



Metabolic, neuromuscular, and performance responses to graded carbohydrate ingestion during exercise.

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The sanest days are mad.

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THESIS ABSTRACT

A dose response relationship between carbohydrate (CHO) ingestion and exercise performance has not been consistently reported. Additionally the underlying metabolic and neuromuscular explanations for an improvement in performance with increasing doses of CHO have not been fully explained. In Chapter 2 of this thesis 20 male cyclists completed 2 h of submaximal exercise followed by a time trial task ($531 \pm 48\text{KJ}$). Three CHO electrolyte beverages, plus a control (water), were administered during a 2 h ride providing 0, 20, 39 or $64 \text{ g}\cdot\text{h}^{-1}$ at a fluid intake rate of $1 \text{ L}\cdot\text{h}^{-1}$. Performance was assessed by time to complete the time trial task, mean power output sustained, and pacing strategy used. Mean task completion time (min:sec \pm SD) for $39 \text{ g}\cdot\text{h}^{-1}$ ($34:19.5 \pm 03:07.1$, $p=0.006$) and $64 \text{ g}\cdot\text{h}^{-1}$ ($34:11.3 \pm 03:08.5$ $p=0.004$) of CHO were significantly faster than control ($37:01.9 \pm 05:35.0$). The mean percentage improvement from control was -6.1% (95% CI: -11.3 to -1.0) and -6.5% (95% CI: -11.7 to -1.4) in the 39 and $64 \text{ g}\cdot\text{h}^{-1}$ trials respectively. The $20 \text{ g}\cdot\text{h}^{-1}$ ($35:17.6 \pm 04:16.3$) treatment did not reach statistical significance compared to control ($p = 0.126$) despite a mean improvement of -3.7% (95% CI -8.8 to 1.5%). These data demonstrate that consuming CHO at a rate between 39 to $64 \text{ g}\cdot\text{h}^{-1}$ is likely to be optimal for most individuals looking to utilise a single source CHO as an ergogenic aid during endurance performances lasting less than 3 hrs.

Attempts have been made to try and understand the acute metabolic regulation that occurs when ingesting increasing amounts of CHO. However, no one study has fully investigated the metabolic mechanisms underlying graded increments of CHO ingestion. In Chapter 3 we aimed to utilise stable isotopes and blood metabolite profiles to examine the integrated physiological responses to CHO ingestion when ingested at rates throughout the range where performance gains appear greatest.

Twenty well-trained male cyclists completed 2 h constant load ride (95% lactate threshold, 185 ± 25 W) where one of three CHO beverages, or a control (water), were administered every 15 min, providing participants with 0, 20, 39 or 64 g CHO·h⁻¹ at a fixed fluid intake rate of 1L·h⁻¹. Dual glucose tracer techniques (6,6,²H₂ glucose and U¹³C labelled glucose) were used to determine glucose kinetics and exogenous carbohydrate oxidation (EXO) during exercise. Endogenous CHO contribution was suppressed in the second hour of exercise when consuming 39 and 64 g·h⁻¹ in comparison to 0 g·h⁻¹ (-7.3%, 95%CI: -13.1 to -1.6 and -11.2%, 95%CI: -16.9 to -5.5 respectively). Additionally, consuming 64 g·h⁻¹ suppressed the endogenous CHO contribution by -7.2% (95%CI: -1.5 to -13.0) compared to the 20 g·h⁻¹ treatment. Exogenous CHO oxidation rate increased by 0.13 g·min⁻¹ (95%CI: 0.10 to 0.15) and 0.29 g·min⁻¹ (95%CI: 0.27 to 0.31) when consuming 39 and 64 g·h⁻¹ in comparison to 20 g·h⁻¹ of CHO. Peak exogenous CHO oxidation rates were 0.34 (0.06), 0.54 (0.09) and 0.78 (0.19) g·min⁻¹ for 20, 39 and 64 g·h⁻¹ respectively. Plasma NEFA concentration was 0.10 (95%CI: 0.07 to 0.13), 0.12 (95%CI: 0.10 to 0.16) and 0.16 (95%CI: 0.13 to 0.19) mmol.L⁻¹ higher when consuming 0 g·h⁻¹ in comparison to 20, 39 and 64 g·h⁻¹ respectively. Both 39 and 64 g·h⁻¹ were effective at sparing endogenous CHO stores of which it is estimated that most of this is liver glycogen sparing, but the measured response was highly variable between individuals. Consuming 39 g·h⁻¹ of CHO appears to be the minimum ingestion rate required to have a significant metabolic effect that results in an increase in performance.

Recent research has indicated a key role of endogenous CHO sensing and oral glucose sensing in maintaining central drive and peripheral function during endurance exercise tasks. Consuming 39 and 64 g·h⁻¹ of CHO elicits the greatest improvements in performance and also demonstrate a similar metabolic response. The improvement

in subsequent time trial performance when consuming 39 and 64 g·h⁻¹ coincided with significant alterations in whole body substrate usage that lead to endogenous CHO sparing at the same ingestion rates. In Chapter 4 we aimed to utilise gold standard neuromuscular function assessment techniques, alongside novel measures, to investigate the effect of consuming different rates of CHO on neuromuscular function during and following prolonged cycling exercise. In a double-blind, randomised cross-over design, well-trained male cyclists (n=20, mean±SD, age 34 ± 10 y, mass 75.8 ± 9 kg, peak power output 394 ± 36 W, $\dot{V}O_{2max}$ 62 ± 9 ml·kg⁻¹·min⁻¹) completed 2 familiarisation trials then 4 experimental trials. Trials involved a 2 h submaximal ride followed by a high intensity time trial task lasting approx. 35 min with each of 0, 20, 39 and 64 g·h⁻¹ CHO ingestion rates during submaximal exercise. Each trial involved pre and post exercise assessments (MVC, Mwave twitch potentiation and force, motor unit recruitment and firing rate assessment using high density EMG) and during exercise (gross EMG amplitude). MVC peak torque values were reduced post exercise by -20.4 nM (95%CI: -26.5 to -14.4) in comparison to pre value on all trials with no differences between trials. The firing rates of early recruited motor units significantly increased by 1.55 pps (95%CI: 0.51 to 2.59) following exercise in comparison to pre-exercise rates. Gross EMG during the 2 h cycling bout revealed a main effect of treatment (p<0.01) but post hoc comparisons provided no clarity and likely reflect methodological issues. Consuming CHO at ingestion rates between 20 and 64 g·h⁻¹ had little to no impact on the neuromuscular function of well-trained cyclists when comparing pre and post fatiguing exercise values. Despite differences in time trial completion time between trials, following exercise to fatigue in an endurance task, no post exercise differences were detected.

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ABBREVIATIONS

CHO - carbohydrate

EMG – electromyography

LT – lactate threshold

MUs – motor units

MUFR – motor unit firing rate

MVC – maximal voluntary contraction

NEFA – non-esterified fatty acids

PPO – peak power output

Ra/Rd – rate of appearance/rate of disappearance of glucose

RMS – root mean squared

SD – standard deviation

TRAP – trapezoid contraction

VL – vastus lateralis

LIST OF PUBLICATIONS / CONFERENCE ABSTRACTS

PUBLICATIONS

See appendix for publications

Newell, M. L., Hunter, A. M., Lawrence, C., Tipton, K. D., & Galloway, S. D. (2014). The Ingestion of 39 or 64 g·h⁻¹ of Carbohydrate is Equally Effective at Improving Endurance Exercise Performance in Cyclists. *International journal of sport nutrition and exercise metabolism*, epub head of print

Newell M, Wallis GA & Galloway SD (2014) Impact of carbohydrate nutrition on exercise metabolism and performance, *Agro Food Industry Hi Tech*, 25 (2), pp. 32-38.

CONFERENCE PRESENTATIONS

See appendix for posters presented at conferences.

Newell, M. L., Hunter, A. M., Lawrence, C., Tipton, K. D., & Galloway, S. D (2014) Carbohydrate and performance: a dose response investigation in the 20 – 64 g.h⁻¹ range, *International Journal of Sport Nutrition and Exercise Metabolism*, Vol. 24, S1 - S10

Newell, M. L., Hunter, A. M., Lawrence, C., Tipton, K. D., & Galloway, S. D (2014) Metabolic and neuromuscular effects of increasing the rate of carbohydrate intake during steady state exercise, *American College of Sports Medicine Integrative Physiology of Exercise Conference*, Miami Beach, Eden Roc Hotel

CHAPTER 1

PART I: GENERAL INTRODUCTION

PART II: GENERAL METHODOLOGY

PART I

1.1 ABSTRACT

Carbohydrate (CHO) ingestion before and during exercise has consistently been reported to extend endurance exercise capacity and improve performance. However, the mechanisms responsible and optimal ingestion rate required are still debated. Feeding CHO has been reported to spare muscle glycogen, spare liver glycogen and have central and peripheral neural actions. A combination of these mechanisms is likely and the nature of the exercise performed is key when interpreting these data. Research on the optimal dose of CHO to improve performance over a range of exercise durations and intensities has been a recent focus. Optimal doses suggested from these studies cover a range (30-80 g·h⁻¹) that likely reflects exercise task, training status, and/or individual variation in response. The ambiguity surrounding the optimal dose for performance, coupled with considerable gaps in the literature coupling dose with physiological examination has led to the development of the aims of this thesis.

1.2 INTRODUCTION AND HISTORICAL BACKGROUND

After almost 100 years of research on CHO metabolism and exercise there are still big questions over mechanisms of action and optimal dose during exercise to maximise performance (Cermak and van Loon 2013; Correia-Oliveira, et al 2013). The purpose of chapter 1 is to highlight these questions and cover key elements for consideration in future research, for applied sport nutrition practice and the aims and scope of this thesis. To achieve this we consider the historical background, effects of CHO on exercise performance/capacity, mechanisms of action, carbohydrate dose, and practical recommendations.

Dietary CHOs were first recognised as an important fuel for muscle during moderate to high intensity exercise (defined for the purpose of the review as 50 – 90% of maximal aerobic capacity) by Krogh and Lindhard (Krogh and Lindhard 1920). They observed that participants felt tired and exercise capacity was reduced following a short term high fat diet. However, three days of a high CHO diet reversed these symptoms of fatigue (Krogh and Lindhard 1920). In addition, other authors reported that blood glucose concentration was lower in the majority of competitors immediately following the 1924 Boston Marathon (Levine, et al 1924; Gordon, et al 1925). The following year blood glucose concentration immediately after a marathon was higher in participants who had consumed confectionary during the race compared to those who consumed nothing (Gordon, et al 1925). Subsequently, when fatigue was associated with low blood glucose concentration feeding a high CHO diet could extend exercise capacity (206 mins vs. 81 mins) (Christensen 1932). The findings led to greater consideration of the potential ergogenic impact that nutrition could have during exercise. These early observations demonstrated that CHO ingestion had an important role in regulating blood glucose and extending exercise capacity. However,

the mechanisms to explain these observations with CHO feeding and elevated blood glucose had not yet been addressed.

The skeletal muscle biopsy technique, developed by the French neurologist Guillaume-Benjamin-Amand Duchenne (Charriere and Duchenne 1865), was reintroduced in the mid 1960's providing insight into muscle glycogen usage during exercise. Bergstrom and Hultman (1967) demonstrated that skeletal muscle glycogen content could be increased following muscle glycogen depleting exercise if a high CHO diet was consumed. Furthermore, increasing the glycogen content of muscle improved exercise capacity above that obtained with normal muscle glycogen content. The application of this new insight was demonstrated when the consumption of a high CHO diet improved 30 km running time over that obtained on a normal diet (Karlsson and Saltin 1971). Similarly, football players covered less distance, ran slower, and walked more often during the second half of a match when starting the game with reduced muscle glycogen stores (Jacobs, et al 1982). It was also noted during these investigations that the consumption of carbohydrate elevated blood glucose concentration whilst sparing muscle glycogen. As such, these observations supported the notion that higher muscle glycogen content, as a result of CHO consumption in the days preceding exercise, improves the capacity to perform moderate to high intensity exercise.

In summary this early work clearly defined an association between muscle glycogen and exercise capacity. The ability to enhance muscle glycogen content via nutritional manipulation is now well established (Hawley, et al 1997) and is still considered to be crucial for optimal performances (Joyner, et al 2011; Atkinson, et al 2011). However, CHO ingestion immediately before, and during exercise is also considered to enhance performance without any initial difference in muscle glycogen content. The remainder

of the present review will focus on quantifying the size of this effect and exploring mechanisms of action and implications for applied practice.

1.3 CHO AND ENDURANCE EXERCISE PERFORMANCE / CAPACITY

The ergogenic effect of CHO feeding during moderate to high-intensity exercise has been extensively investigated and summarised in 4 recent meta-analyses (Temesi, et al 2011; Vandenberghe and Hopkins 2011; Karelis, et al 2010; Stellingwerff and Cox 2014) that are worth consideration. The first meta-analysis investigated the duration of exercise where CHO was having the largest effect. The analysis used Cohen's effect sizes as a means of inferring the magnitude of effect and can be interpreted as; 0.2 small; 0.5 moderate; 0.8 large effects respectively (Karelis, et al 2010). The authors reported that in the 72 studies examined, CHO ingestion had a moderate positive effect size (ES) of ~0.42 on exercise performance / capacity. Feeding CHO during exercise bouts of > 2 h had a significantly greater effect (ES ~0.5) than on those between 30 min and 2 h (ES ~0.35). Interestingly, the effect size was similar in running and cycling (ES ~0.4), and with feeding glucose alone or mixed monomers (ES ~ 0.4). CHO feeding during exercise has been shown to increase power output during a 40 km time trial (TT) lasting ~1 h (Jeukendrup, et al 1997), during the latter stages of a 60 min TT (Anantaraman, et al 1995), during an all-out 15 min TT task following a 45 min submaximal ride (Neufer, et al 1987), and during Wingate sprint performance following a 50 min sub maximal ride (80% $\dot{V}O_{2max}$). However, Mitchell et al (1989) reported no difference in 10 km running time when participants ingested a range of CHO doses (0, 34, 39, 50 g·h⁻¹). Equally, it has been reported that time to exhaustion (TTE) at 80% $\dot{V}O_{2max}$ did not change whether CHO or water was consumed prior to or during exercise (Bonen, et al 1981). Interestingly,

these two studies both examined shorter exercise durations (20-30 mins) than other studies included in the analysis. Consequently, it is generally considered that exercise tasks are required to be > 40 min, irrespective of CHO type or dose, to achieve a moderate beneficial effect on performance / exercise capacity. Nonetheless, new evidence indicates that acute CHO provision may have a beneficial effect on very short task durations (i.e., <10 min) suggesting a need for further study within this context (Galloway, et al 2013).

In the second meta-analysis fifty studies were grouped to illuminate the effects of the exercise protocol, pre-exercise nutritional status (i.e., fed or overnight-fasted), training status, and gender on the efficacy of CHO feeding during exercise (Temesi, et al 2011). Four exercise categories were considered: time to exhaustion (TTE), time trial (TT), submaximal preload with TTE (submax+TTE) and submaximal preload with TT (submax+TT). The inclusion criteria for studies with multiple CHO trials was limited to the single highest glucose concentration trial with ingestion rates between 30 and 80 g.h⁻¹ and solution concentration not exceeding 8%. The results from this analysis are shown in Table 1.

Table 1. Number of studies, effect size (ES) outcomes, and mean percentage improvement in performance from placebo/control as presented in the meta-analysis from Temesi et al (2011).

Mode of Exercise	Number of studies	Grouped mean Cohen's ES*	95% CI of ES, (p-value)	Weighted mean improvement (%)
TTE	19	0.47	0.32 – 0.62, p < 0.01	15.1
Submax+TTE	3	0.44	0.08 – 0.8, p = 0.017	54.2
TT	11	0.30	0.07 – 0.53, p=0.011	2.0
Submax+TT	17	0.53	0.37 – 0.69, p < 0.001	7.5

0.2 small; 0.5 moderate; 0.8 large effect.

The results from Table 1 show that the effect size indicates a moderate effect on all protocols but that weighted mean improvements are greater when a TTE protocol is used (whether alone or after a period of submaximal steady state exercise. As such this lead to the authors concluding that CHO intake is in line with current intake guidelines (30 to 80 g·h⁻¹) during exercise ≥ 1 h, moderately improves exercise performance/capacity irrespective of the exercise protocol used. Additionally, the pre exercise nutritional status of participants (i.e. fasted >8 h, and non-fasted <6 h) appears to have no effect on the subsequent exercise performance / capacity achieved. However, the scope of this meta-analysis was limited and did not comprehensively address all feeding strategies employed in the wider literature, potentially leading to an over-simplified view.

To provide greater scope the third meta-analysis pooled 88 randomised cross over studies investigating the effects of CHO consumption during exercise (Vandenbogaerde and Hopkins 2011). Of the studies included 83% used cycling as the mode of exercise and all studies measured exercise capacity with time to exhaustion (mean duration 106 min) and exercise performance via time trial (mean duration 47 min). The mean percentage change in capacity / performance from control conditions was deduced by compiling the reported additive performance effects of components included in the model. However, a -2% (impairment) to a +6% (improvement) was reported across all studies included in the meta-analysis. Interestingly, for some individuals consuming CHO caused a reduction in exercise performance / capacity. The range in performance outcome is not surprising considering the variety of feeding strategies, exercise interventions, and participant characteristics included in the analysis. Nonetheless, some recent studies report considerable individual variation in performance measures even when the same exercise intervention is used to determine the effect of CHO feeding. Taken together, the variability reported highlights the importance of acknowledging individual responses to CHO intake during exercise (Smith, et al 2010; Smith, et al 2013). Overall, there seems to be a clear moderate positive effect of CHO feeding on exercise performance/capacity throughout the three meta-analyses considered. Several mechanisms have been proposed and investigated to explain the ergogenic action of CHO feeding during sustained moderate-high intensity exercise.

Finally, in the fourth meta-analysis Stellingwerf et al (2014) examined 61 studies (n=679 participants) that examined the effect of CHO supplementation on varying duration exercise. Specifically, studies included utilised a randomised, placebo controlled study design that included no other ingredients other than CHO. The

studies utilised an all-out or endurance based exercise protocol (no team-based performance studies) interventions to determine the efficacy of the CHO interventions used. The results showed that 82% of studies (50 studies) included had a statistically significant ergogenic effect. The authors also reported a significant ($p=0.0036$) correlative relationship between increasing total exercise time and the subsequent percentage increase in performance with CHO (not specific to rate) intake versus placebo. The authors go on to suggest the underlying mechanisms for the improvements in performance for specific exercise durations (<1 h, <2 h, >2 h). They hypothesise that during short duration exercise situations (~1 h), oral receptor exposure to CHO, via either mouthwash or oral consumption then stimulates the pleasure and reward centres of the brain providing a central nervous system (CNS) based mechanism for enhanced performance. For longer duration exercise >2h, where muscle glycogen stores are stressed, the primary mechanism by which carbohydrate supplementation enhances performance is via high rates of CHO delivery ($>90 \text{ g}\cdot\text{h}^{-1}$) resulting in high rates of CHO oxidation. Overall, across the meta-analysis discussed there is a clear moderate positive effect of CHO feeding on exercise performance/capacity throughout the three meta-analyses considered. Several mechanisms have been proposed and investigated to explain the ergogenic action of CHO feeding during sustained moderate-high intensity exercise though none have discussed these mechanisms in any amount of detail.

1.4 MECHANISMS OF ACTION

1.4.1 Muscle glycogen sparing

As indicated in the Introduction fatigue has frequently been associated with low muscle glycogen (Bergstrom, et al 1967; Coyle, et al 1983; Stellingwerff, et al 2007). Nutritional strategies used to increase muscle glycogen stores prior to exercise can lead to enhanced performance and an increase in exercise capacity (Tsintzas, et al 1995; Bjorkman, et al 1984). Similarly, nutritional strategies utilising CHO during exercise may also have a positive effect on endurance performance/capacity by providing an alternative fuel source for muscle energy metabolism and therefore limiting the amount of muscle glycogen utilised (Erickson, et al 1987; Tsintzas, et al 2001; Hargreaves, et al 1984; Stellingwerff, et al 2007; Tsintzas, et al 1995). An overview of the studies investigating the effect of CHO provision before, and during, exercise on working muscle glycogen utilisation (measured from skeletal muscle biopsy samples) is shown in Table 2. Fifty percent of the 16 studies listed report a sparing of muscle glycogen with CHO supplementation during exercise. Further, some reports indicate muscle glycogen sparing may be fibre type specific (Tsintzas, et al 1995) although conflicting evidence has emerged (Stellingwerff, et al 2007). The variability in outcomes may reflect differences in exercise protocol (e.g., continuous vs intermittent running and cycling), location of muscle biopsy, method of analysis (e.g., biochemical vs. histochemical, mixed muscle vs. fibre specific), and inherent variability in the measurement of muscle glycogen. Many of the studies which report muscle glycogen sparing also report an increase in circulating plasma glucose concentration as a major factor in explaining this phenomenon. It is possible that circulating plasma glucose has a role to play but the mechanisms of action continue to be elusive. As such, muscle glycogen sparing can occur in some contexts but this is not always

observed leading to the suggestion that there may be other mechanisms to explain the positive effects of CHO feeding.

Table 2. Summary of studies reporting muscle glycogen usage and carbohydrate ingestion during exercise.

Study	Exercise mode and duration	Intensity	CHO intervention	MG sparing
Glycogen Sparing				
Berstrom and Hultman (1967)	Running, 1 h	950 kpm/min	Glucose infusion (3.5 g·min ⁻¹)	20% total reduction
Bjorkman et al (1984)	Cycling, TTE	68% $\dot{V}O_{2max}$	70 g·h ⁻¹ glucose	Rate of utilisation decreased from 2.3 to 1.3 mmol kg d.w. ⁻¹ min ⁻¹
Errikson et al (1987)	Cycling, 90 min	65-70% $\dot{V}O_{2max}$	1 g·kg BM	31.8% reduction
Hargreaves et al (1984)	Cycling, 4 h	50% $\dot{V}O_{2max}$ + intermittent sprints/30 min.	43 g·h ⁻¹ solid CHO + 400ml H ₂ O	26% total reduction
Stellingwerf et al (2007)	Cycling, 3 h	63% $\dot{V}O_{2max}$	0.7 g·CHO·h ⁻¹	19% total reduction
Tsintzas et al (1995)	Running, 1 h	70% $\dot{V}O_{2max}$	5.5% mixed CHO	28% total reduction
Tsintzas et al (2001)	Running TTE (matched)	70% $\dot{V}O_{2max}$	8 ml·kg BM of 5.5% CHO solution	56% reduction in Type I fibres

Study	Exercise mode and duration	Intensity	CHO intervention	MG sparing
No Glycogen Sparing				
Arkininstall et al (2001)	Running and cycling 1 h	Lactate threshold	8 ml·kg BM pre ex, 2 ml·kg BM every 20 min 6.4% solution	No difference
Chryssanthopoulos et al (2002)	Running 1 h	70% $\dot{V}O_{2max}$	Pre ex meal and 46 g CHO during	No difference
Coyle et al (1986)	Cycling TTE	71% $\dot{V}O_{2max}$	2.0 g·kg BM @ 20min, 4 g·kg.BM each 20 min thereafter.	No difference
Coyle et al (1991)	Cycling 2 h	73% $\dot{V}O_{2max}$	Hyperglycemic infusion clamp (~10 mmol)	No difference
Febbraio et al (1996)	Cycling 2 h + 15 min performance	70% Peak O_2 consumption	Pre ex CHO meal	No difference
Febbraio (2000)	Cycling 2 h + 15 min performance	70% Peak O_2 consumption	Pre ex CHO meal	No difference
Flynn et al (1987)	Cycling 2 h	Complete as much work as possible	Mixed CHO/concentration	No difference
Hargreaves et al (1988)	Cycling 2 h	70% $\dot{V}O_{2max}$	30 g CHO pre and every 30 min	No difference
Mitchell et al (1988)	Cycling 2 h	105min @ 70% $\dot{V}O_{2max}$	6, 12 and 18 g·100ml	No difference

1.4.2 Role of liver glycogen

Moderate to high rates of CHO intake during exercise have been reported to reduce endogenous hepatic glucose output to basal levels (Howlett, et al 1998) or completely inhibit endogenous hepatic glucose output altogether (Jeukendrup, et al 1999). Thus, exogenous CHO oxidation could replace hepatic glycogen as a fuel source. Coyle et al (1986) reported an enhanced maintenance of blood glucose during prolonged exercise with the ingestion of CHO. The authors attributed an enhanced blood glucose concentration with elevated rates of CHO oxidation throughout the bout. Consequently, feeding CHO should allow more liver glycogen to be available towards the latter stages of competitive exercise when exercise intensity, and the requirement for CHO oxidation, are both increased. Casey et al (2000) investigated liver glycogen depletion during exercise and its subsequent repletion over 4 hours post exercise when feeding different types of CHO. Liver glycogen content was measured using ¹³C magnetic resonance spectroscopy following an overnight fast prior to exercise, and following each glycogen resynthesis trial. A weak but positive association between liver glycogen content following resynthesis and subsequent TTE occurred. Therefore, CHO feeding prior to and during exercise, which increases liver glycogen content and/or reduces the rate it is utilised, may be one factor to help explain the mechanisms of action that feeding CHO can have on exercise performance / capacity. However, this mechanism of action may only be relevant when feeding stops but exercise continues. Nevertheless, it is likely the liver has a key regulatory role when achieving optimal performances with CHO supplementation and warrants further investigation.

1.4.3 Neuromuscular Recruitment

Muscle fatigue is defined as any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group (Bigland-Ritchie, et al 1986; Enoka and Stuart 1992). Typically, endurance exercise performance is enhanced with CHO ingestion as muscular force during repeated contractions is maintained thereby delaying fatigue. Investigators have characterised muscle fatigue into central and peripheral factors (Enoka and Stuart 1992). Peripheral fatigue refers to an exercise-induced process that leads to reduced force production at or distal to the neuromuscular junction. Both central and peripheral fatigue have been observed following whole body exercise such as running and cycling (Lepers, et al 2002; Millet, et al 2003; Kremenec, et al 2009). Identifying central and peripheral effects through different measures can infer how the onset of fatigue develops during exercise.

Peripheral Neural effects

One way of measuring peripheral fatigue is by externally stimulating the muscle to contract. The motor response (M-wave) is evoked by direct electrical stimulation of motor efferents. The maximal M-wave, obtained by increasing stimulation intensity until no further increases in the M-wave magnitude is observed, is a steady and accurate measurement of total muscle activation and has been used in many reflex studies (Simonsen and Dyhre-Poulsen 1999; Zehr 2002). The stability of a chosen M-wave magnitude is also used as an indicator of the nerve to stimulating electrode relationship during muscle contraction or limb movement investigations (Zehr 2002). In individual subjects the maximal M-wave magnitude is also known to be altered during the walking or running cycle (Simonsen and Dyhre-Poulsen 1999) and to be affected by the level of voluntary contraction in the test muscle (Nagata and Christianson 1995).

Typically, fatiguing endurance exercise results in a reduction in the twitch, or tetanic force produced, by peripheral nerve stimulation while the muscle is at rest (Lepers, et al 2002; Abbiss, et al 2008). Researchers identified a reduction in force and a delay in signal propagation across the muscle by stimulating the femoral nerve before and after prolonged endurance exercise (Lepers et al 2002). The time course change tends to be dependent on exercise intensity and duration though generally the longer the exercise bout the greater the reduction. However, very little research has involved investigating the effects of CHO ingestion on M wave characteristics. Maintaining the signal propagation across the muscle will only serve to improve innervation of the motor units and therefore innervate a larger muscle mass.

A limited amount of research has been conducted on the peripheral neural effects of CHO ingestion and muscle activity. One study reported attenuation in the rise in muscle neural activity during the latter stages (> 45 min) of exercise at 84% $\dot{V}O_{2max}$ when ingesting a 6.4% CHO solution (Nikolopoulos, et al 2004). Additionally, Abbiss and Peiffer et al (2008) highlighted a correlation between muscle activation of the vastus lateralis (VL) and power output sustained during a 16.1 km TT task. However, CHO feeding had no effect on the percentage activation of the muscle when CHO was consumed. This highlights that it was not central factors influencing fatigue during the trial. Additionally, when CHO was not consumed; muscle activation level was maintained even with a reduction in the power output produced. Taken together these findings suggest a peripheral metabolic nature of the fatigue in the TT task. As such, CHO intake during exercise may be able to directly affect the contractile properties of the active muscle, helping to promote force production and subsequently improve performance, though the exact mechanism to explain the effect is unclear. It should be noted that this interpretation is based on only a few studies, often with poor

methodological control of muscle activity recordings, highlighting a need for future research on this possible mechanism. In contrast, research investigating the potential central neural effects of CHO administration has been much more active.

Central neural effects

More proximal (central) fatigue processes are defined as a progressive exercise-induced failure of voluntary activation of the muscle during contraction (Enoka and Stuart 1992). Under normal circumstances circulating glucose is the only energy source for the central nervous system (Newsholme, et al 1987; Nybo and Nielsen 2001; Pardiidge 1983) and continuous supply is essential as storage of CHO in neuronal tissue is limited (Pardiidge 1983). Previous research has shown decrements in force production following endurance activity which is attributed to both peripheral and central components of fatigue (Nybo et al 2001). Additionally, fatigue was reduced in exercising dogs when glucose was directly infused into the carotid artery (Koslowski, et al 1981). Nybo et al (2003) reported the maintenance of neuromuscular drive when participants consumed a glucose beverage during 3 h of submaximal cycling exercise. Additionally, unpublished results from Glace et al (2014) suggest a suppression of central fatigue characteristics with the ingestion of carbohydrate during prolonged cycling. However, determining the optimal ingestion rate to maintain plasma glucose and maintain neuromuscular function is the ingestion of different amounts of CHO little research has identified an optimal dose for maintaining glucose concentration and therefore maintaining CNS drive to the working muscle.

There is now robust evidence to indicate that CHO can be detected in the oral cavity (Katz, et al 2000; Zheng, et al 2009; Berthoud 2003) which is thought to directly affect the brain (Gant, et al 2010; Chambers, et al 2009). Carter et al (2004) demonstrated

in a pivotal study the detection of CHO in the oral cavity has a positive effect on exercise performance. Participants completed a simulated TT (~1 h) as fast as possible when mouth rinsing either a 6% CHO solution or a placebo solution for 5s every 12.5% of the TT task. CHO mouth rinsing resulted in a significant (2.9%) increase in performance time when compared to the placebo condition.

To isolate the effect of the gastrointestinal (GI) tract Carter et al then infused CHO intravenously to bypass the GI tract and maintain exogenous CHO availability. CHO was infused at $1 \text{ g}\cdot\text{min}^{-1}$ but had no effect on exercise performance compared to placebo (Carter, et al 2004). These observations highlighted the potential importance of oral CHO sensing for performance enhancement during short duration activity. Furthermore, there is great interest in this work since endogenous CHO availability for short duration high intensity efforts is not typically considered a limiting factor for exercise performance in this context.

Ingesting CHO at rates between 20 and $90 \text{ g}\cdot\text{h}^{-1}$ during prolonged endurance activity extends time to fatigue and increases the intensity that can be sustained during a performance task (Stellingwerf and Cox 2014). Coggan and Coyle (1986) reported a reversal of fatigue when CHO was consumed immediately following exhausted prolonged endurance exercise. The CHO ingested following fatigue could not be metabolised before exercise commenced. As such, there is evidence that the central drive to the muscle is altered with the presence of CHO to permit the continuation of exercise that is not metabolically dependant.

The outcomes from these studies have led to speculation regarding the underlying mechanisms explaining CHO mouth sensing, and also CHO ingestion during short duration tasks (<1 h). Some have proposed that oral cavity CHO sensitive receptors

exist which detect exogenous CHO and directly act upon the central nervous system (Gant, et al 2010). An increase in the corticomotor output in the fasted state to both rested and fatigued skeletal muscle when CHO is rinsed in the mouth supports this hypothesis (Gant, et al 2010). Similarly, CHO mouth rinsing significantly improved performance over that of a saccharin sweetened placebo demonstrating that oral sensors are caloric sensitive (Chambers, et al 2009). Additionally, Chambers reported increases in neural activity in brain regions involved in reward and motor control when mouth rinsing a CHO solution. The authors proposed that the increase in brain activity in these regions supports the increase in performance. However, not all studies report an increase in performance with CHO rinsing (Beelen, et al 2009; Whitham and McKinney 2007). The duration of the pre-exercise fast has led some to speculate that nutritional status (fed vs. fasted) of the participant influences the impact of the effect (Rollo and Williams 2011).

1.5 CARBOHYDRATE DOSE RESPONSE – WHAT IS THE OPTIMAL INGESTION RATE?

Many attempts have been made to recommend 'optimal' doses of CHO for endurance performance. However, considerable debate remains as to how much is enough? Some have suggested there may be a dose response relationship between CHO ingestion and endurance exercise performance. Smith et al (2010) fed 15, 30, and 60 g·h⁻¹ during a 2 h submaximal ride prior to a 20 km TT. All CHO conditions significantly improved performance compared to the placebo condition. However, a lack of statistical power (n=12) meant only the 60 g·h⁻¹ ingestion rate significantly improved performance over that achieved with 15 g·h⁻¹. Additionally, Watson and Shirreffs et al (2012) reported that exercise capacity was improved to the same extent

with 32 and 47 g·h⁻¹ of CHO compared to placebo suggesting that a saturation in additional performance gains may occur around 30 g·h⁻¹. Both of these studies suggested a trend for a dose response relationship, but the low sample sizes have made the existence of a dose response relationship difficult to confirm.

Smith et al (2013) increased sample size using a multi-centre study. Fifty five participants spread across four research sites consumed CHO during a 2 h submaximal ride followed by a 20 km TT task. Each participant completed 4 trials, one placebo and three CHO treatments, between 10 and 120 g·h⁻¹ (10 g·h⁻¹ increments). Following some statistical modelling of their data the authors reported an optimal dose of 78 g·h⁻¹ for performances during the TT. However, the linear regression model used for the determination of the optimal feeding strategy utilised was not significant. There are also some concerns over the study design and allocation of treatments across multiple sites. Smith et al also report a 1.7% improvement in performance between 30 and 80 g·h⁻¹ and a rather trivial 0.7% improvement in performance when dose is increased from 40 to 80 g·h⁻¹. Furthermore, Watson et al (2012) observed no further improvements in time to exhaustion when feeding a 6% (47 g·h⁻¹) mixed CHO solution when compared to a 2% (26 g·h⁻¹) mixed solution. Taken together these studies suggest increasing amounts of CHO may result in diminishing returns with respect to performance gains. From a practical perspective, it is important to further clarify the dose-response relationship. There is a need to discriminate between the dose increments where the largest gains in performance are observed, and dose increments where performance gains are marginal but negative effects (such as gastro-intestinal distress) are increased.

Carbohydrate ingestion during exercise has been positively associated with increases in exercise capacity and performance for a number of years. We are only now starting

to understand the mechanisms underpinning these physiologically enhancing effects. The mechanisms are not purely metabolic in nature with peripheral and central neural effects being reported with CHO intake. The optimal dose of CHO for performance is not well defined but appears to be around 30-40 g·h⁻¹. The response appears to be variable between individuals highlighting the need to more fully evaluate the optimal CHO dose for endurance performance and the underlying mechanisms explaining the relationship.

1.6 CONCLUSIONS AND THESIS AIMS

From research conducted to date it is not clear what the optimal ingestion rate of CHO is for the vast majority of individuals in exercise lasting <3 h. Some have suggested higher CHO ingestion rates that 'improve' performance but these performance gains tend to reduce with increasing ingestion CHO rates. In addition, attempts have been made to try and isolate the key physiological (metabolic and neuromuscular) mechanisms that facilitate the improvements in endurance performance observed with CHO consumption. However, it is not clear how these mechanisms are regulated with different CHO ingestion rates. One of these mechanisms, the neuromuscular component, has not been comprehensively investigated. Taking these factors into consideration the aims of the current thesis are as follows:

1. To determine the performance response of trained male cyclists to the ingestion of increasing amounts of CHO during a preloaded time trial task in a suitably powered and controlled trial.
2. To determine the acute differences in metabolic response when ingesting incremental amounts of CHO during a prolonged submaximal exercise bout.
3. To characterise the regulation of neuromuscular control during, and following, prolonged endurance cycling when consuming increasing amounts of CHO.

Part II – General Methodology

1.7 PARTICIPANTS

Twenty well-trained male cyclists were recruited from regional cycling and triathlon clubs. The mean (\pm SD) characteristics of the participants were: age 34.0 (\pm 10.2) years, body mass 74.6 (\pm 7.9) kg, stature 178.3 (\pm 8.0) cm, peak power output (PPO) 393 (\pm 36) W, and $\dot{V}O_{2\max}$ 62 (\pm 9) ml·kg⁻¹·min⁻¹. Participants were required to have been training for >6 h / wk for >3 y. Each individual had the procedures and associated risks explained prior to providing written informed consent to participate in the study, which was approved by the local research ethics committee in accordance with the Declaration of Helsinki. All participants completed all measures across the experimental chapters of this thesis. In some circumstances, not all participants were included in all datasets. Where a deviation in participant numbers occurs a description is included in the relevant sections of the thesis.

1.8 EXPERIMENTAL DESIGN

In a double blind, placebo controlled, randomised cross-over study design participants visited the laboratory 6 times (2 preliminary and 4 intervention) over a six week period (Figure 1.1). They completed one visit per week commencing each trial on the same day at the same time of day on each visit. Following pre-screening, participants completed a preliminary assessment where lactate threshold, $\dot{V}O_{2\max}$, and peak power output were determined and for the first familiarisation of the performance task to be used in subsequent visits. On the second visit participants completed a full familiarisation trial. The familiarisation trial and four subsequent intervention trials involved completing a range of neuromuscular measures before completing a 120 min steady submaximal cycle at 95% lactate threshold (184 \pm 25 W). The 2 h ride was followed by a time trial performance task,

whereupon the participants were instructed to be complete their set work target as quickly as possible. Water was ingested for the familiarisation trial and consumed at a rate of 1 L·h⁻¹. Thereafter, on the intervention trials participants consumed in a random order either: a control (water) 0%, 2%, 3.9% or 6.4% CHO solutions at a fluid ingestion rate of 1 L·h⁻¹. The aforementioned neuromuscular tasks were repeated within 5 min of completing the time trial task. Measures were placed into the categories of performance, metabolism and neuromuscular which corresponds to Chapters 3, 4 and 5 respectively.

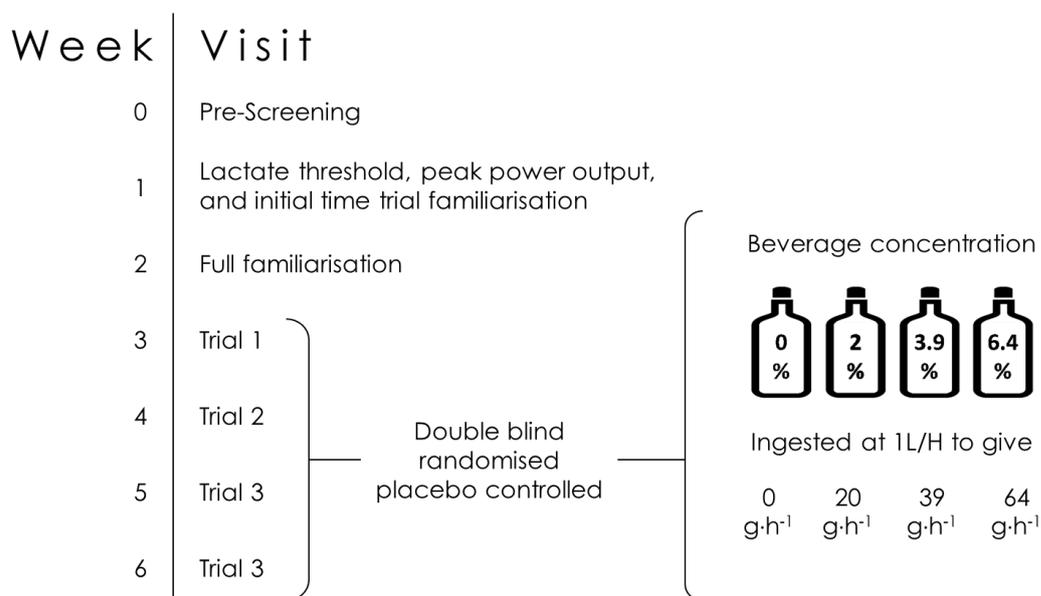


Figure 1.1 Order of visits to the laboratory for participants.

1.9 PRELIMINARY TESTING

On week 1 of 6, following a 10 h overnight fast, participants performed a two section incremental cycle test (Lode Excalibur Sport, Netherlands) to determine maximal oxygen uptake ($\dot{V}O_{2max}$), lactate threshold, and peak power output (Figure 1.1). Section 1 commenced at 120W and each stage increased 30W every 3 min until blood lactate increased more than 2 mmol·L between stages (lactate threshold defined as the final point before the blood lactate concentration increased distinctly from the baseline concentration (Aunola and Rusko 1984). In the last 30 s of each stage, heart rate (Polar Electro, Finland) was recorded and a capillary blood sample (fingertip) was obtained for blood lactate concentration analysis by micro-assay (LactatePro LT-1710, ArkRay Inc., Kyoto, Japan). The reliability and validity of this device has been previously determined (Pyne, et al 2000). This initial stage was followed by a 10 min recovery period. Individual lactate responses were examined independently by two researchers to ensure validity and consistency of the analysis. The mean \pm SD lactate concentration at LT was 2.1 ± 0.4 mmol·L corresponding to an intensity of $52 \pm 6\%$ of PPO for LT which is typical of other studies utilising a similar protocol (Neal, et al 2013).

Participants commenced section 2, starting at an intensity of the penultimate stage of section 1, with each stage lasting 1 min and increased by 30 W until volitional exhaustion. The end time and power output of the stage was used to calculate peak power output (PPO) using the following equation (Kuipers, et al 1985):

$$PPO = W_{final} + ([t/60] \cdot PI)$$

Where, W_{final} = the power output of the final completed stage in (watts), t = the time spent in the final uncompleted stage (seconds), 60 = the duration of each stage (seconds) and PI = the increase in power output between each stage (W). Maximal oxygen uptake ($\dot{V}O_{2max}$) was also measured during this protocol via an automated online gas analysis

machine (Oxycon Pro, Jaeger, Wuerzberg, Germany). $\dot{V}O_{2\max}$ was determined as the highest average $\dot{V}O_2$ captured in a 30 sec period.

To ensure participants were exercising at the correct intensity a 30 min ride was completed at 95% of lactate threshold following 20 min of recovery from the peak power output test. Additional finger prick lactate samples were obtained at 20 and 30 min to ensure lactate values were not drifting up over time.

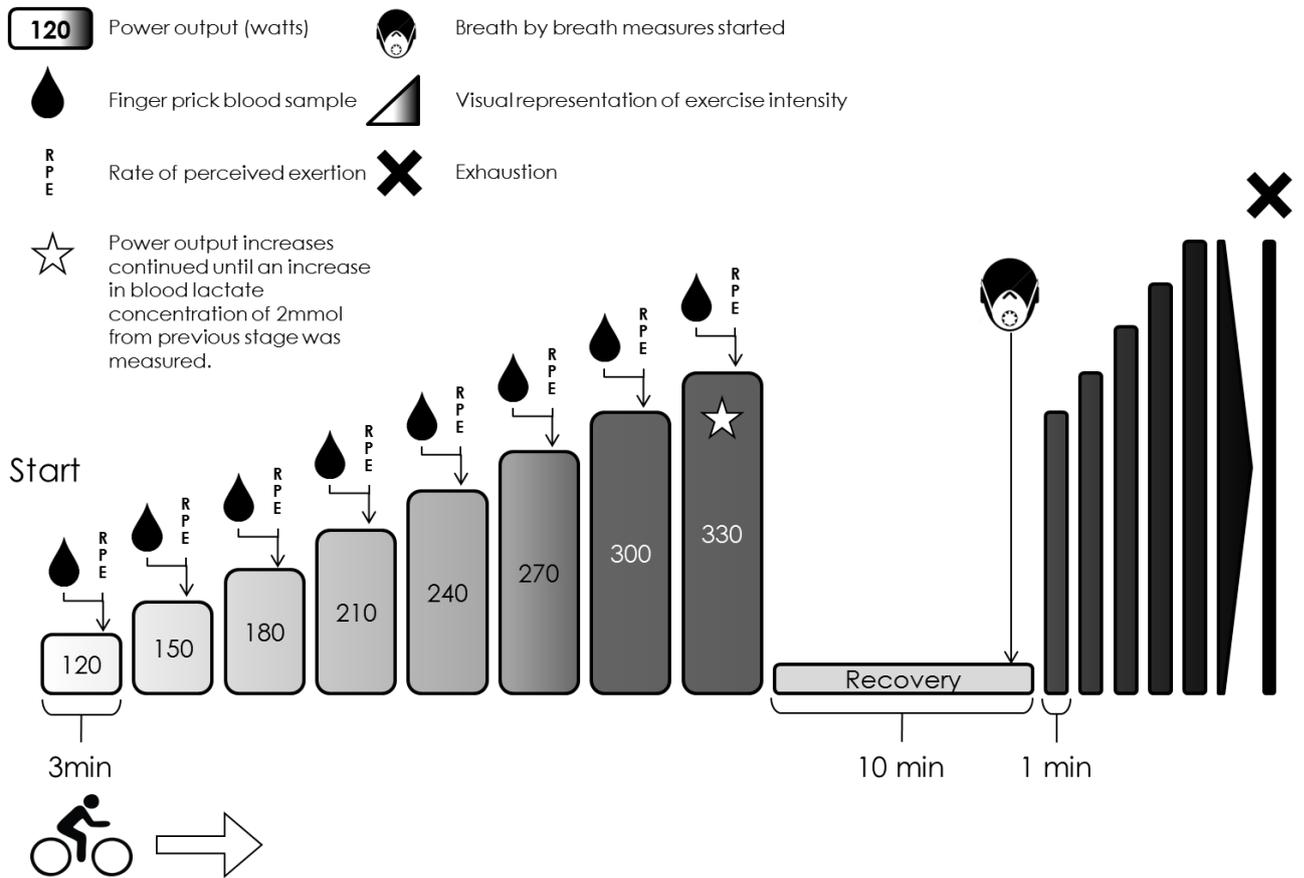


Figure 1.2 Protocol for the 2 stage ramp up test for the determination for lactate threshold, $\dot{V}O_{2max}$ and peak power output.

1.10 PRE TRIAL NUTRITIONAL AND EXERCISE CONTROLS

Participants were provided with a 2 day food diary and asked to record their habitual dietary intake for 2 days prior to the full familiarisation visit. Before each experimental trial visit they were asked to replicate this dietary intake as closely as possible. In addition, participants were asked to record their training activity 48hrs prior to any laboratory visit and to completely refrain from exercise in the 24h period prior to entering the lab.

1.11 FAMILIARISATION AND EXPERIMENTAL TRIALS

On arrival to the laboratory participants emptied bladder and bowel prior to nude body mass measurements. Individuals then changed into cycling attire which was kept consistent throughout all trials to reduce thermoregulatory variability. Participants were cannulated and a primed continuous infusion started (Chapter 3) and a series of neuromuscular measures were performed (Chapter 4). Participants then completed a 2 h submaximal ride at 95% LT (185 ± 25 W) during which one of four beverages (described in full below) were consumed. Each beverage was provided with an initial bolus ingestion of 240 mL two minutes prior to the start of exercise. Subsequently, 220 mL was consumed every 15 min with the final drink provided at 120 min of exercise. Following the 2 h ride, a 5 min recovery period allowed a toilet break and for the equipment to be set up for the performance task. The performance task was a work target simulated time trial specific to the individual (531 ± 48 KJ). A linear factor, $70\% W_{\max}$ divided by preferred cadence (rpm^2), was entered into the cycle ergometer.

The formula used to determine the work target value was:

$$\text{Work target} = (0.7 \cdot \text{PPO}) \cdot 1800$$

The time trial protocol employed has previously been validated and has been shown to be highly reliable (Jeukendrup, et al 1996). Participants were informed of the total amount of work they needed to complete as fast as possible at the start of each time trial. A computer monitor was positioned in front of the participant so they could see how much work they had done at any moment (Figure 2.4). No verbal encouragement or instruction was provided during the time trial task.

1.12 GLUCOSE SOLUTIONS

During the preload ride one of four beverages were consumed: 0% water (familiarisation and control); 2.0%; 3.9%; or 6.4% glucose (single carbohydrate) based, commercially available, CHO beverage. All beverages were maintained at 10°C and were consumed at a rate of 1 L·h⁻¹ providing 0, 20, 39 and 64 g·h⁻¹ of CHO respectively. The 20 g·h⁻¹ solution contained 37 mg of sodium per 100 mL with the 39 and 64 g·h⁻¹ solutions both containing 50 mg per 100 mL. Each beverage was provided with an initial bolus ingestion of 240 mL two minutes prior to the start of exercise. Subsequently, 220 mL was consumed every 15 min with the final drink provided at 120 min of exercise.

CHAPTER 2

THE INGESTION OF 39 OR 64 G·H⁻¹ OF CARBOHYDRATE IS EQUALLY EFFECTIVE AT IMPROVING ENDURANCE EXERCISE PERFORMANCE IN CYCLISTS.

2.1 ABSTRACT

In an investigator-blind, randomised cross-over design, 20 male well-trained cyclists completed four experimental trials. Each trial consisted of a 2h constant load ride ($185 \pm 25\text{W}$) followed by a work-matched time trial task (~ 35 min). Three commercially available CHO beverages, plus a control (water), were administered during the 2h ride providing 0, 20, 39 or $64 \text{ g CHO}\cdot\text{h}^{-1}$ at a fluid intake rate of $1 \text{ L}\cdot\text{h}^{-1}$. Performance was assessed by time to complete the time trial task, mean power output sustained, and pacing strategy used. Mean task completion time (min:sec \pm SD) for $39 \text{ g}\cdot\text{h}^{-1}$ ($34:19.5 \pm 03:07.1$, $p=0.006$) and $64 \text{ g}\cdot\text{h}^{-1}$ ($34:11.3 \pm 03:08.5$ $p=0.004$) of CHO were significantly faster than control ($37:01.9 \pm 05:35.0$). The mean percentage improvement from control was -6.1% (95% CI: -11.3 to -1.0) and -6.5% (95% CI: -11.7 to -1.4) in the 39 and $64 \text{ g}\cdot\text{h}^{-1}$ trials respectively. The $20 \text{ g}\cdot\text{h}^{-1}$ ($35:17.6 \pm 04:16.3$) treatment did not reach statistical significance compared to control ($p = 0.126$) despite a mean improvement of -3.7% (95% CI -8.8 to 1.5%). Mean power output differences mirrored the performance time. There was no interaction between CHO dose and pacing strategy. 39 and $64 \text{ g}\cdot\text{h}^{-1}$ of CHO were similarly effective at improving endurance cycling performance compared to a $0 \text{ g}\cdot\text{h}^{-1}$ control in our well trained cyclists.

2.2 INTRODUCTION

Carbohydrate (CHO) intake during exercise has consistently been shown to improve exercise performance (Smith, et al 2010; Smith, et al 2013) and extend exercise capacity (Galloway and Maughan 2000; Watson, et al 2012). CHO is thought to act in many ways to enhance performance: sparing of muscle glycogen (Bjorkman, et al 1984; Stellingwerff, et al 2007); enhancing and maintaining elevated CHO oxidation rate; maintenance of blood glucose concentration (Coyle, et al 1986); elevated exogenous CHO oxidation rate (Galloway, et al 2001); and central and peripheral neural up-regulation (Carter, et al 2004; Chambers, et al 2009; Nikolopoulos, et al 2004). As a result, CHO feeding strategies are now widely employed in the exercise setting as a means to support athletic performance.

Although the provision of CHO has been shown to improve exercise performance/capacity, the optimal dose of CHO required to maximise athletic performance remains a topic of debate. Currently, guidelines from the ACSM state an optimal dose of CHO during exercise to be within the range of 30 – 60 g·h⁻¹. However, significant improvements in performance and exercise capacity have been reported with ingestion rates as low as 22 g·h⁻¹ (Maughan, et al 1996; Galloway and Maughan 2000) and as high as >100 g·h⁻¹ (Currell and Jeukendrup 2008) highlighting a beneficial impact of CHO ingestion over a much broader range of feeding rates, when compared with water or placebo solutions. Smith et al (2010) indicated that 15, 30 and 60 g·h⁻¹ were all ‘very likely’ to improve power output sustained during a 20km TT when compared to a 0 g·h⁻¹ placebo, with 60 g·h⁻¹ providing the largest effect. Furthermore, 30 g·h⁻¹ was ‘very unlikely’ to further improve performance over 15 g·h⁻¹ whilst 60 g·h⁻¹ was ‘likely’ to improve performance over the 30 g·h⁻¹. However, following post hoc power calculations, the authors indicated that a sample size of 15 to 22 was required to confidently conclude there were no differences in performances across the three doses. In contrast Watson et al (2012) reported no further improvements in time to exhaustion when feeding a 6% (47 g·h⁻¹

¹) mixed CHO solution when compared to a 2% (26 g·h⁻¹) mixed solution. The absence of an additional improvement with the higher CHO dose is surprising considering the improvements in performance reported with higher ingestion rates (Smith, et al 2010). As such, it seems a range of CHO feeding doses increases performance over a 0 g·h⁻¹ condition. However, any additional increases in CHO provision above feeding rates of ~30 g·h⁻¹ do not appear to have a clear significant improvement on performance.

To provide clarity to the optimal dose of CHO for performance additional studies with greater sample sizes have followed up these initial reports. In a recent study Smith et al (2013) expanded on these data and examined fifty five participants spread across four sites. The participants consumed CHO during a 2h submaximal ride followed by a 20km TT task. Each participant completed 4 trials, one placebo and three CHO treatments, between 10 and 120 g·h⁻¹ (10 g·h⁻¹ increments) which consisted of a 1:1:1 ratio of glucose, fructose and maltodextrin. Following some statistical modelling of their data the authors reported an optimal dose of 78 g·h⁻¹ for performances during the TT. However, they reported only a small 1.7% improvement in performance from 30 to 80 g·h⁻¹, and a rather trivial 0.7% improvement in performance from 40 to 80 g·h⁻¹. In addition, the linear regression model used for the determination of the optimal feeding strategy utilised was not significant. Taken together, these studies suggest increasing amounts of CHO result in diminishing returns with respect to performance gains.

These data, coupled with those of Smith et al (2010) and Watson et al (2012), indicate a divide between the optimal feeding rates and compositions reported by investigators and the subsequent measurable and meaningful improvement in performance obtained from increasing amounts of CHO, particularly in the 30 to 60 g·h⁻¹ range. Similarly, the range of responses reported across the feeding rates provided in these studies highlights considerable individual variability. Accordingly, the aim of the current study was to determine the dose response relationship between CHO feeding and exercise

performance in the 0 to 64 g·h⁻¹ range in 20 male cyclists. Furthermore, pacing strategies used during a performance task were investigated to provide insight as to where any changes in performance may be the greatest.

2.3 METHODOLOGY

2.3.1 Participants

Participants described in section 1.7 of this thesis completed all testing described.

2.3.2 Experimental design

In a double blind, placebo controlled, randomised cross-over study design participants visited the laboratory 6 times (2 preliminary and 4 intervention) over a six week period (Figure 1.1). They completed one visit per week commencing each trial on the same day at the same time of day on each visit. Following pre-screening, participants completed a preliminary assessment where lactate threshold, $\dot{V}O_{2max}$, and peak power output were determined and for the first familiarisation of the performance task to be used in subsequent visits. On the second visit participants completed a full familiarisation trial. The familiarisation trial and four subsequent intervention trials involved a 120 min steady submaximal cycle at 95% lactate threshold (184 ± 25 W) followed by a time trial performance task, whereupon the participants were instructed to be complete their set work target as quickly as possible. Water was ingested for the familiarisation trial and consumed at a rate of 1 L·h⁻¹. Thereafter, on the intervention trials participants consumed in a random order either: a control (water) 0%, 2%, 3.9% or 6.4% CHO solutions at a fluid ingestion rate of 1 L·h⁻¹. Performance was determined as the time to complete a work matched simulated time trial task designed to last ~30 min. Pacing strategy was assessed from taking time splits and average power output sustained for each 10% of work completed during the performance task (Figure 2.1).

2.3.3 Preliminary testing

On week 1 of 6, following a 10 h overnight fast, participants performed a two section incremental cycle test (Lode Excalibur Sport, Netherlands) to determine maximal oxygen uptake ($\dot{V}O_{2max}$, lactate threshold, and peak power output (Figure 1.2). Section 1 commenced at 120 W and each stage increased 30W every 3 min until blood lactate increased more than 2 mmol·L between stages (lactate threshold defined as the final point before the blood lactate concentration increased distinctly from the baseline concentration (Aunola and Rusko 1984). In the last 30 s of each stage, heart rate (Polar Electro, Finland) was recorded and a capillary blood sample (fingertip) was obtained for blood lactate concentration analysis by micro-assay (LactatePro LT-1710, ArkRay Inc., Kyoto, Japan). The reliability and validity of this device has been previously determined (Pyne, et al 2000). This initial stage was followed by a 10 min recovery period. Individual lactate responses were examined independently by two researchers to ensure validity and consistency of the analysis. The mean \pm SD lactate concentration at LT was 2.1 ± 0.4 mmol·L corresponding to an intensity of $52 \pm 6\%$ of PPO for LT which is typical of other studies utilising a similar protocol (Neal, et al 2013).

Participants commenced section 2, starting at an intensity of the penultimate stage of section 1, with each stage lasting 1 min and increased by 30 W until volitional exhaustion. The end time and power output of the stage was used to calculate peak power output (PPO) using the following equation (Kuipers, et al 1985):

$$PPO = W_{final} + ([t/60] \cdot PI)$$

Where, W_{final} = the power output of the final completed stage in (watts), t = the time spent in the final uncompleted stage (seconds), 60 = the duration of each stage (seconds) and PI = the increase in power output between each stage (W). Maximal oxygen uptake ($\dot{V}O_{2max}$) was also measured during this protocol via an automated online gas analysis

machine (Oxycon Pro, Jaeger, Wuerzerberg, Germany). $\dot{V}O_{2\max}$ was determined as the highest average $\dot{V}O_2$ captured in a 30 sec period.

To ensure participants were exercising at the correct intensity a 30 min ride was completed at 95% of lactate threshold following 20 min of recovery from the peak power output test. Additional finger prick lactate samples were obtained at 20 and 30 min to ensure lactate values were not drifting up over time.

2.3.4 Familiarisation and experimental trials

On arrival to the laboratory participants emptied bladder and bowel prior to nude body mass measurements. Individuals then changed into cycling attire which was kept consistent throughout all trials to reduce thermoregulatory variability. Participants then completed a 2 h submaximal ride at 95% LT (185 ± 25 W) during which one of four beverages were consumed: 0% water (familiarisation and control); 2.0%; 3.9%; or 6.4% glucose (single carbohydrate) based commercially available CHO beverage. All beverages were maintained at 10°C and were consumed at a rate of $1\text{L}\cdot\text{h}^{-1}$ providing 0, 20, 39 and $64\text{g}\cdot\text{h}^{-1}$ of CHO respectively. The $20\text{g}\cdot\text{h}^{-1}$ solution contained 37 mg of sodium per 100 mL and the 39 and $64\text{g}\cdot\text{h}^{-1}$ solutions both contained 50 mg per 100 mL. Each beverage was provided with an initial bolus ingestion of 240 mL two minutes prior to the start of exercise. Subsequently, 220 mL was consumed every 15 min with the final drink provided at 120 min of exercise. Following the 2 h ride, a 5 min recovery period allowed a toilet break and for the equipment to be set up for the performance task. The performance task was a work target simulated time trial specific to the individual ($531 \pm 48\text{KJ}$). A linear factor, $70\% W_{\text{max}}$ divided by preferred cadence (rpm^2), was entered into the cycle ergometer. The formula used to determine the work target value was:

$$\text{Work target} = (0.7 \cdot \text{PPO}) \cdot 1800$$

The time trial protocol employed has previously been validated and has been shown to be highly reliable (Jeukendrup, et al 1996). Participants were informed of the total amount of work they needed to complete as fast as possible at the start of each time trial. A computer monitor was positioned in front of the participant so they could see how much work they had done at any moment (Figure 2.2). No verbal encouragement or instruction was provided during the time trial task.

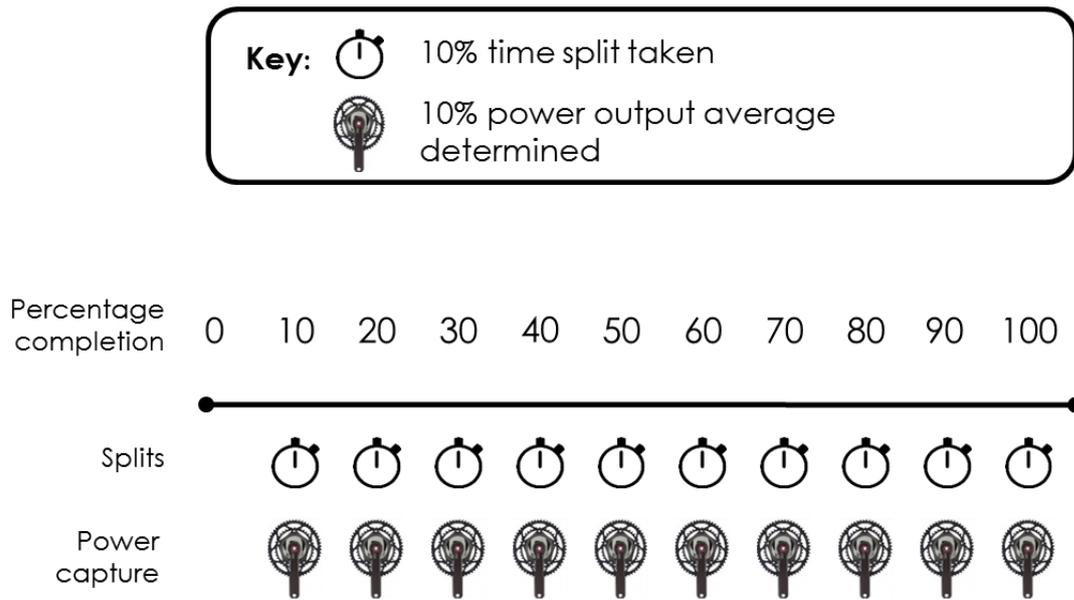


Figure 2.1 Protocol to indicate how the pacing data was obtained from the time trial task.

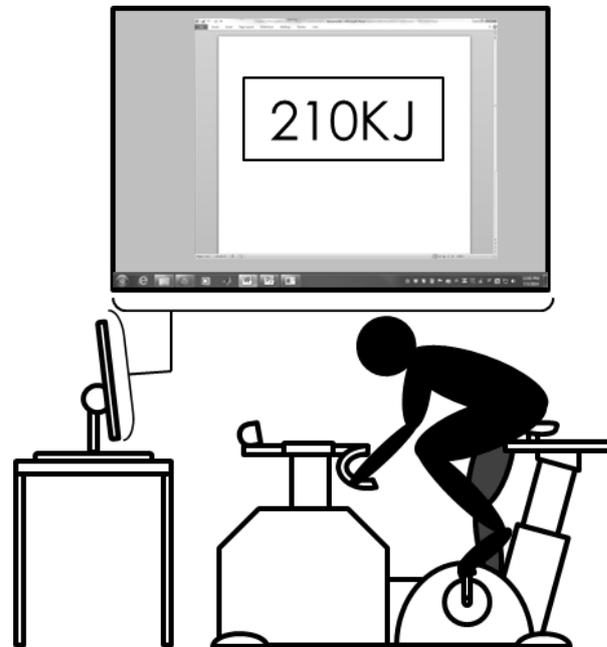


Figure 2.2 Visual representation of the time trial task set-up. The participant was positioned in front of a computer monitor so that they could see the total amount of work they had completed on the screen. Participants were informed of the total amount of work they had to complete at the start of each time trial task.

2.3.5 Data presentation and statistical analysis

All data are presented as mean (\pm SD) unless otherwise stated. Total time to complete the performance task and average power output sustained throughout were compared across all trials. The magnitude of difference from the water control was examined with a one-way ANOVA with Dunnet's post hoc comparisons made. The mean differences between two variables are presented as the mean with associated 95% confidence limits and Cohen's size effects (mean difference; confidence intervals; Cohen's size effects). Cohen's sizes effects can be interpreted as 0.2 = small, 0.6 = moderate, 1.2 = large, 2.0 = very large and 4.0 = extremely large. Performance task time and average power output was compared between treatments using repeated measures regression models. The null hypothesis of no differences between any of the treatments groups was tested using ANOVA with all values compared back to the water control condition. A difference from the control of 3.5% in either time to complete the task or mean power output sustained was considered a large and meaningful difference.

2.4 RESULTS

2.4.1 Performance time and mean power output

Mean task completion time (min:sec \pm SD) for 39 g·h⁻¹ (34:19.5 \pm 03:07.1, $p < 0.01$) and 64 g·h⁻¹ (34:11.3 \pm 03:08.5, $p < 0.01$) CHO solutions were significantly faster than control (37:01.9 \pm 05:35.0) (Figure 2.3). Corresponding percentage change from the 0 g·h⁻¹ condition was similar at 6.1% (95% CI 1 to 11.3%; $p = 0.02$) for the 39 g·h⁻¹ trial, and 7% (95% CI 1 to 12%, $p = 0.01$) for the 64 g·h⁻¹ trial (Figure 2.4). The 20 g·h⁻¹ (35:17.6 \pm 04:16.3) treatment did not reach statistical significance compared to control ($p = 0.13$) despite a mean improvement of 3.7% (95% CI -1.5 to 8.8%). The Cohen's size effect in comparison to the control was 0.6 (95% CI -0.1 to 1.4), 1.0 (95% CI 0.2 to 1.7), and 1.0 (95% CI 0.3 to 1.8) for 20, 39 and 64 g·h⁻¹ treatments respectively indicating moderate and large effects on performance improvement.

In conjunction, there was a significant effect of treatment on mean power output sustained during the time trial between the four experimental trials ($p < 0.01$). There were significant increases of 17W (95% CI 5-30; $p < 0.01$) and 19W (95% CI 6-31; $p < 0.01$) in mean power output sustained throughout the 39 g·h⁻¹ and 64 g·h⁻¹ treatments, respectively (Figure 2.5). Corresponding percentage improvements compared to the 0 g·h⁻¹ trial were similar at 8% (95% CI 1-15%; $p = 0.02$) for the 39 g·h⁻¹ trial, and 9% (95% CI 2-16%; $p = 0.01$) for the 64 g·h⁻¹ trial. There was no statistical difference reported between the 20 g·h⁻¹ treatment and the 0 g·h⁻¹ control ($p = 0.12$) despite a 5.7% (95% CI: -1.2 to 12.6) mean increase in power output sustained. The Cohen's size effect compared to the control was 0.7 (-0.1 to 1.4), 1.1 (0.3 to 1.8), and 1.1 (0.4 to 1.9) for 20, 39 and 64 g·h⁻¹ reflecting moderate and large effects respectfully (Figure 2.6).

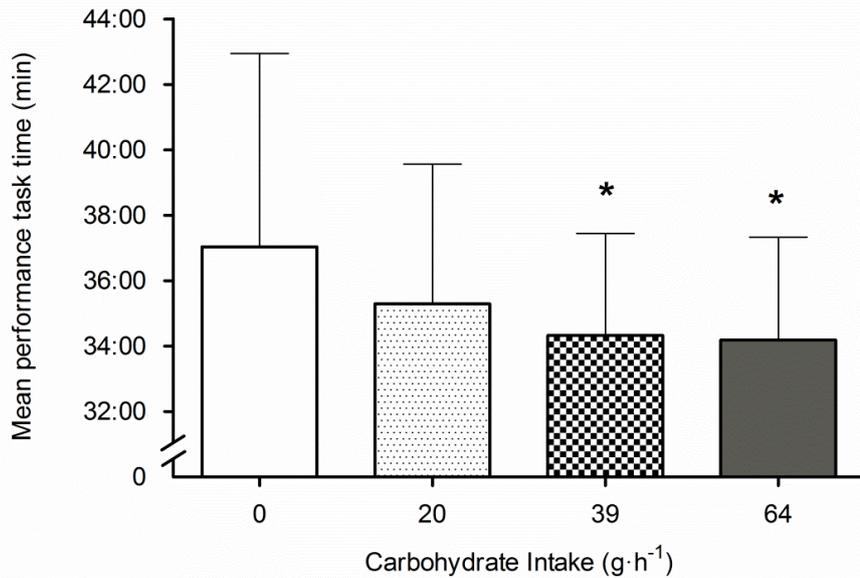


Figure 2.3. Mean performance task time when 0, 20, 39, and 64 g·h⁻¹ of carbohydrate were consumed. Data presented as mean ± standard deviation. Consuming 39 and 64 g·h⁻¹ of CHO resulted in a significantly (*p < 0.05) quicker time trial than 0 g·h⁻¹

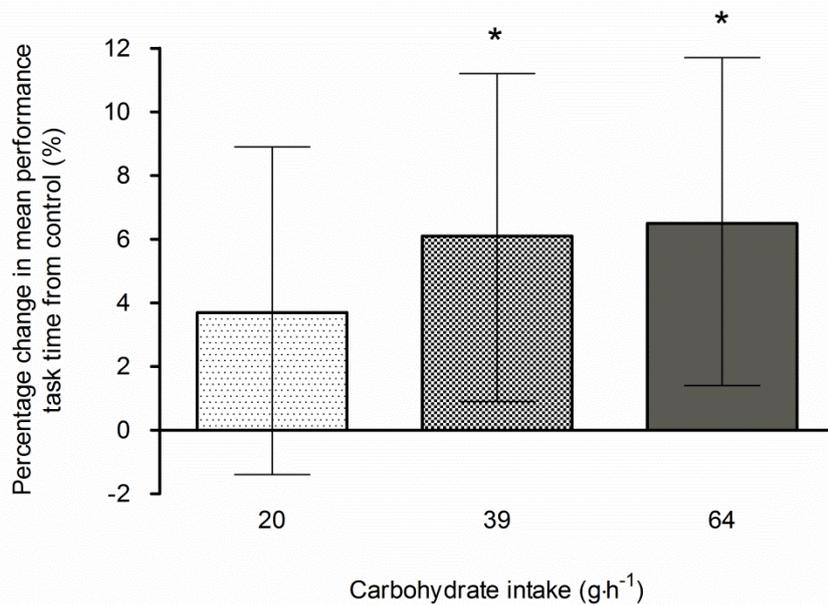


Figure 2.4. Percentage change in mean performance task time from the 0 g·h⁻¹ treatment for 20, 39, and 64 g·h⁻¹ carbohydrate ingestion rates. The percentage change in performance task time was significantly greater in the 39 g·h⁻¹ and 64 g·h⁻¹ trials when compared to 0 g·h⁻¹ (*p < 0.05). Data presented as mean ± 95% confidence intervals.

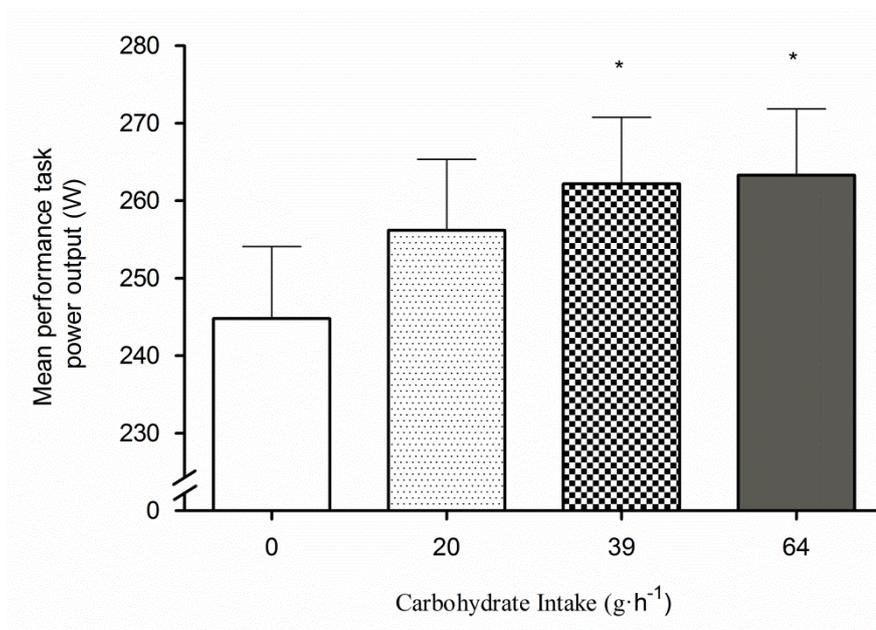


Figure 2.5. Mean performance task power output when 0, 20, 39, and 64 g·h⁻¹ of carbohydrate were consumed. Data presented as mean ± standard deviation. Consuming 39 and 64 g·h⁻¹ of CHO resulted in a significantly (*p < 0.05) higher power output than 0 g·h⁻¹.

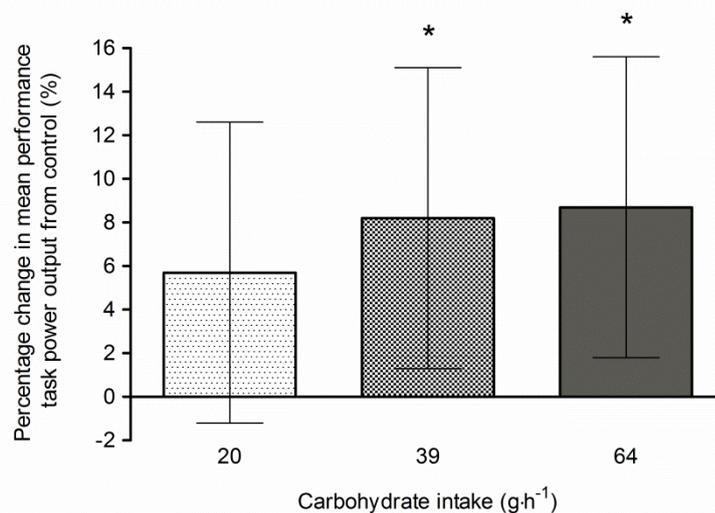


Figure 2.6. Percentage change in mean performance task power output from the 0 g·h⁻¹ treatment for 20, 39, and 64 g·h⁻¹ carbohydrate ingestion rates. The percentage change in power output was significantly greater in the 39 g·h⁻¹ and 64 g·h⁻¹ trials when compared to 0 g·h⁻¹ (*p < 0.05). Data presented as mean ± 95% confidence intervals.

2.4.2 Pacing strategy

The assessment of pacing strategy revealed no interaction between time and treatment ($p=0.80$). This suggests no evidence of any differences in the slopes of the lines between the treatments in the incremental trends of performance time or mean power sustained (Figure 2.7).

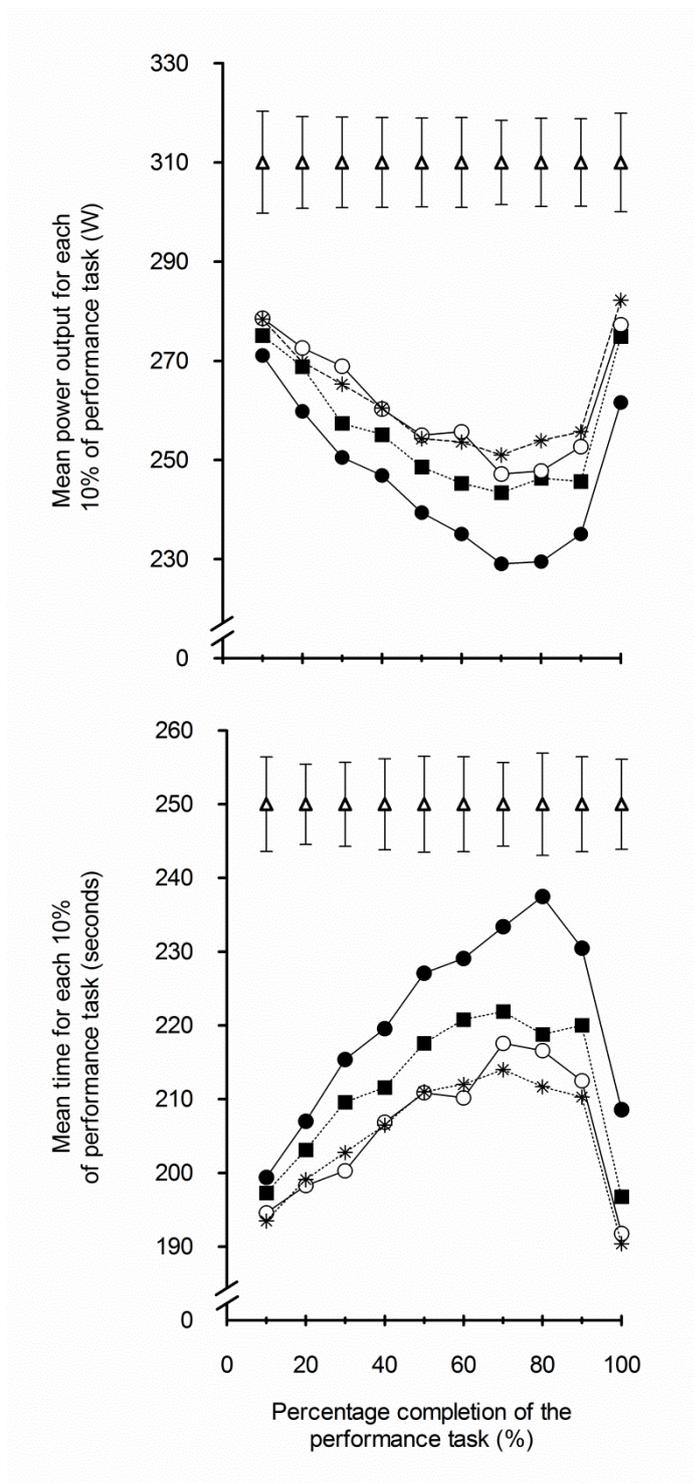


Figure 2.7. Mean power output (A) and time to complete each 10% of the performance task (B) when 0 g·h⁻¹ (-●-), 20 g·h⁻¹ (-■-), 39 g·h⁻¹ (-○-), and 64 g·h⁻¹ (-*-) of carbohydrate was consumed. Data presented as means with the pooled standard error of the mean (open triangle) on an arbitrary value of 310 (Power) and 250 (Time) for ease of viewing.

2.5 DISCUSSION

This study was designed to determine the optimal dose of CHO to maximise endurance exercise performance. We show that CHO provided at rates of 39 and 64 g·h⁻¹ were equally effective at improving performance in 20 well trained male participants compared to a 0 g·h⁻¹ water control. The 20 g·h⁻¹ treatment did not, on average, show evidence of a significant improvement in participants' performance, despite demonstrating a meaningful mean improvement in both performance task time and mean power output over the 0 g·h⁻¹ treatment. As such, our data demonstrate that consuming CHO at rates between 39 to 64 g·h⁻¹ is likely to be optimal for most individuals looking to utilise a single source CHO as an ergogenic aid during endurance performances lasting less than 3hrs.

Previous studies investigating a dose-response relationship between CHO feeding and endurance exercise performance/capacity have reported somewhat conflicting results. Smith et al (2010) provided evidence of a dose-response relationship when feeding glucose in the range of 15 to 60 g·h⁻¹. These authors showed that all trials significantly improved performance of 12 cyclists over the placebo condition, with only the 60 g·h⁻¹ 'likely' to improve performance over the 15 g·h⁻¹. However, the authors highlighted that 15-22 participants would be required to make meaningful comparisons between solutions, leaving no clear picture into the optimal dose of CHO. In a follow up investigation, Smith et al (2013) reported that optimal performance gains with CHO ingestion were likely to occur at rates as high as 78 g·h⁻¹ when consuming multiple forms of CHO. However, the optimal dose for the greatest improvement in performance was unclear in the 40 to 80 g·h⁻¹ range and interpretation is limited by the choice of study design. In contrast, Watson et al (2012) observed no further improvement in exercise capacity when 46 g·h⁻¹ was consumed compared to 31 g·h⁻¹ during prolonged exercise in cool conditions. We add to these data by demonstrating that no further substantial performance gains over a 0 g·h⁻¹

water control are observed when CHO is consumed at $64 \text{ g}\cdot\text{h}^{-1}$ compared with $39 \text{ g}\cdot\text{h}^{-1}$. As such, these results support the hypothesis that a ceiling in performance gains exists when consuming CHO above $40 \text{ g}\cdot\text{h}^{-1}$ during exercise $< 3 \text{ h}$. However, any mechanistic explanation for the outcome would only be speculative due to the limited measures taken throughout the trial: though increased neural drive through oral sensors in the mouth; better maintenance of blood glucose due to greater exogenous glucose availability; enhanced maintenance of exogenous glucose oxidation; and endogenous glycogen sparing; are all potential explanations.

Consuming $20 \text{ g}\cdot\text{h}^{-1}$ of CHO in the present study had a less easily interpretable outcome. When participants consumed $20 \text{ g}\cdot\text{h}^{-1}$ performance did not significantly improve over the water control, while 39 or $64 \text{ g}\cdot\text{h}^{-1}$ of CHO did not significantly differ compared to $20 \text{ g}\cdot\text{h}^{-1}$. Other investigations have reported a significant improvement in performance and/or exercise capacity with quite modest ($\sim 15 \text{ g}\cdot\text{h}^{-1}$) amounts of CHO when compared to a $0 \text{ g}\cdot\text{h}^{-1}$ condition (Galloway and Maughan 2000; Karelis, et al 2010; Maughan, et al 1996; Murray, et al 1989). Consuming $20 \text{ g}\cdot\text{h}^{-1}$ in the present study still produced a mean improvement in performance time of 3.7% compared to $0 \text{ g}\cdot\text{h}^{-1}$, which corresponds to a $\sim 58 \text{ sec}$ reduction in time trial task time. The variance in response is a likely explanation for lack of statistical significance, but it is noteworthy that there is considerable variation in performance responses in all CHO conditions, not just at the $20 \text{ g}\cdot\text{h}^{-1}$. Additionally, some individuals ($n=2$) did not respond positively to any of the CHO ingestion trial, with the control condition being the fastest trial completed. The variability in performances, along with some negative responses to CHO ingestion, highlights the individual nature of CHO feeding as an ergogenic aid.

The range of responses measured in the present study highlights that, for the majority of individuals, there is a ceiling in the performance gains achieved when feeding rates are higher than 40 g.h⁻¹. Any additional performance gains reported appear to result in a minimal increase in performance. However, in elite level athletes, there is evidence there is an enhanced ability to utilise CHO and have a subsequent meaningful improvement in performance (Stellingwerff 2012). In support of this enhanced intake Prof. Louise Burke (personal communication) has recently presented a case study describing a nutritional intervention which enabled an Olympic walker to ingest as much as 90 g.h⁻¹ of multiple transportable CHO. Thus, when providing feeding recommendations, the degree to which an increase in performance translates into a worthwhile change should be considered.

One potential limitation of the current investigation is that participants completed the trial following an overnight fasted to best control and replicate the metabolic state in which they arrive at the laboratory. Overnight fasting is not the current practice for optimal performance for athletes as liver glycogen is reduced following glycogen breakdown in the liver to maintain blood glucose concentrations overnight. However, the glycogen storage capacity of the liver is enhanced following endurance training therefore reducing the impact an overnight fast has on liver glycogen content. Casey et al (2000) reported athletes had an overnight liver glycogen content of 386 mmol.L⁻¹, which is considerably higher than values reported in healthy untrained individuals (~120 to 210 mmol.L⁻¹) (Taylor, et al 1996; Stadler, et al 2013; Magnusson, et al 1992). Therefore, the liver glycogen content of athletes following an overnight fast is unlikely to vastly affect subsequent exercise performance. In studies examining the effect of CHO on performance following a shorter (~3 h) fast, where liver glycogen content is unlikely to be compromised, Hulston and Jeukendrup (2009) reported a significant improvement in performance when consuming a CHO beverage compared to water. Additionally, a recent

meta-analysis indicated the pre exercise nutritional status of participants (fed or fasted) appears to have no effect on the subsequent exercise performance/capacity achieved (Temesi, et al 2011). As such, the findings of this study are still likely to be applicable to those looking to perform in the fed state.

The current investigation only measured performance responses following feeding rates up to $64 \text{ g}\cdot\text{h}^{-1}$ and we are therefore unable to determine responses to higher feeding rates. The upper feeding rate was based on research showing a maximal absorption rate of $\sim 1 \text{ g}\cdot\text{min}^{-1}$ of a single source CHO solution (Jeukendrup, et al 1999). Nevertheless, we cannot be certain that CHO feeding rates above $64 \text{ g}\cdot\text{h}^{-1}$ does not significantly alter subsequent performances as others have reported (Currell and Jeukendrup 2008; Smith, et al 2013). The lack of any further substantial improvement in performance with rates $>39 \text{ g}\cdot\text{h}^{-1}$ in the present study, in addition to reports of a negative impact on performance with higher rates of CHO ingestion, suggests that performance is unlikely to improve with higher rates of single source CHO ingestion during exercise $< 3 \text{ h}$. Future studies should focus on utilising measures and techniques to try and ascertain explanations as to why some feeding rates are more beneficial than others, and which factors contribute to individual variability in response.

2.6 CONCLUSION

The $39 \text{ g}\cdot\text{h}^{-1}$ and $64 \text{ g}\cdot\text{h}^{-1}$ CHO solutions were equally effective in improving the cycling performance of 20 well trained male cyclists over a $0 \text{ g}\cdot\text{h}^{-1}$ water placebo following a 2 h submaximal ride. For most well trained individuals, an optimal feeding rate for maximising the ergogenic effect of CHO for endurance performance is likely to occur at around $40 \text{ g}\cdot\text{h}^{-1}$. There is a wide range of responses to all rates of CHO ingested highlighting the individual nature of the responses observed in individuals using CHO to aid performance.

However, the results of this study highlight that most individuals will respond most positively to CHO ingestion rates around 39 and 64 g·h⁻¹.

CHAPTER 3

METABOLIC RESPONSES DURING
ENDURANCE EXERCISE WITH THE
INGESTION OF VARYING DOSES OF
CARBOHYDRATE

3.1 ABSTRACT

The ingestion of carbohydrate (CHO) during exercise has repeatedly been reported to improve endurance exercise performance and extend exercise capacity. Ingestion rates between 20 and 64 g·h⁻¹ have consistently led to the greatest performance gains compared to a 0 g·h⁻¹ control as confirmed in Chapter 2. Performance gains tend to diminish with ingestion rates above 40 – 60 g·h⁻¹ when consumed during activities <3 h in duration. We aimed to utilise stable isotopes and blood metabolite profiles to examine the integrated physiological responses to CHO ingestion when ingested at rates throughout the range where performance gains appear greatest. In a double-blind, randomised cross-over design, well-trained male cyclists (n=20, mean±SD, age 34 ± 10 y, mass 75.8 ± 9 kg, peak power output 394 ± 36 W, $\dot{V}O_{2max}$ 62 ± 9 ml·kg⁻¹·min⁻¹) completed two familiarisation trials then four experimental trials. Each trial involved a 2 h constant load ride (95% lactate threshold, 185 ± 25 W) where one of three CHO beverages, or a control (water), were administered every 15 min, providing participants with 0, 20, 39 or 64 g CHO·h⁻¹ at a fixed fluid intake rate of 1 L·h⁻¹. Dual glucose tracer techniques were used to determine glucose kinetics and exogenous carbohydrate oxidation (EXO) during exercise. Blood samples were obtained every 15 min. Endogenous CHO contribution was suppressed in the second hour of exercise when consuming 39 and 64 g·h⁻¹ (-7.3%, 95%CI: -1.6 to -13.1 and -11.2%, 95%CI: -5.5 to -16.9 respectively) in comparison to 0 g·h⁻¹. Additionally, consuming 64 g·h⁻¹ suppressed the endogenous CHO contribution by -7.2% (95%CI: -1.5 to -13.0) compared to the 20 g·h⁻¹ treatment. Exogenous CHO oxidation rate increased by 0.13 g·min⁻¹ (95%CI: 0.10 to 0.15) and 0.29 g·min⁻¹ (95%CI: 0.27 to 0.31) when consuming 39 and 64 g·h⁻¹ in comparison to 20 g·h⁻¹ of CHO. Peak exogenous CHO oxidation rates were 0.34 (± 0.06), 0.54 (± 0.09) and 0.78 (± 0.19) g·min⁻¹ for 20, 39 and 64 g·h⁻¹ respectively. NEFA concentration was 0.10 (95%CI: 0.07 to 0.13), 0.12 (95%CI: 0.10 to 0.16) and 0.16 (95%CI: 0.13 to 0.19) mmol.L⁻¹ higher when

consuming 0 g·h⁻¹ in comparison to 20, 39 and 64 g·h⁻¹ respectively. Both 39 and 64 g·h⁻¹ were effective at sparing endogenous CHO stores but the measured response was highly variable between individuals. Consuming 39 g·h⁻¹ of CHO appears to be the minimum ingestion rate required to have a significant metabolic effect that results in an increase in performance.

3.2 INTRODUCTION

In Chapter 2 we observed that consuming 39 and 64 g·h⁻¹ of CHO was equally effective at increasing endurance performance of 20 trained cyclists in comparison to a 0 g·h⁻¹ control. However, there were no metabolic measures presented that could explain why:

1. 39 and 64 g·h⁻¹ were equally effective at improving endurance performance
2. There was no additional increase in performance when consuming 64 vs 39 g·h⁻¹
3. There was no increase in exercise performance when consuming 20 g·h⁻¹ of carbohydrate in comparison to the 0 g·h⁻¹ control
4. There was no clear dose response relationship between CHO ingestion and exercise performance.

During prolonged steady state exercise endogenous glycogen stores and circulating plasma glucose are key substrates for energy provision. Fatigue is often reported to coincide with the depletion of endogenous CHO stores and the dysregulation of circulating plasma glucose concentration (Coyle, et al 1986; Coggan and Coyle 1987; Nybo 2003). Ingesting CHO improves performance and extends exercise duration via a range of proposed mechanisms including: better maintenance of circulating plasma glucose (Coyle, et al 1986), higher rates of exogenous (Smith, et al 2010) and total CHO oxidation, and endogenous glycogen sparing (Jeukendrup, et al 1999). These proposed mechanisms do not occur in isolation but occur together facilitating force production and improving performance and exercise capacity.

Early research indicated that CHO provision during exercise improved performance and extended exercise capacity by maintaining blood glucose concentration that then drove fuel utilisation towards higher rates of CHO oxidation. Coyle et al (1986) reported that feeding CHO maintained blood glucose concentration and CHO oxidation rates were better maintained and in turn exercise capacity increased significantly in comparison to a

water control. In a follow up study (Coggan and Coyle 1987) participants exercised to exhaustion and were then provided with either no CHO, ingested CHO, or infused CHO. Both CHO provision conditions increased exercise duration on commencement of exercise in comparison to no CHO. However, only the infusion condition maintained blood glucose concentration sufficiently to subsequently extend exercise duration above that of the CHO ingestion trial. The authors concluded that the maintenance of blood glucose concentration was critical for maintaining sufficient CHO oxidation levels to extend exercise capacity. Further research has indicated that the better maintenance of CHO oxidation can be primarily explained by an increase in exogenous CHO oxidation rates.

The idea that CHO feeding drives fuel provision towards CHO oxidation is reinforced by evidence demonstrating that the amount of exogenous CHO that is oxidised has the greatest effect on exercise performance (Smith, et al 2010). This has led some researchers to hypothesise that increasing exogenous CHO rates results in ever increasing exercise performance. In 2010, Smith et al demonstrated that the biggest improvement in performance occurred when ingesting 60 g·h⁻¹ in comparison to 15 and 30 g·h⁻¹. The 60 g·h⁻¹ ingestion rate also resulted in the highest exogenous CHO oxidation rates. However, when comparing 60 vs 30 g·h⁻¹ there was a non-significant but 'likely' 2.3% improvement in performance when indicating a meaningful improvement in performance of >1.2%. However, in Chapter 2 of this thesis a percentage change of 2.4% in performance would not have been considered 'likely' or 'meaningful' given the variance in measures obtained. This alternative conclusion to the performance conclusions of Smith et al questions the dose response relationship between high exogenous CHO oxidation rates and optimal endurance performance. The lack of any improvement in performance when feeding 64 in comparison to 39 g·h⁻¹ in Chapter 2 is in direct contrast to the findings of Smith et al (2010). As such, it may not be the absolute peak exogenous

carbohydrate oxidation rate that is the determining performance enhancing factor during exercise lasting less than 3 h.

Feeding CHO during exercise also influences the usage of endogenous glycogen stores. Several studies have assessed the use of endogenous glycogen stores through the use of stable isotopes during 1-2 h of moderate intensity exercise. Jeukendrup et al (1999) provided 30 and 180 g·h⁻¹ of a glucose based CHO beverage during a 2 h moderate intensity exercise bout. They reported reduced fat oxidation rates, increased rate of appearance (Ra) and rate of disappearance (Rd) of glucose, and an increase in the oxidation of exogenous CHO. Endogenous muscle glycogen oxidation rates were not altered with either 30 or 180 g·h⁻¹ of CHO. However, liver glycogen breakdown was significantly reduced when consuming 30 g·h⁻¹, and completely inhibited when consuming 180 g·h⁻¹ of CHO. In addition, Smith et al (Smith, et al 2010) estimated a stepped reduction in the contribution of liver glycogen to total CHO oxidation during the second hour of a submaximal exercise bout whilst consuming 15, 30, and 60 g·h⁻¹ of CHO. However, the reduction in liver glucose contribution was only estimated which may explain why the observed peak exogenous oxidation rates dominated the discussion. Interestingly though, both these studies indicated that muscle glycogen was not spared with any ingestion rate provided.

The amount of CHO to ingest for optimal endurance performance has been widely debated (Chapter 2 and Chapter 3). Though, a consensus has been reached that the maximal exogenous CHO oxidation rate that can be achieved with glucose ingestion is around ~1 g·min⁻¹. As previously mentioned, Smith et al (2010) suggested a dose response relationship between CHO ingestion rate and exercise performance when feeding 0, 15, 30 and 60 g·h⁻¹ of glucose. This study was followed up with a multicentre study which indicated that there is a curvilinear dose response relationship with ingestion rates 0 to 120 g·h⁻¹ with a statistically optimal ingestion rate of 78 g·h⁻¹. However, whether

maximal exogenous oxidation rates driven by higher CHO ingestion rates result in optimal performances during endurance tasks requires further metabolic analysis given the data obtained in Chapter 2 of this thesis. Until now the results of Chapter 2 are the most suitably powered and most statistically robust study design to indicate the lack of a dose response relationship with ingestion rates between 20 and 64 g·h⁻¹. However, from these data alone we are unable to determine what the underlying physiological explanations were for the plateau in performance.

As such we aimed to understand the metabolic responses to submaximal endurance exercise with CHO ingestion rates between 0 and 64 g·h⁻¹. We specifically aimed to: examine glucose kinetics; quantify or estimate the total substrate usage from exogenous and endogenous glycogen stores by utilising stable isotopic tracers; measure key circulating metabolites; quantify the percentage contribution of key substrates throughout the exercise bout. We hypothesised that ingesting greater amounts of CHO would result in a stepped reduction in hepatic glucose production.

3.3 METHODOLOGY

3.3.1 Participants

The same 20 participants described in section 1.7 Chapter 1 took part in the study. Unfortunately, 2 participants had to be removed from all stable isotope and substrate use data due to measurement error. The characteristics of participants included were: height 178.7 (8.1) cm, weight 76.9 (8.4) kg, $\dot{V}O_{2max}$ 61.2 (8.2) ml·kg⁻¹·min⁻¹ PPO 392 (34) W, PO at lactate threshold 206 (30) W.

3.3.2 Pretesting

Following pre-screening participants completed a preliminary assessment where lactate threshold, $\dot{V}O_{2max}$, and peak power output were determined. On week 1 of 6, following a 10h overnight fast, participants performed a two section incremental cycle test (Lode Excalibur Sport, Netherlands) to determine maximal oxygen uptake ($\dot{V}O_{2max}$, lactate threshold (LT), and peak power output as described in Chapter 2. The mean \pm SD lactate concentration at LT was 2.1 ± 0.4 mmol·L⁻¹ corresponding to an intensity of $52 \pm 6\%$ of PPO for LT. An intensity of 95% of LT was used to determine the intensity of exercise for the subsequent trials.

3.3.3 Design

In a double blind, placebo controlled, randomised cross-over study design participants visited the laboratory for 5 experimental trials (1 preliminary and 4 intervention) over a five week period. They completed one visit per week commencing each trial on the same day of the week and at the same time of day. On the first of these trial visits participants completed a full familiarisation. The familiarisation trial and the four subsequent intervention trials consisted of a 120 min steady state submaximal cycle ergometer ride at 95% lactate threshold (184 ± 25 W). Water was ingested before and during the

familiarisation trial and was consumed at a rate of 1 L·h⁻¹. Thereafter, participants consumed in a random order either: 0%, 2%, 3.9% or 6.4% CHO solutions before and during exercise at a fluid ingestion rate of 1L·h⁻¹. The 0% trial was a water control trial. Blood samples, expired gas collection and subjective measures were obtained every 15 min throughout the submaximal ride. Participants were asked to record their habitual dietary intake for 48 h prior to visit 1 and replicate this diary for the two days prior to each subsequent visit. Additionally, participants were asked to arrive at the laboratory following a ~10 h overnight fast.

3.3.4 Familiarisation and Experimental trials

Pre-exercise

On arrival at the laboratory participants emptied their bladder and bowel prior to nude body mass measurements. Individuals then changed into cycling attire which was kept consistent throughout all trials to reduce thermoregulatory variability. Following this, Teflon catheters were placed into an antecubital vein in each arm. One catheter was attached to a three way stop cock to enable the infusion of a constant stable isotope. The second was attached to a 10 cm extension line for multiple venous blood sampling and was kept patent with a saline solution flush following each sample collection. A baseline blood sample was drawn (10 mL) prior to a primed (18.54 $\mu\text{mol}\cdot\text{kg}^{-1}$) continuous (0.32 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) infusion of 6,6,²H₂ glucose via a calibrated syringe pump (Asena GS Syringe Pump; Alaris Medical Systems, Basingstoke, UK) for 60 min at rest. No infusion took place during the familiarisation visit. Further blood samples were drawn at 30 min prior and at the start of exercise for later determination of isotopic enrichments. The concentration of isotopic tracer in the infusate, along with the pre and post syringe weights were both determined to confirm the actual infusion rate achieved.

Immediately pre exercise

Five minutes prior to the start of exercise a resting breath sample was collected into a gas sampling bag (Quintron QT00892 GaSampler, Milwaukee, USA) with 10 mL samples transferred directly in duplicate into vacuated exetainer tubes (Labco, High Wycombe, UK) for the determination of the isotopic ratio of $^{13}\text{C}/^{12}\text{C}$ in the CO_2 of the breath. Two minutes prior to the start of exercise a further blood sample was collected and the first bolus of beverage was provided (240ml). The infusion rate of tracer was doubled at the start of exercise ($0.64 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) to accommodate for the increased turnover of glucose during exercise and to maintain plasma enrichment.

2 h preload ride

Participants then completed a 2 h submaximal ride at 95% LT ($185 \pm 25 \text{ W}$, $59 \pm 7\% \text{VO}_{2\text{max}}$) with a range of measures performed every 15 min. In the last 3 min of each 15 min time segment a breath by breath gas capture was obtained for the determination of $\dot{\text{V}}\text{O}_2$ and $\dot{\text{V}}\text{CO}_2$ (Oxycon Pro, Mannheim, Germany). Immediately following the expired gas collection participants removed the mouth piece and provided a single end-tidal breath sample into a breath sample bag (Quintron QT00892 GaSampler, Milwaukee, USA) for the determination of $^{13}\text{C}/^{12}\text{C}$ ratio. Samples were taken in duplicate and stored as per the baseline sample. Following the breath sampling a 10 mL blood sample (10 mL) was drawn and stored on ice prior to centrifugation. Finally participants were asked to rate their perceived exertion (Borg 1982) and rate their feeling (Hardy and Rejeski 1989) on a +5 to -5 scale. Participants then ingested the next volume of test drink (220ml).

3.3.5 Carbohydrate solutions

The same CHO solutions described in section 1.12 were consumed. Briefly, during the preload ride one of four beverages were consumed: 0% water (familiarisation and control); 2.0%; 3.9%; or 6.4% glucose (single source CHO) based CHO beverages. All beverages

were maintained at 10°C and were consumed at a rate of 1L·h⁻¹ providing 0, 20, 39 and 64 g·h⁻¹ of CHO respectively. The 20 g·h⁻¹ solution contained 37 mg of sodium per 100 mL with the 39 and 64 g·h⁻¹ solutions both containing 50 mg per 100 mL. Each beverage was provided two minutes prior to the start of exercise (240 ml) with subsequent volumes (220 ml) consumed every 15 min. The final drink was provided at 120 min of exercise.

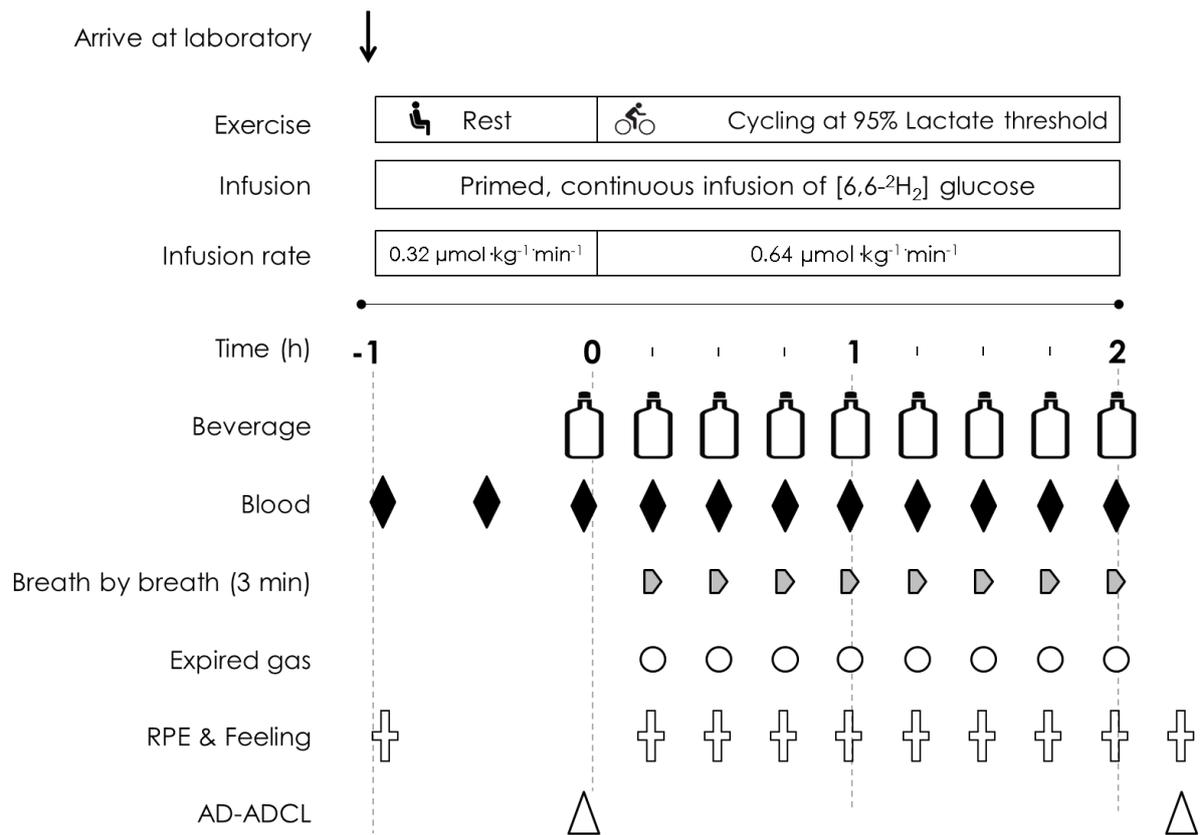


Figure 3.1 Trial visit time line indicating the infusion rate and time course and the frequency of measures taken throughout one visit.

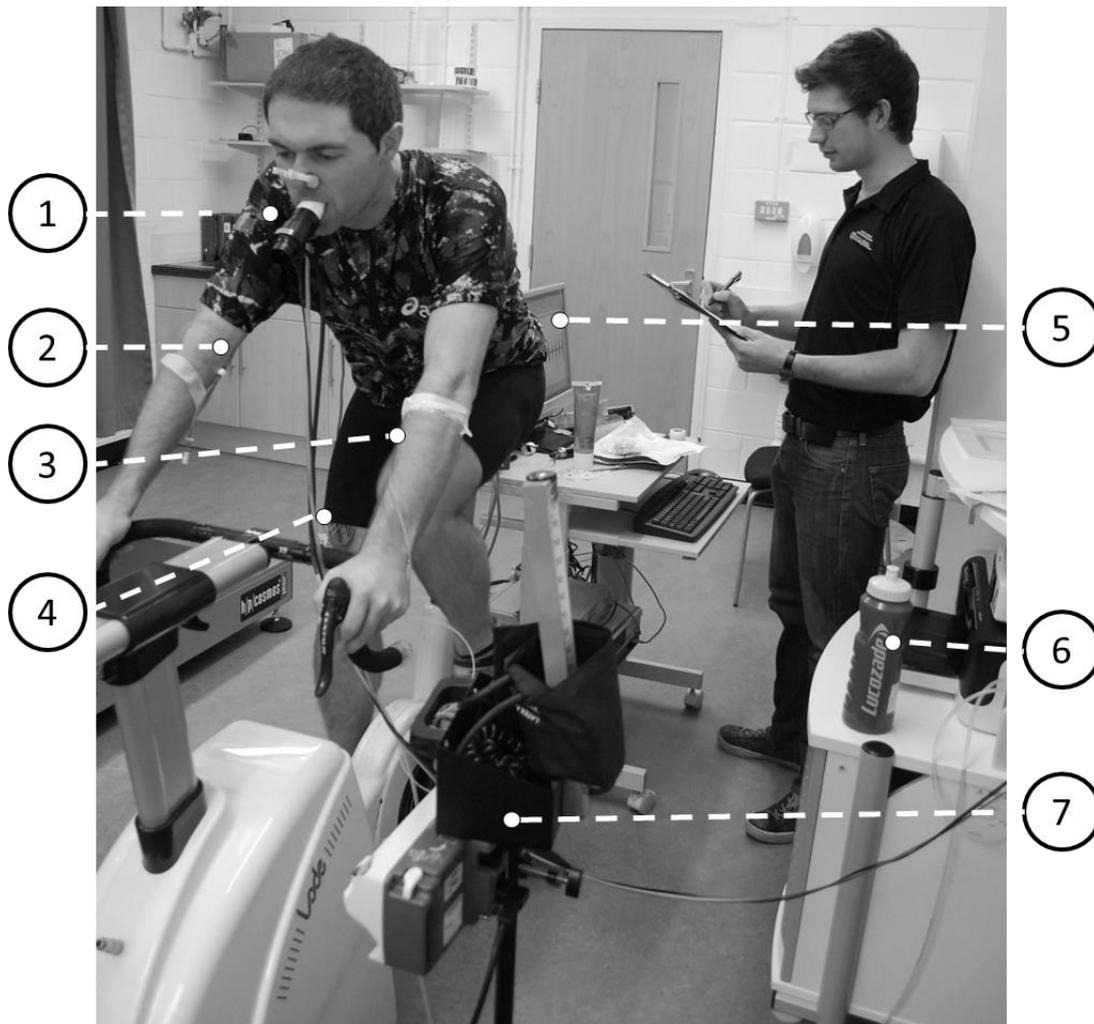


Figure 3.2 Photograph indicating 1. Breath-by-breath oxycon measurement, 2. Blood sampling cannula, 3. Infusion cannula, 4. Vastus lateralis EMG electrode placement, 5. EMG sampling computer, 6. 220 ml for beverage administration, 7. Infusion pump

3.3.6 Analyses / calculations

Blood

Blood samples were collected in EDTA-containing vacutainers and spun in a centrifuge at 3500rpm for 10min at 4 °C. Aliquots of plasma were then frozen and stored at -80 °C until further analysis. Plasma glucose, non-esterified fatty acids, and lactate were analysed using enzymatic methods on an automated analyser (Ilab Aries, Instrumentation Laboratory, Warrington, U.K). Plasma insulin and adrenaline concentrations were analysed using commercially available ELISA kits (Dimedic International, Hamburg, Germany and IBL International, Hamburg, Germany respectively). Both ELISAs were carried out following the manufacturer's instructions.

Plasma samples were derivatised for the analysis of [$^2\text{H}_2$] glucose and [^{13}C] glucose content. 150 μL of plasma and 150 μL of distilled water with added hydrochloric acid (pH 2) was added to a glass vial and mixed vigorously for 10 sec. 3 mL of methanol:chloroform (2.3:1) (500 mL = 348:152) was then added and mixed on a plate shaker (300 rpm) for 3 min. Samples were then centrifuged at 4 °C at 3500 rpm for 15 min. The supernatant was then pipetted into a new glass vial. Here 2 mL of chloroform and 1 mL of distilled (pH 2) water were added and mixed for 15 min on a plate shaker at 300 rpm. Samples were then centrifuged at 4 °C for 15 min at 3500 rpm. The supernatant was then pipetted into a new glass tube. The glass tubes were then transferred to a nitrogen drying rack and incubated at 40 °C for ~2 h until the vials were dry. Once dried 150 μL of butaneboronic acid (10 mg / 1 mL pyridine) was added and mixed on a plate shaker for 15min. Once mixed samples were then incubated at 95 °C for 30 min before 150 μL of acetic anhydride was added and mixed at 300 rpm for 90 min. Samples were then dried under nitrogen gas and incubated at 40 °C until dry. Samples were prepared for the GC-MS and GC-C-IRMS by adding 150 μL of ethylacetate and mixing for 10 min. [$6,6,^2\text{H}_2$] was determined by gas chromatography mass spectroscopy (GCMS) using specific ion monitoring at molecular

weights of 297 and 299 ($[^{12}\text{C}]$ and $[^2\text{H}_2]$ respectively). Plasma $[^{13}\text{C}]$ content was assessed using gas chromatography combustion isotope ratio mass spectroscopy (GC-C-IRMS). Plasma ^{13}C glucose enrichment was determined using the method of Pickert et al (1991), modified for use with gas chromatography–combustion–IRMS (GC-C-IRMS). The glucose derivative (1 μL) was injected into the GC (split ratio 1:15) and analysed by GC-C-IRMS (GC, Trace GC Ultra; C, GC Combustion III; IRMS, Delta Plus XP; all Thermo Finnigan, Herts, UK).

^{13}C Breath samples

Breath samples were collected in duplicate every 15 min into a breath capture bag and a 10ml sample was drawn and stored in a glass evacuated exetainer tube (Labco Ltd., Brow Works, High Wycombe, UK). Breath samples were analysed for $^{13}\text{C}/^{12}\text{C}$ ratio by continuous-flow IRMS (GC, Trace GC Ultra; IRMS, Delta Plus XP; both Thermo Finnigan, Herts, UK).

Substrate oxidation

Expired gas analysis was used to estimate rates of substrate oxidation from $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ every 15 min. These breath measures were averaged every 4 breaths and the mean of these were taken from the last 60 sec of a 3 min sampling period. Whole body substrate oxidation calculations were based on those proposed by Jeukendrup and Wallis (2005):

$$\text{CHO oxidation rate (g}\cdot\text{min}^{-1}) = 4.210 \dot{V}\text{CO}_2 - 2.962 \dot{V}\text{O}_2$$

$$\text{Fat oxidation rate (g}\cdot\text{min}^{-1}) = 1.695 \dot{V}\text{O}_2 - 1.701 \dot{V}\text{CO}_2$$

Once the rate of substrate usage was determined during each 15 min breath by breath capture, the rates calculated in grams per minute were multiplied by 15 and summed from each time point to provide an estimate of the total substrate use during the exercise bout.

3.3.7 Tracer calculations

The isotopic enrichment in the expired breath samples was expressed as mean difference between the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard using the following formula to enable calculation of exogenous carbohydrate oxidation:

$$\text{Exogenous CHO oxidation (g}\cdot\text{min}^{-1}\text{)} = \dot{V}\text{CO}_2 [(R_{\text{exp}} - R_{\text{ref}}) / (R_{\text{exo}} - R_{\text{ref}})] / k$$

Where $\dot{V}\text{CO}_2$ is in litres per minute, R_{exp} is the observed isotopic composition of expired CO_2 , R_{ref} is the isotopic composition of expired CO_2 with the ingestion of the placebo, R_{exo} is the isotopic composition of exogenous glucose ingested in the drink and k ($0.747 \text{ l}\cdot\text{g}^{-1}$) is the volume of CO_2 produced by the complete oxidation of glucose.

3.3.8 Percentage contribution of substrates (second hour of exercise)

Once the total amount of exogenous carbohydrate oxidation had been determined, this rate was extrapolated over the previous 15 min period to determine total grams of exogenous carbohydrate oxidised in each time period from 60 min of exercise onwards. The total exogenous carbohydrate oxidised was subtracted from the total carbohydrate oxidised over the same time period to give an estimate of endogenous carbohydrate oxidation. The endogenous and exogenous carbohydrate oxidised totals were then multiplied by 3.74 to provide total carbohydrate energy expenditure in kcal for each carbohydrate source. The total fat oxidised was multiplied by 9.75 to give total energy expenditure (kcal) for fat oxidation. The total energy expenditure from all three substrates was then summed and each component was expressed as a percentage of the total energy expenditure over the second hour of exercise.

3.3.9 Glucose kinetics

From the $6,6,^2\text{H}_2$ tracer infusion the R_a and R_d of glucose were calculated with the single pool non steady state equations of Steele, as modified for use with stable isotopes

(Proietto 1990). Total Ra represents the total splanchnic glucose from ingested CHO, liver derived glucose, and potentially some from the kidneys.

$$R_a \text{ total} = F - (pV \cdot (C_1 + C_2) / 2 \cdot (E_2 - E_1) / (t_2 - t_1)) / (E_2 + E_1) / 2)$$

$$R_d \text{ total} = R_a \text{ total} - V \cdot (C_1 + C_2 / t_2 - t_1)$$

Where F is the infusion rate ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$); E_1 and E_2 are the [$^2\text{H}_2$] glucose enrichments (MPE) in plasma at time points t_1 and t_2 (min), respectively; C_1 and C_2 are glucose concentrations ($\text{mg} \cdot \text{ml}^{-1}$) at t_1 and t_2 , respectively; and pV is volume of distribution which was set at $40 \text{ mL} \cdot \text{kg}^{-1}$ to coincide with the findings of Wolfe et al (1992).

3.3.10 Estimation of liver glucose contribution

Liver glucose contribution has been estimated from the following calculation:

$$\text{Whole body glucose Ra } (R_{a_{\text{body}}}) \text{ g} \cdot \text{min}^{-1} = R_a \cdot \text{body mass} \cdot 1000$$

$$\text{Estimation of liver glucose contribution to total glucose Ra (\%)} = 100 - ((\text{EXO} / R_{a_{\text{body}}}) * 100)$$

Where R_a is the total R_a previously calculated ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and the body mass is the pre-trial body mass measure taken before each trial (kg). The factor of 1000 is to convert from mg to grams. EXO is the exogenous CHO oxidation rate ($\text{g} \cdot \text{min}^{-1}$) calculated previously. These calculations serve as an estimation of hepatic glucose contribution during the second hour of exercise (Jeukendrup, et al 1999).

3.3.11 Data Presentation and Statistical analysis

All data are presented as mean (\pm SD) unless otherwise stated. Three factor repeated measures analysis of variance was used to determine treatment, time, period (order) main effects and treatment x time interaction effects. Where a significant period effect was observed then period was used as a covariate and the analysis re-run. Significant main and interaction effects were explored using post hoc Tukey's comparisons to indicate where these differences occurred.

3.4 RESULTS

Twenty male competitive cyclists completed all trials in this study. All treatments were tolerated well by all participants and the majority (19 of 20) had no adverse events (AE) from the treatments provided. Tremendous effort was made to ensure all data points were collected, though some data sets had to be removed due to analytical difficulties. As such, all data for substrate oxidation are for n=18 due to absence of ^{13}C samples on one trial for one participant and expired gas analysis analytical problems on one other participant. All other data are for n=20.

Symbols used in Figures to denote treatment by time interaction effects

* - Values significantly different from initial (0min or 15min) time point.

^ - Time point values significantly different from 60min values.

a - 64 g·h⁻¹ treatment significantly different from 0 g·h⁻¹ at marked time point.

b – 39 g·h⁻¹ treatment significantly different from 0 g·h⁻¹ at marked time point.

c – 20 g·h⁻¹ treatment significantly different from 0 g·h⁻¹ at marked time point.

d – 64 g·h⁻¹ treatment significantly different from 20 g·h⁻¹ at marked time point.

e – 39 g·h⁻¹ treatment significantly different from 20 g·h⁻¹ at marked time point.

f – 39 and 64 g·h⁻¹ treatment significantly different from 20 g·h⁻¹ at marked time point.

g – all treatments are significantly different from one another.

3.4.1 Substrate Oxidation

Respiratory exchange ratio

RER data analysis revealed a significant main effect of time ($p < 0.01$), treatment ($p < 0.01$), and period ($p < 0.01$) but no interaction ($p = 0.39$). Period was treated as a covariate for all subsequent analysis. Pairwise comparisons of time indicated that RER values declined with exercise duration. Additional comparisons of treatment indicated that this reduction was greatest when consuming the $0 \text{ g}\cdot\text{h}^{-1}$ treatment (Figure 3.3).

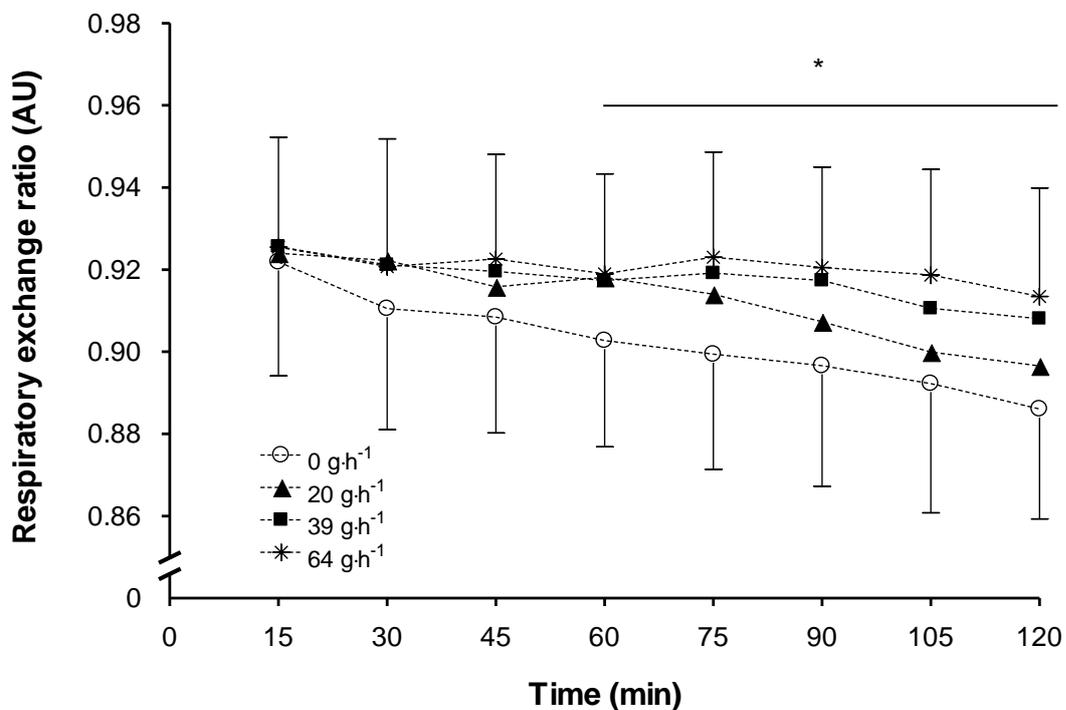


Figure 3.3. Mean (SD) respiratory exchange ratio during submaximal exercise when consuming 0 , 20 , 39 , and $64 \text{ g}\cdot\text{h}^{-1}$ of CHO. RER values are significantly ($*$, $p < 0.01$) lower from time point 60 min onwards in comparison to 15 min with the $0 \text{ g}\cdot\text{h}^{-1}$ treatment eliciting a significantly ($p < 0.01$) lower mean RER over the two hours in comparison to 20 , 39 and $64 \text{ g}\cdot\text{h}^{-1}$.

Whole body carbohydrate oxidation

Analysis of the carbohydrate oxidation data indicated a significant effect of time ($p < 0.01$), treatment ($p < 0.01$), and period ($p < 0.01$) but no interaction effect ($p < 0.58$). Period was treated as a covariate for all subsequent analysis. Pairwise comparisons over time indicate that estimated rate of CHO oxidation was declining over time with measures 90 min onwards significantly lower than 15 min values. Additional treatment pairwise comparisons reveal that the lowest CHO oxidation rate occurred when consuming the 0 $\text{g}\cdot\text{h}^{-1}$ treatment (Figure 3.4).

Estimated total carbohydrate oxidised (g) for 2h and second h of submaximal ride.

Data for the estimated total carbohydrate oxidised during the 2 h cycling period indicated a significant effect of treatment ($p < 0.01$), and period ($p = 0.03$). Period was treated as a covariate for all subsequent analysis. Pairwise comparisons of treatment revealed that consuming 64 $\text{g}\cdot\text{h}^{-1}$ of CHO increased the total amount of CHO oxidised during the 2 h bout by 32.5 g (95%CI: 8.3 to 56.7) in comparison to the 0 $\text{g}\cdot\text{h}^{-1}$ treatment (Figure 3.5). Additional analysis was performed to estimate the total amount of CHO oxidised in the second hour of exercise only. These data indicate that there was a significant effect of treatment ($p < 0.01$) but not period ($p = 0.51$). Pairwise comparisons of treatment revealed that in the second hour of exercise total estimated CHO oxidation increased by 13.9 (95%CI: 1.7 to 26.0) $\text{g}\cdot\text{h}^{-1}$ and 21.6 (95%CI: 9.5 to 33.7) $\text{g}\cdot\text{h}^{-1}$ when consuming 39 and 64 $\text{g}\cdot\text{h}^{-1}$ of CHO, in comparison to the 0 $\text{g}\cdot\text{h}^{-1}$ control (Figure 3.5).

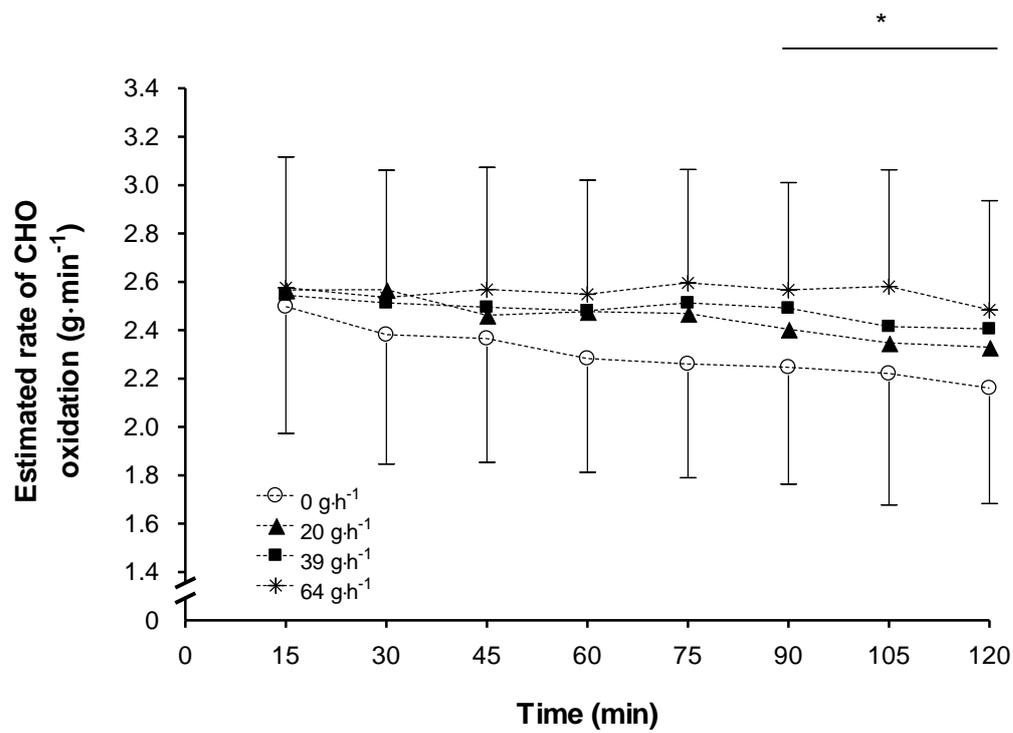


Figure 3.4 Mean (SD) estimated rate of carbohydrate (CHO) oxidation during submaximal exercise when consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. A comparison of time indicated CHO oxidation rates at 90 min onwards were significantly lower than 15 min with the 0 g·h⁻¹ treatment being significantly lower than 20, 39 and 64 g·h⁻¹.

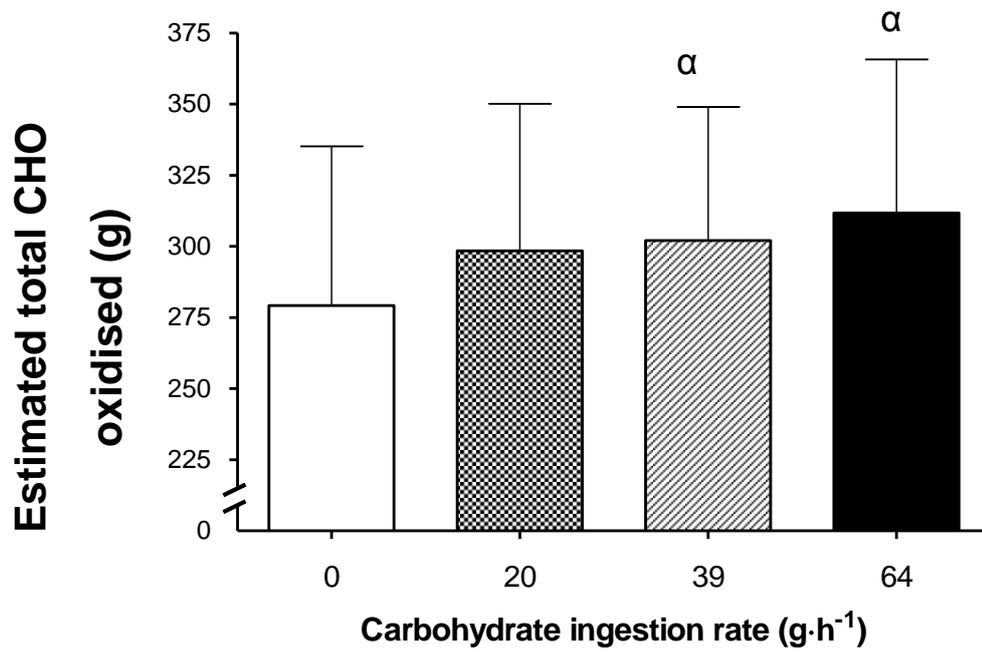


Figure 3.5. Mean (SD) estimated total amount of carbohydrate (CHO) oxidised during the 2 h submaximal ride when consuming 0 (white), 20 (dark grey), 39 (light grey) and 64 (black) g·h⁻¹ of CHO. 'α' indicates treatment was significantly different from 0 g·h⁻¹ treatment.

Fat oxidation

Results for estimated rate of fat oxidation indicated a significant effect of time, treatment and period (all $p < 0.01$) but no interaction effect ($p = 0.82$). Period was treated as a covariate with all further comparisons. Pairwise comparisons of time indicate an increase in fat oxidation rates with increase in exercise duration. Additional pairwise comparisons revealed that consuming the $0 \text{ g}\cdot\text{h}^{-1}$ treatment resulted in the highest mean fat oxidation rates (Figure 3.8).

Estimated total fat oxidised (g) during 2 h and second hour of exercise

Data for the estimated total amount of fat oxidised during the 2 h cycling period revealed no significant period effect ($p = 0.06$) or treatment effect ($p = 0.06$) (Figure 3.5). Additional analysis was performed to estimate the total amount of fat oxidised in the second hour of exercise only. These data indicated that there was a significant effect of treatment ($p < 0.01$) and period ($p = 0.01$), with period subsequently treated as a covariate in all further analyses. Pairwise comparisons indicated that fat oxidation was reduced by -5.0 (95%CI: -9.3 to -0.8) and -6.3 (95%CI: -10.6 to -2.0) $\text{g}\cdot\text{h}^{-1}$ when consuming the 39 and 64 $\text{g}\cdot\text{h}^{-1}$ treatments in comparison to the $0 \text{ g}\cdot\text{h}^{-1}$ control (Figure 3.9).

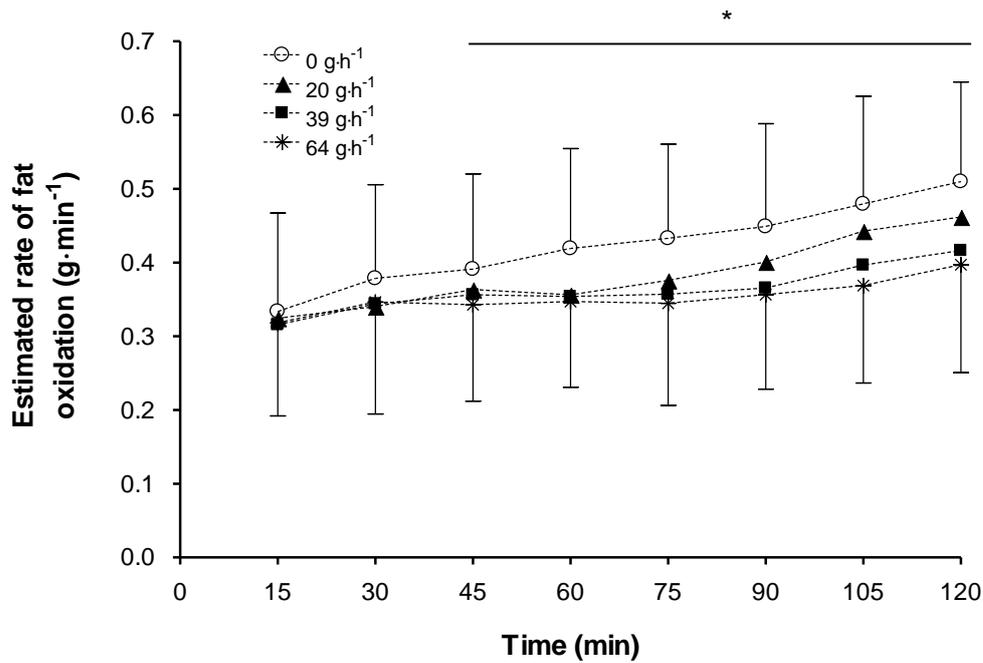


Figure 3.6 Mean (SD) estimated rate of fat oxidation during submaximal exercise when consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. Post hoc comparisons indicated the mean rate of fat oxidation was significantly lower when consuming 39 and 64 g·h⁻¹ of CHO compared to 0 g·h⁻¹. Additionally, time comparisons indicated an increase in fat oxidation from 45 min onwards in comparison to rates at 15 min. Symbol as previously described.

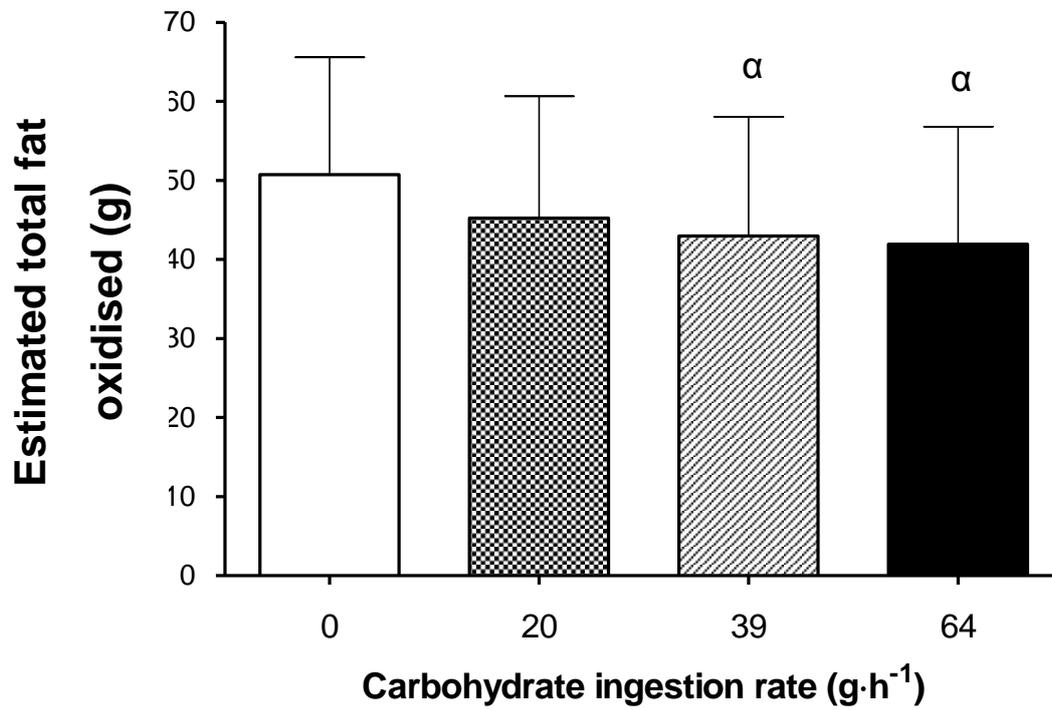


Figure 3.7. Mean (SD) estimated total amount of fat oxidised during the 2h submaximal ride when consuming 0 (white bar), 20 (dark grey bar), 39 (light grey bar) and 64 (black bar) g·h⁻¹ of CHO. Symbols as previously described.

3.4.2 Stable Isotope measures

Exogenous CHO oxidation

Data for exogenous carbohydrate oxidation indicated significant main effects of treatment ($p < 0.01$), time ($p < 0.01$), period ($p < 0.01$) and an interaction effect between treatment and time ($p < 0.01$). Period was included as a covariate for all further comparisons. Pairwise comparisons indicated that all exogenous CHO oxidation rates were significantly different between all treatments from the 60 min time point until the end of exercise. Specifically, exogenous CHO oxidation rates were higher in comparison to the 20 g·h⁻¹ treatment by 0.13 (95%CI: 0.10 to 0.15) and 0.29 (95%CI: 0.27 to 0.31) g·min⁻¹ on the 39 and 64 g·h⁻¹ treatments. Additionally the 64 g·h⁻¹ treatment increased exogenous oxidation rates at 90, 105 and 120 min above the 60 min rates highlighting that exogenous CHO oxidation was still increasing from 60 min onwards on this trial. Similarly, when consuming 39 g·h⁻¹ the values at 120 min were significantly increased above the 60 min values (Figure 3.8).

Percentage contribution of endogenous/exogenous substrates

Percentage contribution of fat oxidised in the second hour of exercise revealed significant effects of treatment ($p < 0.01$) and period ($p < 0.01$). Following inclusion of period as a covariate, pairwise comparisons of treatment indicated the percentage contribution of fat oxidation was significantly lower when consuming 39 (-7.5, 95%CI: -1.6 to -13.4%) and 64 (8.9, 95%CI: 3.1 to 14.8%) g·h⁻¹ in comparisons to consuming 0 g·h⁻¹. Endogenous carbohydrate percentage contribution highlighted significant effects of treatment ($p < 0.01$) but not period ($p = 0.39$). Pairwise comparisons of treatment indicated that endogenous carbohydrate percentage contribution was significantly suppressed in the 39 and 64 g·h⁻¹ trials (-7.3, 95%CI: -1.6 to -13.1 and -11.2, 95%CI: -5.5 to -16.9 respectively) compared to the 0 g·h⁻¹ treatment. Additionally, consuming carbohydrate at 64 g·h⁻¹ suppressed endogenous carbohydrate percentage contribution by -7.2 (95%CI: -1.5 to 13.0) % in comparison to the 20 g·h⁻¹ treatment. Exogenous carbohydrate oxidation percentage

contribution indicated a significant effect of treatment ($p < 0.01$). Pairwise comparisons indicated that all treatments were significantly different from one another (Figure 3.9).

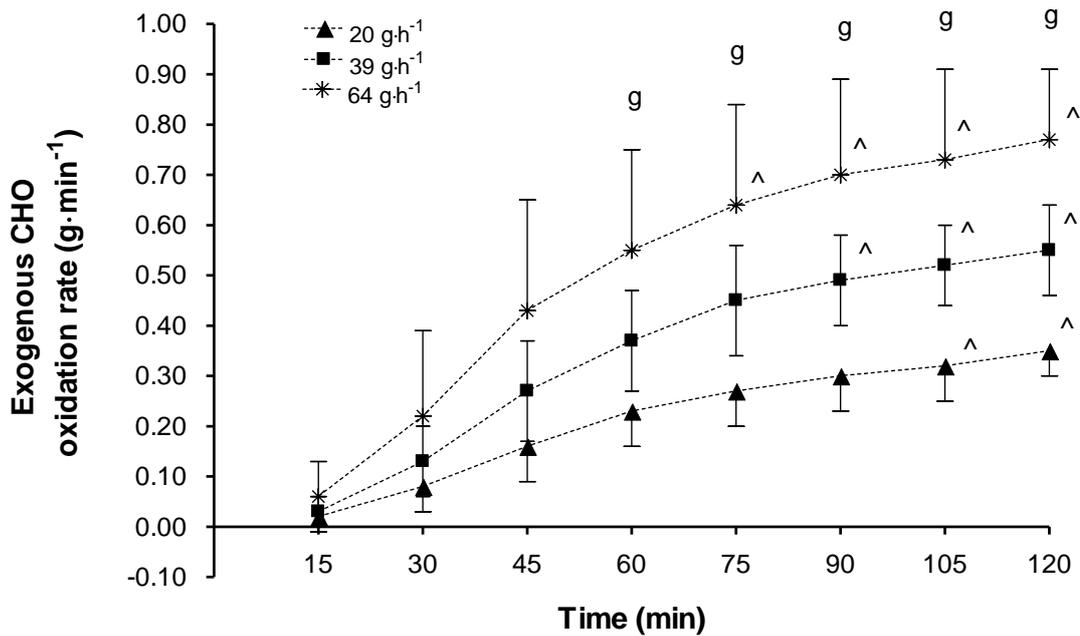


Figure 3.8. Mean (SD) exogenous carbohydrate (CHO) oxidation rates during submaximal exercise while consuming 0, 20, 39 and 64 g·h⁻¹ of CHO. ^ - indicates time point values significantly ($p < 0.01$) different in comparison to 60 min values; g – indicates that all trials are significantly ($p < 0.01$) different from each other at the indicated time point.

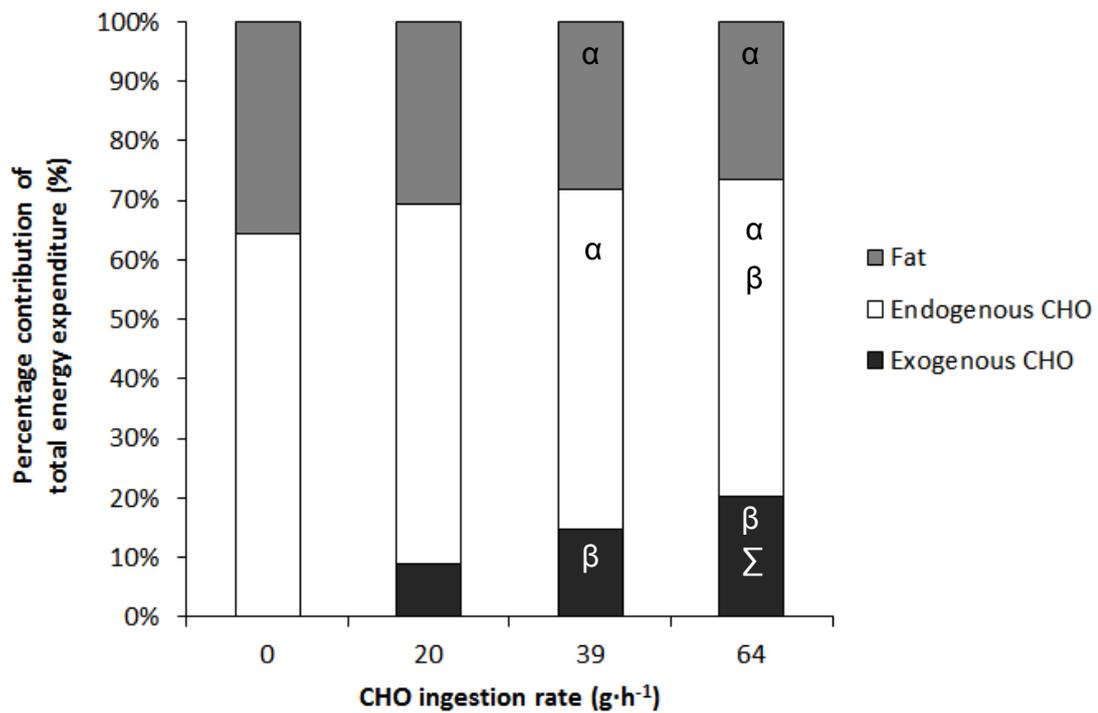


Figure 3.9. Percentage contribution of total carbohydrate oxidation rates from endogenous and exogenous sources during the second hour of exercise. β indicates significantly different from 20 g·h⁻¹, and Σ indicates significantly different from 39 g·h⁻¹, others as previously described.

Glucose Ra and Rd

Glucose Ra values were mirrored by that of the Rd values, and as such statistical analysis for both data sets was almost identical. Analysis of the glucose Ra and Rd indicated significant effects of treatment ($p < 0.01$), time ($p < 0.01$), period ($p < 0.01$) and an interaction of treatment by time ($p < 0.01$). Period was treated as a covariate for all subsequent analysis. Post hoc comparisons revealed that consuming CHO resulted in a significantly higher glucose Ra of 1.98 (95%CI: 1.37 to 2.58), 2.12 (95%CI: 1.52 to 2.72), and 3.65 (95%CI: 3.05 to 4.25) $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for the 20, 39 and 64 $\text{g}\cdot\text{h}^{-1}$ treatments respectively, when compared to the 0 $\text{g}\cdot\text{h}^{-1}$ condition. Post hoc interaction comparisons indicated a significant increase in glucose Ra when consuming 39 and 64 $\text{g}\cdot\text{h}^{-1}$ compared to 0 $\text{g}\cdot\text{h}^{-1}$ at time points from 75 min onwards. Additionally, during the 20 $\text{g}\cdot\text{h}^{-1}$ trial glucose Ra was significantly increased over the control condition at time points from 90 min onwards. Glucose Ra values were significantly increased over the 60min time point value from 75 to 120 min in the 64 $\text{g}\cdot\text{h}^{-1}$, 105 to 120 min with 39 $\text{g}\cdot\text{h}^{-1}$, and only at 120 min in the 20 $\text{g}\cdot\text{h}^{-1}$ Trial (Figure 3.10).

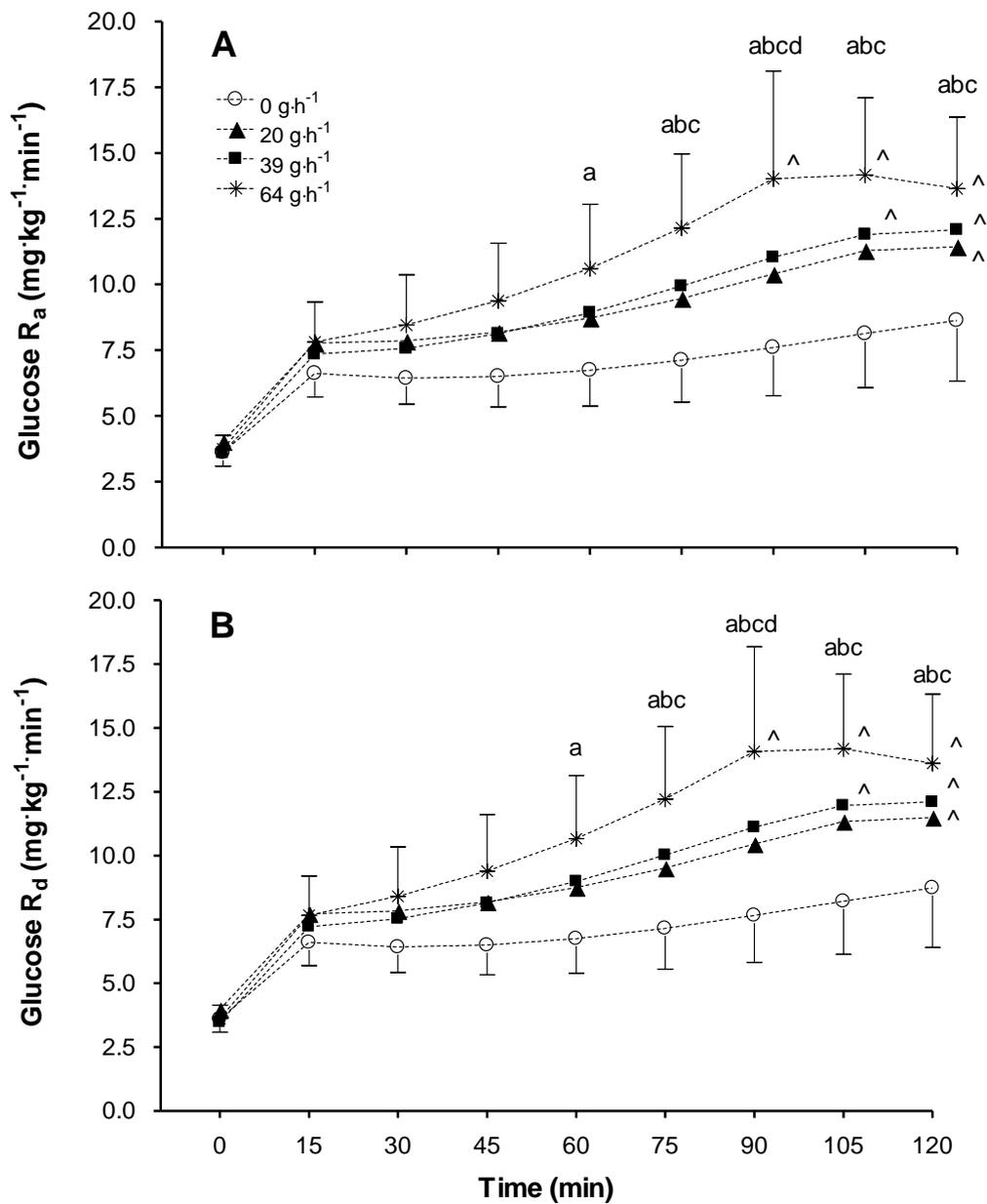


Figure 3.10. Mean (SD) glucose rate of appearance (A) and rate of disappearance (B) during submaximal exercise while consuming 0, 20, 39, and 64 $\text{g}\cdot\text{h}^{-1}$ of CHO. a, b, and c – indicates 64, 39, and 20 $\text{g}\cdot\text{h}^{-1}$ value is significantly different from 0 $\text{g}\cdot\text{h}^{-1}$ at the marked time point, d indicates 64 $\text{g}\cdot\text{h}^{-1}$ is significantly different from 20 $\text{g}\cdot\text{h}^{-1}$ at marked time point.

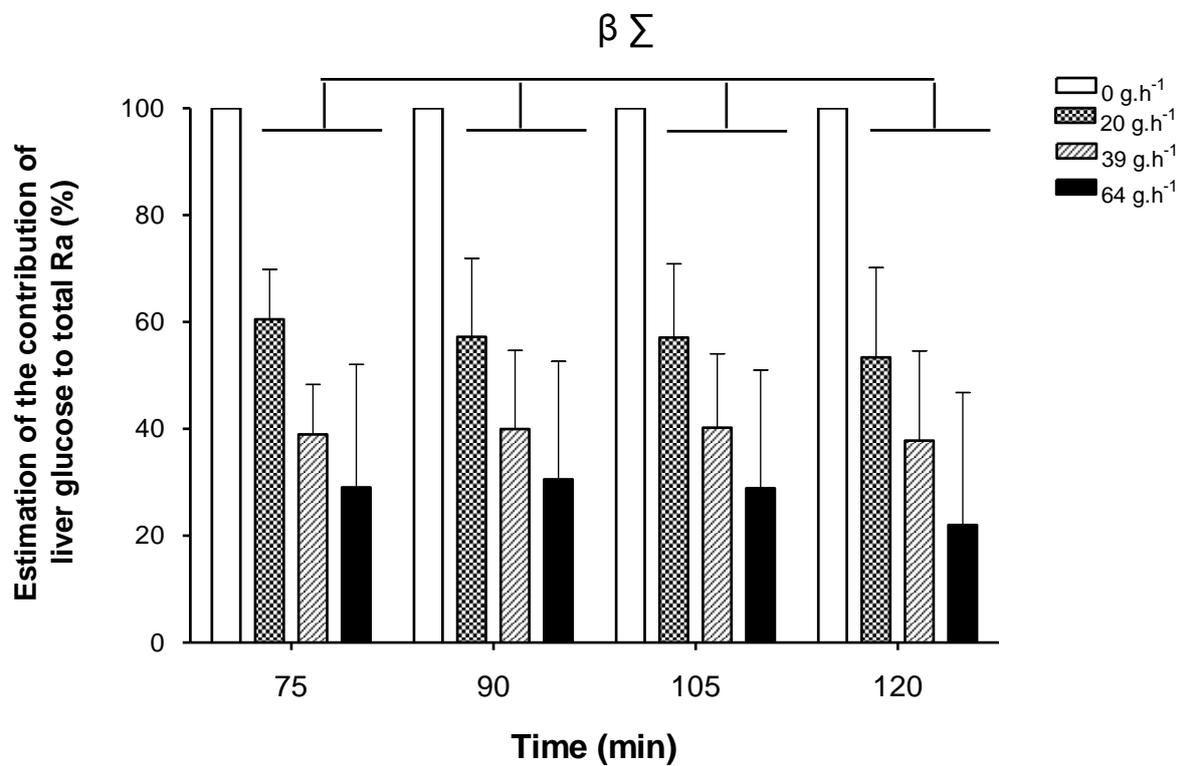


Figure 3.11. Mean (SD) estimation of the contribution of liver glucose to total glucose Ra during submaximal exercise while consuming 20, 39, and 64 g.h⁻¹ of CHO. There was a significant treatment effect whereby all treatments were significantly different from one another. Symbols as previously described.

3.4.3 Blood plasma measures

Glucose

There were main effects of time ($p < 0.01$), treatment ($p < 0.01$), period ($p < 0.01$) and an interaction effect ($p < 0.01$) of treatment by time observed for plasma glucose response. Period was then used as a covariate for all further analysis. Mean glucose concentration was higher when consuming $39 \text{ g}\cdot\text{h}^{-1}$ and $64 \text{ g}\cdot\text{h}^{-1}$ ($0.41 \text{ mmol}\cdot\text{L}^{-1}$ (95% CI: 0.31 to 0.51) and $0.46 \text{ mmol}\cdot\text{L}^{-1}$ (95% CI 0.36 to 0.56), respectively) when pairwise comparisons to the $0 \text{ g}\cdot\text{h}^{-1}$ treatment were made. Consuming 39 and $64 \text{ g}\cdot\text{h}^{-1}$ also resulted in increased mean plasma glucose concentration by 0.23 (95% CI: 0.13 to 0.33) and 0.28 (95% CI: 0.18 to 0.38) $\text{mmol}\cdot\text{L}^{-1}$, respectively, in comparison to consuming $20 \text{ g}\cdot\text{h}^{-1}$. There was no evidence of a difference between 39 and $64 \text{ g}\cdot\text{h}^{-1}$ treatments. Treatment by time interaction analysis revealed that plasma glucose concentration was significantly increased above 0 min in the $64 \text{ g}\cdot\text{h}^{-1}$ treatment from 15 min until the end of the exercise period. Additionally the $39 \text{ g}\cdot\text{h}^{-1}$ treatment significantly increased plasma glucose concentration from the 0 min value at 15, 30, 45 and 60 min, as did the $20 \text{ g}\cdot\text{h}^{-1}$ treatment at 30 and 45 min (3.12A). Finally, the immediate post time trial plasma glucose concentration was: $4.80 (\pm 1.56)$, $4.92 (\pm 1.73)$, $4.77 (\pm 1.65)$ and $4.92 (\pm 1.84)$ when consuming 0, 20, 39, and $64 \text{ g}\cdot\text{h}^{-1}$ respectively.

Insulin

There were main effects of time ($p < 0.01$), treatment ($p < 0.01$), and an interaction effect between treatment and time ($p < 0.01$) on plasma insulin response. There was no effect of period ($p = 0.14$). On average insulin concentration increased by 2.5 (95%CI: 1.3 to 3.7), 5.2 (95%CI: 4.0 to 6.4) and 7.3 (95%CI: 6.1 to 8.5) $\mu\text{IU}\cdot\text{ml}$ when consuming 20, 39 and $64 \text{ g}\cdot\text{h}^{-1}$ CHO, respectively, compared to the $0 \text{ g}\cdot\text{h}^{-1}$ trial. Insulin concentration significantly increased from pre ingestion (0 min) values at 15 to 60 min time points for $64 \text{ g}\cdot\text{h}^{-1}$, and at 30 and 45 min for $39 \text{ g}\cdot\text{h}^{-1}$. Further pairwise comparisons revealed that Insulin concentration was significantly elevated in the 39 and $64 \text{ g}\cdot\text{h}^{-1}$ treatments when compared

to the 0 g·h⁻¹ at time points between 15 to 45 min. At 30 min, consuming 39 and 64 g·h⁻¹ also significantly elevated insulin concentration over that of consuming 20 g·h⁻¹. The 64 g·h⁻¹ treatment also significantly increased insulin concentration at time points 45 and 60 min when compared to the 20 g·h⁻¹ treatment (Figure 3.12B). Finally, the immediate post time trial plasma insulin concentration was: 4.66 (± 5.60), 5.05 (± 4.55), 6.97 (± 7.28), and 6.39 (± 6.15) when consuming 0, 20, 39, and 64 g·h⁻¹ respectively.

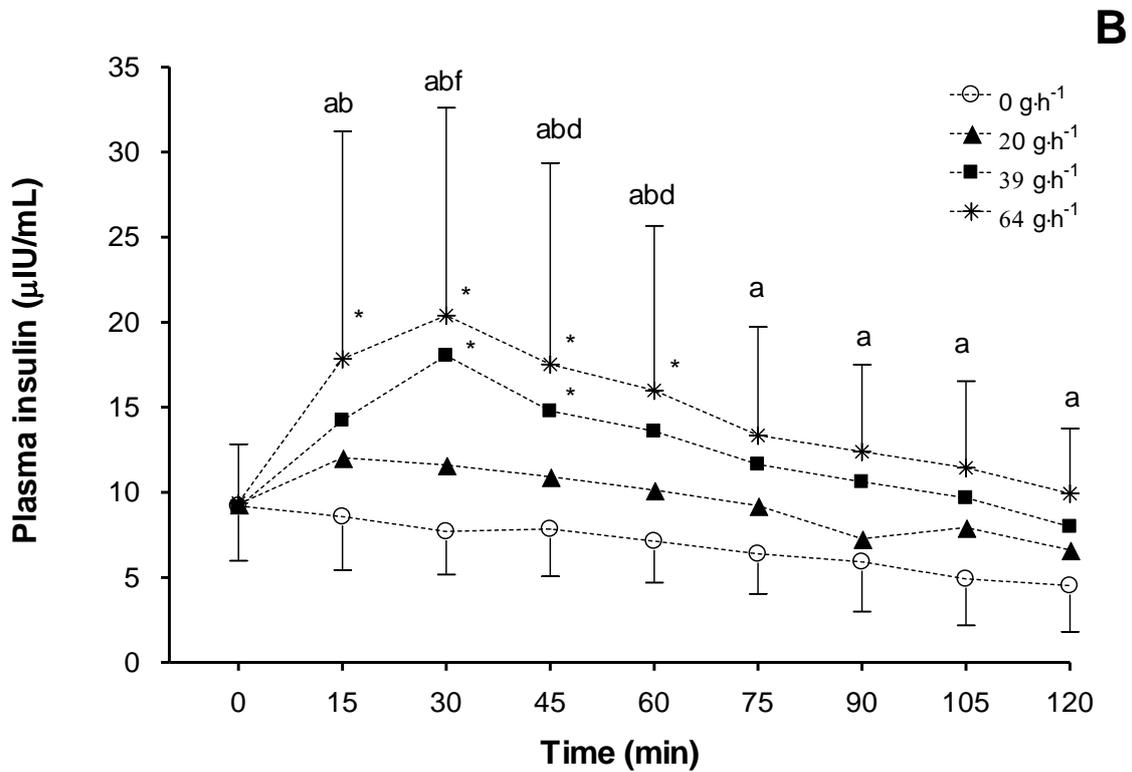
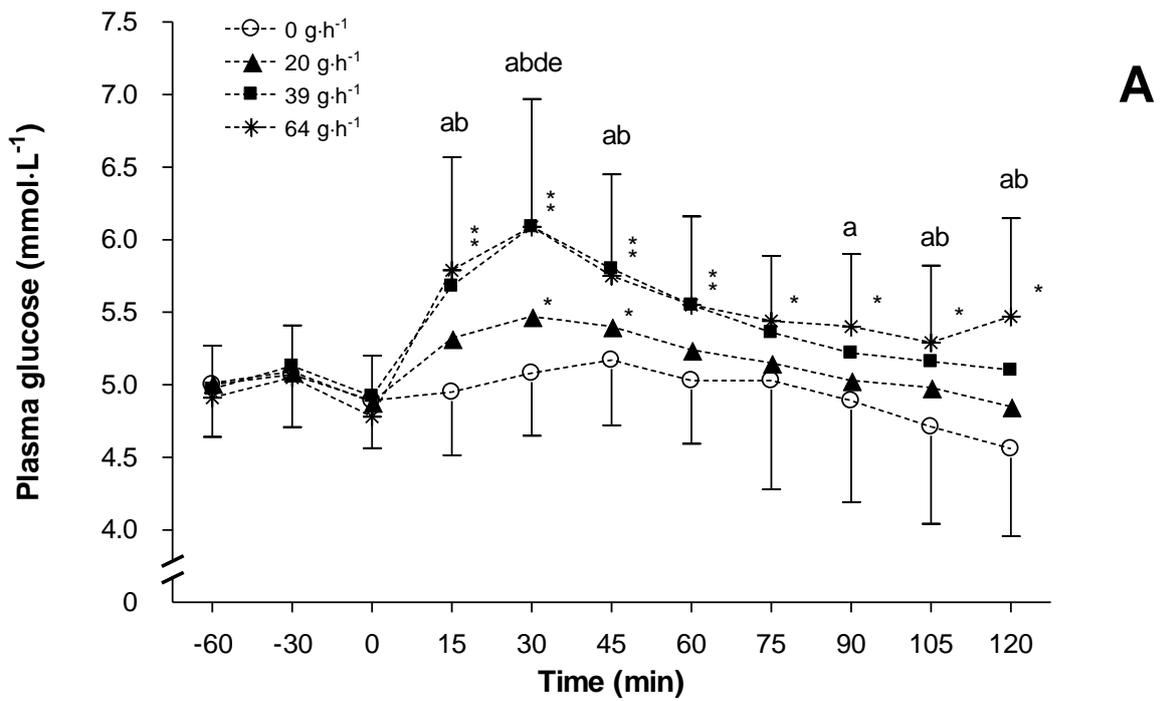


Figure 3.12. Mean (SD) plasma glucose (A) and insulin (B), concentration during rest (-60, -30, and 0 min where appropriate), and during submaximal exercise, (15 to 120 min), while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. * - Values significantly different from 0 min time point. e – Indicates 39 g·h⁻¹ is significantly different from 20 g·h⁻¹ at marked time point, f – indicates 39 and 64 g·h⁻¹ are both different from the 20 g·h⁻¹ at the marked time point.

Non-esterified fatty acids

There were main effects of time ($p < 0.01$), treatment ($p < 0.01$), period ($p < 0.01$) and an interaction of treatment by time ($p < 0.01$). Period was included as a covariate for all further analyses. Pairwise comparisons between treatments revealed that on the 0 $\text{g}\cdot\text{h}^{-1}$ treatment mean NEFA concentration was 0.10 (95%CI: 0.07 to 0.13), 0.12 (95%CI: 0.10 to 0.16) and 0.16 (95%CI: 0.13 to 0.19) $\text{mmol}\cdot\text{L}^{-1}$ higher than when consuming the 20, 39 and 64 $\text{g}\cdot\text{h}^{-1}$ treatments, respectively. Additionally, the NEFA concentration throughout exercise on 20 $\text{g}\cdot\text{h}^{-1}$ was significantly higher (0.06 $\text{mmol}\cdot\text{L}^{-1}$, 95%CI 0.03 to 0.09) than when consuming 64 $\text{g}\cdot\text{h}^{-1}$. When consuming 0 $\text{g}\cdot\text{h}^{-1}$ all NEFA concentrations were significantly increased above the 0 min time point from 60 min onwards. On the 20 $\text{g}\cdot\text{h}^{-1}$ treatment plasma NEFA concentration was elevated compared to the 0 min time point at 90, 105 and 120 min. Additionally, on the 39 $\text{g}\cdot\text{h}^{-1}$ treatment NEFA concentration increased at time points 105 and 120 min compared to 0 min. No increase was observed on 64 $\text{g}\cdot\text{h}^{-1}$ treatment. Post hoc interaction comparisons revealed that mean NEFA concentration in the 0 $\text{g}\cdot\text{h}^{-1}$ treatment was significantly elevated compared to the 64 and 39 $\text{g}\cdot\text{h}^{-1}$ treatments from the 45 min time point until the end of exercise. Additionally, the 20 $\text{g}\cdot\text{h}^{-1}$ treatment was significantly different from 64 $\text{g}\cdot\text{h}^{-1}$ at 90, 105 and 120 min. Finally, the 20 $\text{g}\cdot\text{h}^{-1}$ treatment significantly elevated plasma NEFA concentration in comparison to the 0 $\text{g}\cdot\text{h}^{-1}$ at time point 90min (Figure 3.13A). Finally, the immediate post time trial plasma NEFA concentration was: 0.83 (± 0.48), 0.67 (± 0.31), 0.56 (± 0.32), and 0.46 (± 0.36) when consuming 0, 20, 39, and 64 $\text{g}\cdot\text{h}^{-1}$ respectively.

Adrenaline

Analysis of adrenaline concentration revealed there was a main effect of period ($p < 0.01$) time ($p < 0.01$), treatment ($p < 0.01$), but no interaction ($p = 0.10$). Period was treated as a covariate for all subsequent analysis. Pairwise comparisons of time indicated adrenaline concentrations were increasing over the duration of the exercise bout. Additionally,

comparisons between treatment indicated that adrenaline concentration was highest on the 0 g·h⁻¹ treatment in comparison to the 39 and 64 g·h⁻¹ treatments (Figure 3.13B). Finally, the immediate post time trial plasma adrenaline concentration was: 4.88 (± 4.27), 4.54 (± 2.74), 4.32 (± 3.49), 3.21 (± 1.93) respectively when consuming 0, 20, 39, and 64 g·h⁻¹ respectively.

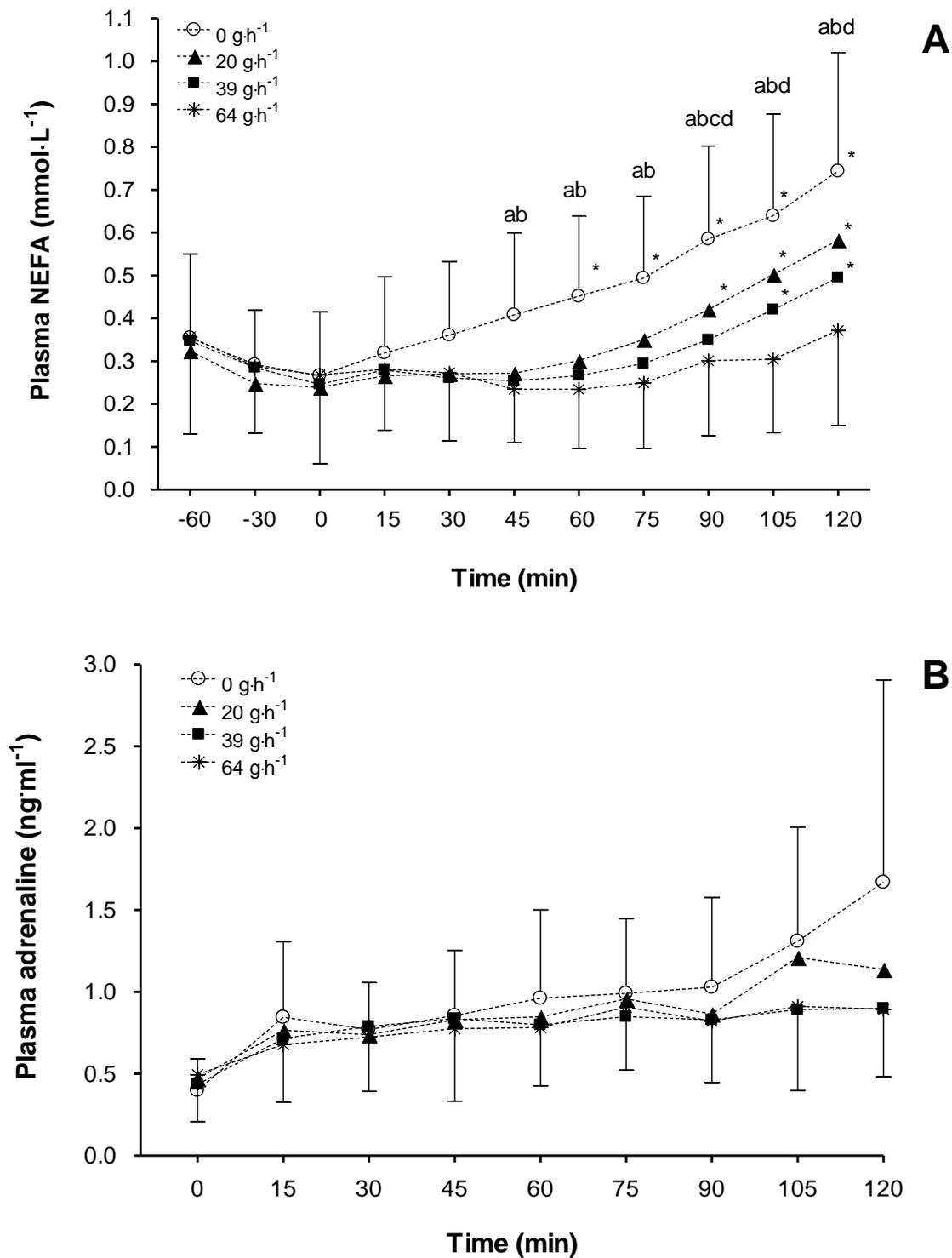


Figure 3.13. Mean (SD) NEFA (A) and adrenaline (B), concentration during rest (-60, -30, and 0 min where appropriate), and during submaximal exercise, (15 – 120 min), while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. * - Values significantly different from 0min time point within the treatment at the marked time point. All other significance markers as previously described.

Lactate

Plasma lactate concentration response revealed a significant effect of time ($p < 0.01$) and an effect of treatment ($p = 0.02$) but no interaction ($p = 0.84$), and no period effect ($p = 0.57$). Post hoc comparisons of time indicated that all exercising lactate concentrations were elevated above resting values, though there was no significant difference between trials (Figure 3.14). Finally, the immediate post time trial plasma lactate concentration was: $6.2 (\pm 3.30)$, $6.6 (\pm 3.4)$, $6.0 (\pm 0.3)$, and $6.6 (\pm 3.2)$ respectively when consuming 0, 20, 39, and $64 \text{ g}\cdot\text{h}^{-1}$.

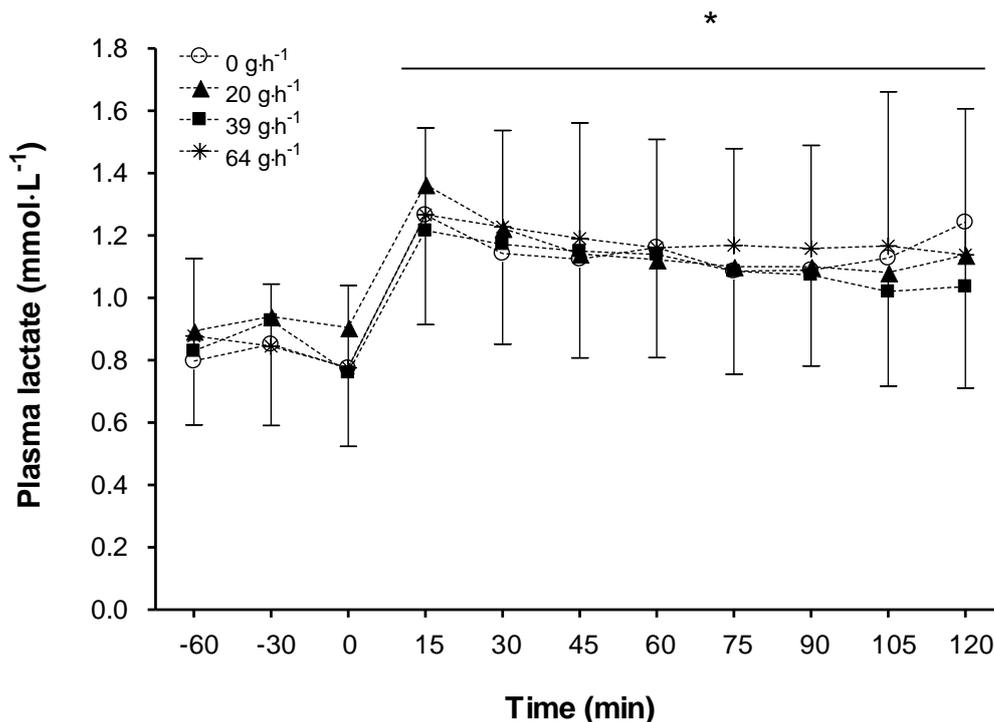


Figure 3.14. Mean (SD) plasma lactate concentration at rest (-60, -30 and 0 min) and during submaximal steady state exercise (15 - 120 min) when consuming 0, 20, 39, and $64 \text{ g}\cdot\text{h}^{-1}$ of CHO. A significant effect of time indicated that all time points from 15 min onwards were significantly elevated above values at 0 min (*)

3.4.4 Heart rate

There were significant effects of time, treatment and period ($p < 0.01$) but no interaction effect ($p = 1.00$). Period was treated as a covariate for all subsequent analysis. Pairwise comparisons indicated that heart rate tended to increase with increasing exercise duration though no differences between treatments occurred (Figure 3.15).

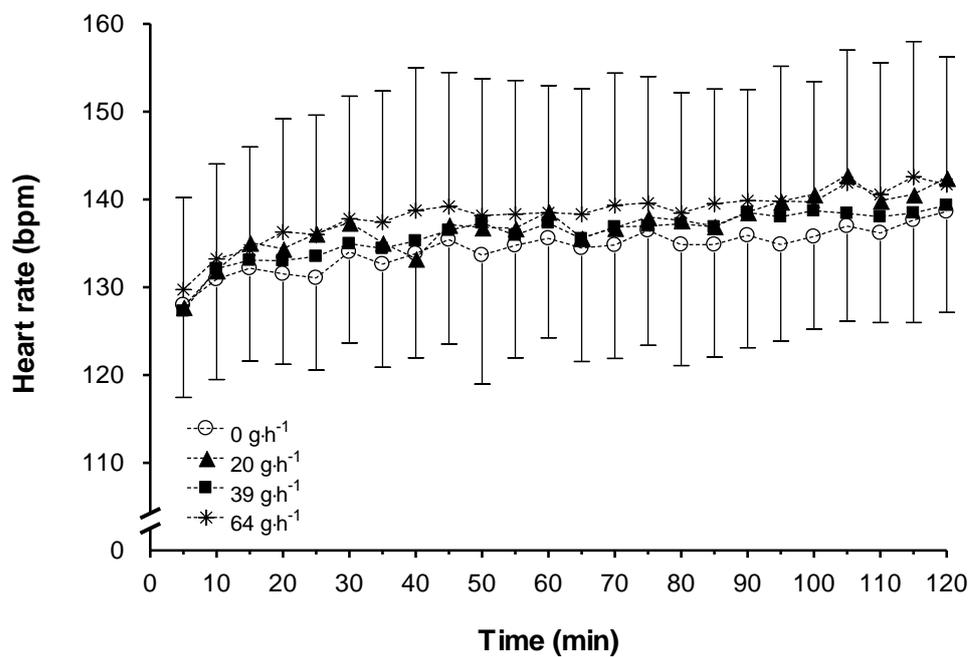


Figure 3.15. Mean (SD) heart rate during the 2 h sub maximal exercise bout whilst consuming 0, 20, 39 and 64 g·h⁻¹ of CHO.

3.4.5 Subjective measures

Rate of perceived exertion

There was a significant effect of time ($p < 0.01$) and period ($p = 0.02$), but not treatment ($p = 0.83$) or interaction ($p = 0.94$) effects on RPE response to exercise. Period was treated as a covariate for all further comparisons. Post hoc comparisons indicated that RPE scores increased with increasing exercise duration (Figure 3.16).

Feeling scale

Analysis of feeling scores indicated a significant effect of time ($p < 0.01$) but there was a trend for treatment ($p = 0.051$) effect, and no effect of period ($p = 0.062$) or interaction ($p = 0.95$). Pairwise comparisons of time indicated that feeling scores increased with increasing exercise duration (Figure 3.17).

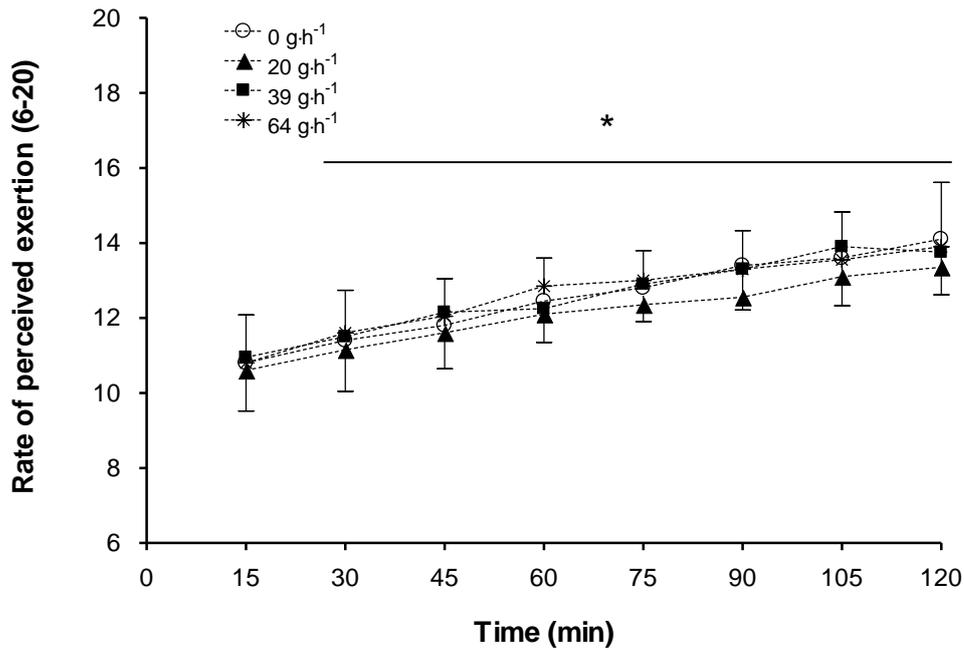


Figure 3.16. Mean (SD) rate of perceived exertion during submaximal exercise while consuming 0, 20, 39 and 64 g·h⁻¹ of CHO. * indicates a significant time effect whereby all RPE scores were significantly ($p < 0.01$) elevated above the 15 min time point.

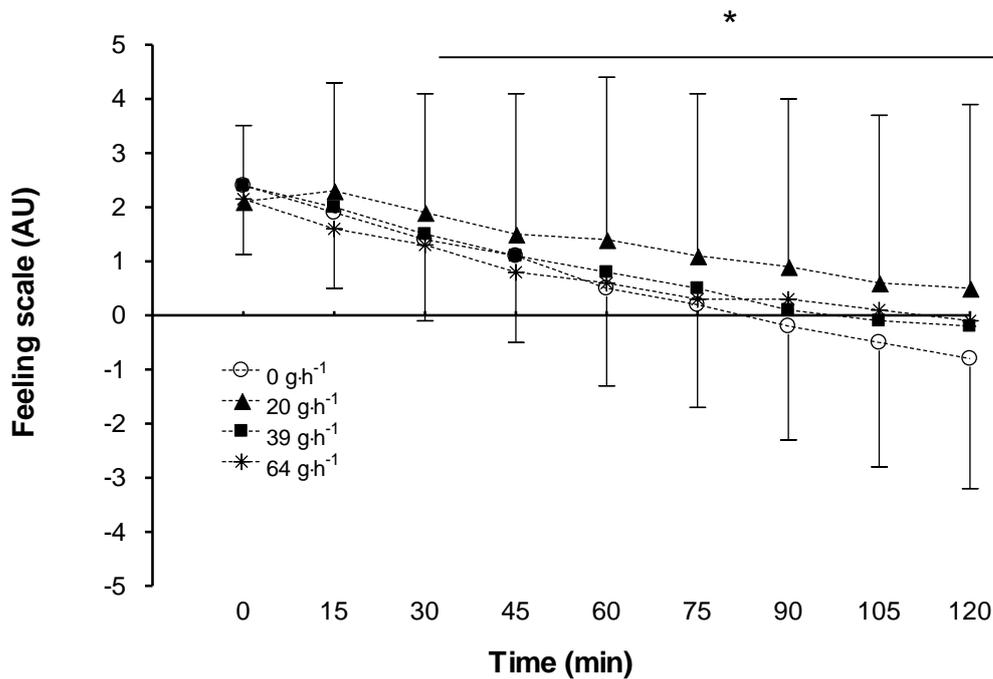


Figure 3.17. Mean (SD) feeling scores during submaximal exercise while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. * indicates a significant effect of time indicating that feeling scores at 30 minutes onward were significantly lower than scores at 0 min.

3.5 DISCUSSION

During this investigation we aimed to primarily characterise the metabolic response of trained cyclists to the ingestion of graded amounts of CHO during a 2 h submaximal ride. The study was designed in an attempt to explain the differences in performance outcomes reported in Chapter 2. We observed that increasing rates of CHO ingestion (particularly at 39 and 64 g·h⁻¹ ingestion rates) during non-exhaustive submaximal exercise resulted in: 1) increased circulating blood glucose and insulin concentration, 2) Increased rate of plasma glucose turnover, 3) increased rate of total carbohydrate oxidation, 4) Increased contribution of exogenous CHO oxidation to total energy contribution, and 5) a reduction in the contribution of endogenous carbohydrate stores to total energy provision. These significant alterations in fuel selection with the ingestion of 39 and 64 g·h⁻¹ of CHO compliment the enhancement in performance previously reported in Chapter 2. In addition, the ingestion of 64 g·h⁻¹ had no added effect on the key metabolic responses to exercise, other than an increased rate of exogenous substrate oxidation, in comparison to the 39 g·h⁻¹ treatment. Taken together these findings indicate that consuming 39 g·h⁻¹ of CHO may be an optimal ingestion strategy for trained cyclists.

Carbohydrate ingestion during prolonged non-exhaustive exercise has been previously reported to maintain blood glucose at a higher concentration for longer in comparison to ingestion of a non-CHO control (Coyle, et al 1986; Coggan and Coyle 1987; Jeukendrup 2004). Generally, higher CHO ingestion rates maintain plasma glucose concentration at a higher level than with lower ingestion rates (Smith, et al 2010). In the present investigation mean plasma glucose concentration was significantly elevated when consuming 39 and 64 g·h⁻¹ in comparison to the 0 g·h⁻¹ control and 20 g·h⁻¹ treatments. However, there was no significant difference in plasma glucose concentration between the two highest ingestion rates. These observations are similar to that of Smith et al (2010) who observed an increase in blood glucose concentration of 0.2 mmol·L⁻¹ with 15 g·h⁻¹ of

CHO administration and more marked increases of 0.4 and 0.6 mmol·L⁻¹ when ingesting 30 and 60 g·h⁻¹ in the second hour of 2 h of exercise, in comparison to a 0 g·h⁻¹ control. Similarly, Wallis et al (2007) observed that consuming 30 g·h⁻¹ of CHO during a 2 h submaximal (50% $\dot{V}O_{2max}$) ride resulted in a lower plasma glucose concentration towards the end of exercise when compared to ingesting 60 and 90 g·h⁻¹. In both these examples and our observations there is a plateau in the increase in blood glucose concentration with CHO ingestion rates greater than 30 g·h⁻¹ reaching 6 mmol·L, suggesting that changes in substrate delivery, the hormonal response, and/or substrate oxidation combine to establish a new homeostasis. The regulation of the magnitude of increase in blood glucose concentration with feeding rate is likely to be facilitated by insulin to help maintain blood glucose within the normal range.

Many investigators have observed a marked increase in plasma insulin concentration with the ingestion of CHO during submaximal exercise and a subsequent alteration of fuel utilisation (Smith, et al 2010; Wallis, et al 2007; Jeukendrup, et al 1999). We report that a minimum ingestion rate of 39 g·h⁻¹ is required to observe a significant increase in plasma insulin concentration in comparison to the control. However, no further significant increase in plasma insulin concentration was observed when comparing 64 g·h⁻¹ and 39 g·h⁻¹ ingestion rates. The significant increases in insulin concentration when consuming 39 and 64 g·h⁻¹ of CHO also coincided with a significantly lower: NEFA concentration (Figure 3.13A), total fat oxidation during the second hour of exercise (Figure 3.7), and lower hepatic glucose output (Figure 3.13). Consuming 39 g·h⁻¹ appears to be the minimum ingestion rate required to elicit an insulin response sufficient to alter both lipolysis and hepatic glucose output and have a significant positive effect on performance (Chapter 2). The 20 g·h⁻¹ treatment reduces hepatic glucose output from estimates made (figure 3.9) but the performance outcome reported in Chapter 2 is too variable for it to be a significant improvement in performance. Recently, Smith et al (2010) reported 60 g·h⁻¹ of CHO

during exercise was required to significantly increase insulin concentration during the first 30 minutes of ingestion when compared to 15 g·h⁻¹ and placebo treatments (Smith, et al 2010). In the present investigation we highlight the threshold effect of consuming 39 g·h⁻¹ on insulin production. Any additional CHO ingestion (64 g·h⁻¹) has no further significant increase in insulin production. Additionally, elevated insulin concentration when ingesting 39 and 64 g·h⁻¹ indicates that plasma glucose regulation through its disposal from the plasma pool is insulin dependent at higher ingestion rates (Jeukendrup, et al 1999). An elevated insulin concentration when consuming 39 and 64 g·h⁻¹ vs 0 g·h⁻¹ from 60min onwards indicates that glucose disposal from contraction mediated mechanisms at CHO ingestion rates does not appear to be solely sufficient to regulate plasma glucose concentration. As such, insulin plays a much wider systemic role and as such has significant effects on the site specific disposal of circulating plasma glucose.

By utilising stable glucose isotopes researchers have been able to quantify the movement of glucose into and out of the plasma pool during exercise when carbohydrate is consumed (Wolfe 1992). During exercise, blood glucose can be maintained or increased by the augmented release of glucose from the liver. Ingested CHO can also regulate plasma glucose concentration. Furthermore, exercise intensity is a key driver of the rate of glycogen breakdown in the liver (Coggan and Coyle 1987; Zinker, et al 1993; Romijn, et al 1993; Bosch, et al 1994). In the present investigation we observed a significant increase in glucose Ra with each increase in CHO ingestion rate at all-time points after 60 min of exercise. The peak glucose Ra occurred with the highest ingestion rate. Glucose Ra was closely matched by glucose Rd throughout the 2 h bout. The comparable Rd indicates that the glucose entering the plasma pool was also disposed of at a similar rate irrespective of the CHO ingestion rate. We have also demonstrated that increasing CHO ingestion suppresses estimated liver glucose output when calculated from the difference between total Rd and the exogenous oxidation rates of the ingested CHO.

Estimations of hepatic glucose output in the current investigation show that all CHO ingestion rates reduced hepatic glucose output. However, higher ingestion rates of 39 and 64 g·h⁻¹ of CHO both suppress hepatic glucose output to a greater extent than the 20 g·h⁻¹ treatment. The metabolic fate of glucose during exercise with CHO ingestion of various rates has been well defined (Coggan, et al 1995; Jeukendrup, et al 1999; Rose and Richter 2005) with most studies observing a matching of the Rd glucose and the rate of plasma glucose oxidation. Jeukendrup et al (1999a) reported that 96-100% of glucose Rd is accounted for by plasma glucose oxidation when ingesting both 36 and 180 g·h⁻¹ of CHO. However, there was a greater amount of glucose contributed from the liver in the lower ingestion rate where the higher ingestion rate was almost exclusively metabolism of the ingested glucose. Here we report that consuming 39 g·h⁻¹ of CHO suppresses liver glucose output to a similar extent as consuming 64 g·h⁻¹ treatment, thereby conserving liver glycogen content which has been positively associated with improvements in subsequent endurance performance (Casey, et al 2000). The lack of any additional performance improvement between 39 and 64 g·h⁻¹ from Chapter 2 may therefore be partly explained due to the absence of any additional preservation of liver glycogen between these two trials. The matching of the insulin response with the two highest ingestion rates indicates that the insulin response stimulated is inherently linked to whole body regulation.

The movement of glucose out of the plasma pool is likely facilitated by the increased insulin concentration when ingesting 39 and 64 g·h⁻¹ of CHO. The majority of glucose Rd during exercise with CHO ingestion is reported to be directly into the muscle where it is oxidised (Jeukendrup et al 1999a) or disposed of by a non-oxidative means (glycogen synthesis). However, it is likely that glucose disposal into the muscle from contraction mediated up regulation of GLUT-4 translocation at the prescribed intensity alone is not sufficient to fully dispose of all glucose entering the plasma pool with higher CHO ingestion

rates (Jensen and Richter 2012). Typically, insulin facilitates glucose disposal through storage of glucose as glycogen in both the liver and the muscle. As nearly 100% of plasma glucose Rd is explained by plasma glucose oxidation, storage seems unlikely under these circumstances. Though, not all studies report such high percentages of plasma glucose oxidation from glucose Rd (Roberts, et al 1997; Colberg, et al 1994). The elevated insulin response with higher ingestion rates coupled with a discrepancy between glucose Rd and plasma glucose oxidation may be explained by glycogen synthesis in the liver (and/or contracting muscle) facilitating the regulation of blood glucose concentration. There are methodological issues associated with the execution of the stable isotope method in the studies that do not match glucose Rd with plasma oxidation which is a strong consideration in the interpretation of these data (Jeukendrup et al 1999). Nevertheless, when an estimation of the contribution of liver glucose to total Ra is made we find that absolute ingestion rate of the 64 g·h⁻¹ treatment exceeds the total glucose Ra. Therefore, it is possible that glycogenolysis is not only blunted when consuming high rates of CHO but that the consumed CHO contributed to glycogen synthesis from the surplus ingested glucose provided. As such, the elevated insulin concentration observed when consuming 39 and 64 g·h⁻¹ of CHO is likely required to regulate plasma glucose concentration by facilitating transport into the muscle and by glycogen synthesis in the liver and muscle (Nielsen and Wojtaszewski 2004). Additionally, regardless of whether or not there was increased glycogen synthesis when consuming 64 vs 39 g·h⁻¹, this did not translate into an additional improvement in performance (Chapter 2). Collectively, the increase in glucose Ra/Rd, elevated plasma glucose concentration, and increase in circulating insulin concentration with CHO ingestion have also been attributed to increasing whole body CHO oxidation during exercise.

Increasing the ingestion rate of CHO during submaximal exercise has been reported to augment whole body CHO oxidation rates (Coyle, et al 1986; Coggan and Coyle 1987;

Bosch, et al 1994). Other studies indicate that the maintenance of CHO oxidation with CHO feeding also increases exercise performance. In the present investigation whole body CHO oxidation in the second hour of exercise was significantly elevated when consuming 39 and 64 g·h⁻¹ of CHO in comparison to the control treatment. Consuming 20 g·h⁻¹ of CHO had no effect on total CHO oxidation rates when compared to the control. Increased plasma glucose concentration, increased glucose disposal, and elevated insulin concentration are key regulators of fuel utilisation during exercise and are likely facilitating the increased reliance on CHO for fuel provision with higher ingestion rates. These metabolic changes, coupled with the reduction in whole body fat oxidation when consuming 39 and 64 g·h⁻¹ demonstrates a shift in substrate preference to CHO oxidation. Ingesting 64 g·h⁻¹ in comparison to 39 g·h⁻¹ did not further increase whole body CHO oxidation. Of note, only considering the total amount of CHO oxidised fails to acknowledge the source of the CHO being utilised and thereby limits any interpretation and provides an oversimplified view of whole body fuel selection.

Exogenous CHO oxidation rates increase when CHO is ingested but only up to ingestion rates of ~ 60 g·h⁻¹ when a single source CHO is ingested (Jeukendrup, et al 1999; Jentjens, et al 2005; Harvey, et al 2007). Any additional increases in exogenous CHO oxidation rates require the ingestion of multiple forms of CHO in the form of glucose and fructose monomers (Currell and Jeukendrup 2008). Single source CHO ingestion at rates ≤60 g·h⁻¹ results in a stepped increase in exogenous CHO oxidation with increasing CHO ingestion rates from 15 to 60 g·h⁻¹ (Smith 2010). Smith et al (2010) observed peak exogenous CHO rates of 0.22, 0.39 and 0.65 g·min⁻¹ at the end of a 2 h submaximal ride when consuming 15, 30, and 60 g·h⁻¹. These EXO oxidation rates contributed significantly to total energy expenditure from EXO CHO sources in the second hour of exercise by 3.9, 7.8 and 12.4% respectively. Furthermore, the increase in exogenous oxidation rate reported was matched with an increase in exercise performance following the 2 h

submaximal exercise bout with the ingestion of 15, 30 and 60 g·h⁻¹. These results suggest that the ingestion of CHO during submaximal exercise increases exogenous CHO oxidation rates which may spare endogenous CHO stores. Any sparing of endogenous stores would make them available during a subsequent maximal performance task by helping to maintain CHO oxidation rates.

The results of the present investigation are in good agreement with Smith et al (2010) with stepped peak exogenous CHO oxidation rates of 0.38, 0.59, and 0.70 g·min⁻¹ when ingesting 20, 39 and 64 g·h⁻¹ of CHO respectively. The exogenous CHO percentage contributions to total energy expenditure (8.9, 14.6, and 20.1% for 20, 39 and 64 g·h⁻¹ respectively) also increased in a graded manner. However, the increase in exogenous CHO oxidation rate with higher CHO consumption did not correspond to a subsequent improvement in exercise performance as reported in Chapter 2. Consuming 39 and 64 g·h⁻¹ of CHO significantly increased exogenous CHO oxidation rates above that of the 20 g·h⁻¹, but only improved performance above that of the control condition. Therefore, increasing CHO ingestion increases total CHO oxidation which is driven by elevated exogenous CHO oxidation rates. Better maintenance of CHO oxidation in the second hour of exercise, and an increase in the contribution of EXO CHO to CHO oxidation rates when ingesting 39 and 64 g·h⁻¹ of CHO, resulted in an improvement in performance as reported in Chapter 2. Though, no clear link between the highest exogenous CHO oxidation rates measured and subsequent performance has been made (Karelis, et al 2010; Cermak and van Loon 2013). Some have speculated that an increase in the exogenous CHO oxidation rate reduces the amount of endogenous glycogen stores utilised for energy production during exercise. There may be a ceiling effect reached when consuming 39 g·h⁻¹ of CHO which may reflect the ideal balance of substrate provision for an increase in CHO oxidation and subsequent liver glycogen sparing.

It seems clear that CHO provision during submaximal exercise leads to a reduction in the oxidation of endogenous glycogen which has been observed to occur in a dose response manner i.e. the more CHO provided the greater the sparing that occurs (Jeukendrup et al 1999, Smith et al 2010). The majority of recent observations report a reduction in the contribution of liver glycogen to total energy expenditure with CHO ingestion (Cermak et al 2013). Few studies report a reduction in muscle glycogen use though some have reported this in running exercise, and in specific fibre types but debate still exists in the literature. Very high ingestion rates of CHO ($180 \text{ g}\cdot\text{h}^{-1}$) have been reported to completely suppress hepatic glucose output, with CHO ingestion reducing the endogenous contribution to total energy expenditure (Jeukendrup 1999). Lower CHO ingestion rates result in a more modest endogenous CHO sparing (Jeukendrup 1999, McConnell et al 1994, Smith 2010). In the present investigation we observed a significant reduction in the contribution of endogenous CHO to total energy expenditure of 7 and 11 % when consuming 39 and $64 \text{ g}\cdot\text{h}^{-1}$. No significant sparing of endogenous CHO occurred when consuming $20 \text{ g}\cdot\text{h}^{-1}$ whereas ingesting $64 \text{ g}\cdot\text{h}^{-1}$ of CHO did not result in any further significant sparing of these stores over $39 \text{ g}\cdot\text{h}^{-1}$ contradicting these previous studies. Ingesting $39 \text{ g}\cdot\text{h}^{-1}$ of CHO is the minimum ingestion rate required to elicit a significant reduction in the contribution of endogenous CHO stores to total energy provision. Also, $39 \text{ g}\cdot\text{h}^{-1}$ may be a threshold above which additional CHO may be unnecessary for a ride lasting <3hrs.

The alteration of fuel provision with increasing CHO ingestion may be explained in a number of ways. It is possible the increase in insulin concentration was only sufficient when ingesting at least $39 \text{ g}\cdot\text{h}^{-1}$ to facilitate the endogenous CHO suppression observed. Though the lack of any further small non-significant increase in insulin concentration when ingesting $64 \text{ g}\cdot\text{h}^{-1}$ may not be sufficient for additional endogenous CHO suppression to occur. Additionally, no further increase in circulating blood glucose concentration was

observed when consuming $64 \text{ g}\cdot\text{h}^{-1}$ in comparison to the $39 \text{ g}\cdot\text{h}^{-1}$ which may also explain the lack of any additional endogenous CHO sparing. Alternatively any additional increase in insulin concentration when ingesting $64 \text{ g}\cdot\text{h}^{-1}$, whilst not significant in comparison to consuming $39 \text{ g}\cdot\text{h}^{-1}$, may have been sufficient enough to blunt lipolysis and therefore fatty acid oxidation.

The blunting of fat oxidation subsequently drives fuel utilisation towards a CHO substrate dependant state. In support of this hypothesis other researchers have reported the suppression of adipose tissue lipolysis increases glycogen utilisation in exercising humans (van Loon, et al 2005). Additionally the higher rates of exogenous CHO oxidation in the present study at $64 \text{ g}\cdot\text{h}^{-1}$ lends some additional support to this as the ingested CHO is oxidised rather than contributing to storage (figure 3.6). Of note is the relatively modest increase in CHO ingestion between 39 and $64 \text{ g}\cdot\text{h}^{-1}$ in comparison to considerably higher ingestion rates that do show an increase in endogenous sparing (Jeukendrup, et al 1999). Consuming $20 \text{ g}\cdot\text{h}^{-1}$ of CHO did not significantly increase insulin or glucose concentrations. The lack of an increase in these two parameters ultimately resulted in no change in the contribution of energy provision in the second hour of exercise despite a decrease in contribution of the liver glycogen to total glucose Ra. As such, for significant endogenous glycogen sparing that results in improved performance a minimum ingestion of $39 \text{ g}\cdot\text{h}^{-1}$ is required to increase: plasma glucose and plasma insulin concentration, whole body CHO oxidation rate, and exogenous CHO rate.

3.6 CONCLUSIONS

Researchers have been aiming to identify the optimal ingestion rate of CHO to elicit the greatest improvements in performance. From Chapter 2 we reported that ingesting 39 and $64 \text{ g}\cdot\text{h}^{-1}$ of CHO were equally effective at improving endurance performance in comparison to a control condition. From the data presented in this chapter we have

observed that the ingestion of $39 \text{ g}\cdot\text{h}^{-1}$ of CHO sufficiently alters substrate usage during a submaximal exercise bout leading to the preservation of endogenous glycogen stores, most likely from a hepatic source. The ingestion of less ($20 \text{ g}\cdot\text{h}^{-1}$) is not sufficient for these changes to occur while the ingestion of $64 \text{ g}\cdot\text{h}^{-1}$ does not appear to have any additional benefit. A lack of any additional change when consuming $64 \text{ g}\cdot\text{h}^{-1}$ in these metabolic measures is likely responsible for a lack of any additional improvement in performance on this trial, as reported in Chapter 2. From these observations we would conclude that an ingestion rate of $39 \text{ g}\cdot\text{h}^{-1}$ is the minimum, or possibly optimal ingestion rate, required to elicit a sufficient alteration in fuel provision during submaximal exercise to elicit a subsequent beneficial impact on exercise performance over water alone. The findings of this investigation are confined to exercise durations lasting $<3 \text{ h}$.

CHAPTER 4

Neuromuscular

responses to varying the

ingestion rate of

carbohydrate during

exercise

4.1 ABSTRACT

Recent research has indicated a key role of endogenous CHO sensing and oral sensing maintaining central drive and peripheral function during endurance exercise tasks. In Chapter 2 we observed a significant improvement in cycling performance when consuming 39 and 64 g·h⁻¹ of CHO. The improvement in performance coincided with significant alterations in whole body substrate usage that lead to endogenous CHO sparing at the same ingestion rates. We aim to utilise gold standard neuromuscular function assessment techniques, alongside new novel measures, to investigate the effect of consuming different rates of CHO on neuromuscular function during prolonged cycling exercise. In a double-blind, randomised cross-over design, well-trained male cyclists (n = 20, mean ± SD, age 34 ± 10 y, mass 75.8 ± 9 kg, peak power output 394 ± 36 W, $\dot{V}O_{2max}$ 62 ± 9 ml·kg⁻¹·min⁻¹) completed 2 familiarisation trials then 4 experimental trials. Each trial involved pre and post exercise assessments (maximal voluntary contractions (MVC), M-wave twitch potentiation and force, motor unit recruitment and firing rate assessment) and during exercise (gross EMG amplitude). The exercise bouts consistent of 2 h submaximal cycling (95% lactate threshold, 185 ± 25 W) during which one of three CHO beverages, or a control (water), were administered every 15 min, providing participants with 0, 20, 39 or 64 g CHO·h⁻¹ at a fixed fluid intake rate of 1 L·h⁻¹. The 2h bout was followed by a maximal time trial task. There was no effect of CHO ingestion on any of the pre to post exercise measures conducted. MVC peak torque values reduced post exercise by -20.4 (95%CI: -26.5 to -14.4) in comparison to pre value. The firing rates of early recruited motor units significantly increased by 1.55 pps (95%CI: 0.51 to 2.59) following exercise in comparison to pre-exercise rates. Gross EMG during the 2 h cycling bout revealed a main effect of treatment (p<0.01) but post hoc comparisons provided no clarity. In conclusion consuming CHO at ingestion rates between 20 and 64 g·h⁻¹ has little to no impact on the neuromuscular function of well-trained cyclists when comparing pre and post exercise

values. There does not appear to be a neuromuscular explanation for the improvement in performance observed when consuming 39 and 64 g·h⁻¹.

4.2 INTRODUCTION

Metabolic factors that regulate fuel provision during submaximal exercise (Chapter 3) provide some explanations for improved performance observed when consuming 39 and 64 g·h⁻¹ of CHO (Chapter 2). Specifically, the sparing of endogenous CHO stores in the liver may go some way to explaining any improvement observed. However, ingesting CHO during endurance exercise to improve performance has a greater physiological impact than just the traditional metabolic outcomes (Chapter 1). In chapter 1 we presented emerging evidence detailing the impact that CHO ingestion has on muscle function such as: preservation of neuromuscular drive to the muscle attenuating muscular fatigue (Nikolopoulos, et al 2004), effect of CHO sensing within the oral cavity having a direct effect on the central nervous system (Carter, et al 2004), and potential evidence for an endogenous CHO 'glycostat' enabling feedback of endogenous CHO stores to regulate neuromuscular drive (Rauch, et al 2005). All of these actions of CHO ingestion have been reported to result in delayed fatigue or increased sustainable exercise intensity leading to improved performance or increased exercise capacity. However, limited evidence exists to explain whether there is an optimal rate of CHO ingestion to impact upon neuromuscular function.

Evidence has emerged that there is intrinsic sensing of endogenous glycogen stores that regulates exercise intensity. Rauch et al (2005) manipulated pre exercise muscle glycogen levels by providing a CHO loading diet. The loading significantly increased muscle glycogen content from 104 ± 11 to 151 ± 9 mmol·kg⁻¹ wet weight. Participants then completed a 2 h steady state ride followed by a 1 h maximal time trial; the authors reported improved performance when participants were CHO loaded. Most participants (7 of 8) were able to adjust their pacing strategy so they finished the performance task with remarkably similar muscle glycogen levels irrespective of the starting muscle glycogen content. The biggest difference between finishing muscle glycogen content between high

and low pre exercise muscle glycogen was 6 mmol·kg wet weight. The authors therefore suggested existence of a metabolic signal from the exercising muscle. They posit that this signal is sensed by the CNS to determine an optimal pacing strategy specific to the endogenous glycogen availability, a phenomenon they termed the 'glycostat'. Put simply, sensing of low glycogen stores could feed back to the CNS to alter neuromuscular recruitment and thus regulate exercise intensity – something that appears to be occurring in the pacing data shown in Figure 2.7 Chapter 2.

In Chapter 3 we observed a significant sparing of liver glycogen stores with all ingestion rates between 20 and 64 g·h⁻¹. Higher rates of CHO ingestion (39 and 64 g·h⁻¹) resulted in a greater sparing of liver glycogen sufficient enough to improve exercise performance during a 35 min task. In the study by Rauch et al (2005) participants consumed 60 g·h⁻¹ of glucose during the 2 h submaximal ride to prevent hypoglycaemia during the exercise task. The glycogen sensing signal was thought to be specific to the muscle but the study design did not measure liver glycogen content. Casey et al (2000) identified the importance of both liver and muscle glycogen stores on exercise capacity. Following prolonged endurance exercise (83 min at 70% $\dot{V}O_{2max}$) participants consumed a CHO beverage of 1 g·kg⁻¹ BW to resynthesise endogenous glycogen stores and rested for 4 h before completing a TTE task at 70% $\dot{V}O_{2max}$. The authors observed a modest but significant correlation ($r=0.55$, $p<0.05$) between post recovery liver glycogen content and time to exhaustion when exercise was recommenced. Increased TTE of ~10 min with glucose ingestion suggests that CNS sensing of the re-synthesised liver glycogen stores must have occurred which may have facilitated the increase in exercise capacity participants were able to sustain.

Taking these studies together, if such a glycostat exists in muscle, then sensing of liver glycogen to regulate exercise exertion during prolonged endurance cycling is quite possible. Coupled with the observation that liver glycogen sparing with higher CHO

ingestion rates (Chapter 3) may explain the improvement in performance (Chapter 2). However, investigating to what extent graded amounts of CHO ingested would have on neuromuscular function has not yet been determined.

To facilitate our understanding of contractile properties of muscles or muscle groups, following a fatiguing exercise protocol, new technologies have been developed to explain the impact that any intervention has had on neuromuscular function (De Luca 2008; De Luca and Contessa 2012). Stock et al (2012) have used this new technique to good effect to observe a decrease in the mean firing rate of motor units recruited at 50% of MVC following a prolonged protocol of fatiguing isometric MVC contractions. As such, prolonged exercise consistently alters the recruitment patterns of the active muscle providing a sensitive measure of motor unit firing rate.

During prolonged steady state cycling at a fixed submaximal intensity researchers have observed significant increases in sEMG, and therefore the total number of motor units recruited, over time (Abbiss, et al 2008; St Clair Gibson, et al 2001). The increase in sEMG observed during prolonged exercise can be attenuated with sufficient provision of CHO (8 ml·kg⁻¹ of a 6.4% carbohydrate followed by 2 ml·kg⁻¹ of the same solution every 15 min during exercise) during endurance exercise bouts of ~1 h (Nikolopoulos, et al 2004). However, it is not clear if CHO provision is the determining explanation for preventing increased sEMG amplitude. We would expect that increasing CHO ingestion rates would increase delivery of CHO to the working muscle and delay the onset of the increased sEMG (Jeukendrup, et al 1999; Abbiss, et al 2008) based on the findings of chapter 3. Though, a clear determination of the effect of different CHO ingestion rates on sEMG is yet to be established.

The aim of the present chapter was to assess the neuromuscular response to prolonged endurance cycling when ingesting CHO at different rates by investigating how motor unit

activation characteristics changed following prolonged exhaustive endurance exercise. Additionally, we investigated time course changes in gross motor unit recruitment during a 2 h submaximal cycling bout whilst feeding CHO at different rates. Based on the findings presented in chapter 2 we hypothesised that consuming 39 and 64 g·h⁻¹ would result in the preservation of neuromuscular function in comparison to consuming no CHO at all.

4.3 METHODS

4.3.1 Participants

The same twenty well-trained male cyclists described in section 1.7 took part in this dataset.

4.3.2 Experimental protocol

In a double blind, placebo controlled, randomised cross-over study design participants visited the laboratory 6 times (2 preliminary and 4 intervention) over a six week period (Figure 1.1). They completed one visit per week commencing each trial on the same day at the same time of day on each visit. Following pre-screening, participants completed a preliminary assessment as described in section 3.3.2. On the second visit participants completed a full familiarisation trial. The familiarisation trial and four subsequent intervention trials were identical and involved participants completing a peripheral stimulation protocol for the determination of M-wave, 3 maximal voluntary isometric MVC, and a controlled 20 sec progressive submaximal contraction. Following these initial assessments participants completed a 120 min steady submaximal cycle at 95% lactate threshold (184 ± 25 W). During the cycling bout water was ingested for the familiarisation trial and consumed at a rate of $1 \text{ L}\cdot\text{h}^{-1}$. Thereafter, on the intervention trials participants consumed in a random order either: a control (water) 0%, 2%, 3.9% or 6.4% CHO solutions at a fluid ingestion rate of $1 \text{ L}\cdot\text{h}^{-1}$. During the 2 h bout gross EMG measures were captured from the vastus lateralis muscle for 30 sec every 15 min. The preload ride was followed by a time trial performance task (described in section 2.3.4) to induce exhaustive fatigue, whereupon the participants were instructed to be complete their set work target as quickly as possible. Following the time trial task participants the repeated the same pre exercise neuromuscular assessments. All post cycling measures were completed in the same order.

4.3.3 Pre exercise

On arrival to the laboratory participants emptied their bladder and bowel prior to nude body mass measurements. Individuals then changed into cycling attire, which was kept consistent throughout all trials to reduce thermoregulatory variability. A pair of EMG electrodes (VERMED A10005-60 performance plus ECG diagnostic electrodes, Vermont, USA) were placed on the vastus lateralis of the participant's right leg according to SENIAM guidelines. Skin preparation involved removal of hair, cleaning of the skin with alcohol swabs, and abrasion with emery paper. A reference electrode was positioned on the elbow of the participant's right arm and secured with micropore tape. A bipolar electrode configuration was used in accordance with the surface EMG for the non-invasive assessment of muscles guidelines (Hermens et al., 2000). Following electrode placement participants were seated in the chair of a dynamometer with their right leg performing all measures in the trial.

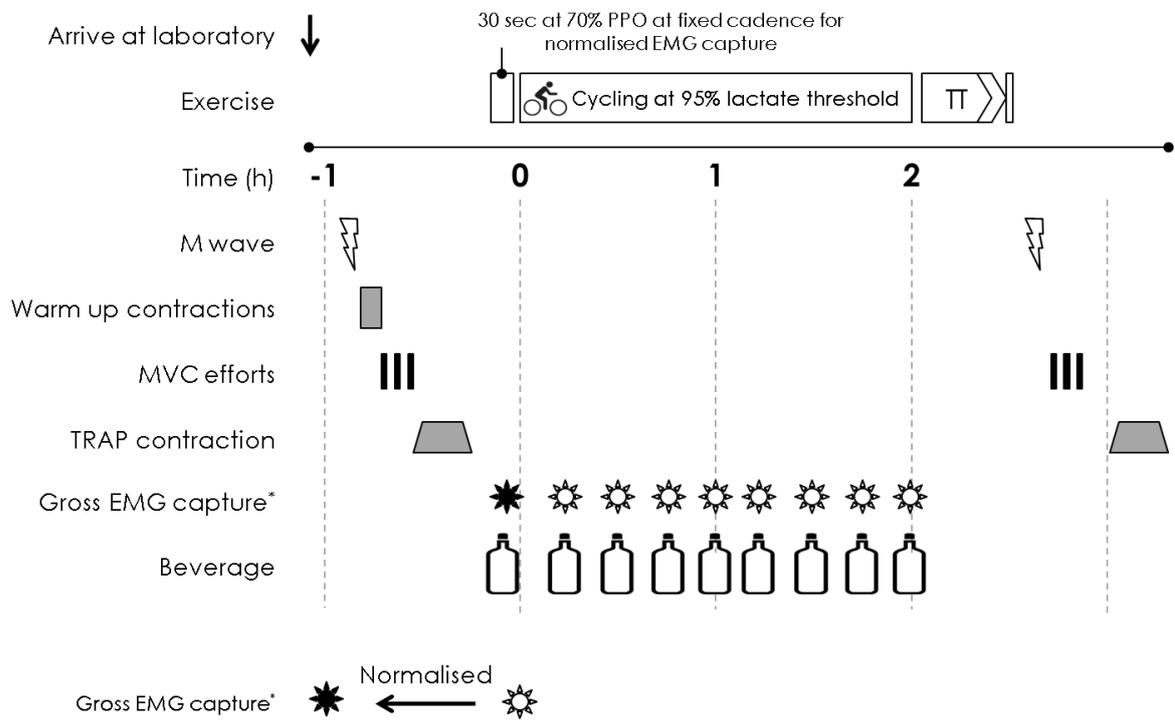


Figure 4.1 Timeline of measures during treatment visits.

4.3.4 M-wave assessment

The stimulation protocol was performed with the participant's right leg at 60° of knee flexion (0° equalling full extension; Biodex 3 dynamometer, Biodex Medical Systems, Shirley, New York, USA). Participants were secured in the dynamometer chair with single straps across the upper left leg to minimise movement. The dynamometer position (axis, seat, and attachment settings) were recorded during the familiarisation visit and repeated across each trial day for each participant. The lateral femoral epicondyle was positioned in line with the dynamometer axis and the dynamometer attachment strap was positioned above the lateral malleolus. For delivery of the electrical stimulation the positive electrode was placed on the iliofemoral fold 1 cm lateral to the femoral artery. The position was determined by detecting the pulse in the femoral artery and moving 1 cm laterally to this point. The electrode was secured in place with a metal pressure algometer with pressure applied by the participant themselves at a 90° angle to the torso. A second electrode (dermatrode, 5 cm diameter) was placed centrally on the gluteus maximus to close the circuit. The stimulation protocol commenced with the lowest current amplitude applied and progressively increased until the electrical stimulation delivered no longer caused an increase in the force produced. A 15 sec recovery period separated each stimulation induced to avoid any potentiation effects.

4.3.5 Maximal Voluntary contractions (MVC)

Participants were asked to perform a 5 sec dynamometer-based knee extension MVC. Initially participants completed a standardised warm-up procedure consisting of six 5 sec isometric contractions of which three were performed at a self-determined intensity of 50% and three at 75% of perceived maximum effort. Each contraction was followed by a 30 sec recovery periods. Three MVCs were performed with the participant's right leg at 60° of knee flexion (0° equalling full extension; Biodex 3 dynamometer, Biodex Medical Systems, Shirley, New York, USA). A one min recovery period separated every maximal

isometric effort. During MVCs participants were secured in the dynamometer chair with straps across the upper body, waist, and non-dominant leg to minimise movement. Participants were asked to contract maximally as quickly as possible from the start of the MVC and were strongly verbally encouraged throughout all contractions to ensure maximal efforts were achieved (Campenella, et al 2000). MVC measures were repeated following the performance task with the identical protocol described above. No warm-up contractions were completed on the post exercise measures due to the short transfer time from the cycle ergometer to the Biodex following the endurance exercise performance task.

4.3.6 Submaximal trapezoidal contractions (TRAP)

TRAP contractions consisted of a 3 sec rest period, a 7 sec linear ramp up from 0 to 70% of pre-exercise MVC force, a 10 sec steady state force period maintaining 70% of MVC with visual feedback to aid in maintaining consistent force production, and finally a 7 sec linear reduction in force from 70 to 0 % of pre MVC force production followed by a 3 sec rest period (figure 4.2). Participants achieved the required force production via a live visual force feedback provided on a computer monitor. Participants were verbally encouraged to ensure they attained the correct force level and maintained it during the steady state phase. Previous investigations utilising a similar technique have been limited to MU detection at much lower force levels (around 30%) (Fling, et al 2009) but can now be accurately measured up to 100% (Naweb et al 2010). The higher isometric force generated during the TRAP contraction would allow the effect of CHO ingestion and endurance exercise to be examined on a larger range of motor units. One-minute recovery periods separated MVC and TRAP efforts. Both types of isometric efforts were performed at a knee joint flexion angle of 60° (full extension equalling 0°), this angle was selected as it has previously been demonstrated as optimal for peak isometric force

production (Knapik, et al 1983). The M-wave, MVC and TRAP measures described were all performed again following the time trial task.

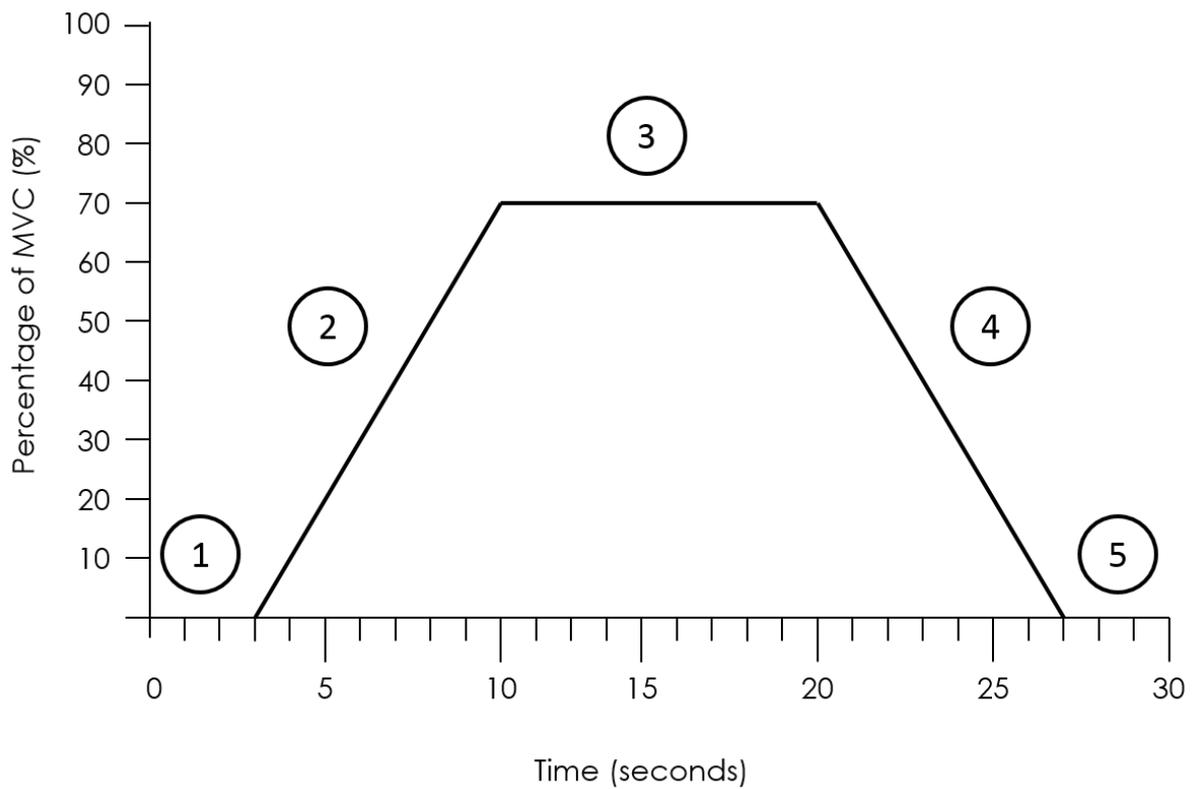


Figure 4.2 Submaximal trapezoid force trace that participants were required to follow during their submaximal contraction. 1. 3 sec rest period for baseline collection, 2. Linear increase in force production from 0 to 70% of pre exercise MVC, 3. Ten sec steady state isometric hold, 4. Linear reduction in force from 70% to 0% of pre exercise MVC force, 5. Final resting position.

4.3.7 Immediately pre exercise

Participants positioned themselves onto the cycle ergometer (Lode Excalibur, Netherlands) and were asked to cycle for 45 sec at 70% of PPO at a fixed self-selected cadence. The self-selected cadence was determined from the average cadence they sustained during a 30 min pretesting ride described in section 1.9. The cadence was recorded and participants were asked to maintain this cadence for all dynamic gross EMG captures thereafter. All EMG signals obtained during the 2 h pre load ride were normalised to the activity recorded at 70% of PPO captured prior to exercise.

4.3.8 Exercise protocol

The exercise protocol was identical that that already described in section 1.11. Briefly participants completed the cycling exercise bout at 95% of the predetermined lactate threshold for 2 h. Throughout this submaximal ride 30 sec EMG captures were performed every 15 min. Participants were asked to hold their cadence at their predetermined preferred cadence whilst the EMG captures were being performed.

4.3.9 Glucose solutions

Participants consumed the same glucose solutions as described in section 1.12

4.3.10 Data processing

High density EMG (HDEMG) from the vastus lateralis (VL) was measured and amplified during the TRAP contractions with a modified Bagnoli 16-channel EMG system (Delsys, Boston, USA). Before placing the electrode an identical skin preparation was performed as described for the sEMG. A five pin sensor was placed on the VL between the SENIAM recommended site for VL bipolar surface electrode configuration and the muscle belly. The HDEMG electrode position was altered to ensure a minimum 4:1 signal to noise ratio was achieved prior to any measurement taken. The sensor was pressed firmly onto the

skin avoiding piercing the dermis and was secured with micropore tape. A reference electrode (5 cm diameter, HE-R, Dermatode, American Imex, Irvine) was secured to the patella of the right leg.

The HDEMG system recorded four separate bipolar EMG signals from the 5-pin sensor probe array at a sampling frequency of 20 kHz. The four signals from each isometric TRAP effort were filtered with a band width of 20 Hz to 1750 Hz (De Luca and Contessa 2012). VL HDEMG and force data from the Biodex 3 dynamometer were synchronously recorded via software (EMGworks® 4.0 Acquisition software, Delsys, Boston, USA) integrated with the HDEMG system. Voltage data measured from the Biodex was calibrated within the EMGworks® software during the dynamometer calibration process, before each testing session, to allow force data to be captured during MVC and TRAP efforts.

4.3.11 EMG signal decomposition, analysis and accuracy

HDEMG signal decomposition

All HDEMG motor unit firing rate and MVC force data performed on the VL were processed with EMGworks® 4.0 Analysis software (Delsys, Boston, USA). To decompose surface HDEMG signals collected into constituent motor unit action potential trains (MUAPTs) Precision Decomposition III (PD III) algorithms were used. These algorithms use the artificial intelligence framework known as Integrated Processing and Understanding of Signals to separate the action potentials of different MUs from the overall surface EMG signal. The PD III system involves four separate stages that takes the surface EMG signal input ($x(t)$) and produces MUAPTs of the individual motor units ($y(j)(t)$, $j= 1, 2, \dots, N$) identified within the input signal. Finally, Matlab software (Mathworks, Inc., Natick, USA) was used to produce absolute MUFDR data from each of the identified time periods during the TRAP efforts.

Firing rate and motor unit number analysis

Firing rates of MUs from the decomposed HDEMG signals were divided into 3 groups (tertiles) depending on their order of recruitment: 1) early recruited, 2) mid-recruited and 3) late recruited MUs. The three tertiles were determined by arbitrarily dividing the total number of motor units detected by three. By splitting the MUs three distinct populations of MUs could be examined of which each group was expected to display unique firing rate characteristics. If the total number of motor units detected was not divisible by three additional motor units were added to the late recruited group. For example, if 28 MU were detected 9 MU each would be allocated to the early and mid-recruited group and 10 MU to the late recruited group. To determine the most reliable portion of the TRAP efforts 3 sec epochs throughout the duration of the contraction were examined to investigate the firing rates of each tertile. The reliability of the MUFR of each epoch was identified in the pre intervention TRAP efforts to determine the suitability of using each time period for the analysis. The total number of MU detected during each TRAP effort was also compared between treatments in the pre intervention contraction.

Decomposition accuracy

The accuracy of the decomposition for each TRAP effort completed was assessed with 'reconstruct and test' analysis software. The 'reconstruct and test' analysis (Nawab et al 2010) is considered the most suitable way of validating the decomposition of the HDEMG signals (Stock et al 2011). The analysis assesses the level of firing rate accuracy of each MU and the number of errors per second during the entire submaximal TRAP effort. An inclusion threshold of >85% accuracy throughout the entire TRAP effort was required in order for the MU to be included in the analysis (Stock et al 2012). Accuracy levels during the plateau phase of the TRAP efforts were typically >90%.

EMG SAMPLING

EMG was sampled at a rate of 2000 Hz and anti-aliased with a 500 Hz low pass filter. A 10 Hz high pass filter was also applied. The Biopac MP100 system had an input impedance and common mode rejection ratio of $2M\Omega$ and >110 dB, respectively. EMG signals were root mean square processed. Average root mean square was calculated for a moving window 200 ms time period across the entire waveform for each activity (Hägg et al., 2004). Root mean square processing was conducted by the software used to operate the EMG system (AcqKnowledge® 3.8.1, Biopac Systems Inc, California, USA), in accordance with the manufacturer's guidelines (Acqknowledge® software guide, 2008).

4.3.12 STATISTICAL ANALYSIS

All data are presented as mean (\pm SD) unless otherwise stated. Three factor repeated measures analysis of variance was used to determine treatment, time, period (order) main effects and treatment x time interaction effects. Where a significant period effect was observed then period was used as a covariate and the analysis re-run. Significant main and interaction effects were explored using post hoc Tukey's comparisons to indicate where these differences occurred.

4.4 RESULTS

All 20 participants successfully completed the trial. Unfortunately, some measures could not be completed on all 20 individuals. The M-wave and HDEMG datasets consists of 14 participants due to technical restraints with the stimulator on the first 6 participants and file decomposition problems with the HDEMG system. All other datasets presented are based on all 20 participants.

4.4.1 M-Wave

M-Wave peak to peak twitch potential revealed no main effect of treatment ($p=0.71$), time ($p=0.91$) or interaction ($p=0.15$) (Figure 4.3). Analysis was then performed on the peak torque generated when a twitch potential was provoked. The analysis revealed no significant effect of treatment ($p=0.42$), time ($p=0.77$), or period ($p=0.84$) (Figure 4.4).

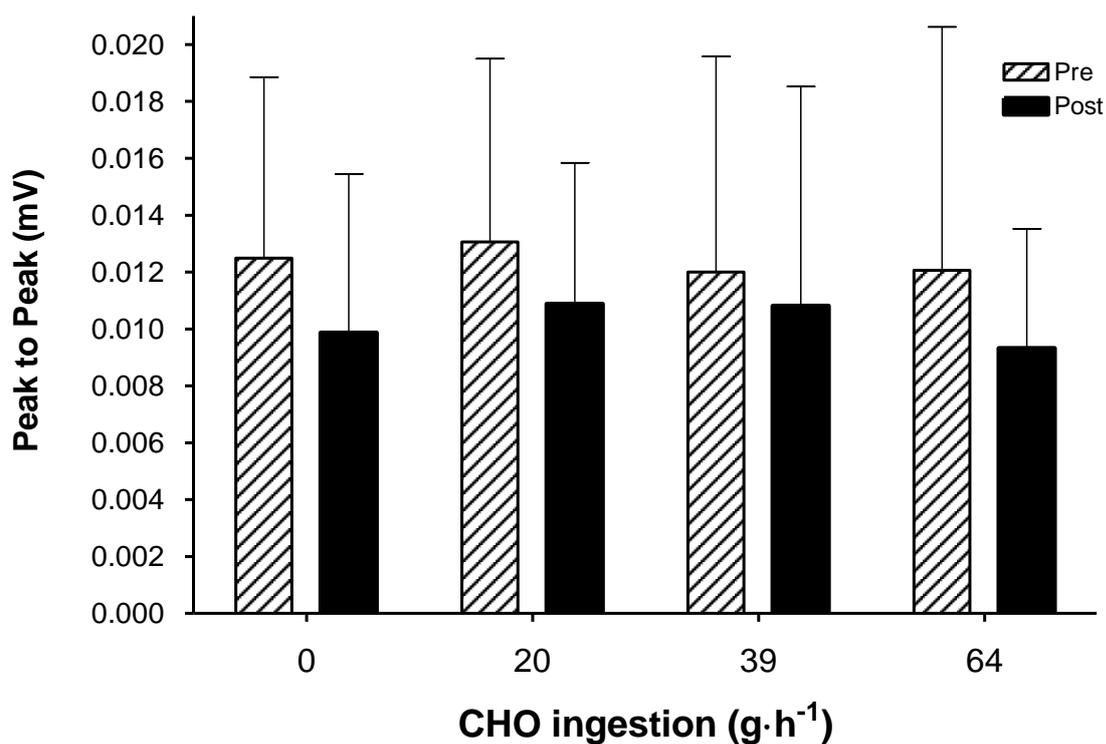


Figure 4.3 Mean (SD) Peak to peak assessment during peripheral femoral stimulation pre and post a 2 h submaximal cycling bout while consuming 0, 20, 39, and 64 $\text{g}\cdot\text{h}^{-1}$ of CHO and maximal time trial performance task.

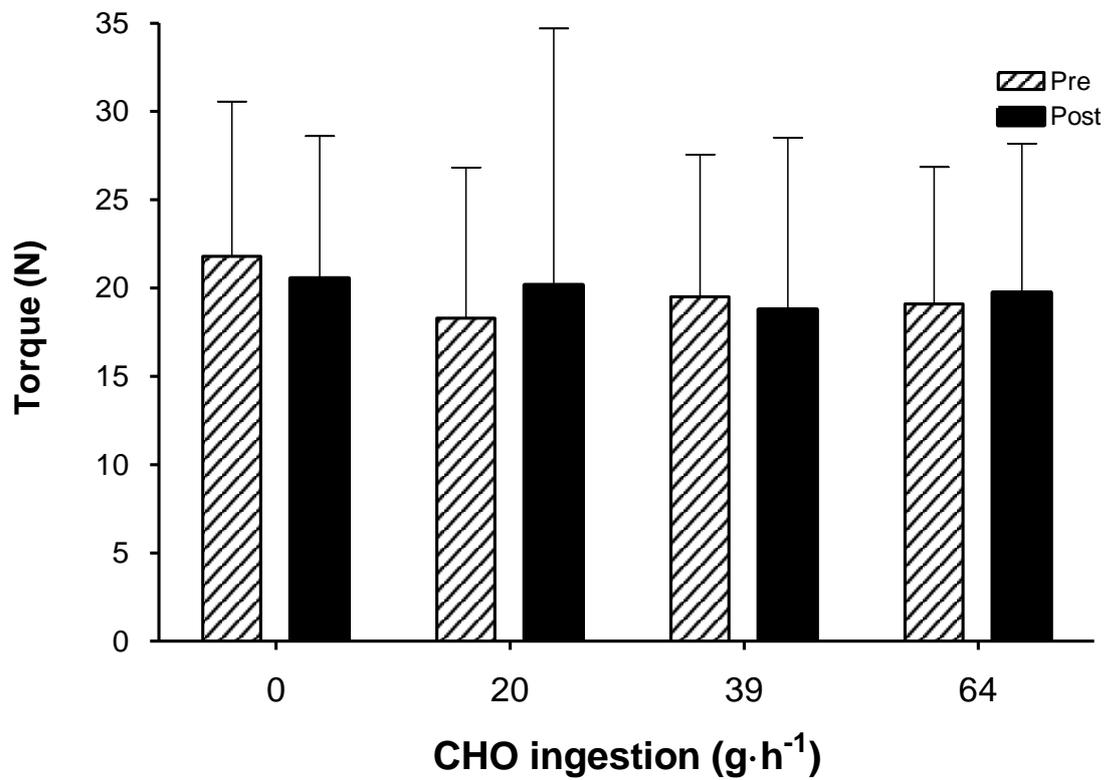


Figure 4.4 Mean (SD) Maximal torque production following peripheral femoral stimulation prior to and following post a 2 h submaximal cycling bout while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO and maximal time trial performance task.

4.4.2 MVC

MVC peak force revealed a main effect of time ($p < 0.001$) and period ($p < 0.001$) but no main effect of treatment ($p = 0.14$) or interaction ($p = 0.86$). Period was included as a covariate and the analysis repeated. A significant main effect of time ($p < 0.01$) indicate a significant reductions in MVC force of -20.4 (95%CI: -26.5 to -14.4) Nm (Figure 4.5).

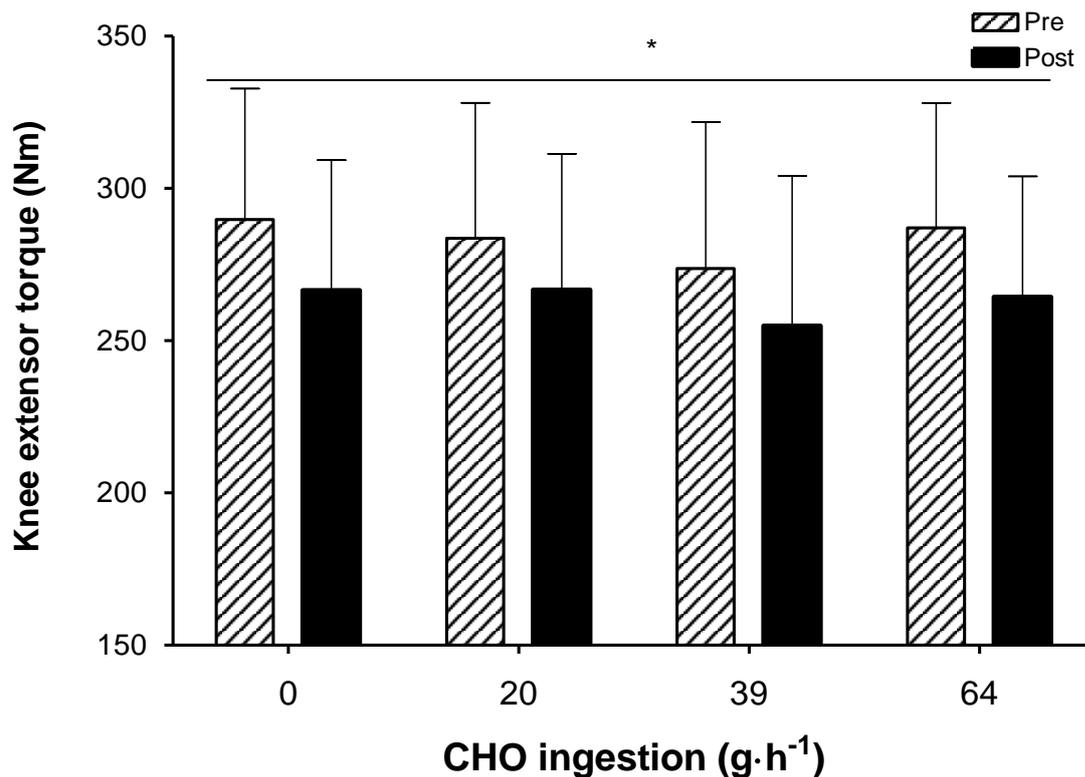


Figure 4.5 Mean (SD) maximal knee extensor torque produced during an MVC performed prior to and following a prolonged cycling exercise bout of variable intensity while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. There was a significant ($*p < 0.01$) effect of time where knee extensor torque was significantly lower following the prolonged cycling exercise bout.

4.4.3 Motor unit firing characteristics

The total number of motor units detected during the submaximal TRAP contractions showed no main effect of time ($p=0.51$), treatment ($p=0.84$) or interaction ($p=0.97$) (Figure 4.6). The firing rates of early recruited motor units indicated a significant effect of time ($p<0.01$). Post hoc comparisons indicated that early recruited motor units fired significantly faster (1.55, 95%CI: 0.51 to 2.59, pulses per second) post exercise in comparison to pre exercise values. The increase was irrespective of the amount of CHO consumed ($p=0.81$). There were no other significant effects of time, treatment, or interaction of time and treatment for the firing rates of mid or late recruited motor units (Figure 4.7).

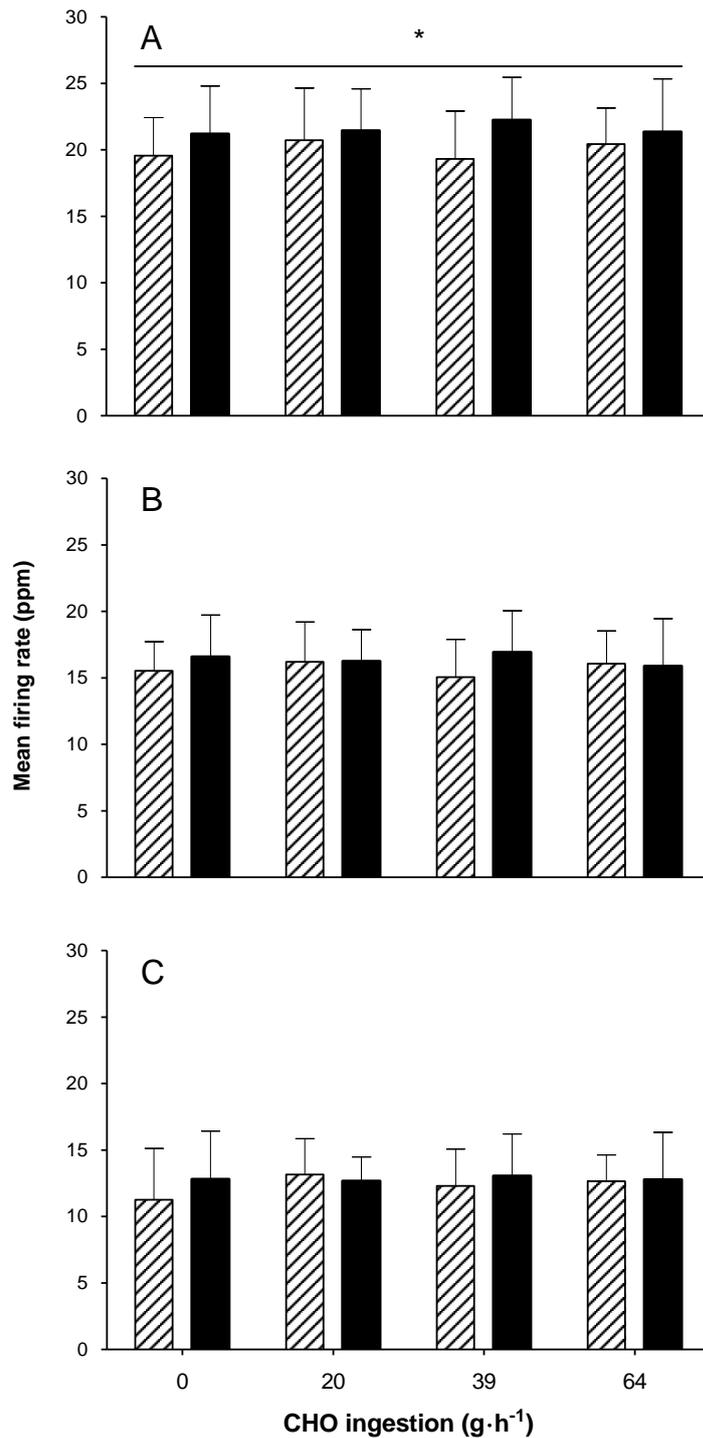


Figure 4.6 Mean motor unit firing rate for early (A), mid (B), and late (C) recruited motor units during the TRAP submaximal isometric contraction prior to (Stripes) and following (solid) a prolonged cycling exercise bout of variable intensity while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. * indicates a significant ($p < 0.01$) effect of time.

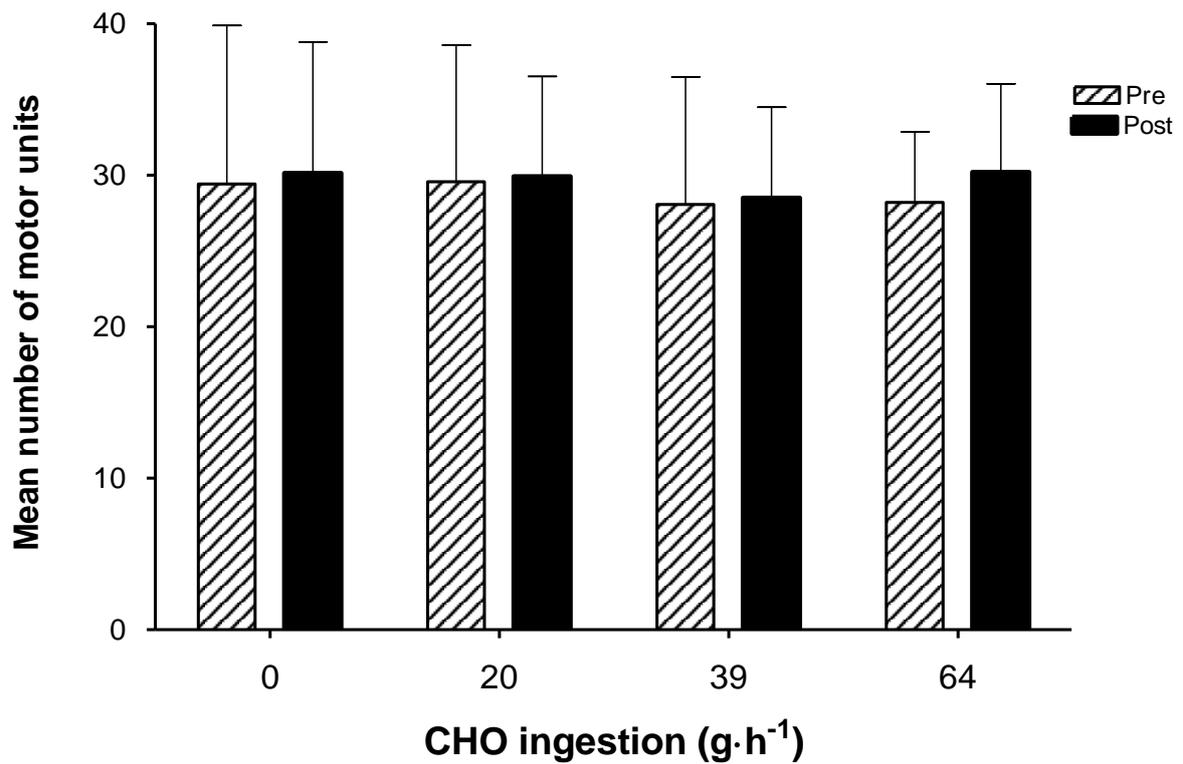


Figure 4.7 Mean number of motor unit recruited during the submaximal trapezoidal contraction pattern before and following the ingestion of 0, 20, 39 and 64 g·h⁻¹ of CHO during 2h of exercise and a time trial task.

4.4.4 EMG amplitude

Gross EMG response revealed a main effect of treatment ($p < 0.01$) but not for time ($p = 0.57$) or interaction ($p = 0.85$) (Figure 4.8).

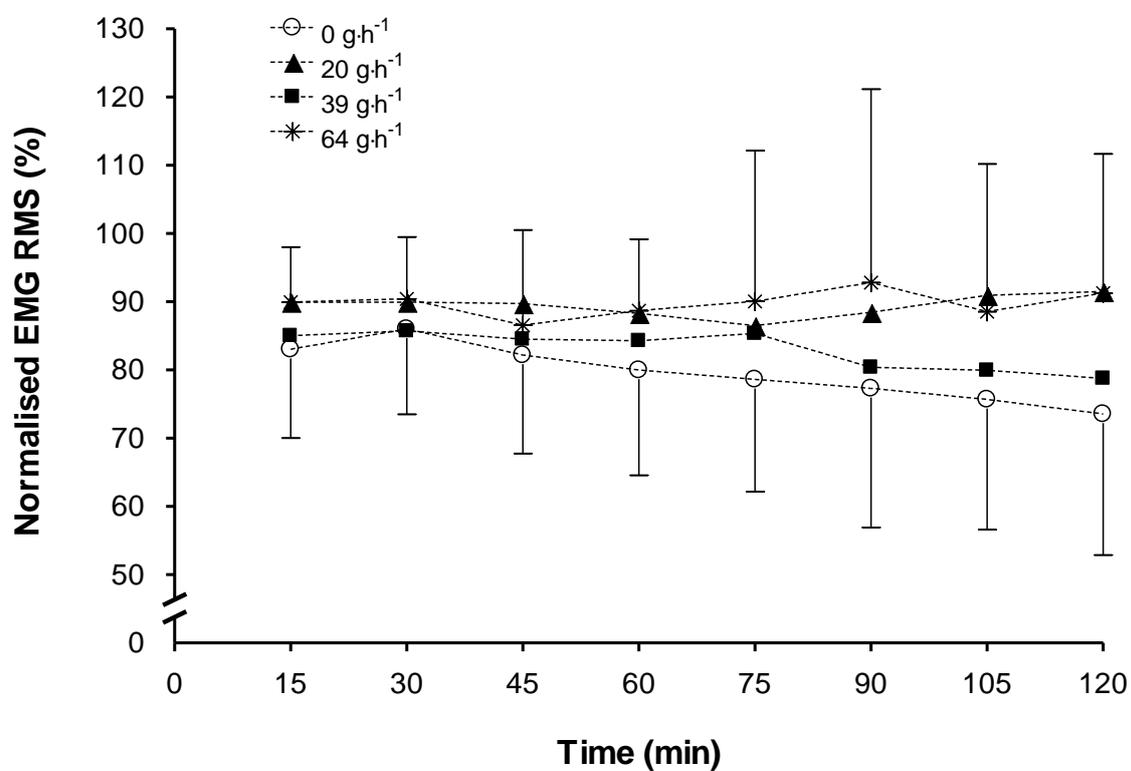


Figure 4.8. Mean (SD) normalised EMG root mean square (RMS) captured from the vastus lateralis during submaximal cycling exercise at a fixed cadence while consuming 0, 20, 39, and 64 g·h⁻¹ of CHO. The EMG signal is normalised to EMG activity collected at 70% of PPO.

4.5 DISCUSSION

In this study we demonstrated an increase in the firing rates of early recruited motor units following prolonged endurance exercise irrespective of the ingestion rate of CHO provided. The MUFR analysis was performed at the same absolute contraction force (70% of pre MVC) pre and post exercise highlighting that central drive to early recruited MUs was altered by increasing firing rate to achieve the same absolute force following exercise. Whereas, firing rates of mid and late recruited motor units remained unchanged following the interventions. The exercise performed induced significant reductions in MVC torque from pre to post exercise highlighting the fatiguing nature of the activity. However, ingesting CHO had no effect on the amount of torque produced. No other significant differences in the contractile properties of the vastus lateralis muscle from pre to post exercise, or with the ingestion of different rates of CHO, were observed. During the 2 h submaximal steady state exercise bout sEMG amplitude was not influenced by the dose of CHO ingested. In summary, these data highlight that prolonged cycling exercise of varying intensity to fatigue alters central drive and subsequent firing characteristics of lower limb knee extensor muscles. Ingestion of different amounts of CHO during the first 2 h of prolonged endurance exercise had little to no influence in altering neuromuscular recruitment, and did not alter fatigue status of the muscle assessed using a range of peripheral and central fatigue measures.

Prolonged cycling activity in the present investigation reduced maximal MVC values by ~8% following exercise in comparison to pre exercise values (figure 4.5). However, none of the CHO ingestion rates provided had any influence on this reduction. A reduction in MVC following prolonged endurance exercise has been previously reported (Bigland-Ritchie, et al 1986; Place, et al 2004; Decorte, et al 2012) and to a similar extent (Bentley, et al 2000; Lepers, et al 2000; Lepers, et al 2001; Lepers, et al 2002) demonstrating the exercise bout performed induced muscular fatigue to a similar extent to studies already

reported. The total amount of time participants were exercising for did significantly reduce when ingesting 39 and 64 g·h⁻¹ of CHO as a higher power output could be maintained throughout the time trial task (chapter 2, figure 2.7). Taken together, it seems apparent that participants reached the same level of fatigue following the exercise bout. Therefore, our data support the notion that the absolute amount of work completed appears to be the determining factor for fatigue rather than the intensity the work was completed at (Rauch, et al 2005).

CHO ingestion did not preserve maximal force generation following prolonged exercise which may be explained by the lack of muscle glycogen sparing with any ingestion rate consumed (chapter 3). This finding is in good agreement with the 'glycostat' phenomenon presented by Rauche et al (2008). Participants finished an exhaustive exercise bout with remarkably similar muscle glycogen content even when pre exercise muscle glycogen was altered and CHO was consumed. In chapter 3 of this thesis we have estimated that little or no muscle glycogen was spared with any CHO ingestion rate provided.

Glycogen depletion correlates well with a number of key measures explaining force reduction (Fitts 1994) and specifically the successful cross bridge formation and activation (Keyser 2010). Prolonged glycogen depleting exercise has been reported to reduce the calcium sensitivity of type II glycolytic fibres (Hvid, et al 2013). Previous studies have also shown that intramyofibrillar glycogen pool is preferentially depleted during high intensity exercise (Nielsen, et al 2011) and correlates with muscle fatigue (Nielsen, et al 2010). In both cases a lack of endogenous glycogen sparing with any CHO ingestion rate would not have prevented these processes from occurring and as such go some way to explaining the similar reduction in MVC force observed on all trials. However, it does not explain why some higher threshold MU are still recruited at 70% of MVC, though, contractions at 70% of MVC may still exclude higher threshold MUs from being activated thereby limiting the interpretation for maximal contractions. De luca et al (2007)

demonstrated that new motor units are still recruited past the 70% of MVC level when describing the 'onion skin' principle. It is possible, though speculative, that the amount of glycogen stored in close proximity to the active muscle could have an influence on the ability to generate force following prolonged endurance exercise (Nielsen, et al 2011). However, this narrow hypothesis is only one example explaining the multifaceted nature of fatigue.

The M-wave formation following peripheral stimulation of the muscle is often used as an indicator of neuromuscular propagation failure as it permits a direct assessment of muscle fibre electrical conductance characteristics (Cupido, et al 1996). In the current investigation we observed no difference in M-wave amplitude (figure 4.3), force generation (figure 4.4) from pre to post exercise, or with different CHO ingestion rates contrasting the existing literature (Lepers, et al 2002; Decorte, et al 2012). In these previous studies the duration of time taken to elicit a change in M-wave characteristics was ~4 h in one case (Lepers, et al 2002) or at a much higher cycling intensity (Decorte et al 2010). The lack of any observable difference suggests that the reduction in MVC post exercise was likely due to more centrally driven factors of fatigue, rather than signal propagation distally of the motor neurone junction. We did observe considerable variability in performing the measure, which likely affected the outcome of these data. Individual variation between participants such as adiposity, hydration status, sweat rates and other contravening factors may explain some of the variance. Nevertheless, it is entirely plausible that the exercise intervention simply had no effect on M wave characteristics highlighting the centrally driven nature of the fatigue induced.

Blood lactate concentration (6.2, 6.6, 6.0, 6.6 for 0, 20, 39, and 64 g·h⁻¹ respectively) was elevated immediately following the time trial task (section 3.4.3, Chapter 3). Generally, a decrease in extracellular pH causes a delay in M-Wave latency (Kremenec, et al 2009) which we would expect with the elevated post TT lactate concentrations. Interestingly,

even under these circumstances there is no reduction in evoked twitch force indicating no alteration in the E-C uncoupling patterns from pre to post exercise. As such this supports the notion that the fatigue induced is central rather than peripheral typical of locomotor endurance activity of this duration (Thomas et al 2015).

The centrally governed control of motor unit utilisation appeared to mirror the MVC data as firing rates of early recruited motor units were increased following exercise but were unaffected by CHO ingestion (Figure 4.6). An eloquent study conducted by De Luca et al (2008) reported that MUs are recruited in a predictable and reliable fashion according to the “onion skin” principle. The principle describes an inverse relationship between MU firing rate and excitability threshold i.e. earlier recruited MUs and thus lower excitement threshold MUs (lower force) fire at a faster rate (DeLuca 2008) than the later recruited MUs (higher force). Stock et al (2011) utilised the same MU measurement techniques from DeLuca and observed a decrease in MU firing rates following an acute isometric fatiguing protocol in comparison to resting values. The decrease in MU firing rates highlighted a relationship between acute isolated fatigue and decreased firing rates of MU. In the present study a statistical comparison between the mean firing rates of early, mid and late recruited motor units regardless of time or treatment indicated a significant reduction in motor unit firing rate from early to mid (-4.7, 95%CI: -5.5 to 3.9 pps), and from mid to late (-3.5, 95%CI: -4.3 to -2.7 pps) demonstrating an ordered recruitment pattern like that described in the onion skin principle. Also, we observed no difference in the total number of MU recruited at any time point suggesting that the same motor units were recruited pre and post exercise. Taken together our results demonstrate that MUs recruited pre and post exercise are similar and demonstrate good agreement with the “onion skin” principle. The firing rates of early recruited MUs increase post exercise to generate the same absolute amount of force in comparison to pre exercise firing rates. It is possible that some form of afferent feedback from the muscle is regulating the motor

unit firing rate drive in the motor cortex. If slow firing, late recruited units begin to fail then a compensatory affect may occur with an increase in early recruited fibres. The central control of motor unit firing appears to be altered following endurance exercise but is completely unaffected by CHO ingestion rates between 20 and 64 g·h⁻¹.

We are the first to report increased firing rates of early recruit motor units following long duration cycling activity. Additionally, we are also the first to report that CHO ingestion has no effect on the recruitment pattern of motor units following prolonged work matched endurance exercise to fatigue. Recently Thomas et al (2015) suggested that following different durations (4, 20, and 40 km cycling time trials) of cycling exercise – the type of fatigue induced was task dependant. Shorter duration TT tasks (~6 min) demonstrated a prevalence of peripheral fatigue whereas longer duration tasks (>30 min) highlighted a key role of central fatigue (decrease in voluntary activation). In the present investigation exercise fatigue was induced following prolonged, metabolically challenging, endurance cycling exercise. We speculate that the central drive to the motor units is altered as the type 2 fibres fatigued during the TT task. Stock et al (2011) would not have induced central fatigue during isolated contraction exercise hence the shift to lower firing rates.

The utilisation of endogenous glycogen stores (chapter 3), and the lack of muscle sparing observed when feeding CHO (chapter 3), likely provides some afferent feedback similar to the 'glyco-stat' phenomenon (Rauche, et al 2008). The CNS sensing of endogenous stores likely reduces central drive and explains the alteration in firing patterns of low threshold early recruited motor units. Others have highlighted the capacity for different motor unit recruitment patterns in different muscle groups that comprise of different percentage contributions of fibre types (Stock, et al 2012). As such, the interpretation of the motor unit recruitment data of the VL should be considered as a snap shot into motor unit recruitment of the lower limb following exhaustive endurance exercise.

During the 2 h cycling bout surface EMG recorded was around 80-90% of the activation level recorded at 70% of PPO and was consistent throughout the 2 h bout. We did not observe a characteristic progressive increase in sEMG amplitude throughout the 2 h bout previously reported (St Clair Gibson, et al 2001; Abbiss, et al 2008). It is possible that the 2 h submaximal ride was not long enough or at a sufficient intensity in order to cause a sufficient increase in sEMG amplitude. It may be possible that the onset of fatigue occurred at some point during the time trial task. If we had continued to measure sEMG during the TT task we may have observed an increase in sEMG amplitude.

4.6 CONCLUSION

In conclusion ingesting CHO at rates of 20 to 64 g·h⁻¹ has no effect on preserving neuromuscular function following fatigue induced by variable intensity, prolonged, exhaustive endurance exercise. Following endurance exercise there is an increase in the firing rate of early recruited motor units during submaximal muscular contractions likely due to an alteration in the central drive to the muscle. Though, the recruitment characteristics of early, mid and late recruited motor units are not altered with CHO ingestion or following prolonged endurance exercise

CHAPTER 5

SYNOPSIS OF FINDINGS

5.1 INTRODUCTION

CHO ingestion has been shown to improve endurance exercise performance but the exact mechanisms and the precise understanding of what underpins this enhancement in performance, and optimal ingestion rates, remains difficult to ascertain. Researchers have proposed a dose-response relationship between CHO ingestion and exercise performance suggesting: an increase in exogenous CHO oxidation rates, whole body CHO oxidation rates, improved maintenance of blood glucose concentration and improved neuromuscular function to facilitate muscular contraction. However, no comprehensive assessment has been conducted to adequately determine the mechanisms and confirm the hypotheses. As such this gap in the literature formed the basis of this thesis and resulted in the development of the following aims:

1. To determine the performance response of trained male cyclists to the ingestion of increasing amounts of carbohydrate during a preloaded time trial task in a suitably powered and controlled trial.
2. To determine the acute differences in metabolic regulation when ingesting incremental amounts of carbohydrate during a prolonged submaximal exercise bout.
3. To characterise the regulation of neuromuscular control during, and following, prolonged endurance cycling when consuming increasing amounts of carbohydrate.

5.2 AIM 1 – PERFORMANCE RESPONSES

In Chapter 2 of this thesis we determined the performance responses of trained male cyclists when ingesting 0, 20, 39 and 64 g·h⁻¹ of CHO during a preload submaximal ride followed by a time trial task. The result was 39 and 64 g·h⁻¹ of CHO were equally effective at improving exercise performance by around 6.5 % when compared to a 0 g·h⁻¹ control treatment. Additionally, >20 g·h⁻¹ appears to be the minimal ingestion rate required in order to elicit an improvement in endurance performance. Here we add to the literature as few studies have utilised a suitably powered, double blind, randomised control trial to determine this relationship. Additionally, we utilised a realistic meaningful performance improvement criteria of >3.5% that surpasses the day to day variability of the performance task performed. Any improvement in performance measured is a direct reflection of the intervention provided rather than potentially lost in the variability of the test employed.

The results of Chapter 2 throw into question what is considered a meaningful improvement in performance when assessing the effectiveness of a CHO ingestion intervention. Current guidelines promote the ingestion of higher CHO ingestion rates (~60 g·h⁻¹) to maximise performance gains in tasks lasting <3 h (Smith et al 2012, Stellingwerff and Cox, 2014). However, we observed a plateau in performance with more modest ingestion rates of 39 g·h⁻¹ in trained club level athletes. What constitutes a meaningful improvement in performance depends on the duration of the performance task and the target audience of the outcome. It is likely that a 0.7% improvement in performance for an elite athlete's personal best would be a meaningful improvement. Though, a 0.7% improvement in performance over a 20km time trial for amateur athletes, as was the difference in performance time when comparing 50 and 78 g·h⁻¹ of CHO (Smith et al 2012), is unlikely to be meaningful, repeatable or out with

the expected day to day error associated with the performance task. As yet, no endurance cycling performance task has been reported to have a day to day coefficient of variation of less than ~2-3% (Jeukendrup 1996). We contribute to the literature as we have reported, with statistical rigor, that the majority of individuals experience a ceiling in performance gains achieved when feeding a single source CHO at rates higher than 40 g·h⁻¹ when total exercise duration is less than 3 h. ...

The multicentre Smith et al (2012) study suffered with study design constraints when attempting to increase the number of ingestion rates examined (13 different ingestion rates). The 0 g·h⁻¹ control was the only ingestion rate that was repeated between the study sites. Furthermore, participants only consumed 3 of the possible 12 CHO ingestion rates meaning performance data was compared across 13 groups. Taken together these design elements restrict the interpretation of the data as we are unable to determine the graded performance response from all 56 participants. These limitations points, coupled with the suggestion that 0.7% improvement in performance would be considered worthwhile and repeatable for most individuals, raise questions on the validity of the study results and places restrictions on the interpretation of this data.

The practical implementation of these findings suggest that individuals should aim to consume around 40 g·h⁻¹ of a single source CHO in order to optimally improve their performance during exercise tasks lasting less than 3 h. Potential further gains in performance are likely to be minimal when comparing between 39 and 64 g·h⁻¹. Additionally, individuals should consume a minimum of >20 g·h⁻¹ for a measurable and consistent improvement in endurance performance. Taken together, athletes and performance nutritionists should consider how replicable and measurably beneficial these gains in performance are likely to be to the individual performing the task.

5.2.1 Non responders

For 18 out of the 20 participants that completed the performance task an improvement of some description occurred when they consumed CHO. For 2 of the 20 participants their fastest performance occurred when they consumed only water (Figure 5.1). Closer inspection of their key metabolic profiles (CHO oxidation rate, fat oxidation rate, insulin response, and percentage reduction in liver glucose Ra) indicates they respond in a similar fashion to the mean response measured. As such, it is not immediately clear why these individuals did not respond positively to CHO ingestion. Though some researchers have identified gut trainability and genetic determination as being potential explanations for responders and non-responders

Some researchers have proposed that individuals who regularly consume CHO, or have a high daily intake of CHO, may increase their capacity to absorb it – a so called ‘trainable gut’ (Cox, et al 2010; Jeukendrup and McLaughlin 2011). In a recent study endurance-trained cyclists or triathletes (n=16) were paired and randomly allocated to two groups: high-CHO group (n = 8) or an energy-matched low-CHO group (n = 8) for 28 days. During the 28 days both groups completed a structured training programme that was matched. Daily CHO intake for both groups was $5 \text{ g}\cdot\text{kg}^{-1}$ body weight with the high CHO group receiving an additional $1.5 \text{ g}\cdot\text{kg}^{-1}$ ($25 \text{ kJ}\cdot\text{kg}^{-1}$) of CHO (flavoured glucose drink) for every hour of exercise performed daily. The low group also received an additional $25 \text{ kJ}\cdot\text{kg}^{-1}$ for each hour but from protein and fat sources. Breath by breath gas analysis was conducted during a 100min submaximal (63% PPO, $\sim 70\% \dot{V}O_{2\text{peak}}$) ride where substrate use analysis was performed prior to and following the 28 day training and dietary intervention. Following the high CHO treatment resulted in enhanced total CHO oxidation after a month of “gut training” providing some evidence that the intestine is trainable. Exogenous CHO is limited mainly by the rate

of absorption and subsequent transport of ingested CHO (Jeukendrup et al 1999). Furthermore, no difference in muscle GLUT-4 concentration was found between high and low CHO groups in the Cox et al (2010) study. Taken together a possible explanation is that high CHO intake can increase intestinal absorption capacity. In the context of non-responders it is possible that those participants that did not respond positively to any CHO ingestion had low habitual CHO ingestion excluding them from these aforementioned proposed adaptations to gut CHO absorption.

Further research to identify key markers (genetic, physiological) that would predispose an individual to responding positively to CHO ingestion during endurance exercise would also be beneficial. Recently, researchers identified stable markers of gene expression regulating CHO metabolism which were determined by geographic ancestry (Schisler, et al 2009). This type of research indicates that is possible to differentially investigate key genes regulating CHO metabolism to a specific group of individuals. Some researchers have hypothesised that it is not possible to be at the top level of endurance athletic performance without the ability to maximally oxidise exogenous CHO (Joyner, et al 2011). The identification of key genes that are associated with superior athletic performance continues to increase posing ethical questions along the way (Tamburrini and Tännsjö 2005; Brown 2015). It is likely that in the future genetic profiling of athletes may help direct athletes and coaches on their nutritional intake during events.

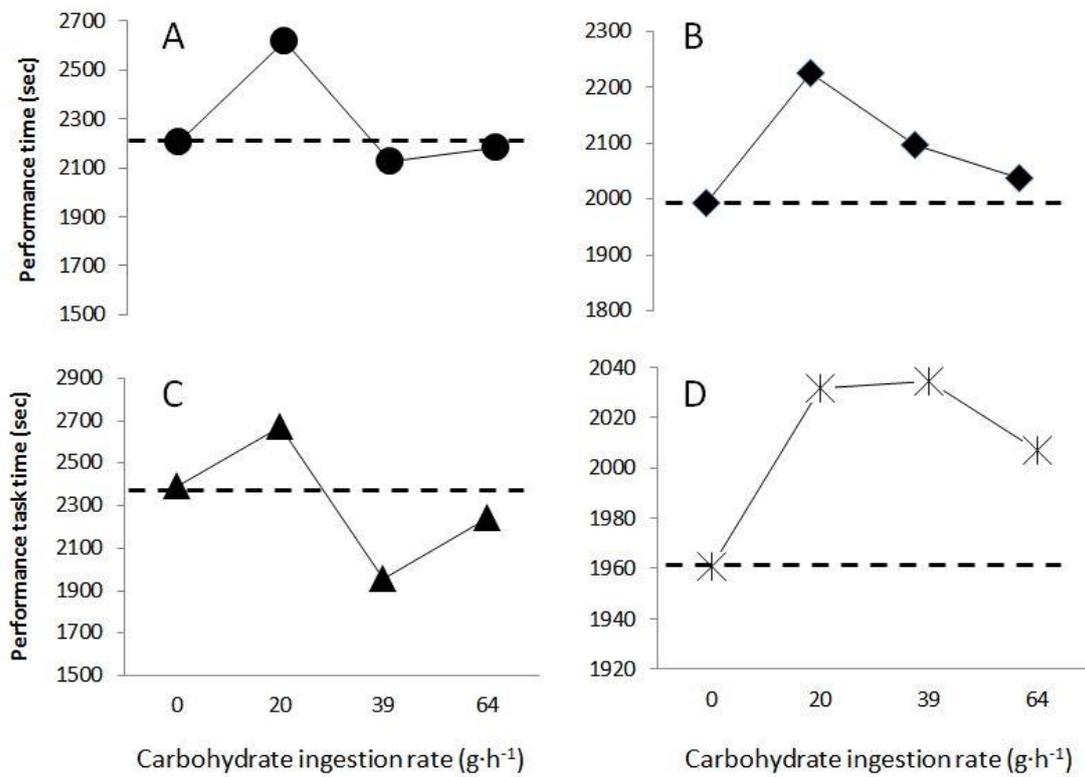


Figure 5.1 Individual performance times of 4 participants who had at least 1 negative response to ingesting carbohydrate in comparison to consuming water. Graphs B and D indicate the two participants whose fastest performance time occurred when drinking water.

5.2.2 Optimal preparation

Individuals completed all trials in an overnight fasted state. We make reference to some of the considerations when interpreting this data in Chapter 2 though some further consideration is warranted. Trained individuals have a greater liver and muscle glycogen storage capacity. Following an overnight fast it is unlikely that liver glycogen storage is fully depleted in a trained individual, as might be the case for an untrained individual (Casey, et al 2000). However, when considering 'optimal' performance, athletes typically prepare for major events by maximising their endogenous glycogen stores by carbohydrate loading (Hawley, et al 1997). CHO loading has been shown to increase endogenous glycogen stores above the level of their normal habitual dietary intake - therefore maximising endogenous glycogen availability and improving subsequent exercise performance (Chen, et al 2008).

We argue in Chapter 2 that the performance outcomes associated with increasing dose of CHO are applicable following an overnight fast for the majority of trained individuals most of the time. Additionally, we reported in Chapter 1 that a meta-analysis performed suggested that fed vs fasted state exercise did not influence the outcome of CHO ingestion studies (Temesi et al 2011) though the scope of the meta-analysis is quite restrictive. Studies indicate that the amount of CHO ingested has the greatest influence on running performance (Chen, et al 2008) however; there is still a lack of knowledge on the effects of pre exercise CHO loading on the guidelines for CHO intake during exercise. Actual measured practice during competitive events simply does not match that of the recommendations presented here (Chapter 2) and elsewhere (Smith et al 2012, Stellingwerff and Cox, 2014). During an elite level triathlon actual CHO ingestion rates were around $\sim 25 \text{ g}\cdot\text{h}^{-1}$ for men and $23 \text{ g}\cdot\text{h}^{-1}$ for women (Cox, et al 2010) when consuming a range of different CHO rich sports

nutritional products. However, in a case study by Stellingwerff (2012) the mean CHO ingestion rate was $60 \pm 15 \text{ g}\cdot\text{h}^{-1}$ (10.1% solution) when consuming both glucose and fructose from a range of different CHO sources (drinks, gels, bars). It seems that, for many individuals the ingestion rates consumed are lower than the $40 \text{ g}\cdot\text{h}^{-1}$ where we see performance gains and considerably lower than the previously reported 'optimal' ingestion rates (Smith, et al 2013; Stellingwerff and Cox 2014). However, in an elite scenario – tolerability following a nutritional intervention specifically designed to enhance the race day CHO ingestion rate may actually increase the CHO ingestion rate possible. Nevertheless, further research that investigates CHO ingestion during exercise in the fed state, and simulates CHO loading prior to starting, would be prudent to provide a much more pragmatic approach to the optimal feeding strategy guidelines.

5.2.3 Mode of exercise

During this thesis we chose cycling activity because it is very controlled, easy to execute, relatively inexpensive and very easily replicable. Interestingly, around 83% of all the articles cited in the most recently published CHO ingestion review paper (Stellingwerff and Cox, 2014) used cycling as the mode of activity; with the remaining 17% choosing running. As such, the current guidelines are biased towards cycling activity and are potentially mis-representing other types of endurance activity. Cycling is limited to the lower body musculature therefore limiting the total muscle mass recruited during exercise limiting the total work rate possible and substrate usage potential. Far more research into the optimal feeding rates of specific sports would provide further insight into what is considered optimal for sports with different demands. Furthermore, the effects of gastro-intestinal movement, gastrointestinal comfort, and blood flow and substrate delivery are likely to be dramatically different depending on the demands of the sport.

5.3 AIM 2: METABOLIC REGULATION

In Chapter 3 of this thesis we aimed to understand the complex regulation of substrate provision during steady state submaximal exercise when consuming CHO at different rates. We identified that despite consuming 39 or 64 g·h⁻¹ there were similar effects on substrate utilisation when compared to ingesting 0 g·h⁻¹ during 2 h of submaximal cycling: reduced fat oxidation, decreased circulating NEFA, elevated whole body CHO oxidation, increased circulating insulin and glucose concentrations. However, consuming 64 g·h⁻¹ also resulted in elevated exogenous CHO oxidation and decreased hepatic glucose contribution to total Ra above that of 39 g·h⁻¹. These differences between 39 and 64 g·h⁻¹ did not result in any additional improvement in performance.

The increased rate of exogenous CHO oxidation when consuming 64 g·h⁻¹ appears to increase the dependency on CHO as a whole body fuel source subsequently suppressing whole body fat oxidation and reducing the impact of any hepatic glycogen sparing that has occurred. Jeukendrup et al (1999) reported a similar phenomenon though fed considerably higher rates of CHO (180 g·h⁻¹) likely compromising performance outcomes. The net gain in endogenous CHO stores from the increase in hepatic glucose suppression, driven by the elevated insulin response when consuming 64 g·h⁻¹ may be offset by the increase in whole body CHO oxidation rate. The increased dependency on CHO as a fuel could thus explain why there are no significant additional gains in performance when consuming 64 g·h⁻¹ over 39 g·h⁻¹, as there is no net gain of endogenous glycogen due to higher oxidation rates. As such, it seems that 39 g·h⁻¹ provides a sufficient insulin response to significantly reduce the contribution of hepatic glucose to total Ra, increase CHO oxidation and yet also

maintain fat oxidation so that the benefit of endogenous sparing is maintained until the performance task.

Table 2 in Chapter 1 provides numerous examples of muscle glycogen sparing following exercise with CHO ingestion. In Chapter 3 of this thesis we show that the majority of endogenous CHO sparing could be explained by an estimation of the reduction in hepatic glucose release. There have been reports in the literature that consuming water alone during extended exercise is able to spare muscle glycogen. Hargreaves et al (1996) reported muscle glycogen was spared by 16% (318 ± 46 and 380 ± 53 mmol/kg dry weight for water and no water, respectively; $P < 0.05$) when replacing fluid losses with water. Additionally, Logan Sprenger et al (2012) reported muscle glycogenolysis was 31% greater in the no water trial (252 ± 49 vs. 330 ± 33 mmol·kg⁻¹ dry muscle). It seems that maintenance of hydration status also promotes muscle glycogen sparing which could also have an effect on the glycogen sparing capacity of the muscle. As such, consuming 0 g·h⁻¹ (water) in the present investigations may well have contributed to the sparing of muscle glycogen. Sparing on the control treatment would therefore limit the chance of muscle glycogen sparing and therefore limit the chance of muscle glycogen to contribute to performance enhancement in the present work. Unfortunately we were unable to corroborate muscle glycogen usage with muscle biopsies. However, table 2 indicates just as many studies report no muscle sparing with CHO ingestion and would seem that capacity to blunt liver glucose output is a key mechanism that could impact upon performance responses to CHO feeding in endurance tasks lasting <3 h.

5.3.1 Higher rates and different carbohydrates

Some researchers have reported that much higher rates of multiple source CHO ingestion improves performance to a greater extent than lower rates and single source CHO (Jeukendrup and Currell, Smith et al 2012). The work of Smith et al (2010, 2012) has been discussed extensively throughout this thesis but no empirical metabolic explanation for the 'optimal' ingestion rate of $78 \text{ g}\cdot\text{h}^{-1}$ was provided in their performance study. Jeukendrup and Currell compared the substrate usage and performance outcomes of consuming $1.8 \text{ g}\cdot\text{min}^{-1}$ ($108 \text{ g}\cdot\text{h}^{-1}$) of glucose and glucose + fructose. We know that consuming $108 \text{ g}\cdot\text{h}^{-1}$ of glucose alone is detrimental to performance (Smith et al 2012) likely due to increased GI distress (Triplett et al 2010) so the comparison on performance is misleading as the glucose ingestion rate would likely inflate the beneficial performance effect of the glucose fructose trial. However, Baur et al (2014) demonstrate that consuming high rates of glucose fructose ($93 \text{ g}\cdot\text{h}^{-1}$) when compared to ingesting moderate rates ($60 \text{ g}\cdot\text{h}^{-1}$) of single source glucose does not significantly improve performance during a 30km TT. Unfortunately, comprehensive substrate usage measures using stable isotopes were not obtained nor did the authors report the insulin response induced by each of the interventions. However, the feeding interventions were conducted in the postprandial state suggesting, as previously indicated, that higher CHO ingestion rates of any CHO composition may not have the additional performance gains that some researchers have alluded to.

5.3.2 The future of CHO metabolism research

Some researchers have already suggested a link between habitual CHO intake and the ability to oxidise CHO (Cox, et al 2010). Additionally, others have indicated an increase in the ability to oxidise fat when undertaking training with low muscular glycogen availability (Yeo, et al 2008). In the present chapter we have observed an

ingestion rate ($64 \text{ g}\cdot\text{h}^{-1}$) where CHO ingestion drives its own oxidation but does not result in a net gain in CHO preservation ($64 \text{ g}\cdot\text{h}^{-1}$) that is sufficient to improve performance. Therefore, it is worth considering at what point does CHO ingestion rate and subsequent oxidation rate become more beneficial than endogenous preservation and substrate flexibility (the ability to utilise fats and CHO simultaneously) observed when consuming $39 \text{ g}\cdot\text{h}^{-1}$. An intervention which follows individuals on different long term dietary interventions (high/low CHO) followed by a high and low acute CHO feeding dose would provide some insight into beneficial training with or without CHO and the effect this has on ability to utilise CHO versus ability to continue metabolising fats alongside CHO oxidation (figure 5.2). This proposal differs from the previous aforementioned research as the CHO ingestion rate during exercise would be altered to investigate the effect of the nutritional intervention on the ability to oxidise different rates of CHO.

5.3.3 Individual recommendations from peak exogenous CHO oxidation rates.

Some well-funded athlete support industry bodies have started to determine the peak exogenous CHO oxidation rate possible with some of their supported athletes (personal communication). Here, athletes make multiple visits to the lab to ingest CHO solutions at different ingestion rates. The solutions are spiked with U^{13}C glucose (Chapter 3) for the determination of exogenous CHO oxidation. Recommendations for 'optimal' ingestion rates are then made based on the peak exogenous CHO oxidation rate measured.

However, as we have demonstrated, this may not be a practical method for determining the optimal feeding strategy, particularly during events where the intensity increases or feeding opportunities are reduced such as: the latter stages of a marathon, the run phase of a triathlon or the final 20km of a cycling event. Determining the amount of endogenous CHO sparing achieved coupled with the increase in CHO oxidation that does not compromise fat oxidation or undo the sparing achieved would be a more accurate means of determining how effective a feeding strategy would be. Wallis et al (2005) managed to make an estimate of liver glycogen sparing when measuring the ^{13}C plasma enrichment. This approach would provide an estimate of glycogen sparing without changing the assessment protocol too much. However, this type of nutritional assessment is likely to be limited to exceptionally well-funded professional athletes.

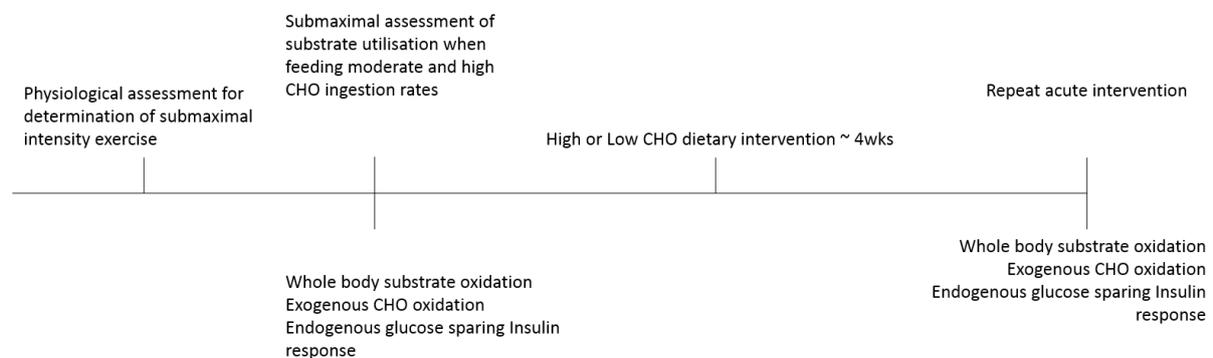


Figure 5.2 Time line of proposed habitual dietary metabolism cross over study.

Clear that peak oxidation rates are not the only explanation for an improvement in performance when consuming higher rates of CHO.

5.4 AIM 3: NEUROMUSCULAR INVESTIGATION

In Chapter 4 we performed a comprehensive neuromuscular assessment prior to and following endurance cycling exercise whilst consuming CHO at different rates, an investigation that has not been previously performed. We were one of the first to utilise the non-invasive HDEMG system before and after endurance based exercise providing insight into the recruitment of motor units and the individual firing rates of those units. Here, we observed an increase in the firing rate of early recruited motor units following exhaustive endurance exercise. However, consuming CHO at ingestion rates between 20 and 64 g·h⁻¹ did not alter these recruitment patterns. We also observed no change in M-wave peak to peak twitch potential or force generation indicating that CHO dose does not influence peripheral signal propagation. Additionally, we identified no changes in gross EMG amplitude during the 2h submaximal bout.

The increase in motor unit firing rate of early recruited motor units provides some insight into the regulation of force development following prolonged exhaustive endurance exercise. Early recruited, highly oxidative fibres, low force motor units vary the rate of firing to accommodate for the reduction in force generation possible (figure 4.5) following the metabolic stress of exhaustive endurance exercise. The reduction in MVC suggests the endurance exercise performed was sufficient to reduce peak force and compromise the ability to generate maximal force. It is possible, though highly speculative, that the increase in firing rates of these early recruited units was in order to compensate for the loss of later recruited motor units that may not have been recruited at 70% of MVC (the intensity of the contraction performed when assessing motor unit firing rate characteristics). Irrespective of the impact of exercise ingesting CHO at rates between 20 and 64 g·h⁻¹ had no effect on the contractile properties and recruitment patterns of motor units. As such, it seems that the increase in firing rate

of early recruited motor units may occur irrespective of substrate provision during the exercise conducted.

Timing of the measure

Performing the motor unit characteristics assessment following the 2 h bout would have been beneficial – though our study design did not permit this type of assessment as we did not wish to delay the start of the time trial task following the 2 h submaximal ride. Though, on reflection we only observed very subtle changes in firing rate characteristics following the exhaustive protocol in comparison to resting values. A future study specifically designed to determine the time course changes in motor unit recruitment following endurance exercise with CHO ingestion may adopt a study design with longer exercise duration or increased intensity. However, consideration would need to be made as to how long and how hard the exercise should be as these factors are likely to influence the ‘optimal’ ingestion rate possible.

The original hypothesis for the neuromuscular component in CHO stemmed from: 1. the increased in performance attained when conducting mouth rinsing during shorter duration higher intensity exercise and 2. The increase in brain activity following MRI with CHO mouth rinsing (Gant, et al 2010). As such it could be possible that the performance gains are attained only during exercise bouts less than 1 h. A study by Jeffers et al (2015) is currently in press describing some of the neuromuscular actions measured following CHO mouth rinse. They report some attenuation of neuromuscular fatigue however this does not correspond with an increase in exercise performance.

5.4.1 Range of measures

We utilised a wide range of fundamental neuromuscular assessment techniques however we have not exhausted the range of measures available. It is possible that other more sensitive techniques may determine a difference. For example, transcranial magnetic stimulation would provide additional insight into the development of central fatigue with different CHO ingestion rates (Davranche, et al 2015). Coupled with the MUFR assessment measure it may then be possible to drill down into what exactly the changes in central activation are and how they affect the outcome measure of motor unit activation.

5.4.2 Central cognitive function

Laboratory based cycling activity is not a cognitively challenging task when compared to other sports that require key decision making tasks that usually determine the outcome of the game/match/play. The latest CHO feeding research is recognising the benefits of CHO ingestion on maintaining cognitive function with fatigue during exercise. For example McRae et al (2012) reported that return success was greater during the second set of a simulated tennis match ($p < .05$) when consuming a carbohydrate electrolyte beverage in comparison to a placebo.

Higher cognitive functioning is important in sports where decision making is key. Performance in this regard is not the number of Watts that can be maintain in an isolated linear task, such as a time trial, but rather at what critical point in a race should those Watts be applied in order to attain the best competitive advantage over a competitor. Indoor cycling simulation devices are becoming more and more complex and 'life like' providing an opportunity in which to try and simulate race winning decision making tasks. It may be possible to apply this particular scenario to cycling and assess

whether participants responded positively or negatively to a particular simulated situation.

5.5 Concluding summary of novel findings

When using CHO during exercise recent studies on well-trained but not elite athletes demonstrate the effect of diminishing returns in exercise performance gains with increasing amount of CHO ingested. As such, well-trained individuals can be recommended to consume more modest amounts (30-40 g·h⁻¹) of CHO without fear of a reduction in performance gains when exercise lasts <3 h. It is likely a combination of enhanced endogenous CHO sparing, maintained CHO oxidation rates, and sustained rates of fat oxidation when ingesting 39 g·h⁻¹ resulted in improved endurance performance. At higher CHO ingestion rates blunting of fatty acid oxidation may counter the benefit of greater CHO delivery. CHO ingestion at any rate also does not appear to affect neuromuscular function during exercise bouts less than 3 h. There is potential that elite athletes may well tolerate higher ingestion rates and can start to increase the amount of CHO they are consuming should they respond positively to the moderate ingestion rate of CHO. However, it should be noted that not all individuals will respond positively to CHO intake and practitioners and nutritionists alike should be aware of this possibility.

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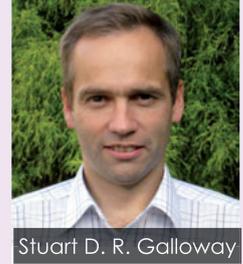
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Impact of carbohydrate nutrition on exercise metabolism and performance

KEYWORDS: Carbohydrate, performance, metabolism, glycogen

Abstract Carbohydrate (CHO) ingestion before and during exercise has consistently been reported to increase endurance exercise capacity/performance but the mechanisms responsible and optimal dose required are still debated. Feeding CHO is believed to spare muscle glycogen, spare liver glycogen, have central neural actions, and peripheral neural effects. A combination of these mechanisms is likely and the nature of the exercise performed is key when interpreting these data. Research on the optimal dose of CHO to improve performance over a range of exercise durations and intensities has been a recent focus. Optimal doses suggested from these studies cover a range (30-80 g•h⁻¹) that likely reflects exercise task, training status, and/or individual variation in response.

INTRODUCTION AND HISTORICAL BACKGROUND

After almost 100 years of research on CHO metabolism and exercise there are still big questions over mechanisms of action and optimal dose during exercise to maximise performance (1, 2). The purpose of the present review is to highlight these questions and cover key elements for consideration in future research and for applied sport nutrition practice. To achieve this we consider the historical background, effects of CHO on exercise performance/capacity, mechanisms of action, carbohydrate dose, and practical recommendations. Dietary carbohydrates (CHO) were first recognised as an important fuel for muscle during moderate to high intensity exercise (defined for the purpose of the review as 50 – 90% of maximal aerobic capacity) by Krogh and Lindhard (3). They observed that participants felt tired and exercise capacity was reduced following a short term high fat diet. However, three days of a high CHO diet reversed these symptoms of fatigue (3). In addition, other authors reported that blood glucose concentration was lower in the majority of competitors immediately following the 1924 Boston Marathon (4, 5). The following year blood glucose concentration immediately after a marathon was higher in participants who had consumed confectionary during the race compared to those who consumed nothing (5). Subsequently, when fatigue was associated with low blood glucose concentration feeding a high CHO diet could extend exercise capacity (206 mins vs. 81 mins) (6). The findings led to greater consideration of the potential ergogenic impact that nutrition could have during exercise. These early observations demonstrated that CHO ingestion had an important role in regulating blood glucose and extending exercise capacity. However, the mechanisms to explain these observations with CHO feeding and elevated

blood glucose had not yet been addressed.

The skeletal muscle biopsy technique, developed by the French neurologist Guillaume-Benjamin-Amand Duchenne (7), was reintroduced in the mid 1960's providing insight into muscle glycogen usage during exercise. Bergstrom and Hultman (8) demonstrated that skeletal muscle glycogen content could be increased following muscle glycogen depleting exercise if a high CHO diet was consumed. Furthermore, increasing the glycogen content of muscle improved exercise capacity above that obtained with normal muscle glycogen content. The application of this new insight was demonstrated when the consumption of a high CHO diet improved 30 km running time over that obtained on a normal diet (9). Similarly, football players covered less distance, ran slower, and walked more often during the second half of a match when starting the game with reduced muscle glycogen stores (10). It was also noted during these investigations that the consumption of carbohydrate elevated blood glucose concentration whilst sparing muscle glycogen. As such, these observations supported the notion that higher muscle glycogen content, as a result of CHO consumption in the days preceding exercise, improves the capacity to perform moderate to high intensity exercise.

In summary this early work clearly defined an association between muscle glycogen and exercise capacity. The ability to enhance muscle glycogen content via nutritional manipulation is now well established (11) and is still considered to be crucial for optimal performances (12, 13). However, CHO ingestion immediately before, and during exercise is also considered to enhance performance without any initial difference in muscle glycogen content. The remainder of the present review will focus on quantifying the size of this effect and exploring mechanisms of action and implications for applied practice.

CARBOHYDRATE AND ENDURANCE EXERCISE PERFORMANCE / CAPACITY

The ergogenic effect of CHO feeding during moderate to high-intensity exercise has been extensively investigated and summarised in 3 recent meta-analyses (14-16) that are worth consideration. The first meta-analysis investigated the duration of exercise where CHO was having the largest effect. The analysis used Cohen's effect sizes as a means of inferring the magnitude of effect and can be interpreted as; 0.2 small; 0.5 moderate; 0.8 large effects respectively (14). The authors reported that in the 72 studies examined, CHO ingestion had a moderate positive effect size (ES) of ~0.42 on exercise performance / capacity. Feeding CHO during exercise bouts of > 2 h had a significantly greater effect (ES ~0.5) than on those between 30 min and 2 h (ES ~0.35). Interestingly, the effect size was similar in running and cycling (ES ~0.4), and with feeding glucose alone or mixed monomers (ES ~ 0.4). CHO feeding during exercise has been shown to increase power output during a 40km time trial (TT) lasting ~1 h (17), during the latter stages of a 60min TT (18), during an all-out 15 min TT task following a 45 min submaximal ride (19), and during Wingate sprint performance following a 50 min sub maximal ride (80% VO_{2max}). However, Mitchell et al (20) reported no difference in 10 km running time when participants ingested a range of CHO doses (0, 34, 39, 50 $g \cdot h^{-1}$). Equally it has been reported that time to exhaustion (TTE) at 80% VO_{2max} did not change whether CHO or water was consumed prior to or during exercise (21). Interestingly, these two studies both examined shorter exercise durations (20-30 mins) than other studies included in the analysis. Consequently, it is generally considered that exercise tasks are required to be > 40 min, irrespective of CHO type or dose, to achieve a moderate beneficial effect on performance / exercise capacity. Nonetheless, new evidence indicates that acute CHO provision may have a beneficial effect on very short task durations (i.e., <10 min) suggesting a need for further study within this context (22).

In the second meta-analysis fifty studies were grouped to illuminate the effects of the exercise protocol, pre-exercise nutritional status (i.e., fed or overnight-fasted), training status, and gender on the efficacy of CHO feeding during exercise (15). Four exercise categories were considered: time to exhaustion (TTE), time trial (TT), submaximal preload with TTE (submax+TTE) and submaximal preload with TT (submax+TT). The inclusion criteria for studies with multiple CHO trials was limited to the single highest glucose concentration trial with ingestion rates between 30 and 80 $g \cdot h^{-1}$ and solution concentration not exceeding 8%. The results from this analysis are shown in Table 1.

The results from this meta-analysis imply that CHO intake in line with current intake guidelines (30 to 80 $g \cdot h^{-1}$) during exercise ≥ 1 h, moderately improves exercise performance/capacity irrespective of the exercise protocol used. Additionally, the pre exercise nutritional status of participants (i.e. fasted >8 h, and non-fasted <6 h) appears to have no effect on the subsequent exercise performance / capacity achieved. However, the scope of this meta-analysis was limited and did not comprehensively address all feeding strategies employed in the wider literature, potentially leading to an over-simplified view.

To provide greater scope the third meta-analysis will consider pooled 88 randomised cross over studies

Mode of Exercise	Number of studies	Grouped mean Cohen's ES*	95% CI of ES, (p-value)	Weighted mean improvement (%)
TTE	19	0.47	0.32 – 0.62, p < 0.01	15.1
Submax+TTE	3	0.44	0.08 – 0.8, p = 0.017	54.2
TT	11	0.30	0.07 – 0.53, p=0.011	2.0
Submax+TT	17	0.53	0.37 – 0.69, p < 0.001	7.5

Table 1. Number of studies, effect size (ES) outcomes, and mean percentage improvement in performance from placebo/control as presented in the meta-analysis from Temesi et al (2011). *0.2 small; 0.5 moderate; 0.8 large effect.

investigating the effects of CHO consumption during exercise (16). Of the studies included 83% used cycling as the mode of exercise and all studies measured exercise capacity with time to exhaustion (mean duration 106 min) and exercise performance via time trial (mean duration 47 min). The mean percentage change in capacity / performance from control conditions was deduced by compiling the reported additive performance effects of components included in the model. However, a -2% (impairment) to a +6% (improvement) was reported across all studies included in the meta-analysis. Interestingly, for some individuals consuming CHO caused a reduction in exercise performance / capacity. The range in performance outcome is not surprising considering the variety of feeding strategies, exercise interventions, and participant characteristics included in the analysis. Nonetheless, some recent studies report considerable individual variation in performance measures even when the same exercise intervention is used to determine the effect of CHO feeding. Taken together the variability reported highlights the importance of acknowledging individual responses to CHO intake during exercise (23, 24). Overall, there seems to be a clear moderate positive effect of CHO feeding on exercise performance/capacity throughout the three meta-analyses considered. Several mechanisms have been proposed and investigated to explain the ergogenic action of CHO feeding during sustained moderate-high intensity exercise.

MECHANISMS OF ACTION

Muscle glycogen sparing

As indicated in the Introduction fatigue has frequently been associated with low muscle glycogen (8, 25, 26). Nutritional strategies used to increase muscle glycogen stores prior to exercise can lead to enhanced performance and an increase in exercise capacity (27, 28). Similarly, nutritional strategies utilising CHO during exercise may also have a positive effect on endurance performance/capacity by providing an alternative fuel source for muscle energy metabolism and therefore limiting the amount of muscle glycogen utilised (26-31). An overview of the studies investigating the effect of CHO provision before, and during, exercise on working muscle glycogen utilisation (measured from skeletal muscle biopsy samples) is shown in Table 2. 50% of the 16 studies listed report a sparing of muscle glycogen with CHO supplementation during exercise. Further, some reports indicate muscle glycogen sparing may be fibre type specific (26, 27) although conflicting evidence has emerged (26). The variability in outcomes may reflect differences in exercise protocol (e.g., continuous vs intermittent running and cycling), location of muscle biopsy, method of analysis (e.g., biochemical vs. histochemical, mixed muscle vs. fibre

Study	Exercise mode and duration	Intensity	CHO intervention	MG sparing
Glycogen Sparing				
Berstrom and Hultman (1967)(8)	Running, 1 h	950 kpm/min	Glucose infusion (3.5 g·min ⁻¹)	20% total reduction
Bjorkman et al (1984)(28)	Cycling, TTE	68% VO _{2max}	70 g·h ⁻¹ glucose	Rate of utilisation decreased from 2.3 to 1.3 mmol kg d.w. ⁻¹ min ⁻¹
Eríksson et al (1987)(32)	Cycling, 90 min	65-70% VO _{2max}	1 g·kg BM	31.8% reduction
Hargreaves et al (1984)(30)	Cycling, 4 h	50% VO _{2max} + intermittent sprints/30 min.	43 g·h ⁻¹ solid CHO + 400ml H ₂ O	26% total reduction
Stellingwerf et al (2007)(26)	Cycling, 3 h	63% VO _{2max}	0.7 g CHO·h ⁻¹	19% total reduction
Tsintzas et al (1995)(27)	Running, 1 h	70% VO _{2max}	5.5% mixed CHO	28% total reduction
Tsintzas et al (2001)(31)	Running TTE (matched)	70% VO _{2max}	8 ml·kg BM of 5.5% CHO solution	56% reduction in Type I fibres
No Glycogen Sparing				
Arkininstall et al (2001)(33)	Running and cycling 1 h	Lactate threshold	8 ml·kg BM pre ex, 2 ml·kg BM every 20 min 6.4% solution	No difference
Chryssanthopoulos et al (2002)(34)	Running 1 h	70% VO _{2max}	Pre ex meal and 46 g CHO during	No difference
Coyle et al (1986)(35)	Cycling TTE	71% VO _{2max}	2.0 g·kg BM @ 20min, 4 g·kg BM each 20 min thereafter.	No difference
Coyle et al (1991)(36)	Cycling 2 h	73% VO _{2max}	Hyperglycemic infusion clamp (~10 mmol)	No difference
Febbraio et al (1996)(37)	Cycling 2 h + 15 min performance	70% Peak O ₂ consumption	Pre ex CHO meal	No difference
Febbraio (2000)(38)	Cycling 2 h + 15 min performance	70% Peak O ₂ consumption	Pre ex CHO meal	No difference
Flynn et al (1987)(39)	Cycling 2 h	Complete as much work as possible	Mixed CHO/concentration	No difference
Hargreaves et al (1988)(40)	Cycling 2 h	70% VO _{2max}	30 g CHO pre and every 30 min	No difference
Mitchell et al (1989)(41)	Cycling 2 h	105min @ 70% VO _{2max}	6, 12 and 18 g·100ml	No difference

Table 2. Summary of studies reporting muscle glycogen usage and carbohydrate ingestion during exercise

specific), and inherent variability in the measurement of muscle glycogen. Many of the studies which report muscle glycogen sparing also report an increase in circulating plasma glucose concentration as a major factor in explaining this phenomenon. It is possible that circulating plasma glucose has a role to play but the mechanisms of action continue to be elusive. As such, muscle glycogen sparing can occur in some contexts but this is not always observed leading to the suggestion that there may be other mechanisms to explain the positive effects of CHO feeding.

Role of liver glycogen

Moderate to high rates of CHO intake during exercise have been reported to reduce endogenous hepatic glucose output to basal levels (42) or completely inhibit endogenous hepatic glucose output altogether (43). Thus, exogenous CHO oxidation could replace hepatic glycogen as a fuel source. Coyle et al (1986) reported an enhanced maintenance of blood glucose during prolonged exercise with the ingestion of CHO. The authors attributed an enhanced blood glucose concentration with elevated rates of CHO oxidation throughout the bout. Consequently, feeding CHO should allow more liver glycogen to be available towards the latter stages of competitive exercise when exercise intensity, and the requirement for CHO

oxidation, are both increased. Casey et al (44) investigated liver glycogen depletion during exercise and its subsequent repletion over 4 hours post exercise when feeding different types of CHO. Liver glycogen content was measured using ¹³C magnetic resonance spectroscopy following an overnight fast prior to exercise, and following each glycogen resynthesis trial. A weak but positive association between liver glycogen content following resynthesis and subsequent TTE occurred. Therefore, CHO feeding prior to and during exercise, which increases liver glycogen content and/or reduces the rate it is utilised, may be one factor to help explain the mechanisms of action that feeding CHO can have on exercise performance / capacity. However, this mechanism of action may only be relevant when feeding stops but exercise continues. Nevertheless, it is likely the liver has a key regulatory role when achieving optimal performances with CHO supplementation and warrants further investigation.

Peripheral neural effects

A limited amount of research has been conducted on the peripheral neural effects of CHO ingestion and muscle activity. One study reported attenuation in the rise in muscle neural activity during the latter stages (> 45 min) of exercise at 84% VO_{2max} when ingesting a 6.4% CHO solution (45). Additionally, Abbiss and Peiffer et al (46) highlighted a correlation between muscle activation of the VL and power output sustained during a 16.1 km TT task. However, CHO feeding had no effect on the percentage activation of the muscle when CHO was consumed. This highlights that it was not central factors influencing fatigue during the trial. Additionally, when CHO was not consumed; muscle activation level was maintained

even with a reduction in the power output produced. Taken together these findings suggest a peripheral metabolic nature of the fatigue in the TT task. As such, CHO intake during exercise may be able to directly affect the contractile properties of the active muscle, helping to promote force production and subsequently improve performance, though the exact mechanism to explain the effect is unclear. It should be noted that this interpretation is based on only a few studies, often with poor methodological control of muscle activity recordings, highlighting a need for future research on this possible mechanism. In contrast, research investigating the potential central neural effects of CHO administration has been much more active.

Central neural effects (oral glucose sensing)

There is now robust evidence to indicate that CHO can be detected in the oral cavity (47-49) which is thought to directly affect the brain (50, 51). Carter et al (52) demonstrated that detection of CHO in the oral cavity also has an effect on exercise performance. Participants completed a simulated TT (~1 h) as fast as possible when mouth rinsing either a CHO or placebo solution. CHO mouth rinsing caused a significant (2.9%) increase in performance compared to the placebo condition. To isolate the effect of the gastrointestinal (GI) tract Carter et al then infused CHO

intravenously to bypass the GI tract and maintain exogenous CHO availability. CHO was infused at $1 \text{ g} \cdot \text{min}^{-1}$ but had no effect on exercise performance compared to placebo (53). These observations highlighted the potential importance of oral CHO sensing for performance enhancement during short duration activity. Furthermore, there is great interest in this work since endogenous CHO availability for short duration high intensity efforts is not typically considered a limiting factor for exercise performance in this context. The outcomes from these studies have led to speculation regarding the underlying mechanisms explaining CHO mouth sensing, and also CHO ingestion during short duration tasks (<1 hr). Some have proposed that oral cavity CHO sensitive receptors exist which detect exogenous CHO and directly act upon the central nervous system (50). An increase in the corticomotor output in the fasted state to both rested and fatigued skeletal muscle when CHO is rinsed in the mouth supports this hypothesis (50). Similarly, CHO mouth rinsing significantly improved performance over that of a saccharin sweetened placebo demonstrating that oral sensors are caloric sensitive (51). Additionally, Chambers reported increases in neural activity in brain regions involved in reward and motor control when mouth rinsing a CHO solution. The authors proposed that the increase in brain activity in these regions supports the increase in performance. However, not all studies report an increase in performance with CHO rinsing (54, 55). The duration of the pre-exercise fast has led some to speculate that nutritional status (fed vs. fasted) of the participant influences the impact of the effect (56).

CARBOHYDRATE DOSE RESPONSE – WHAT IS THE OPTIMAL INGESTION RATE?

Many attempts have been made to recommend 'optimal' doses of CHO for endurance performance. However, considerable debate remains as to how much is enough? Some have suggested there may be a dose response relationship between CHO ingestion and endurance exercise performance. Smith et al (14) fed 15, 30, and $60 \text{ g} \cdot \text{h}^{-1}$ during a 2 h submaximal ride prior to a 20 km TT. All CHO conditions significantly improved performance compared to the placebo condition. However, a lack of statistical power ($n=12$) meant only the $60 \text{ g} \cdot \text{h}^{-1}$ ingestion rate significantly improved performance over that achieved with $15 \text{ g} \cdot \text{h}^{-1}$. Additionally, Watson and Shirreffs et al (57) reported that exercise capacity was improved to the same extent with 32 and $47 \text{ g} \cdot \text{h}^{-1}$ of CHO compared to placebo suggesting that a saturation in additional performance gains may occur around $30 \text{ g} \cdot \text{h}^{-1}$. Both of these studies suggested a trend for a dose response relationship, but the low sample sizes have made the existence of a dose response relationship difficult to confirm. Smith et al (2013) increased sample size using a multi-centre study. Fifty five participants spread across four sites consumed CHO during a 2 h submaximal ride followed by a 20 km TT task.

Each participant completed 4 trials, one placebo and three CHO treatments, between 10 and $120 \text{ g} \cdot \text{h}^{-1}$ ($10 \text{ g} \cdot \text{h}^{-1}$ increments). Following some statistical modelling of their data the authors reported an optimal dose of $78 \text{ g} \cdot \text{h}^{-1}$ for performances during the TT. However, the linear regression model used for the determination of the optimal feeding strategy utilised was not significant. There are also some concerns over the study design and allocation of treatments across multiple sites. Smith et al report a 1.7% improvement in performance between 30 and $80 \text{ g} \cdot \text{h}^{-1}$ and a rather trivial 0.7% improvement in performance when dose is increased from 40 to $80 \text{ g} \cdot \text{h}^{-1}$. Taken together these studies suggest increasing amounts of CHO may result in diminishing returns with respect to performance gains. From a practical perspective, it is important to further clarify the dose-response relationship. There is a need to discriminate between the dose increments where the largest gains in performance are observed, and dose increments where performance gains are marginal but negative effects (such as gastro-intestinal distress) are increased. As such, we recently conducted a study where participants ingested 0 (water control) 20, 39 and $64 \text{ g} \cdot \text{h}^{-1}$ of glucose during



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a 2 h submaximal ride which was followed by simulated time trial (~ 35 min). Our unpublished data indicate that 39 and 64 g.h⁻¹ were equally effective at improving endurance exercise performance (6.1% and 6.5% respectively) over that of a water control. Additionally, although not statistically significant, consuming 20 g.h⁻¹ led to a meaningful mean improvement of 3.7% (~1 min) when compared to the control trial. The results of our study are in line with that of Watson et al (57) highlighting a probable saturation in performance gains with increasing rates of CHO ingestion. Similarly, as shown in other studies, there is considerable variability in the inter-individual response to the different CHO rates ingested. As such, a focus is required on the underlying mechanisms to understand why some individuals respond positively to CHO supplementation and some do not. This may involve individual variation in the tolerability of the solution ingested and in the ability to oxidise the ingested CHO available, in addition to differences in the putative central or peripheral neural mechanisms of CHO feeding outlined above.

PRACTICAL APPLICATION

CHO is crucial for optimal performances and maintaining glycogen stored during heavy periods of training. Consuming a high CHO diet will ensure elevated muscle and liver glycogen contents prior to exercise which may facilitate an increase in exercise performance. When using CHO during exercise recent studies on well-trained but not elite athletes demonstrate the effect of diminishing returns in exercise performance gains with increasing amount of CHO ingested. As such, well-trained individuals can be recommended to consume more modest amounts (30-40 g h⁻¹) of CHO without fear of a reduction in performance gains. Elite athletes may well tolerate higher ingestion rates and can start to increase the amount of CHO they are consuming should they respond positively to increasing amounts of CHO. However, it should be noted that not all individuals will respond positively to CHO intake and practitioners and nutritionists alike should be aware of this possibility.

CONCLUSION

Carbohydrate ingestion during exercise has been positively associated with increases in exercise capacity and performance for a number of years. We are only now starting to understand the mechanisms underpinning these physiologically enhancing effects. The mechanisms are not purely metabolic in nature with peripheral and central neural effects being reported with CHO intake. The optimal dose of CHO for endurance performance is not well defined but appears to be around 40 g.h⁻¹. However, this value is likely to be highly individual specific so athletes should take an active role in determining what is optimal for them.

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The Ingestion of 39 or 64 g·hr⁻¹ of Carbohydrate is Equally Effective at Improving Endurance Exercise Performance in Cyclists

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In an investigator-blind, randomized cross-over design, male cyclists (mean \pm SD) age 34.0 (\pm 10.2) years, body mass 74.6 (\pm 7.9) kg, stature 178.3 (\pm 8.0) cm, peak power output (PPO) 393 (\pm 36) W, and $\text{VO}_{2\text{max}}$ 62 (\pm 9) ml·kg⁻¹·min⁻¹ training for more than 6 hr/wk for more than 3y ($n = 20$) completed four experimental trials. Each trial consisted of a 2-hr constant load ride at 95% of lactate threshold (185 \pm 25W) then a work-matched time trial task (~30min at 70% of PPO). Three commercially available carbohydrate (CHO) beverages, plus a control (water), were administered during the 2-hr ride providing 0, 20, 39, or 64g·hr⁻¹ of CHO at a fluid intake rate of 1L·hr⁻¹. Performance was assessed by time to complete the time trial task, mean power output sustained, and pacing strategy used. Mean task completion time (min:sec \pm SD) for 39g·hr⁻¹ (34:19.5 \pm 03:07.1, $p = .006$) and 64g·hr⁻¹ (34:11.3 \pm 03:08.5 $p = .004$) of CHO were significantly faster than control (37:01.9 \pm 05:35.0). The mean percentage improvement from control was -6.1% (95% CI: -11.3 to -1.0) and -6.5% (95% CI: -11.7 to -1.4) in the 39 and 64g·hr⁻¹ trials respectively. The 20g·hr⁻¹ (35:17.6 \pm 04:16.3) treatment did not reach statistical significance compared with control ($p = .126$) despite a mean improvement of -3.7% (95% CI -8.8–1.5%). No further differences between CHO trials were reported. No interaction between CHO dose and pacing strategy occurred. 39 and 64g·hr⁻¹ of CHO were similarly effective at improving endurance cycling performance compared with a 0g·hr⁻¹ control in our trained cyclists.

Keywords: nutrition, metabolism, time trial

Carbohydrate (CHO) intake during exercise has consistently been shown to improve exercise performance (Smith et al., 2013; Smith et al., 2010) and extend exercise capacity (Galloway & Maughan, 2000; Watson, Shirreffs, & Maughan, 2012). CHO is thought to act in many ways to enhance performance: sparing of muscle glycogen (Bjorkman, Sahlin, Hagenfeldt, & Wahren, 1984; Stellingwerff et al., 2007); enhancing and maintaining elevated CHO oxidation rate; maintenance of blood glucose concentration (Coyle, Coggan, Hemmert, & Ivy, 1986); elevated exogenous CHO oxidation rate (Galloway, Wootton, Murphy, & Maughan, 2001); and central and peripheral neural up-regulation (Carter, Jeukendrup, & Jones, 2004; Chambers, Bridge, & Jones, 2009; Nikolopoulos, Arkinstall, & Hawley, 2004). As a result, CHO feeding strategies are now widely employed in the exercise setting as a means to support athletic performance.

Although the provision of CHO has been shown to improve exercise performance/capacity, the optimal dose of CHO required to maximize athletic performance remains a topic of debate. Currently, guidelines from the ACSM state an optimal dose of CHO during exercise to be within the range of 30–60 g·hr⁻¹. However, significant improvements in performance and exercise capacity have been reported with ingestion rates as low as 22 g·hr⁻¹ (Galloway & Maughan, 2000; Maughan, Bethell, & Leiper, 1996) and as high as >100 g·hr⁻¹ (Currell & Jeukendrup, 2008) highlighting a beneficial impact of CHO ingestion over a much broader range of feeding rates, when compared with water or placebo solutions. Smith et al. (2010) indicated that 15, 30 and 60 g·hr⁻¹ were all *very likely* to improve power output sustained (7.4, 8.3 and 10.7% respectively) during a 20 km TT when compared with a 0 g·hr⁻¹ placebo, with 60 g·hr⁻¹ providing the largest effect. Furthermore, 30 g·hr⁻¹ was *very unlikely* to further improve performance over 15 g·hr⁻¹ while 60g·hr⁻¹ was *likely* to improve performance over the 30g·hr⁻¹ with a mean percentage improvement of only 2.3%. However, following post hoc power calculations, the authors indicated that a sample size of 15–22 was required to confidently conclude there were no differences in performances across the three doses. In contrast,

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Watson et al. (2012) reported no further improvements in time to exhaustion when feeding a 6% (~47 g·hr⁻¹) mixed CHO solution when compared with a 4% (~27 g·hr⁻¹) mixed solution, though a small increase of 20 g·hr⁻¹ may have missed any potential increase. Nevertheless, the absence of an additional improvement with the higher CHO dose is surprising considering the improvements in performance reported with higher ingestion rates (Smith et al., 2010). As such, it seems a range of CHO feeding doses increases performance over a 0g·hr⁻¹ condition. However, any additional increases in CHO provision above feeding rates of ~30 g·hr⁻¹ do not appear to have a clear significant improvement on performance.

To provide clarity to the optimal dose of CHO for performance additional studies with greater sample sizes have followed up these initial reports. In a recent study Smith et al. (2013) expanded on these data and examined fifty five participants spread across four sites. The participants consumed CHO during a 2 hr submaximal ride followed by a 20km TT task. Each participant completed 4 trials, one placebo, and three CHO treatments, between 10 and 120g·hr⁻¹ (10 g·hr⁻¹ increments) which consisted of a 1:1:1 ratio of glucose, fructose, and maltodextrin. Following some statistical modeling of their data the authors reported an optimal dose of 78 g·hr⁻¹ for performances during the TT. However, they reported only a small 1.7% improvement in performance from 30 to 80 g·hr⁻¹, and a rather trivial 0.7% improvement in performance from 40 to 80 g·hr⁻¹. In addition, the linear regression model used for the determination of the optimal feeding strategy used was not significant. Taken together, these studies indicate that the largest gains in performance occur between 0 and 40 g·hr⁻¹ of CHO ingestion with only relatively small increases in performance with ingestion rates up to 80 g·hr⁻¹.

These data, coupled with those of Smith et al. (2010) and Watson et al. (2012), indicate a divide between the optimal feeding rates and compositions reported by investigators and the subsequent measurable and meaningful improvement in performance obtained from increasing amounts of CHO, particularly in the 30–60 g·hr⁻¹ range. Similarly, the range of responses reported across the feeding rates provided in these studies considered this individual variability. Accordingly, the aim of the current study was to determine the dose response relationship between CHO feeding and exercise performance in the 0–64 g·hr⁻¹ range in 20 male cyclists. We hypothesize that performance gains compared with a 0 g·hr⁻¹ will be smaller with increasing doses of CHO administered.

Methodology

Twenty trained male cyclists were recruited from regional cycling and triathlon clubs. The mean (\pm SD) characteristics of the participants were: age 34.0 (\pm 10.2) years, body mass 74.6 (\pm 7.9) kg, stature 178.3 (\pm 8.0) cm, peak power output (PPO) 393 (\pm 36) W, and VO_{2max} 62 (\pm 9) ml·kg⁻¹·min⁻¹. Participants were required to have

been training for more than 6 hr per week for more than 3 years. Each individual had the procedures and associated risks explained before providing written informed consent to participate in the study, which was approved by the local research ethics committee in accordance with the Declaration of Helsinki.

Design

In an investigator blind, placebo controlled, randomized cross-over study design participants visited the laboratory 6 times (2 preliminary and 4 intervention) over a 6-week period. They completed one visit per week commencing each trial on the same day at the same time of day on each visit. The laboratory was maintained at a constant 19 \pm 1°C for all visits. Following prescreening, participants completed a preliminary assessment where lactate threshold, VO_{2max}, and, peak power output were determined. Following a 20-min break participants then completed the first familiarization of the performance task to be used in subsequent visits. On the second visit participants completed a full familiarization trial. The familiarization trial and four subsequent intervention trials involved a 120-min steady state submaximal cycle at 95% lactate threshold (185 \pm 25 W, 59 \pm 7% VO_{2max}) followed by a time trial performance task, whereupon the participants were instructed to be complete their set work target as quickly as possible. The steady state intensity was set at 95% lactate threshold to ensure a similar metabolic demand of the exercise for all participants. Water was ingested for the familiarization trial and consumed at a rate of 1 L·h⁻¹. Thereafter, on the intervention trials participants consumed in a random order either: a control (water) 0%, 2%, 3.9% or 6.4% CHO solutions, in counter balanced randomized order, at a fluid ingestion rate of 1L·h⁻¹, thus providing carbohydrate at 0, 20, 39, or 64g·hr⁻¹. Performance was determined as the time to complete a work matched simulated time trial task designed to last ~30 min. Pacing strategy was assessed from taking time splits and average power output sustained for each 10% of work competed during the performance task.

Preliminary Testing

On week 1 of 6, following a 10 hr overnight fast, participants performed a two-section incremental cycle test (Lode Excalibur Sport, Netherlands) to determine maximal oxygen uptake (VO_{2max}, lactate threshold, and peak power output. Section 1 commenced at 120 W and each stage increased 30W every 3 min. The wattage continued to increase until the blood lactate concentration increased more than 2 mmol·L⁻¹ from the previous stage. The lactate threshold was defined as an increase of more than 1 mmol·L⁻¹ between stages (Aunola & Rusko, 1984). In the last 30 s of each stage, heart rate (Polar Electro, Finland) was recorded and a capillary blood sample (fingertip) was obtained for blood lactate concentration analysis by microassay (LactatePro LT-1710, ArkRay Inc., Kyoto, Japan). The reliability and validity of this device has

been previously determined (Pyne, Boston, Martin, & Logan, 2000). This initial stage was followed by a 10 min recovery period. Individual lactate responses were examined independently by two researchers to ensure validity and consistency of the analysis. The mean \pm SD lactate concentration at LT was 2.1 ± 0.4 mmol·L corresponding to an intensity of $52 \pm 6\%$ of PPO for LT which is typical of other studies utilizing a similar protocol (Neal et al., 2013).

Participants commenced section 2, starting at an intensity of the penultimate stage of section 1, with each stage lasting 1 min and increased by 30 W until volitional exhaustion. The end time and power output of the stage was used to calculate peak power output (PPO) using the following equation (Kuipers, Verstappen, Keizer, Geurten, & Van Kranenburg, 1985):

$$\text{PPO} = W_{\text{final}} + [(t/60) \cdot \text{PI}]$$

Where, W_{final} = the power output of the final completed stage in (watts), t = the time spent in the final uncompleted stage (seconds), 60 = the duration of each stage (seconds) and PI = the increase in power output between each stage (W). Maximal oxygen uptake ($\text{VO}_{2\text{max}}$) was also measured during this protocol via an automated online gas analysis machine (Oxycon Pro, Jaeger, Wuerzberg, Germany). $\text{VO}_{2\text{max}}$ was determined as the highest average VO_2 captured in a 30sec period.

Familiarization and Experimental Trials

Participants were asked to record their dietary intake for 2 days before the full familiarization, and were asked to replicate their diaries for all subsequent visits. In addition, participants were asked to refrain from intense exercise for 48 hr, and to rest completely 24 hr before any laboratory visit. On arrival to the laboratory participants emptied bladder and bowel before nude body mass measurements. Individuals then changed into cycling attire which was kept consistent throughout all trials to reduce thermoregulatory variability. Participants then completed a 2 hr submaximal ride at 95% LT (185 ± 25 W, cadence 80–95 rpm) during which one of four beverages were consumed: 0% water (familiarization and control); 2.0%; 3.9%; or 6.4% glucose (single carbohydrate, glucose monomers and polymers) based commercially available CHO beverage. All beverages were maintained at 10 °C and were consumed at a rate of $1\text{L}\cdot\text{h}^{-1}$ providing 0, 20, 39, and $64\text{g}\cdot\text{hr}^{-1}$ of CHO respectively. The 20 $\text{g}\cdot\text{hr}^{-1}$ solution contained 37 mg of sodium per 100 ml and the 39 and $64\text{g}\cdot\text{hr}^{-1}$ solutions both contained 50 mg per 100 ml. Such a small difference in sodium content is unlikely to have had any effect on the subsequent exercise performance. Each beverage was provided with an initial bolus ingestion of 240 ml 2 min before the start of exercise. Subsequently, 220 ml was consumed every 15 min with the final drink provided at 120 min of exercise. Following the 2 hr ride, a 5min recovery period allowed a toilet break and for the equipment to be set up for the performance task. The performance task was a work target simulated time trial specific to the individual ($531 \pm 48\text{KJ}$). A linear

factor, $70\% W_{\text{max}}$ ($275.4 \pm 24.8\text{W}$) divided by preferred cadence (rpm^2), was entered into the cycle ergometer. The formula used to determine the work target value was:

$$\text{Work target} = (0.7 \cdot \text{PPO}) \cdot 1800$$

The time trial protocol employed has previously been validated and has been shown to be highly reliable (A. Jeukendrup, Saris, Brouns, & Kester, 1996). Participants did not receive any verbal encouragement throughout the time trial task and the task was completed in silence.

Data Presentation and Statistical Analysis

All data are presented as mean (\pm SD) unless otherwise stated. Total time to complete the performance task and average power output sustained throughout were compared across all trials. The magnitude of difference from the water control was examined with a one-way ANOVA with Dunnett's post hoc comparisons made. The mean differences between two variables are presented as the mean with associated 95% confidence limits and Cohen's size effects (mean difference; confidence intervals; Cohen's size effects). Cohen's size effects can be interpreted as 0.2 = small, 0.6 = moderate, 1.2 = large, 2.0 = very large and 4.0 = extremely large. Performance task time and average power output was compared between treatments using repeated measures regression models. The null hypothesis of no differences between any of the treatments groups was tested using ANOVA with all values compared back to the water control condition. A difference from the control of 3.5% in either time to complete the task or mean power output sustained was considered a large and meaningful difference.

Results

Performance Time and Mean Power Output

Mean task completion time (min:sec \pm SD) for $39\text{g}\cdot\text{hr}^{-1}$ ($34:19.5 \pm 03:07.1$, $p < .01$) and $64\text{g}\cdot\text{hr}^{-1}$ ($34:11.3 \pm 03:08.5$, $p < .01$) CHO solutions were significantly faster than control ($37:01.9 \pm 05:35.0$) (Figure 1). Corresponding percentage change from the 0 $\text{g}\cdot\text{hr}^{-1}$ condition was similar at 6.1% (95% CI 1–11.3%; $p = .02$) for the 39 $\text{g}\cdot\text{hr}^{-1}$ trial, and 7% (95% CI 1–12%, $p = .01$) for the 64 $\text{g}\cdot\text{hr}^{-1}$ trial (Figure. 2). The 20 $\text{g}\cdot\text{hr}^{-1}$ ($35:17.6 \pm 04:16.3$) treatment did not reach statistical significance compared with control ($p = .13$) despite a mean improvement of 3.7% (95% CI -1.5 – 8.8%). Furthermore, the 20 $\text{g}\cdot\text{hr}^{-1}$ treatment did not differ significantly from the 39 or $64\text{g}\cdot\text{hr}^{-1}$ treatments. The Cohen's size effect in comparison with the control was 0.6 (95% CI -0.1 – 1.4), 1.0 (95% CI 0.2 – 1.7), and 1.0 (95% CI 0.3 – 1.8) for 20, 39 and $64\text{g}\cdot\text{hr}^{-1}$ treatments respectively indicating moderate and large effects on performance improvement.

In conjunction, there was a significant effect of treatment on mean power output sustained during the time

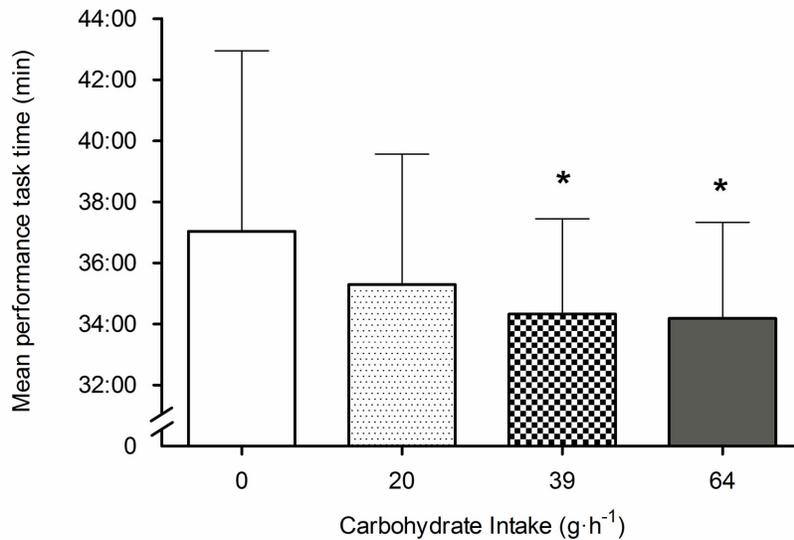


Figure 1 — Mean performance task time when 0, 20, 39, and 64 g·hr⁻¹ of carbohydrate were consumed. Data are presented as mean \pm standard deviation. Statistical significance from 0 g·hr⁻¹ is denoted by *($p < .05$).

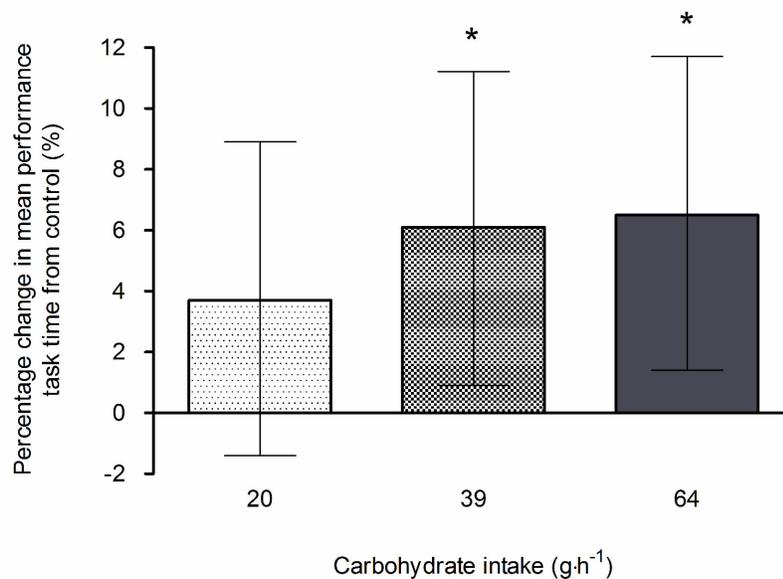


Figure 2 — Percentage change in mean performance task time from the 0 g·hr⁻¹ treatment for 20, 39, and 64 g·hr⁻¹ carbohydrate ingestion rates. The percentage change in performance task time was significantly greater in the 39 g·hr⁻¹ and 64 g·hr⁻¹ trials when compared to 0 g·hr⁻¹ *($p < .05$). Data are presented as mean \pm 95% confidence intervals.

trial between the four experimental trials ($p < .01$). There were significant increases of 17W (95% CI 5–30; $p < .01$) and 19W (95% CI 6–31; $p < .01$) in mean power output sustained throughout the 39 g·hr⁻¹ and 64 g·hr⁻¹ treatments, respectively. Corresponding percentage improvements compared with the 0g·hr⁻¹ trial were similar at 8% (95% CI 1–15%; $p = .02$) for the 39 g·hr⁻¹ trial, and 9% (95% CI 2–16%; $p = .01$) for the 64 g·hr⁻¹ trial. There was no statistical difference reported between the 20g·hr⁻¹ treatment and the 0 g·hr⁻¹ control ($p = .12$) despite a 5.7% (95% CI: -1.2–12.6) mean increase in power output sustained.

The Cohen's size effect compared with the control was 0.7 (-0.1–1.4), 1.1 (0.3–1.8), and 1.1 (0.4–1.9) for 20, 39 and 64 g·hr⁻¹ reflecting moderate and large effects respectively.

Pacing Strategy

The assessment of pacing strategy revealed no interaction between time and treatment ($p = .80$). This suggests no evidence of any differences in the slopes of the lines between the treatments in the incremental trends of performance time or mean power sustained (Figure 3).

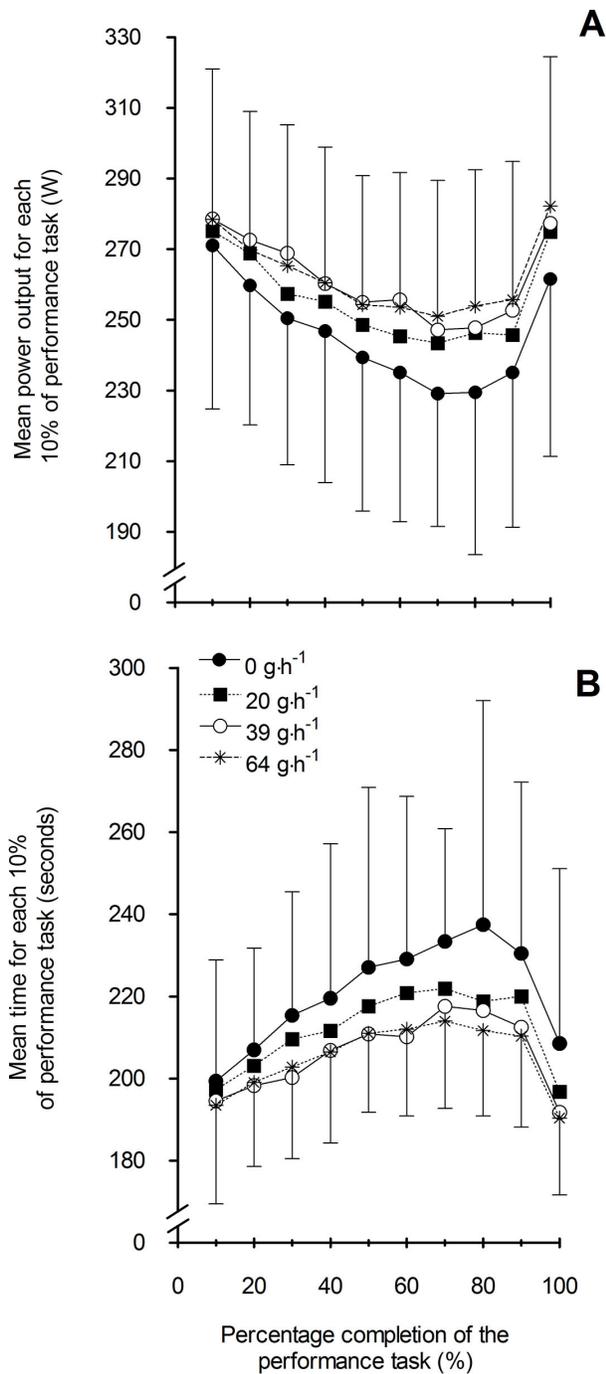


Figure 3 — Mean power output (A) and time to complete each 10% of the performance task (B) when 0 g·hr⁻¹ (closed circle), 20 g·hr⁻¹ (closed square), 39 g·hr⁻¹ (open circle), and 64 g·hr⁻¹ (star) of carbohydrate was consumed. Data are presented as mean ± standard deviation.

Discussion

This study was designed to determine the optimal dose of CHO to maximize endurance exercise performance. We show that CHO provided at rates of 39 and 64 g·hr⁻¹

were equally effective at improving performance in 20 trained male participants compared with a 0 g·hr⁻¹ water control. The 20 g·hr⁻¹ treatment did not, on average, show evidence of a significant improvement in participants' performance, despite demonstrating a mean improvement in both performance task time and mean power output of 3.7% over the 0 g·hr⁻¹ treatment. As such, our data demonstrate that a plateau in performance gain occurs when consuming a single source CHO beverage at rates between 39–64 g·hr⁻¹ during endurance tasks lasting less than 3 hr.

Previous studies investigating a dose-response relationship between CHO feeding and endurance exercise performance/capacity have reported somewhat conflicting results. Smith et al. (2010) provided evidence of a dose-response relationship when feeding glucose in the range of 15–60 g·hr⁻¹. These authors showed that all trials significantly improved performance of 12 cyclists over the placebo condition, with only the 60 g·hr⁻¹ likely to improve performance over the 15 g·hr⁻¹. However, the authors highlighted that 15–22 participants would be required to make meaningful comparisons between solutions, leaving no clear picture into the optimal dose of CHO. In a follow up investigation, Smith et al. (2013) reported that optimal performance gains with CHO ingestion were likely to occur at rates as high as 78 g·hr⁻¹ when consuming multiple forms of CHO. However, the optimal dose for the greatest improvement in performance was unclear in the 40–80 g·hr⁻¹ range and interpretation is limited by the choice of study design. In contrast, Watson et al. (2012) observed no further improvement in exercise capacity when 46 g·hr⁻¹ was consumed compared with 31 g·hr⁻¹ during prolonged exercise in cool conditions. We add to these data by demonstrating that the vast majority of the performance gains occur when ingesting 39 g·hr⁻¹ with greater amounts of CHO ingestion (64 g·hr⁻¹) providing negligible additional performance gains. As such, these results support the hypothesis that a ceiling in performance gains exists when consuming CHO above 40 g·hr⁻¹ during exercise less than 3 hr; however, any mechanistic explanation for the outcome would only be speculative due to the limited measures taken throughout the trial: though increased neural drive through oral sensors in the mouth; better maintenance of blood glucose due to greater exogenous glucose availability; enhanced maintenance of exogenous glucose oxidation; and endogenous glycogen sparing within the liver; are all potential explanations.

Consuming 20 g·hr⁻¹ of CHO in the current study had a less easily interpretable outcome. When participants consumed 20 g·hr⁻¹ performance did not significantly improve over the water control, while 39 or 64 g·hr⁻¹ of CHO did not significantly differ compared with 20 g·hr⁻¹. Other investigations have reported a significant improvement in performance and/or exercise capacity with quite modest (~15 g·hr⁻¹) amounts of CHO when compared with a 0 g·hr⁻¹ condition (Galloway & Maughan, 2000; Karelis et al., 2010; Maughan et al., 1996; Murray, Seifert, Eddy, Paul, & Halaby, 1989). Consuming 20 g·hr⁻¹ in the current study still produced a mean improvement in performance time of 3.7% compared with 0 g·hr⁻¹, which

corresponds to a ~58s reduction in time trial task time. The variance in response is a likely explanation for lack of statistical significance, but it is noteworthy that there is considerable variation in performance responses in all CHO conditions, not just at the 20 g·hr⁻¹. In addition, some individuals ($n = 2$) did not respond positively to any of the CHO ingestion trial, with the control condition being the fastest trial completed. No gastrointestinal discomfort was reported by any participant when consuming any of the beverages raising an interesting research question regarding the nonresponse of some individuals when consuming CHO during exercise. The variability in performances, along with some negative responses to CHO ingestion, highlights the individual nature of CHO feeding as an ergogenic aid.

The range of responses measured in the current study highlights that, for the majority of individuals, there is a ceiling in the performance gains achieved when feeding rates are higher than 40 g·hr⁻¹. Any additional performance gains reported appear to result in a minimal increase in performance. However, in elite level athletes, there is evidence there is an enhanced ability to use CHO and have a subsequent meaningful improvement in performance (Stellingwerff, 2012). In support of this enhanced intake Prof. Louise Burke (personal communication) has recently presented a case study describing a nutritional intervention which enabled an Olympic walker to ingest as much as 90g·hr⁻¹ of multiple transportable CHO. Furthermore, there may be further additional improvements in exercise performance with multiple transportable CHO with increasing exercise duration i.e., bouts >3h (Stellingwerff & Cox, 2014). Thus, when providing feeding recommendations, the degree to which an increase in performance translates into a worthwhile change should be considered.

One potential limitation of the current investigation is that participants completed the trial following an overnight fasted to best control and replicate the metabolic state in which they arrive at the laboratory. Overnight fasting is not the current practice for optimal performance for athletes as liver glycogen is reduced following glycogen breakdown in the liver to maintain blood glucose concentration overnight. However, the glycogen storage capacity of the liver is enhanced following endurance training therefore reducing the impact an overnight fast has on liver glycogen content. Casey et al. (2000) reported athletes had an overnight liver glycogen content of 386 mmol·L⁻¹, which is considerably higher than values reported in healthy untrained individuals (~120–210mmol·L⁻¹) (Magnusson, Rothman, Katz, Shulman, & Shulman, 1992; Stadler et al., 2013; Taylor et al., 1996). Therefore, the liver glycogen content of athletes following an overnight fast is unlikely to vastly affect subsequent exercise performance. In studies examining the effect of CHO on performance following a shorter (~3h) fast, where liver glycogen content is unlikely to be compromised, Hulston and Jeukendrup (2009) reported a significant improvement in performance when consum-

ing a CHO beverage compared with water. In addition, a recent meta-analysis indicated the pre exercise nutritional status of participants (fed or fasted) appears to have no effect on the subsequent exercise performance/capacity achieved (Temesi, Johnson, Raymond, Burdon, & O'Connor, 2011). As such, the findings of this study are still likely to be applicable to those looking to perform in the fed state.

The current investigation only measured performance responses up to feeding rates of 64 g·hr⁻¹ and we are therefore unable to determine responses to higher feeding rates. The upper feeding rate was based on research showing a maximal absorption rate of ~1 g·min⁻¹ of a single source CHO solution (A. E. Jeukendrup et al., 1999). Nevertheless, we cannot be certain that CHO feeding rates above 64 g·hr⁻¹ do not significantly alter subsequent performances as others have reported (Currell & Jeukendrup, 2008; Smith et al., 2013). The lack of any further substantial improvement in performance with rates more than 39 g·hr⁻¹ in the current study, in addition to reports of a negative impact on performance with higher rates of CHO ingestion, suggests that performance is unlikely to improve with higher rates of single source CHO ingestion during exercise less than 3 hr. Future studies should focus on utilizing measures and techniques to try and ascertain explanations as to why some feeding rates are more beneficial than others, and which factors contribute to the individual variability in response.

Finally, we decided to use water as a control solution as athletes are likely to be consuming water rather than a color sweetened matched placebo in their current practice. Some individuals may have preconceived ideas that a flavored drink alone would have a beneficial effect on performance. The nonsignificant increase in performance when consuming the 20 g·hr⁻¹ treatment compared with control may simply be due to a placebo effect, if participants felt they had something, rather than nothing. Similarly, the increases in performance with CHO feeding in light of the water control could be artificially elevated. Nevertheless, if athletes are utilizing water as their main hydration strategy then the performance gains are likely to be realistic.

Conclusions

The 39 g·hr⁻¹ and 64 g·hr⁻¹ CHO solutions were equally effective in improving the cycling performance of 20 trained male cyclists over a 0 g·hr⁻¹ water placebo during an exercise task less than 3 hr. For most trained individuals, an optimal feeding rate for maximizing the ergogenic effect of CHO for endurance performance is likely to occur at around 40 g·hr⁻¹. There is a wide range of responses to all rates of CHO ingested highlighting the individual nature of the responses observed in individuals using CHO to aid performance. However, the results of this study highlight that most individuals will respond most positively to CHO ingestion rates around 39 and 64 g·hr⁻¹.

Novelty Statement

The vast majority of performance improvement with CHO ingestion occurred when ingesting 39 g·hr⁻¹, with any additional CHO intake (64 g·hr⁻¹) providing a minimal additional performance gain. As such, both 39 and 64 g·hr⁻¹ of carbohydrate, ingested during 2 hr of endurance exercise, are equally effective at improving subsequent TT task performance in comparison with a water control.

Practical Application

Cyclists performing tasks lasting between 2–3 hr should consider consuming around 40–60 g·hr⁻¹ of single source carbohydrate and increase their intake within this range depending on individual comfort and experience.

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Carbohydrate feeding: a dose response investigation in the 20-64g·h⁻¹ range

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UNIVERSITY OF STIRLING



INTRODUCTION

Recent studies have examined the response of ingesting graded doses of carbohydrate (CHO) on endurance exercise performance. Optimal performances have been reported at doses between 60 to 80 g·h⁻¹. However, the evidence for a dose-response in the 20-64 g·h⁻¹ range using single or multiple source CHO drinks is still unclear. We aimed to further investigate the dose-response of CHO in a dose range where the largest improvements in performance have been reported.

METHODS

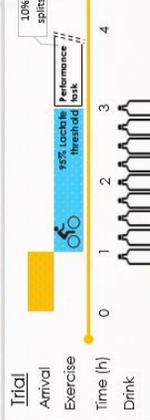
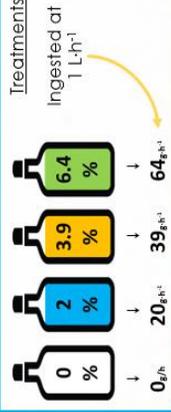
Participants (n=20)

Age: 24 (± 10) yrs
 stature: 178.3 (± 6.0) cm
 mass: 73.0 (± 8.8) kg
 V_{O₂max}: 61.2 (± 8.4) ml·kg⁻¹·min⁻¹
 PFO: 374 (± 31) W
 Power at LT: 204 (± 29) W

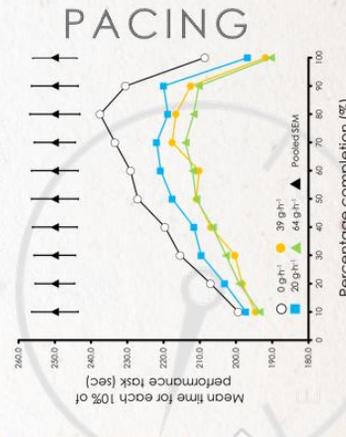
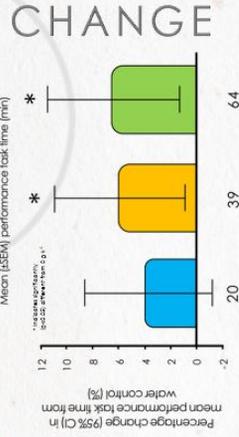
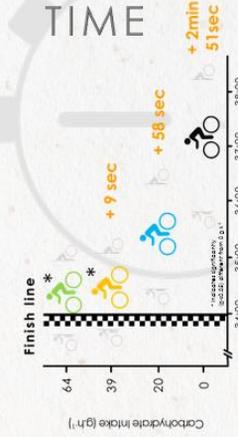
Visits

0 Pre-screening
 1 Maximal testing
 2 Familiarisation
 3 Trial 1
 4 Trial 2
 5 Trial 3
 6 Trial 4
 Investigator blind randomised placebo controlled

Treatments



RESULTS



DISCUSSION

- 39g·h⁻¹ and 64 g·h⁻¹ were equally effective at improving simulated time trial performance compared to a 0 g·h⁻¹ water control.
- 20 g·h⁻¹ did not significantly improve performance over the control condition despite a meaningful mean improvement in time trial time of 3.7%.
- There is considerable variation in performance outcome to all doses of carbohydrate ingested. This variation highlights the individual nature of the responses observed when using carbohydrate to aid performance.
- Future work will provide insight into the metabolic and neuromuscular mechanisms for individual differences in performance with increasing doses of CHO.

PRACTICAL APPLICATION

For most individuals an optimal feeding rate for maximising the ergogenic effect of carbohydrate on endurance performance is likely to occur between 39 and 64g·h⁻¹.

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Metabolic and neuromuscular effects of increasing the rate of carbohydrate intake during steady state exercise

Michael L Newell¹, Gareth A Wallis², Angus M Hunter¹, Kevin D Tipton¹, Claire Lawrence³ and Stuart D R Galloway¹

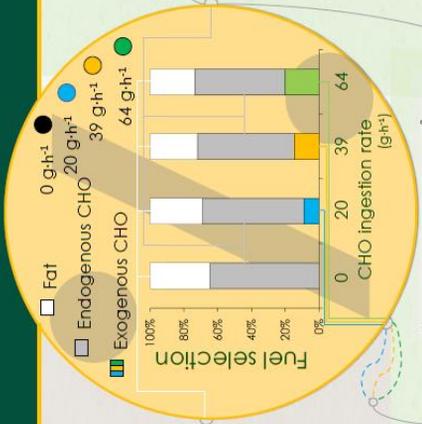
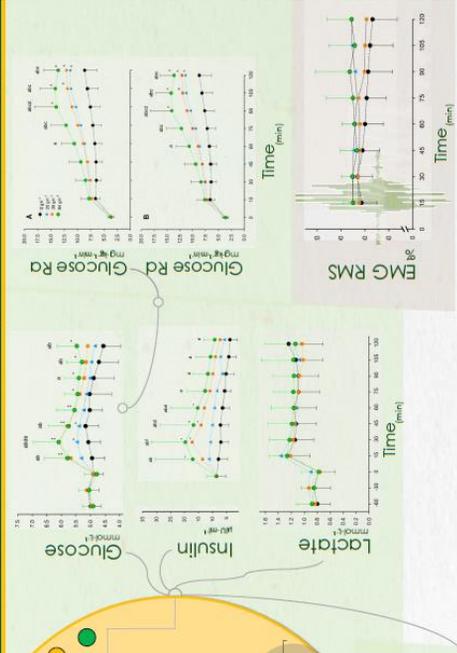
Let us know what you think...
@MikeLNewell #IPE_CHO

The ingestion of carbohydrate (CHO) during exercise improves endurance exercise performance and extends exercise capacity.

Ingestion rates between 20 and 64 g h⁻¹ have consistently led to the greatest performance gains compared to a 0 g h⁻¹ control. Performance gains tend to diminish with ingestion rates above 40–60 g h⁻¹ during exercise <3h.

Few studies have explored the combined effect of CHO ingestion on metabolic and peripheral neural actions during exercise.

We aimed to utilise stable isotope and neuromuscular function techniques to examine the integrated physiological responses to CHO feeding.



CONCLUSIONS

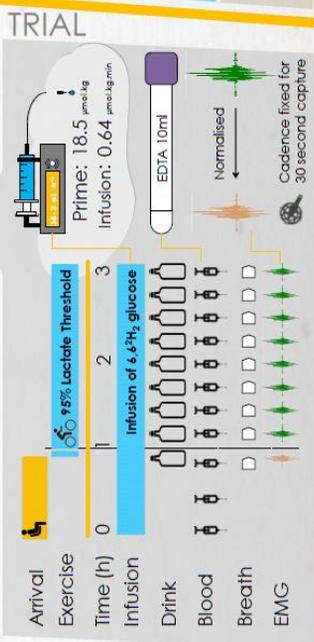
Consuming 39 and 64 g h⁻¹ of CHO suppressed endogenous CHO oxidation by 7.3% and 11.2% compared to the control.



Elevated glucose and insulin concentration play a key role in sparing endogenous CHO.

Exogenous CHO oxidation increases with each feeding rate but only consuming 39 and 64 g h⁻¹ of CHO was sufficient to increase total CHO oxidation rates.

CHO ingestion of any rate had no effect on knee extensor muscular recruitment during steady state exercise.



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