJ).

Previous work using the HMW glucose polymer showed more rapid glycogen resynthesis when compared to a solution containing simple sugars (Piehl-Aulin et al., 2000), furthermore another study showed an improvement in work output during a subsequent bout using the same solutions utilised in the present study (Stephens et al., 2008). It was hypothesised that the performance effect was due to an increased availability of muscle glycogen at the onset of the second exercise test due to a greater rate of rise in blood glucose and insulin during recovery that was observed after ingestion of HMW compared to LMW potentially enhancing muscle glycogen resynthesis.

In contrast to expectations however, the present study demonstrated no difference in muscle glycogen resynthesis during recovery from exhaustive exercise when comparing ingestion of the glucose polymers that differed

markedly in molecular weight. Notably, the study by Stephens et al (2008) showed a greater rate of rise in blood glucose and insulin in the first 40 min of a 2 hr recovery from glycogen-depleting exercise after ingestion of the HMW compared to the LMW glucose polymer. This effect was not observed in the present study as differences in either blood glucose or serum insulin concentration were not present at any point. As the experimental protocols and volunteers were similar in both studies, a potential disparity may lie with the test solutions used in the studies which were different versions derived from different native starches. This may have accounted for the difference in blood glucose, serum insulin concentration and exercise performance responses. The HMW glucose polymer has been developed since previous investigations to a more soluble formula based on amylopectin barley starch to improve palatability. This was deemed to be an improvement by the manufacturer due to a marked improvement in solubility being achieved without any change in the characteristics of the carbohydrate. As the study by Stephens and colleagues and the present study used different versions of the HMW glucose polymer and have observed different rates of rise in blood glucose and serum insulin concentration following ingestion, it is feasible that the physical properties of the product may have been altered and this warrants further investigation.

Although a 70% greater rate of muscle glycogen resynthesis was demonstrated after 2 hr recovery from exhaustive exercise with the HMW glucose polymer in an earlier study (Piehl-Aulin et al., 2000), this was in comparison to a solution containing a monomer and oligomer of glucose with a high osmolality that is atypical of common post-exercise recovery beverages. In addition, 75g was fed

every 30 min during the recovery whereas a single bolus was provided in the present study thus rates of resynthesis cannot be directly comparable. The HMW glucose polymer used in the study by Piehl-Aulin et al (2000) was an earlier version of the current product that was derived from potato starch and not the same version used in the study by Stephens et al (2008). Therefore, a main aim of the current study was to document muscle glycogen resynthesis when isoenergetic glucose polymers with low and high molecular weights were ingested after exhaustive exercise. In contrast with Piehl-Aulin and colleagues, muscle glycogen resynthesis was not enhanced with the HMW glucose polymer. Exhaustive exercise caused considerable muscle glycogen depletion in both the present study (39, 24 and 19 mmol.kg<sup>-1</sup>) and the study by Piehl-Aulin (53 and 58 mmol.kg<sup>-1</sup>), therefore a reasonably high rate of muscle glycogen resynthesis would be expected (Zachwieja et al., 1991). A rate of 50.2 mmol.kg<sup>-1</sup>.h<sup>-1</sup> with the HMW glucose polymer was seen in the study by Piehl-Aulin et al (2000) and rates of 59 and 61 mmol.kg<sup>-1</sup>.h<sup>-1</sup> were seen for the LMW and HMW glucose polymer respectively in the present study, although a greater amount of carbohydrate at consistent intervals was provided in the former (300g) compared to a smaller single feeding in the present study (100g). From the literature the rates of muscle glycogen resynthesis reported in the present study can be considered high, since other studies following similar protocols, although with repeated carbohydrate feeding during recovery, have reported similar, albeit slightly lower resynthesis rates (Vollestad et al., 1989; Casey et al., 1995; Battram et al., 2004; Shearer et al., 2005; Pedersen et al., 2008). Current opinion indicates that increasing the amount of carbohydrate fed to >1.2 g.kg<sup>-1</sup> bw does not further augment muscle glycogen resynthesis

above feeding  $\sim 1\text{g}\cdot\text{kg}^{-1}\text{ bw}$  (Jentjens and Jeukendrup, 2003; Betts and Williams 2010). Accordingly it is important to consider that  $\sim 1\text{g}\cdot\text{kg}^{-1}\text{ bw}$  of carbohydrate was provided in the aforementioned studies, whereas absolute amounts were provided in the studies pertaining to the HMW glucose polymer, the current study being equivalent to  $1.4\text{ g}\cdot\text{kg}^{-1}\text{ bw}$  (range 1.1-1.8), suggesting that a sufficient amount of carbohydrate for optimal muscle glycogen resynthesis was provided. However, all participants in the study by Stephens and colleagues improved exercise performance after ingestion of the high compared to the low molecular weight glucose polymer with a similar relative carbohydrate intake (mean of  $1.2\text{ g}\cdot\text{kg}^{-1}\text{bw}$ ) suggesting muscle glycogen in this study was markedly different at the onset of the second bout of exercise.

It may be pertinent to consider that the studies by Piehl-Aulin and colleagues did not find any differences in blood glucose or serum insulin following ingestion of the HMW glucose polymer and the LMW glucose solution. Considering the difference in muscle glycogen content observed after 2 hr between the high and low molecular weight glucose solution which suggests greater substrate availability in the former, blood glucose concentration would be expected to be higher in this condition. However, as previously noted by Stephens et al. (2008), the use of venous blood sampling at relatively distant time points may have concealed any distinction in blood glucose and serum insulin response. Furthermore, in the study by Leiper and colleagues, again no differences were seen in blood glucose or insulin between the high or low molecular weight solutions, however, this study was done at rest rather than post-exercise and the lack of differences in blood glucose concentration between trials was attributed to large inter-individual differences. When no

carbohydrate is ingested post-exercise, NEFA concentration is maintained  $>1$  mmol.l<sup>-1</sup> (Krssak et al., 2000), as evidenced by the results from the present study when flavoured water was ingested after the glycogen-depleting exercise, reflecting an increased reliance on fat metabolism. With carbohydrate ingestion, NEFA concentration declines in response to increases in circulating insulin (Kimber et al., 2003). In response to glucose polymer ingestion a parallel decline in free fatty acid concentration was observed in both polymer trials over the 2 hr that was consistent with a similar pattern of increase in circulating glucose and insulin. The randomisation of the experimental trials should negate any trial order effects, however blood glucose and insulin concentrations after exhaustive exercise tended to be lower in LMW than HMW and CON. This can be attributed to three individuals measuring lower concentrations in both after the exercise in the LMW trial, however this was not found to be different compared to HMW and CON. The reason for the lower concentrations is not clear but may be due to dietary effects and non-adherence to the prescribed diet.

The contribution of liver glycogen to exercise performance in the second bout should not be discounted. The liver is extremely sensitive to dietary intake as evidenced by a starvation study that reported depletion of liver glycogen from 270 to 30 mmol.kg<sup>-1</sup> (Nilsson and Hultman 1973) and exercise studies that have reported increases in hepatic glycogen with carbohydrate intake (Jue et al., 1989; Casey et al., 2000). Although the present study only measured skeletal muscle glycogen, blood glucose uptake for hepatic glycogenesis will have accounted for a portion of the ingested glucose polymer. To this end, a pilot study was conducted to ascertain if <sup>13</sup>C NMR spectroscopy could be used

to monitor post exercise changes in liver glycogen concentration (Appendix 3). This pilot study reported that despite identical experimental protocols undertaken on 2 visits separated by 1 week, measurement of liver glycogen repletion with  $^{13}\text{C}$  MRS after ingestion of 100g of carbohydrate was not reproducible and thus the low sensitivity of this technique may not be suitable to accurately measure post exercise changes in liver glycogen content.

The 'all out' performance time trial is a reproducible measure of endurance performance that was shown to present a CV of 1.6% in recreationally active individuals such as the cohort in this chapter (Jeukendrup et al., 1996). The test consists of 15 min of cycling performed at the highest work intensity possible and considering that other studies have shown glycogenolysis in the order of 150-250 mmol.kg<sup>-1</sup> in tests of the same duration at 80-85%  $\dot{V}O_2$  max (Chelsey et al., 1998; Dyck et al., 1993), the muscle glycogen degradation observed during the test in the present study seems low. It may be due to the self-paced test that the volunteers were not exercising at their respective maximum ability, however the heart rates measured during the performance test reflect a high exercise intensity. Additionally blood lactate concentrations at the end of the test were significantly higher in both glucose polymer trials compared to the control reflecting greater reliance on carbohydrate metabolism. Nonetheless it is surprising that muscle glycogen degradation was not different across all trials. Debate still exists as to whether glycogenolysis in exercising muscle is regulated by glycogen content with studies either supporting an association (Hespeel and Richter 1992; Hargreaves et al., 1997; Vandenberghe et al., 1999) or showing no effect of glycogen on rates of glycogenolysis (Ren et al., 1990; Spriet et al., 1990; Spencer and Katz 1991; Vandenberghe et al., 1995). It is

worth considering that in contrast to the other studies reporting glycogen use during 15 min high intensity cycling, the performance test in the present study was undertaken in a glycogen-reduced state ( $\sim 140 \text{ mmol.kg}^{-1}$ ) and low glycogen has been associated with a reduced rate of glycogenolysis during exercise (Hargreaves et al., 1995; Shearer et al., 2001). As the role of glycogen content in mediating the rate of glycogenolysis during exercise is still in contention, the results from the current study indicate that performance in the subsequent exercise bout may also be affected by factors other than muscle glycogen content. It has been shown that glycogenolysis in the muscle is attenuated in the glycogen-reduced state but is associated with increased muscle glucose uptake (Howarth et al., 2010). Therefore a contribution from hepatic glucose output derived from resynthesised hepatic glycogen and the higher blood glucose in the glucose polymer trials could potentially account for the exercise improvement compared to the control trial despite similar muscle glycogen utilisation.

Understandably, in the current study there was no difference in exercise performance after ingestion of the high and low molecular weight glucose polymers in line with similar blood glucose and serum insulin responses and more importantly, with almost identical muscle glycogen resynthesis during recovery. Although the absolute work outputs obtained in the performance tests were slightly higher in the current investigation than the study by Stephens et al (2008), the latter described a 20% improvement in performance with the HMW glucose polymer compared to the control condition whereas the improvement seen in the present study with both glucose polymers to the control was only 11%. This difference is more in line with the improvement

observed in Stephen's study following ingestion of the LMW glucose polymer compared to the control (9%). Considered with the discrepancy in blood glucose and serum insulin between these similar investigations, it appears that favourable properties of the HMW glucose polymer have been diminished during modification of the product to a more soluble powder.

Alterations to the production of the HMW glucose polymer to improve solubility and therefore the utility and popularity of the product as a practical nutritional supplement have occurred gradually such that the investigations from Piehl-Aulin et al (2000), Leiper et al (2000), Stephens et al (2008) and the present study have utilised different versions. The progression of the HMW glucose polymer from a gel-like substance derived from potato starch, to a more soluble solution derived from barley starch was achieved by modifying the manufacturing process from granulation to agglomeration of the acid-hydrolysed starch. The product has deviated from the original version with gel-forming properties that was reported to confer advantageous effects on gastric emptying and muscle glycogen resynthesis (Piehl-Aulin et al., 2000; Leiper et al., 2000). Indeed, Leiper and colleagues noted it was unusual that a solution with the propensity to form a gel did not retard gastric emptying but enhanced it. There is minimal research into gastric emptying of gels versus liquid carbohydrate supplements, however, recently it has been suggested that exogenous carbohydrate oxidation during exercise is maintained at a high rate of  $1.4 \text{ g}\cdot\text{min}^{-1}$  when 108 g per hour was administered as either a drink or a gel (Pfeiffer et al., 2010). Future investigation is warranted into restoring the HMW glucose polymer to its original formulation to further ascertain any

effects on post exercise gastric emptying, muscle glycogen resynthesis and recovery of exercise performance.

In conclusion, ingestion of high or low molecular weight glucose polymers with corresponding low and high osmolalities elicited similar rates of muscle glycogen resynthesis during a short recovery from glycogen-depleting exercise. The results from this study contradict a previous investigation that showed an enhanced work output and a greater rate of rise in blood glucose and insulin concentration with ingestion of the HMW glucose polymer in comparison to the LMW glucose polymer. The present study did not report this pattern suggesting that modifications to the HMW glucose polymer may have altered the characteristics of the product. Further investigation of the physical properties of the polymer in regards to viscosity and gel-forming tendencies is warranted. Additionally, most studies investigating post-exercise muscle glycogen resynthesis have measured at relatively large intervals (~2 hr) and with few measurements, thus future investigation into earlier and more frequent determination of muscle glycogen may be beneficial.

## **Chapter 4**

Temporal changes in post-exercise skeletal muscle glycogen resynthesis following ingestion of glucose polymers with different molecular weights as measured by ultra high-field nuclear magnetic resonance spectroscopy

## 4.1 Introduction

Muscle glycogen content is commonly determined using the muscle biopsy technique however recent developments in the technology used in  $^{13}\text{C}$  magnetic resonance spectroscopy have allowed it to be used for the same purpose. The potential for assessment of skeletal muscle metabolism using  $^{13}\text{C}$  spectroscopy is aided by the ability for continuous monitoring without relying on invasive procedures (Price et al., 1999; Roden 2001). However the widespread use of the spectroscopy methodology in the area of exercise physiology is limited by the high economic cost and availability of the equipment, technological development and suitable expertise (Roden and Shulman 1999).

The suitability of the method for post-exercise physiology is reliant on the sensitivity of muscle glycogen detection, which inherently possesses low signal to noise ratio (SNR) due to the low natural abundance of the  $^{13}\text{C}$  nucleus giving longer acquisition times (Boesch, 2007). Increased field strength provides increased signal (SNR is proportional to field strength) thus due to recent developments that have allowed MRS of humans at 7.0 T, the increased SNR available allows acquisition times to be shortened. This could potentially increase the number of more accurate measurements that can be made in the same time (Stephenson et al., 2011). As previous investigations using  $^{13}\text{C}$  MRS have been restricted to few measurement points due to longer acquisition times, in this way it is possible that more effective real-time monitoring of post-exercise muscle glycogen resynthesis can be achieved with 7.0 T field strength. Briefly,  $^{13}\text{C}$  spectroscopy involves positioning the volunteer in a strong magnetic field with a radio frequency coil laid over the specific tissue of interest. Initially a scout image is obtained of the tissue to verify positioning of

the coil. An oscillating magnetic field (pulse sequence) is emitted from the coil which then generates the resonance signals from  $^{13}\text{C}$ -containing compounds i.e. glycogen, within the tissue which are then picked up by the radiofrequency coil. The signals from the spectral resonance of the different compounds are converted by Fourier analysis to a frequency-intensity display (spectra) and the integral area of a specific peak is calculated in relation to an external standard of known concentration. It has been reported that all glycogen molecules *in vivo* are visible (Sillerud and Shulman 1983; Gruetter et al., 1991) thus their  $^{13}\text{C}$  intensities have been used to determine glycogen concentrations (Taylor et al., 1992; Gruetter et al., 1994), and to monitor glycogen repletion in skeletal muscle or hepatic tissue (Jue et al., 1989). The sensitivity of the method is compromised by the inherent low natural abundance of the  $^{13}\text{C}$  nucleus thus the development of higher strength magnets that improve the peak definition have improved the potential for measurements of  $^{13}\text{C}$  containing compounds such as glycogen. The visibility of glycogen can be enhanced by using 1- $^{13}\text{C}$ -enriched glucose to increase the  $^{13}\text{C}$  signal, a method that has been used to monitor the incorporation of newly formed glycogen molecules from infused glucose under glucose clamp conditions (Shulman et al., 1990). Unfortunately this method is expensive and not readily available. In addition, it is impossible to differentiate between glucose from infusion and glucose from other sources, which makes quantification difficult.

Application of  $^{13}\text{C}$  NMR to exercise studies was initially shown in a small study of glycogen levels in the gastrocnemius muscle of two runners before and after a 13 mile run that showed a 70% depletion of muscle glycogen after the race and a restoration to 80% after 19 hours recovery (Avison et al., 1988).

This was deemed consistent with a study using muscle biopsies following running at 80%  $\dot{V}O_2$  max for 16.1 km that caused muscle glycogen depletion to 62% which had then recovered to 76% after 24 hr (Costill et al., 1971). However the experimental protocols were different and thus the methods of  $^{13}\text{C}$  MRS and muscle biopsies could not be directly compared. Early natural abundance glycogen studies lacked the sensitivity to monitor post exercise fluctuations in glycogen; however changes in muscle glycogen as a result of exercise and recovery have been documented more successfully since then (Jue et al., 1989). A notable contribution to the understanding of the local control of muscle glycogen resynthesis was achieved with an exercise protocol of calf-raises and  $^{13}\text{C}$  NMR of the gastrocnemius muscle. Reduction of muscle glycogen to 25% of its resting value was associated with a higher initial glycogen resynthesis rate compared to less pronounced reduction (50 and 75% of resting levels) suggesting the extent of depletion exerts some control over the rate of resynthesis. Furthermore, this study indicated a biphasic pattern of glycogen resynthesis by demonstrating that suppressing insulin release after reduction to 25% of basal levels ( $<35 \text{ mmol.l}^{-1}$ ) did not affect the rate of glycogen resynthesis until a threshold of  $>35 \text{ mmol.l}^{-1}$  was reached, at which point resynthesis was impaired (Price et al., 1994).

Subsequently, experimental protocols involving glycogen-depleting exercise and subsequent patterns of glycogen restoration during recovery have been undertaken with assistance from  $^{13}\text{C}$  NMR spectroscopy (Casey et al., 2000; Krssak et al., 2000; Rothman et al., 2000; Zehnder et al., 2001). Until recently when higher field strengths became available, the acquisition times with 1.5-3.0 T magnets were longer, thus in the aforementioned studies only few

measurements were made (minimum of 1 hr intervals up to 6 hr intervals) thus muscle glycogen has only been determined at few time points. In addition the between subject variability associated with the method at lower field strengths is high. For example, muscle biopsy studies have found variation in post exhaustive exercise muscle glycogen content of  $\sim 10 \text{ mmol.kg}^{-1}$  (Casey et al., 1995; van Hall et al., 2000; Kimber et al., 2003) compared to natural abundance MRS that has showed larger variation ( $>30 \text{ mmol.l}^{-1}$ ) following a similar exercise and carbohydrate feeding protocol (Casey et al., 2000). Thus the increased signal available with 7.0 T MRS may also reduce the variability of the method lending the technique to be more suitable for post exercise metabolism.

Very few studies have attempted to validate and record reproducibility of  $^{13}\text{C}$  MRS of glycogen. Thus far an *in vivo* direct comparison of MRS with muscle biopsies has been performed by Taylor et al (1992) which reported a close correlation between 6  $^{13}\text{C}$  MRS measurements and 3 biopsies of the gastrocnemius ( $R=0.95$ ,  $p<0.0001$ ). In addition the mean concentrations were near-equal (MRS=  $87.4 \text{ mmol.l}^{-1}$  vs. biopsy=  $88.3 \text{ mmol.l}^{-1}$ ) with multiple measurements allowing an estimation of the reproducibility (MRS= CV of  $4.3 \pm 2.1\%$  vs. biopsy=  $9.3 \pm 5.9\%$ ). Similarly, a good agreement ( $R=0.95 \pm 0.05$ ) was found between the glycogen peak with *in vivo* exposed rabbit liver between MRS and biochemical analysis (Gruetter et al., 1994). The between subject variation in MRS studies completed with field strengths up to 3.0 T has been reported to be large (Van Den Bergh et al., 1996; Casey et al., 2000) thus it is worth considering that as little work has been done since to measure

reproducibility and variability of the MRS method, inter-subject variability in glycogen levels may be high at lower field strengths.

Given that MRS can determine natural abundance muscle glycogen and with the advent of improved technology and thus the sensitivity of the measurement, it has become possible to monitor changes in content with a much lower time period between measurements. The study described in Chapter 3 reported muscle glycogen content as a result of post-exercise feeding of HMW and LMW glucose polymers but only after a recovery period of 2 hr. Considering the magnitude of depletion reported in that study, the insulin-independent phase of muscle glycogen resynthesis would be likely to proceed (Price et al., 1994) thus a single measurement point in recovery may have missed subtle earlier changes in muscle glycogen. The advantage of MRS lies in the ability to sequentially quantify muscle glycogen with smaller time resolution between measurement points (Ivy et al., 2002). The higher strength magnets now available have improved the resolution of the spectra obtained and reduced measurement times therefore it was suggested that post-exercise glycogen content following feeding of the HMW and LMW glucose polymers could be monitored with ultra high-field MRS.

## 4.2 Aims and hypotheses

This chapter aims to ascertain the suitability of ultra-high field  $^{13}\text{C}$  NMR spectroscopy in post-exercise recovery of muscle glycogen and the application of the method to sequentially monitor fluctuating muscle glycogen levels in the *vastus lateralis* with carbohydrate feeding. Additionally, in Chapter 3, muscle glycogen content was measured using muscle biopsies, however a time interval of 2 hrs was present between measurements thus early differences between treatments may have been missed. An advantage of magnetic resonance spectroscopy, particularly with high strength magnets, is that multiple measures can be made without discomfort to the volunteer. To determine temporal changes in muscle glycogen content after ingestion of the HMW and LMW glucose polymers used in Chapter 3, a  $^{13}\text{C}$  MRS study was performed using a 7.0 T magnet. Two studies are described here in sections 4.3 and 4.4 to address these aims.

It is hypothesised that feeding of the HMW glucose polymer will elicit temporal changes in muscle glycogen content of a higher magnitude compared to the LMW glucose polymer such that muscle glycogen resynthesis proceeds at a faster rate in the early post exercise period.

### **4.3 Skeletal muscle glycogen measured using ultra high-field spectroscopy**

#### *4.3.1 Introduction*

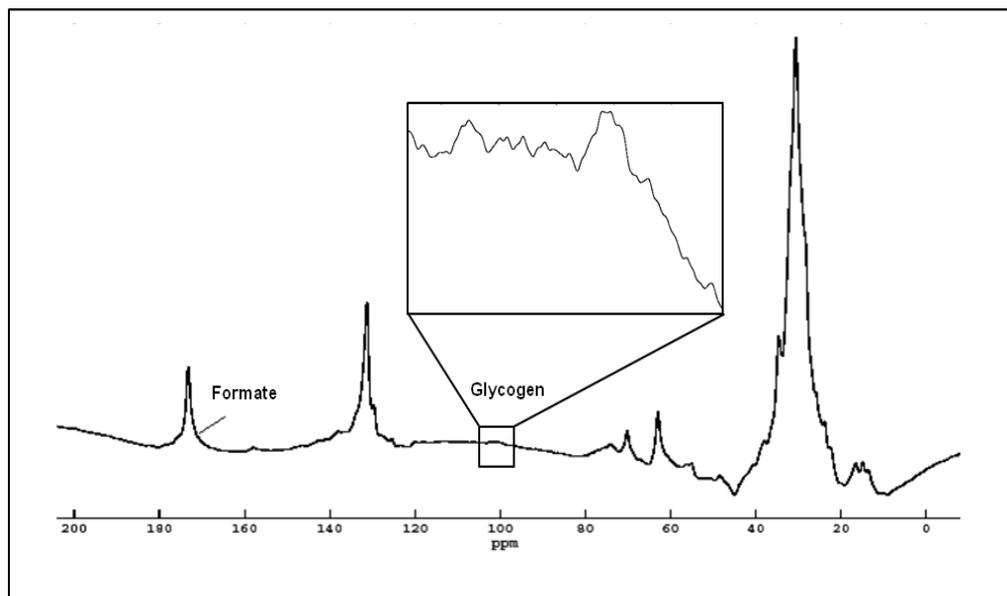
Recent developments in MRS technology have allowed the development of high-field spectrometers such that human experiments can now be carried out in a magnet of 7.0 tesla strength. The technique has been employed for studies investigating recovery processes from both prolonged and intermittent exercise that had previously been limited by hardware capability. Acquisition of spectra for glycogen using  $^{13}\text{C}$  spectroscopy is limited by the low natural abundance of the nucleus leading to long measurement times. Additionally lower field strength MRS is associated with large between-subject variation. Increasing the magnetic field strength leads to proportional increases in SNR, this is seen in example spectra obtained from both 3.0 and 7.0 T (Figures 4.1 and 4.2) that demonstrates improved spectral resolution with ultra-high field spectroscopy thus enhancing the accuracy of the measurement.

The increased signal available with the higher field strength of 7.0 T also allows for shorter acquisition times giving a greater sensitivity and allowing a smaller time resolution between measurements. It has been demonstrated that the signal to noise ratio (SNR) for the C1 peak of glycogen at 100.4 ppm is increased by 60% at 7.0 compared to 3.0 T (Stephenson et al., 2011). This equates to a time period 2.5 times longer for 3.0 T than 7.0 T to obtain the same SNR at both field strengths. A shorter time resolution for spectra acquisition would potentially allow for multiple measurements in a short period thus the technique may be suitable for early post exercise changes in metabolites. The improved accuracy as a result of higher magnet strength is

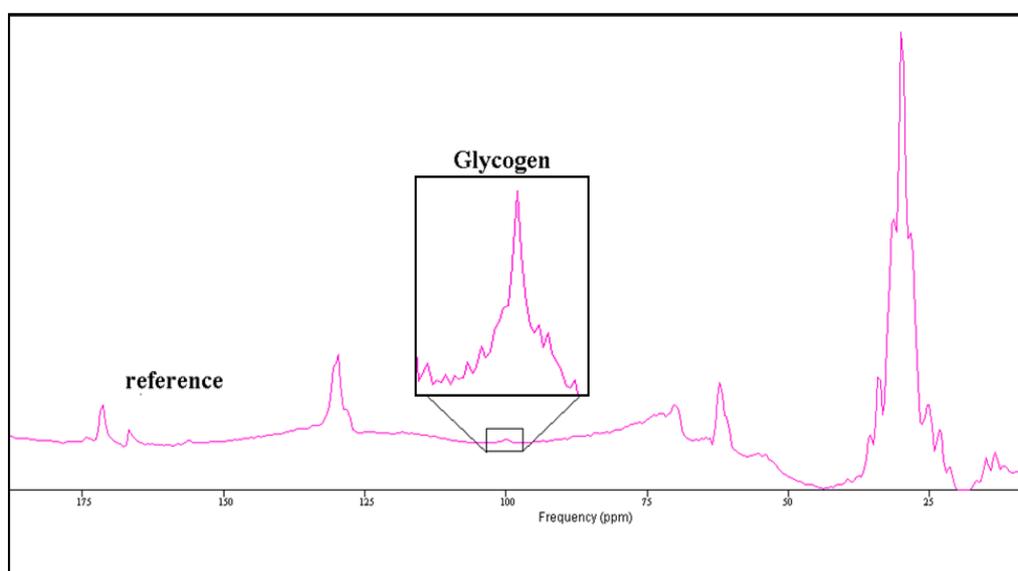
likely to reduce the variation present in the method. As previous spectroscopy studies investigating post exercise glycogen resynthesis have been undertaken using field strengths up to a 3.0 T which have been subjected to large variation, a pilot study was initially conducted to assess the suitability of high-field MRS (7.0 T) in monitoring post exercise muscle glycogen changes.

#### *4.3.2 Aims and hypotheses*

To investigate the efficacy of the method, this pilot study was performed to detect different magnitudes of muscle glycogen depletion following exercise of varied intensity and subsequent resynthesis as a result of carbohydrate feeding. Given that the proportion of fuel metabolism met by carbohydrate metabolism increases with exercise intensity, it should be observed that greater muscle glycogen depletion occurs at 75% compared to 50%  $\dot{V}O_2$  max. To date there is limited research into muscle metabolism using high-field spectroscopy; furthermore, examination of the thigh muscle in respect to glycogen resynthesis at a higher resolution has not been common. This pilot study provided an opportunity to investigate post-exercise changes in muscle glycogen with ultra high field spectroscopy and assess the suitability of a  $^{13}\text{C}$  MRS protocol in a context traditionally occupied by muscle biopsy techniques. Lower field strengths have been associated with large between subject variability thus an aim of the pilot study was to assess if variability of muscle glycogen after exercise of a high and low intensity and during recovery with carbohydrate feeding is lowered by using ultra high-field spectroscopy.



**Figure 4.1** Example of  $^{13}\text{C}$  spectra of glycogen at 100.4 ppm acquired at 3.0 T



**Figure 4.2** Example of  $^{13}\text{C}$  spectra of glycogen at 100.4 ppm acquired at 7.0 T

### 4.3.3 Methods

#### 4.3.3.1 Subject characteristics

Six, healthy, recreationally active male volunteers (Mean  $\pm$  SD; Age:  $26 \pm 1.5$  yrs; BMI:  $23.7 \pm 0.9$  kg.m<sup>-2</sup>) were recruited to the study. All volunteers were informed of the study procedures and risks before providing written informed consent to participate in the study. In addition, an initial exposure to the scanner was completed by volunteers to familiarise them with the sensation of the measurement procedure. The volunteers then underwent preliminary exercise testing during 3 visits to establish  $\dot{V}O_2$  max and relative workloads that corresponded to 50% and 75% of this value as described in Chapter 2. Mean  $\dot{V}O_2$  max was  $53.4 \pm 2.7$  ml.kg<sup>-1</sup>min<sup>-1</sup>.

#### 4.3.3.2 Study design and protocol

Volunteers attended the Sir Peter Mansfield Magnetic Resonance Centre on 2 separate occasions separated by a minimum of 1 week. On arrival at the facility they had been fasted overnight and had abstained from alcohol, caffeine or strenuous exercise for the previous 24 hr. Dietary intake was recorded the day preceding the first visit and subsequently repeated the day before the second visit. Volunteers firstly underwent 2 baseline scans on the front and back of the thigh respectively to determine resting glycogen levels in the quadriceps and hamstrings muscle groups (~20 min). Immediately following the scanning measurements volunteers began cycling exercise for a period of 60 min on a cycle ergometer (Lode Excalibur, Lode, Groningen, The Netherlands) at an intensity of either 50% or 75%  $\dot{V}O_2$  max. All volunteers completed the 2 sessions at both intensities in a randomised order to negate any order effects.

At the end of the exercise period, a post-exercise scan was performed on the front of the thigh (~11 min) to determine glycogen levels in this muscle group following exercise, after which the volunteer was briefly removed from the scanner. A 1 litre solution containing 100g of a commercially available glucose polymer was then given to the volunteer in a non-transparent bottle to be consumed within 3 min. (Vitargo, Swecarb AB, Kalmer, Sweden). Immediately after consumption the volunteer was re-positioned in the scanner and a  $^{13}\text{C}$  scanning procedure for glycogen commenced for a period of 2 hr. As the radiofrequency coil did not differ from its original position, scanning was initiated with the volunteer in a prone position for measurement of the Quadriceps after which the volunteer was briefly removed from the scanner and positioned in a supine position to obtain measurements in the hamstrings. This procedure was repeated for the duration of the scanning period such that glycogen measurements were acquired at 20, 80 and 120 min for the Quadriceps muscle group and at 50 and 100 min in the Hamstrings muscle group.

#### *4.3.3.3 Sampling and analysis*

Measurement of glycogen concentration was determined using  $^{13}\text{C}$  MRS. Spectra were acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling. Scout images were obtained to ensure accurate positioning of the coil between measurements. A total of 8 spectra, each with 80 averages, were collected at each time point (scanning time: 11 mins). Averaging the signal completed analysis of the  $^{13}\text{C}$  spectra and 50Hz Lorentzian line broadening was added before a phase correction was

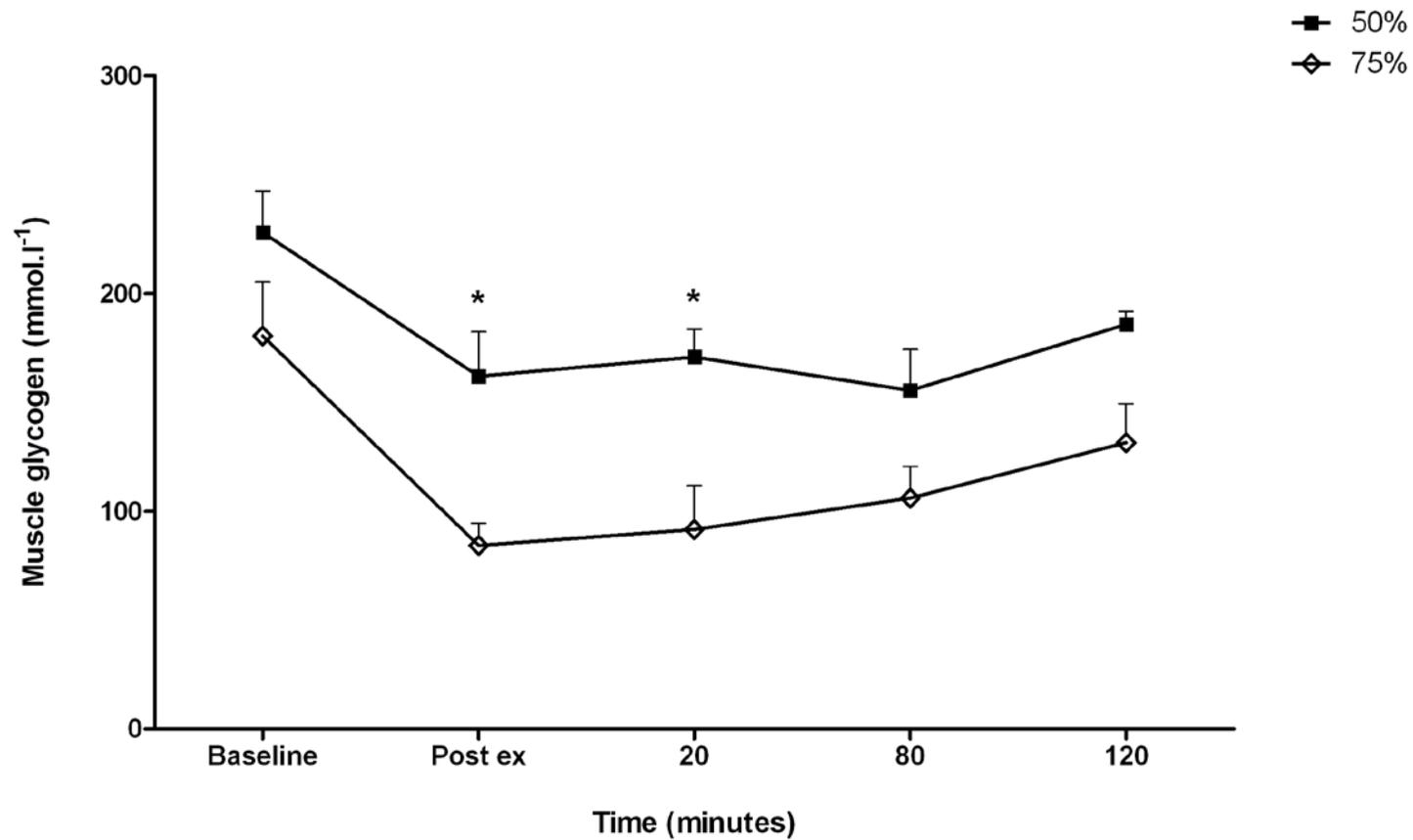
applied using jMRUI. The ratio of glycogen to external reference peak areas was determined using in-house software built in Matlab.

#### 4.3.4 Results

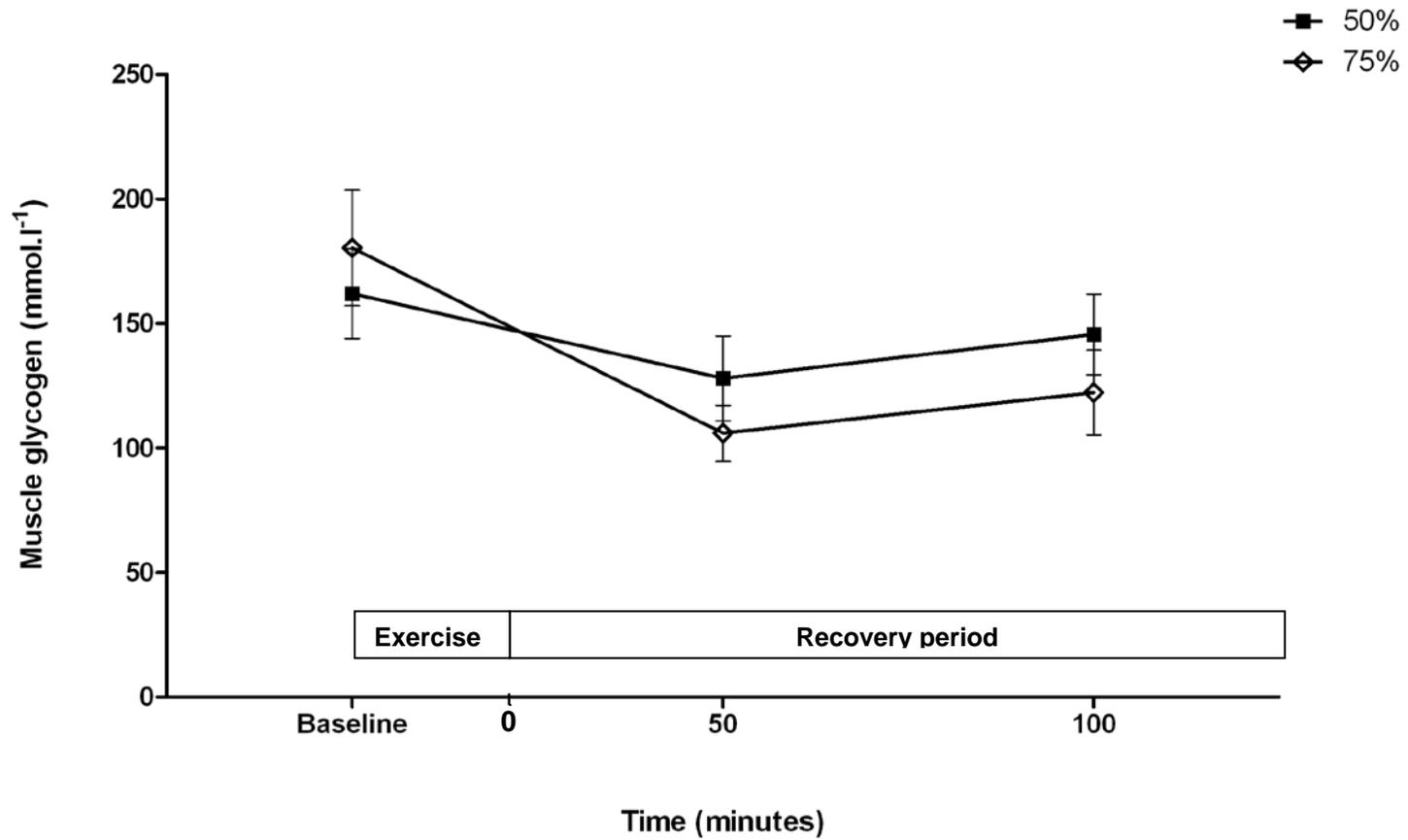
Baseline scans showed no difference in resting glycogen levels between the 2 visits in the Quadriceps ( $180.5 \pm 60.3$  and  $228 \pm 46.2$  mmol.l<sup>-1</sup>) and the Hamstrings ( $162 \pm 44.2$  and  $180.3 \pm 56.8$  mmol.l<sup>-1</sup>). It was observed that on average basal glycogen levels were higher in the Quadriceps compared to the Hamstrings ( $204$  vs.  $171$  mmol.l<sup>-1</sup>) although this was not significant.

Volunteers completed 60 min cycling on 2 occasions at 50 ( $50.8 \pm 0.7$ ) or 75 ( $74.9 \pm 1.9$ ) %  $\dot{V}O_2$  max. Changes in muscle glycogen concentration after exercise and during recovery are shown in Figures 4.3 and 4.4 for the Quadriceps and Hamstrings respectively. One hour of exercise at 50%  $\dot{V}O_2$  max decreased muscle glycogen to 72% of the baseline level whereas the same duration of exercise at 75%  $\dot{V}O_2$  max induced a greater decrease in glycogen to 48% in the Quadriceps muscles ( $p < 0.05$ ). Muscle glycogen remained lower in the Quadriceps in the 75% exercise condition at 20 min into recovery ( $p < 0.05$ ). Supplementation with 100g of the glucose polymer caused increases in muscle glycogen in both conditions however a greater increase occurred following exercise at 75% intensity where glycogen increased from  $84 \pm 10$  to  $132 \pm 22$  mmol.l<sup>-1</sup> compared to the 50% intensity visit which showed a smaller increase from  $162 \pm 20$  to  $186 \pm 9$  mmol.l<sup>-1</sup>. At the end of the 2 hr scanning period muscle glycogen had recovered to 93% and 85% of baseline levels in the 50 and 75% exercise intensity visits respectively and no differences were observed at 80 and 120 min between both conditions.

Muscle glycogen in the Hamstring muscle group was not determined post exercise but at 50 min after ingestion of the glucose polymer solution, at which point muscle glycogen was lower at 80% and 63% of baseline in the 50% and 75% exercise conditions. After 100 min the glycogen levels had recovered to 94% and 76% for the 50% and 75% intensity visits however no significant differences were noted for both conditions at any time point.



**Figure 4.3** Changes in muscle glycogen content of the Quadriceps from baseline, after 60 min exercise at either 50 or 75%  $\dot{V}O_2$  max and during a 2 hr recovery from feeding of 100g carbohydrate. \*  $P < 0.05$  50% vs. 75%. Values are mean  $\pm$  sem, n=6.



**Figure 4.4** Changes in muscle glycogen content in the Hamstrings from the resting level and at 50 and 100 min into recovery from 60 min exercise at 50 or 75%  $\dot{V}O_2$  max followed by feeding of 100g of carbohydrate. Values are mean  $\pm$  sem, n=6.

#### 4.3.5 Summary

Exercise intensity is a fundamental determinant of substrate oxidation (van Loon et al., 2001), and prolonged exercise at 75%  $\dot{V}O_2$  max would be expected to rely predominantly on muscle glycogen for ATP production. Consequently, the results from this pilot study are consistent with the hypothesis in that exercise at a higher exercise intensity induced greater reduction of muscle glycogen reserves compared to a lower exercise intensity (48 vs. 72%) and that repletion was initially faster following depletion to a lower level. Total muscle glycogen resynthesis over the recovery period was lower at 23.8 mmol.l<sup>-1</sup> after exercise at the lower intensity in comparison to 47.1 mmol.l<sup>-1</sup> achieved after the higher exercise intensity. These results are in agreement with previous literature (Zachwieja et al., 1991; Price et al., 2000) that found more rapid muscle glycogen resynthesis when the magnitude of muscle glycogen reduction was greater potentially due to higher glycogen synthase activity in the more glycogen reduced state (Zachwieja et al., 1991). In the present study the rate of muscle glycogen resynthesis was approximately doubled after muscle glycogen depletion to a lower level in the 75% compared to 50% exercise intensity (23.6 vs. 11.9 mmol.l<sup>-1</sup>.h<sup>-1</sup>). Cycling exercise involves both concentric and eccentric motion with the Quadriceps and Hamstrings acting as agonists and antagonists alternatively during the 'down phase' and 'pull up' of the movement (Raasch et al., 1997). It is noted that both the Quadriceps and Hamstrings drive the cycling movement at different phases of the whole closed-loop motion (Raasch and Zajac 1999) thus are recruited at varying angles, nevertheless to overcome resistance at the 'down phase' the quadriceps are considered the principle muscle group involved in cycling. It was not

possible in the pilot study to simultaneously determine muscle glycogen at set time points in both muscle groups as an interleaved scanning protocol was used. Post exercise muscle was only reported in the Quadriceps due to the length of scanning time and the need to provide the test solution close to the end of exercise. After 2 hr, muscle glycogen had recovered to a higher percentage of the basal level (85%) in the Quadriceps compared to the Hamstrings (76%) indicating a preferential resynthesis in the exercised muscle although this cannot be confirmed due to a lack of post-exercise glycogen measurement in the Hamstrings.

The pilot study has demonstrated a pattern of muscle glycogen resynthesis, observed after an exercise protocol that partially depleted muscle glycogen. It is reported that supplementing 100g of carbohydrate immediately post-exercise is sufficient to restore depleted glycogen after 1 hr of exercise at 50% but not 75%  $\dot{V}O_2$  max. Notably, the between subject variation in this pilot study is lower than a similar exercise and feeding study completed at 3.0 T that showed post-exercise muscle glycogen values of  $166 \pm 35$ ,  $153 \pm 39$  and  $159 \pm 31$  mmol.l<sup>-1</sup> following exercise of ~83 min at 70%  $\dot{V}O_2$  max (Casey et al., 2000). In comparison the present study showed muscle glycogen depletion to  $84 \pm 10$  mmol.l<sup>-1</sup>. The pilot study reported the anticipated patterns of muscle glycogen degradation and repletion therefore it can be concluded that ultra high field <sup>13</sup>C NMR spectroscopy is a suitable, non-invasive technique to investigate post exercise glycogen changes and could potentially be used to determine subtle, temporal alterations in glycogen concentration.

## **4.4 Temporal changes in muscle glycogen content during recovery from prolonged exercise following ingestion of high and low molecular weight glucose polymers**

### *4.4.1 Introduction*

As mentioned previously, a potential advantage of  $^{13}\text{C}$  NMR spectroscopy at higher field strengths is the ability to sequentially monitor fluctuating levels of metabolites. The formation of muscle glycogen has been shown to proceed by means of prior depletion in muscle (Zachwieja et al., 1991), a process that is enhanced with carbohydrate administration (Ivy et al., 1988). An increase in muscle glycogen post exercise has been shown to occur specifically in response to a low level of glycogen in the exercised muscle (Bergstrom et al., 1966) but is maintained via an increased insulin sensitivity coupled with glucose provision (Price et al., 1994). Although a wealth of research has sought to resolve the extent of muscle glycogen resynthesis during the post exercise period in a variety of metabolic situations, it is notable that these studies have typically reported muscle glycogen concentrations after intervals of 60 min or more.

Post exercise muscle glycogen resynthesis with a HMW glucose polymer has been reported by Piehl-Aulin et al (2000) in comparison to a LMW glucose solution and by the study in Chapter 3 in comparison to an isoenergetic LMW glucose polymer. Although the former reported a 70% greater rate of muscle glycogen resynthesis during recovery from glycogen-depleting exercise no differences were seen in the latter; however both studies only reported muscle glycogen changes in recovery after 2 hr. Given that the HMW glucose polymer

has been shown to empty from the stomach faster than the LMW solution resulting in a greater amount of carbohydrate being delivered to the small intestine in the first 10 min post ingestion (Leiper et al., 2000) it is feasible that earlier changes in muscle glycogen may have been missed in Chapter 3 due to the large sampling interval. Also when considering that a greater rate of rise in glucose and insulin in the first 30 min following exhaustive exercise has been reported after consumption of the high compared to a low molecular weight glucose polymer (Stephens et al., 2008), it is reasonable to suggest a greater substrate availability in the immediate post exercise period with HMW glucose polymer ingestion. It can therefore be suggested that there may be earlier differences in muscle glycogen resynthesis that are not reflected in the few and sparse measurements previously obtained with muscle biopsy studies.

Traditionally the measurement of muscle glycogen content has been obtained using muscle biopsies, as such the number of measurements that can be taken is limited due to the invasive nature of the procedure (Price et al., 1999). Considering the recent advances in technology, the ability to detect early subtleties in post exercise muscle glycogen content is an attractive feature of  $^{13}\text{C}$  MRS.

#### *4.4.2 Aim and Hypotheses*

As previous determination of muscle glycogen resynthesis with ingestion of the HMW glucose polymer has been determined at large sampling intervals of 2 hr, it remains to be determined if an enhanced response occurs at earlier time points during recovery. Despite similar blood glucose concentrations observed during recovery in the study described in Chapter 3, it is possible that increased muscle glucose uptake due to contraction-mediated increase in muscle membrane permeability may have obscured any differences in blood glucose during the early post-exercise period. Therefore, sampling with a single muscle biopsy at 2 hr in the study described in Chapter 3 may have missed subtle earlier changes in muscle glycogen content as a result of post-exercise ingestion of the HMW compared to the LMW glucose polymer. The previously reported augmented gastric emptying and blood glucose and insulin responses with HMW glucose polymer ingestion led to the expectation that muscle substrate availability will occur earlier. Thus muscle glycogen resynthesis may proceed at a faster rate in the early post-exercise period.

This study aims to report sequential changes in muscle glycogen concentration as a result of post-exercise ingestion of a high or low molecular weight glucose polymer throughout the first hour following glycogen-depleting cycling exercise.

Accordingly, the hypothesis is that muscle glycogen concentration after 60 min of recovery is expected to be highest with the HMW glucose polymer and both glucose polymer conditions are anticipated to result in greater resynthesis in comparison to a control solution with negligible carbohydrate content.

#### 4.4.3 Methods

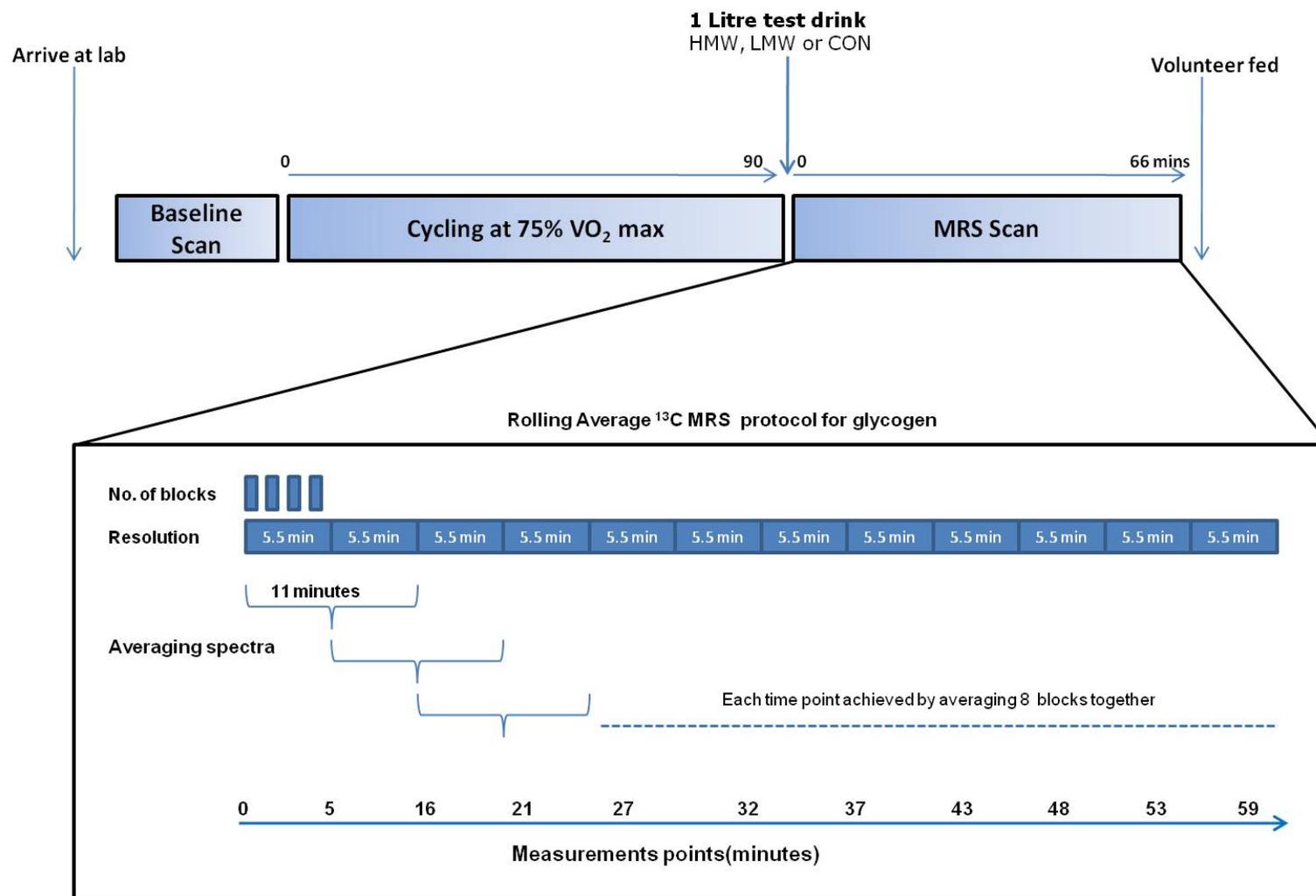
##### 4.4.3.1 Subject characteristics

Nine, healthy, non-smoking, non-vegetarian male volunteers (Mean  $\pm$  SD; Age:  $25 \pm 4$  yrs; BMI:  $24.2 \pm 1.9$ ) were recruited to the study. All volunteers were informed of the study procedures and risks before providing written informed consent to participate in the study. Volunteers were familiarised with lying inside the MRS scanner and the sensation of the magnetic field before beginning any study procedures. Preliminary exercise testing was completed on 3 separate visits to establish  $\dot{V}O_2$  max and a relative workload that corresponded to 75% of this value as described in Chapter 2. Mean  $\dot{V}O_2$  max was  $45.7 \pm 5.8$  ml.kg<sup>-1</sup>min<sup>-1</sup>.

##### 4.4.3.2 Study design and protocol

On 3 separate visits separated by a minimum of 1 week, volunteers arrived at the facility in the morning following an overnight fast and having abstained from alcohol, caffeine and strenuous exercise for the previous 24 hr. Dietary intake on the days preceding the study was maintained the same and intake was recorded for 3 days following a visit to minimise any dietary compensatory effects as a result of strenuous exercise. On arrival, volunteers were placed in the desired position within the scanner and the exact placement of the thigh muscle in the coil was marked on the skin using indelible ink. Volunteers were measured in a prone position lying with their mid-section supported on a foam pad and the upper right leg contained within a radio-frequency coil. A baseline scan (~11 min) was then conducted to establish resting muscle glycogen levels. At the end of the scan the volunteers were removed from the scanner and

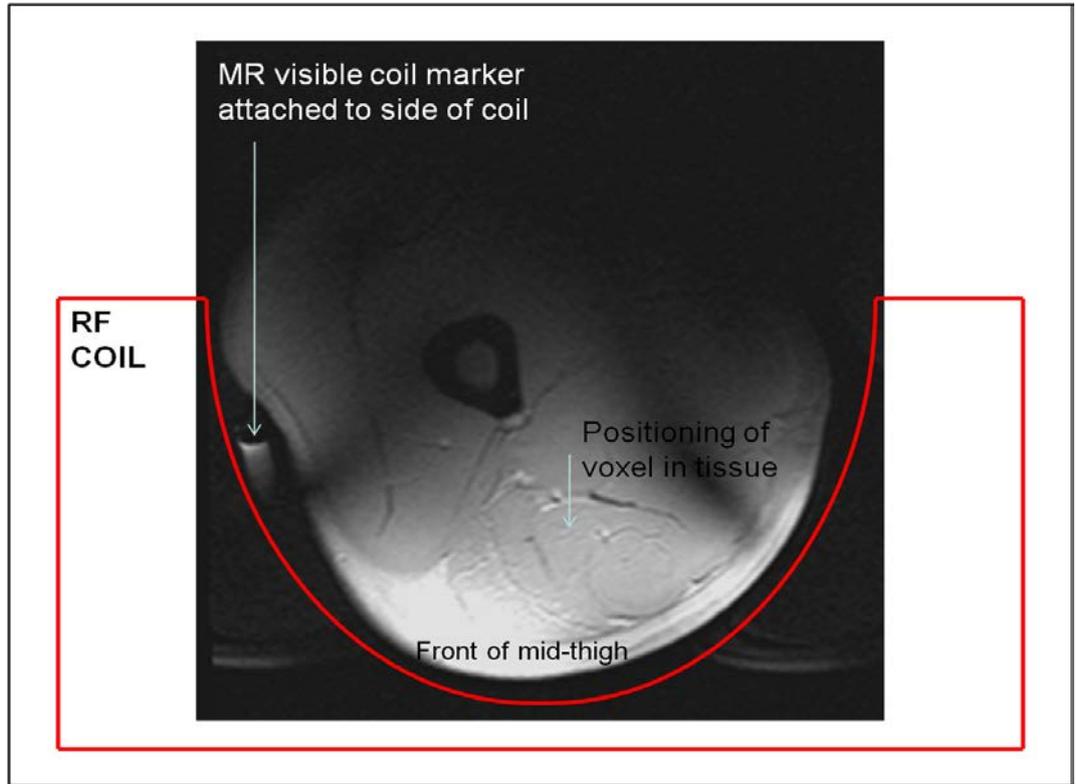
mounted the cycle ergometer to begin cycling at an intensity of 75%  $\dot{V}O_2$  max. Each volunteer, allowing 5 min rest breaks that were taken when they were unable to maintain cycling at the required workload, continued exercising until they reached a total exercise time of 90 min. Volunteers were verbally encouraged to sustain their effort during the exercise and water was provided *ad libitum* on the first visit and replicated thereafter on further visits. Once 90 min of exercise was completed, volunteers were provided with one of three, 1 litre solutions in a non-transparent bottle to ensure volunteers were unable to detect which solution was being consumed. The solutions contained 100g of a HMW glucose polymer, 100g of a LMW glucose polymer or a carbohydrate-free control. The test solutions were identical to the solutions described in Chapter 3 and more detail is provided in Chapter 2. Ingestion of the solutions was achieved within 3 min, volunteers were then immediately re-positioned in the scanner and a continuous  $^{13}\text{C}$  glycogen scanning protocol was initiated, lasting for 1 hr. The scanning protocol produced 11 time point measurements as a rolling average. This was done by running scans continually in a series of 4 blocks with 10 averages in each giving 1 spectra per block in a time resolution of 5.5 min. To get one measurement time point, 8 blocks were averaged together thus 8 spectra were generated with an 11 min resolution. The 8 blocks overlapped such that the measurements are representative of a rolling concentration and a pattern of glycogen resynthesis would be visible. At the end of the measurement period volunteers were removed from the scanner, fed and allowed to leave the facility. A schematic representative of the experimental protocol is shown in Figure 4.5.



**Figure 4.5** Schematic of  $^{13}\text{C}$  MRS experimental protocol

#### 4.4.3.3 Sampling and analysis by $^{13}\text{C}$ MRS

An image of the tissue was firstly obtained with MRI from the 7.0 T system for accurate voxel placement (~10 seconds). Measurement of glycogen concentration was determined by  $^{13}\text{C}$  spectra that were acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling. A total of 8 spectra, each with 80 averages (scanning time: 11 min), were collected at baseline. During recovery 42 spectra, each with 80 averages, were collected in a continuous period of ~66 min which were then averaged together in blocks of 8 with a 4 block rolling offset. Following spectral averaging into the respective time periods, 50 Hz Lorentzian line broadening was added and a phase correction was applied using jMRUI. The ratio of glycogen to external reference peak areas was determined using in-house software built in Matlab. Absolute concentrations were calculated by measuring the volume of glycogen containing tissue within the sensitive area of the coil using the acquired MR images. The position of the tissue within the coil is shown in the MRI image depicted in Figure 4.6. Analysis was completed with the investigator blinded to the time and condition each spectrum was acquired in.



**Figure 4.6** Position of tissue within a radiofrequency coil

#### 4.4.4 Results

##### 4.4.4.1 Glycogen-reducing exercise

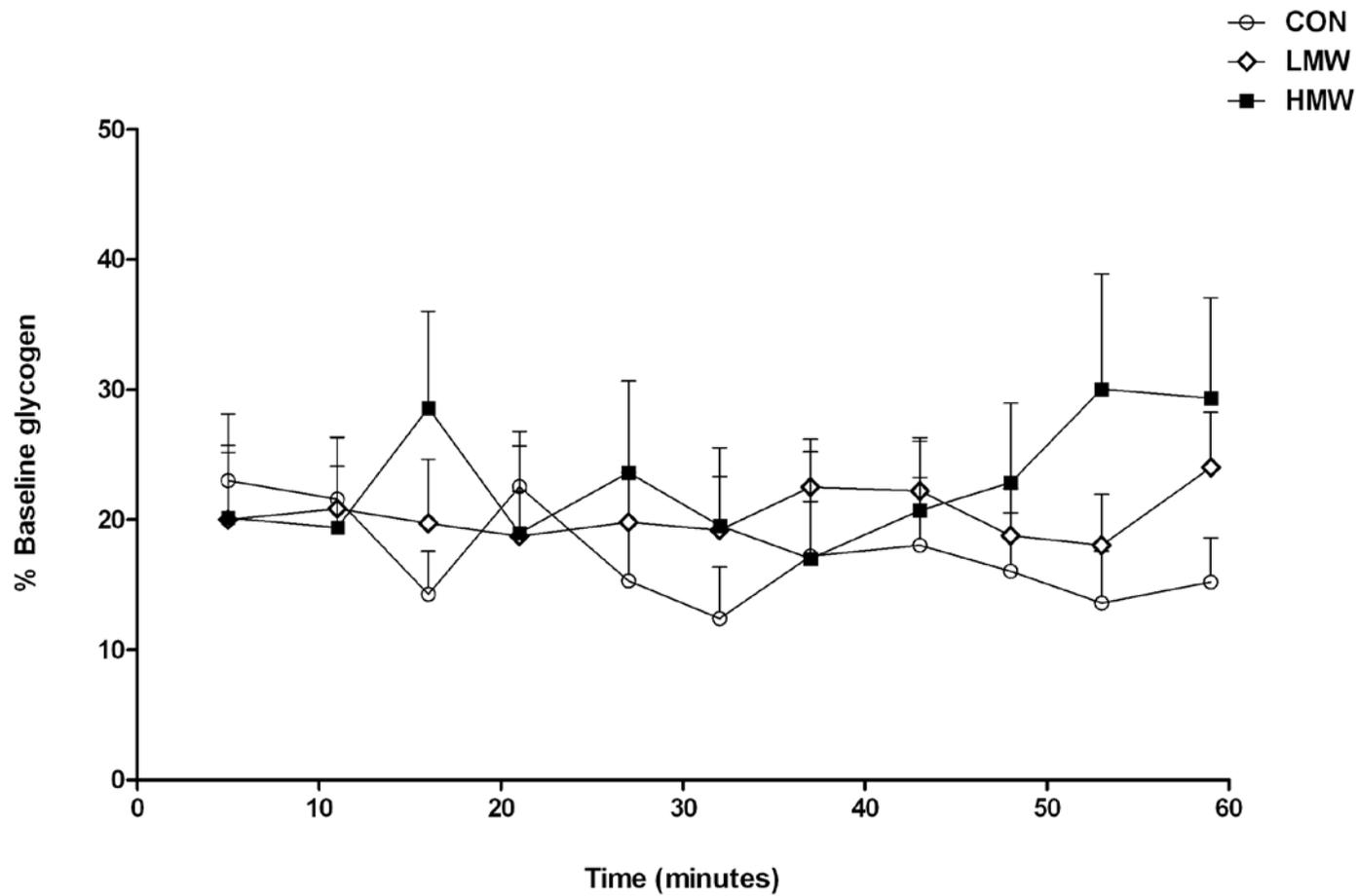
All volunteers completed the 90 min cycling period on 3 occasions at a workload of  $194 \pm 15.5$  watts, which corresponded to a workload of  $76 \pm 0.5\%$   $\dot{V}O_2$  max.

##### 4.4.4.2 Percentage changes in muscle glycogen

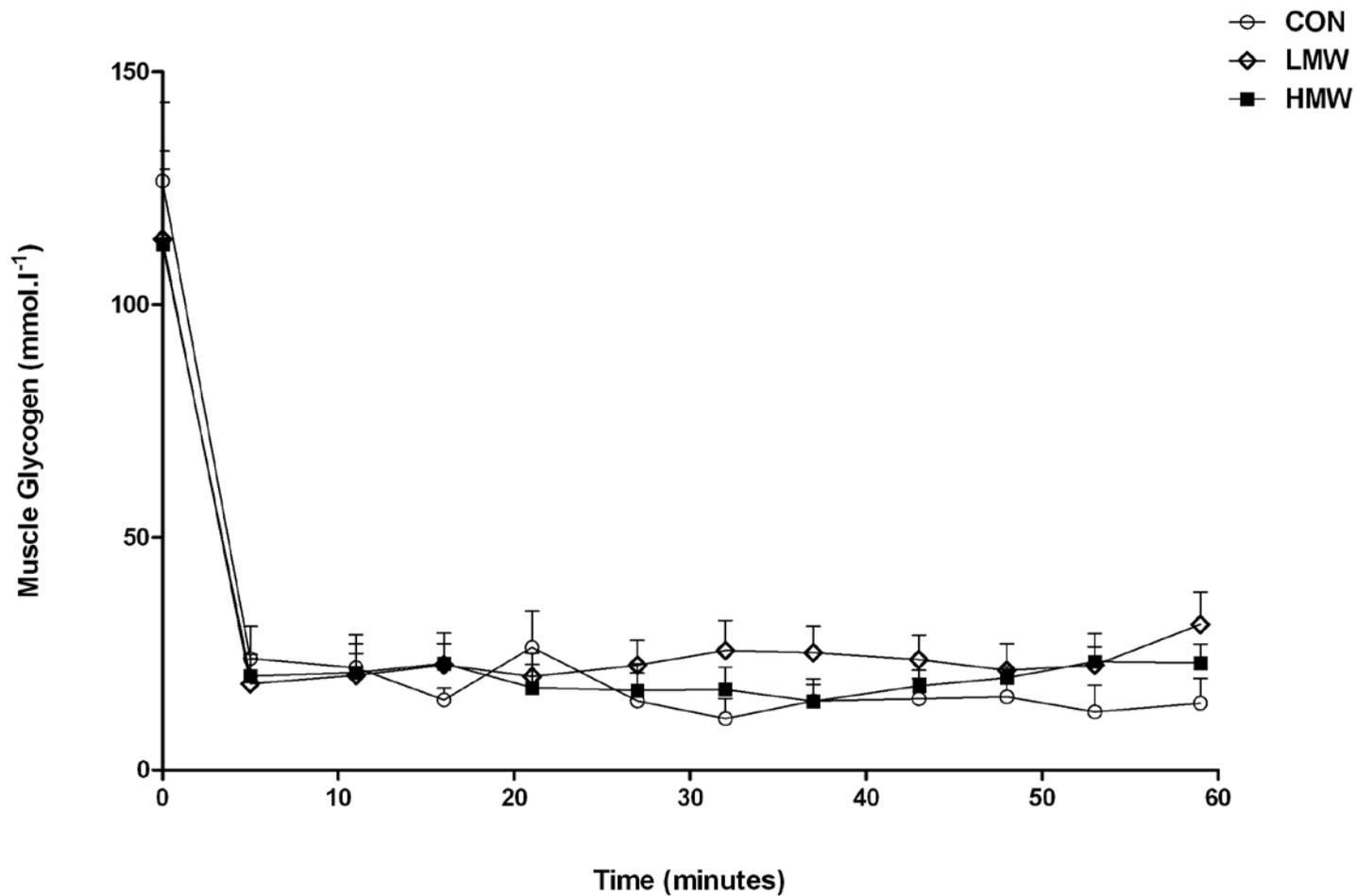
The 90 min exercise period decreased muscle glycogen concentration in the Quadriceps muscle group to 20% of the resting level in the LMW and HMW conditions and 23% in the CON condition (Figure 4.7). At the end of the 60 min measurement period muscle glycogen had further decreased in the control condition to 15% of the basal value whereas it had increased to 24% (LMW) and 29% (HMW) in the glucose polymer trials. No differences in muscle glycogen content were observed between the 3 conditions at any measurement point.

##### 4.4.4.3 Absolute glycogen values

Baseline muscle glycogen content did not differ between the 3 conditions at  $126 \pm 17$  mmol.l<sup>-1</sup> (CON),  $114 \pm 15$  mmol.l<sup>-1</sup> (LMW) and  $113 \pm 19$  mmol.l<sup>-1</sup> (HMW). The exercise period decreased glycogen concentration to  $24 \pm 7$  mmol.l<sup>-1</sup> (CON),  $19 \pm 5$  mmol.l<sup>-1</sup> (LMW) and  $20 \pm 5$  mmol.l<sup>-1</sup> (HMW). At the end of the 1 hr recovery period, glycogen had decreased to  $14 \pm 5$  mmol.l<sup>-1</sup> in the control condition whereas an increase to  $31 \pm 7$  mmol.l<sup>-1</sup> was observed in the LMW glucose polymer condition. A marginal increase to  $23 \pm 4$  mmol.l<sup>-1</sup> was demonstrated in the HMW glucose polymer condition. Muscle glycogen concentration did not differ between conditions (Figure 4.8).



**Figure 4.7** Muscle glycogen concentration as a percentage of resting levels as measured by  $^{13}\text{C}$  MRS during a 60 min period of rest after 90 min cycling and feeding of either 100g of a low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. Values are mean  $\pm$  sem, n=9.



**Figure 4.8** Muscle glycogen concentration as measured by <sup>13</sup>C MRS at the resting level, during a 60 min period of rest after 90 min cycling and feeding of either 100g of a low (LMW) or high (HMW) molecular weight glucose polymer or a sugar-free control. Values are mean  $\pm$  sem, n=9.

## 4.5 Discussion

This chapter has initially focused on the viability of ultra-high field  $^{13}\text{C}$  MRS to monitor muscle glycogen resynthesis following post-exercise carbohydrate feeding. A further aim was to compare subtle post exercise changes in muscle glycogen at multiple time points with ingestion of a HMW and LMW glucose polymer during resting recovery from glycogen-depleting exercise. In particular the temporal changes in muscle glycogen concentration during the initial post exercise period were determined to highlight any differences in muscle glycogen resynthesis between feeding the glucose polymers. Muscle glycogen content was only previously determined with a single biopsy at 2 hr post-ingestion of the HMW and LMW glucose polymers in Chapter 3 and given that the rate of muscle glycogen resynthesis slows as the content increases (Ivy et al., 1998), it is possible that early differences may have been missed. Although NMR spectroscopy is an emerging technique, it has been previously used with exercise protocols for the purposes of glycogen resynthesis determination (Price et al., 1999). The advancement in the magnetic fields up to 7.0 T has improved the sensitivity of the method subsequently shortening acquisition times and reducing the variation in the measurement.

The main study in this chapter demonstrated minor increases in muscle glycogen during 1 hr of recovery from glycogen-depleting exercise when either HMW or LMW was ingested. The small increments observed with glucose polymer feeding (100g) tended to be higher but were not significantly different from a flavoured water control solution with negligible energy content. Changes in muscle glycogen in the Quadriceps muscles were marginal despite

reducing initial muscle glycogen to a low level (20% of basal level) by means of prior exercise, and provision of a large bolus of carbohydrate (100g). Despite an increased signal to noise ratio achieved with 7.0 T spectroscopy, an absence of an increase in muscle glycogen when fed a large amount of carbohydrate suggests that there may be an issue with detection of the glycogen molecules when the concentration is low. The study described in Chapter 3 reported muscle glycogen resynthesis of  $\sim 120 \text{ mmol.kg}^{-1}$  which assuming a normal baseline content of recreationally active males to be  $\sim 350\text{-}400 \text{ mmol.kg}^{-1}$  corresponds to an initial depletion to  $<10\%$  followed by a repletion after 2 hr to  $\sim 40\%$  of baseline with supplementation of the glucose polymers. Similarly Piehl-Aulin et al (2000) reported muscle glycogen resynthesis of  $\sim 100 \text{ mmol.kg}^{-1}$  thus repletion approximates 40% of assumed baseline values after 2 hr. It has been reported from a biopsy study that muscle glycogen after feeding  $\sim 80\text{g}$  of carbohydrate following exhaustive exercise increased from  $74 \pm 55$  post-exercise to  $133 \pm 38 \text{ mmol.kg}^{-1}$  after 1 hr of recovery (Pedersen et al., 2008). Even though baseline glycogen was not reported, assuming a range of  $300\text{-}500 \text{ mmol.kg}^{-1}$  this magnitude of resynthesis represents a resynthesis of to an estimated 26-44% of baseline. A study by Battram et al (2004) reported a muscle glycogen resynthesis rate of  $72 \pm 14 \text{ mmol.kg}^{-1}$  with ingestion of 75g of carbohydrate in the first 30 min following exhaustive cycling. However no control groups were reported in both these studies. Concurrently, a muscle glycogen resynthesis rate of  $\sim 50 \text{ mmol.kg}^{-1}$  has been reported in the first 90 min of recovery from glycogen-depleting exercise with ingestion of  $\sim 124\text{g}$  of sucrose compared to a rate of  $18 \text{ mmol.kg}^{-1}$  with water ingestion (van Hall et al., 2000). Interestingly this study calculated that (assuming that 6kg of muscle

in the leg is depleted to the same extent as the vastus lateralis which was biopsied), leg glucose uptake and the amount of glucose required to cause the glycogen resynthesis seen in 90 min of recovery were almost 100% matched. This suggests a metabolic priority to divert ingested glucose for storage. Given that exercise studies have reported muscle glycogen resynthesis rates of 25-50 mmol.kg<sup>-1</sup>.h<sup>-1</sup> (Betts and Williams, 2010) with provision of ~1-1.2 g.kg<sup>-1</sup> bw of carbohydrate and withholding carbohydrate has been shown to result in minimal resynthesis (Ivy et al., 1988), increases in muscle glycogen after 1 hr above that of the control trial would be expected in the present study.

It is worth noting that the design of the radiofrequency coil necessitated a volunteer scanning position of lying prone to ensure constant contact between the limb and coil surface. This created pressure to the stomach area such that volunteers complained of gastric distress and gut fullness following consumption of the test solutions and assuming the position in the scanner. The emptying of saline and water is delayed on the left side or when supine than when lying on the right side or standing (Burn-Murdoch et al., 1980; Anvari et al., 1995). However this effect was not thought to occur with ingested glucose due to stimulation of intestinal receptors hindering gravity effects on the antrum and pylorus in the stomach (Hunt et al., 1965; Burn-Murdoch et al., 1980). Subsequently, gastric emptying of a liquid meal has been shown to be reduced by 50% when consumed in a supine compared to a seated position (Spiegel et al., 2000). Although gastric emptying of liquids has been shown to either be unaffected (Treier et al., 2006) or to be slowed (Horowitz et al., 1993) when lying in the left decubitus position, and no differences shown when seated compared to a standing position (Jones et al., 2006), there does not

appear to be any data available at present on the effects of a prone posture on the emptying of liquids. Accordingly it cannot be discounted that the posture used in the present study may have affected gastric emptying and thus glucose delivery as a substrate for glycogen resynthesis. The right limb was measured in the present study in all volunteers in order to avoid a slight left decubitus position as this has been shown to delay gastric emptying of a zero-energy solution when compared to a sitting position (Anvari et al., 1995). Further work is warranted to investigate the effects of the volunteer's posture (particularly in a prone position) on gastric emptying and the 'pharmacokinetics' of ingested nutrients during MRS experiments.

Previous investigations utilising glycogen-depleting exercise and muscle glycogen determination with  $^{13}\text{C}$  NMR spectroscopy have reported increases in muscle glycogen concentration after carbohydrate feeding. Casey et al. (2000) showed a rate of muscle glycogen resynthesis of  $40 \text{ mmol.l}^{-1}$  in the first hour following 83 min of cycling exercise at  $70\% \dot{V}O_2 \text{ max}$  and provision of  $\sim 76 \text{ g}$  of glucose, however, in agreement with the present study, no difference between trials of glucose, sucrose or a flavoured control were observed. Importantly the variation in this study between subjects was much higher than reported in the present study perhaps accounting for a lack of differences between trials. One study that recognised the potential of MRS for temporal monitoring of glycogen compared feeding  $108\text{g}$  of carbohydrate with an isoenergetic carbohydrate-protein mixture, they subsequently observed an increased glycogen storage in the first 40 min following 2 hr of cycling at an intensity of  $65\text{-}75\% \dot{V}O_2 \text{ max}$  (Ivy et al., 2002). The high carbohydrate condition ( $108\text{g}$ ) that could be considered similar to the glucose polymer trials

in the present study induced an increase in muscle glycogen of  $\sim 12 \text{ mmol.l}^{-1}$  in that period. However, in the studies by Casey et al (2000) and Ivy et al (2002) and other studies that have measured post exercise muscle glycogen (Krssak et al., 2000; Rothman et al., 2000; Stevenson et al., 2009), the extent of muscle glycogen depletion from basal level following prolonged exercise is not as pronounced as in the present study (20%).

In conclusion, the time course of muscle glycogen resynthesis in the first hour following glycogen-depleting exercise did not differ following ingestion of either a high or low molecular weight glucose polymer. Additionally this response could not be distinguished from temporal changes when an energy free control was consumed suggesting an influence of a prone posture on the delivery of ingested carbohydrate. This chapter has shown that natural abundance ultra-high field spectroscopy is a viable technique for measuring temporal fluctuations in glycogen content above that of lower field spectroscopy due to its increased sensitivity and reduced variation. Future investigations of muscle glycogen resynthesis should firstly ascertain the effects of a prone posture on the rate of gastric emptying of energy-containing liquids as this may have affected the typical pattern. Additionally this study may benefit from a longer measurement period during recovery to determine further changes at 2 hr to compare more directly to muscle biopsy studies. To date there is little information directly comparing MRS with the biopsy method in terms of fluctuating muscle glycogen levels therefore future work could consider the benefit of both methodologies in application to exercise studies.

## **Chapter 5**

Post-exercise blood glucose and serum insulin responses following ingestion of an agglomerated and granulated version of a high molecular weight glucose polymer

## 5.1 Introduction

Post-exercise carbohydrate feeding is effective for resynthesis of depleted muscle and liver glycogen stores as it increases blood glucose concentration which in turn stimulates insulin release. Skeletal muscle is the primary site for insulin-mediated glucose disposal and strategies to augment blood glucose and therefore insulin concentration following exercise are common (van Hall et al., 2000a; van Hall et al., 2000b; van Loon et al., 2000a; van Loon et al., 2000b; Kaastra et al., 2006). The level of blood glucose is a function of the rate of appearance from hepatic output as a result of digestion and the rate of disappearance through glucose uptake by skeletal muscle mediated by insulin release (Wasserman 1995; Rose and Richter 2005). Thus an increase in blood glucose concentration is typically observed following carbohydrate feeding that declines as a function of corresponding increasing insulin concentration. When no carbohydrate is given following exercise, a minimal muscle glycogen resynthesis is reported most likely due to low circulating blood glucose and insulin (Ivy et al., 1988).

A unique glucose polymer with a very HMW and low osmolality has been previously studied in relation to the post exercise period and enhancement of muscle glycogen resynthesis. Earlier studies have found an enhanced rate muscle glycogen resynthesis in the initial 2 hr following glycogen-depleting exercise with the HMW glucose polymer compared to a LMW glucose solution (Piehl-Aulin et al., 2000). Accordingly a further study with the same solutions reported a faster gastric emptying rate with the HMW glucose polymer (Leiper et al., 2000). When comparing the HMW glucose polymer with an isoenergetic LMW glucose polymer, an improved performance in a second exercise bout

undertaken 2 hr after glycogen-depleting exercise in all study participants was reported with the former (Stephens et al., 2008). A notable observation in this study was that ingestion of the HMW glucose polymer caused a greater rate of rise in blood glucose and serum insulin during the first 40 min of resting recovery thus the improved performance effect was ascribed to greater muscle glycogen resynthesis enhancing muscle glycogen availability at the onset of the second bout. The study in Chapter 3 replicated the experimental protocol of the study by Stephens et al (2008) to quantify changes in muscle glycogen, however, not only was muscle glycogen not different between the HMW and LMW glucose polymer there were no observed differences in blood glucose or serum insulin. As both studies followed an identical protocol it is possible that there may be differences in the HMW glucose polymer product that was utilised.

In the interim between earlier studies (Piehl-Aulin et al., 2000; Leiper et al., 2000) and the studies in this thesis, the formulation of the product has graduated from a less soluble, gelatinous liquid to a more soluble, less viscous solution. Additionally the native starch used has gradually deviated from potato to corn and later to barley starch (personal communication with Hans Rydin, Swecarb AB, Kalmer, Sweden). To further improve the solubility of the powder and thus enhance the practicality of using the product, the HMW glucose polymer is currently manufactured using agglomeration instead of granulation following acid hydrolysis of the starch (personal communication with Stefan Bengtsson, Swecarb AB, Kalmer, Sweden). Both methods are used to prevent segregation of powder constituents by bonding particles together; however granules of powder are formed with granulation whereas

agglomeration causes finer particles to adhere to each other creating larger masses. The agglomeration process is most commonly used to improve properties such as flow ability and solubility (Salmon et al., 2006).

The studies from the previous chapters did not demonstrate any differences in muscle glycogen, blood glucose and insulin concentrations or exercise performance between the HMW glucose polymer and an isoenergetic LMW glucose polymer despite previous research showing clear differences, particularly in blood glucose and serum insulin responses post ingestion. This suggests that changes in the manufacturing process of the HMW glucose polymer used in the studies in Chapters 3 and 4, which is a different version to the polymer used in previous investigations, have altered favourable physical characteristics.

## 5.2 Aims and hypotheses

As the manufacturing method of the HMW glucose polymer has been altered from granulation to agglomeration, it was necessary to investigate whether this has diminished the effect on postprandial blood glucose and insulin responses previously reported but not seen in Chapter 3. The following study was conducted to ascertain if the modified, more soluble version of the HMW polymer used in Chapter 3 (agglomerated), caused different postprandial blood glucose and insulin responses from an original, less soluble version that has been manufactured using a different process (granulation) when fed post exercise.

The present study compared blood glucose and serum insulin concentrations over a 2 hr resting recovery period after glycogen-depleting exercise and following ingestion of the 2 versions of the HMW glucose polymers. Any effect on a secondary 'all out' exercise bout following the 2 hrs recovery was also determined.

Post exercise ingestion of the agglomerated HMW glucose polymer described in Chapter 3 had shown a similar rate of rise in blood glucose and serum insulin to a LMW glucose polymer in contrast to a previous investigation (Stephens et al., 2008). It was hypothesised that ingestion of the original granulated HMW glucose polymer would replicate the pattern of blood glucose and serum insulin response observed with HMW polymer ingestion in the study by Stephens et al (2008) during the 2 hr resting recovery. In this way it would elicit a greater rate of rise in these postprandial responses compared to the more soluble, agglomerated HMW glucose polymer.

## 5.3 Methods

### 5.3.1 Subjects

Seven healthy, non-smoking, non-vegetarian male volunteers participated in the study (Mean  $\pm$  SD; Age:  $22 \pm 3.3$  yrs; BMI:  $23.3 \pm 2.2$  kg.m<sup>-2</sup>). The volunteers were all engaged in regular physical activity including cycling. All the volunteers were fully informed regarding the study procedures and provided written consent to participate. Following acceptance into the study, volunteers underwent the preliminary exercise tests described in Chapter 2. These tests established maximal oxygen uptake ( $\dot{V}O_2$  max) and the subsequent workload corresponding to 75% of this maximum value for each volunteer. Mean  $\dot{V}O_2$  max was  $47.31 \pm 4.9$  ml.kg<sup>-1</sup>min<sup>-1</sup>.

### 5.3.2 Study design and protocol

Volunteers visited the laboratory for a total of 3 main experimental trials separated by a minimum of 1 week. Preceding the 3 trials, each volunteer had undergone the preliminary tests described in Chapter 2. Before arrival at the laboratory for each trial, volunteers refrained from consuming alcohol or caffeine for a period of 24 hr and did not engage in any strenuous exercise during that time. On the day preceding all trials, volunteers consumed an identical diet that was monitored using food diaries. Volunteers were instructed to complete a food diary for the 3 days after the first visit and to consume an identical diet following the second visit. This food intake was monitored with a daily food diary.

On arrival at the laboratory in the morning following an overnight fast, volunteers rested semi-supine on a bed whilst a retrograde cannula was inserted

into a superficial vein on the back of the hand and attached to a 0.9% saline drip for patency throughout the trial. Volunteers then mounted the cycle ergometer and began the exhaustive cycling protocol described in Chapter 2. The time to reach exhaustion was recorded and volunteers were required to at least match the time from the previous trial. Once exhaustion was reached, the exercise was terminated and a 3 ml blood sample was immediately drawn, after which the subject was given a non-transparent bottle containing a 1 litre test solution to consume within 3 min. Details of the 3 test solutions that were investigated in this study are described in Chapter 2. The solutions contained either 100g of an original, granulated version of the HMW glucose polymer (GRAN) or 100g of the agglomerated, improved solubility HMW glucose polymer used in Chapter 3 (AGG), both derived from barley starch (Vitargo, Swecarb AB, Kalmer, Sweden). The third solution was a flavoured water control (CON), also used in Chapter 3. The 3 experimental trials were completed in a randomised order to avoid any order effects of the test solutions.

Once the solution was consumed, the volunteers dismounted the ergometer and rested semi-supine on a bed with the cannulated hand contained in a hand-warming unit. For 2 hr they remained resting, during which a 3 ml arterialised-venous blood sample was withdrawn from the cannula every 5 min in the first hour and every 10 min in the second hour. To assess whether ingestion of the individual test solutions had different effects on gastrointestinal factors influencing appetite and satiety, a visual analogue scale (VAS) was given to the volunteers to complete every 20 min throughout the 2 hr resting recovery period (Appendix 4). Subjective measures relating to feelings of satiety were

recorded on a sliding scale of 1 to 100, (Stubbs et al., 2000). With the individual ratings, a composite satiety score referring to overall feelings of satiety (CSS) was calculated using the formula below from ratings of hunger, fullness, desire to eat and prospective food consumption (PFC) to indicate overall satiety (Stubbs et al., 2000).

$$\text{CSS} = \frac{\text{Full} + (100 - \text{Desire}) + (100 - \text{Hunger}) + (100 - \text{PFC})}{4}$$

After the 2 hr resting recovery period, volunteers re-mounted the ergometer and began an 'all out' maximal performance test at the highest exercise intensity they could sustain (Chapter 2). The time of the test was extended from 15 to 20 min to increase the work demand and highlight any differences in work output due to muscle glycogen content. Volunteers were instructed to complete the test at the maximum effort that could be maintained and were frequently provided with verbal encouragement and time elapsed. At the completion of the performance test a 3 ml blood sample was immediately drawn. Once recovered from the exercise, volunteers were fed and left the laboratory.

### *5.3.3 Sample collection and analysis*

During the 2 hr resting recovery, arterialised-venous blood samples (3 ml) were drawn from the cannula at 5 min intervals (0-60 min) and 10 min intervals (60-120 min). From these samples, a small volume (~1ml) was used to immediately analyse blood glucose concentration (YSI 2300 STATplus, Yellow Springs Instruments, OH, USA), after which the remainder was transferred into a plain tube. The samples were left to clot for a minimum of 15 min and then spun in a

centrifuge at 3000 rpm after which the supernatant was extracted and stored at -80°C for later determination of insulin concentration.

#### *5.3.4 Statistical analysis*

Results were analysed using GraphPad Prism 5.03 (GraphPad Software, Inc, California, USA). Paired t-tests were carried out to determine differences in AUC of serum insulin. Other statistical methods used are described in Chapter 2. Significance was accepted when P values were less than 0.05 and all values unless stated otherwise are expressed as mean  $\pm$  SEM.

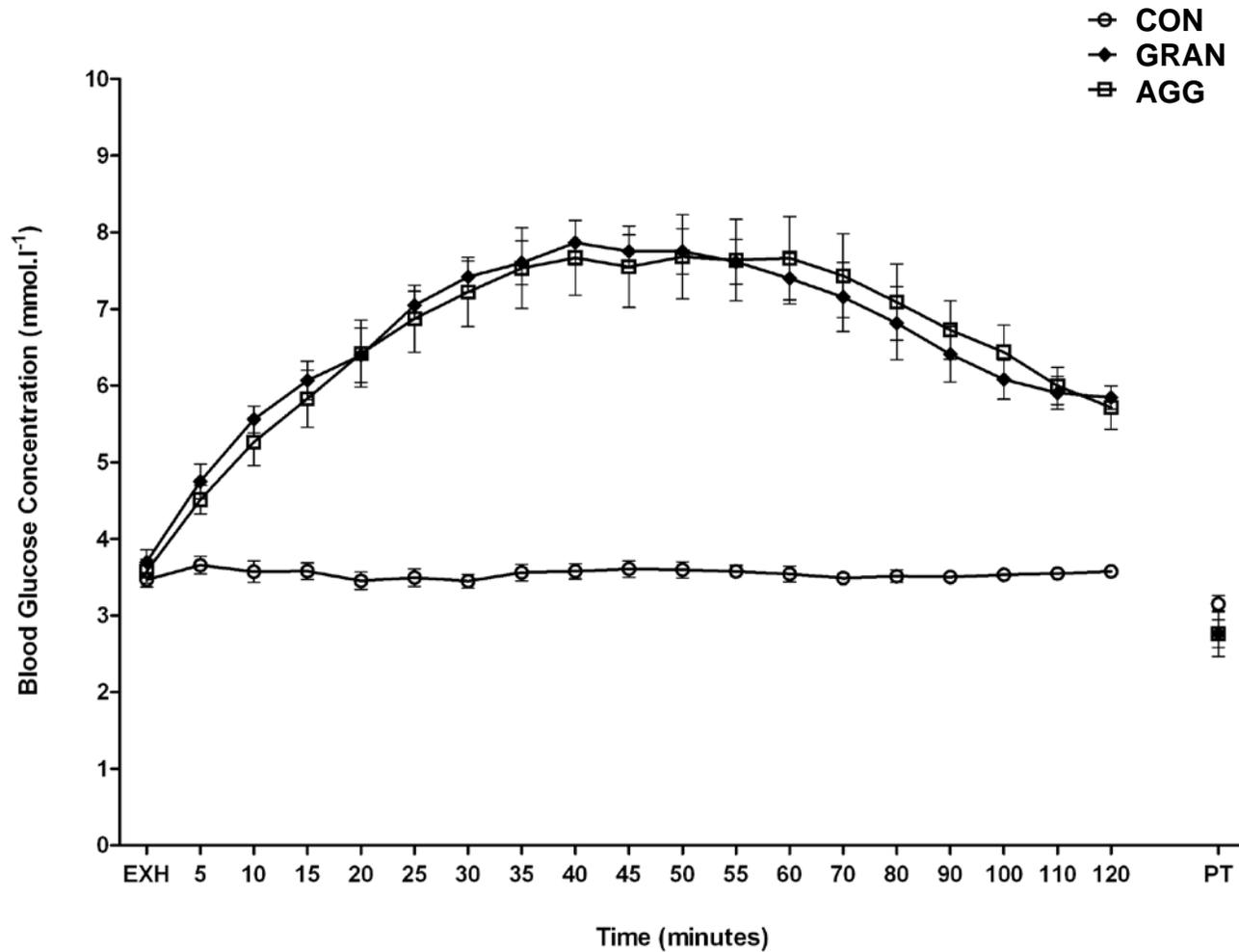
## 5.4 Results

### 5.4.1 Exercise time to exhaustion

Volunteers cycled to exhaustion at a workload of  $199 \pm 18$  watts which was estimated to be an exercise intensity of  $75\% \dot{V}O_2 \text{ max}$  ( $77 \pm 2\%$ ). Mean exercise time to reach exhaustion was similar between all 3 test conditions at  $92 \pm 7$  min (CON),  $91 \pm 4$  min (GRAN) and  $95 \pm 7$  min (AGG).

### 5.4.2 Blood glucose response

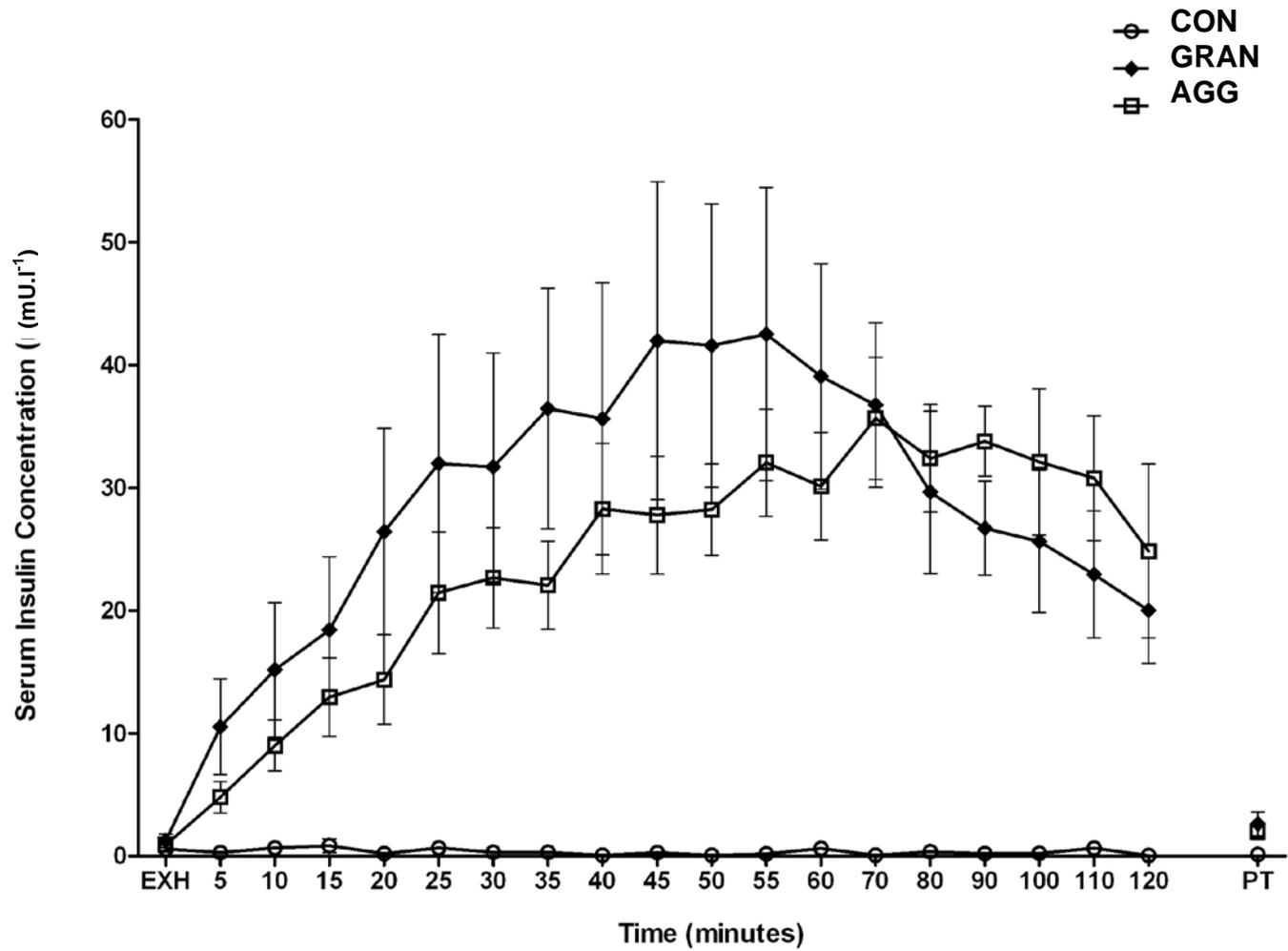
Blood glucose concentration during the 2 hr resting recovery is shown in Figure 5.1. The blood glucose concentration at the point of exhaustion was comparable between all conditions at  $3.5 \pm 0.1 \text{ mmol.l}^{-1}$  (CON),  $3.7 \pm 0.2 \text{ mmol.l}^{-1}$  (GRAN) and  $3.6 \pm 0.2 \text{ mmol.l}^{-1}$  (AGG). After ingestion of CON, blood glucose remained between 3 and 4  $\text{mmol.l}^{-1}$  for the duration of the resting recovery period. Ingestion of the glucose polymers caused a similar increase in blood glucose concentration to peaks of  $7.9 \pm 0.3 \text{ mmol.l}^{-1}$  at 40 min (GRAN) and  $7.7 \pm 0.6 \text{ mmol.l}^{-1}$  at 50 min (AGG). Although the blood glucose concentration was significantly greater from 10-120 min for both glucose polymers when compared to control, no differences were observed when comparing carbohydrate interventions.



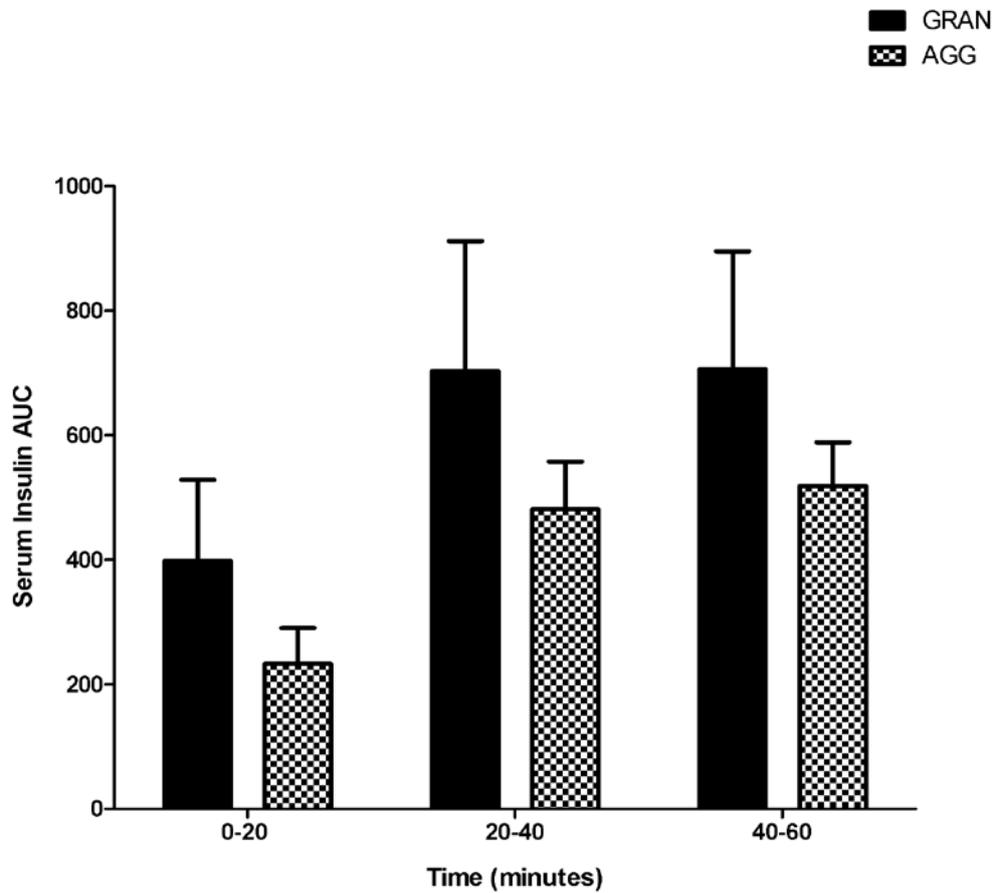
**Figure 5.1** Blood glucose concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of a less soluble, granulated version (GRAN) or a more soluble, agglomerated version (AGG) of a HMW glucose polymer, or a sugar-free control. Values are mean  $\pm$  sem, n=7.

#### 5.4.3 Serum insulin response

Serum insulin concentration during the 2 hr resting recovery period is shown in Figure 5.2. At exhaustion serum insulin concentration was similarly low in all conditions at  $0.6 \pm 0.4$  mU.I<sup>-1</sup> (CON),  $1.2 \pm 0.5$  mU.I<sup>-1</sup> (GRAN) and  $0.9 \pm 0.5$  mU.I<sup>-1</sup> (AGG). Following ingestion of CON serum insulin remained at a very low concentration ( $<1$  mU.I<sup>-1</sup>) during the 2 hr resting recovery period. Following ingestion of the glucose polymers, serum insulin peaked at  $42.5 \pm 17$  mU.I<sup>-1</sup> at 55 min and  $35.7 \pm 5$  mU.I<sup>-1</sup> at 70 min for GRAN and AGG respectively. No significant differences were observed in serum insulin concentration between the glucose polymer conditions at any time point. There was a clear trend for a more rapid and larger serum insulin response following ingestion of GRAN compared to AGG in the first hour post exercise as the AUC was higher ( $P<0.05$ ) (Figure 5.3). The AUC for serum insulin in the first 55 min post exercise up to the first observed peak concentration was higher in GRAN compared to AGG ( $P<0.0001$ ). The calculated time to reach half of the peak concentration was also earlier for GRAN (18 min) compared to AGG (29 min).



**Figure 5.2** Serum insulin concentration during a 2 hr resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of a less soluble, granulated version (GRAN) or a more soluble, agglomerated version (AGG) of a HMW glucose polymer, or a sugar-free control. Values are mean  $\pm$  sem, n=6.



**Figure 5.3** Serum insulin concentration AUC during the first hour of a resting recovery period following glycogen-depleting exercise and supplementation with a 1 litre solution containing either 100g of a less soluble, granulated version (GRAN) or a more soluble, agglomerated version of a HMW glucose polymer (AGG). Values are mean  $\pm$  sem, n=6.

#### *5.4.4 Satiety questionnaires*

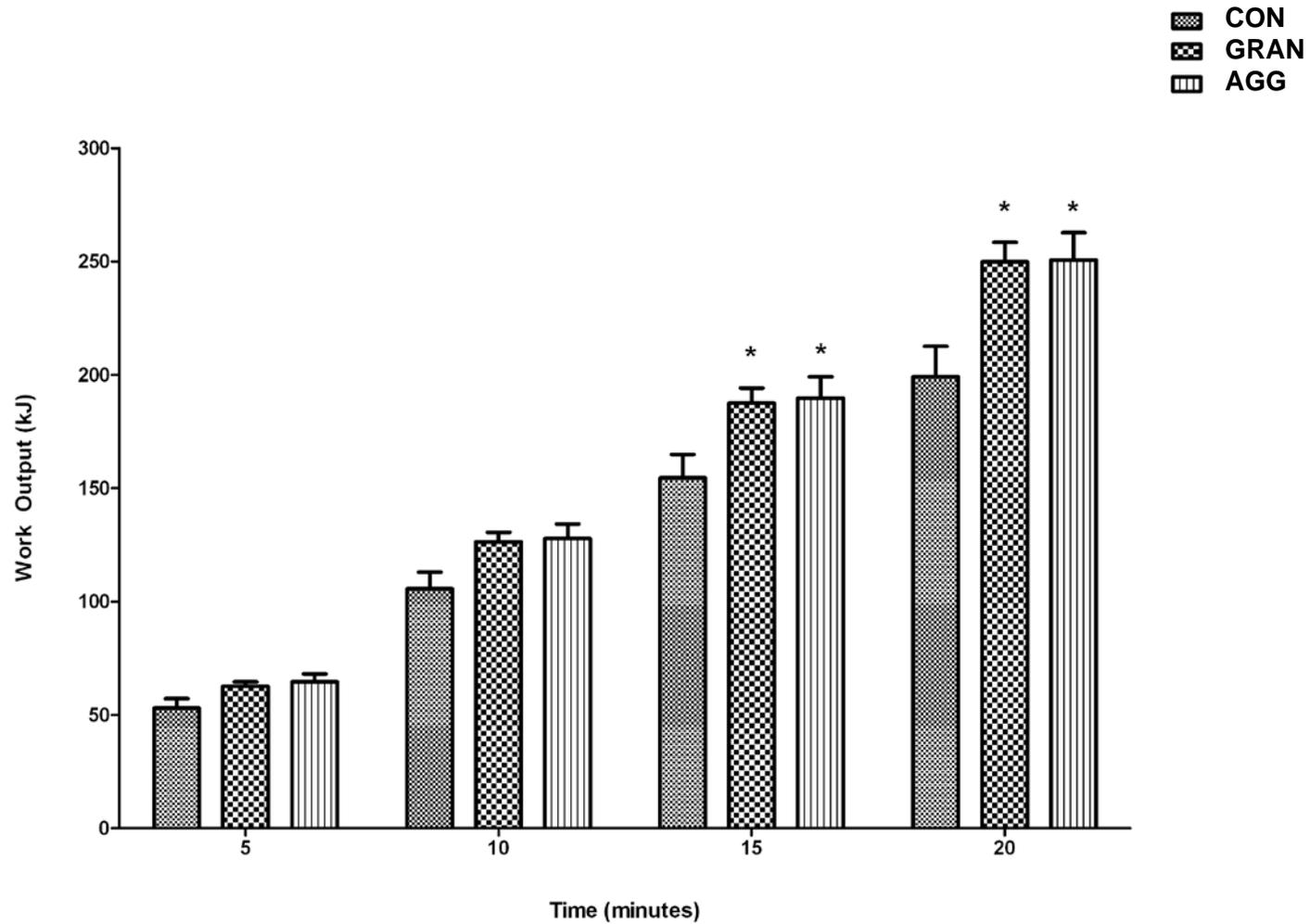
During the 2 hr resting recovery there were no differences in subjective feelings of satiety between the 3 trials (Table 5.1). The CSS in CON declined from  $30 \pm 9$  at 20 min post-drink ingestion to  $16 \pm 4$  at 2 hr. The scores at the same time points for the glucose polymers were  $26 \pm 8$  and  $29 \pm 7$  (GRAN) and  $34 \pm 8$  and  $30 \pm 7$  (AGG). Although scores related to level of hunger increased over the recovery period in all trials, there was a trend for higher scores after ingestion of CON compared to both glucose polymer trials; however there was no difference in VAS scores between the 3 conditions. Concurrently scores related to fullness declined over the 2 hr recovery period and were not different between trials.

|      | CSS    |        |        | HUNGER  |         |        | FULLNESS |         |        |
|------|--------|--------|--------|---------|---------|--------|----------|---------|--------|
| TIME | CON    | GRAN   | AGG    | CON     | GRAN    | AGG    | CON      | GRAN    | AGG    |
| 20   | 30 ± 9 | 26 ± 8 | 34 ± 8 | 69 ± 11 | 82 ± 7  | 67 ± 8 | 33 ± 8   | 40 ± 11 | 41 ± 9 |
| 40   | 28 ± 7 | 31 ± 8 | 31 ± 7 | 73 ± 7  | 74 ± 10 | 67 ± 9 | 33 ± 9   | 43 ± 12 | 35 ± 7 |
| 60   | 28 ± 7 | 29 ± 7 | 28 ± 6 | 69 ± 10 | 67 ± 13 | 71 ± 9 | 32 ± 9   | 28 ± 10 | 31 ± 4 |
| 80   | 26 ± 7 | 30 ± 7 | 29 ± 7 | 75 ± 8  | 72 ± 9  | 70 ± 8 | 26 ± 9   | 36 ± 9  | 38 ± 8 |
| 100  | 23 ± 6 | 28 ± 7 | 26 ± 6 | 79 ± 6  | 74 ± 7  | 77 ± 6 | 30 ± 11  | 31 ± 9  | 32 ± 8 |
| 120  | 16 ± 4 | 29 ± 7 | 30 ± 7 | 87 ± 4  | 73 ± 10 | 72 ± 7 | 24 ± 6   | 29 ± 5  | 36 ± 7 |

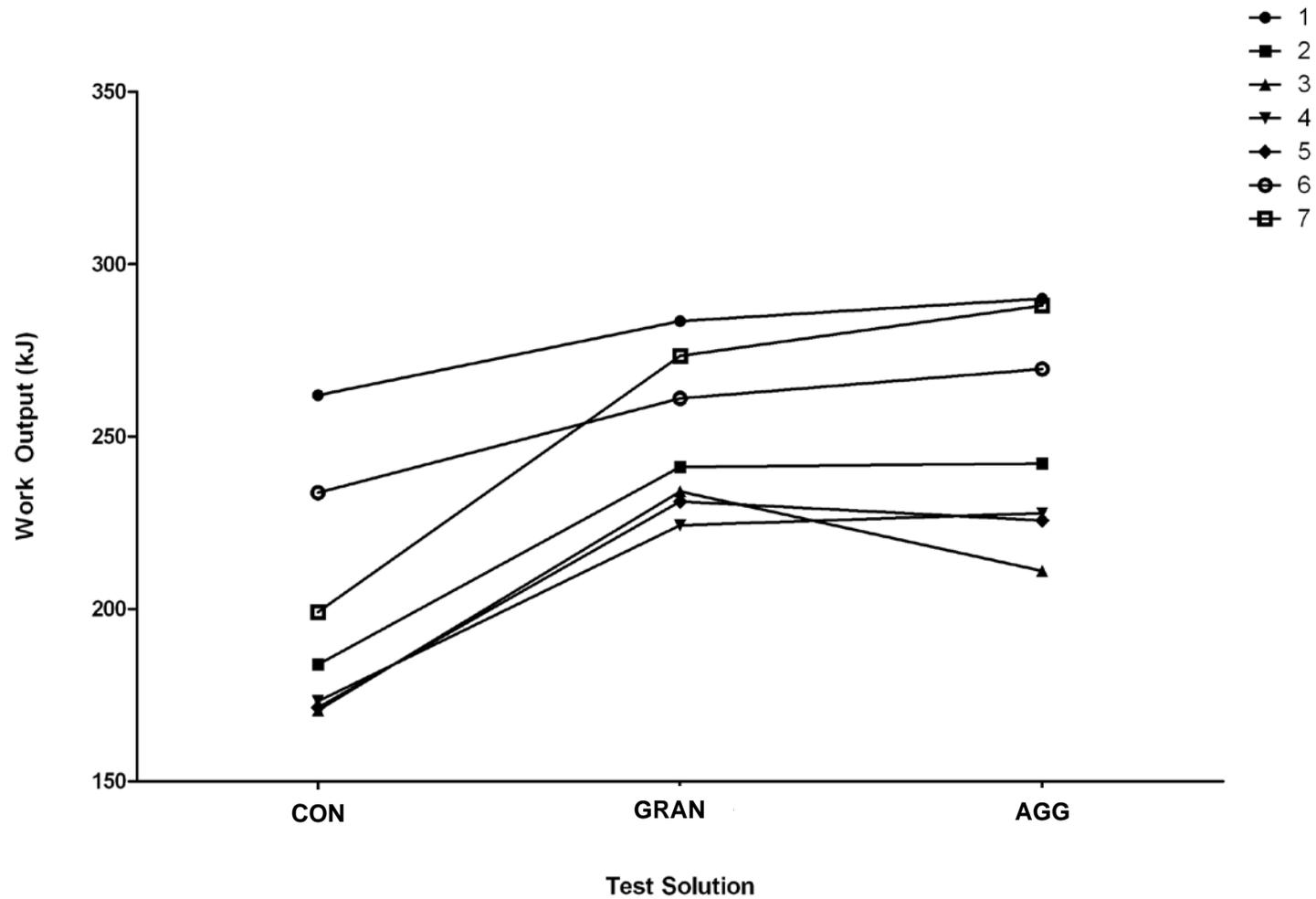
**Table 5.1** Visual analogue scores related to feelings of Satiety (CSS), Hunger and Fullness during a 2 hr recovery period from glycogen-depleting exercise and ingestion of 100g of a granulated or agglomerated version of a HMW glucose polymer or a flavoured control. Values are mean ± sem, n=7.

#### 5.4.5 Exercise performance

Work output during a 20 min maximal performance test was recorded immediately after the 2 hr rest period as a measure of exercise capacity (Figure 5.4). Average work output following consumption of CON was  $199 \pm 14$  kJ. Exercise performance was higher after both glucose polymer conditions when compared to CON ( $P < 0.001$ ) but was not different from each other (GRAN =  $250 \pm 9$  kJ vs. AGG =  $251 \pm 12$  kJ). Performance from all individuals is shown in Figure 5.5. Further analysis revealed that the significant difference in the glucose polymer conditions to the control condition was evident at 15 and 20 min and there was no difference between any trial during the first 10 min of the performance test. Heart rate at 20 min was  $168 \pm 4$  bpm (CON),  $182 \pm 2$  bpm (GRAN) and  $181 \pm 3$  bpm (AGG).



**Figure 5.4** Average exercise performance at 5 min intervals during a 20 min cycling performance test undertaken after 2 hr rest from glycogen-depleting exercise and ingestion of either 100g of a granulated (GRAN) or agglomerated version (AGG) of a HMW glucose polymer or a flavoured control. Values are means  $\pm$  sem, n=7. 150



**Figure 5.5** Individual exercise performance during a 20 min cycling performance test undertaken 2 hr after glycogen-depleting exercise and ingestion of either 100g of a granulated (GRAN) or agglomerated (AGG) version of a HMW glucose polymer, or a flavoured control. Values are means, n=7. 151

## 5.5 Discussion

The study in this chapter investigated the post-exhaustive exercise postprandial blood glucose and serum insulin responses to a less soluble granulated version and a more soluble agglomerated version of a HMW glucose polymer. The results from this study demonstrate that exercise performance in a secondary bout was the same (250 kJ) with ingestion of both glucose polymers after 2 hr recovery. A greater AUC of serum insulin was observed in the first 55 min post exercise and an earlier peak concentration (55 vs. 70 min) was reported with GRAN compared to AGG however, overall in the 2 hr recovery period no differences in serum insulin concentration were observed between the polymers. Potentially the influence of incretin hormones on stimulation of insulin release may account in part for the insulin response observed in the initial 55 min post-drink ingestion. A rise in plasma levels of GIP and GLP-1 is present within minutes of oral nutrient ingestion (Drucker 2006) and the potentiation of insulin secretion by these gut hormones is considerable accounting for an estimated 70% of total insulin secretion in response to an oral glucose load (Baggio and Drucker 2007). A variation in the delivery rate of infused glucose to the small intestine within a physiological range ( $0.71\text{-}3\text{ kcal}\cdot\text{min}^{-1}$ ) has been shown to elicit marked differences in insulin and incretin responses (O'Donovan et al., 2004). When this rate of glucose delivery was increased to  $6\text{ kcal}\cdot\text{min}^{-1}$  the magnitude of insulin and GLP-1 release was greater (Chaikomin et al., 2005), suggesting a dose-response effect of intestinal glucose delivery, GLP-1 secretion and insulin secretion. Thus given the potent insulintropic effect of incretin hormones, it is reasonable to consider that rapid gastric emptying and the presence of glucose in the small intestine would be

associated with a rise in circulating levels of GIP and GLP-1. It is perhaps a limitation of this study that these hormones were not measured and future work should monitor incretin levels in conjunction with gastric emptying measurements to give a more complete assessment of the digestive effects of the HMW glucose polymer.

Blood glucose concentration also did not differ between the glucose polymer conditions. However as the concentration reflects both the rate of appearance and disappearance of glucose (Rose and Richter, 2005), an enhanced uptake via insulin-mediated glucose disposal may have obscured any early disparity in blood glucose concentration. Subjective feelings of hunger increased in all trials over the 2 hr recovery period but tended to be higher and feelings of fullness lower after ingestion of the control solution compared to the glucose polymer trials as would be expected, nevertheless the two energy-containing solutions elicited similar patterns of response.

It appears possible that physical properties of the product may have affected the digestibility and subsequent bioavailability of glucose following ingestion. Previous investigations have shown markedly different glycaemic and insulinaemic profiles following ingestion of a HMW glucose polymer compared to a LMW glucose polymer when fed post-exercise (Stephens et al., 2008). In contrast, this difference was absent in the study described in Chapter 3, despite an identical experimental protocol with similar subjects to that reported by Stephens et al (2008). Consequently, the present study was conducted to determine if the change in the manufacturing process of the HMW glucose polymer has altered the digestibility and is therefore responsible for the similar blood glucose and insulin profiles reported in the study in

Chapter 3. An earlier version of the HMW glucose polymer was provided that had not been agglomerated to improve solubility, however it was also derived from barley starch which is in contrast to the products used in prior studies that have used the HMW glucose polymer derived from corn starch (Stephens et al., 2008), 98-99% amylopectin waxy maize starch (Rowlands et al., 2005) or potato starch (Leiper et al., 2000; Piehl Aulin et al., 2000). The continual evolution of the HMW glucose polymer from potato starch that formed a viscous paste to the more soluble barley starch product currently available has made direct comparison between the studies difficult. It was not possible to compare the potato or corn starch products to the newest formulation, however the versions used in this chapter have undergone different manufacturing processes thus can be considered representative of 'earlier' and 'recent' formulations.

Post-exercise ingestion of both versions of the HMW glucose polymer produced a similar rate of rise in blood glucose over a 2 hr recovery period. This was not mirrored by the insulin response which, although not significant, displayed a trend for a more rapid and greater rate of rise following ingestion of the granulated solution. The immediate post-exercise state is characterised by an increased capacity for muscle glucose uptake (Goodyear et al., 1990; Richter et al., 2004), particularly when muscle glycogen content is low (Price et al., 1994). Residual effects of contraction-induced muscle glucose uptake and an increased sensitivity to insulin post-exercise are conducive to the increased capability of the muscle to transport glucose from the blood (Richter et al., 1984; Cartee et al., 1989). The concentration of glucose in the blood at any time is a reflection of both the rate of appearance from digestive and

hepatic output and disappearance via muscle glucose uptake. Considering the enhanced capacity for increased muscle glucose uptake following exercise (Cartee et al., 1989; Goodyear et al., 1990; Richter et al., 2001), it is reasonable to suggest that increased muscle glucose uptake in the post-exercise period may impact on blood glucose concentration.

Exercise performance was unaffected by the different glucose polymers, showing near-identical accumulated work output during a 20 min performance test following ingestion of GRAN and AGG solutions (249.8 vs. 250.6 kJ respectively). Both of these were different to work output accrued during CON (199 kJ) that was presumably limited by muscle glycogen availability as evident by differences in accumulated work output at 15 and 20 min compared to both HMW glucose polymers. It is likely then that both the granulated and agglomerated glucose polymers were effective in restoring muscle glycogen to a similar level, however this was not measured. It appears that the early greater rise seen in the insulin response was not sufficient to induce enhanced muscle glycogen resynthesis after ingestion of the granulated HMW glucose polymer, as indicated by the near-identical work output performed in the subsequent bout in both glucose polymer trials.

In conclusion it appears that the change in manufacturing from granulation to agglomeration may be in part responsible for the contrasting blood glucose and serum insulin responses reported in Chapter 3 compared with those reported by Stephens et al (2008). Further work should be directed towards elucidating the more physical properties present in the agglomerated and granulated versions of the HMW glucose polymer.

## **Chapter 6**

Determination of physical characterisation and viscosity properties of an agglomerated and granulated version of a HMW glucose polymer

## 6.1 Introduction

In the previous chapters contained in this thesis, direct studies using human volunteers have sought to ascertain whether a unique glucose polymer with a HMW and low osmolality can enhance muscle glycogen resynthesis and exercise performance above that of an isoenergetic glucose polymer with a LMW and higher osmolality. The study described in Chapter 3 demonstrated no differences in blood glucose or serum insulin during a 2 hr resting recovery from glycogen depleting exercise following ingestion of the HMW and the LMW glucose polymers. However, previous investigation using the same polymers and an identical experimental protocol (Stephens et al., 2008) showed a clear greater rate of rise in both responses thus there may be a potential physical difference in the HMW glucose polymer versions used. As a result this hypothesis was challenged by the study described in Chapter 5 by using the same experimental protocol and comparing the more soluble, agglomerated HMW glucose polymer and an earlier formulation of the polymer that was created using a different manufacturing process (granulation as opposed to agglomeration). A more rapid serum insulin response was noted in the initial 55 min after ingestion of the granulated compared to the agglomerated version despite similar absolute insulin concentrations consistent with the blood glucose response and near-identical exercise performance. Thus although the blood glucose and serum insulin results from Stephens et al (2008) were not wholly replicated in chapter 5, it remains to be determined whether there are physical differences present in the agglomerated and granulated versions

related to viscosity and flow behaviour as a result of changes in the manufacturing process.

From the manufacturer's summary, the presence of novel bonds in the form of beta linkages, more specifically  $\beta$  1-6 bonds and also the  $\beta$  1-4 bonds that are more commonly found in cellulose is suggested. In addition, the osmolality of the product is quoted to be 20 mosmol.kg<sup>-1</sup> solution, this is half the osmolality suggested for a comparable maltodextrin with a dextrose equivalent (DE) of 5, therefore it can be implied that the HMW glucose polymer will have a DE of <5. Osmolality and DE are related to the molecular weight in that the DE is inversely proportional to the number average molecular weight (Rong et al., 2009). The association between a low DE whilst maintaining a low osmolality is an intriguing characteristic of maltodextrins and underpins their favourable application in post-exercise recovery.

## 6.2 Aims and hypotheses

The *in vivo* blood glucose response to ingestion of the granulated and agglomerated versions of the HMW glucose polymer was not different; however the insulin response did show an earlier time to peak concentration and greater rise in the first 55 min post exercise with the less soluble granulated solution. A trend for a more rapid rate of rise in insulin following ingestion with the granulated version suggests that the more soluble, agglomerated HMW glucose polymer has presently been altered to a point where favourable properties have diminished. It remains to be determined if the change in manufacturing process to improve solubility caused any fundamental changes in the physical characteristics of the solutions.

The aim of the work in this chapter is to use physical techniques to establish the physical properties in terms of the molecular size and flow behaviour of the agglomerated and granulated versions of the HMW polymer that could potentially account for the *in vivo* observations. The viscosity behaviour of a macromolecule in solution is a commonly used approach for characterisation and in this instance may detail any physical differences in the HMW glucose polymers that may manifest within *in vivo* responses.

It is hypothesised that the granulated and agglomerated versions of the HMW glucose polymer will display different characteristics in terms of viscosity and flow behaviour.

## 6.3 Materials and methods

The work contained in this chapter was carried out in the Food Sciences Division of the School of Biosciences at Sutton Bonington campus, University of Nottingham.

### 6.3.1 Molecular size

The molecular weights of the granulated (GRAN) and agglomerated (AGG) versions of the HMW glucose polymer were determined by size exclusion chromatography with an on-line multi angle laser light scattering detector (SEC-MALLS). A third sample of the granulated polymer was included to determine any effect of constructing the solution using a mechanical whisk instead of mixing by hand. Size can be used to determine the molecular weight distribution but because light scattering measurements on a polydisperse polymer will yield a weight average molecular weight, the mean molecular weight obtained from SEC-MALLS is generally represented as the weight average ( $M_w$ ). Briefly, the polymer is dissolved in a solvent and pumped through a gel permeation column to segregate the polymer molecules according to individual molecular size. The time for a molecule to pass along the column to the end is known as the elution time (the larger the polymer molecule, the more rapidly it elutes the column). Since the elution time depends on other factors in addition to molecular size, to obtain an absolute value of molecular weight it is necessary to couple this with light scattering. Light from a laser is scattered by the molecules in solution and absorbed by photodiode detectors at a range of angles. The signal from the light-scattering detector is proportional to the molecular mass multiplied by the concentration thus when concentration is detected by a refractive index detector which is also in-line, it is possible to

determine the molecular mass of each peak or component of a peak from the column.

### 6.3.2 *Intrinsic viscosity*

An increase in viscosity of a solution is usually seen when a polymer is added, with higher molecular weight molecules generally causing more viscous solutions. Intrinsic viscosity is the ratio of a solution's specific viscosity to the solute concentration, which is then extrapolated to zero concentration. It reflects the volume swept out by the polymer in solutions divided by the polymers molecular weight. For polymers such as starch polysaccharides which adopt coil like conformations in solutions the volume swept out (occupying space of the molecules in motion) increases with molecular weight  $M$  to  $M^{1.5-2.0}$ . It therefore follows that the intrinsic viscosity which proportional to this volume divided by  $M$  will increase with  $M^{0.5-1.0}$ .

The intrinsic viscosity of both granulated polymer solutions (hand-mixed and whisked) and the agglomerated solution was measured using 2 methods. Intrinsic viscosity was firstly determined using an online viscometer coupled to the SEC-MALLS system. Intrinsic viscosity was also determined using dilute solution viscometry with a rolling ball viscometer. In this method viscosity is calculated by monitoring the time elapsed for a 5 mm metal ball to migrate from one end of a thin, glass capillary tube filled with the sample solution to the opposite end, at a variety of angles. The times recorded are then contrasted against the transit time for a 'pure' solution such as distilled water to calculate relative and specific viscosity. Intrinsic viscosity is calculated from the relative viscosity and concentration.

### 6.3.3 Rheology

Rheological measurements are suggested to be a useful method for characterisation of a material considering that flow behaviour can be sensitive to changes in molecular weight. Most frequently measured is viscosity, defined as the measure of the internal friction of a fluid that resists the tendency to flow. The rheological properties, in the form of viscosity, of the HMW glucose polymer were identified with a series of experiments using a Rapid Visco Analyser (RVA). The RVA measures viscosity by applying a pre-determined shear rate (rpm) to a small container of sample (~25g) and recording torque, which is then converted to rheological measurements. Briefly, the RVA consists of an aluminium sample container that is stirred using a one way plastic mixing paddle. The rate of stirring can be programmed and the temperature of the sample can be manipulated with an attached water bath. The viscosity measured in this way is a less fundamental parameter than the intrinsic viscosity because the shear rate is not well defined and the starch concentrations are higher but it is a useful way of following changes with temperature and time e.g. the effect of enzyme activity, and comparing samples.

#### *RVA Experiment 1:*

Initially, the viscosity was compared between AGG and GRAN (hand mixed and whisked). A 15% solution of each polymer was formed and a sample of ~25g was weighed into the aluminium canister. The RVA was pre-programmed to a constant shear rate and temperature of 160 rpm and 37°C for 2 min. A second program was then run on identical samples but following a standard starch profile. This profile increased the temperature from ~20°C up

to ~60°C and back down to 20°C over a period of 13 min at 160 rpm. This experiment was run twice on each polymer and the results averaged.

*RVA Experiment 2:*

The effect of temperature on viscosity at a range of concentrations was measured using the granulated HMW glucose polymer and a commercial potato starch preparation (Paselli SA2, AVEBE U.A, The Netherlands). The potato starch is representative of a commercial maltodextrin with a similar DE and native starch to the product described in the patent application for the HMW glucose polymer. Solutions of 30%, 35%, 40% and 45% were formed by adding distilled water to dry powder of both products and mixing by hand until a uniform paste was evident. Samples of ~25 g from each solution were then subjected to the standard starch profile as in experiment 1 for a period of 15 min.

*RVA Experiment 3:*

The addition of starch-degrading enzymes to a polymer solution will evoke hydrolysis of the alpha linkages resulting in smaller polymer molecules and thus a lowering of viscosity, observed by a decline in the viscosity curve as measured by the RVA (Ferry et al., 2004). This experiment aimed to determine the presence or absence of  $\alpha$  1-4 and  $\beta$  1-4 glycosidic bonds in the HMW glucose polymer by measuring changes in viscosity after addition of degrading enzymes. The addition of amylase to a starch preparation would be expected to induce a rapid hydrolysis of the  $\alpha$  1-4 linkages present that would be manifested in a decline in viscosity (Ferry et al., 2004). The solutions tested were GRAN, AGG and the Potato starch (Paselli SA-2). A 40% solution from

each polymer was formed and mixed using a plastic spatula until a smooth solution was achieved, of these a sample (~25g) was transferred to the aluminium canister. The RVA profile was pre-set to measure viscosity at 160 rpm for 10 min at 40°C. Firstly, a sample of each was run through the profile without the addition of any enzyme to act as a control. After which another sample was run in the RVA immediately after 50µl of amylase was pipetted into the canister. This experiment was then repeated but 50µl of cellulase was added. Due to the nature of the cellulase enzyme that meant it only became active above temperatures >50°C, the temperature of the RVA in this run was increased to 60°C.

## 6.4 Results

### 6.4.1 Molecular size and intrinsic viscosity

The weight average molecular weight was measured in conjunction with the intrinsic viscosity by the SEC-MALLS system. Table 6.1 shows the values obtained. The error for molecular weight and intrinsic viscosity was 1% and 0.8-1% respectively.

|  | <b>AGG</b> | <b>GRAN-<br/>WHISKED</b> | <b>GRAN-<br/>HAND MIXED</b> |
|--|------------|--------------------------|-----------------------------|
| <b>Molecular Weight<br/>(g.mol<sup>-1</sup>)</b>   | 853,000    | 1,040,000                | 1,040,000                   |
| <b>Intrinsic Viscosity<br/>(ml.g<sup>-1</sup>)</b> | 22.9       | 23.3                     | 22.8                        |

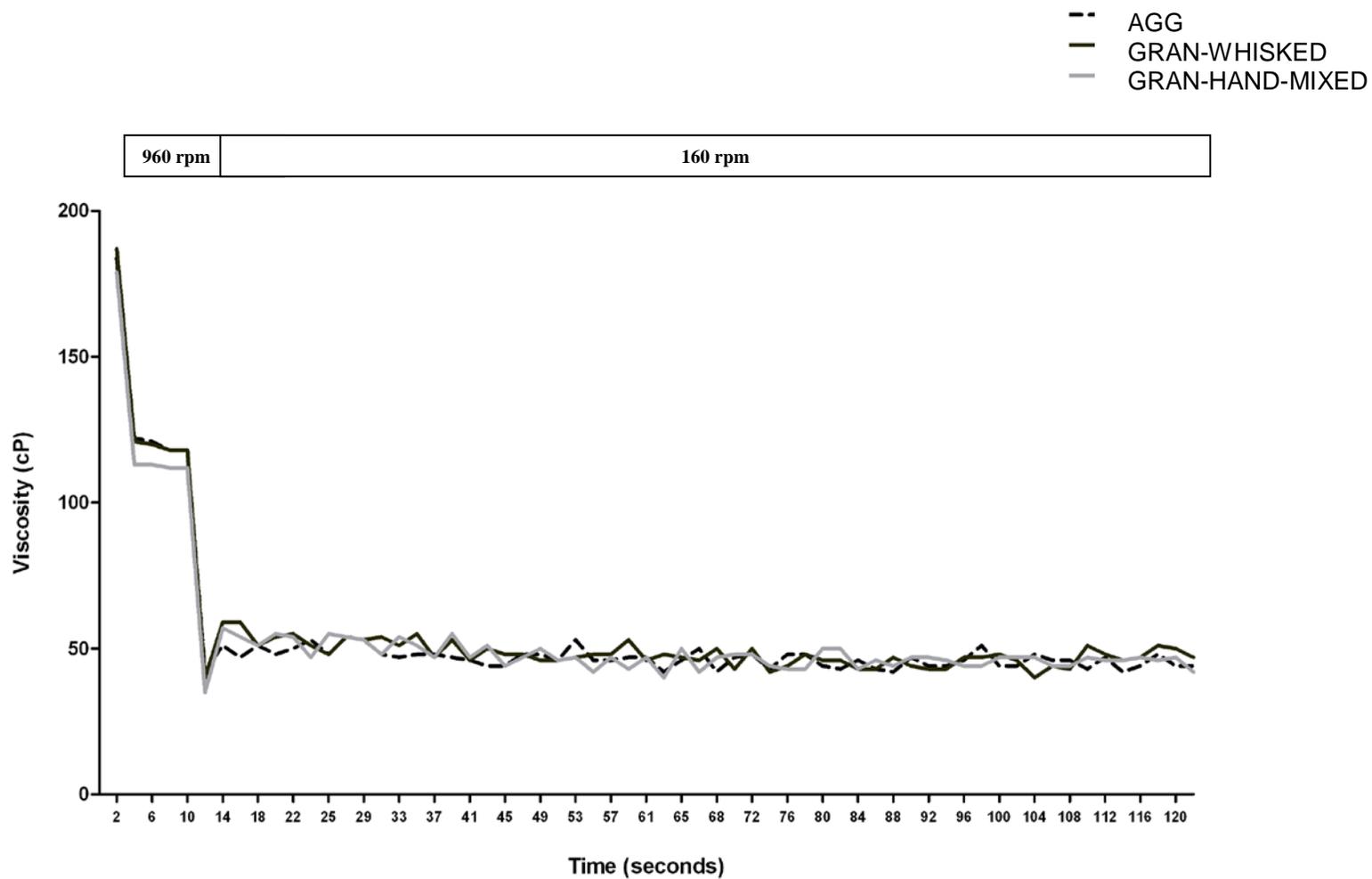
**Table 6.1** SEC-MALLS results

## 6.4.2 Rheological measurements

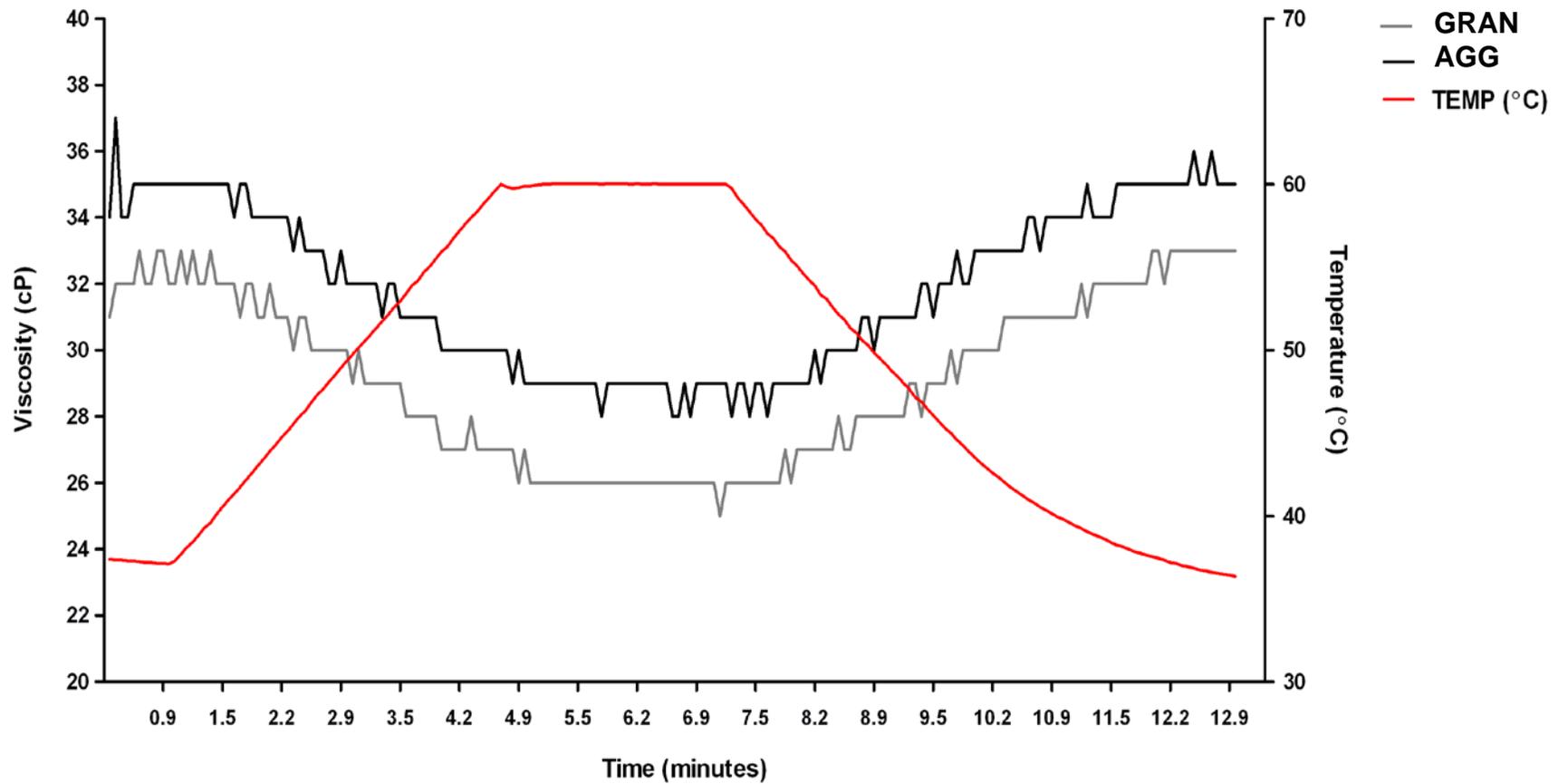
### *Experiment 1*

The viscosity of a 15% solution was compared between AGG and GRAN and concurrently between 2 methods of formulating the granulated polymer (mechanical whisk and hand-mixing). The profile of the RVA initiated by stirring at 960 rpm for 10 sec which corresponds to the high initial velocity of ~180 centipoise (cP), after which a steady state of stirring began at 160 rpm (Figure 6.1). This state corresponded with an average viscosity of  $47 \pm 2.9$  cP and  $47 \pm 4.2$  cP for the AGG and whisked GRAN solutions respectively and  $48 \pm 4$  cP for the hand mixed GRAN solution.

The effect of heating and cooling the agglomerated and granulated polymers on viscosity was then investigated using a pre-programmed starch profile on the RVA consisting of 13 min constant stirring at 160 rpm (Figure 6.2). Increasing the temperature from 37°C to 60°C had a similar effect on both polymers by decreasing viscosity slightly from 32 to 26 cP (GRAN) and 35 to 29 cP (AGG). A subsequent decrease in temperature back to 37°C restored viscosity levels back to the initial level in both polymers.



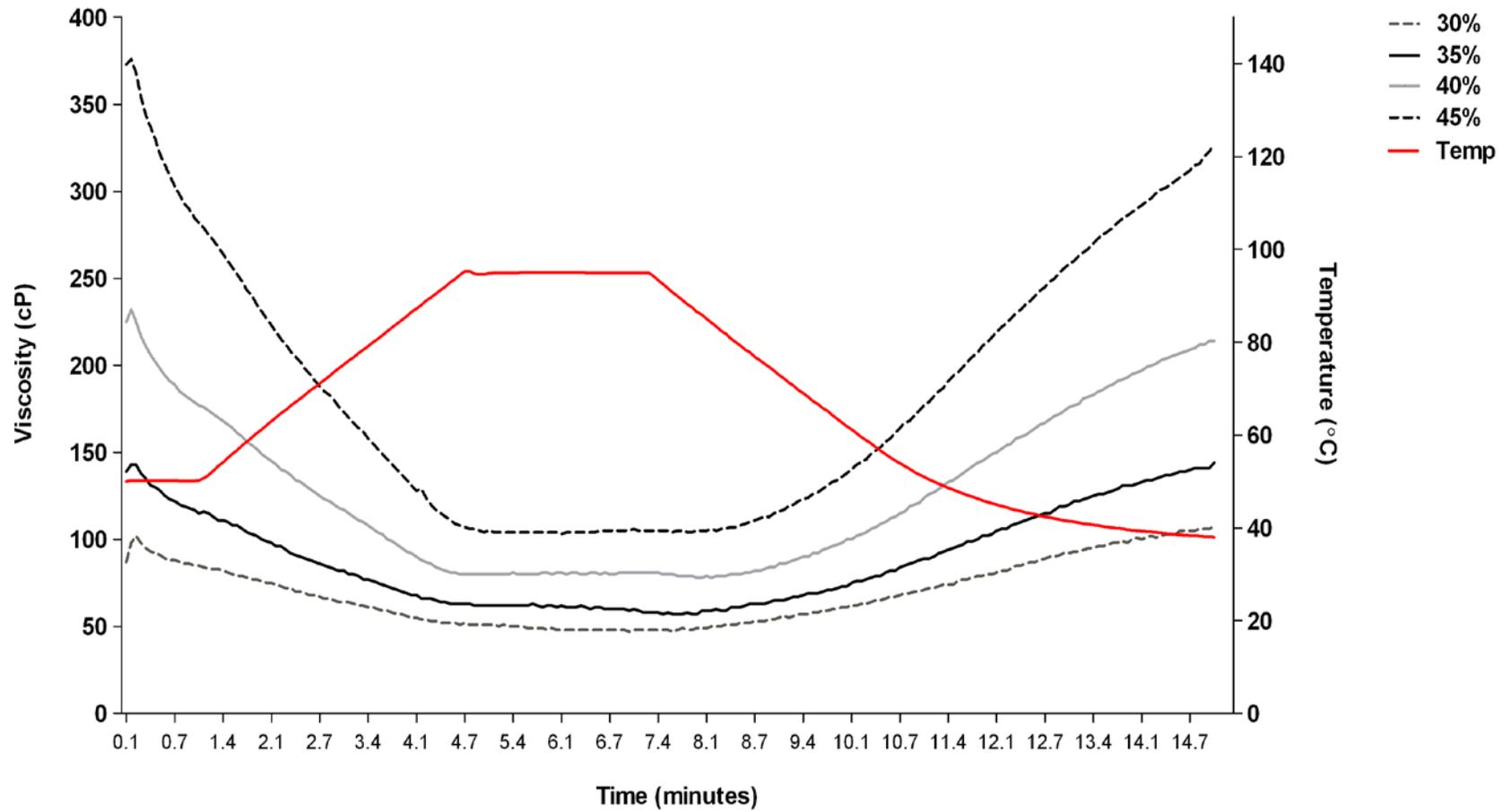
**Figure 6.1** Viscosity of an agglomerated (AGG) and a granulated (GRAN; hand-mixed and mechanically mixed) version of a HMW glucose polymer as measured with an RVA.



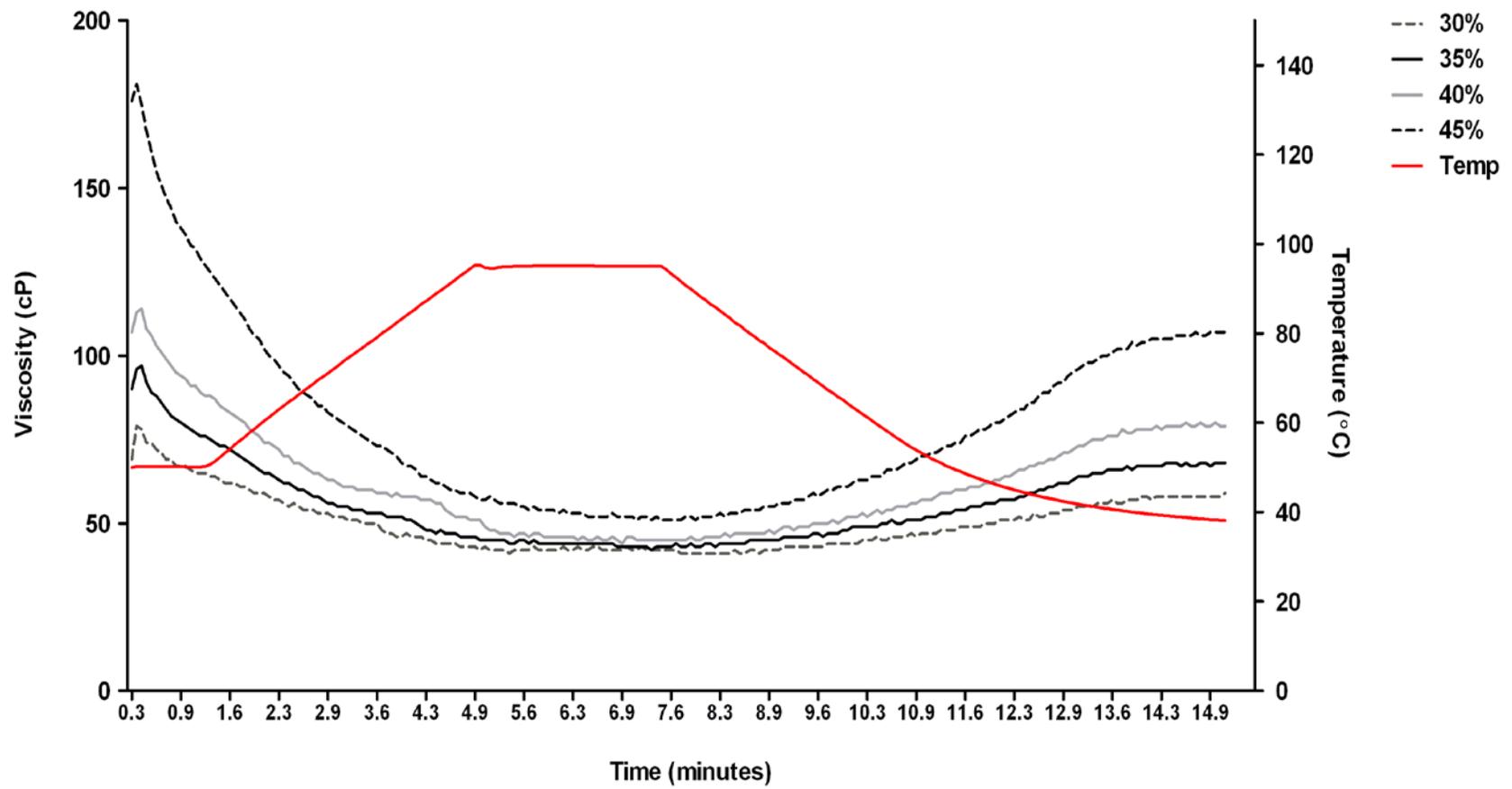
**Figure 6.2** Changes in viscosity of a granulated (GRAN) and agglomerated (AGG) version of a HMW glucose polymer due to heating and cooling

### *Experiment 2*

The RVA was then used to determine a viscosity-concentration relationship for GRAN (Figure 6.3) and a commercial potato starch (Figure 6.4). The potato starch product was utilised as a representative of the primary native starch version of the HMW glucose polymer as described in the patent application. The standard starch RVA profile was used on 4 increasing concentrations and an inverse relationship was observed between temperature and viscosity. Increasing the concentration of the solution from 30 to 45% increased initial viscosity and increasing the temperature from 50°C to 95°C concurrently decreased viscosity in both the HMW glucose polymer and the potato starch indicating slight degradation. As the temperature returned to 50°C the viscosity returned towards a level approaching the initial measure at this temperature. However it was notable that the potato starch did not recover to this level to the same extent. At the highest concentration (45%) the final viscosity at 50°C was 11% lower than the initial viscosity at the same temperature for the granulated HMW glucose polymer compared to the potato starch which was 38% lower.



**Figure 6.3** Changes in viscosity of a granulated version of a HMW glucose polymer due to heating and cooling at a range of concentrations

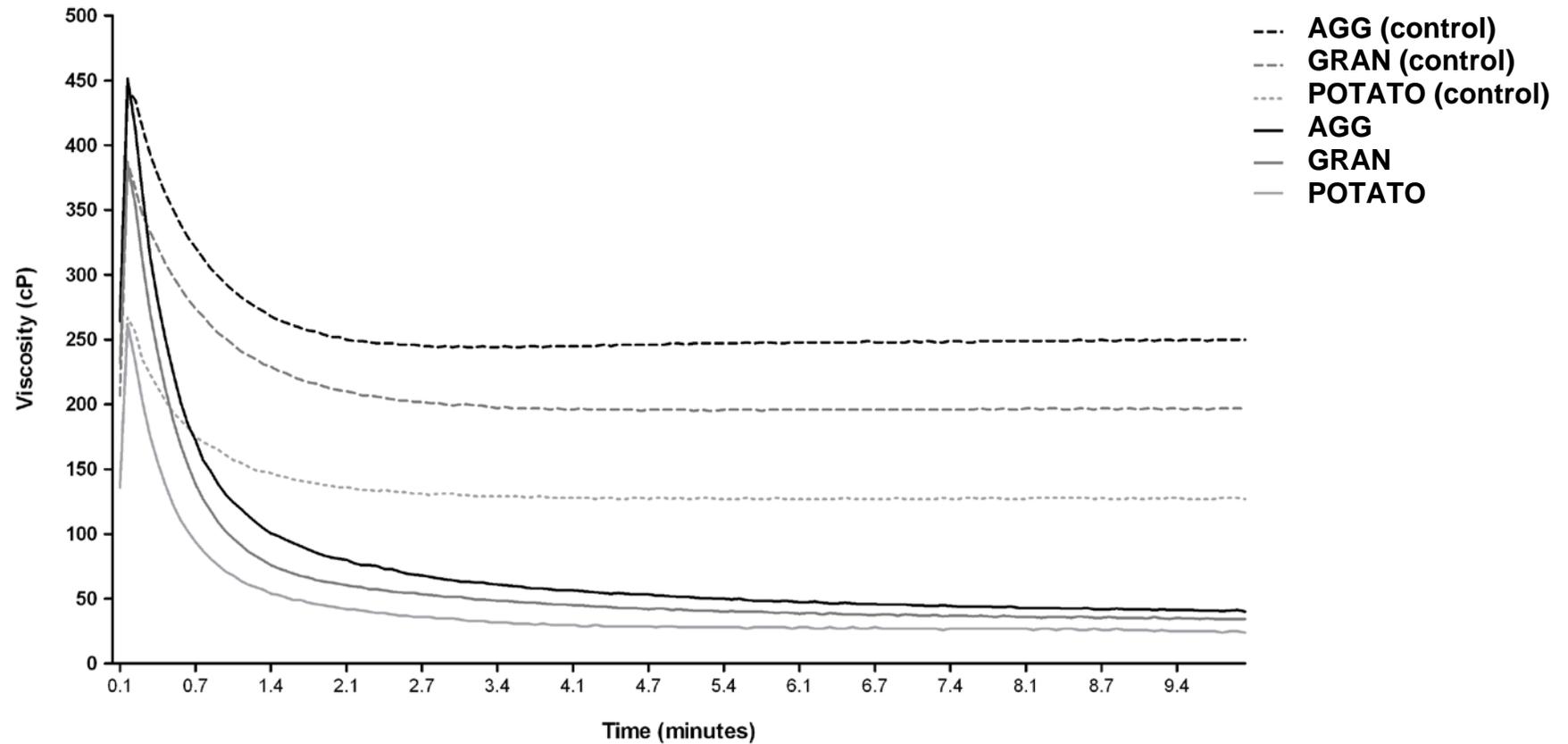


**Figure 6.4** Changes in viscosity of a potato starch preparation due to heating and cooling at a range of concentrations

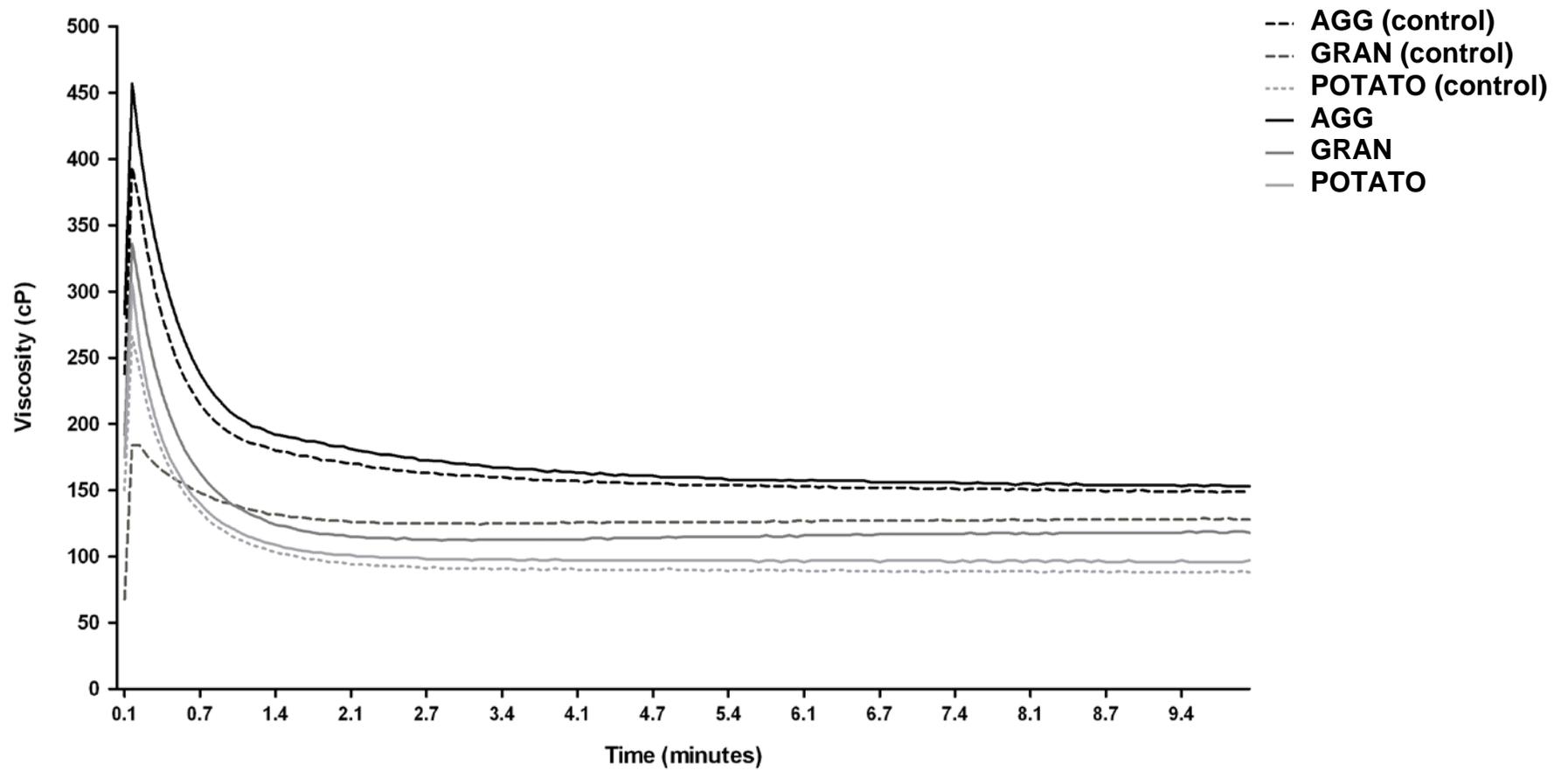
### *Experiment 3*

The addition of amylase to a 40% solution of GRAN, AGG and the potato starch caused a reduction in viscosity evident immediately that continued declining throughout the 10 min measurement period (Figure 6.5). Aside from a brief peak and decline in viscosity observed due to the initiation of the mixing action, the level observed when no enzyme was added (CONTROL) was higher compared to the amylase conditions. The average viscosity during the final minute was 249.8 cP (AGG), 196.8 cP (GRAN) and 127.4 cP (POTATO) in the control conditions, in comparison to the solutions subjected to amylase where viscosity had reduced in all conditions to 41.3 cP (AGG), 34.9 cP (GRAN) and 25.1 cP (POTATO). Although there was a clear distinction in the control viscosity, the similar viscosity seen at the end of the experiment in the 3 solutions that were degraded by amylase implies that starch hydrolysis was complete in all conditions.

In contrast to amylase, the addition of a pure cellulase did not alter viscosity in any of the conditions (Figure 6.6). Viscosity in the final minute was unchanged between the cellulase trial and the control trial for AGG (154 vs. 149 cP), GRAN (118 vs. 128 cP) and POTATO (96 vs. 88 cP).



**Figure 6.5** Changes in viscosity of a granulated (GRAN) and agglomerated (AGG) version of a HMW glucose polymer compared to a potato starch preparation with (control) and without the addition of amylase



**Figure 6.6** Changes in viscosity of a granulated (GRAN) and agglomerated (AGG) version of HMW glucose polymer compared to a potato starch preparation with (control) and without the addition of cellulase

## 6.5 Discussion

In order to ascertain if there were any fundamental differences in the physical composition of the granulated and agglomerated HMW glucose polymers, further work was conducted to investigate the rheological properties of the products. Molecular size of the polymers was purported to be between 500,000 and 700,000  $\text{g}\cdot\text{mol}^{-1}$ , however analysis using the SEC-MALLS system determined the weight average molecular weight to be higher at 853,000  $\text{g}\cdot\text{mol}^{-1}$  for the AGG and 1,040,000  $\text{g}\cdot\text{mol}^{-1}$  for GRAN. Thus, altering the native starch and agglomerating the powder of the HMW glucose polymer appears to have further increased molecular weight. The method of constructing the solutions (mechanically vs. manually) did not affect measurements of molecular weight or intrinsic viscosity.

The viscosity of a fluid is a measure of its resistance to flow and the viscosity behaviour of a macromolecular substance in solution is a common approach for physical characterisation. Viscometry can be investigated both at the 'Rheology' or more concentrated end which informs about structure of gels and suspensions or at the more dilute end that determines intrinsic viscosity and the molecular properties (Harding 1997). Intrinsic viscosity is defined as the capability of a polymer in solution to enhance the viscosity of a solution and increases as a function of increasing molecular weight of the solution. Intrinsic viscosity can be used to determine the average molecular weight and degree of polymerisation. The values of intrinsic viscosity for GRAN and AGG were calculated in parallel with the molecular weight and were not different suggesting a similar structure of the polymer molecules.

Rheology analysis of the 2 versions of the HMW glucose polymer was conducted initially to assess if there were differences in a concentrated solution (15%) and whether mixing the solution by hand altered the consistency and therefore the viscosity (granulated only). Viscosity measured by a RVA was near-identical for the AGG and GRAN solutions and between the GRAN solutions mixed by hand or whisk. There was also a similar pattern seen in viscosity when samples of the agglomerated and granulated HMW glucose polymers were heated to 60° and then cooled suggesting both versions behave similarly in response to temperature deviations. Prolonged heating and stirring can dissolve starch thereby reducing viscosity and altering the rheology. When starch is subsequently cooled for a long enough period it will thicken or gel (retrogradation) and re-arrange itself again to a more crystalline structure. These observations indicate an absence of retrogradation as the viscosity recovers to its initial level in both glucose polymers after heating and cooling. This was confirmed using Differential Scanning Calorimetry (DSC) which measures thermal transitions of polymers to record the presence of melting or crystallisation. This technique was run on samples of the agglomerated and granulated HMW glucose polymers and did not indicate the presence of either endothermic or exothermic events (data not shown) suggesting the polymer is amorphous in structure and does not alter its configuration with extremes of temperature.

The temperature dependence of liquid viscosity in GRAN and AGG was first demonstrated with the RVA experiment that increased temperature up to 60° and showed a concomitant decrease in viscosity. This relationship was again demonstrated at an increasing range of concentrations with the granulated

HMW glucose polymer in comparison to a potato starch polymer. The potato starch represented a low DE (<5) maltodextrin with a common native starch to the initial preparation of the unique HMW glucose polymer that is now unavailable. The initial viscosity of the potato starch was markedly lower than the HMW glucose polymer and although both followed a similar pattern of viscosity in response to an increase in temperature, the potato starch was unable to recover viscosity to the same level upon cooling. The low viscosity due to cooling suggests some retrogradation, indeed it was visually observed that when left at room temperature the potato starch formed a thick, glutinous paste whereas the HMW glucose polymer remained as a liquid. The amylose content of starch is the dominant factor for short-term retrogradation as when dry native starch is suspended in water and heated the granules absorb water and swell causing amylose to leach out and gelation to occur (Miles et al., 1985; Orford et al., 2006). Subsequently upon cooling the structure is partially re-formed.

Starch is extracted from a variety of tuberous plants as well as cereal grains and the physical properties are related to the structural characteristics of the granules including granule size, distribution and the ratio of amylose to amylopectin (Wischmann et al., 2007). In gelatinised unmodified starch the viscosity is related to granule size which for potato is higher (~10-70  $\mu\text{m}$ ) than for cereal starches such as corn (5-25  $\mu\text{m}$ ), however it should be appreciated that even though the molecular weights of the processed starches may seem high (Table 6.1), in preparing these materials the structure of the original starch granules will be lost and the polysaccharide will be extensively degraded. Although the organisation of the original starch will be lost, the difference in

ratio of amylopectin to amylose would be expected to be important. All starch is comprised of the essentially linear fraction amylose and the highly branched fraction amylopectin, the proportions of which will vary with plant species (Stawski 2008). The ratio of the starch components influences various physicochemical properties such as swelling capacity and water solubility (Sandhu et al., 2005). Amylose and amylopectin content changes with the origin of starch however, normal starch consists of about 75% amylopectin and 25% amylose and potato starch has been reported to range between 12 and 20% (Talja et al., 2008), 18 and 24% (Karim et al., 2007) and 27% (Stawski 2008). Amylose is digested slower than amylopectin therefore blunting blood glucose concentrations after a meal high in amylose (Holt and Miller 1995). The HMW glucose polymer was noted to contain 78% amylopectin and 22% amylose in its initial version used in earlier studies (Leiper et al., 2000; Piehl Aulin et al., 2000), however its most current version is derived from amylopectin barley starch thus would be expected to digest faster than a starch containing amylose (Morita et al., 2007). Similar blood glucose and serum insulin concentrations were observed in the study in Chapter 3 between a HMW and LMW glucose polymer suggesting a similar pattern of digestion and absorption. Thus the starch proportions of the HMW glucose polymer may not have been a primary determinant of postprandial glycaemic responses and other factors need to be considered.

The role of starch chemical properties in digestibility is not yet fully understood and as the evolution of the HMW glucose polymer to its current formulation has involved altering the native starch used, it may be prudent to consider factors affecting the bioavailability of starch. The rate and extent of

carbohydrate digestion are principal determinants of postprandial glucose and insulin responses in healthy humans (Wolever and Bolognesi 1996; Wolever and Mehling 2003), moreover, rapidly digestible starch as determined by in vitro rates of hydrolysis provokes more rapid, greater changes in these responses compared to more slowly digestible starch (Seal et al., 2003; Ells et al., 2005). The physicochemical properties of a food such as the botanical origin and degree of food processing will largely determine the gastrointestinal handling and subsequent utilisation of carbohydrate (Englyst and Englyst 2005). These characteristics relate to the type and integrity of the starch granules and the structural properties of the food matrix. Subsequently, the glycaemic response is due largely to the rate at which starch is absorbed during transit through the small intestine and the level of dispersion that can be achieved in the food matrix to aid this process. It was shown that treatment of both HMW glucose polymer solutions with amylase induced a similar decline in viscosity indicating similar susceptibility to enzymatic degradation and the blood glucose profiles suggest that both are rapidly absorbed thus it is unlikely that they differ much structurally. The addition of cellulose did not alter viscosity indicating an absence of  $\beta$  1-4 bonds, however since it is claimed in the original patent that novel bonding including  $\beta$  1-4 and  $\beta$  1-6 bonds are present this would suggest that the latest versions of the product have deviated away from its original formulation. Taking the observations from this study in respect to molecular weight and viscosity the studied formulations of the HMW glucose polymer do not appear to be substantially different. An obvious difference between the different versions of the HMW glucose polymer between previous investigations is the type of starch used. Considering the

susceptibility of different starches to enzyme hydrolysis can vary (Kim et al., 2008), and that potato starch granules have a lower viscosity and greater swelling ability resulting in a lower temperature for retrogradation, this might affect the digestibility. Indeed, retrogradation was observed in the potato starch after heating and the effects of this on intestinal absorption are not clear. Further work would be required to monitor the effects of viscosity and gel-forming ability of ingested carbohydrate solutions on gastric emptying and glucose absorption.

In summary, the modifications to improve solubility that have been applied to the HMW glucose polymer do not appear to have affected the viscosity of the product or altered the physical properties. In addition the HMW glucose polymer appears to have a higher molecular weight than first thought. In Chapter 5 the postprandial glycaemic responses following ingestion of both an granulated and agglomerated version of the HMW glucose polymer were similar, although there was a trend for a more rapid early insulin response (<55 min of recovery). The study in Chapter 3 demonstrated similar work output in a secondary exercise bout when muscle glycogen at the onset of the activity was near-identical. A dependence on muscle glycogen as the primary substrate for energy production during moderate-high intensity exercise has been demonstrated (Romijn et al., 1993; van Loon et al., 2001), thus it can be assumed that performance in the exercise test would be reliant on muscle glycogen reserves. In the present study no effect was observed on exercise performance after a short recovery period between the HMW glucose polymers but both were enhanced above a control solution. In addition, blood glucose and insulin concentrations were not different between the two HMW glucose

polymers over the 2 hr recovery period. Thus muscle glycogen availability at the onset of the second exercise bout, although not measured, can be implied to be similar. In conclusion, the two versions of the HMW glucose polymer elicited different insulinaemic responses immediately post-exercise but not overall during a 2 hr recovery period and no differences in blood glucose concentration were observed. Therefore the differences in postprandial glucose and insulin responses and in exercise performance between the study described in chapter 3 and that of Stephens et al (2008) cannot solely be explained by changes in the manufacturing process. Deviations away from the properties of the original starch which may have as yet undiscovered favourable effects on digestion and absorption of the starch could be at least partly responsible for the differences between studies.

## **Chapter 7**

### **General Discussion**

## 7.1 Overview of thesis

Numerous work has sought to enhance the rate of short term muscle glycogen resynthesis by means of manipulating the type, form and frequency of carbohydrate feeding (Jentjens and Jeukendrup., 2003). A common method is to feed carbohydrate in association with insulinotropic amino acids to augment the plasma insulin response in parallel with carbohydrate availability (van Hall et al., 2000; van Loon et al., 2001; Ivy et al., 2002). As muscle glycogen synthesis proceeds only minimally without increases in circulating blood glucose and insulin concentrations (Ivy et al., 1998) it follows that increasing the magnitude of responses would be beneficial for accelerating muscle glycogen resynthesis. However with research studies that have fed carbohydrate and protein together it appears that there is only an enhanced effect present when sub-optimal ( $<0.8\text{g}\cdot\text{kg}^{-1}\text{ bw}$ ) levels of carbohydrate are fed (van Loon et al., 2000; van Hall et al., 2000; Jentjens et al., 2001; Betts et al., 2010). Thus glucose availability is limiting and increases in glucose with the concurrent increasing insulin concentration is crucial for muscle glycogen resynthesis. The studies in this thesis have sought to further investigate the potential for a unique high molecular weight ( $500,000\text{-}700,000\text{ g}\cdot\text{mol}^{-1}$ ) glucose polymer to enhance recovery of muscle glycogen during a short recovery from glycogen-depleting exercise. To this end, *in vivo* healthy volunteer studies were completed, the key observations of which will be discussed here. Previous observations regarding feeding the HMW glucose polymer reported a 70% greater muscle glycogen content at 2 hr post exercise above that of LMW glucose solution (Piehl-Aulin et al., 2000) . It was

subsequently reported that this effect may be due to a more rapid gastric emptying rate achieved with the HMW glucose polymer that resulted in greater carbohydrate delivery to the small intestine in the first 10 min following ingestion (Lieper et al., 2000). As this indicates that carbohydrate delivery is enhanced after ingestion of the HMW glucose polymer, it follows that recovery of exercise capacity would be improved due to a greater magnitude of muscle glycogen resynthesis during recovery from prolonged exercise. Indeed Stephens et al (2008) did demonstrate an enhanced exercise performance (10%) after ingestion of the HMW glucose polymer compared to an isoenergetic glucose polymer with a markedly lower molecular weight ( $\sim 900 \text{ g}\cdot\text{mol}^{-1}$ ) when performed 2 hr following glycogen-depleting exercise. It was implied that muscle glycogen availability was greater due to a significantly higher rate of rise in blood glucose and serum insulin in the initial 40 min of recovery. It was therefore of interest to quantify pre-exercise muscle glycogen content as a result of HMW glucose polymer ingestion to correlate with an improved exercise work output. However the results of Stephens et al (2008) were not replicated in the study described in Chapter 3 despite identical experimental procedures being performed using similar volunteers. There were no differences in blood glucose and serum insulin concentration in addition to similar muscle glycogen resynthesis in 2 hr with the same solutions. As expected exercise performance was not different in the subsequent exercise bout as glycogen was near-identical at the onset of the test ( $\sim 142 \text{ mmol}\cdot\text{kg}^{-1}$ ). Muscle glycogen degradation during exercise at 70%  $\dot{V}O_2$  max has been reported to be  $\sim 2 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (Betts et al., 2008) which would equate to  $\sim 30\text{-}40 \text{ mmol}\cdot\text{kg}^{-1}$  depending on body weight during the 15 min exercise

performance test. This magnitude of utilisation was demonstrated in the study described in Chapter 3 thus the improvement in exercise performance with glucose polymers compared to control may be due to increased blood glucose oxidation, increased hepatic output or an influence from an increased central drive. Further work would be warranted to determine substrate utilisation from endogenous and exogenous sources during the secondary exercise bout.

It was suggested that the large sampling interval may have missed early post-exercise differences in muscle glycogen. Muscle biopsy studies have typically biopsied at 2 hr intervals or more (Ivy et al., 2002), and so it was suggested that ultra-high field  $^{13}\text{C}$  MRS may be able to monitor early changes in muscle glycogen with less temporal resolution. A pilot study reported in Chapter 4 found that the between subject variability in muscle glycogen was greatly reduced compared to a similar study using a lower field strength (Casey et al., 2000) therefore it was suggested that ultra-high field spectroscopy would be a suitable methodology to use in temporal monitoring of muscle glycogen. Up to 1 hr post glycogen-depleting exercise there were no differences apparent in muscle glycogen between ingestion of the HMW and LMW glucose polymers and a zero-energy control. Furthermore only marginal increases were noted in the 3 trials. Considering muscle biopsy studies have shown muscle glycogen resynthesis during the first hour post-exercise with carbohydrate feeding (Battram et al., 2005; Pedersen et al., 2008) this result was unexpected. It was then postulated that the subject positioning within the scanner may have inhibited typical gastric emptying patterns and thus compromised carbohydrate delivery to the muscle. To date there is some research regarding the effects of posture on the emptying of liquid and solid meals (Anvari et al., 1997; Spiegel

et al., 2000), however the influence of a prone posture as adopted in Chapter 4 has not yet been studied.

In conflict with suggestion from previous work (Stephens et al., 2008) it appears that ingestion of the HMW glucose polymer does not augment muscle glycogen resynthesis during recovery from prolonged exercise compared to a LMW glucose polymer. A noteworthy disparity between the study of Stephens et al (2008) and the present research was the pattern of postprandial glucose and serum insulin response. There was not a greater rate of rise in blood glucose and plasma insulin after ingestion of the HMW compared to the LMW glucose polymer, suggesting that there may be a difference in the HMW glucose polymer that was used. It was reported that the HMW glucose polymer used in the study by Stephens et al (2008) was manufactured using a granulation process for powder production compared to the most recent versions used in the studies reported in Chapters 3 and 4 which in contrast was produced via agglomeration. Further investigation was carried out to directly compare the post-exercise blood glucose and serum insulin concentrations during recovery from post-exhaustive exercise when ingesting both the granulated and agglomerated versions of the HMW glucose polymer. Although blood glucose response was near-identical with both versions, a greater insulin response was noted in the first 55 min of recovery for the granulated version. However, exercise performance was similar and absolute concentration of insulin was not different between the granulated and agglomerated versions. Thus the alterations in manufacturing are not sufficient to fully explain potential differences in the *in vivo* carbohydrate bioavailability as suggested by previous research and the studies reported in this thesis. Indeed further analysis

of the HMW granulated and agglomerated versions did not show any differences in viscosity, molecular weight or degradation by enzymes. Accordingly there is not strong evidence to suggest that the HMW glucose polymer has markedly differed in consistency or in its *in vivo* postprandial responses. However the versions used in this analysis were both derived from barley starch whereas the version used by Stephens et al (2008) originated from corn starch therefore they cannot be directly comparable to the latter. It is therefore still unconfirmed and meriting further study whether the deviation of the HMW glucose polymer away from various botanical origins may have caused subsequent variation in *in vivo* responses.

## **7.2 Key research questions**

At the beginning of the thesis the following questions were posed.

*Does consumption of a unique, high molecular weight glucose polymer elicit enhanced muscle glycogen resynthesis after a short time period as a result of an augmented glucose and insulin response in comparison to an isoenergetic low molecular weight glucose polymer?*

Considering that Stephens et al (2008) reported an improved exercise performance in all eight participants after two hours recovery from glycogen-depleting exercise and ingestion of a very high compared to a low molecular weight glucose polymer it can reasonably be assumed that muscle glycogen availability at the onset of exercise was higher. The release of insulin in response to an oral glucose load stimulates post-exercise muscle glycogen resynthesis by mediating a decrease in the extent of phosphorylation of glycogen synthase thereby increasing the proportion of the enzyme in its active form and promoting an increased permeability of the muscle membrane to glucose transport (Cohen et al., 1978; Ivy and Kuo 1998; Maarbjerg et al.,

2011). For that reason an elevated postprandial glucose and insulin response would be favourable during recovery from glycogen-depleting exercise (Richter et al., 1989; De Bock et al., 2005; Beelen et al., 2010) when increased fat oxidation indicates that resynthesis of muscle glycogen is a metabolic priority (Kiens and Richter 1998; Kimber et al., 2003). Taking the observations of studies that have augmented postprandial insulin levels via the ingestion of carbohydrate with protein yet have not demonstrated concomitant enhanced muscle glycogen resynthesis above carbohydrate ingestion alone (van Hall et al., 2000; van Loon et al., 2000; Jentjens et al., 2001), it appears that increasing insulin concentration independently is not effective for muscle glycogen recovery. Accordingly, a simultaneous delivery of glucose with higher carbohydrate intake ( $>1 \text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) and increased circulating insulin concentrations may be more effective during recovery.

Stephens et al (2008) reported that the rate of rise in both blood glucose and serum insulin over the first hour of recovery from glycogen-depleting exercise was higher after ingestion of the HMW glucose polymer. However this pattern was not replicated in the study in Chapter 3 despite involving identical exercise protocols that were completed with similar volunteers. The study in Chapter 3 demonstrated a similar pattern in blood glucose and insulin response over a two hour recovery, thus muscle glycogen was near-identical 2 hr post-ingestion between the LMW and HMW glucose polymers. Given that a clear difference between the study by Stephens and colleagues and the study in Chapter 3 exists in the pattern of postprandial responses it can be deduced that effects on digestion and absorption of the ingested solution are likely to be different. The rate of hydrolysis of different types of starch has been shown to correlate with

postprandial glycaemic responses *in vivo* and *in vitro* (Bjorck et al., 1994; Berti et al., 2004; Gee and Johnson 2006). Thus enzymatic hydrolysis of ingested starch can be a rate limiting factor for carbohydrate digestion due to the physicochemical properties of the starch affecting the susceptibility to amylase (Slaughter et al., 2001). Reasons for differing rates of starch digestion include particle size (Jenkins et al., 1988), degree of gelatinisation (Bruen et al., 2011), the ratio of amylose to amylopectin (Bjorck et al., 1994) and various process methods that influence starch structure. Analysis, in chapter 6, of two versions of the HMW glucose polymer that were thought to differ in solubility did not demonstrate any differences in molecular weight, viscosity or degradation in response to amylase. A trend for a greater insulin response was observed with an earlier less soluble, granulated version of the HMW glucose polymer *in vivo* although this was not translated into an improvement in performance in a second exercise bout in the study in chapter 5. Accordingly, as the first formulations of the polymer were derived from potato starch, it is possible that retrogradation effects and a lower viscosity may have favourable effects on post-exercise starch ingestion.

*Is early phase, post-exercise muscle glycogen resynthesis different when consuming the high or low molecular weight glucose polymers?*

Depletion of muscle glycogen during exercise activates glycogen synthase (Nielsen and Richter 2003), an effect which is exacerbated further when the magnitude of depletion is higher resulting in a faster rate of glycogen resynthesis in the immediate post exercise period (Price et al., 1994). The majority of studies have only measured post exercise muscle glycogen resynthesis after time intervals of 1 or 2 hr (for review see (Jentjens and

Jeukendrup 2003; Betts and Williams 2010), thus measures of muscle glycogen at smaller time resolutions earlier in post exercise may be valuable. The utilisation of  $^{13}\text{C}$  MRS has potential for non-invasive repetitive determination of muscle content (Ivy et al., 2002), however at very low levels the visibility of the glycogen molecule appears to be compromised. Muscle glycogen resynthesis with the provision of carbohydrate is more effective than if carbohydrate is restricted (Ivy et al., 1988), therefore as no distinction between muscle glycogen content over a period of 60 min was found between ingestion of the HMW, LMW or an energy-free control solution in chapter 4 implies that other factors may be affecting the measurement.

*Is a subsequent bout of maximal effort exercise following a short recovery from prior glycogen-depleting exercise improved after consumption of the high molecular weight glucose polymer above that of the low molecular weight glucose polymer?*

Exercise performance in a secondary bout following a prolonged exercise session has been previously correlated with muscle glycogen recovery during a short recovery period (Williams et al., 2003). Time to fatigue during a moderate to high prolonged exercise is related to the pre-exercise muscle glycogen (Bergstrom et al., 1967) and during high-intensity exercise there is a rapid rate of glycogenolysis (Gaitanos et al., 1993), thus the ability to sustain performance in a maximal cycling performance test would be expected to be limited by muscle glycogen availability (Widrick et al., 1993; Balsom et al., 1999). A validated time trial is thought to be a more sensitive measure of exercise performance compared to exercise to exhaustion protocols (Jeukendrup et al., 1996). However it is interesting to note that two studies in this thesis employed exhaustive exercise protocols to deplete endogenous

muscle glycogen stores and demonstrated similar overall time (~90-100 min) in all conditions in both studies. Assuming that individual baseline muscle glycogen levels were not markedly different and not supercompensated, this would imply that exhaustive exercise is sensitive to muscle glycogen levels as fatigue coincided with glycogen depletion (Chapter 3). It could thus present an alternative to the 'all-out' maximal test in ascertaining recovery of endurance capacity. Accordingly, an improved exercise performance in a subsequent exercise bout after a short recovery with carbohydrate ingestion has been demonstrated when exhaustive exercise protocols in the second bout have been employed (Wong and Williams, 2000; Wong et al., 2000). However, increased muscle glycogen storage during a short recovery has not been associated with an improved exercise time to exhaustion in a second bout (Fallowfield et al., 1995; Casey et al., 2000). Thus as the high intensity 'maximal' exercise protocol is highly reliant on muscle glycogen availability, in conditions when the time available for muscle glycogen recovery is limited, it would be expected to accurately reflect differences in substrate availability. Exercise performance by all subjects in the study by Stephens and colleagues was improved by an overall 10% after ingestion of the HMW glucose polymer, whereas exercise performance was only enhanced with both glucose polymers above that of a carbohydrate-free control as described in Chapter 3. This magnitude of improvement was at a comparable level with the LMW glucose polymer in Stephens et al (2008), however any further enhancement with the HMW glucose polymer was absent. Considering muscle glycogen was the same for both HMW and LMW in the study described in Chapter 3 this is not unexpected.

### 7.3 Limitations and directions for further research

Importantly, the work in this thesis suggests that the HMW glucose polymer does not appear to convey any benefit to aid recovery of muscle glycogen above that of a standard commercial glucose polymer. Whether this is due to alterations that have been made to the HMW polymer remains to be elucidated, however it is not likely to be due to differences in the manufacturing process to improve solubility. A major limitation for the work was the inability to compare versions of the HMW glucose polymer that differed in native starch. As the properties of starches vary considerably between different sources in regards to particle size, swelling properties, viscosity and gelling behaviour (Swinkels et al., 1985) it is possible that *in vivo* responses can vary as a result. Thus any future investigation to be conducted in post exercise feeding of the HMW glucose polymer should take into account the gradual alterations in the product. It would be advantageous to return the HMW glucose polymer to its original form to investigate a similar experimental protocol to Chapter 3 as considering the results from Stephens et al (2008) it would be expected that muscle glycogen resynthesis may be enhanced above that of a LMW weight glucose polymer. Further investigation is also warranted as to the *in vivo* postprandial blood glucose and serum insulin responses as a result of feeding starches from different origins. On the possibility that the HMW glucose polymer could be returned to its original formulation, further investigation would be warranted into the post-exercise gastric emptying rate and intestinal absorption following ingestion to further assess carbohydrate availability. Although the available literature on gastric emptying during exercise is considerable, there appears to be little information available regarding post

exercise gastric emptying. Additionally considering the potent effect of incretin hormones on insulin secretion, there appears to be potential value in ascertaining the effect of HMW glucose polymer ingestion on glucose delivery and incretin release.

The work in this thesis has also utilised MRS at an ultra-high field to monitor temporal changes in muscle glycogen resynthesis. The study described in Chapter 3 reported no difference in muscle glycogen resynthesis between ingestion of the HMW and LMW glucose polymer, nonetheless both were markedly higher compared to a control solution in contrast to the study described in Chapter 4. Accordingly it was reported that increases in the magnitude of muscle glycogen content with 100g of carbohydrate feeding compared to a zero-energy control were not as expected in light of results from muscle biopsy studies. Potentially the method of measurement itself may be influencing physiological processes that facilitate post-exercise muscle glycogen resynthesis. As noted previously, there is currently no information available as to the effect of a prone posture on gastric emptying. A method of measuring the emptying rate of liquids using MRI has been previously reported with good reproducibility (Fruehauf et al., 2011), thus further work could consider measuring the gastric emptying rate of liquids in a supine and prone position between carbohydrate-containing and energy-free solutions.

It has been shown that post-exercise muscle glycogen resynthesis is most rapid in the first 30 min post exercise (Price et al., 1994; van Hall et al., 2000). Considering that a greater serum insulin response was seen in the first hour post-exercise in Chapter 5 and Stephens et al (2008) noted greater rates of rise in both glucose and insulin within 40 min post-exercise, it may be interesting to

compare muscle glycogen resynthesis and recovery of exercise capacity with HMW glucose polymer (original formulations) ingestion when recovery time is <1 hour.

#### **7.4. Conclusions**

The rate of post-exercise muscle glycogen resynthesis will dictate the time required to recover from prolonged, glycogen-depleting exercise, thus when the time available for recovery is compromised such as in many athletic competitive contexts optimising the rate of muscle glycogen resynthesis is essential. Ingestion of a very HMW glucose polymer with a corresponding low osmolality did not enhance muscle glycogen resynthesis after a 2 hr recovery period from glycogen-depleting exercise compared to an isoenergetic LMW glucose polymer. However, a high rate of muscle glycogen resynthesis during a short recovery from glycogen-depleting exercise was reported, a level that has only been reported once previously in the literature (Pedersen et al., 2008).

A lack of an enhanced rate of rise in blood glucose and serum insulin levels indicates that properties of the HMW glucose polymer may have been lost. Evolution of the HMW glucose polymer from a potato starch based thicker solution to a more soluble amylopectin barley starch does not appear to have affected the starch structure however favourable effects on the bioavailability of glucose following ingestion may have been affected. Further investigation should focus on returning the HMW glucose polymer to its original formulation and directly comparing post-exercise responses to ingestion, particularly gastric emptying and muscle glycogen resynthesis.

Importantly, this research has been conducted in a relatively novel area regarding the HMW glucose polymer. As such the study by Stephens et al

(2008) remains the only investigation to report an improved exercise performance in a secondary exercise bout. The 'maximal' exercise test is self-paced thus is subject to individual motivation which may have influenced the result. Nevertheless the markedly enhanced blood glucose and insulin responses that were observed in Stephens's study were not replicated in this thesis. It is possible that there were different individual responses that could account for this result however it does not appear that there were any observable differences in the volunteer demographic or training status between the present study and that of Stephens et al (2008). Clearly additional investigation into the HMW glucose polymer would be beneficial to more robustly ascertain whether it has potential effects on post-exercise metabolism.

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## **Appendix 1**

## **Appendix 1: Procedure for Insulin assay (Coat-a-Count)**

**Principle:** Insulin is a polypeptide hormone secreted from the beta cells of the pancreas that facilitates the storage and production of glucose. Presence of insulin in the blood plasma is a direct response to the circulating blood glucose which will decline in parallel with decreasing blood glucose concentration. The plasma concentration of insulin is determined using an immunoassay where <sup>125</sup>I-labelled insulin competes with insulin within a sample for sites on an insulin-specific antibody. The antibody is bound to the walls of a polypropylene tube therefore decanting the tube terminates the competition and isolates the antibody-bound fraction of the radio-labelled insulin. The tube is counted in a gamma counter to create a number that can be converted via a calibration curve to an insulin concentration.

**Assay procedure:** The assay takes place over two days

### **Preparing the Calibrators (standards)**

Remove 1 set of calibrators A to G from the fridge and uncap the vials  
Label one set of eppendorfs A to G (6 per letter), include one labelled AB\*

Add 6 ml of deionised water to vial A and 3 ml to vials B to G then recap and mix the vials

Leave the vials to stand for 30 minutes before storing in a bag and freezing at -20°C.

These standards can be used for up to 30 days

\*If some of the samples are from fasting subjects, insulin will not be detected unless the lowest standard is approximately 3 mIU/L. An additional point on the standard curve can be created by mixing 1 ml each of vials A and B to produce AB.

## Day 1

Take 1 set of calibrators, a high and low quality control and the samples out of the fridge, put in a rack and leave to thaw

Make a note of the samples in the order in which they will be analysed

Remove the green antibody-coated tubes out of the fridge (1 per sample plus 20-22 additional for the calibrators and QC's). Label the tubes as follows;

- 2 plain tubes TC (total control)
- 2 plain tubes NSB (non-specific binding)
- 2 green tubes each for each calibrator (A to G)
- 1 green tube for low and high QC (beginning of assay)
- 1 green tube for each sample
- 1 green tube for low and high QC (end of assay)

Prepare the radioactive label. Take the vial of  $^{125}\text{-I}$  Insulin out of the fridge (1 vial is sufficient for 108 assay tubes). Measure out 100 ml of deionised water, add to the gel concentrate and swirl gently

Transfer the radioactive label to a beaker for pipetting

When the samples have thawed, mix each one by vortexing. Mix the calibrators and QC's by gentle inversion

Using a 200  $\mu\text{l}$  pipette, pipette the following;

- Nothing in the TC tubes
- 200  $\mu\text{l}$  of calibrator A into the NSB tubes and each A tube (A measures zero binding- total binding of labelled insulin)
- 200  $\mu\text{l}$  of each calibrator AB to G, QC's and samples into their corresponding tubes

Using an automatic pipette, add 1 ml of the radioactive label from the beaker to each tube

Cap the TC tubes and mix all the other tubes using a vortex

Cover the entire rack with cling film and leave at room temperature for 18-24 hours

## **Day 2**

Take out the TC tubes and put them in a spare rack

Lay out blue bench roll at several sheets thickness in a radioactive area and keep the tap running

Decant the rack over the sink then leave it to stand upside down for 2 minutes

Blot against the blue paper and then tap the rack firmly upside down repeatedly until no more moisture is visible on the paper. Turn the rack back up and blot the tubes

Put the TC's back into the rack and count the tubes on a gamma counter using programme 24. Check the standards are correct and edit if not

The gamma counter automatically plots the standard curve from the calibrators and calculates the insulin concentrations from the samples. If the samples are below the lowest standards the concentration can be calculated using a spreadsheet by assuming linearity continues at lower concentrations.

## **Appendix 2**



MR Volunteer Safety Screening Questionnaire:

|              |                  |                      |
|--------------|------------------|----------------------|
| NAME         | Date of Scan     | Date of Birth        |
| ADDRESS      | Volunteer Number |                      |
|              | Ethics Code      |                      |
| Phone number | Weight           | Height if applicable |

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? Y/N
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)* Y/N
4. Have you ever had any surgery? Please give brief details over. Y/N  
*(We do not need to know about uncomplicated caesarian delivery, vasectomy or termination of pregnancy)*
5. Do you have any foreign bodies in your body (e.g. shrapnel)? Y/N
6. Have you ever worked in a machine tool shop without eye protection? Y/N
7. Do you wear a hearing aid or cochlear implant? Y/N
8. Could you be pregnant? (Pregnancy tests are available in the female toilets) Y/N
9. Have you ever suffered from tinnitus? Y/N
10. Do you wear dentures, a dental plate or a brace? Y/N
11. Are you susceptible to claustrophobia? Y/N
12. Do you suffer from blackouts, epilepsy or fits? Y/N
13. Do you have any tattoos? Where? Y/N
14. Do you have any body piercing jewellery that cannot be removed? Y/N
15. Do you have any skin patches (trans-dermal patches)? Y/N
16. Do you have a coil in place (IUD) for contraception? Do you know what type? Y/N
17. Do you have any condition that may affect your ability to control your temperature (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease? Are you taking diuretics, beta-blockers, calcium blockers, amphetamines, muscle relaxants or sedatives?) Y/N
18. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc before entering the magnet hall.? *(lockers available by the changing rooms)* Y/N
19. Is there anything else you think we should know? Y/N

|   |              |
|---|--------------|
| <b>I have read and understood all the questions</b>     |              |
| <b>Signature:</b>                                       | <b>Date:</b> |
| Verified by:<br><b>SPMMRC/B&amp;BC Staff Signature:</b> | <b>Date:</b> |

## **Appendix 3**

## **A pilot study to determine the reproducibility of fluctuations in liver glycogen content as a result of exhaustive exercise and carbohydrate feeding using $^{13}\text{C}$ MRS.**

The recent developments in  $^{13}\text{C}$  MRS have allowed repetitive measurement of liver glycogen content with minimum discomfort to volunteers. However to date little research has been completed to discover the day-to-day reproducibility of the method thus if changes in liver glycogen content are small it is possible that the variation may obscure real differences. The study in Chapter 3 has measured post exercise muscle glycogen resynthesis following ingestion of 2 glucose polymers with markedly different molecular weights. As liver glycogen content has been shown to decrease with exercise and increase as a result of carbohydrate feeding (Casey et al., 2000), it follows that there may be potential differences in liver glycogen resynthesis following post-exercise feeding of a high or low molecular weight glucose polymer.

### ***Aim***

The aim of this pilot study was to measure the reproducibility on 2 occasions of liver glycogen determination by  $^{13}\text{C}$  MRS at 3.0 T. It was expected that the pattern of post exercise depletion and subsequent repletion would be similar on 2 visits with an identical exercise protocol and carbohydrate supplement.

### ***Methods***

#### *Subjects*

Three healthy, non-smoking, non-vegetarian male volunteers (Mean  $\pm$  SD; Age:  $28 \pm 2$  yrs; BMI:  $22.7 \pm 2.9$  kg.m<sup>-2</sup>) participated in the study. The volunteers were all currently engaged in some form of regular physical activity

and were familiar with cycling exercise. All the volunteers were informed of the study procedures and associated risks of participation before providing written consent. Volunteers then attended the laboratory on 3 separate occasions for preliminary testing using an online system (Vmax 29, SensorMedics, Yorba Linda, CA, USA), to establish maximal rate of oxygen consumption ( $\dot{V}O_2$  max) and workload corresponding to 75% of  $\dot{V}O_2$  max as described in Chapter 2. Mean  $\dot{V}O_2$  max was  $55.8 \pm 5.3$  ml.kg<sup>-1</sup>min<sup>-1</sup>.

### *Experimental protocol*

The volunteers visited the lab on 2 occasions separated by 1 week after an overnight fast and having refrained from strenuous exercise, alcohol and caffeine for the previous 24 hr. They had followed an identical food intake the day preceding a visit and food intake for 3 days after the first visit and food intake for the 3 days after the first visit was controlled for total energy intake and carbohydrate content (55%) by means of a food diary. The volunteers immediately began the exhaustive exercise protocol on a cycle ergometer (Lode Excalibur, Lode, Groningen, The Netherlands) and exercise time to fatigue was monitored on the first visit to be replicated on the second visit. Water intake was *ad libitum* on the first visit and repeated for the second visit. At the termination of the exhaustive exercise volunteers were transported to the Sir Peter Mansfield Magnetic Resonance Centre on the University of Nottingham campus by vehicle. The time elapsed between the end of exercise and the baseline scan was ~18 min. They immediately underwent a baseline <sup>13</sup>C MRS scan to determine post exercise liver glycogen content (~23 min). Following the scan they were temporarily removed from the scanner to

consume a 1 litre beverage containing 100g of a HMW glucose polymer (Vitargo, Swecarb AB, Kalmar, Sweden) within 2-3 min. The volunteers were then re-positioned in the scanner and underwent a continuous scanning protocol to determine liver glycogen content (120 min).

#### *Liver glycogen analysis*

Liver glycogen content was determined using  $^{13}\text{C}$  spectroscopy using a 3.0 T whole body magnetic resonance scanner with a 1m diameter bore (Philips Healthcare, The Netherlands). A surface coil was used with a carbon coil for transmission and reception, and quadrature proton coils for  $^1\text{H}$  decoupling. Volunteers were placed in a supine position with the surface coil placed over the abdomen above the liver and the position marked with indelible ink to ensure repeat positioning. An initial survey image was taken to assess the liver position for coil placement. The  $^{13}\text{C}$  spectra were acquired using a proton-decoupled pulse acquire sequence with adiabatic pulses and narrowband decoupling. Quantitation of liver glycogen was achieved using the Matlab version of MRUI and a phantom containing a known glycogen concentration.

#### *Statistical analysis*

A paired t-test (GraphPad Prism 5.03, GraphPad Software, Inc, California, USA) was undertaken on the 3 sets of data to determine if the first and second visit responses were different.

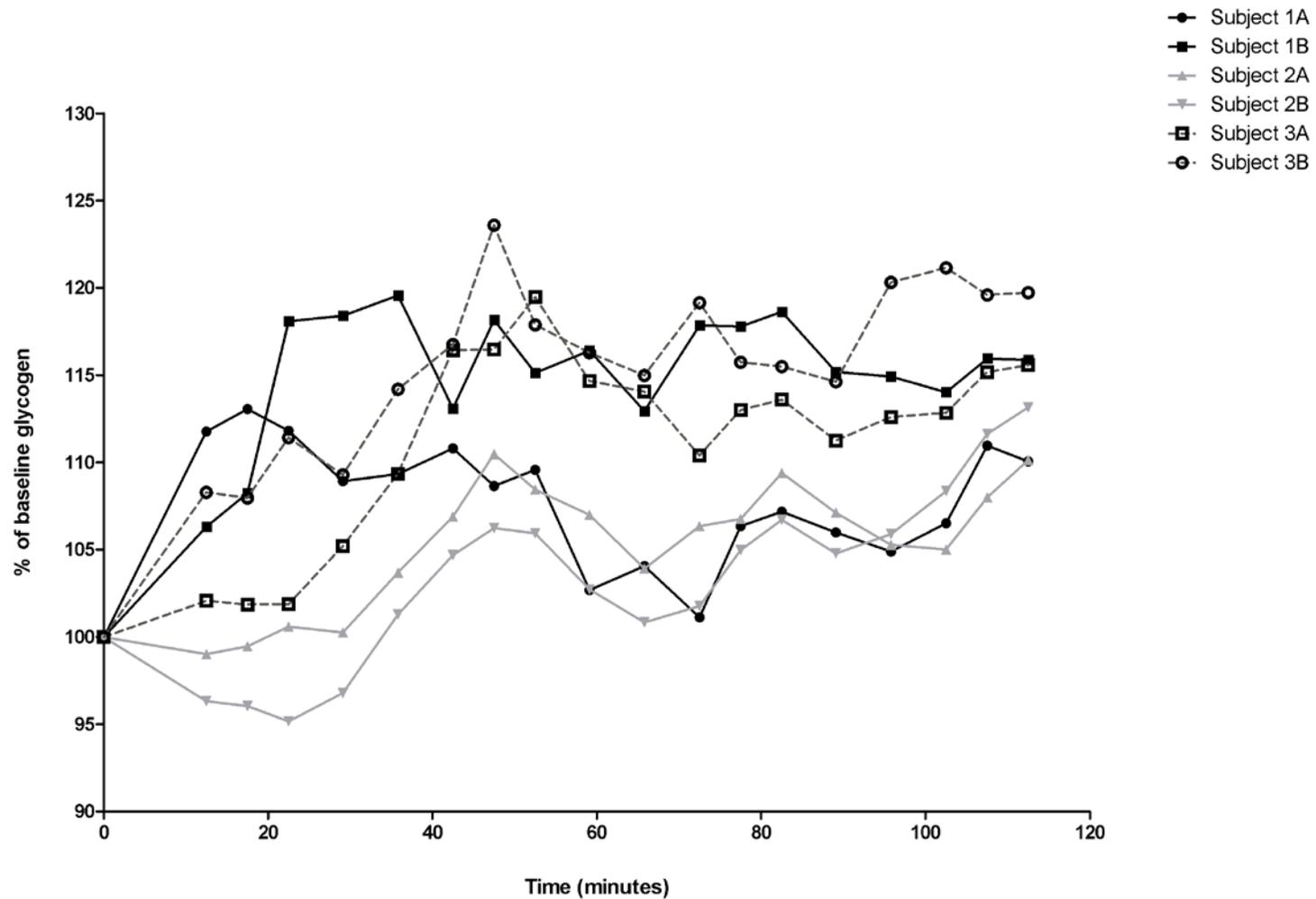
## **Results**

### *Exhaustive exercise*

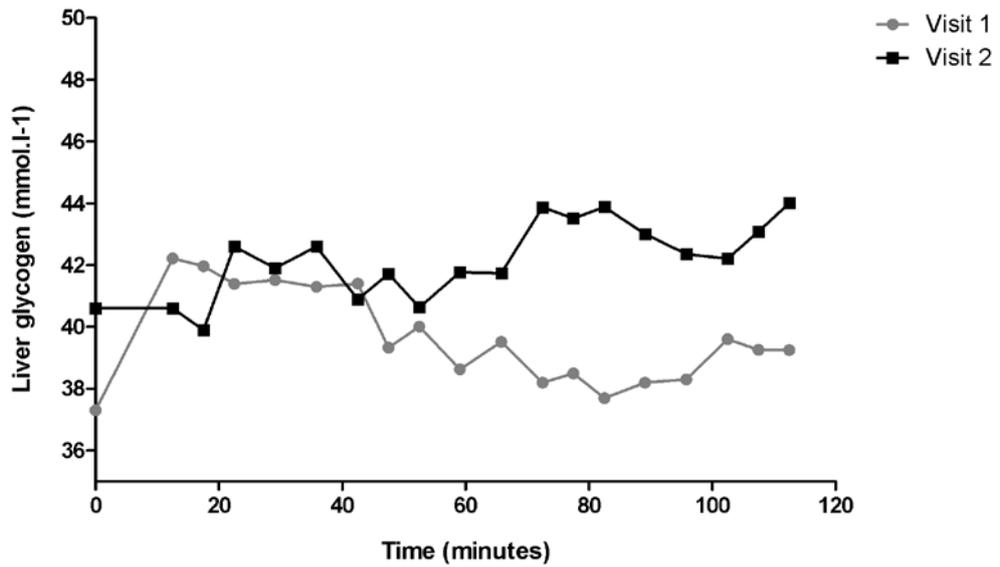
The volunteers completed the exhaustive exercise bout at a workload of  $218 \pm 16$  watts which corresponded to an exercise intensity of  $75.8 \pm 3\% \dot{V}O_2$  max. The cycling time to exhaustion was  $91 \pm 16$  min.

### *Liver glycogen*

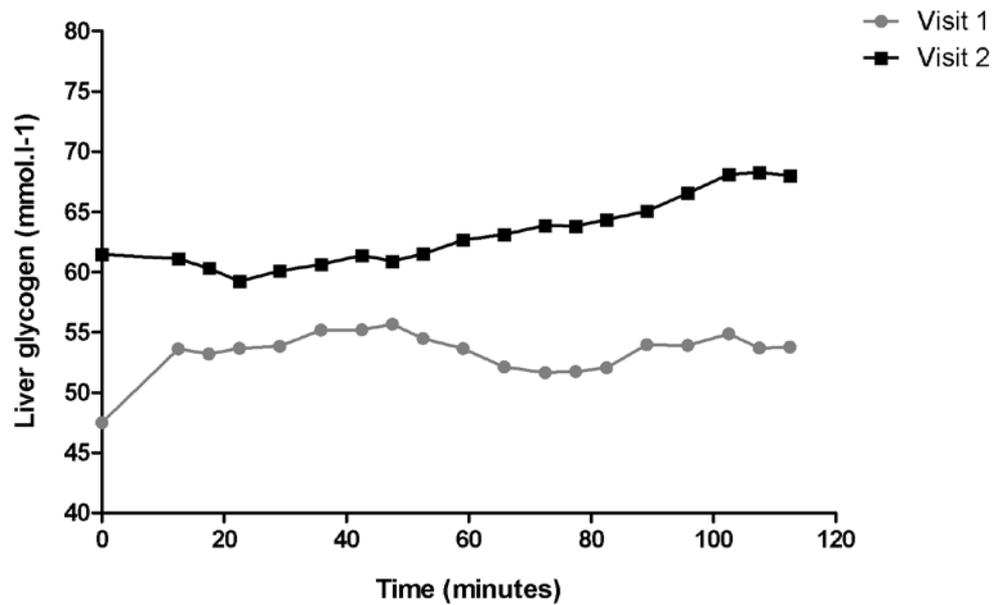
The fluctuations in liver glycogen on both visit 1 and 2 are shown for the volunteers in Figure 1. Liver glycogen content increased in all 3 volunteers 2 hrs after post exercise ingestion of 100g of carbohydrate (increase of 6, 11 and 4% in volunteers A, B and C respectively). Post exercise liver glycogen was similar on both visits for volunteer A ( $37.3$  vs.  $40.6$   $\text{mmol.l}^{-1}$ ) but markedly different for volunteer B ( $47.5$  vs.  $61.5$   $\text{mmol.l}^{-1}$ ) and C ( $52.3$  vs.  $46.5$   $\text{mmol.l}^{-1}$ ). On average there was a 2.5% difference in visit 1 and 2 for volunteer A (Figure 2) which was less than volunteer B (Figure 3) and C (Figure 4) which varied by 19% and 11% respectively. The pattern of liver glycogen content of visit 1 compared to visit 2 was deemed to be different in volunteer A ( $P < 0.05$ ) and as well as volunteer B and C ( $P < 0.0001$ ).



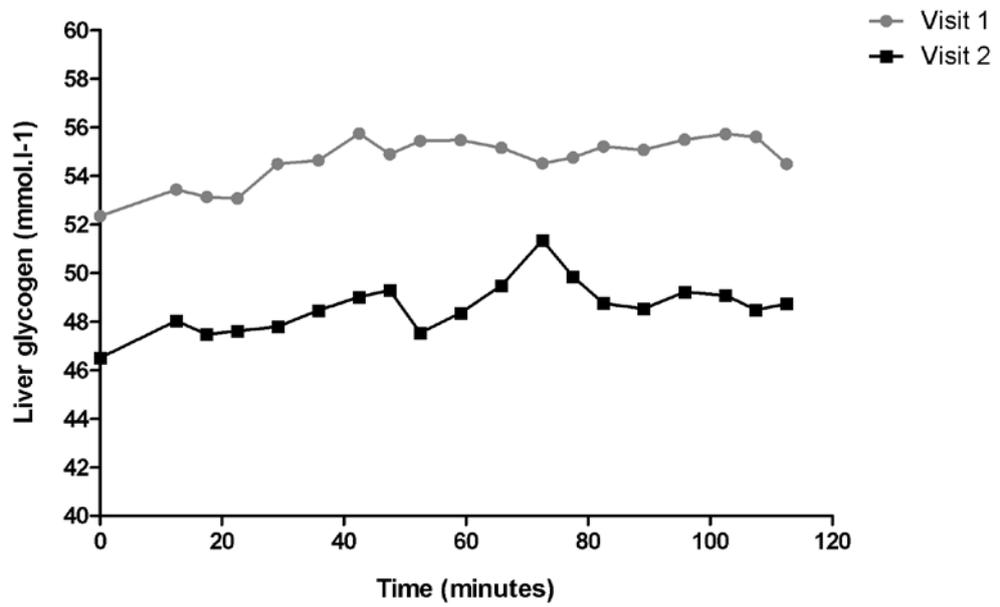
**Figure 1.** Liver glycogen as a % of baseline levels during 2 hr recovery from exhaustive exercise and 100g of carbohydrate. N=3 230



**Figure 2.** Liver glycogen content in Subject A over 2 hr recovery from exhaustive exercise and 100g of carbohydrate feeding



**Figure 3.** Liver glycogen content in Subject B over 2 hr recovery from exhaustive exercise and 100g of carbohydrate feeding



**Figure 4.** Liver glycogen content in Subject C over 2 hr recovery from exhaustive exercise and 100g of carbohydrate feeding

## **Conclusions**

This pilot study has demonstrated that liver glycogen content assessed by  $^{13}\text{C}$  MRS using a 3.0T system can not be considered reproducible as variation of between 2 and 19% was reported between 2 identical exercise protocols performed a week apart with carbohydrate feeding. It has previously reported that between subject post exercise liver glycogen content is variable using the 3.0T magnetic scanner. Chokkalingam et al (2007) reported post exercise glycogen content of  $303 \pm 59$ ,  $210 \pm 51$  and  $145 \pm 63$   $\text{mmol.l}^{-1}$  after 3 bouts of 40 min cycling exercise. Casey et al (2000) also showed large variation in liver glycogen content post exercise and during carbohydrate feeding (40-60  $\text{mmol.l}^{-1}$ ). Considering that muscle glycogen was near-identical at  $\sim 142$   $\text{mmol.kg}^{-1}$  dw 2 hr after feeding 100g of HMW and LMW glucose polymers following exhaustive exercise in Chapter 3, any subtle differences in liver glycogen may not be detected with the same solutions using this spectroscopy due to insensitivity of the method and biological variation.

## **References**

Casey, A., Mann, R., Banister, K., Fox, J., Morris, P.G., Macdonald, I.A. and Greenhaff, P.L. (2000) Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by  $(^{13}\text{C})$  MRS. *Am J Physiol Endocrinol Metab.* 278(1): E65-75.

Chokkalingam, K., Tsintzas, K., Snaar, J.E., Norton, L., Solanky, B., Leverton, E., Morris, P., Mansell, P. and Macdonald IA. (2007) Hyperinsulinaemia during exercise does not suppress hepatic glycogen concentrations in patients with type 1 diabetes: a magnetic resonance spectroscopy study. *Diabetologia.* 50(9):1921-9.

## **Appendix 4**

Subject Code: .....  
VAS number/Time: .....

Date: ...../...../.....

Please make a vertical mark through the horizontal line to show how you feel at the moment.  
Left and right extremes represent minimum and maximum values.

**1. How Clear headed do you feel?**

Not at all \_\_\_\_\_ Extremely

**2. How strong is your Desire to eat?**

Not at all \_\_\_\_\_ Extremely

**3. How energetic do you feel?**

Not at all \_\_\_\_\_ Extremely

**4. How full do you feel?**

Not at all \_\_\_\_\_ Extremely

**5. How happy do you feel?**

Not at all \_\_\_\_\_ Extremely

**6. How hungry do you feel?**

Not at all \_\_\_\_\_ Extremely

**7. How nauseous do you feel?**

Not at all \_\_\_\_\_ Extremely

**8. How thirsty do you feel?**

Not at all \_\_\_\_\_ Extremely

**9. How much food do you think you could eat?**

Not at all \_\_\_\_\_ Extremely