OPTIMIZING MUSCLE GLYCOGEN RESYNTHESIS POSTEXERCISE: SHOULD PROTEIN BE ADDED TO A CARBOHYDRATE DRINK?

OPTIMIZING MUSCLE GLYCOGEN RESYNTHESIS POSTEXERCISE: SHOULD PROTEIN BE ADDED TO A CARBOHYDRATE DRINK?

By

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ABSTRACT

During the first few hours of recovery from prolonged exercise, co-ingestion of protein (PRO) with carbohydrate (CHO) will increase the rate of muscle glycogen resynthesis if the rate of CHO intake is sub-optimal and/or when the feeding intervals are > 1 h apart (Burke et al., 2004). It remains controversial whether the higher rate of glycogen resynthesis is attributable to a PRO-mediated increase in insulin release or simply the result of higher energy intake (Jentjens et al., 2001). The optimal rate of CHO ingestion necessary to maximize glycogen resynthesis remains unknown, although some studies have recommended a peak CHO intake of ≥1.2 g/kg/h. **PURPOSE:** We examined whether the addition of PRO or "extra CHO" to a drink that provided 1.2 g CHO/kg/h would increase muscle glycogen resynthesis during recovery from prolonged exercise. **METHODS:** Six men (22± 1 yr; $V0_{2peak}$= 48±8 ml/kg/min) were studied during a 4-h recovery period on 3 separate occasions after a standardized 2-h exercise protocol designed to substantially lower muscle glycogen. Subjects randomly consumed 1 of 3 drinks during recovery from each trial: 1.2 gCHO/kg/h (CHO), 1.6 gCHO/kg/h (CHO/CHO) or 1.2 gCHO + 0.4 gPRO/kg/h (CHO/PRO). Drinks were consumed immediately post-exercise and at 15 min intervals for 3 hrs. **RESULTS:** Biopsies obtained at 0 and 4 hrs post-exercise revealed no difference in muscle glycogen resynthesis rates between trials (CHO: 22.7±6.6; CHO/CHO: 25.0±3.0; CHO/PRO: 24.6±4.2 mmol/kg dry wt/h) despite differences in energy intake between trials. The ingestion of additional CHO or PRO did not induce changes in blood [insulin] and blood [glucose] compared to a 1.2 gCHO/kg/h beverage. Muscle [lactate] increased from

immediately post-exercise to 4 hrs into recovery in all 3 trials (main effect for time, $P \leq 0.05$). All subjects reported some degree of gastro-intestinal(GI) distress after 3 and 4 hrs of recovery but there were no differences between treatments (main effect for time, $P \leq 0.05$ vs. 0 hrs). **CONCLUSION:** Ingesting 1.2 gCHO/kg/h met or exceeded the threshold necessary to optimize muscle glycogen synthesis during the first 4 hrs of recovery from prolonged, strenuous exercise in recreationally active men. The ingestion of ≥ 1.2 gCHO/kg/h at a concentration of ~20% solution induced GI distress in some individuals and may hamper subsequent same-day performance.

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TABLE OF CONTENTS

Abstract..... iii
Acknowledgements..... v
Table of contents..... vi
List of Tables..... viii
List of Figures..... ix

Chapter 1: Review of Literature

1.1. Introduction..... 1
1.2. Muscle glycogen resynthesis..... 2

1.2.1 Skeletal muscle glucose transport..... 4

1.2.1.1. Contraction-stimulated glucose uptake..... 6
1.2.1.2. Insulin-mediated glucose uptake..... 7
1.2.1.3. Increases in GLUT-4 transporter number vs. increases in transporter intrinsic activity..... 9
1.2.1.4. The effect of post-exercise muscle glycogen concentration on muscle glycogen resynthesis..... 10

1.2.2 Glycogen synthase regulation of muscle glycogen resynthesis..... 11

1.2.2.1. Glycogenin..... 11
1.2.2.2. Glycogen synthase I and D..... 13
1.2.2.3. The effects of glycogen concentration on glycogen synthase activity..... 15
1.2.2.4. Glycogen synthase localization..... 17
1.2.2.5. Glycogen phosphorylase..... 18

1.2.3 The limiting factor..... 19

1.3. Training status and muscle glycogen resynthesis..... 20
1.4. Carbohydrate supplementation following exercise..... 22

1.4.1 Effect of timing of CHO intake..... 22
1.4.2 Effect of amount of CHO intake..... 25
1.4.3 Method and type of CHO intake..... 27

1.5. Effect of protein supplementation on muscle glycogen resynthesis post-exercise..... 28

1.5.1 Insulinotropic amino acids and proteins..... 28
1.5.2 CHO-PRO supplements vs. CHO alone supplements..... 28
1.5.3 The effects of insulin on muscle glycogen resynthesis..... 31

1.6. Summary..... 32

Chapter 2: **Optimizing muscle glycogen resynthesis postexercise: should protein be added to a carbohydrate drink?**

2.1. Introduction..... 41
2.2. Methods..... 44

2.2.1 Subjects..... 44
2.2.2 Preliminary testing..... 44
2.2.3 General design..... 45
2.2.4 Experimental protocol..... 46
2.2.5 Beverages..... 48
2.2.6 Analyses..... 49

2.2.6.1. Blood analyses..... 49
2.2.6.2. Muscle analyses..... 50

2.2.7 Gastro-intestinal (GI) questionnaires..... 50
2.2.8 Statistical analyses..... 51

2.3. Results..... 52

2.3.1 Muscle metabolites..... 52
2.3.2 Serum insulin, Blood glucose and Blood lactate..... 55
2.3.3 Gastro-intestinal(GI) complaints..... 58

2.4. Discussion..... 59
2.5. Conclusion..... 72
2.6 References..... 73

Appendix I. Subject information and consent forms, Subject recruitment poster..... 85
Appendix II. REB approval form..... 98
Appendix III. Gastro-intestinal (GI) questionnaire..... 101
Appendix IV. Subject characteristics, Drink order..... 103
Appendix V. Raw data – Muscle metabolites, Blood metabolites, GI questionnaire, Statistical summary tables..... 106

LIST OF TABLES

Table 1. Glycogen concentration and glycogen synthase activity in normal and I_{FnLn} I mice before and after stimulation..... 16

Table 2. Summary data of carbohydrate/protein studies..... 34

Table 3. Subject characteristics..... 44

Table 4. Composition of beverages..... 49

Table 5. Muscle glycogen concentrations for muscle biopsy samples taken from vastus lateralis immediately after exercise and after 4-h recovery period..... 52

Table 6. Muscle metabolites immediately after exercise (0) and after 4-h recovery period(4-h)..... 54

Table 7. Serum insulin and blood metabolites, pre-exercise to 240 min into recovery..... 57

LIST OF FIGURES

Figure 1. Factors affecting muscle glycogen resynthesis post-exercise..... 4

Figure 2. Overview of the glycogen resynthesis pathway..... 14

Figure 3. The effect of insulin on the relation between glycogen synthase I and activity and glycogen in the isolated rat diaphragm..... 16

Figure 4. Muscle glycogen resynthesis rates 2, 4, 8, and 24 hrs into recovery from exercise..... 24

Figure 5. Muscle glycogen resynthesis rates with different amounts of carbohydrate intake..... 26

Figure 6. Percent recovery of depleted glycogen stores of the vastus lateralis from 0 to 40, 40 to 120, and 120 to 240 min of recovery..... 30

Figure 7. Schematic representation for each trial day..... 46

Figure 8. Muscle glycogen depletion protocol..... 47

Figure 9. Muscle glycogen concentration immediately after exercise and after 4-hour recovery period..... 53

Figure 10. Muscle glycogen resynthesis rate (mmol/kg dry wt/h) over the first 4-hrs of recovery..... 53

Figure 11. Serum insulin concentration pre-exercise and in 4-hr recovery from exercise..... 55

Figure 12. Area under insulin curve..... 56

Figure 13. Blood glucose concentration pre-exercise and in 4-hr recovery from exercise..... 56

Figure 14. Gastro-intestinal problems at 0, 2, 3, and 4 hrs into recovery from glycogen-depleting exercise..... 58

CHAPTER 1:

REVIEW OF LITERATURE

1.1. INTRODUCTION

Muscle glycogen is an essential source of energy during prolonged moderate-to-intense exercise. A reduction in muscle glycogen stores before and during exercise has been shown to impair aerobic endurance performance and to increase the perception of fatigue during exercise (Bergström et al., 1967a; Hultman, 1967; Ivy et al., 1991). Whereas fat stores (primarily adipose tissue and intramuscular triglycerides) are relatively plentiful in the human body, carbohydrate (CHO) stores (primarily liver and muscle glycogen) are limited. Muscle glycogen stores in a typical well-fed human are 500-700g and provide ~2000-2500 kcal of energy (van Hall et al., 2000a). Muscle glycogen concentrations have been shown to fall as much as ~525 mmol glucosyl units/kg dry wt. during prolonged exercise (van Hall, 2000a) and high exercise intensity cannot be maintained when muscle glycogen stores are depleted.

The replenishment of muscle glycogen stores following exhaustive exercise is an important factor determining the time necessary for recovery. Classic research by Bergström and Hultman (1967) demonstrated that muscle glycogen synthesis occurred most rapidly in muscle depleted of its glycogen stores. Complete restoration of muscle glycogen can occur within 24 hrs post-exercise provided that sufficient CHO is consumed (Burke et al., 2004). The rate of muscle glycogen resynthesis post-exercise may be influenced by factors such as amount of CHO or other nutrients consumed, type of CHO consumed, and timing of CHO consumed. These factors become even more influential

when athletes require strategies that will maximize muscle glycogen storage within very short periods, for example between training sessions or events on the same day. Maximizing the muscle glycogen resynthesis rate between exercise bouts leads to better performance outcomes in the second bout (Williams et al., 2003).

The following review of literature focuses on the process of muscle glycogen resynthesis following exercise. The main topics considered are the factors that limit muscle glycogen resynthesis post-exercise and the effect of nutritional manipulation on post-exercise muscle glycogen resynthesis. Insights into how factors affecting muscle glycogen resynthesis following exercise may be linked to disease states such as Type I (insulin dependent diabetes mellitus[IDDM]) and Type II (insulin independent diabetes mellitus[IIDM]) diabetes and McArdle's Disease will also be briefly discussed.

1.2. MUSCLE GLYCOGEN RESYNTHESIS

The resynthesis of skeletal muscle glycogen following glycogen-lowering exercise, occurs in a biphasic pattern (Ivy et al., 1988b; Maehlum et al., 1977; Price et al., 1994, 1996). The first, a contraction-induced phase of muscle glycogen resynthesis, is rapid (12-30 mmol/kg dry wt/h), is insulin independent and lasts 30-60 min. In contrast, the second phase is much slower (3-5 mmol/kg dry wt/h), is insulin dependent and can continue for greater than 24 hrs if supplementation persists (Ivy et al., 1988b; Maehlum et al., 1977; Price et al., 1994, 1996). Glycogen "supercompensation" or an elevation in muscle glycogen levels above normal, is also possible (Host et al., 1998a). Phase I and Phase II have been shown to be completely independent of each other (Maehlum et al.,

1977; Price et al., 1996). For example, Maehlum et al. (1977) compared rates of muscle glycogen resynthesis in insulin dependent diabetics (IDDM) and non-diabetic control subjects after systemic exercise and found that IDDM patients who were injected with insulin had similar glycogen resynthesis rates as non-diabetic controls. However, when the diabetics were not administered insulin, their rate of resynthesis ceased when muscle glycogen levels reached ~160 mmol/kg dry wt (Maehlum et al., 1977). Price et al. (1996) showed that inhibition of insulin secretion by infusion of somatostin completely eliminated the slow phase of muscle glycogen resynthesis in normal healthy human gastrocnemius muscle after single leg toe raises but did not significantly alter Phase I.

Although it is widely accepted that glucose is the primary substrate for muscle glycogen resynthesis, the role of other substrates such as fat and protein (PRO) on the rate of post-exercise muscle glycogen resynthesis has also been studied recently (see section 1.5). With respect to CHO availability, it has been shown that muscle glycogen resynthesis is limited by a combination of factors including glucose absorption and/or delivery in the bloodstream, extraction of glucose by other tissues, the glucose transport capacity of the muscle and the activity level of a key anabolic enzyme known as glycogen synthase (Figure 1). In turn, both glucose transport and glycogen synthase activity are partially regulated by the pancreatic hormone insulin. Finally, the amount of glycogen depletion and/or the immediate post-exercise muscle concentration of glycogen has also been shown to regulate post-exercise muscle glycogen resynthesis. The following sections will address the research pertaining to each of these regulating factors.

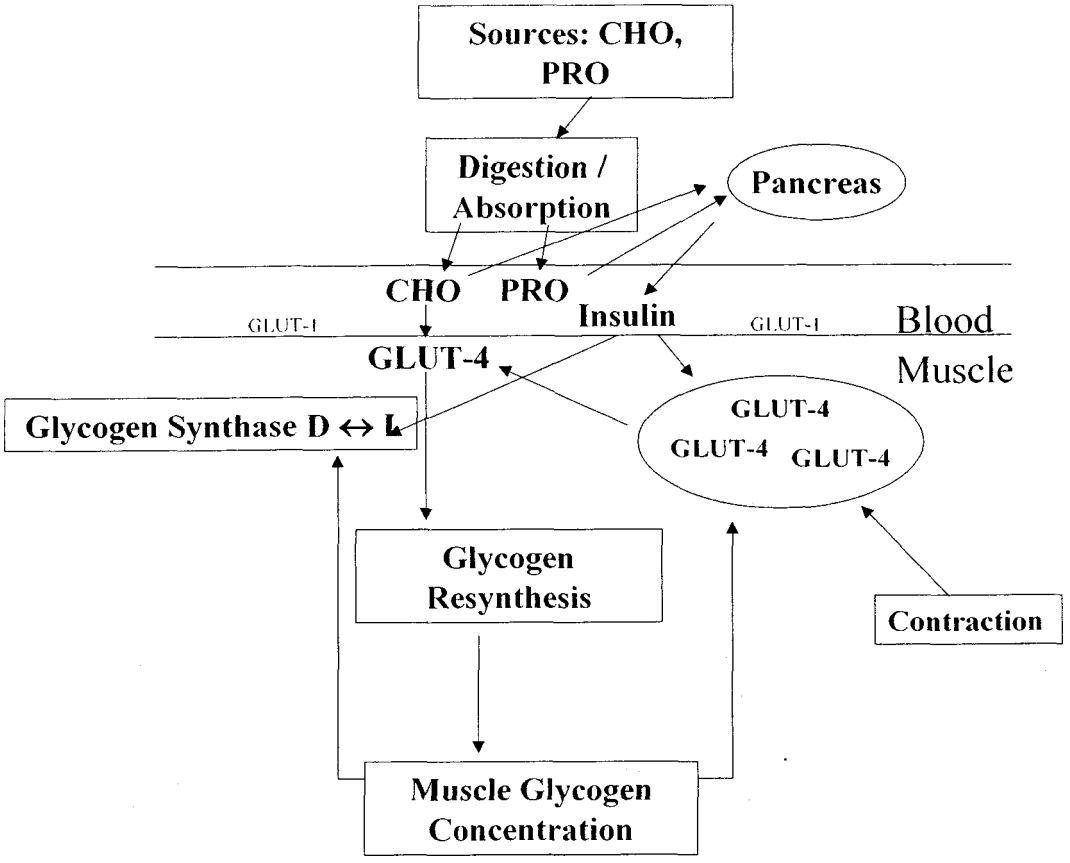


Figure 1. Factors affecting muscle glycogen resynthesis post-exercise. CHO, carbohydrate; PRO, protein; glycogen synthase D, glycogen synthase inactive form; glycogen synthase I, glycogen synthase active form; GLUT-1 and GLUT-4, muscle membrane glucose transporters.

1.2.1 Skeletal muscle glucose transport

In humans, glucose transport across the skeletal muscle membrane occurs by facilitated diffusion, an energy-independent process that uses two isoforms (GLUT-1 and GLUT-4) of a family of glucose transporter carrier proteins for transport of glucose across the muscle membrane (Hayashi et al., 1997). GLUT-1 has been shown to be expressed in human skeletal muscle cells during gestation, however its expression is markedly reduced around birth and is further reduced to undetectable levels within the

first year of life (Gaster et al., 2000). Clarification on whether GLUT-1 is expressed in human skeletal muscle is hampered by the unavoidable presence of erythrocytes and perineurial sheaths in the muscle tissue homogenates which express GLUT-1 at very high concentrations (Gaster et al., 2000). In contrast, Berggren et al. (2005) have demonstrated using cultured human skeletal muscle cells that sedentary individuals have lower basal glucose uptake than endurance-trained individuals and that there was a trend for endurance-trained individuals to have higher skeletal muscle GLUT-1 expression. These observations are similar to findings by Henry et al. (1995) who have reported decreased GLUT-1 content in cell cultures obtained from diabetic subjects who displayed reduced basal glucose uptake. In addition, Phillips et al. (1996) demonstrated that the non-insulin-dependent glucose transporter (GLUT-1) is increased in skeletal muscle (131%) after endurance training. It is therefore a controversial issue whether GLUT-1 does play a significant role in basal glucose uptake by the skeletal muscle. However, it is well known that GLUT-1 does not play a major role in mature human muscle glucose uptake during exercise under non-pathological conditions (Gaster et al., 2000).

The GLUT-4 isoform is the primary glucose transporter in human skeletal muscle during exercise and feeding conditions. At rest, the GLUT-4 isoform is located intracellularly and is translocated to the muscle membrane under the effects of both muscle contraction and/or insulin (Lund et al., 1995). Although the major mediators of glucose transport are insulin and contraction, it is quite possible that other factors such as catecholamines, hypoxia, growth factors and corticosteroids can alter glucose transport.

Intracellular GLUT-4 transporters reside in small tubulo-vesicular organelles (Hirshman et al., 1990). These vesicles also contain the vesicle-associated membrane protein-2 [VAMP-2] (Volchuk et al., 1994), cellubrevin [VAMP-3] (Volchuk et al., 1994), gp 160 (Coderre et al., 1995b) and Rab 4 (Sherman et al., 1996). In the early 1990s, Douen et al. (1990b) found that while acute insulin treatment increased GLUT-4 transporters in a plasma membrane fraction of the rat hind limb muscle and decreased GLUT-4 in an intracellular membrane fraction, exercise also increased the GLUT-4 transporters in the plasma membrane but in contrast to insulin did not significantly decrease GLUT-4 in the intracellular fraction. Thus, it was proposed that muscle contraction mobilizes GLUT-4 proteins from a different intracellular pool than does insulin. In contrast, some investigators found that Rab 4, guanosine triphosphate (GTP)-binding proteins, which are thought to act as molecular switches catalyzing membrane trafficking events, are stimulated to redistribute from the intracellular pool to the muscle membrane under the effects of insulin (Sherman et al., 1996). However, the activity of these proteins is not influenced by muscle contraction. Therefore, instead of two different intracellular pools for GLUT-4, there may exist one common sub-cellular location of transporters with different molecular "switches" for mobilization.

1.2.1.1. Contraction-stimulated glucose uptake

The finding that it is possible to inhibit the effects of insulin without inhibiting the effects of contraction on skeletal muscle glucose uptake (Yeh et al., 1995) and that contraction-activated glucose transport is normal in insulin resistant rat muscle (Brozinick

et al., 1992) suggests that there are distinct signaling mechanisms for insulin-stimulated and contraction-stimulated translocation of glucose transporters in skeletal muscle. Contraction-stimulated glucose transport has long been considered to be regulated by the increase in cytoplasmic calcium (Nolte et al., 1995). Dantrolene, a substance that prevents the release of calcium from the sarcoplasmic reticulum, inhibits muscle glucose transport (Nolte et al., 1995 & Youn et al., 1991). However, increases in cytoplasmic calcium following contraction are transient; therefore, if calcium does regulate exercise-stimulated glucose transport, it must do so indirectly by activating intracellular signaling molecules. Inhibition of protein kinase C (PKC), a type of intracellular signaling protein, using polymyxin B has been shown to decrease contraction-stimulated glucose transport (Young et al., 1991). However, studies using polymyxin B must be interpreted with caution since polymyxin B is not a specific inhibitor of PKC and can in fact decrease contractility of muscle fibers (Young et al., 1991). Substances such as nitric oxide (Balon et al., 1994; Balon et al., 1997) have also been shown to influence muscle glucose transport following contraction. It is thought that these substances act in a paracrine / autocrine fashion to activate cell-signaling molecules such as PKC (Balon et al., 1997). Other less defined and less studied mechanisms of glucose transport that are stimulated by muscle contraction are the C-Jun NH_2-terminal kinase (JNK) and p38 kinase signaling cascades (Goodyear et al., 1996).

1.2.1.2. Insulin-mediated glucose uptake

In skeletal muscle, insulin binds to the vascular endothelium to the extracellular part of a receptor called insulin-receptor tyrosine kinase (IRTK). Binding of insulin

causes activation and autophosphorylation of the IRTK receptor which subsequently activates insulin receptor substrate (IRS). IRS then binds PI3 kinase, a process that has been shown to be essential for insulin-stimulated glucose uptake (Holman et al., 1997; Shepherd et al., 1998). Catalytic products of PI3 kinase such as phosphatidylinositol 3,4,5 – triphosphate are thought to act as second messenger systems activating downstream kinases such as phosphoinositide-dependent kinase-1 (PDK–1), protein kinase B (PKB or Akt-1) and protein kinase C (PKC) (Alessi and Downes, 1998; Holman et al., 1997; Shepherd et al., 1998). How PKC, PDK–1 and PKB then stimulate glucose transport is unknown. Evidence for this mechanism of insulin-stimulated glucose transport comes from Lund et al. (1995) who showed that pharmacological blockade of PI3 kinase inhibited insulin-stimulated GLUT-4 translocation.

Following exercise, increased insulin "sensitivity" of skeletal muscle glucose uptake is commonly observed. Insulin sensitivity is defined as the insulin concentration that elicits 50% of the maximal glucose uptake response (Hansen et al., 1998). This increased insulin sensitivity to glucose uptake is however restricted to the exercised muscle only (Richter et al., 1984; Richter et al., 1989). CHO deprivation post-exercise inhibits the reversal of the increased insulin sensitivity in rat muscle (Cartree et al., 1989). It is thus likely that the rate at which CHO is taken up into the skeletal muscle has a direct effect on the reversal of the increased insulin sensitivity of skeletal muscle glucose uptake post-exercise. Evidence shows that increased insulin binding or insulin signaling are not mechanisms by which muscle develops increased insulin sensitivity to glucose transport post-exercise (Goodyear et al., 1995; Richter et al., 1989; Treadway et al., 1989). In fact,

the potential for insulin to activate PI3 kinase in vivo has been shown in some studies to be diminished in previously exercised muscles despite an increase in insulin-stimulated glucose uptake (Goodyear et al., 1995). Thus IRS-1 associated PI3 kinase is not the sole mediator of insulin stimulated glucose uptake by skeletal muscle post-exercise.

1.2.1.3. Increases in GLUT-4 transporter number vs. increases in transporter intrinsic activity

Investigations on isolated plasma membranes from rat gastrocnemius muscle by Goodyear et al. (1990) found a 2-fold increase in glucose transporters and a 4-fold increase in glucose transport immediately after exercise. It was concluded that after an acute bout of exercise the glucose transport system of rat skeletal muscle plasma membranes' is characterized by increases in both glucose transporter number and glucose transporter intrinsic activity. The study by Goodyear et al. (1990) and other similar studies (Brozinick et al., 1994; Douen et al., 1990a) that employed the traditional Klip or the Hirshman-modified Grimditch technique of preparing sub-cellular fractions of skeletal muscle for GLUT-4 detection have been criticized on the basis that some degree of cross contamination between plasma and intracellular membranes may have occured. More recently however, using a new membrane impermeable glucose transporter exofacial photolabelling with bis-mannose derivative (ATB-BMRA) method for GLUT-4 determination and subsequent immunoprecipitation with GLUT-4 antibodies near perfect correlations between plasma membrane GLUT-4 protein concentration and glucose transport activity induced by insulin and contraction have been found (Lund et al., 1993). The increase in glucose transport or membrane permeability to glucose is thus accepted to

be due to an increased number of GLUT-4 molecules at the muscle membrane and not due to an increase in transporter turnover number or intrinsic activity (Gao et al., 1994; Lund et al., 1995). Instead, exercise probably increases the average turnover number of all transporters in the plasma membrane by recruiting the more active GLUT-4 transporters instead of the GLUT-1 transporters.

Whether the increase in muscle glucose transport with the combination of a maximal contraction stimulus and a maximal insulin stimulus is greater, equal or less than the effect of either contraction or insulin alone remains controversial (Douen et al., 1990a; Gao et al., 1994). Furthermore it is known that skeletal muscle glucose transport follows saturation kinetics and for the most part studies have shown that increases in glucose transport due to both contraction and insulin increase the maximal velocity (V_{max}) of transport without changing the Michaelis-Menton constant (K_m) for glucose (Hansen et al., 1995).

1.2.1.4. The effect of post-exercise muscle glycogen concentration on muscle glycogen resynthesis

While skeletal muscle insulin sensitivity increases after exercise (Hansen et al., 1998; Wojtaszewski et al., 2000), insulin does not act independently as the sole factor mediating glucose transport during the insulin dependent phase of glycogen synthesis. Jensen et al. (1997) showed that maximal insulin-stimulated glucose transport rates in different rat muscles were inversely related to the glycogen content of the muscles. Furthermore, Derave et al. (2000) showed that PKB-α / Akt-1 is the only upstream kinase in the insulin-stimulated glucose transport mechanism that is modulated by muscle

glycogen content in fast-twitch muscle. Therefore, while some investigators propose that the regulation of glucose transport is at the translocation level, i.e. GLUT-4 is attached to the glycogen molecule (Coderre et al., 1995a), Derave et al. (2000) proposed that regulation is more upstream at the signaling level.

1.2.2. Glycogen synthase regulation of muscle glycogen resynthesis

The classical immutable metabolic pathway for glycogen synthesis is described as follows. After entering the muscle cell, glucose is converted to glucose-6-phosphate (G-6-P) by the enzyme hexokinase, phosphoglucomutase then converts G-6-P to glucose-1-phosphate (G1P). Under anabolic conditions, i.e. insulin and/or high energy state, G1P subsequently reacts with uridine triphosphate to form uridine diphosphate-glucose (UDP-glucose) which acts as a glucosyl unit carrier. Glycogen synthase (GS) catalyses a reaction where by UDP-glucose is transferred to the terminal glucose residue at the non-reducing end of an amylose chain to form an α (1→4) glycosidic linkage. Once the glycogen molecule is sufficiently large, amylo (1,4→1,6) transglycosylase creates α (1→6) linkages in a glycogen molecule by transferring terminal oligosaccharide fragments six or seven glycosyl units long from the end of the glycogen molecule to the 6-hydroxyl group of other residues in the same or another chain (Jentjens and Jeukendrup, 2003). This creates the characteristic structure of glycogen.

1.2.2.1. Glycogenin

Since the late 1980's, the relatively simple classic model of muscle glycogen synthesis has been modified in radical ways. The one long-standing dilemma of the classic model was that it failed to answer the question as to where the glucose primer

originated from. It is now known that the primer is a self-glucosylating protein, glycogenin, which contains a sequence of 332 amino acids of which tyrosine-194 is the site of autoglucosylation or formation of a glucose-tyrosine bond (Smythe et al., 1988). Autoglucosylation of glycogenin has been shown to create a chain averaging 7-11 glucose residues long, that is maltooctaose (Lomako et al., 1990a). Smythe et al. (1990; 1991) suggest that a "tight" 1:1 glycogenin – synthase complex is required for efficient priming. The latter statement is in response to a study that demonstrated that the extent of utilization of a glycogenin primer is limited when homogenous muscle glycogenin is first allowed to self-glucosylate and then synthase is added (Lomako et al., 1988).

Once glycogenin is primed, glycogen can be produced. Glycogen is now reliably divided into two distinct forms: proglycogen (molecular mass ~400 kDa) in muscle and "depot glycogen" or macroglycogen (molecular mass 10^7 Da) in muscle (Lomako et al., 1991). Glycogen oscillates between macroglycogen and proglycogen but not usually to the glycogenin form. There is no free glycogenin in muscle (Lomako et al., 1990b; 1991). Therefore, the supply of glycogenin limits the amount of glycogen that can accumulate in the muscle. On a molar basis, proglycogen is greatly in excess over macroglycogen (4.4:1) and thus the conversion of proglycogen to macroglycogen theoretically could significantly increase the mass of glycogen in skeletal muscle by freeing up more glycogenin molecules (Alonso et al., 1995). The possibility of a lower proglycogen / macroglycogen ratio may explain the mechanism behind glycogen supercompensation. The possibility that the amount of primer varies in different

nutritional states may explain the aberrant rates and extents of glycogen synthesis in diseased states such as diabetes.

1.2.2.2. Glycogen synthase I and D

Glycogen synthase exists in two enzymatically inter-convertible forms: glycogen synthase I, which is considered the active form and is not dependent on glucose-6-phosphate (G-6-P) for activation; and glycogen synthase D, which is considered the inactive form and is stimulated in the presence of glucose-6-phosphate. The conversion of glycogen synthase from its I→D form requires its phosphorylation in the presence of Mg^{2+} and its reverse reaction from D→I is the product of dephosphorylation of the enzyme protein (Danforth, 1965).

The activity of glycogen synthase is typically expressed an "activity ratio", the ratio of enzyme in the I-form to total enzyme activity (I /I +D) (Kochan et al., 1979). Glycogen synthase has been shown to exist in multiple phosphorylated forms along the spectrum from its active I form to its inactive D form. Some argue that glycogen synthase has one to six possible phosphorylation sites (Kochan et al., 1979) while others believe that it has as many as nine phosphorylation sites (Roach et al., 1977). Nevertheless, the principle remains the same: the more phosphorylated the enzyme, the closer it is to its less active D form. Dephosphorylation of glycogen synthase is accomplished by protein phosphatase (Kochan et al., 1979). The discovery of intermediary forms of glycogen synthase was based on studies that demonstrated that although some forms of glycogen synthase may all have low activity ratios, they may have different levels of sensitivity to glucose-6-phosphate. A more sensitive way to

measure the activity of glycogen synthase is thus to measure its fractional velocity. Fractional velocity (FVx) is calculated by dividing the velocity of the enzyme at any given concentration of glucose-6-phosphate by the velocity at saturating levels of glucose-6-phosphate (Kochan et al., 1979).

Although glycogen synthase is commonly depicted as one enzyme either in its active or in its inactive form, researchers have recently revealed the possible existence of two different forms of glycogen synthase: proglycogen synthase and macroglycogen synthase (Alonso et al., 1995) (Figure 2).

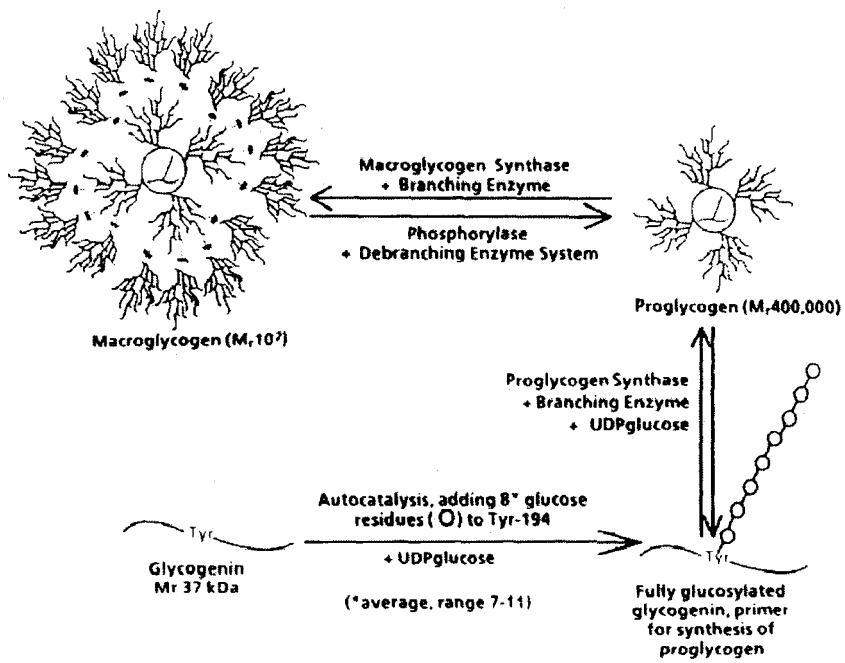


Figure 2. Overview of the glycogen resynthesis pathway. Glycogenin autocatalytically adds glucose from UDP-glucose to its Tyr-194 and then an average of a further seven residues to form the protein-bound maltosaccharides that serve as the primer for the synthesis of proglycogen by a form of glycogen synthase (proglycogen synthase), distinct in its properties from the "classical" synthase (macroglycogen synthase) that takes proglycogen to macroglycogen (Taken from Alonso et al., 1995).

In a study by Lomako et al. (1993) it was demonstrated that astrocytes exposed to NH^{4+} were able to synthesize proglycogen but not macroglycogen. The difference between the two forms of glycogen synthase may be due to differing phosphorylation states of one enzyme or even the existence of synthases as different gene products. Differing phosphorylation states of glycogen synthase may not only affect dependence of glycogen synthase on G-6-P as described previously but it may also alter the affinity of the enzyme for UDP-glucose and the primer substrate: glycogen for proglycogen synthase and proglycogen for macroglycogen synthase (Alonso et al., 1995).

1.2.2.3. The effects of glycogen concentration on glycogen synthase activity

Glycogen concentration seems to exert control over metabolic events in muscle (Hargreaves, 2004). Not only does it regulate glucose transport as previously discussed but it also mediates glycogen synthase activity. Danforth (1965) studied glycogen synthase in mice from an I_{FnLn} strain. Like people with McArdles disease, these mice have high levels of muscle glycogen. They are unable to convert phosphorylase b to phosphorylase a due to a lack of phosphorylase kinase and are thus unable to breakdown glycogen (Lyon and Porter, 1963). It was shown that 5 min of stimulation causes little increase in the I form of glycogen synthase in the I_{FnLn} mice compared to the normal mice. The results in Table 1 show that the amount of glycogen synthase I activity in the I_{FnLn} mice is exactly what is predicted if the assumption is correct that the increase in the glycogen synthase I activity is proportional to the decrease in glycogen concentration.

Strains of mouse	At Rest		5 minutes after stimulation	
	Glycogen	Synthase I	Glycogen	Synthase I
	mg/g/muscle	% total	mg/g/muscle	% total
I_{FnLn}	10.7	3.5	8.4	11.7
C_{57} (normal mice)	4.0	24.8	0.9	75.0

Table 1. Glycogen concentration and glycogen synthase activity in normal and I_{FnLn} mice before and after stimulation (Adapted from Danforth, 1965).

Insulin in the presence or absence of glucose is also a regulator of glycogen synthase activity. Studies (Danforth, 1965; Nielson et al., 2001) have shown that insulin increases glycogen synthase I activity in rat muscles by altering the position but not the general shape of the curve representing the relationship between glycogen content and glycogen synthase fractional velocity (Figure 3).

When glycogen levels are low, glycogen synthase activity in the un-stimulated

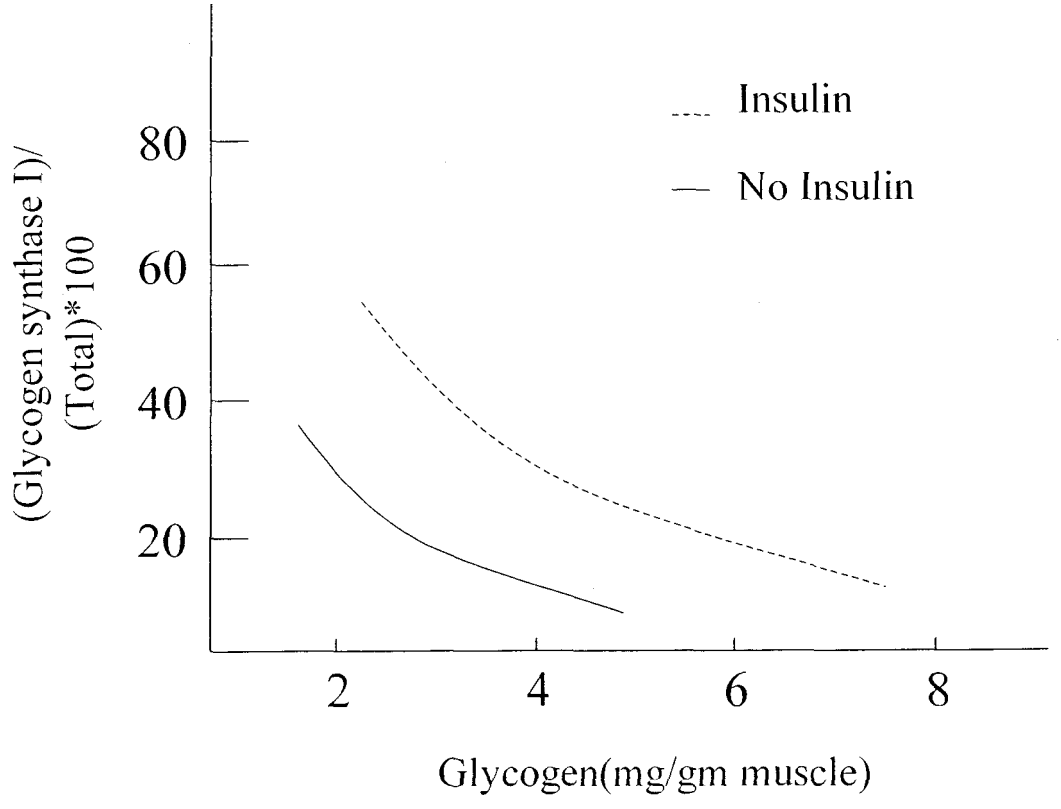


Figure 3. The effect of insulin on the relation between glycogen synthase I activity and [glycogen] in the isolated rat diaphragm (Modified from Danforth, 1965).

muscle is still higher than maximally insulin-stimulated glycogen synthase activity in muscle with high glycogen concentration (Nielson et al., 2001). This indicates that glycogen is a more potent regulator of glycogen synthase activity than insulin. It is only reasonable that muscle glycogen would exert control over metabolic events in the muscle. If no such mechanism existed, humans who can synthesize but not degrade glycogen would accumulate glycogen indefinitely, which does not happen! Danforth (1965) succinctly stated: "The most efficient arrangement for assuring proper supply is one in which the product reacts with its synthetic pathway in a manner that regulates its own synthesis". The results presented herein support the notion that glycogen content negatively feeds back to regulate its own synthesis.

1.2.2.4. Glycogen synthase localization

Glycogen synthase has been shown to be associated with both glycogen particles (Meyer et al., 1970) and myofibrils (Lane et al., 1989). When glycogen levels are decreased, glycogen synthase translocates from a glycogen-enriched membrane fraction to the cytoskeleton (Nielson et al., 2001). The functional consequences of this change in sub-cellular location of glycogen synthase are only speculative, however Nielsen et al. (2001) suggested that it may be advantageous to have the glycogen synthesis site of a muscle close to the contractile apparatus especially when glycogen stores are almost depleted. In addition, these authors speculated that glycogen synthase sub-cellular localization might be necessary for insulin to activate glycogen synthase (Nielson et al., 2001).

The effects of insulin on the downstream PKB (Akt-1) are enhanced by lowering glycogen levels (Derave et al., 2000; Jensen et al., 1997). Whether muscle glycogen concentration mediates the insulin-induced deactivation of glycogen synthase kinase 3 (GSK3) activity, a kinase used to deactivate / phosphorylate glycogen synthase, downstream from PKB remains to be investigated. Villar-Palasi (1969) showed that the conversion of glycogen synthase from its inactive D form into its active I form is inhibited at physiological concentrations of glycogen in vitro, possibly suggesting deactivation of glycogen synthase phosphatase. Newgard et al. (2000), suggested that glycogen levels might regulate glycogen synthase activity by glycogen binding to the glycogen targeting subunits of protein phosphatase-1 (PP1) which also binds glycogen synthase, glycogen phosphorylase and glycogen kinase. While glycogen synthase binding to PP1 seems to be important for its activity, the extent to which glycogen binding to PP1 inhibits binding of glycogen synthase remains unstudied as of yet. As for the effects of muscle contraction on glycogen synthase activity, Nielson et al. (2001) demonstrated that muscle contraction does not have an independent effect on glycogen synthase activity. The increase in glycogen synthase activity following contraction is simply due to the decreased glycogen content of the muscle (Nielson et al., 2001).

1.2.2.5. Glycogen phosphorylase

It has been shown that high levels of active glycogen phosphorylase (GP), the enzyme that catalyzes glycogen degradation, can co-exist with glycogen deposition and that glycogen phosphorylase is not affected by glucose or by insulin (Montell et al., 1999). Therefore, the stimulation of glycogen synthesis by insulin is not due to the

deactivation of glycogen phosphorylase. In addition, Montell et al. (1999) showed that glucose-6-phosphate levels have no impact on glycogenic flux.

1.2.3. The limiting factor

Some investigators propose that glucose transport is the limiting factor for glycogen resynthesis following intense exercise (Nakatani et al., 1997; Ren et al., 1993, 1994) while others argue that glycogen synthase activity is the limiting factor (Iozzo et al., 2001; Manchester et al.,1996; Richter et al., 1988). Fisher et al. (2002) have concluded that both the rate of glucose transport and glycogen synthase activity can be limiting for glycogen accumulation depending on the circumstances. When glycogen synthase activity is increased in rats by the application of GF-109203, there is no increase in glycogen accumulation compared to controls (Fisher et al. 2002). Lithium treatment in rats was shown to result in 50% higher glycogen accumulation compared to the fact that it does not prevent the return in glycogen synthase to fasting levels after 4 hrs (Fisher et al., 2002). In addition, it has also been shown that trained muscles accumulate higher glycogen stores due to their higher muscle GLUT-4 content despite no changes in glycogen synthase activity (Nakatani et al., 1997). What most researchers fail to acknowledge is that perhaps neither glucose transport nor glycogen synthase limits glycogen synthesis. There is strong evidence to suggest "that the production of active glycogen primer in the muscle cell has the potential to be the overall rate-limiting process in glycogen formation, capable of overriding the better understood hormonally controlled mechanisms of protein phosphorylation / dephosphoryation that regulate the activity of glycogen synthase"(Alonso et al., 1995).

1.3. TRAINING STATUS AND MUSCLE GLYCOGEN RESYNTHESIS

Trained athletes have higher muscle glycogen resynthesis rates post-exercise than sedentary individuals. For example, Greiwe et al. (1999) reported a mean increase in muscle glycogen resynthesis rate post-exercise from 21.8 mmol/kg dry wt/h (untrained) to 50.5 mmol/kg dry wt/h (trained) in subjects after they performed high-intensity stationary cycling 3 days/wk and continuous running 3 days/wk for 10 wk. Adaptations at the muscular level that may contribute to such a training induced increase in muscle glycogen resynthesis rate include: increased muscle GLUT-4 content (Hickner et al., 1997; Philips et al., 1996), improved insulin signaling (Houmard et al., 1999; Kirwan et al., 2000) and increased muscle blood flow (Ebeling et al., 1993).

It has been reported in several investigations that muscle GLUT-4 content is higher in trained vs. untrained individuals (Hickner et al., 1996; Phillips et al., 1996). This increase in muscle GLUT-4 content is present after only 2 hrs of training for 5 days and increases even more thereafter (Hickner et al., 1997). Increases in muscle GLUT-4 concentration with training however completely reverse within 40 hrs after the last exercise bout after both 5 days and 5 weeks of training (Host et al., 1998b). To date, only one study has measured both increases in muscle glycogen resynthesis and increases in muscle GLUT-4 content immediately after exercise in trained vs. untrained participants (Hickner et al., 1997). Hickner et al. (1997) reported that muscle GLUT-4 content immediately post-exercise was threefold higher in trained than in untrained individuals ($p<0.05$) and correlated with glycogen accumulation rates ($r = 0.66$, $p<0.05$).

Exercise training has also been shown to enhance insulin signal transduction (Houmard et al., 1999; Kirwan et al., 2000). Kirwan et al. (2000) found that when 8 trained and 8 sedentary individuals underwent a 2-hr hyperinsulinemic (40 $mU/m^2/min$)-euglycemic (5.9 mM) clamp procedure and were infused with [6,6-^{2}H] glucose tracer to measure hepatic glucose output:

1- Insulin-stimulated PI3-kinase activation was greater ($p<0.004$) in the trained compared with the sedentary group (3.8±0.5 vs. 1.8±0.2 fold increase from basal).
2- Insulin-mediated glucose disposal rates (GDR) were greater ($p<0.05$) in the exercise-trained compared with the sedentary group (9.22±0.95 vs. 6.36±0.57 mg/ kg fat free mass/min).
3- And, increases in GDR were positively related to PI3-kinase activation ($r = 0.60$, $p<0.02$).

In conclusion, it is thought that regular exercise leads to increased insulin-stimulated IRS-1 associated PI3-kinase activation in human skeletal muscle post-exercise and that this causes increased insulin-mediated glucose uptake post-exercise.

Lastly, it has been suggested that blood flow may be a limiting factor for glycogen resynthesis post-exercise in sedentary individuals. Ebeling et al. (1993) showed that trained individuals had a 64% higher basal forearm blood flow than sedentary individuals. These higher blood flows in association with a 2.2 fold greater basal A-V difference in the trained individuals lead to a 3.3 fold greater average basal glucose disposal rate in the trained athletes vs. the sedentary individuals. More studies are necessary to clarify

whether blood flow is in fact a limiting factor for muscle glycogen resynthesis post-exercise in sedentary individuals.

1.4. CARBOHYDRATE (CHO) SUPPLEMENTATION FOLLOWING EXERCISE

1.4.1. Effect of timing of CHO intake

In the absence of CHO supplementation following strenuous exercise, the muscle glycogen storage rate is very low. Most studies report average basal muscle glycogen storage rates of approximately 2.14 mmol/kg dry wt/h (Ivy et al., 1988b). Substrates for muscle glycogen resynthesis in the absence of CHO may include blood glucose, muscle lactate and/or amino acids such as alanine and glutamine. Both skeletal muscle and the liver have the capacity to convert lactate into glycogen through a process known as gluconeogenesis (Astrand et al., 1986). In addition, liver gluconeogenesis can convert alanine into glucose while renal gluconeogenesis can convert glutamine into glucose (Rennie, 1996). The glucose product can then be transported to skeletal muscle via the bloodstream where it is then assembled into glycogen.

The rate of muscle glycogen resynthesis with CHO supplementation immediately after exercise has been reported to be between 20-50 mmol/kg dry wt/h (Jentjens and Jeukendrup, 2003). The highest rates of muscle glycogen synthesis occur during the first 2 hrs after exercise and are due to the activation of glycogen synthase, the exercise induced increase in insulin sensitivity and the exercise-induced GLUT-4 translocation to

the muscle membrane (Ivy et al., 1988b). These high rates of glycogen synthesis (~33 mmol/kg dry wt/h) slow to more typical rates (~4.3 mmol/kg dry wt/h) after 2 hrs (Ivy et al., 1988b).

Delaying the ingestion of the CHO supplement by 2 hrs following exercise leads to a 45% slower rate of glycogen storage and an overall lower muscle glycogen concentration at 4 hrs post-exercise compared to when CHO is administered immediately after exercise (Ivy et al., 1988a) (Figure 4). This decreased rate of glycogen resynthesis occurs despite elevated blood glucose and insulin concentrations and no difference in glycogen synthase activity (Ivy et al., 1988a). This suggests that muscle glucose uptake is limiting and the latter is possibly the result of a decreased insulin sensitivity of muscle as time after exercise increases. It is interesting to note that muscle glycogen resynthesis was only measured during the first 4 hrs of recovery from exercise in the Ivy et al. (1988a) study. It would have been optimal to have 6 hrs of recovery in order to measure total muscle glycogen recovery 4 hrs post-supplement in each group rather than 4 hrs post-exercise. It is possible that although the peak resynthesis rate was lower in the delayed supplementation group, total glycogen storage 4 hrs post-supplement was the same in both groups. Parkin et al. (1997) showed there was no difference in glycogen storage after 8 and 24 hrs recovery whether CHO supplements were given immediately after exercise or delayed for 2 hrs (Figure 4).

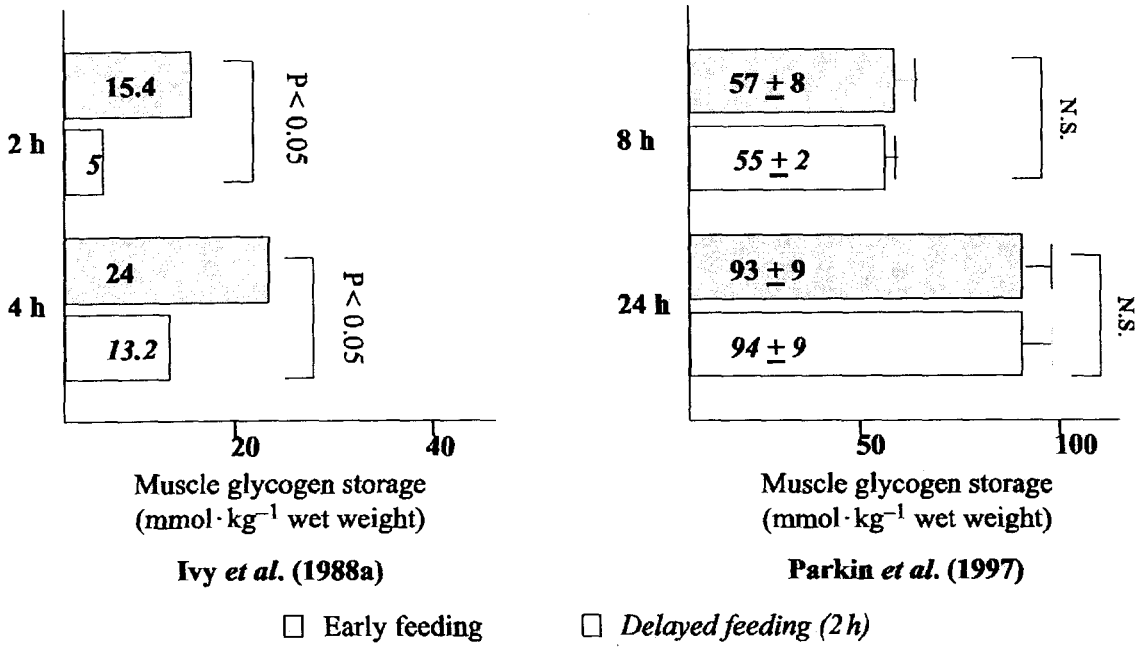


Figure 4. Muscle glycogen resynthesis rates 2, 4, 8, and 24 hrs into recovery from exercise following immediate CHO feeding (early feeding) and delayed feeding (2h) (Taken from Burke et al., 2004).

Furthermore, Garcia-Roves and colleagues (2003) found that the increase in GLUT-4 protein and the approximate 2-fold greater glucose transport activity in response to insulin is still present in rats 66 hrs post-exercise if rats aren't fed CHO following exercise. When these same rats were fed CHO 66 hrs post-exercise, their total muscle glycogen concentration 18 hrs post-feeding was equal to that of rats 24 and 48 hrs post-exercise who were fed CHO diets immediately following exercise (Garcia-Roves et al., 2003). Elevated GLUT-4 mRNA levels at 42 hrs post-exercise in these rats suggests that the persistence in GLUT-4 in CHO-fed rats may be due to an increase in GLUT-4 protein synthesis. On the other hand, it is also possible that persistence of GLUT-4 in CHO-free rats was due to inhibition of GLUT-4 proteolysis because, it was demonstrated that

GLUT-4 mRNA decreased to basal levels 66 hrs post-exercise while GLUT-4 protein content of the muscle membrane remained above basal levels beyond 66 hrs (Garcia-Roves et al., 2003).

Overall, it would appear that when recovery between exercise bouts is <8 hrs immediate CHO supplementation is necessary to obtain maximum glycogen storage. However, when recovery time is > 8 hrs, athletes may choose their preferred meal schedule as long as total CHO intake goals are achieved.

1.4.2. Effect of amount of CHO intake

Blom et al. (1987) demonstrated that increasing CHO consumption from 0.35 to 0.7 g CHO/kg body wt/h post-exercise did not lead to an increased muscle glycogen storage rate. However, when CHO supplementation was reduced to 0.18 g CHO/kg body wt/h, the rate of muscle glycogen storage decreased by 150% from 24.8 to 9.0 mmol/kg dw/h (Blom et al., 1987). Furthermore, Ivy et al. (1988b) reported that the doubling of a CHO supplement from 0.75 g CHO/kg body wt/h to 1.5 g CHO/kg body wt/h for 4 hrs after exercise did not significantly increase the muscle glycogen storage rate (19.6 vs. 22.0 mmol/kg dw/h). In contrast, a recent study by van Loon et al. (2000a) showed that when the rate of CHO ingestion was increased from 0.8 to 1.2 g CHO/kg body wt/h, the rate of glycogen storage increased from 16.6 to 35.4 mmol/kg dw/h. The difference between this study and the Blom et al. (1987) and Ivy et al. (1988b) studies is that in the van Loon et al. (2000a) study, CHO supplements were given at 30 min intervals rather than at 2 hr intervals which suggests the interval between food doses may affect the glycogen resynthesis rate. It has also been reported that delayed gastric emptying is not

likely to be the cause of lower glycogen storage rates with 2 hr vs. 30 min supplement intervals because, a large supplement bolus has actually been shown to empty faster from the stomach than a small bolus (Rehrer et al., 1994).

In summary, many studies have reported very high glycogen synthesis rates (40-43 mmol/kg dw/h) when 1.0-1.85 g CHO/kg body wt/h was consumed at intervals ranging from 15-60 minutes over a 3-4 h recovery period (Jentjens and Jeukendrup, 2003). However, there is no consensus regarding the exact amount of CHO that must be consumed post-exercise in order to maximize muscle glycogen recovery. This variability is likely the results of differences in study designs such as time of CHO supplementation, type of CHO ingested, training status of participants and the recovery time over which muscle glycogen resynthesis was measured (Figure 5).

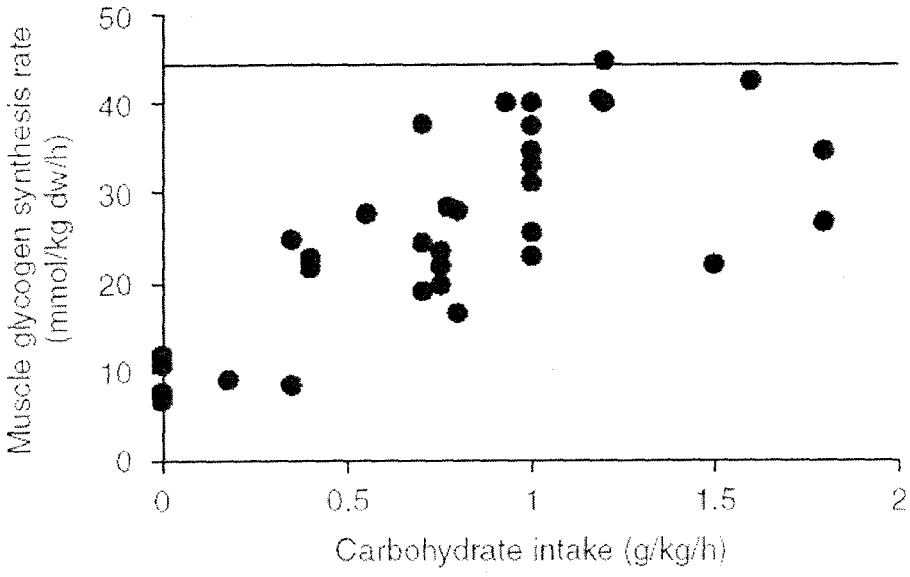


Figure 5. Muscle glycogen resynthesis rates with different amounts of CHO intake (Taken from Jentjens and Jeukendrup, 2003).

1.4.3. Method and type of CHO intake

Infusion rather than oral ingestion of 3 g CHO/kg body wt does not lead to any additional advantage for the restoration of muscle glycogen following exercise (Reed et al., 1989). In other words, delayed gastric emptying of CHO is not limiting for muscle glycogen resynthesis. Nevertheless, it has been shown that infusion of larger amounts of glucose such as 8 g CHO/kg body wt, results in substantially greater glycogen storage rates (Roch-Norlund et al., 1972). Infusion of such large doses of glucose however is not practical for most individuals since it is too expensive and requires an intravenous line.

Surprisingly, it has been reported that solid glucose supplements are just as effective as liquid glucose treatments in promoting muscle glycogen storage (Reed et al., 1989. Studies have shown that glucose (high Glycemic Index (GI)) and sucrose (moderate GI) supplementation are twice as effective as fructose (low GI) supplementation in promoting muscle glycogen resynthesis post-exercise (Blom et al., 1987). The GI, "is a measure for the blood response observed after a certain food product is ingested with a certain amount of glucose (mostly 50g), compared with the blood glucose response observed when an equal amount of pure glucose or white bread is ingested. The GI reflects the rate of digestion and absorption of a CHO-rich food (or a single CHO feeding)" (Jentjens and Jeukendrup, 2003). Fructose is not as effective due to it being preferentially taken up and metabolized by the liver before it has the chance to reach the muscle while glucose circumvents being taken up by the liver due to the high K_m of liver glucokinase (Casey et al., 2000) and coincidently goes directly to the muscle where it is either oxidized or stored as glycogen (Blom et al., 1987). When comparing

high CHO diets that differ in GI, Kiens et al. (1990) reported that the high CHO - high GI diet resulted in 61% higher muscle glycogen resynthesis rates compared with the high CHO -low GI diet (40 vs. 24 mmol/kg dry wt/h).

1.5. EFFECT OF PROTEIN SUPPLEMENTATION ON MUSCLE GLYCOGEN RESYNTHESIS POST-EXERCISE

1.5.1 Insulinotropic amino acids and proteins

Pancreatic insulin release is not only regulated by glucose but has also been shown to be stimulated by certain amino acids and protein (PRO) mixtures. In particular, drinks containing leucine, phenylalanine and arginine or leucine, phenylalanine and wheat protein hydrosylate have large insulinotropic effects when ingested in combination with CHO (van Loon et al., 2000b). The insulin response to the addition of PRO to CHO has been shown to be greater than the additive responses of CHO and PRO alone (Zawadzki et al., 1992). As noted by van loon et al. (2000b) the potential for PRO to stimulate insulin secretion from the pancreas is important because it raises the possibility that PRO may be used as a diagnostic tool to evaluate Type I insulin dependant diabetics. In addition, the use of PRO may be beneficial for elevating insulin concentrations in vivo without the need for intravenous infusions.

1.5.2. CHO-PRO supplements vs. CHO alone supplements

Ingestion of a CHO-PRO supplement during recovery from prolonged exercise has been reported to enhance the rate of muscle glycogen resynthesis – and thus the potential to perform repeated daily bouts of strenuous exercise – as compared to CHO

alone (Ivy et al., 2002). However, the precise amount and timing of CHO necessary to optimize muscle glycogen resynthesis post-exercise both in combination with PRO and alone remains controversial. For example, some studies report that ingestion of 1.0-1.2 g CHO/kg body wt/h for 3 or 4 hrs immediately post-exercise is the amount of CHO required to achieve maximum muscle glycogen resynthesis rates post-exercise (Carrithers et al., 2000; Jentjens et al. 2001; Tarnopolsky et al., 1997; van Hall et al., 2000b). However, these same studies cannot confirm that consumption of 1.0-1.2 gCHO/kg body wt/h provides optimal early muscle glycogen storage post-exercise because in these studies, higher CHO consumption rates such as 1.6 gCHO/kg body wt/h were not studied. Most importantly, these studies report that at CHO feeding rates between 1.0-1.2 g CHO/kg body wt/h associated PRO supplementation is not beneficial. The experimental design of each of the latter studies is different in terms of recovery time, timing and number of drinks and type of CHO and PRO ingested when comparing them to studies that have shown beneficial effects of CHO-PRO supplements on muscle glycogen resynthesis (Ivy et al., 2002; van Loon et al., 2000; Zawadzki et al., 1992). Refer to Table 2 for a summary of CHO/PRO studies.

Regardless of differences in experimental designs between studies, most evidence suggests that feeding a high amount of CHO at frequent intervals negates the benefits of added PRO when it comes to muscle glycogen resynthesis over a 4 hr period. Nevertheless, there is absolutely no evidence in the literature to show that this is true when it comes to muscle glycogen resynthesis post-exercise within the first hour of feeding. In fact, Ivy et al. (2002) showed that PRO is significantly more effective in

enhancing muscle glycogen storage compared to both an isoenergetic CHO feeding and an iso-CHO feeding in the first hour after supplementation (Figure 6).

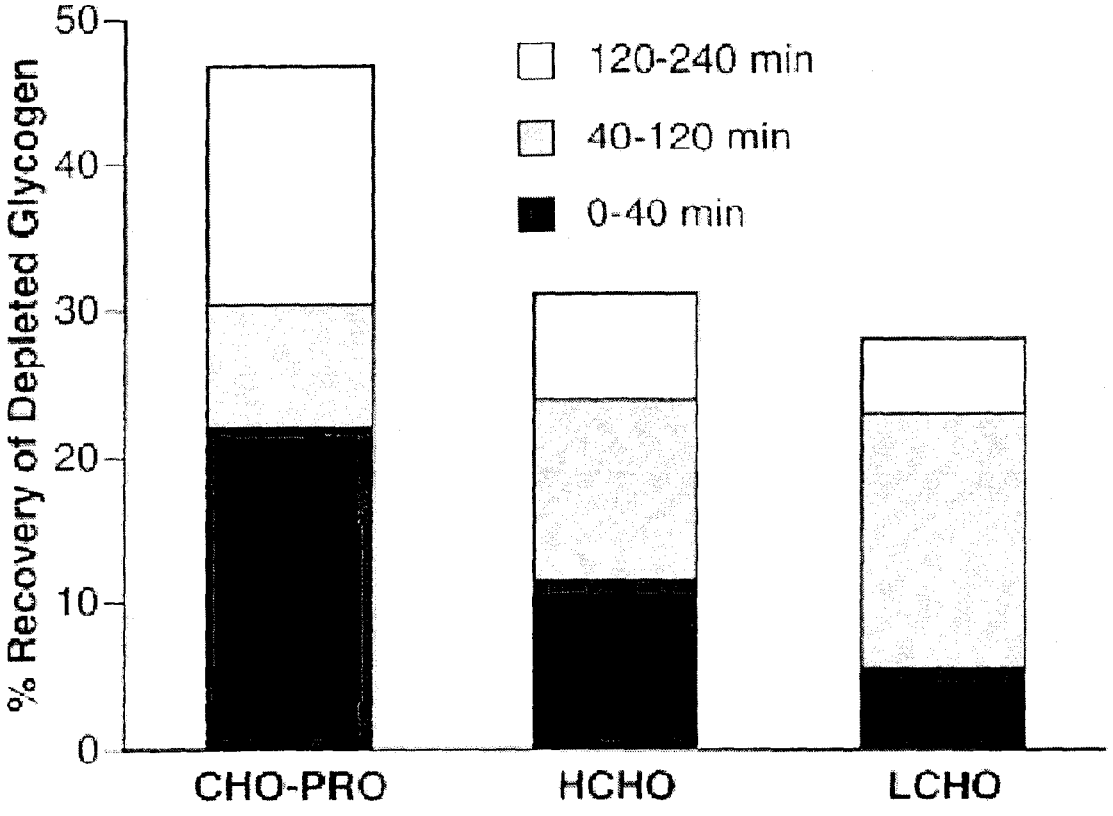


Figure 6. Percent recovery of depleted glycogen stores of the vastus lateralis from 0 to 40, 40 to 120, and 120-240 min of recovery. Initial and post-exercise muscle glycogen stores were not different among treatments (Ivy et al., 2002).

The only other study to measure muscle glycogen resynthesis post-exercise between 0 and 1 hr was the study by Jentjens et al. (2001). Close examination of immediate post-exercise muscle glycogen concentrations in the Jentjens et al. (2001) study reveals that there was an average difference of ~70 mmol/kg dw between the CHO and the CHO/PRO trials, the CHO/PRO trial having the highest post-exercise muscle glycogen concentration. When comparing these to the reported average resynthesis rates

between 0 and 1 hr post-exercise (CHO - 35±34 mmol/kg dw/h; CHO/PRO - 35±31 mmol/kg dw/h) it could be argued that the CHO/PRO trial had the same resynthesis rate from 0 to 1 hr post-exercise despite having a higher muscle glycogen concentration immediately post-exercise (Jentjens et al., 2001). There is recent evidence to suggest that muscle glycogen resynthesis is faster in muscle that is more heavily depleted (Zachwieja et al., 1991). This could mean that PRO is in fact beneficial even when high amounts of CHO are consumed per hr and more precisely, beneficial between 0 and 1 hr post-exercise. It is also unknown whether glycogen synthase activity is elevated at 1 hr post-exercise in a CHO/PRO trial vs. a CHO only trial. This leads to a second important question regarding the effectiveness of CHO/PRO supplements: What is the precise mechanism of action to explain the potential beneficial effect of PRO ingestion on muscle glycogen resynthesis post-exercise? Does insulin play a role?

1.5.3. The effects of insulin on muscle glycogen resynthesis

As described above, PRO and amino acids can stimulate insulin release from the pancreas. Many CHO/PRO supplement studies have demonstrated increased plasma insulin concentrations post-exercise when individuals consumed CHO/PRO supplements vs. CHO only supplements (Jentjens et al., 2001; Van Hall et al., 2000a; van Hall et al., 2000b; van Loon et al., 2000; Zawadzki et al., 1992). However, higher muscle glycogen resynthesis rates post-exercise do not correlate with higher plasma insulin concentrations. For example, in the Jentjens et al. (2001) study, there was no difference in muscle glycogen resynthesis rates between the CHO and CHO/PRO trials despite plasma insulin concentration being higher in the CHO/PRO trials. It was also shown in both the Ivy et

al. (2002) and the van Loon et al. (2000a) studies that higher muscle glycogen resynthesis rates were obtained in the CHO/PRO trials vs. CHO only trials despite no differences in plasma insulin concentrations among trials. When CHO supplementation is not adequate to maximize plasma insulin concentrations, PRO supplementation may stimulate a further increase in insulin. It does seem as though studies that used low hourly CHO ingestion rates and/or 15-30 min drink intervals have higher plasma insulin concentrations in their CHO/PRO trials vs. CHO trials (Jentjens et al., 2001; van Hall et al., 2000a, van Loon et al., 2000b; Zawadzki et al., 1992).

A simple mechanism by which PRO may contribute to higher muscle glycogen synthesis rates post-exercise is by increasing the total energy intake of a CHO drink. As of yet, no study as investigated whether protein oxidation may be increased post-exercise when ingesting a CHO/PRO vs. a CHO only drink. It is possible although highly unlikely that PRO oxidation spares muscle glucose in recovery from exercise and results in higher muscle glucose availability for glycogen storage. This situation would likely occur only in trials where CHO supplementation is inadequate to maximize post-exercise muscle glycogen resynthesis.

1.6. SUMMARY

Great strides have been made in deducing the factors that limit muscle glycogen resynthesis post-exercise including glucose transport and glycogen synthase activity. Most studies have focused on the potential for CHO ingestion to augment glycogen

resynthesis. New research suggests that PRO in addition to its role in whole body PRO kinetics, may also play a role in glycogen resynthesis post-exercise. Various attempts have been made to compare the effects of typical CHO only beverages vs. CHO/PRO beverages on the muscle glycogen storage rate post-exercise. In general, the data appears to suggest that the addition of PRO to a CHO drink is only beneficial in terms of increasing muscle glycogen synthesis when the CHO ingested is not sufficient to promote optimal levels of muscle glycogen resynthesis post-exercise (Burke et al., 2004). No investigation however has studied in tandem the optimal amount of CHO that must be consumed to reach the threshold of muscle glycogen resynthesis and the CHO consumption rate that PRO no longer is of any additional benefit in terms of muscle glycogen resynthesis post-exercise. Furthermore, while PRO does increase muscle glycogen resynthesis post-exercise when CHO ingestion is sub-optimal, no attempt has been made to link this optimal muscle glycogen resynthesis rate to increments in exercise performance. Lastly, more emphasis must be put on carrying out studies in which timing and type of CHO and PRO consumed are kept standardized in order to more specifically study the effects of amount of CHO ingested on muscle glycogen resynthesis post-exercise and subsequent exercise performance.

Table 2. Summary data of carbohydrate/protein studies.
GRR, glycogen resynthesis rate; PI, plasma insulin; AG, arterial glucose; PG, plasma glucose; GU, glucose uptake; AUC, area under curve; CHO, carbohydrate; PRO, protein; * Significantly different from 1.; # Significantly different from 2.; ∅ Significantly different from 3.

Study	Trials	Amount of nutrients	Depletion Protocol	Recovery Time	Biopsy Times	Results
Bowtell et al. (1999).	Three trials: 1. CHO 2. Placebo (Glutamine) 3. CHO-PRO	- 330 mL (18.5% glucose polymer) ~ 61.05g CHO 1. 18.5% (wt/vol) glucose polymer solution containing dextrose, maltose, *maltotriose*, tetrasaccharide, pentasaccharide, and higher sugars. 2. Placebo: 8 g glutamine. 3. 18.5% glucose polymer containing 8 g glutamine.	- 70% $V0_2$ max for 30 min, 16 * 1 min intervals (2 min rests). 45 min at 70% $V0_2$ max.	- 2 hrs	- 0, 1, and 2 hrs (3 total).	GRR: 1. 21.57±4.84 mmol/kg dw/h 2. 17.55±4.62 mmol/kg dw/h 3. 19.30±8.04 mmol/kg dw/h PI: 1. 10 µU/mL → 75 µU/mL *∅ 2. 10 µU/mL → 10 µU/mL 3. 10 µU/mL → 110 µU/mL PG: 1. 4.2 mM → 8.0 mM *∅ 2. 4.2 mM → 4.2 mM 3. 4.2 mM → 8.0 mM
Burke et al. (1995).	Three trials: 1. CHO Control 2. CHO control plus added FAT and PRO 3. CHO matched energy diet	- 4 meals (0, 4, 8, and 21 hrs) 1. Meals composed of high glycemic index CHO foods, 7 g CHO/kg/day divided into 4 meals. 2. Control diet plus 1.6 g FAT/kg/day and 1.2 g PRO/kg/day. 3. Control diet plus 4.8 g CHO/kg/day (111.8 g CHO/kg/day – a bit higher than the recommended 8-10 g CHO/kg/day).	- 2 hrs on personal bike on a windtrainer plus 4*30-s all out sprints with 2 min recovery.	- 24 hr profiles	- 0 and 24 hrs (2 total).	GRR: 1. 14.2 g/kg dw/h 2. 13.4 g/kg dw/h 3. 14.65 g/kg dw/h PI: (no difference in meal or total insulin areas between diets). PG: (FAT/PRO diet resulted in a significantly reduced total incremental glucose area compared with the other diets).

Carrithers et al. (2000).	Three trials: 1. CHO 2. CHO/PRO 3. CHO/AA	- eight meals total (at time 0 and every 30 min till 210 min), eucaloric. 1. CHO: 301.6 g [α-D-glucose]. 2. CHO: 214.5 g, PRO: 61.3 g, FAT: 1.5 g [dextrose, fructose, maltodextrine, and vegetable oil, caseinate, milk, whey protein]. 3. CHO: 258.5g, PRO:43.1g[sucrose, fructose, dextrose, L-lysine, L-leucine, L-valine, L-phenyalanine, L-threonine, L-histidine, L-isoleucine, and L-methionine.	- 75 min at 70% of $V0_2$ peak on cycle ergometer + six 1 min sprints at 125% $V0_2$ peak, 1 min recovery.
Ivy et al. (2002).	Three trials: 1. CHO/PRO 2. HCHO 3. LCHO	- two bolus drinks: at t=10 min and t=120 min into recovery (472 mL each). 1. 80 g CHO + 28 g PRO + 6 g FAT. 2. 108 g CHO, 6 g FAT. 3. 80 g CHO + 6 g FAT. (*no details about actual CHO or PRO type).	- 2 hrs at 65-75% $V0_2$ max + ~15 *1-min sprints.
Jentjens et al. (2001).	Two trials: 1. CHO 2. CHO/PRO	- 3.5 mL / kg at 0, 30,60,90,120, and 150 min. 1. 1.2 g/kg/h CHO (50% glucose + 50% maldextrin). 2. 1.2 g/kg/h CHO(50% glucose +50% maldextrin) + 0.4 g/kg/h of protein hydrosylate (wheat protein hydrosylate, free leucine and phenylalanine).	- 90% / 50% W_{max} till exhaustion (2 min blocks), 80% / 50% W_{max} till exhaustion (min blocks), 70% / 50% W_{max} till exhaustion (2 min blocks).

- 4hrs	- 0 + 4hrs (2 total).	GRR: 1. 31.0 mmol/kg/h 2. 28 mmol/kg/h 3. 29.5 mmol/kg/h PI: No difference between trials, all went from 5 μU/mL to ~40-60 μU/mL. AUC: no difference. PG: CHO trial greater at 90 min only ($p<0.05$). AUC: no difference between trials at any time.
- 4hrs	- 13C-NMR Pre and at 0, 20, 40, 60, 120, 180, and 240 min post-exercise (8 total).	GRR: 1. ~48 mmol/L/4h [#○] 2. ~33 mmol/L/4h 3. ~30 mmol/L/4h PI: (no difference at any time point: 5 μU/mL (t=0)→33 μU/mL (t=180 min). PG: 1. 70 mg/dl→100 mg/dl [#○] 2. 70 mg/dl→130 mg/dl 3. 70 mg/dl→130 mg/dl
- 3 hrs	- 0, 60 and 180 min (3 total).	GRR: 1. 35±34 mmol/kg dw/h (0-1 h), 42 mmol/kg/h (1-3h), 40±10 mmol/kg dw/h (0-3 h). 2. 35±31 mmol/kg dw/h (0-1h), 21±16 mmol/kg/h (1-3 h), 25±16 mmol/kg dw/h (0-3). PI:

Roy et al. (1998).	Three trials: 1. CHO/PRO/FAT 2. CHO only 3. Placebo	- 2 drinks (0 and 1 hr) 1. 66% CHO/ 23% PRO/ 11%FAT (iso-energetic to CHO only drink). 2. CHO only (1g/kg) (56% sucrose – 44% glucose polymer from corn syrup solids). 3. Placebo (sucralose).	- Resistance Exercise. - Three sets of each exercise. - bench press, sit-ups, knee extension, latissmus pulldowns, bicep curls, leg press, and military press.	- 4.5 h
Tarnopolsky et al. (1997).	Three trials: 1. CHO/PRO/FAT 2. CHO 3. Placebo	- 2 drinks (0+ 1 hr) 1. 0.75 g CHO/kg (56% sucrose, 46% glucose polymer from corn syrup solids)+ 0.1 g PRO/kg (milk protein) + 0.02 g FAT/kg (canola, sunflower and corn oil, with vitamins, minerals and flavoring). 2. 1 g CHO/kg (56% sucrose, 46% glucose polymer from corn syrup solids).	- 65% of $V0_2$ peak for 90 min.	- 4 hrs

		1. 9.7±0.9 μU/mL→ ~50 μU/mL 2. 9.1±1.8 μU/mL→ ~ 130 μU/mL (*insulin significantly higher in CHO-PRO vs. CHO during final 60 min.) (AUC significantly different between 60-180 min and 0-180 min). PG: 1. ~3.4mmol/L (0 h)→ ~7.0 mmol/L(1 h)→5 mmol/L (3 hr). 2. ~3.4mmol/L (0 h)→ ~7.0 mmol/L(1 h)→5.6 mmol/L (3hr).
rs	- 0 and 4 hrs (2 total).	GRR: 1. 23.0±4.5 mmol/kg dw/h 2. 19.3±6.1 mmol/kg dw/h 3. 2.0±2.3 mmol/kg dw/h PI: (AUC) 1. 28.9±2.7 μIU/h/L 2. 33.6±4.6 μIU/h/L 3. 10.1±1.2 μIU/h/L*# PG: (AUC) 1. 5.87±0.27 mM/h/L 2. 5.59±0.35 mM/h/L 3. 4.2 mM/h/L*#
	-0 and 4 hrs (2 total).	GRR: 1. Men: 25.5 mmol/kg dw/h, Women: 23.5 mmol/kg dw/h 2. Men: 40.0 mmol/kg dw/h, Women: 34.5 mmol/kg dw/h 3. Men: 3.0 mmol/kg dw/h, Women: 12.0 mmol/kg dw/h*# PI: (AUC) 1. 21.66 μIU/L/h 2. 26.2 0μIU/L/h 3. 6.34 μIU/L/h*# PG:

		3. sucralose.		
Van Hall et al. (2000a).	Four trials: 1. Control (glucose only) 2. CHO + Free glutamine 3. CHO + wheat hydrosylate with high glutamine 4. CHO + wheat hydrsylate with normal glutamine	- Drinks at 0, 1, and 2 hrs. 1. 0.8 g/kg BW CHO. 2. 0.8 g/kg BW CHO + 0.3 g/kg free glutamine. 3. 0.8 g/kg BW CHO + 0.3 g/kg wheat protein hydrosylate with high glutamine. 4. 0.8 g/kg BW CHO + 0.3 g/kg normal glutamine.	- 90% / 50% W_{max} till exhaustion (2 min blocks), 80% / 50% W_{max} till exhaustion (min blocks), 70% / 50% W_{max} till exhaustion (2 min blocks).	- 3 hrs
Van Hall et al. (2000b).	Three trials: 1. CHO/PRO 2. CHO 3. H_20	- 600 mL immediately and 150 mL every 15 min for 4 hrs 1. 1.67 g/kg BW/L sucrose and 0.5 g/kg BW/L whey protein hydrosylate. 2. 1.67 g/kg BW/L sucrose. 3. H_20.	- 90% / 50% W_{max} till exhaustion (2 min blocks), 80% / 50% W_{max} till exhaustion, shower, 14 min @ 40% W_{max} and 4 min at 60, 70, and 80% W_{max}.	- 4 hrs

	(AUC) 1. 4.75 mM/h 2. 5.35 mM/h 3. 4.06 mM/h*#	
- 15 min and 3hrs (2 total).	GRR: 1. 28 mmol/kg dw/h 2. 26 mmol/kg dw/h 3. 33 mmol/kg dw/h 4. 34 mmol/kg dw/h	
	PI: 1. 10 μU/mL → 40 μU/mL 2. 10 μU/mL → 62 μU/mL 3. 10 μU/mL → 85 μU/mL 4. 10 μU/mL → 95 μU/mL (significantly higher between 30 and 150 min in both protein hydrosylates trials).	
	PG: (Almost doubled 30 min after ingestion in all trials but gradually decreased thereafter: 4.2 mmol/L → 7.5 mmol/L → 4.5 mmol/L).	
- 0, 90 and 240 min (3 total).	GRR: 1. 50 mmol/kg dw/h (0-1.5 hrs), 30 mmol/kg dw/h (1.5- 4 hrs). 2. 50 mmol/kg dw/h (0-1.5 hrs), 30 mmol/kg dw/h (1.5-4 hrs). 3. 18 mmol/kg dw/h (0-1.5 hrs), 8 mmol/kg dw/h (1.5-4 hrs).*#	
	PI: 1. 70-80 mU/L#◎ 2. 40-50 mU/L 3. 5 mU/L	
	AG: 1. 8.7 ± 0.3 mmol/L (20 min after 1st bolus) to 6.3 mmol/L, GU: 1.6→0.9 mmol/min. 2. 9.6±0.2 mmol/L (20 min after 1st bolus) to 6.3 mmol/L, GU: 1.6→0.9 mmol/min.	

van Loon et al. (2000a).	Three trials: 1. CHO 2. CHO-PRO 3. CHO-CHO	- 3.5 mL/kg at t=0 min and every 30 min for 5 hrs. 1. 0.8 g CHO/kg/h (50% glucose + 50% maldextrin). 2. 0.8 g CHO /kg/h + 0.4 AA/kg/h (25% mass % free leucine, 25% mass % phenyl, and 50% mass % a wheat-gluten protein hydrosylate). 3. Isoenergetic amount of 1.2 g CHO/kg/h.	- 90% / 50% W_{max} till exhaustion (2 min blocks), 80% / 50% W_{max} till exhaustion (min blocks), 70% / 50% W_{max} till exhaustion (2 min blocks).	- 5 hr
Varnier et al. (1995).	Three trials: 1. Saline 2. Glutamine 3. Alanine + glycine	- all substances were infused intravenously 1. 3.75 ml/min saline and [U-^{13}C] labeled glucose (prime 9 mg/kg; 10 mg/kg/h). 2. 3.75 ml/min glutamine (prime 30 mg/kg; 50 mg/kg/h) and [U-^{13}C] labeled glucose (prime 9 mg/kg; 10 mg/kg/h). 3. 3.75 ml/min alanine and glycine (primes 18.3 and 15.4 mg/kg; infusions 30.5 and	- 30 min at ~70% $V0_2$max, 1 min bouts at twice the above workload (2 min rest intervals), 45 min at 70% $V0_2$ max.	- 2hrs

		3. ~4.2 mmol/L (20 min after 1st bolus) to ~5.2 mmol/L). GU: 0.7→0.00 mmol/min.*#
s	- 0 and 300 min (2 total).	GRR: 1. 16.6 ±7.8 µmol glucosyl units/ g dw/h 2. 35.4 ±5.1 µmol glucosyl units/ g dw/h* 3. 44.8 ±6.8 µmol glucosyl units/ g dw/h* PI: (no difference in plasma insulin concentrations across trials[5 mU/L→83 mU/L]). AUC: significantly higher in both CHO-PRO and CHO-CHO trials than in control trial (15.9±2.2 and 12.3±1.8 compared with 8.6±0.9 U*h/L#∅). PG: (no difference in plasma glucose concentration across trials [4.2 mmol/L→7mmol/L]). AUC: 1. 500 mmol/5h/L 2. 258 mmol/5h/L*∅ 3. 592 mmol/5h/L
	- 0,1, and 2 hrs (2 total).	GRR: 1. 3.4 µmol/g dw/2 hrs 2. 11.98 µmol/g dw/2 hrs 3. 3.4 µmol/g dw/2 hrs PI: (All were the same; 10 µU/mL (immediately post-exercise)→ 15 µU/mL (in recovery). PG: (All trials were the same; 4.5 mmol/L).

		25.7 mg/kg/h) and the same amount of unlabeled glucose as [U-^{13}C] glucose given to the other groups.				
Williams et al. (2003).	Two trials: 1. CHO/PRO 2. CHO	- 2 drinks total (0 and 2 hrs), 355 ml each 1. Total: 106 g CHO(~0.40 g CHO/kg/h) + 28 gPRO (~0.10 gPRO/kg/h). 2. 6% Gatorade. Total: 42g CHO (~0.15 gCHO/kg/h).	- 2 hrs at 65-75% $V0_2$ max + 1-4 five min sprints at 75% $V0_2$ max.	- 4 hrs	- 0 and 240 min (2 total)	GRR: 1. 33.7 ± 4.5 mmol/kg dw/h[#] 2. 17.4 ± 5.2 mmol/kg dw/h PI: 1. 6.90 mU/L→47 mU/L→14 mU/L[#] 2. 6.90 mU/L→23 mU/L→13 mU/L PG: 1. 3.4 mmol/L→5.8 mmol/L→3.6 mmol/L[#] 2. 3.4 mmol/L→5.0 mmol/L→3.4 mmol/L
Yaspelkis et al. (1999).	Two trials: 1. CHO 2. CHO/AA	- 4 drinks (0, 1, 2, and 3 hrs) 1. 23% (w/v) maltodextrin mixture, received 1.0 g CHO/kg BW with each drink. 2. Received 0.08 g arginine/kg BW with each drink (1.83 g arginine hydrochloride in 100 mL of the CHO supplement).	- 2 hrs total, alternated exercise intensity between 60% and 75% every 15 min for 90 min, final 30 min consisted of cycling for 10 min at 60%, 10 min at 75%, 5 min at 50%, and 5 min at 80% VO_2 max.	- 4 hrs	- 0 and 4 hrs (2 total).	GRR: 1. 25.57 mmol/kg dw/h 2. 34.88 mmol/kg dw/h PI: (no difference between trials, 7.0 µU/mL (t=0) → 85 µU/mL (t=240 min). PG: (no difference between trials, ~3.5 mM (t=0) → ~7.0 mM (t=60 min) → ~5.5mM (t=240 min). *At 180 and 240 min post-exercise, CHO oxidation was significantly higher in the CHO trial vs. the CHO/PRO trial. Total CHO oxidation: 1. 35.8±4.1g CHO 2. 24.3±3.3g CHO
Zawadzki et al. (1992).	Three trials: 1. CHO 2. PRO 3. CHO/PRO	- two bolus drinks at 0 and 180 min into recovery. 1. 112 g CHO (dextrose-maldextrin mixture, 21% wt/vol). 2. 40.7 g PRO (milk	- 2 hrs total, [15 min at 60-65% $V02_{max}$ and 15 min at 70-75% $VO2_{max}$]*3, 10 min at 60-65% $VO2_{max}$, 10 min at 70-75% $VO2_{max}$, 5 min at 50% $VO2_{max}$,	- 4 hrs	- 0 and 4 hrs post-exercise (2 total).	GRR: 1. 22.0 mmol/kg/dw/h[#] 2. 10.0 mmol/kg dw/h 3. 28.5 mmol/kg dw/h*[#] PI: 1. 5 µU/mL (t=0)→60µU/mL (t=150).

and whey protein isolate mixture, 7.6 % wt/vol).
3. CHO/PRO.

and 5 min at 80-85% $VO2_{max}$

2. 5 µU/mL (t=0)→10µU/mL (t=150).
3. 5 µU/mL (t=0)→88µU/mL (t=150).*#

PG:
1. 3.67±0.11 mM→6.2 mM
2. 3.67±0.11 mM→4.4 mM
3. 3.67±0.11 mM→7.06 mM*#

CHAPTER 2

OPTIMIZING MUSCLE GLYCOGEN RESYNTHESIS POSTEXERCISE: SHOULD PROTEIN BE ADDED TO A CARBOHYDRATE DRINK?

2.1 INTRODUCTION

Muscle glycogen is an important source of fuel for athletes and the need to replenish muscle glycogen stores is critical for recovery and subsequent exercise performance. It is well established that ingesting carbohydrate (CHO) during recovery will increase the rate of muscle glycogen resynthesis (Burke et al., 2004; Jentjens and Jeukendrup, 2003), but investigators have also considered the possibility that ingesting other nutrients with CHO will enhance the rate of muscle glycogen resynthesis. Several studies have reported that co-ingesting PRO with CHO has a beneficial effect on early (≤ 4 hrs) muscle glycogen resynthesis post-exercise. A number of studies have reported higher muscle glycogen resynthesis when PRO was added to a CHO drink following exercise (Zawadzki et al., 1992; van Loon et al., 2000; Ivy et al., 2002), whereas others have found no effect (Carrithers et al., 2000; Jentjens et al., 2001; Roy and Tarnopolsky, 1998; Tarnopolsky et al., 1997; van Hall et al., 2000b). The equivocal data are likely due to differences in study designs including the frequency of CHO ingestion, type of CHO and amount of CHO ingested. For example, in studies that showed that the addition of PRO to a CHO supplement increases muscle glycogen storage, feeding intervals of 2 hrs were used (Ivy et al., 2002; Zawadzki et al., 1992). Most but not all studies that did not show a beneficial effect of PRO used feeding intervals of 15-30 min and fed a high hourly amount of CHO within the first four hours of recovery (Carrithers et al., 2000; Jentjens et

al., 2001; van Hall et al., 2000b). In a recent review of this topic, Burke et al., (2004) concluded that "the evidence is compelling that the co-ingestion of protein with carbohydrate will increase the efficiency of muscle glycogen storage when the amount of carbohydrate ingested is below the threshold for maximal glycogen storage." Burke et al. (2004) also noted that controversy exists regarding the "optimal" post-exercise nutritional strategy for glycogen resynthesis and posed the question: "At what intake of carbohydrate is the threshold for glycogen storage reached over the first 4 h of post-exercise recovery".

Several investigators have suggested that ingesting CHO at a rate of 1.2 g/kg/h in frequent intervals will maximize the rate of muscle glycogen resynthesis post-exercise and obviate any additional effect of PRO (Jentjens et al., 2001; van Hall et al. 2000b). However, no study has systematically evaluated this hypothesis and the questions that remain to be clarified are: what rate of CHO ingestion maximizes the rate of muscle glycogen resynthesis post-exercise? Secondly, does the addition of PRO to a CHO drink provide any additional benefit to muscle glycogen storage when the rate of CHO intake exceeds 1.2 gCHO/kg/h? The specific mechanism of action for the proposed effect of PRO ingestion on glycogen resynthesis also remains unclear. It has been suggested that the benefit of PRO on muscle glycogen storage is simply a matter of an increase in energy intake while others suggest a distinct mechanism of action of PRO on the muscle glycogen storage pathway. For example, it has been reported that PRO may increase muscle glycogen storage via increasing blood insulin concentration that in turn increases muscle glucose uptake. However, a study by Ivy et al., 2002, demonstrated that if PRO does in fact increase muscle glycogen storage, it does so during the insulin-independent

phase of glycogen storage post-exercise and therefore is clearly not regulated by insulin concentration.

The purpose of the present study was to investigate whether post-exercise CHO intake supplemented with PRO could enhance the muscle glycogen resynthesis rate when the CHO intake is close to the maximal consumption rate (~1.2g CHO/kg/h) observed to produce the maximal muscle glycogen resynthesis rate post-exercise. We examined whether the addition of 0.4 g/kg/h of PRO or an extra 0.4 g/kg/h of CHO to 1.2 gCHO/kg/h would increase the rate of muscle glycogen resynthesis as compared to 1.2 gCHO/kg/h. Our initial intention was also to examine both changes in skeletal muscle metabolism as well as exercise performance, by having subjects perform a second exercise challenge 4 hrs after the an initial bout of glycogen depleting exercise. However, gastro-intestinal (GI) problems during some trials precluded an objective analysis of this sort. With respect to skeletal muscle metabolism, we tested the hypothesis that ingesting 0.4 g/kg/h of additional energy — either in the form of CHO or PRO — would result in a higher rate of muscle glycogen resynthesis post-exercise, as compared to the control trial that provided 1.2 gCHO/kg/h.

2.2 METHODS

2.2.1. Subjects

Six recreationally active men volunteered to participate in the study. Participation in the study necessitated that participants have the ability to perform a 750 kJ cycling time-trial (simulated 30 km bike race). The protocol and the potential benefits and risks associated with participation were fully explained to each subject before they signed an informed consent document. The experimental protocol was approved by the McMaster University and the Hamilton Health Sciences Research Ethics Boards. Subject characteristics are listed in Table 3.

Table 3. Subject Characteristics

Age, yr	22 ± 1
Body mass. kg	90.1 ± 4.8
$V0_2$ peak, ml/kg/min	48.7 ± 2.9
Wpeak, W	348 ± 14
Heart Rate maximum, beats/min	187 ± 2

Values are mean ± SE; n = 6 subjects. VO_2peak, peak 0_2 consumption; Wpeak, peak power output.

2.2.2. Preliminary Testing

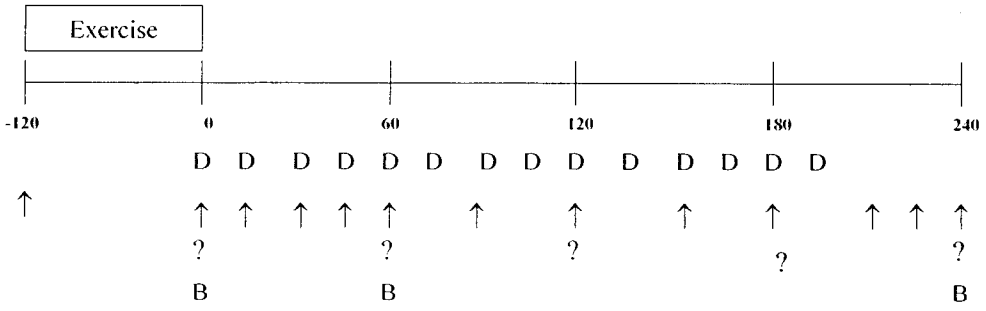
At least one week prior to the start of the first experimental trial, subjects performed an incremental exercise test to fatigue on a cycle ergometer in order to determine their peak oxygen uptake (VO_2peak) and peak power output (Wpeak, i.e., peak power at VO_2peak). The exercise test was performed on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) using an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburg,

PA). Subjects started with a 5-min warm-up at 50 W, followed by a ramp increase in workload of 1 Watt (W) every 2 seconds until volitional exhaustion. The inability of the subject to maintain a pedal cadence above 40 RPM was used to determine the end of the test since according to manufacturer's specifications power outpout is inconsistent below 40 RPM. VO_2 peak was considered to be the highest VO_2 averaged over a 30-second period and W_{peak} was considered to be the highest power averaged over a 30-second period during the exercise test to exhaustion. Participants also performed two 750 kJ familiarization time-trials (simulated 30 km bike race). Each time-trial was performed on a separate day and at least one week prior to the start of the first experimental trial.

2.2.3. General Design

After preliminary testing, each subject completed three experimental trials separated by at least 7 days. To minimize differences in resting muscle glycogen between trials, subjects were instructed to consume the same type of food for 48 hrs prior to each experimental trial and this was subsequently confirmed by analyses of diet records. In addition, subjects were asked to avoid any vigorous exercise and abstain from alcohol for 48 hrs prior to each experimental trial. On the morning of each experimental trial, subjects consumed a standardized breakfast 3 hrs prior to a standardized cycle ride that was designed to reduce muscle glycogen content. Following the cycle ride subjects received one of three different drinks that contained different amounts of CHO and PRO in order to examine the effect of drink composition on muscle glycogen resynthesis. Initially, we set out to use a randomized counter-balanced design to determine the order of drinks consumed by each subject. However, due to methodological problems with the

catheters for isotope infusions, the initial order of drinks had to be modified for some of the subjects (Refer to Appendix IV). During a 4-h recovery period following the exercise bout, subjects remained seated or lying supine while reading or watching videos. Muscle biopsy and blood samples were collected periodically during recovery as shown in Fig.7 and described in detail below.



B Muscle biopsy D Drink

↑ Blood sample ? Questionnaire

Figure 7. Schematic representation for each trial day.

2.2.4. Experimental protocol

Subjects reported to the McMaster University Exercise Metabolism Laboratory at 9:30 a.m. on the morning of each experimental trial. After subjects were weighed, a catheter was inserted into an antecubital vein and a resting blood sample was obtained. Subjects then received a primed constant infusion of the stable isotope labeled amino acids, L-[ring-$^{2}H_{5}$]-phenylalanine and 1-^{13}C-leucine, as well as a bolus infusion of $NaH^{13}CO_{3}$ for the subsequent determination of muscle protein kinetics. The muscle protein data are unrelated to the present thesis and will not be discussed further. Subjects

then mounted an ergometer and cycled for 2 hrs using an interval protocol designed to reduce muscle glycogen content (modified from Kuipers et al., 1987). The protocol consisted of alternating 10 min "easy" and "hard" workloads, beginning with 10 min @ 50% Wpeak, then 80% Wpeak for 10 min, then 50% Wpeak for 10 min, then 75% Wpeak for 10 min and so on until their last workload at 55% Wpeak (Fig. 8). If subjects were unable to finish one of the hard 10 min workloads, the remaining time was added to the next low workload @ 50% Wpeak. After the first depletion protocol, each subject repeated the identical protocol (with any workload adjustments kept standard) during the remaining two trials.

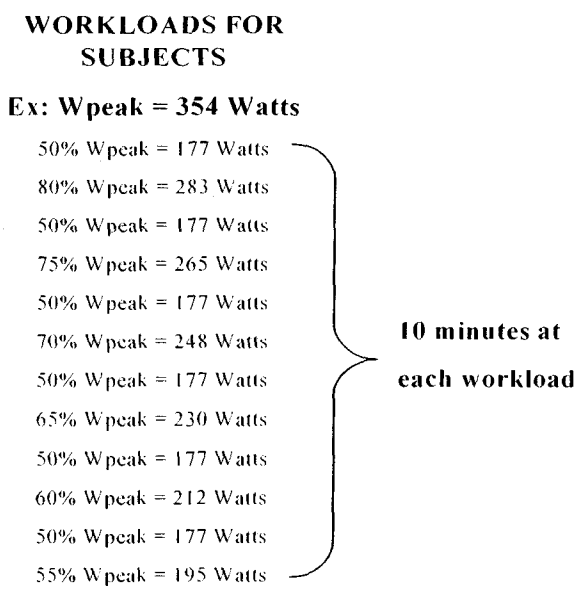


Figure 8. Muscle glycogen depletion protocol.

During the glycogen-depletion exercise, the subjects were cooled with standing floor fans and water was provided ad libitum. Each subject consumed 1.65-2.20 liters of water during the depletion ride. Each subject was required to drink the same amount of water in their 2nd and 3rd depletion rides as they consumed in their first depletion ride.

Immediately following cessation of the depletion exercise protocol, the lateral portion of one thigh was prepared for the extraction of needle biopsy samples as

described by Bergström (1975). One small incision was made through the skin and fascia over the vastus lateralis muscle under local anesthesia (2% lidocaine) and a post-exercise biopsy was obtained. The muscle biopsy procedure was repeated after 1 and 4 hrs of recovery, however only data from the immediate post-exercise and 4 h recovery sample will be presented here. The muscle samples obtained were immediately frozen in liquid nitrogen and subsequently stored at -80°C prior to subsequent analyses.

After the first biopsy, a second catheter was inserted into an antecubital vein of the arm opposite to that used for the first catheter. A post-exercise blood sample was obtained before subjects received the first drink. Blood samples were taken at 15 min intervals for the first hour then every 30 min up to 4 hrs post-exercise. All blood samples were stored on ice before being processed on the same day.

2.2.5. Beverages

After the post-exercise blood sample was obtained, subjects received a 188 mL drink every 15 min until 180 min into recovery. The drinks were formulated to provide: 1) 1.2g CHO/kg/h, 2) 1.6g CHO/kg/h or 3) 1.2g CHO/kg/h + 0.4 g PRO/kg/h. The CHO source was maltodextrin (Glucidex ® IT 19, ©Roquette Frère, S.A.) and the PRO source was a hydrolyzed whey protein concentrate (American Casein Company, HLA-198). To make the drinks comparable in taste, 5 g of sucralose (Splenda, USA), half a teaspoon of sodium, and an orange powder flavoring was added to 750 mL of each beverage. The PRO drinks also contained trace amounts of L-[ring-$^{2}H_{5}$]-phenylalanine. An example of the composition of a test drink for an 80 kg male is presented in Table 2.

Table 4. Composition of beverages

	Trial		
	CHO	CHO/CHO	CHO/PRO
L-[ring-$^2H_5$]-phenylalanine, g/h	0	0	0.09
Whey Protein, g/h	0	0	32
Maltodextrin, g/h	91	123	91
Sucralose, g/h	5	5	5
Sodium Chloride (NaCl), g/h	1	1	1
Water, L/h	0.75	0.75	0.75

Example beverage composition based on a 80 kg individual. CHO, low carbohydrate only beverage (1.2g CHO/kg/h); CHO/CHO, high carbohydrate only beverage (1.6 g CHO/kg/h); CHO/PRO, carbohydrate plus protein mixture beverage (1.2g CHO/kg/h + 0.4 g PRO/kg/h).

2.2.6. Analyses

2.2.6.1. Blood analyses

Blood samples were collected into heparinized and non-heparinized tubes. 200 µl of heparinzed whole blood was added to 1000 µl of 0.6 N perchloric acid (PCA), centrifuged, and the supernatant collected and stored at -30°C. The PCA extract was used for the fluorometric determination of glucose and lactate (Hitachi F-2500 fluorescence spectrophotometer) using enzyme assays described by Passoneau and Lowry (1993). The non-heparinized tubes were centrifuged and the resulting serum was collected and stored at -30°C for subsequent serum insulin analysis. A radioimmunoassay kit (RIA) (Coat-A-Count, Diagnostic Products, Los Angels, CA) was used to analyze the serum insulin concentration of each sample in duplicate. Serum insulin samples, after a 24-h incubation, were analyzed via a gamma counter. Area under the curve (AUC) for insulin was measured as total area over 4 hrs minus baseline.

2.2.6.2 Muscle Analyses

The frozen wet muscle biopsy samples (~50 mg wet wt) were freeze-dried for 1 day, powdered and dissected free of connective tissue and blood. All samples were stored at -80°C prior to analyses.

The extraction procedure to measure glycogen concentration was adapted from Harris and colleagues (1974) and used 500 µl of 2.0 N HCL added to ~2 mg freezed-dried muscle. Samples were incubated at 100°C for 2 hrs, and then 500 µl of 2.0 NaOH was added. The extract was stored at -80°C until analysis. For all samples, two separate pieces of muscle were extracted and used to determine glycogen content. The coefficient of variation for the glycogen assay was calculated using the method error technique described by Sale (1991). The inter-assay variability, based on 23 muscle samples that were analyzed on two separate days, was 14%.

For the determination of all other muscle metabolites except glycogen, a ~10 mg portion of freeze-dried muscle was extracted on ice using 0.5 M PCA containing 1mM EDTA, neutralized with 2.2 M $KHC0_3$, vortexed, centrifuged and the supernantant was used for metabolite analyses. Creatine (Cr), phosphocreatine (PCr), adenosine triphosphate (ATP), lactate (La), and glycogen (glucose assay) concentrations were determined with an Hitachi F-2500 fluorescence spectrophotometer (Hitachi, Ltd. Tokyo, Japan) using fluorometric enzyme assays described by Passoneau and Lowry (1993).

2.2.7. Gastro-intestinal (GI) Questionnaires

Subjects were asked to complete a questionnaire immediately after the glycogen-depletion exercise and each hour during the 4-h recovery period. The questionnaire was

designed to assess the response to drink ingestion with respect to bloating, belching, cramping, vomiting, diarrhea, urge to defecate, urge to urinate, as well as the presence of body chills, nausea, headache, and dizziness (Jentjens et al., 2001). Each question was scored on a 10-point scale (1 = not at all, 10 = extremely bad) and thus the maximum distress score at any time point was 110. Because there were no GI problems immediately after exercise or at 1-h post-exercise, scores were all "1", only the results from the 0, 2, 3, and 4 h questionnaires were reported.

2.2.8. Statistical analyses

Muscle and blood data were analyzed using a two-factor (time x treatment) repeated measures analysis of variance (ANOVA) (SigmaStat, Systat Software Inc., Point Richmond, CA, USA). When a significant main effect was identified, data were subsequently analyzed using a Tukey HSD post hoc test. Integrated area under the curve calculations were performed using GraphPad Prism 3.0 (GraphPad Software, Inc.,San Diego, CA) and analyzed using a one-factor(treatment) repeated-measures ANOVA. Significance for all analyses was set at $P \leq 0.05$. All values are expressed as means ± standard error of the mean (SEM).

2.3 RESULTS

2.3.1 Muscle Metabolites

The concentration of muscle glycogen was similar between treatments immediately after exercise (Table 5, Figure 9). Over the 4-h recovery period, muscle glycogen concentration increased in all trials ($P \leq 0.05$; main effect for time). However, there was no difference in muscle glycogen synthesis rate between trials over the 4-h recovery period (Figure 10).

Table 5. Muscle glycogen concentrations for muscle biopsy samples taken from vastus lateralis immediately after exercise and after 4-h recovery period.

	After Exercise	After 4 hrs of Recovery	Δ
Glycogen (mmol/kg dry wt)			
CHO	97±31	188±36	91±12
CHO/CHO	84±26	184±35	100±26
CHO/PRO	63±18	162±23	98±17

Values are ± SEM. Δ, difference between after exercise and 4 h after exercise recovery glycogen concentrations. CHO, low carbohydrate only beverage (1.2 gCHO/kg/h); CHO/CHO, high carbohydrate only beverage (1.6 gCHO/kg/h); CHO/PRO, carbohydrateplus protein mixture beverage (1.2g CHO/kg/h +0.4 g PRO/kg/h).

There was no significant difference in the concentration of muscle [lactate], [creatine], [PCr], or [ATP] between trials at any time-point post-exercise (Table 6). However, muscle [lactate] increased over the 4-h recovery period in all trials (main effect for time, $P \leq 0.05$).

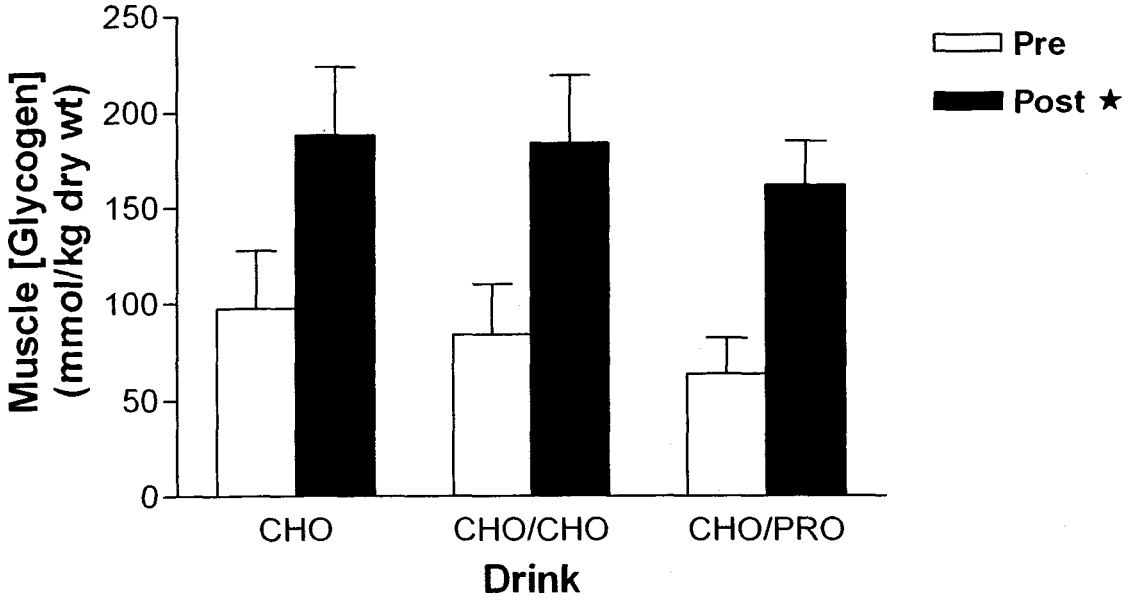


Figure 9. Muscle glycogen concentration immediately after exercise and after 4-h recovery period. ★ $P \leq 0.05$ (main effect for time).

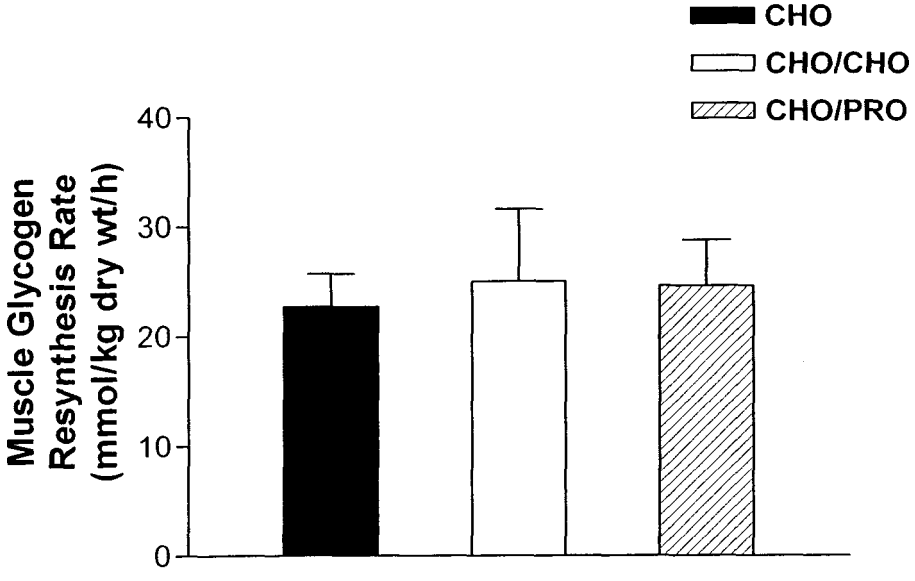


Figure 10. Muscle glycogen resynthesis rate (mmol/kg dry wt/h) over the first 4-hrs of recovery.

Table 6. Muscle metabolites immediately after exercise (0) and after 4-h recovery period (4-h).

	CHO		CHO/CHO		CHO/PRO	
	0	4-h	0	4-h	0	4-h
Lactate	25.9±5.1	36.1±7.8*	13.3±2.2	23.4±5.6*	14.2±1.7	27.4±7.4*
Creatine	58.1±6.7	65.7±6.1	46.9±4.2	53.4±3.9	57.3±6.8	54.5±6.3
PCr	63.6±5.4	57.5±3.9	73.6±5.8	61.2±6.7	65.8±5.6	61.0±5.5
ATP	22.8±1.4	22.1±1.9	22.8±0.9	22.7±1.9	24.2±1.5	21.4±2.5

Values are mean ± SEM. n = 6. CHO, low carbohydrate only beverage (1.2 gCHO/kg/h); CHO/CHO, high carbohydrate only beverage (1.6 gCHO/kg/h); CHO/PRO, carbohydrate plus protein mixture beverage (1.2g CHO/kg/h +0.4 g PRO/kg/h).
*Significantly different from time 0
(main effect for time, $P \leq 0.05$).

2.3.2 Serum insulin, blood glucose and blood lactate

Serum [insulin] increased during recovery compared to immediately post-exercise (main effect, $P<0.05$) however there were no differences between trials (Figure 11). The area under the curve for insulin was not different between trials (Figure 12) (79 ± 17 μIU x min/mL (CHO) vs. 90 ± 22 μIU x min/mL (CHO/CHO) vs. 104 ± 18 μIU x min/mL (CHO/PRO). Blood [glucose] increased from 0 to 60 min post-exercise in all trials (main effect for time, $P\leq0.05$), however there were no differences between treatments (Figure 13). After 60 min blood [glucose] progressively decreased and was lower ($P\leq0.05$) between 120-240 min post-exercise (Figure 13) (Table 7). Blood [lactate] remained unchanged throughout the entire 4-hr recovery period (Table 7).

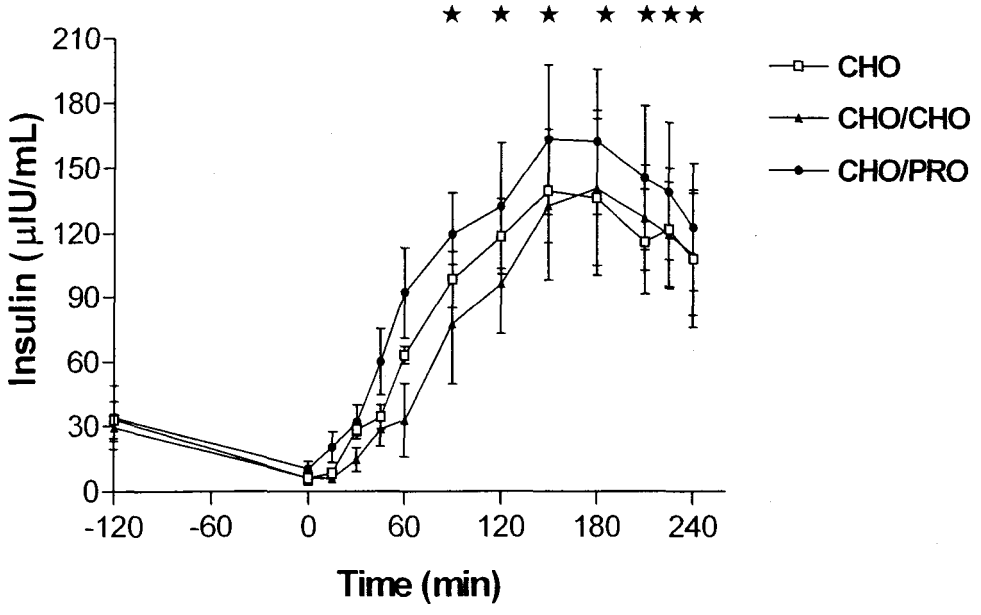


Figure 11. Serum insulin concentration (± SEM) pre-exercise and in 4-h recovery from exercise. ★ $P \leq 0.05$ vs. Time 0 (main effect for time).

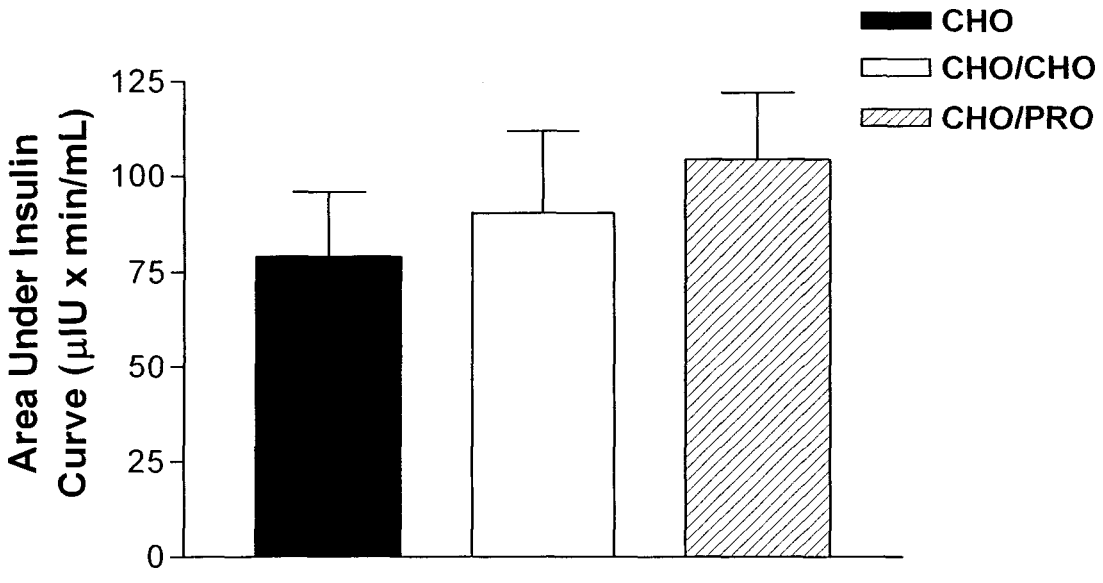


Figure 12. Area under insulin curve.

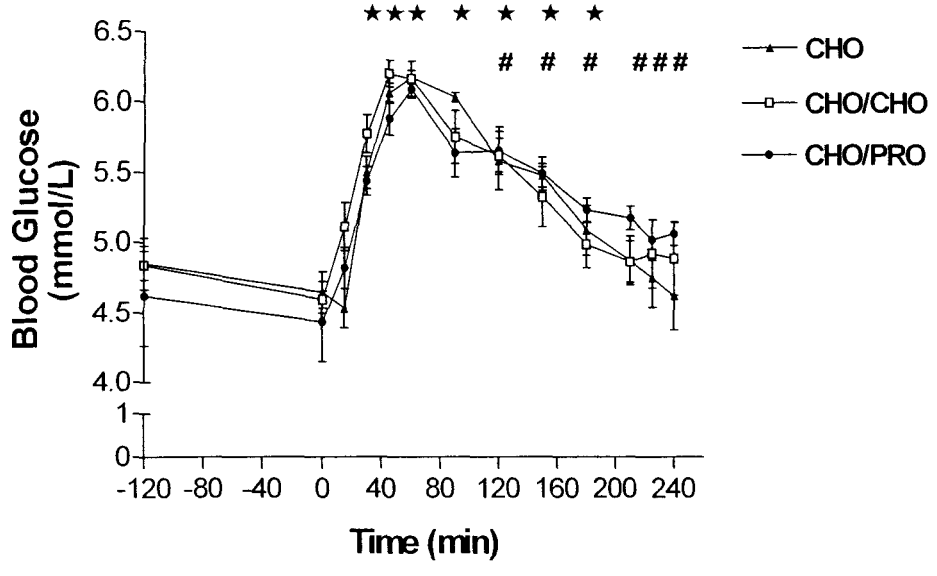


Figure 13. Blood glucose concentration (± SEM) pre-exercise and in 4-hr recovery from exercise. * $P \leq 0.05$ vs. Time 0 (main effect for time). $^{\#}$ $P \leq 0.05$ vs. Time 60 min (main effect for time).

Table 7. Serum insulin and blood metabolites summary, pre-exercise to 240 min into recovery.

	Insulin (uIU/mL)			Blood glucose (mmol/L)			Blood lactate (mmol/L)		
Time (min)	CHO	CHO/CHO	CHO/PRO	CHO	CHO/CHO	CHO/PRO	CHO	CHO/CHO	CHO/PRO
Pre	29±1	33±6.3	34±15	4.8±0.2	4.8±0.1	4.6±0.4	2.0±0.2	2.7±0.6	2.0±0.3
0	6±3	4±0	10±4	4.6±0.1	4.6±0.1	4.4±0.3	2.0±0.3	2.1±0.5	2.3±0.3
15	6±1	8±2	20±7	4.5±0.1	5.1±0.2	4.8±0.1	1.7±0.3	2.3±0.5	1.8±0.3
30	14±3	28±5	32±8	5.5±0.1	5.8±0.1	5.4±0.1	1.5±0.3	1.7±0.4	1.5±0.2
45	29±6	34±8	60±15	6.1±0.1	6.2±0.1	5.9±0.1	2.0±0.3	1.7±0.3	1.4±0.2
60	33±4	63±17	92±21	6.2±0.0	6.2±0.1	6.1±0.1	2.2±0.4	1.6±0.4	1.5±0.2
90	77±13	98±28	119±19	6.0±0.0	5.7±0.2	5.6±0.2	2.1±0.3	2.2±0.7	1.9±0.4
120	96±18	118±23	132±29	5.6±0.2	5.6±0.1	5.6±0.2	2.0±0.3	1.0±0.4	1.7±0.3
150	133±24	140±35	163±35	5.5±0.1	5.3±0.2	5.5±0.1	1.7±0.3	1.7±0.3	1.5±0.3
180	141±36	136±36	162±34	5.1±0.2	5.0±0.2	5.2±0.1	1.5±0.3	1.4±0.4	1.9±0.7
210	127±25	116±25	145±33	4.9±0.2	4.9±0.1	5.2±0.1	2.0±0.3	1.7±0.4	1.4±0.2
225	119±28	122±24	139±32	4.8±0.2	4.9±0.2	5.0±0.1	2.2±0.4	1.4±0.3	1.6±0.2
240	110±32	108±29	123±30	4.6±0.2	4.9±0.3	5.1±0.1	2.1±0.3	1.8±0.3	1.6±0.3

Values are mean ± SEM. n = 6. CHO, low carbohydrate only beverage (1.2 gCHO/kg/h); CHO/CHO, high carbohydrate only beverage (1.6 gCHO/kg/h); CHO/PRO, carbohydrate plus protein mixture beverage (1.2g CHO/kg/h +0.4 g PRO/kg/h).

2.3.3. Gastrointestinal(GI) complaints

Subjects reported significant GI distress, including bloating and the need to vomit, at 3 and 4hrs into recovery compared to immediately post-exercise (main effect for time, $P \leq 0.05$) (Figure 14). There was no effect of beverage type on GI distress.

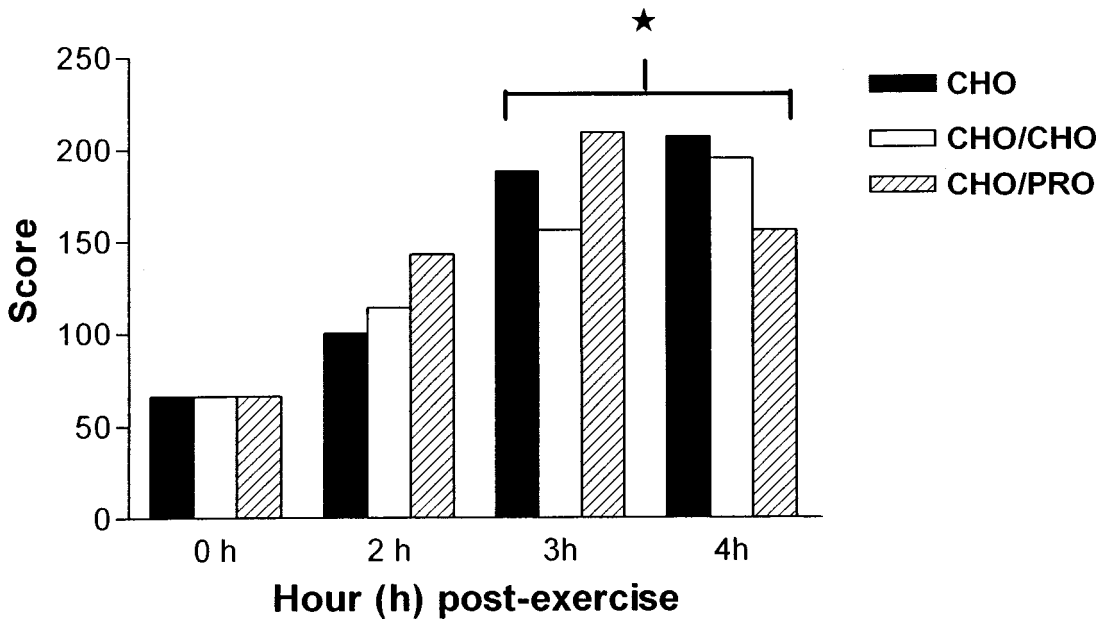


Figure 14. Gastro-intestinal problems at 0, 2, 3, and 4 hrs into recovery from glycogen-depleting exercise. Maximum score per hour for all 6 subjects combined was 660. Based on 11 questions rated 1-10. ★ $P \leq 0.05$ vs. Hour 0 (main effect for time).

2.4 DISCUSSION

The main finding of the present study was that a CHO intake of 1.2 g/kg/h was sufficient to maximize the rate of muscle glycogen resynthesis over 4 hrs of recovery after glycogen-depleting exercise in recreationally-active men. Ingestion of an additional 0.4 g/kg/h of CHO or PRO did not induce greater muscle glycogen resynthesis rates when drinks were consumed every 15 min between 0 and 300 min post-exercise. Thus, the answer to Burke et al.'s (2004) question: "At what intake of carbohydrate is the threshold for glycogen storage reached over the first 4 hrs of post-exercise recovery?" is: 1.2 gCHO/kg/h, at least in recreationally-active young men.

Muscle glycogen resynthesis post-exercise

Our drinks were formulated to provide a minimum of 1.2 gCHO/kg/h since the highest rates of muscle glycogen resynthesis have been reported after subjects ingested CHO at this rate following strenuous exercise (Jentjens et al., 2001; van loon et al., 2000). Our study was specifically designed to determine whether drinks that provided a higher rate of energy intake, either from PRO or CHO, would augment muscle glycogen resynthesis rate. Specifically, we compared a drink that provided 1.2 gCHO/kg/h with two higher energy drinks that were matched in terms of total energy but differed with respect to the source of the additional energy (PRO or CHO). Muscle glycogen resynthesis post-exercise was not significantly different between the CHO, CHO/CHO and CHO/PRO trials (Figure 10). These findings are consistent with previous data that showed ingestion of 1.2 gCHO/kg/h + 0.4 gPRO/kg/h did not increase muscle glycogen resynthesis to a greater extent then ingestion of 1.2 gCHO/kg/h alone (Jentjens et al.,

2001). Previously, van Loon and colleagues (2000) reported higher post-exercise muscle glycogen resynthesis rates with ingestion of 1.2 gCHO/kg/h and 0.8 gCHO/kg/h + 0.4 gPRO/kg/h compared to a 0.8 gCHO/kg/h. However, both van Loon et al. (2000) and Jentjens et al. (2001) did not establish that the highest level of CHO consumption in their studies was the optimal amount of CHO required to maximize early post-exercise muscle glycogen resynthesis. Therefore, it was unclear at what CHO intake PRO did not provide any additional benefits in terms of muscle glycogen resynthesis post-exercise. Burke et al. (2004) alluded to the fact that PRO ingestion does not increase muscle glycogen resynthesis post-exercise when CHO intake is adequate and/or optimal. The term "adequate and/or optimal" has never been clearly defined. The present study reveals that 1.2 gCHO/kg/h is the maximum amount of CHO required to reach the threshold for post-exercise muscle glycogen resynthesis in recreationally active men when supplement drinks are ingested in frequent intervals (~15 min) immediately after exercise. We found that co-ingestion of PRO or additional CHO provided no additional benefit. The data presented herein are thus in agreement with Burke et al. (2004) who stated: "Most evidence suggests that feeding a high amount of carbohydrate at frequent intervals negates the benefits of added protein".

Blood insulin concentration and its role in muscle glycogen resynthesis post-exercise

Muscle glycogen resynthesis post-exercise occurs in two phases: an insulin-independent phase which is rapid and occurs within the first 60 min post-exercise and an insulin dependent phase which is slower and can last up to 24 hrs post-exercise (Jentjens and Jeukendrup, 2003). Addition of PRO to a CHO drink has been suggested to increase

muscle glycogen resynthesis post-exercise via a stimulatory effect of PRO on blood insulin concentration which promotes glucose uptake and consequently an increase in muscle glycogen storage (Zawadzki et al. 1992). However, most studies suggest that if PRO does in fact increase muscle glycogen storage post-exercise, insulin is not the mechanism by which such a process occurs (Carrithers et al., 2000; Ivy et al., 2002; Roy et al., 1998; Tarnopolsky et al., 1997; van Loon et al., 2000). The insulin data presented in this study demonstrate that there might be a threshold for blood insulin concentration when it comes to ingesting supplement drinks. There was no significant difference in area under the insulin curve between either of the three trials although there was a trend for the CHO/PRO trial to have higher blood insulin values at all time-points in recovery. Regardless, muscle glycogen storage rates were not different between trials.

We chose to measure glycogen in the immediate and 4-h biopsies only given that the muscle biopsy technique may not be sensitive enough to detect changes over a 1 h time period. Ivy et al. (2002), using ^{13}C-NMR to measure muscle glycogen concentration, reported that PRO may increase muscle glycogen storage post-exercise via an insulin-independent mechanism that occurs within the first hour of recovery. However, in the study by Ivy et al. (2002), the differences between treatments after one hour of recovery were still apparent after 4 hrs of recovery. Therefore, by measuring the increase in glycogen over 4 hrs post-exercise, any effect after 1 hour would still be detectable, however we could not examine the mechanisms that may have been responsible for potential differences between treatments during the early phase of

recovery. To date, no research has been done at the molecular level in terms of elucidating how PRO may increase muscle glycogen within the first hour post-exercise.

Glucose storage in muscle

The results of this study reveal that despite ingesting more CHO in the 1.6 gCHO/kg/h trial than in the 1.2 gCHO/kg/h trial, subjects did not have a higher muscle glycogen storage rate. The question remains, where was the extra glucose processed? It is possible that the liver cleared more glucose in the CHO/CHO than in the CHO trial but, without obtaining a liver biopsy or access to ^{13}C magnetic resonance spectroscopy, this cannot be determined. To date, only one study has used interleaved ^{13}C liver and muscle spectra to quantify changes in human liver and muscle glycogen content with exercise and during recovery. In this lone study, subjects cycled at 70% $V0_{2\,max}$ for 83±8 min and consumed 1g/kg body mass of glucose immediately post-exercise. Measurement of liver glycogen over 4 hrs of recovery showed that 30% of the glucose consumed was extracted by the liver before being made available to the relatively large mass of skeletal muscle (Casey et al., 2000). Whether the liver would extract more glucose from the blood if more glucose was consumed as in the present study remains to be investigated.

Second, the extra glucose consumed may have remained in the muscle as simple glucosyl units not stored as glycogen. Muscle glucose was not measured in this study but considering the range for muscle glucose concentration in muscle is typically between 2-4 mmol/kg dry wt under any condition (Chryssanthopoulos et al., 2004), it is unlikely to have had a major impact. Third, it is possible that extra glucose retained in the muscle was converted into muscle lactate or oxidized via pyruvate dehydrogenase (PDH). In this

study, the muscle lactate concentrations immediately post-exercise were higher than typical resting muscle lactate values of ~3-6 mmol/kg dry wt. found in the literature (Chryssanthopoulos et al., 2004; Hermansen and Vaage, 1977; Putman et al., 1995) and they did not return to resting values during recovery. In all three trials, muscle lactate concentration increased from immediately post-exercise to 4-hrs post-exercise (main effect for time, $P<0.05$). The transformation of glucose to pyruvate then to lactic acid may be a mechanism by way extra glucose in the muscle was eliminated.

Data from several studies indicate an increase in whole-body fat oxidation after exercise as demonstrated by a decrease in the respiratory exchange ratio (RER) (Kiens & and Richter, 1998; Kimber et al. 2003). It seems paradoxical that fat oxidation remained elevated in these studies (Kiens & and Richter, 1998; Kimber et al. 2003) despite the presence of elevated insulin levels due to CHO ingestion during recovery. However, one study highlighted that the inhibitory effect of insulin on fatty acid oxidation was dampened during recovery from a marathon (Tuominen et al., 1996). Whether this tendency to store glycogen, reduce CHO oxidation and burn more fat in recovery from exercise also occurs when ample CHO is provided during recovery remains to be determined. If this tendency does occur regardless of CHO availability, it might explain why muscle lactate did not decrease to baseline values during recovery in the present study. Build-up of lactic acid may have been the result of too much glucose in the muscle at any one time and the inability to up-regulate CHO oxidation during recovery. More research is needed in this domain.

Training status of participants and muscle glycogen resynthesis

The participants in this study were recreationally active men with $V0_2$ peak values that ranged from 38-57 mL O_2/kg/min. The mean muscle glycogen resynthesis rates of each of the three trials were similar (22.7 ±3.0, 25.0 ±6.6, 24.6 ±4.2 mmol/kg dw/h) to previously reported data in untrained subjects (Greiwe et al., 1999). Greiwe et al. (1999) showed muscle glycogen resynthesis rate post-exercise increased two-fold from ~22 to ~50 mmol/kg dry wt/h after endurance training 6 days/wk for 10 wks. Most studies that have investigated the effect of PRO on muscle glycogen resynthesis post-exercise have reported muscle glycogen resynthesis rates of 30-50 mmol/kg dw/h (Ivy et al., 2002; Jentjens et al., 2001; van Hall et al., 2000; van Loon et al., 2000). The subjects studied in these earlier investigations were trained athletes with $V0_2$ peak values of between 60-65 mL O_2/kg/min. Thus, the lower muscle glycogen resynthesis rates reported in this study are probably due to the lower fitness level of our subjects compared to other studies and are more comparable with those from a study by Hickner et al. (1997) in which untrained subjects ($V0_{2max}$= ~38 mL O_2/kg/min) had a mean muscle glycogen resynthesis rate of ~ 22 mmol/kg dry wt/h within the first 6 hrs of recovery. In the Hickner et al. (1997) study subjects consumed 1.4 gCHO/kg/h at 0, 2 and 4 hrs into recovery.

Training status is unlikely to play a role in gastric emptying of CHO and CHO/PRO beverages during recovery from muscle glycogen depleting exercise. A study by Rehrer et al. (1989) showed that although drink composition and exercise intensity did significantly alter gastric emptying rates at rest and during exercise, there was no difference in gastric emptying rates between trained and untrained subjects.

Gastro-intestional(GI) problems

The primary factors affecting the gastric emptying rate are the energy content and volume of the fluid that is ingested. Dehydration and intense exercise can also slow gastric emptying (Leiper et al., 2005; Van Nieuwenhoven et al., 2005). In this study, 3 out of 6 subjects reported severe bloating and an urge to vomit around 180 min into recovery. This urge to vomit and bloating feeling occurred across all three trials and was surprisingly not correlated to the PRO content of the drink. Jentjens et al. (2001) reported two subjects with high scores regarding nausea, belching, and bloated feeling only after consuming CHO+PRO beverages. Subjects in the present study drank 750 ml of a 17-22% glucose polymer solution. Similar concentrated drinks have been used in other studies both during and in recovery from exercise and no bloating and/or vomiting has been reported (Table 2).

Hydration and CHO guidelines set out in a Joint Position Statement by the American College of Sports Medicine, American Dietetic Association and Dietitians of Canada (Manore et al., 2000) during and in recovery from exercise are as follows:

- Two hours before exercise, 400 to 600 mL (14 to 22 oz) of fluid should be consumed.
- During exercise, 150 to 350 mL (6 to 12 oz) of fluid should be consumed every 15 to 20 min depending on tolerance.
- After exercise, the athlete should drink adequate fluids to replace sweat losses during exercise. The athlete needs to drink at least 450 to 675 mL (16 to 24 oz) of fluid for every pound (0.5 kg) of body weight lost during exercise.

- After exercise, the dietary goal is to provide adequate energy and carbohydrates to replace muscle glycogen and to ensure rapid recovery. If an athlete is glycogen-depleted after exercise, a carbohydrate intake of 1.5 g/kg body weight during the first 30 min and again every 2 h for 4 to 6 h will be adequate to replace glycogen stores.

Unfortunately, we were unable to weigh subjects with their wet clothes off after the exercise protocol due to practical limitations related to the stable isotopic measurements (indwelling venous lines). Each individual consumed between 1.65- 2.2 liters of water during the exercise protocol and ingested a standardized amount of fluid during recovery. Assuming each individual lost at least 1kg over the 2-h exercise protocol after considering the weight of water they consumed during the exercise, this means they should have consumed ~1350 mL within the first two hours post-exercise (Manore et al., 2000). Our subjects consumed 1500 mL of solution in the first 2 hours and 2437 mL in the first 3 hours. Our hydration values are thus slightly higher than those recommended in the Joint Position Statement by the American College of Sports Medicine, American Dietetic Association and Dietitians of Canada (Manore et al., 2000). However, fluid volumes and CHO and PRO consumption rates similar to the ones consumed in this study have been used in other studies investigating the effect of PRO on muscle glycogen resynthesis post-exercise (Jentjens et al., 2001; Van Hall et al., 2000; van Loon et al., 2000). These studies have not reported any GI problems among their subjects despite the fact that most of their subjects were lighter then the subjects in the present study.

Post-exercise CHO and CHO/PRO supplementation and exercise performance

In all the studies in Table 2, exercise performance 4-hrs into recovery was not studied. One of the initial aims of this study was to investigate whether or not the addition of PRO to a CHO drink affected subsequent exercise performance in addition to muscle glycogen resynthesis post-exercise. One study by Williams et al. (2003) showed that when athletes consumed a CHO/PRO beverage post-exercise instead of the traditional 6% carbohydrate-electrolyte sports beverage, they had a 55% greater time to exhaustion during a subsequent exercise bout at 85% $V0_{2max}$. A second study by this same group reported that a CHO/PRO drink consumed post-exercise had a 17% greater plasma glucose response, a 92% greater insulin response, and a 128% greater storage of muscle glycogen compared with a traditional 6% carbohydrate-electrolyte sports beverage (Williams et al., 2003). However, in these two studies by Williams et al. (2003), the CHO/PRO beverage not only differed in PRO content from the CHO beverage, but also contained over double the amount of CHO. Therefore, it is unknown whether the findings of Williams et al. (2003) are due to the PRO content of the CHO/PRO beverage or simply the product of consuming a higher amount of CHO. No study has examined both muscle glycogen resynthesis and exercise performance 4-hrs into recovery in tandem. Unfortunately, while we planned to examine both variables in our study, this was not feasible given the GI distress during the 4-hr recovery period. Subjects were too bloated by the drinks they consumed in recovery that they neither felt comfortable or able to complete a performance ride at the end of the trial. Each subject

had prior to the first trial performed two 750 kJ familiarization time-trials (simulated 30-km bike race) in preparation for this end of trial performance ride.

Study limitations

The present study establishes that the ingestion of no more than 1.2 g/kg/h of CHO is needed to maximize post-exercise muscle glycogen resynthesis in young healthy men. Nonetheless, there were limitations inherent to our work. First, our study examined muscle glycogen resynthesis in biopsy samples of mixed muscle. Using the muscle biopsy technique, the assumption is made that the muscle samples at different time-points are homogenous to each other in terms of number of type I and type II muscle fibers but we can not be completely certain. To date, only a few studies have attempted to determine glycogen synthesis rates in single human muscle fibers and these have reported conflicting results. Some studies have reported higher glycogen synthesis rates in type I fibres than type II fibres (Casey et al., 1995, Essen et al., 1974) when using biochemical methods, while others have found higher glycogen synthesis rates in type II fibres when using histochemical methods to determine muscle glycogen concentrations (Piehl et al., 1974, Vollestad et al., 1989). We did not perform single fiber analyses in the present study and thus our data may reflect some sampling error consistent with the muscle biopsy technique. We did measure glycogen on two separate pieces of muscle within a given biopsy in order to obtain a more representative measure of mixed muscle glycogen. However, our design could not completely overcome the fact that the needle biopsy technique only provides a "snapshot" of a relatively small piece of tissue which may not be representative of the entire muscle.

A second limitation of our study was the relatively low number of subjects, which meant that relatively large differences between treatments were necessary in order to detect significant effects. Our decision to test only 6 subjects was due in part to economic realities and the fact that this project was part of a much larger study that included stable isotopic tracers and also examined muscle protein kinetics. In order to compensate in part for the low power, we employed a repeated measures design and measured glycogen (our primary outcome variable) on two separate pieces of muscle for each biopsy in order to reduce measurement variability. Moreover, other studies have detected differences in muscle glycogen resynthesis rates between drink treatments using 5-7 subjects. For example, Ivy et al. (2002) showed that a CHO-PRO supplement was significantly more effective for the rapid replenishment of muscle glycogen after exercise than a CHO supplement of equal CHO or caloric content in a group of 7 men. A study by van Hall et al. (2000b) only had five subjects and reported no difference in muscle glycogen resynthesis rates post-exercise between a CHO and a CHO/PRO beverage of equal CHO content. In the van Hall et al. (2000b) study there was no trend for a CHO/PRO beverage to increase muscle glycogen resynthesis compared to a CHO beverage. Finally, our statistical analyses in the present study did not reveal any "trends" in that one treatment was consistently higher than the others with respect to mean glycogen resynthesis rates.

A final, and unexpected limitation of our study was the inherent variability and severe GI distress experienced by some of our participants. Three out of the six subjects experienced GI distress on at least one trial, which forced us to alter our original design and omit the planned exercise performance tests at 4 hrs of recovery. Whether the GI

distress was caused by the volume of liquid ingested or the energy content or composition of the beverages remains unclear. Nonetheless, a paradoxical and practical implication of the present work is that manipulations designed to optimize glycogen resynthesis post-exercise might impair the subjects' desire to perform strenuous exercise. This is obviously an important point for coaches and athletes to consider when formulating their post-exercise nutritional strategy.

Future directions

With the advent of new technologies such as ^{13}C-NMR, it will be possible to differentiate smaller changes in muscle glycogen concentration over smaller time intervals such as 1 hour. If PRO does have an effect on muscle glycogen synthesis within the first hour of recovery, it is hopeful that such technologies as ^{13}C-NMR will help distinguish this effect. Furthermore, there is much research that needs to be done to back-up data showing that PRO does increase muscle glycogen synthesis post-exercise when an insufficient amount of CHO is consumed. What is protein's mechanism of action is still a controversial question. It is unlikely that increased blood insulin concentration is what causes increased muscle glycogen storage however; there is little evidence to demonstrate other possible mechanisms of PRO's influence on muscle glycogen storage. As research into phase I of muscle glycogen resynthesis, the non-insulin dependent phase, gains momentum as part of research being done in the search for a treatment for diabetes, it is likely that more insights into measurement techniques will further the knowledge in the domain of PRO's effect on muscle glycogen storage.

A well-designed study comparing CHO and CHO/PRO beverages must be done that incorporates both the study of muscle glycogen synthesis post-exercise and its effects on a second bout of intense exercise early in recovery from a first bout of exercise. Importance must be put on keeping CHO content between trials identical and only studying the effects of differing PRO content of the beverages on early muscle glycogen storage. A pre-exercise muscle biopsy should also be incorporated into the experimental protocol. This would help distinguish confounding factors affecting the rate of muscle glycogen resynthesis post-exercise. One such factor may be the amount of glycogen depletion.

2.5 CONCLUSION

The main finding of the present study was that the addition of 0.4 g/kg/h of PRO or CHO to a drink that provided 1.2 gCHO/kg/h did not promote a higher glycogen storage rate over the first 4 hrs of recovery from prolonged strenuous exercise in recreationally-active men. Thus, 1.2 gCHO/kg/h was the maximum amount of CHO needed to attain the threshold for glycogen resynthesis within the first 4 hrs of recovery. The consumption of extra CHO or PRO beyond 1.2 gCHO/kg/h did not induce changes in blood insulin and blood glucose concentration compared to the effects of a 1.2 gCHO/kg/h beverage. However, the consumption of 1.2 + gCHO/kg/h in a ~20% solution does promote GI problems in some recreationally active individuals and may hamper subsequent same-day performance.

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APPENDIX I

SUBJECT INFORMATION AND CONSENT FORMS

SUBJECT RECRUITMENT POSTER

McMaster University

Exercise Metabolism Research Group
Department of Kinesiology
Ivor Wynne Centre, Room A103
1280 Main Street West
Hamilton, Ontario, Canada
L8S 4K1

Phone: 905-525-9140
EMRG Laboratory: ext. 27037
Dr. MJ Gibala: ext. 23591
Dr. MJ MacDonald: ext. 23580
Dr. SM Phillips: ext. 24465
Fax: 905-523-4025

EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

CONSENT TO PARTICIPATE IN RESEARCH

You are asked to participate in a research study being conducted by the investigators listed below at McMaster University, Hamilton, Ontario. Prior to your participation, you are asked to read and complete this form and the accompanying forms which outline the purpose, procedures, and risks associated with the study, and also provide other essential information regarding your rights and responsibilities as a subject. The accompanying forms are entitled "Invasive Procedures" and "Subject Screening Questionnaire." All experimental procedures will be conducted in the Metabolism Research Laboratory, Room A103, Ivor Wynne Centre.

LIST OF PRIMARY INVESTIGATORS

Name	Campus Address	Phone Number
Dr. Martin Gibala	Kinesiology, AB122	905-525-9140 ext. 23591
Dr. Stuart Phillips	Kinesiology, AB116	905-525-9140 ext. 24465
Ms. Natalie Moreau	Kinesiology, A103	905-525-9140 ext. 27037
Ms. Krista Howarth	Kinesiology, A103	905-525-9140 ext. 27037

PROJECT TITLE

"The protein effect: the effect of protein-carbohydrate ingestion on early post-exercise glycogen synthesis and subsequent performance."

FUNDING SPONSOR

Natural Sciences and Engineering Research Council of Canada (NSERC).

PURPOSE OF THE STUDY

Our laboratory is interested in the effect of exercise and nutrition on the regulation of energy provision in human skeletal muscle. One strategy that we employ is the manipulation of nutrient intake before, during or following exercise. It is known that carbohydrate (CHO) ingestion during recovery is necessary to obtain optimal muscle glycogen re-synthesis. However, it remains controversial whether a protein-CHO supplement — as compared to CHO alone — is superior for optimal muscle glycogen re-synthesis. Protein ingestion may also confer added benefits with respect to muscle recovery. Finally, the optimal amount of CHO ingestion for maximal glycogen resynthesis is unclear. In the present study we plan to comprehensively examine the effect of a protein-CHO ingestion versus CHO alone on: 1) muscle glycogen synthesis in recovery from a muscle glycogen depleting exercise; (2) muscle protein metabolism during recovery; and (3) subsequent performance early in recovery from muscle glycogen depleting exercise.

DESCRIPTION OF TESTING AND EXPERIMENTAL PROCEDURES

Overview: Following routine medical screening and a couple familiarization visits to the laboratory (in order to become oriented with testing procedures and equipment), you will arrive at the laboratory the evening before your trial. At this time, you will be given controlled diet to follow until the following morning. The morning of the trials, you will report to the laboratory and perform a muscle glycogen depletion cycle ride, followed by 4 hours of recovery. Finally, after 4 hours of recovery, you will perform a "time trial" exercise test in which you will be asked to complete a fixed amount of work in as fast a time as possible (i.e., a simulated race). During the 4 hr recovery period from the depletion ride, you will receive one of three supplement drinks in random order: (1) 1.2 g/kg/hr of CHO; (2) 1.6 g/kg/h of CHO; or (3) 1.2 g/kg/h CHO + 0.4 g/kg/h protein. The entire experimental protocol will be repeated on three occasions, separated by 1 week, such that all subjects will complete all three experimental conditions. Venous blood samples and muscle biopsy samples will be obtained during recovery as detailed below.

VO2peak Test. This test involves cycling on a stationary bike (cycle ergometer) at progressively higher workloads while the amount of oxygen taken up by your body is determined from a mouthpiece connected to a gas analyzer. This test will be used to assess the fitness level of the participants.

Experimental Exercise Trial. After controlling your diet and exercise habits for the last 15-16 hrs you will report to the Room A103 of the Ivor Wynne Centre at ~9:00 am. A catheter will be inserted into a forearm vein and infusion of a stable (i.e. non-radioactive) tracer will be started at approx. 10 a.m.. During this time a second catheter will also be inserted into a vein on the opposite forearm. This second catheter will be used for venous blood sampling. Finally, a muscle biopsy will be obtained at 0, 60, and 240 minutes into recovery from the vastus lateralis (thigh) muscle. Nine biopsy samples in total will be obtained over the course of the entire study, three per trial. ***The details and risks associated with the stable isotope infusion, venous blood sampling and***

muscle biopsy procedures are thoroughly described on the attached forms entitled "Description of Medical Procedures." A baseline blood sample will be obtained at rest, and you will then perform a prolonged exercise bout on a cycle ergometer in order to deplete muscle glycogen. Venous blood samples (~5 mL per sample) will be obtained periodically during the 4 hr recovery period. You will periodically ingest small amounts of a drink that contains either CHO alone or CHO+protein during the recovery period. A biopsy sample will be obtained at the end of the depletion ride after 1 and 4 hours of recovery. Breath samples will be obtained periodically throughout recovery through a mouthpiece. After the 4 hr recovery period, you will perform a time-trial on the cycle ergometer.

Glycogen Depletion Ride. This involves cycling on an ergometer at a moderate work intensity (~75% VO2peak) until the point of volitional exhaustion (~60-120 min), resting 3 minutes and repeating the protocol a second time. You will then perform 1 minute sprints separated by 1 minute each until exhaustion. Following this test you will consume one of the three supplement drinks over a 4 hour recovery period.

500 kJ Time Trial Test. This test involves performing a fixed bout of exercise (500 kJ; equivalent to a simulated 20 km cycle ride) in as fast a time as possible. You will be able to self-select the intensity of exercise by adjusting your pedal cadence during the test.

Supplement Drinks. The supplement drinks will contain carbohydrate in the form of maltodextrin and splenda and protein in the form whey hydrolysate. Maltodextrin is made of linked glucose molecules while whey is a protein found in normal milk.

DESCRIPTION OF POTENTIAL RISKS AND DISCOMFORTS

Please refer to the attached form entitled "Description of Medical Procedures" for a complete description of the invasive medical procedures to be performed during the study and the potential risks and discomforts associated with these procedures.

REMUNERATION

You will receive an honorarium of $450.00 in order to compensate for your time commitment and effort. Remuneration is normally provided within one week following completion of the study.

PROVISION OF CONFIDENTIALITY

Any information that is obtained in connection with this study will remain confidential, and appropriate measures will be taken by all investigators to ensure privacy. The results from this study will be used for educational purposes and may be published in scientific journals, presented at scientific meetings or disseminated using other appropriate methods. Regardless of presentation format, subjects will not be identified by name and your personal data will be identified by a code number only. Upon

completion of the study, you will have access to your own data and the group data for your own interest.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions which you do not want to and still remain in the study. The investigators also reserve the right to withdraw you from this research project if circumstances arise which warrant doing so. Should you withdraw from the study prior to its completion, a partial honorarium payment will be made based on the relative proportion of the study which was completed.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. The nature of the exercise stresses and invasive procedures to be employed in this study have been approved by the McMaster University / Hamilton Health Sciences Research Ethics Board (REB). If you have questions regarding your rights as a research participant, contact:

REB Secretariat	Telephone:	905-525-9140, ext. 24765
McMaster University	Fax:	905-540-8019
1280 Main Street W., CNH-111	E-mail:	grntoff@mcmaster.ca
Hamilton, ON L8S 4L9		

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE

I have read and understand the information provided for the study as described herein and in the accompanying forms entitled "Description of Medical Procedures" and "Subject Screening Questionnaire." My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Name of Participant

_____
Name of Legal Representative (if applicable)

_____ _____
Signature of Participant or Legal Representative Date

SIGNATURE OF INVESTIGATOR

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

_____ _____
Signature of Investigator Date

McMaster University

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

SUBJECT SCREENING QUESTIONNAIRE

Your responses to this questionnaire are confidential and you are asked to complete it for your own health and safety. If you answer "YES" to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

Name: _____ Date: _____

Address: _____

Phone Number: _____ E-mail: _____

1. Have you ever been told that you have a heart problem?

   YES NO

2. Have you ever been told that you have a breathing problem such as asthma?

   YES NO

3. Have you ever been told that you have kidney problems?

   YES NO

4. Have you ever been told that you sometimes experience seizures?

   YES NO

5. Have you ever had any major joint instability or ongoing chronic pain such as in the knee or back?

   YES NO

6. Have you ever had any allergies to medication?

YES NO

7. Have you ever had any allergies to food or environmental factors?

YES NO

8. Have you ever had any stomach problems such as ulcers?

YES NO

9. When you experience a cut do you take a long time to stop bleeding?

YES NO

10. When you receive a blow to a muscle do you develop bruises easily?

YES NO

11. Is there any major medical condition with which you have been diagnosed and are under the care of a physician (e.g. diabetes, high blood pressure)?

YES NO

12. Are you currently taking ANY medication or have you taken any medication in the last two days? If yest, please indicate medication(s).

YES NO

13 Have you ever taken part is a study which involved needle biopsy sampling or the placement of arterial or venous catheters? If yes, please provide details in space below

YES NO

Please use the space below to transmit any additional comments you may have.

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF INVASIVE MEDICAL PROCEDURES

The study in which you are invited to participate involves several invasive medical procedures. Prior to your involvement in the study, you are asked to read this form which outlines the potential medical risks inherent to these procedures. In addition, you must also complete the "Subject Screening Questionnaire" which is designed to identify any medical reason which might preclude your participation as a subject.

Muscle Biopsy Procedure

This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. The area over the muscle to be sampled will be cleaned and a small amount of local anesthetic ("freezing") will be injected into and under the skin over the vastus lateralis (quadriceps) muscle. A small incision (~4 mm) in the skin will then be made in order to create an opening through which to put the biopsy needle into the muscle. There is a small amount of bleeding from the incision, but this is minimal. The incision will be covered with sterile gauze and surgical tape. At those times during the experiment when a biopsy is required, the bandage will be removed and the biopsy needle will be inserted into your thigh through the incision. A small piece of muscle (~50-100 mg; about the size of the eraser on the end of a pencil) will quickly be obtained and then the needle will be removed. During the time that the sample is being taken (~5 sec), you may feel the sensation of deep pressure in the muscle and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise. If a biopsy sample is required during an exercise trial, the exercise bout is briefly interrupted in order to obtain the sample, and the muscle may feel a little "tight" during the first few seconds as you begin to exercise again.

Following the exercise bout, the biopsy sites will be closed with sterile bandage strips or a suture (stitch) and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". You should not take any aspirin-based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics such as Ibuprofen or Tylenol are acceptable alternatives. It is also beneficial to keep your limb elevated when you are sitting, and the periodic application of an ice pack will help to reduce any swelling and residual soreness. The following day your muscle may feel uncomfortable upon movement, e.g., going down stairs. The tightness in the muscle usually disappears within 1-2 days, and subjects routinely begin exercising normally within 2-3 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided.

Potential Risks. The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discolouration of the skin. On occasion a small lump of fibrous tissue may form under the site of the incision, but this normally disappears within 2-3 months. As with any incision there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately. In very rare occasions there can be damage to a superficial sensory nerve which will result in temporary numbness in the area. There is also an extremely remote chance (1 in ~1,000,000) that you will be allergic to the local anesthetic.

It is the collective experience of members in our laboratory that, in healthy young subjects, 1 in ~2,500 have experienced a local skin infection; 1 in ~1,000 have experienced a small lump at the site of the biopsy (in all cases this disappeared within approximately one week using gentle massage over the area of the lump); 1 in ~2,000 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a quarter that lasted up to 4 months), and 1 in ~100 have experienced mild bruising around the site of incision that lasted for ~4-5 days. There is also a theoretical but extremely small risk of damage to a small motor nerve branch leading to the muscle which is being sampled.

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF INVASIVE MEDICAL PROCEDURES

Forearm Venous Catheterization and Stable Isotope Infusion

A small catheter will be inserted into a forearm vein with the assistance of a small needle, which is subsequently removed. A small amount of an amino acid solution will be infused through the catheter. The amino acid will be dissolved in saline (a salt solution similar to your blood). The amino acid will be 'labelled' with a stable isotope of carbon, hydrogen, or nitrogen. An isotope is slightly heavier form of these elements. Since the isotope is stable (i.e., non-radioactive), it poses no health risk to you due to radioactive exposure. Additionally, a certain fraction of all of the carbon, hydrogen, and nitrogen within your body is already in the same form as that of the stable isotope. Hence, the infusion of the stable isotope-labelled amino acid will simply result in a slight increase in the amount of stable isotope within your body, we refer to this as "enriching" the amount of stable isotope within your body. This enrichment will not remain high, however, and will be back to pre-infusion levels within 24 hours. All of the infused solutions are prepared under sterile conditions and are filtered through a very selective filter prior to entering your body.

Potential Risks. Similar to the catheterization procedures described above, there is a theoretical risk of infection, although the catheter is inserted under completely sterile conditions. There is also a theoretical risk that you could have a rapid drop in blood pressure due to bacterial contamination of the infusate. The risks of this drop in blood pressure are impossible to estimate. There are no calculatable risks associated with receiving the stable isotope-labelled amino acid.

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EXERCISE METABOLISM RESEARCH GROUP (EMRG)
DEPARTMENT OF KINESIOLOGY, MCMASTER UNIVERSITY

DESCRIPTION OF INVASIVE MEDICAL PROCEDURES

The study in which you are invited to participate involves several invasive medical procedures. Prior to your involvement in the study, you are asked to read this form which outlines the potential medical risks inherent to these procedures. In addition, you must also complete the "Subject Screening Questionnaire" which is designed to identify any medical reason which might preclude your participation as a subject.

Venous Catheterization and Blood Sampling

A small Teflon catheter will be inserted into a forearm vein with the assistance of a small needle, which is subsequently removed. The discomfort of this procedure is transient and is very similar to having an injection by a needle, or when donating blood. Once the needle is removed there should be no sensation from the catheter. During the course of the experiment, blood will be drawn periodically from the catheter. In any one experiment the total blood loss is typically less than 100 ml, which is approximately 1/6 of the blood removed during a donation to a blood bank. It is not enough of a blood loss to affect your physical performance in any way. After each blood sample has been taken, the catheter is "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. This is a salt solution that is very similar in composition to your own blood and it will not affect you. Following removal of the catheter, pressure will be placed on the site in order to minimize bleeding and facilitate healing.

Potential Risks. The insertion of a venous catheter for blood sampling is a common medical practice and involves minimal risk provided proper precautions are taken. The catheter is inserted under completely sterile conditions, however there is a theoretical risk of infection. There is also chance of bleeding if adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration which could last up to a few weeks. There is also the remote risk that trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the bloodstream and become lodged in a smaller vessel. However, we have never experienced such a complication in our laboratory after several thousand venous catheter placements.

Participants Needed For

An Exercise Study

Purpose: -To examine the effect of nutrition on recovery from strenuous exercise.
-Study will involve muscle biopsies and blood sampling.

Required: Males 18-30 years old, recreationally active or trained.

Subjects will be compensated for their time and effort.

Contact Natalie Moreau at moreauna@univmail.mcmaster.ca or call the EMRG lab at (905)525-9140 ext. 27037 and ask for Nat.

Natalie Moreau
905-525-9140 ext. 26825
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APPENDIX II

REB APPROVAL FORM

McMaster University
Health Sciences

Hamilton Health Sciences
EXEMPLARY CARE • ACADEMIC EXCELLENCE

RESEARCH ETHICS BOARD

AMENDMENT REQUEST

REB Project #: 00-92

Locally Responsible Investigator: Dr. Martin Gibala

Title of Study: TCA Cycle and Amino Acid Metabolism in Skeletal Muscle

Document(s) Amended with version # and date:

Information/Consent - Consent form

Research Ethics Board Review

(this box to be completed by REB Chair only)

[X] **Amendment approved as submitted**

[ ] **Amendment approved conditional on changes noted in "Conditions" section below**

[ ] **New enrolment suspended**

[ ] **Study suspended pending further review**

Level of Review:

[X] Full Research Ethics Board

[ ] Research Ethics Board Executive Committee

Conditions:

The Hamilton Health Sciences/McMaster Health Sciences Research Ethics Board operates in compliance with the ICH Good Clinical Practice Guidelines and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.

J. Holland/kn

Date: 20 July, 2004

F. Jack Holland, MD, FRCP, FRCP(C), Chair
Hamilton Health Sciences/McMaster Health Sciences Research Ethics Board

All Correspondence should be addressed to the REB Chair and forwarded to:
REB Secretary, Henderson Campus, 90 Wing, Room #1
711 Concession Street Hamilton ON L8V 1C3
Telephone: 905-527-4322, ext. 42013
Fax: 905-574-5645

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28 June 2004

Dr. Jack Holland
Chair, Hamilton Health Sciences Research Ethics Board
c/o Deborah Mazzetti
Room 1, 90 Wing
Henderson Hospital
711 Concession St.
Hamilton, ON L8V 1C3

Dear Dr. Holland:

Re: Addendum to HHS REB Project Number 00-92
Project Title: "TCA Cycle and Amino Acid Metabolism in Skeletal Muscle"
Principal Investigator: M.J. Gibala

Please find below details regarding an amendment to the protocol and consent form for the above cited Project, which received REB approval in April, 2000. The proposed changes are:

(1) I would like to conduct a comprehensive study designed to examine the effect of nutient intake during recovery from exhaustive exercise on skeletal muscle carbohydrate and amino acid metabolism. The project is a logical follow-up to a study we conducted last summer that examined the effect of pre-exercise nutritional status on muscle metabolism.

(2) The nature of the exercise stresses and invasive medical procedures to be employed in the proposed study (i.e., venous catheterization, stable isotope tracer infusion and muscle biopsy sampling) are routinely applied in my laboratory, and are similar to those outlined in the original REB application for Project Number 00-92 or previously approved addendums. With respect to the stable isotope infusions, I will again collaborate with my colleague Dr. Stuart Phillips, who has extensive experience in this regard and REB approval to utilize these techniques (e.g., HHS REB Project # 00-91).

(3) The level of financial remuneration has been adjusted to $350.00 per subject, given the amount of time and effort required in order to complete all facets of the study. Ten subjects will be recruited for the proposed study.

(4) Natalie Moreau, an MSc student under my supervision, will be the primary student investigator for this project. My PhD student Krista Howarth will also be a collaborator.

I have also attached a copy of the revised Informed Consent From that is specific to this study, including the description of invasive medical procedures. Please do not hesitate to contact me if you have any questions or concerns.

Sincerely,

Martin J. Gibala, Ph.D.
Associate Professor

APPENDIX III

GASTRO-INTESTINAL(GI) QUESTIONNAIRE

Date: _____
Subject: _____

THE EFFECT OF CARBOHYDRATE AND PROTEIN ON MUSCLE GLYCOGEN RESYTHESIS POST-EXERCISE

Study Questionnaire

On a scale of 1 to 10, 1 = absent and 10 = strongly present, score the presence of the following gastro-intestinal discomforts and other complaints during your trial.

Time: ___ Hour

1. Nausea	1	2	3	4	5	6	7	8	9	10
2. Bloated feeling	1	2	3	4	5	6	7	8	9	10
3. Need to belch	1	2	3	4	5	6	7	8	9	10
4. Gastrointestinal cramps	1	2	3	4	5	6	7	8	9	10
5. Need to vomit	1	2	3	4	5	6	7	8	9	10
6. Diarrhea	1	2	3	4	5	6	7	8	9	10
7. Urge to defecate	1	2	3	4	5	6	7	8	9	10
8. Urge to urinate	1	2	3	4	5	6	7	8	9	10
9. Headache	1	2	3	4	5	6	7	8	9	10
10. Dizziness	1	2	3	4	5	6	7	8	9	10
11. Body Chills	1	2	3	4	5	6	7	8	9	10

Other comments:

_____

_____

APPENDIX IV

RAW DATA – SUBJECT CHARACTERISTICS

DRINK ORDER

THESIS SUBJECT CHARACTERISTICS

SUBJECT	AGE (yr)	WEIGHT (kg)	HR MAX (beats/min)	$V0_{2PEAK}$ (mL/min)	$V0_{2PEAK}$ (mL/kg/min)	W_{PEAK} (W)
1	21	102.5	177	5887	57.4	389
2	22	92.1	191	3961	43.0	354
3	23	77.2	192	4082	52.9	291
4	25	83.5	187	4031	48.3	337
5	21	80.0	190	4206	52.6	373
6	20	105.2	187	3998	38.0	341
MEAN	22	90.1	187	4361	48.7	348
STDV	2	11.8	5	752	7.2	34
SEM	1	4.8	2	307	2.9	14

DRINK ORDER

SUBJECT	1ST TRIAL	2ND TRIAL	3RD TRIAL
1	CHO/CHO	CHO/PRO	CHO
2	CHO	CHO/CHO	CHO/PRO
3	CHO	CHO/PRO	CHO/CHO
4	CHO/PRO	CHO	CHO/CHO
5	CHO/PRO	CHO	CHO/CHO
6	CHO	CHO/CHO	CHO/PRO

APPENDIX V

RAW DATA – MUSCLE METABOLITES, BLOOD METABOLITES, GASTRO-INTESTINAL(GI) QUESTIONNAIRE

STATISTICAL SUMMARY TABLES

MUSCLE GLYCOGEN (mmol/kg dry wt)

CHO			CHO/CHO			CHO/PRO		
Post Exercise Time (hr)			Post Exercise Time (hr)			Post Exercise Time (hr)		
Subject	0	4	**Subject**	0	4	**Subject**	0	4
S1	110.5	195.0	**S1**	185.2	245.6	**S1**	106.6	205.7
S2	133.6	177.1	**S2**	119.3	206.6	**S2**	127.4	180.6
S3	15.0	111.6	**S3**	10.8	96.3	**S3**	6.5	50.1
S4	222.2	356.9	**S4**	101.2	222.2	**S4**	57.4	195.6
S5	53.2	148.2	**S5**	27.7	57.8	**S5**	40.5	169.0
S6	49.2	140.7	**S6**	57.8	274.1	**S6**	40.5	168.5
Mean	**97.3**	**188.2**	**Mean**	**83.7**	**183.8**	**Mean**	**63.2**	**161.6**
SD	75.0	87.6	**SD**	64.8	86.6	**SD**	45.3	56.6
SEM	30.6	35.7	**SEM**	26.5	35.4	**SEM**	18.5	23.1

MUSCLE GLYCOGEN (mmol/kg dry wt)
Anova: two factor (Drink x Time)

Summary of all effects

Source o variation	**DF**	**SS**	**MS**	**F**	**P**
Subjects	5	93814	18762.8		
Drink	2	5842.83	2921.42	0.718	0.511
Drink x S	10	40700.7	4070.07		
Time	1	83818	83818	49.499	<0.001
Time x S	5	8466.6	1693.32		
Drink x T	2	141.973	70.987	0.086	0.918
Residual	10	8253.93	825.393		
Total	35	241038	6886.8		

Tukey HSD test
Comparisons for factor: Time

omparisc	**ifferenc of Means**	**p**	**q**	**P**	**P<0.050**
4 vs. 0	96.504	2	9.95	0.001	Yes

MUSCLE GLYCOGEN RESYNTHESIS OVER 4-HOURS(mmol/kg dry wt)

Subject	CHO	CHO/CHO	CHO/PRO
S1	84.5	60.5	99.1
S2	43.6	87.3	53.3
S3	96.6	85.5	43.6
S4	134.6	121.0	138.2
S5	95.0	30.1	128.6
S6	91.5	216.3	127.9
Mean	**91.0**	**100.1**	**98.4**
SD	29.1	64.5	41.0
SEM	11.9	26.3	16.7

MUSCLE GLYCOGEN RESYNTHESIS OVER 4-HOURS(mmol/kg dry wt)
Anova: one factor (Drink)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	16933.204	3386.641		
Between Treatments	2	283.946	141.973	0.086	0.918
Residual	10	16507.856	1650.786		
Total	17	33725.005			

MUSLE GLYCOGEN RESYNTHESIS RATE (mmol/kg dry wt/h)

Subject	CHO	CHO/CHO	CHO/PRO
S1	21.1	15.1	24.8
S2	10.9	21.8	13.3
S3	24.2	21.4	10.9
S4	33.7	30.2	34.5
S5	23.8	7.5	32.1
S6	22.9	54.1	32.0
Mean	**22.7**	**25.0**	**24.6**
SD	16.1	7.3	10.2
SEM	6.6	3.0	4.2

MUSLE GLYCOGEN RESYNTHESIS RATE (mmol/kg dry wt/hr)
Anova: one factor (Drink)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	1058.325	211.665		
Between Treatments	2	17.747	8.873	0.086	0.918
Residual	10	1031.741	103.174		
Total	17	2107.813			

CREATINE (mmol/kg dry wt)

CHO			CHO/CHO			CHO/PRO		
Post Exercise Time (hr)			Post Exercise Time (hr)			Post Exercise Time (hr)		
Subject	0	4	**Subject**	0	4	**Subject**	0	4
S1	39.9	57.1	**S1**	57.6	53.9	**S1**	57.0	73.1
S2	52.8	83.7	**S2**	57.3	49.9	**S2**	79.0	45.1
S3	60.6	43.9	**S3**	31.8	47.9	**S3**	34.8	37.1
S4	75.6	60.6	**S4**	39.7	46.6	**S4**	68.9	47.0
S5	78.4	69.4	**S5**	50.2	72.3	**S5**	63.0	73.8
S6	41.4	79.6	**S6**	44.6	50.1	**S6**	41.1	50.9
Mean	**58.1**	**65.7**	**Mean**	**46.9**	**53.5**	**Mean**	**57.3**	**54.5**
SD	16.5	14.9	**SD**	10.2	9.6	**SD**	16.8	15.4
SEM	6.7	6.1	**SEM**	4.2	3.9	**SEM**	6.8	6.3

CREATINE (mmol/kg dry wt)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subjects	5	2210.6	442.12		
Drink	2	830.29	415.143	4.153	0.069
Drink x Sub.	10	999.73	99.973		
Time	1	129.77	129.77	0.858	0.397
Time x Subj	5	756.51	151.302		
Drink x Tim	2	197.38	98.69	0.478	0.633
Residual	10	2064	206.404		
Total	35	7188.3	205.381		

PHOSPHOCREATINE (mmol/kg dry wt)

CHO			CHO/CHO			CHO/PRO		
Post Exercise Time (hr)			Post Exercise Time (hr)			Post Exercise Time (hr)		
Subject	0	4	**Subject**	0	4	**Subject**	0	4
S1	77.5	72.2	**S1**	89.3	32.7	**S1**	86.9	64.6
S2	72.4	63.8	**S2**	73.6	59.9	**S2**	54.5	66.8
S3	43.5	58.0	**S3**	74.1	72.4	**S3**	77.7	61.0
S4	60.7	47.8	**S4**	87.1	76.4	**S4**	63.0	69.1
S5	53.9	47.5	**S5**	67.7	54.1	**S5**	60.8	34.4
S6	73.6	55.9	**S6**	50.1	71.7	**S6**	51.8	70.1
MEAN	**63.6**	**57.5**	**MEAN**	**73.6**	**61.2**	**MEAN**	**65.8**	**61.0**
SD	13.2	9.5	**SD**	14.2	16.3	**SD**	13.7	13.4
SEM	5.4	3.9	**SEM**	5.8	6.7	**SEM**	5.6	5.5

PHOSPHOCREATINE (mmol/kg dry wt)
Anova: two factor (Drink x Time)

Summary of all effects

Source o Variation	**DF**	**SS**	**MS**	**F**	**P**
Subjects	5	1067.76	213.551		
Drink	2	284.923	142.461	0.888	0.441
Drink x S	10	1603.5	160.35		
Time	1	542.657	542.657	2.359	0.185
Time x S	5	1149.99	229.997		
Drink x T	2	101.031	50.516	0.297	0.749
Residual	10	1700.83	170.083		
Total	35	6450.68	184.305		

ATP (mmol/kg dry wt)

CHO			CHO/CHO			CHO/PRO		
Post Exercise Time (hr)			Post Exercise Time (hr)			Post Exercise Time (hr)		
Subject	0	4	**Subject**	0	4	**Subject**	0	4
S1	18.9	22.1	**S1**	25.0	24.1	**S1**	24.7	20.7
S2	22.1	22.3	**S2**	21.4	19.0	**S2**	23.5	21.3
S3	18.9	17.7	**S3**	20.9	24.3	**S3**	25.7	20.0
S4	23.9	26.1	**S4**	26.2	26.2	**S4**	25.3	27.3
S5	26.2	28.2	**S5**	22.3	15.5	**S5**	28.4	11.3
S6	26.8	15.9	**S6**	21.2	27.4	**S6**	17.7	28.0
Mean	**22.8**	**22.1**	**Mean**	**22.8**	**22.8**	**Mean**	**24.2**	**21.4**
SD	3.5	4.7	**SD**	2.2	4.6	**SD**	3.6	6.0
SEM	1.4	1.9	**SEM**	0.9	1.9	**SEM**	1.5	2.5

ATP (mmol/kg dry wt)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subjects	5	81.975	16.395		
Drink	2	1.18	0.59	0.0419	0.959
Drink x Su	10	140.686	14.069		
Time	1	13.068	13.068	0.809	0.41
Time x Su	5	80.797	16.159		
Drink x T	2	11.88	5.94	0.244	0.788
Residual	10	243.259	24.326		
Total	35	572.846	16.367		

MUSCLE LACTATE (mmol/kg dry wt)

CHO			CHO/CHO			CHO/PRO		
Post Exercise Time (hr)			Post Exercise Time (hr)			Post Exercise Time (hr)		
Subject	0	4	**Subject**	0	4	**Subject**	0	4
S1	22.77	17.34	**S1**	11.83	17.34	**S1**	11.92	58.78
S2	19.99	57.12	**S2**	13.10	16.44	**S2**	9.33	15.07
S3	31.49	52.39	**S3**	19.91	31.43	**S3**	10.05	32.63
S4	45.10	20.20	**S4**	6.84	11.70	**S4**	14.19	16.29
S5	28.52	50.63	**S5**	19.16	47.99	**S5**	17.91	32.68
S6	7.61	18.77	**S6**	8.91	15.54	**S6**	21.84	9.01
Mean	**25.91**	**36.07**	**Mean**	**13.29**	**23.41**	**Mean**	**14.21**	**27.41**
SD	12.54	19.10	**SD**	5.31	13.81	**SD**	4.86	18.19
SEM	5.12	7.80	**SEM**	2.17	5.64	**SEM**	1.99	7.42

MUSCLE LACTATE (mmol/kg dry wt)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Subjects	5	1506.3	301.261		
Drink	2	1067.7	533.83	3.656	0.064
Drink x	10	1460.1	146.015		
Time	1	1097	1097.05	6.665	0.049
Time x S	5	823.02	164.604		
Drink x	2	14.588	7.294	0.0439	0.957
Residual	10	1659.9	165.988		
Total	35	7628.6	217.961		

Tukey HSD test
Comparisons for factor: Time

Compar	**Diff of Means**	**p**	**q**	**P**	**P<0.050**
4 vs. 0	11.041	2	3.651	0.05	Yes

SERUM INSULIN (uIU/mL)

CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	7.18	3.62	6.44	11.21	14.98	35.15	62.29
S2	63.77	3.20	5.68	12.17	51.12	36.76	94.17
S3	42.89	2.48	8.73	16.81	27.21	35.32	67.61
S4	29.73	20.62	5.56	24.33	36.74	45.81	82.74
S5	16.06	5.13	3.11	5.36	14.66	17.19	31.37
S6	15.89	2.46	6.69	16.82	26.70	26.31	126.41
Mean	**32.94**	**3.74**	**8.32**	**28.33**	**34.18**	**63.09**	**98.30**
SD	15.49	0.74	3.84	13.47	19.28	42.03	68.17
SEM	6.32	0.30	1.57	5.50	7.87	17.16	27.83

CHO/CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	21.56	4.33	9.47	34.20	12.63	62.91	84.35
S2	52.78	2.62	5.91	44.77	55.95	121.26	171.44
S3	27.28	4.31	9.71	38.69	55.21	46.75	92.67
S4	44.94	3.66	14.85	13.78	23.80	20.27	19.99
S5	39.52	3.10	5.20	11.55	16.26	22.76	35.78
S6	11.57	4.39	4.80	27.01	41.25	104.58	185.59
Mean	**29.25**	**6.25**	**6.03**	**14.45**	**28.57**	**32.75**	**77.43**
SD	21.06	7.11	1.83	6.44	13.85	9.82	32.11
SEM	8.60	2.90	0.75	2.63	5.65	4.01	13.11

CHO/PRO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	9.09	10.24	22.34	31.09	52.18	92.29	109.13
S2	4.14	26.78	51.02	39.95	87.08	145.14	191.02
S3	16.89	4.07	12.44	29.28	32.73	54.55	142.55
S4	103.07	11.82	26.59	63.77	120.49	149.61	127.74
S5	35.67	3.72	2.60	6.92	18.00	16.10	49.53
S6	35.64	4.74	6.34	20.59	49.27	94.71	97.28
Mean	**34.08**	**10.23**	**20.22**	**31.93**	**59.96**	**92.07**	**119.54**
SD	36.28	8.80	17.65	19.18	37.62	51.60	47.35
SEM	14.81	3.59	7.20	7.83	15.36	21.07	19.33

SERUM INSULIN (uIU/mL) continued

CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	45.97	99.94	103.06	89.68	92.29	123.49
S2	125.44	143.73	198.06	204.96	213.84	251.01
S3	143.98	221.64	250.11	165.22	163.39	104.30
S4	67.66	97.04	63.40	79.39	55.83	49.23
S5	58.61	58.63	26.99	54.56	36.55	25.40
S6	134.56	176.64	202.79	168.08	153.82	106.95
Mean	**118.42**	**139.74**	**136.48**	**115.82**	**121.74**	**107.95**
SD	56.89	85.67	88.05	60.13	60.12	69.99
SEM	23.23	34.98	35.95	24.55	24.54	28.57

CHO/CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	129.57	137.57	131.61	113.06	166.88	86.33
S2	168.92	228.10	210.12	159.59	133.34	167.74
S3	95.90	104.77	130.96	120.85	103.69	96.00
S4	63.80	51.94	34.75	38.43	53.57	58.24
S5	55.81	59.83	50.39	62.03	64.05	25.99
S6	196.50	256.24	261.07	200.96	208.89	213.40
Mean	**96.04**	**132.94**	**140.73**	**126.98**	**119.29**	**110.06**
SD	43.27	59.65	88.81	60.22	68.82	78.69
SEM	17.66	24.35	36.26	24.58	28.09	32.12

CHO/PRO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	154.04	166.37	184.84	197.87	196.32	216.27
S2	243.70	298.81	305.87	284.18	267.76	214.09
S3	59.31	108.62	87.68	88.86	76.78	77.03
S4	77.75	76.49	184.65	95.90	129.70	75.17
S5	84.24	104.40	96.20	70.83	90.83	96.05
S6	175.42	224.03	113.54	135.06	73.86	57.24
Mean	**132.41**	**163.12**	**162.13**	**145.45**	**139.21**	**122.64**
SD	71.21	84.92	82.38	81.64	77.96	72.73
SEM	29.07	34.67	33.63	33.33	31.83	29.69

SERUM INSULIN (uIU/mL)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subject	5	202235.57	40447.1		
Drink	2	23909.771	11954.9	1.486	0.272
Drink x Subject	10	80444.773	8044.48		
Time	12	598267.08	49855.6	19.325	<0.001
Time x Subject	60	154792.96	2579.88		
Drink x Time	24	11426.556	476.107	0.45	0.987
Residual	120	127044.43	1058.7		
Total	233	1198121.1	5142.15		

Tukey HSD Test
Comparisons for factor: Time

Comparison	Diff of Means	p	q	P	P<0.050
180.000 vs. 0.000	139.711	13	11.67	<0.001	Yes
180.000 vs. 15.000	134.923	13	11.27	<0.001	Yes
180.000 vs. 30.000	121.542	13	10.152	<0.001	Yes
180.000 vs. -120.000	114.355	13	9.552	<0.001	Yes
180.000 vs. 45.000	105.546	13	8.816	<0.001	Yes
180.000 vs. 60.000	83.812	13	7.001	<0.001	Yes
180.000 vs. 90.000	48.025	13	4.011	0.213	No
180.000 vs. 240.000	32.897	13	2.748	0.764	Do Not Test
180.000 vs. 120.000	30.828	13	2.575	0.832	Do Not Test
180.000 vs. 225.000	19.705	13	1.646	0.994	Do Not Test
180.000 vs. 210.000	17.031	13	1.423	0.998	Do Not Test
180.000 vs. 150.000	1.181	13	0.0986	1	Do Not Test
150.000 vs. 0.000	138.53	13	11.571	<0.001	Yes
150.000 vs. 15.000	133.742	13	11.171	<0.001	Yes
150.000 vs. 30.000	120.361	13	10.054	<0.001	Yes
150.000 vs. -120.000	113.174	13	9.453	<0.001	Yes
150.000 vs. 45.000	104.365	13	8.717	<0.001	Yes
150.000 vs. 60.000	82.631	13	6.902	<0.001	Yes
150.000 vs. 90.000	46.844	13	3.913	0.245	Do Not Test
150.000 vs. 240.000	31.716	13	2.649	0.804	Do Not Test
150.000 vs. 120.000	29.647	13	2.476	0.865	Do Not Test

Comparisons for factor: Time (continued)

Comparison	Diff of Means	p	q	P	P<0.050
150.000 vs. 225.000	18.524	13	1.547	0.996	Do Not Test
150.000 vs. 210.000	15.85	13	1.324	0.999	Do Not Test
210.000 vs. 0.000	122.68	13	10.247	<0.001	Yes
210.000 vs. 15.000	117.892	13	9.847	<0.001	Yes
210.000 vs. 30.000	104.511	13	8.73	<0.001	Yes
210.000 vs. -120.000	97.324	13	8.129	<0.001	Yes
210.000 vs. 45.000	88.515	13	7.394	<0.001	Yes
210.000 vs. 60.000	66.782	13	5.578	0.012	Yes
210.000 vs. 90.000	30.994	13	2.589	0.826	Do Not Test
210.000 vs. 240.000	15.866	13	1.325	0.999	Do Not Test
210.000 vs. 120.000	13.797	13	1.152	1	Do Not Test
210.000 vs. 225.000	2.674	13	0.223	1	Do Not Test
225.000 vs. 0.000	120.005	13	10.024	<0.001	Yes
225.000 vs. 15.000	115.217	13	9.624	<0.001	Yes
225.000 vs. 30.000	101.837	13	8.506	<0.001	Yes
225.000 vs. -120.000	94.65	13	7.906	<0.001	Yes
225.000 vs. 45.000	85.84	13	7.17	<0.001	Yes
225.000 vs. 60.000	64.107	13	5.355	0.02	Yes
225.000 vs. 90.000	28.32	13	2.366	0.898	Do Not Test
225.000 vs. 240.000	13.192	13	1.102	1	Do Not Test
225.000 vs. 120.000	11.123	13	0.929	1	Do Not Test
120.000 vs. 0.000	108.883	13	9.095	<0.001	Yes
120.000 vs. 15.000	104.095	13	8.695	<0.001	Yes
120.000 vs. 30.000	90.714	13	7.577	<0.001	Yes
120.000 vs. -120.000	83.527	13	6.977	<0.001	Yes
120.000 vs. 45.000	74.718	13	6.241	0.003	Yes
120.000 vs. 60.000	52.985	13	4.426	0.112	No
120.000 vs. 90.000	17.197	13	1.436	0.998	Do Not Test
120.000 vs. 240.000	2.069	13	0.173	1	Do Not Test
240.000 vs. 0.000	106.814	13	8.922	<0.001	Yes
240.000 vs. 15.000	102.026	13	8.522	<0.001	Yes
240.000 vs. 30.000	88.645	13	7.404	<0.001	Yes
240.000 vs. -120.000	81.458	13	6.804	<0.001	Yes
240.000 vs. 45.000	72.649	13	6.068	0.004	Yes
240.000 vs. 60.000	50.916	13	4.253	0.148	Do Not Test
240.000 vs. 90.000	15.128	13	1.264	0.999	Do Not Test
90.000 vs. 0.000	91.685	13	7.658	<0.001	Yes
90.000 vs. 15.000	86.898	13	7.258	<0.001	Yes
90.000 vs. 30.000	73.517	13	6.141	0.004	Yes
90.000 vs. -120.000	66.33	13	5.54	0.013	Yes

Comparisons for factor: Time (continued)

Comparison	Diff of Means	p	q	P	P<0.050
90.000 vs. 45.000	57.521	13	4.805	0.057	No
90.000 vs. 60.000	35.787	13	2.989	0.654	Do Not Test
60.000 vs. 0.000	55.898	13	4.669	0.074	No
60.000 vs. 15.000	51.11	13	4.269	0.145	Do Not Test
60.000 vs. 30.000	37.73	13	3.152	0.575	Do Not Test
60.000 vs. -120.000	30.543	13	2.551	0.84	Do Not Test
60.000 vs. 45.000	21.733	13	1.815	0.985	Do Not Test
45.000 vs. 0.000	34.165	13	2.854	0.717	Do Not Test
45.000 vs. 15.000	29.377	13	2.454	0.872	Do Not Test
45.000 vs. 30.000	15.996	13	1.336	0.999	Do Not Test
45.000 vs. -120.000	8.809	13	0.736	1	Do Not Test
-120.000 vs. 0.000	25.356	13	2.118	0.951	Do Not Test
-120.000 vs. 15.000	20.568	13	1.718	0.991	Do Not Test
-120.000 vs. 30.000	7.187	13	0.6	1	Do Not Test
30.000 vs. 0.000	18.169	13	1.518	0.997	Do Not Test
30.000 vs. 15.000	13.381	13	1.118	1	Do Not Test
15.000 vs. 0.000	4.788	13	0.4	1	Do Not Test

AREA UNDER THE INSULIN CURVE (uIU x min/mL)

Subject	CHO	CHO/CHO	CHO/PRO
S1	58.8	89.3	119.7
S2	115.3	141.4	178.8
S3	118.6	80.5	71.1
S4	41.5	31.7	93.5
S5	28.5	37.1	56.8
S6	112.0	161.1	104.4
Mean	79.1	90.2	104.1
SD	52.9	40.8	43.0
SEM	21.6	16.7	17.6

AREA UNDER THE INSULIN CURVE (uIU x min/mL)
Anova: one factor (Drink)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Between Subjects	5	23770.484	4754.097		
Between Treatments	2	1879.076	939.538	1.204	0.34
Residual	10	7805.983	780.598		
Total	17	33455.543			

BLOOD GLUCOSE (mmol/L)

CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	4.8	4.42	4.32	5.55	6.07	6.25	6.01
S2	4.67	4.53	4.43	5.36	6.16	6.27	6.11
S3	5.59	4.28	4.27	5.16	5.86	6	6.09
S4	4.77	5.18	5.15	5.99	6.3	6.29	6.01
S5	5.04	5	4.31	5.48	6.11	6.14	5.88
S6	4.23	4.45	4.70	5.46	5.85	6.06	6.06
Mean	**4.85**	**4.64**	**4.53**	**5.50**	**6.06**	**6.17**	**6.03**
SD	0.45	0.36	0.34	0.28	0.18	0.12	0.08
SEM	0.18	0.15	0.14	0.11	0.07	0.05	0.03

CHO/CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	4.78	4.64	5.61	5.38	6.05	6.43	5.4
S2	4.51	4.57	5.08	6.34	6.51	6.39	6.49
S3	4.69	4.46	4.96	5.76	5.99	6.17	5.78
S4	5.18	4.6	5.52	5.84	6.33	5.67	5.16
S5	5.08	4.85	5.05	5.56	5.95	5.9	5.75
S6	4.78	4.44	4.44	5.75	6.35	6.39	5.91
Mean	**4.84**	**4.59**	**5.11**	**5.77**	**6.20**	**6.16**	**5.75**
SD	0.25	0.15	0.42	0.32	0.23	0.31	0.46
SEM	0.10	0.06	0.17	0.13	0.09	0.13	0.19

CHO/PRO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	2.99	3.12	4.36	5.38	5.63	5.92	6
S2	5.27	4.78	5.28	5.66	6.44	6.37	6.27
S3	4.36	4.36	4.68	5.51	5.72	6.04	5.6
S4	5.27	5.2	5.19	5.74	5.9	6.03	5.47
S5	5.13	4.6	4.83	5.09	5.79	6.01	5.21
S6	4.70	4.54	4.56	5.25	5.79	6.12	5.27
Mean	**4.62**	**4.43**	**4.82**	**5.44**	**5.88**	**6.08**	**5.64**
SD	0.88	0.70	0.36	0.25	0.29	0.16	0.42
SEM	0.36	0.29	0.15	0.10	0.12	0.06	0.17

BLOOD GLUCOSE (mmol/L) continued

CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	4.66	5.13	4.34	4.32	3.9	4.33
S2	6.05	5.63	5.37	5.2	4.87	5.04
S3	6.03	5.69	5.28	4.43	4.64	3.55
S4	5.49	5.46	5.15	4.95	5.25	5.05
S5	5.64	5.55	4.83	4.91	4.58	4.74
S6	5.63	5.40	5.54	5.40	5.23	4.99
Mean	**5.58**	**5.48**	**5.09**	**4.87**	**4.75**	**4.62**
SD	0.51	0.20	0.44	0.42	0.50	0.59
SEM	0.21	0.08	0.18	0.17	0.20	0.24

CHO/CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	5.1	4.34	4.41	4.21	3.94	3.89
S2	5.74	5.65	5.33	5.18	5.09	5.11
S3	5.46	5.66	5.26	4.72	4.48	5
S4	5.8	5.14	4.63	5.14	5.12	5.86
S5	5.74	5.46	4.9	4.95	5.32	4.7
S6	5.88	5.69	5.37	4.96	5.56	4.74
Mean	**5.62**	**5.32**	**4.98**	**4.86**	**4.92**	**4.88**
SD	0.29	0.52	0.40	0.36	0.60	0.64
SEM	0.12	0.21	0.16	0.15	0.24	0.26

CHO/PRO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	5.93	5.72	5.13	4.9	4.77	4.92
S2	6.21	5.68	5.51	5.47	5.62	5.45
S3	5.06	5.62	5.3	5.11	4.88	4.97
S4	5.35	4.97	5.14	5.33	4.79	5.11
S5	5.81	5.6	5.4	5.01	5.29	4.97
S6	5.56	5.35	4.90	5.22	4.75	4.93
Mean	**5.65**	**5.49**	**5.23**	**5.17**	**5.02**	**5.06**
SD	0.42	0.29	0.22	0.21	0.36	0.20
SEM	0.17	0.12	0.09	0.09	0.15	0.08

BLOOD GLUCOSE (mmol/L)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subject	5	7.538	1.508		
Drink	2	0.169	0.0843	0.681	0.528
Drink x Subject	10	1.238	0.124		
Time	12	58.9	4.908	35.875	<0.001
Time x Subject	60	8.209	0.137		
Drink x Time	24	3.89	0.162	1.368	0.138
Residual	120	14.22	0.119		
Total	233	94.17	0.404		

Tukey HSD test
Comparisons for factor: Time

Comparison	Diff of Means	p	q	P	P<0.050
60.000 vs. 0.000	1.579	13	18.116	<0.001	Yes
60.000 vs. -120.000	1.367	13	15.685	<0.001	Yes
60.000 vs. 15.000	1.317	13	15.105	<0.001	Yes
60.000 vs. 240.000	1.283	13	14.72	<0.001	Yes
60.000 vs. 225.000	1.243	13	14.255	<0.001	Yes
60.000 vs. 210.000	1.169	13	13.407	<0.001	Yes
60.000 vs. 180.000	1.037	13	11.891	<0.001	Yes
60.000 vs. 150.000	0.706	13	8.099	<0.001	Yes
60.000 vs. 30.000	0.566	13	6.493	0.002	Yes
60.000 vs. 120.000	0.517	13	5.933	0.006	Yes
60.000 vs. 90.000	0.332	13	3.811	0.281	No
60.000 vs. 45.000	0.0917	13	1.051	1	Do Not Test
45.000 vs. 0.000	1.488	13	17.065	<0.001	Yes
45.000 vs. -120.000	1.276	13	14.634	<0.001	Yes
45.000 vs. 15.000	1.225	13	14.054	<0.001	Yes
45.000 vs. 240.000	1.192	13	13.668	<0.001	Yes
45.000 vs. 225.000	1.151	13	13.203	<0.001	Yes
45.000 vs. 210.000	1.077	13	12.356	<0.001	Yes
45.000 vs. 180.000	0.945	13	10.839	<0.001	Yes
45.000 vs. 150.000	0.614	13	7.048	<0.001	Yes
45.000 vs. 30.000	0.474	13	5.442	0.016	Yes

Comparisons for factor: Time

Comparison	Diff of Means	p	q	P	P<0.050
45.000 vs. 120.000	0.426	13	4.881	0.05	Yes
45.000 vs. 90.000	0.241	13	2.759	0.759	Do Not Test
90.000 vs. 0.000	1.247	13	14.306	<0.001	Yes
90.000 vs. -120.000	1.035	13	11.875	<0.001	Yes
90.000 vs. 15.000	0.985	13	11.294	<0.001	Yes
90.000 vs. 240.000	0.951	13	10.909	<0.001	Yes
90.000 vs. 225.000	0.911	13	10.444	<0.001	Yes
90.000 vs. 210.000	0.837	13	9.597	<0.001	Yes
90.000 vs. 180.000	0.704	13	8.08	<0.001	Yes
90.000 vs. 150.000	0.374	13	4.289	0.14	No
90.000 vs. 30.000	0.234	13	2.683	0.79	Do Not Test
90.000 vs. 120.000	0.185	13	2.122	0.95	Do Not Test
120.000 vs. 0.000	1.062	13	12.184	<0.001	Yes
120.000 vs. -120.000	0.85	13	9.753	<0.001	Yes
120.000 vs. 15.000	0.8	13	9.172	<0.001	Yes
120.000 vs. 240.000	0.766	13	8.787	<0.001	Yes
120.000 vs. 225.000	0.726	13	8.322	<0.001	Yes
120.000 vs. 210.000	0.652	13	7.475	<0.001	Yes
120.000 vs. 180.000	0.519	13	5.958	0.005	Yes
120.000 vs. 150.000	0.189	13	2.167	0.943	Do Not Test
120.000 vs. 30.000	0.0489	13	0.561	1	Do Not Test
30.000 vs. 0.000	1.013	13	11.623	<0.001	Yes
30.000 vs. -120.000	0.801	13	9.192	<0.001	Yes
30.000 vs. 15.000	0.751	13	8.612	<0.001	Yes
30.000 vs. 240.000	0.717	13	8.227	<0.001	Yes
30.000 vs. 225.000	0.677	13	7.761	<0.001	Yes
30.000 vs. 210.000	0.603	13	6.914	<0.001	Yes
30.000 vs. 180.000	0.471	13	5.397	0.018	Yes
30.000 vs. 150.000	0.14	13	1.606	0.995	Do Not Test
150.000 vs. 0.000	0.873	13	10.017	<0.001	Yes
150.000 vs. -120.000	0.661	13	7.586	<0.001	Yes
150.000 vs. 15.000	0.611	13	7.006	<0.001	Yes
150.000 vs. 240.000	0.577	13	6.621	0.001	Yes
150.000 vs. 225.000	0.537	13	6.156	0.003	Yes
150.000 vs. 210.000	0.463	13	5.308	0.021	Yes
150.000 vs. 180.000	0.331	13	3.791	0.288	No
180.000 vs. 0.000	0.543	13	6.226	0.003	Yes
180.000 vs. -120.000	0.331	13	3.795	0.287	No
180.000 vs. 15.000	0.28	13	3.214	0.545	Do Not Test
180.000 vs. 240.000	0.247	13	2.829	0.728	Do Not Test

Comparisons for factor: Time

Comparison	Diff of Means	p	q	P	P<0.050
180.000 vs. 225.000	0.206	13	2.364	0.898	Do Not Test
180.000 vs. 210.000	0.132	13	1.517	0.997	Do Not Test
210.000 vs. 0.000	0.411	13	4.709	0.068	No
210.000 vs. -120.000	0.199	13	2.278	0.92	Do Not Test
210.000 vs. 15.000	0.148	13	1.698	0.991	Do Not Test
210.000 vs. 240.000	0.114	13	1.313	0.999	Do Not Test
210.000 vs. 225.000	0.0739	13	0.848	1	Do Not Test
225.000 vs. 0.000	0.337	13	3.862	0.263	Do Not Test
225.000 vs. -120.000	0.125	13	1.43	0.998	Do Not Test
225.000 vs. 15.000	0.0741	13	0.85	1	Do Not Test
225.000 vs. 240.000	0.0406	13	0.465	1	Do Not Test
240.000 vs. 0.000	0.296	13	3.396	0.457	Do Not Test
240.000 vs. -120.000	0.0842	13	0.965	1	Do Not Test
240.000 vs. 15.000	0.0336	13	0.385	1	Do Not Test
15.000 vs. 0.000	0.263	13	3.011	0.644	Do Not Test
15.000 vs. -120.000	0.0506	13	0.58	1	Do Not Test
-120.000 vs. 0.000	0.212	13	2.431	0.879	Do Not Test

BLOOD LACTATE (mmol/L)

CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	1.4	1.38	1.54	0.72	0.95	4	1.84
S2	2.02	2.55	2.19	2.08	2.6	1.9	2.36
S3	1.94	2.59	2.23	1.73	2.91	1.97	1.91
S4	1.89	1.31	1.07	1.24	1.88	2.09	2.61
S5	2.89	2.69	2.59	2.37	2.52	2.07	3.04
S6	1.89	1.37	0.73	0.61	1.20	1.38	0.76
Mean	**2.01**	**1.98**	**1.73**	**1.46**	**2.01**	**2.24**	**2.09**
SD	0.49	0.69	0.73	0.72	0.80	0.90	0.79
SEM	0.20	0.28	0.30	0.29	0.33	0.37	0.32

CHO/CHO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	1.86	1.82	2.4	0.5	0.71	1.68	2
S2	2.81	1.59	1.67	2.42	2.12	1.28	2.3
S3	5.37	4.19	4.41	2.89	2.92	3.25	5.31
S4	1.97	1.38	1.24	1.31	1.39	1.07	1.04
S5	2.87	2.66	2.73	2.19	1.95	1.89	1.87
S6	1.64	1.20	1.16	1.12	1.06	0.33	0.65
Mean	**2.75**	**2.14**	**2.27**	**1.74**	**1.69**	**1.58**	**2.20**
SD	1.38	1.13	1.22	0.90	0.80	0.98	1.65
SEM	0.56	0.46	0.50	0.37	0.33	0.40	0.67

CHO/PRO

	Pre and Post-exercise Time (hr)						
Subject	**Pre**	**0**	**15**	**30**	**45**	**60**	**90**
S1	0.65	1.04	0.72	0.92	0.75	1.59	3.24
S2	2.87	3.1	2.36	1.38	1.76	1.69	2.8
S3	1.61	2.91	1.66	1.43	1.51	1.91	1.73
S4	1.9	1.9	1.51	1.11	1.3	0.94	1.35
S5	2.95	2.34	2.61	2	2.17	2.23	1.7
S6	1.89	2.38	1.96	2.44	0.93	0.80	0.57
Mean	**1.98**	**2.28**	**1.80**	**1.55**	**1.40**	**1.53**	**1.90**
SD	0.85	0.74	0.67	0.57	0.53	0.56	0.97
SEM	0.35	0.30	0.27	0.23	0.22	0.23	0.40

BLOOD LACTATE (mmol/L) continued

CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	1.38	1.54	0.72	0.95	4	1.84
S2	2.55	2.19	2.08	2.6	1.9	2.36
S3	2.59	2.23	1.73	2.91	1.97	1.91
S4	1.31	1.07	1.24	1.88	2.09	2.61
S5	2.69	2.59	2.37	2.52	2.07	3.04
S6	1.37	0.73	0.61	1.20	1.38	0.76
Mean	**1.98**	**1.73**	**1.46**	**2.01**	**2.24**	**2.09**
SD	0.69	0.73	0.72	0.80	0.90	0.79
SEM	0.28	0.30	0.29	0.33	0.37	0.32

CHO/CHO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	1.97	2.95	2.12	2.96	2.31	2.8
S2	1.66	2.05	1.84	1.7	1.65	1.91
S3	0.11	1.52	2.19	2.16	1.85	2.59
S4	0.3	1.44	0.33	1.14	0.6	1.25
S5	1.94	1.63	1.66	2	1.82	1.82
S6	0.32	0.43	0.09	0.33	0.23	0.49
Mean	**1.05**	**1.67**	**1.37**	**1.72**	**1.41**	**1.81**
SD	0.89	0.82	0.92	0.90	0.81	0.85
SEM	0.36	0.34	0.38	0.37	0.33	0.35

CHO/PRO

	Post-exercise Time (hr)					
Subject	**120**	**150**	**180**	**210**	**225**	**240**
S1	1.71	1.53	1.43	1.84	2.26	2.57
S2	2.36	1.54	1.73	1.72	2.05	1.75
S3	1.66	1.28	4.91	1.19	1.27	1.53
S4	1.09	0.81	1.01	1.62	1.33	1.15
S5	2.5	3.13	1.99	1.56	1.69	1.85
S6	0.92	0.84	0.08	0.56	0.78	0.74
Mean	**1.71**	**1.52**	**1.86**	**1.42**	**1.56**	**1.60**
SD	0.64	0.85	1.64	0.47	0.55	0.63
SEM	0.26	0.35	0.67	0.19	0.22	0.26

BLOOD LACTATE (mmol/L)
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subject	5	58.364	11.673		
Drink	2	1.948	0.974	0.695	0.522
Drink x Subject	10	14.008	1.401		
Time	12	10.717	0.893		
Time x Subject	60	32.732	0.546	1.637	0.105
Drink x Time	24	12.938	0.539		
Residual	120	45.521	0.379	1.421	0.111
Total	233	176.229	0.756		

GASTRO-INTESTINAL(GI) QUESTIONNAIRE

GI DISTRESS SCORE BY SUBJECT AND BY HOUR

	Actual rating (1-10)											
	CHO				CHO/CHO				CHO/PRO			
Hour Post-Exercise	0	2	3	4	0	2	3	4	0	2	3	4
Subject 1												
Nausea	1	1	1	1	1	1	4	7	1	1	7	1
Bloated feeling	1	1	4	5	1	1	7	10	1	5	7	8
Need to belch	1	1	6	7	1	1	3	7	1	6	7	6
GI cramps	1	1	1	1	1	1	4	6	1	4	5	5
Need to vomit	1	1	1	1	1	1	1	1	1	1	4	1
Diarrhea	1	1	1	1	1	1	1	1	1	1	1	1
Urge to defecate	1	1	1	1	1	1	1	1	1	4	5	5
Urge to urinate	1	1	1	6	1	1	1	1	1	1	1	5
Headache	1	1	1	1	1	1	1	1	1	1	1	1
Dizziness	1	1	1	1	1	1	6	1	1	1	1	1
Body chills	1	1	1	1	1	7	7	7	1	1	1	1
Total (out of 110)	11	11	19	26	11	17	36	43	11	26	40	35
Subject 2												
Nausea	1	1	3	5	1	4	5	8	1	6	6	4
Bloated feeling	1	4	8	7	1	5	5	8	1	6	6	4
Need to belch	1	3	6	6	1	4	5	8	1	9	6	7
GI cramps	1	1	1	1	1	4	4	8	1	5	4	2
Need to vomit	1	1	8	8	1	2	8	7	1	4	8	5
Diarrhea	1	1	1	1	1	1	1	1	1	1	1	1
Urge to defecate	1	1	1	1	1	1	1	1	1	1	1	1
Urge to urinate	1	3	6	5	1	4	4	4	1	2	3	3
Headache	1	1	5	4	1	3	4	4	1	4	3	3
Dizziness	1	2	4	5	1	3	5	6	1	5	5	5
Body chills	1	1	2	3	1	6	7	6	1	5	3	3
Total (out of 110)	11	19	45	46	11	37	49	61	11	48	46	38

Subject 3												
Nausea	1	2	8	8	1	2	3	3	1	5	7	2
Bloated feeling	1	2	9	10	1	2	2	3	1	5	7	2
Need to belch	1	4	9	10	1	2	2	3	1	2	2	1
GI cramps	1	1	9	10	1	1	1	3	1	1	4	1
Need to vomit	1	4	10	10	1	3	3	3	1	5	7	2
Diarrhea	1	1	4	4	1	1	1	3	1	1	1	1
Urge to defecate	1	1	2	2	1	1	1	3	1	1	1	1
Urge to urinate	1	3	7	8	1	2	3	4	1	4	6	1
Headache	1	1	3	3	1	1	2	3	1	1	1	3
Dizziness	1	3	6	6	1	3	3	3	1	2	3	3
Body chills	1	6	6	6	1	1	1	3	1	3	3	2
Total (out of 110)	11	28	73	77	11	19	22	34	11	30	42	19
Subject 4												
Nausea	1	1	1	1	1	1	1	1	1	1	5	2
Bloated feeling	1	2	2	2	1	2	3	2	1	2	2	4
Need to belch	1	4	2	1	1	1	1	1	1	1	3	1
GI cramps	1	1	1	1	1	1	1	1	1	1	1	1
Need to vomit	1	1	1	1	1	1	1	1	1	1	3	1
Diarrhea	1	1	1	1	1	1	1	1	1	1	1	1
Urge to defecate	1	1	1	1	1	1	1	1	1	1	7	1
Urge to urinate	1	1	1	3	1	1	3	4	1	1	7	4
Headache	1	1	1	1	1	1	1	1	1	1	3	2
Dizziness	1	1	2	3	1	1	1	1	1	2	5	3
Body chills	1	1	1	1	1	1	1	1	1	1	1	1
Total (out of 110)	11	15	14	16	11	12	15	15	11	13	38	21
Subject 5												
Nausea	1	1	1	1	1	1	1	1	1	1	1	1
Bloated feeling	1	1	1	1	1	3	2	3	1	1	2	3
Need to belch	1	1	1	1	1	1	1	2	1	1	1	1
GI cramps	1	1	1	1	1	1	1	2	1	1	1	1
Need to vomit	1	1	1	1	1	1	1	2	1	1	1	1
Diarrhea	1	1	1	1	1	1	1	1	1	1	1	1
Urge to defecate	1	1	1	1	1	1	1	1	1	1	1	1
Urge to urinate	1	3	3	5	1	5	1	5	1	4	5	5
Headache	1	1	1	1	1	1	1	1	1	1	1	1
Dizziness	1	1	1	1	1	1	1	1	1	1	1	1
Body chills	1	1	1	1	1	1	1	1	1	1	1	1
Total (out of 110)	11	13	13	15	11	17	12	20	11	14	16	17

Subject 6												
Nausea	1	1	1	1	1	1	1	1	1	1	1	4
Bloated feeling	1	2	6	4	1	2	6	6	1	2	6	6
Need to belch	1	1	2	4	1	1	1	1	1	1	1	1
GI cramps	1	1	1	1	1	1	1	1	1	1	1	1
Need to vomit	1	1	6	3	1	1	6	3	1	1	6	6
Diarrhea	1	1	1	1	1	1	1	1	1	1	1	1
Urge to defecate	1	1	1	5	1	1	1	1	1	1	1	1
Urge to urinate	1	3	3	5	1	1	2	5	1	1	2	3
Headache	1	1	1	1	1	1	1	1	1	1	1	1
Dizziness	1	1	1	1	1	1	1	1	1	1	6	1
Body chills	1	1	1	1	1	1	1	1	1	1	1	1
Total (out of 110)	11	14	24	27	11	12	22	22	11	12	27	26

GASTRO-INTESTINAL(GI) QUESTIONNAIRE

SUM OF GI DISTRESS SCORES PER HOUR

	CHO				CHO/CHO				CHO/PRO			
Hour Post-exercise	0	2	3	4	0	2	3	4	0	2	3	4
Subject												
1	11	11	19	26	11	17	36	43	11	26	40	35
2	11	19	45	46	11	37	49	61	11	48	46	38
3	11	28	73	77	11	19	22	34	11	30	42	19
4	11	15	14	16	11	12	15	15	11	13	38	21
5	11	13	13	15	11	17	12	20	11	14	16	17
6	11	14	24	27	11	12	22	22	11	12	27	26
Total (out of 660)	66	100	188	207	66	114	156	195	66	143	209	156

GASTRO-INTESTINAL(GI) QUESTIONNAIRE DATA
Anova: two factor (Drink x Time)

Summary of all effects

Source of Variation	DF	SS	MS	F	P
Subject	5	4415.24	883.047		
Drink	2	38.528	19.264	0.0897	0.915
Drink x Subject	10	2147.14	214.714		
Time	3	4919.38	1639.79	12.422	<0.001
Time x Subject	15	1980.04	132.003		
Drink x Time	6	617.917	102.986	1.446	0.23
Residual	30	2136.42	71.214		
Total	71	16254.7	228.939		

Tukey HSD Test
Comparisons for factor: Time

Comparison	Diff of Means	p	q	P	P<0.050
4.000 vs. 0.000	19.833	4	7.324	<0.001	Yes
4.000 vs. 2.000	11	4	4.062	0.051	No
4.000 vs. 3.000	0.222	4	0.0821	1	No
3.000 vs. 0.000	19.611	4	7.242	<0.001	Yes
3.000 vs. 2.000	10.778	4	3.98	0.057	No
2.000 vs. 0.000	8.833	4	3.262	0.141	No