

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# A 0.8 Fructose:Maltodextrin Ratio Enhances Endurance Performance and Exogenous Carbohydrate Oxidation

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

Master of Science in Exercise and Sport Science

at Massey University, Wellington, New Zealand

Wendy Jean O'Brien

2011

## Acknowledgements

Firstly, I would like to sincerely thank my subjects for their time (4:30 am became a well known time of day and getting to work late, a common occurrence), energy (10 repeated-sprints are not easy in anyone's language, let alone before breakfast) and commitment (you never let me down and stuck strictly to the often frustrating restrictive diet) to this study. It was a pleasure to work with every one of you, and without you I would have no data and nothing to write up. My thanks also go to the families of my participants for their support of the early starts and dietary restrictions.

To Jim Clarke, David Gleason, Andy Hollings, Alex Thillier, and others who helped in the lab - I could not have carried out this study without such a willing, capable, reliable and fun team of people supporting me. And thanks also to your partners, for their tolerance of the ~4 am alarm for months on end.

I would also like to thank my supervisor, Dr David Rowlands. With your guidance and expertise I have learnt more than I ever expected, and through your patience and attention to detail you have kept me honest and shown me what it means to do good science. Thanks also to my second supervisor Assoc. Prof. Steve Stannard.

I would also like to acknowledge my friends and family for your undying support and encouragement; for your innate ability not to glaze over during my endlessly rants about the intricacies of my study; and for knowing when NOT to ask when I'd be finished or what my plans were at the end of it all. Also, to my flatmates, for letting me believe I never woke you when leaving the house at 4:30 am or when sneaking to bed in the wee small hours.

## Abstract

**Introduction:** A ratio of fructose to glucose/maltodextrin of approximately 0.8 in a carbohydrate-electrolyte solution ingested during endurance exercise was recently seen to substantially increase exogenous-carbohydrate oxidation, gut comfort and performance. However, it remains to be determined if the apparent fructose:glucose ratio optima is robust when the possible confounders of differences in solution osmolality and carbohydrate concentration are removed from consideration via clamping, and if the 0.8 ratio also promotes faster fluid absorption.

**Methods:** In a randomised double-blind crossover, 12 male cyclists rode 2 h at 57.5% peak power, then performed 10 repeated-maximal-sprints, while ingesting artificially sweetened water or one of three isosmotic 11.25% carbohydrate-salt solutions at 800 mL·h<sup>-1</sup>, comprising fructose and, maltodextrin/glucose, at the respective mean rates (g·min<sup>-1</sup>): 1.0, 0.5 (0.5-Ratio); 0.67, 0.83 (0.8-Ratio); 0.83, 0.67 (1.25-Ratio). Each solution was also spiked with 5 g D<sub>2</sub>O at 30 min into the 2-h preload. <sup>14</sup>C-enriched fructose and naturally <sup>13</sup>C-enriched maltodextrin/glucose permitted fructose and glucose oxidation rate evaluation by liquid scintillation and mass spectrometry, respectively, and indirect calorimetry.

**Results:** Mean exogenous-fructose and mean exogenous-glucose oxidation rates were 0.27 (SD%, 46), 0.39 (56) and 0.46 g·min<sup>-1</sup> (53), and 0.65 (30), 0.71 (14) and 0.58 (28) g·min<sup>-1</sup> in 0.5-, 0.8- and 1.25-Ratio, respectively; representing oxidation efficiencies (%) for fructose of 56 (12), 60 (7) and 56 (10), for glucose of 67 (16), 86 (11) and 89 (21), and for total exogenous-carbohydrate of 70 (9), 74 (6) and 64 (9), respectively. Relative to 0.5- and 1.25-Ratios, total exogenous-carbohydrate oxidation rate with 0.8-Ratio was very likely 6.4% (90% confidence limits; ±3.1%) and almost certainly 12.7% (±2.6%) higher, respectively, while respective differences in total-exogenous carbohydrate oxidation efficiency was 4.1±1.8% and 8.8±1.9%. Endogenous-carbohydrate oxidation with 1.25-Ratio was very likely higher relative to 0.5- and 0.8-Ratio conditions (31.3%; ±26.6% and 37.3%; ±27.8%, respectively) but comparisons of fat and total-carbohydrate oxidation rates were unclear among carbohydrate solutions. Mean sprint power with 0.8-Ratio was moderately higher than 0.5-Ratio (2.9%; 99% confidence limits ±2.8%) and 1.25-Ratio (3.1%; ±2.7%), and almost certainly higher than Water (11.9%; ±3.0%); repeated-sprint fatigue (slope) was possibly attenuated with 0.8-Ratio compared to 0.5- and 1.25-Ratio (2.1%; ±5.7% and 1.7%; ±5.5%, respectively). Blood D<sub>2</sub>O enrichment differences were possibly small or inconclusive among all solutions. Differences in gastrointestinal comfort during the 2-h ride were trivial/unclear among the carbohydrate conditions, however, increases in abdominal cramping were likely greater with 0.8-Ratio during the performance test.

**Conclusions:** Substantial enhancement of endurance performance results from ingestion of 0.8 ratio fructose:maltodextrin/glucose solutions, which is associated with increased exogenous-carbohydrate oxidation efficiency driven largely by a greater contribution from exogenous-fructose oxidation. Further research is required to determine the effect on fluid absorption and the physiological site responsible for the 0.8 ratio effect.

# Table of Contents

Acknowledgements .....	ii
Abstract.....	iii
Table of Contents .....	v
List of Figures.....	ix
List of Tables .....	x
1 Introduction .....	1
2 Review of the Literature .....	3
2.1 Introduction.....	3
2.2 Carbohydrate Ingestion.....	4
2.2.1 The Significance of Carbohydrate Ingestion during Exercise.....	4
2.2.2 Intestinal Absorption and Transport of Exogenous Carbohydrate .....	5
2.2.3 Differences in Multiple- vs. Single-Transportable Carbohydrates.....	6
2.2.4 Multiple-Transportable Carbohydrate Ratio .....	7
2.2.5 Measurement of Exogenous Carbohydrate Oxidation.....	8
2.3 Fluid Absorption.....	9
2.3.1 Physiological Influences on Fluid Absorption .....	10
2.3.2 Fluid/Carbohydrate Interaction .....	10
2.3.3 Effect of Solution Osmolality and Concentration on Gastric Emptying and Fluid Absorption .....	11
2.4 Gastrointestinal Comfort.....	12
2.4.1 What is Gastrointestinal Distress?.....	12
2.4.2 The Importance of Gastrointestinal Comfort in the Context of Exercise Performance and Sports Drinks .....	13

2.4.3	Factors Contributing to Gastrointestinal Comfort .....	13
2.5	Performance Benefits of Multiple-Carbohydrate Ingestion.....	14
2.6	Summary .....	15
3	Methods .....	17
3.1	Subjects.....	17
3.2	Experimental Design.....	17
3.3	Protocols .....	18
3.3.1	Preliminary testing and familiarisation.....	18
3.3.2	Training and diet .....	18
3.3.3	Experimental trial .....	19
3.3.4	Performance test .....	19
3.3.5	Breath sampling.....	20
3.4	Carbohydrate Solutions.....	20
3.5	Fluid Absorption .....	21
3.6	Psychometric Scales .....	21
3.7	Plasma Biochemistry .....	22
3.8	Expired Breath .....	22
3.8.1	<sup>13</sup> C analysis.....	22
3.8.2	<sup>14</sup> C analysis.....	23
3.8.3	Substrate Oxidation .....	23
3.9	Sample Size .....	24
3.10	Statistical Analysis.....	25
3.10.1	General method .....	25

3.10.2	Presentation of data .....	25
3.10.3	Estimate precision and statistical inference .....	25
4	Results .....	27
4.1	Performance .....	27
4.2	Stable and Radioactive Isotope Measurements.....	29
4.3	Substrate Oxidation.....	30
4.3.1	Exogenous fructose oxidation .....	30
4.3.2	Exogenous glucose oxidation .....	30
4.3.3	Total exogenous carbohydrate oxidation.....	30
4.3.4	Endogenous and total carbohydrate oxidation.....	33
4.3.5	Endogenous fat oxidation .....	33
4.4	Gastrointestinal Comfort and Exertion .....	35
4.4.1	Gastrointestinal comfort .....	35
4.4.2	Perceived exertion and muscle tiredness .....	36
4.5	Drink Taste .....	37
4.5.1	Palatability.....	37
4.5.2	Sweetness .....	37
4.6	Fluid Absorption.....	39
5	Discussion.....	40
5.1	Future Research .....	46
6	Conclusion .....	47
7	References .....	48
	Appendix A – Recruitment Email and Flyer .....	56



Appendix B – Information Sheet .....	58
Appendix C – General Health Questionnaire .....	66
Appendix D – Consent Form .....	69
Appendix E – Ethics Committee Approval.....	70
Appendix F – Food and Training Diary.....	72
Appendix G – Performance Test Data Sheet .....	75
Appendix H – <sup>14</sup> C-Fructose Dose .....	76
Appendix I – Psychometric Scales .....	79
Appendix J – Blood D <sub>2</sub> O Analysis Method .....	81

## List of Figures

<b>Figure 1.</b> Pattern of sprint mean power during the repeated-sprint performance test. Data are back log-transformed least-squares means. Bar represents the back-transformed composite between-subject coefficient of variation. ....	27
<b>Figure 2.</b> The effect of solution composition on performance test mean power. Point data are the back log-transformed least-squares means. Bars are the 99% confidence interval. Thresholds for small (0.3%), moderate (2.79%), large (4.96%), very large (7.5%), and extremely large (12.4%) effects are shown as dashed lines within the shaded zones. R, Ratio. ....	28
<b>Figure 3.</b> Breath $^{14}\text{CO}_2$ activity and $^{13}\text{C}$ enrichment during the 2-h ride. Data are raw means with the between-subject standard deviation, offset from the sampling point for visual clarity. The Water trial provided the $^{14}\text{C}$ and $^{13}\text{C}$ background, and standard deviations for $^{14}\text{C}$ are obscured. ....	29
<b>Figure 4.</b> The pattern of substrate oxidation during the 60 <sup>th</sup> -120 <sup>th</sup> min of the 2-h ride. Data are back log-transformed least-squares means. Bars represent the back-transformed composite between-subject coefficient of variation. ....	31
<b>Figure 5.</b> The pattern of efficiency of oxidation for exogenous fructose, exogenous glucose, and total exogenous carbohydrate during the 60 <sup>th</sup> -120 <sup>th</sup> min of the 2-h ride. Data are back log-transformed least-squares means. Bars represent the back-transformed composite between-subject coefficient of variation. ....	32
<b>Figure 6.</b> Ratings of gastrointestinal comfort during the 2-h ride and the repeated-sprint performance test. Data are least-squares means and bars the composite between-subject standard deviation. ....	36
<b>Figure 7.</b> Ratings of drink sweetness during the 2-h ride and the repeated-sprint performance test. Data are least-squares means and bars the composite between-subject standard deviation. ....	38
<b>Figure 8.</b> Blood $\text{D}_2\text{O}$ enrichment ( $\delta^2\text{H}$ vs. VSMOW, ‰) following ingestion of 5.00 g $\text{D}_2\text{O}$ at 30-min of exercise (VSMOW – Vienna Standard Mean Ocean Water). Data are raw means and bars are standard deviations, offset from the sampling point for visual clarity. ....	39
<b>Figure 9.</b> Integrated model of oxidation rate and oxidation efficiency of exogenous fructose, exogenous glucose and total carbohydrate ingested in the three experimental fructose:glucose/maltodextrin ratios during endurance exercise. Point data are back log-transformed means and standard deviations. Curves are quadratics derived from a within-subject mixed model of the back log-transformed data. ....	42

## List of Tables

<b>Table 1.</b> Composition and carbohydrate concentration of test solutions. ....	21
<b>Table 2.</b> Oxidation rate of endogenous and exogenous substrates during the 60 <sup>th</sup> to 120 <sup>th</sup> min of the 2-h ride. ....	33
<b>Table 3.</b> Summary of the effect of solution composition on substrate oxidation rate from the 60 <sup>th</sup> to 120 <sup>th</sup> min period of the 2-h ride. ....	34

# 1 Introduction

It is now established that the ingestion of solutions containing multiple transportable carbohydrates (e.g., fructose and glucose) during prolonged exercise can substantially increase gastric emptying, intestinal fluid absorption (Shi, Summers *et al.* 1995; Jeukendrup and Moseley 2010), and exogenous-carbohydrate oxidation rates (Adopo, Peronnet *et al.* 1994; Jentjens, Moseley *et al.* 2004; Jentjens, Venables *et al.* 2004; Wallis, Rowlands *et al.* 2005), and improve endurance performance (Currell and Jeukendrup 2008; Triplett, Doyle *et al.* 2010), relative to single carbohydrate solutions (e.g., glucose). Non-competitive intestinal transport of multiple-transportable carbohydrates and associated paracellular transport synergies (Shi, Summers *et al.* 1995), or undefined hepatic metabolism, may be responsible for these beneficial effects of coingestion. In addition to carbohydrate type, the fructose:glucose ratio of the coingested carbohydrate influences intestinal absorption and exogenous-carbohydrate oxidation rate. Using a triple-lumen perfusion model, Shi *et al.* (1995) reported faster carbohydrate and fluid absorption with fructose and glucose or sucrose with effective fructose:glucose ratios of 0.7-1.0:1 compared to solutions with effective ratios of 0.5:1. In support of these findings, Rowlands *et al.* (2008) and O'Brien and Rowlands (2011) observed that during prolonged endurance exercise, a 0.8 ratio of fructose:maltodextrin yielded the highest exogenous-carbohydrate oxidation rate, when compared to 0.5 and ~1.2 ratio solutions.

To determine the individual oxidation rates and efficiency (ingestion/oxidation ratio) of exogenous fructose and glucose, Rowlands *et al.* (2008) employed a dual tracer approach -  $^{14}\text{C}$  tracer for fructose and the property of naturally  $^{13}\text{C}$ -enriched maltodextrin. Maltodextrin ingestion was clamped at  $0.6 \text{ g}\cdot\text{min}^{-1}$ , while fructose co-ingested at 0.3, 0.5 and  $0.7 \text{ g}\cdot\text{min}^{-1}$  produced ratios of 0.5, 0.83 and 1.17, respectively. Oxidation efficiency for glucose was substantially greater at 0.5 fructose:maltodextrin, and likely higher for fructose at 0.83 ratio, compared to the other ratio solutions. Since net exogenous-carbohydrate oxidation efficiency decreased with increasing fructose dose, the increase in concentration and osmolality of solutions may have influenced carbohydrate absorption in the small intestine independent of ratio effects (Shi, Summers *et al.* 1995; Gisolfi, Summers *et al.* 1998). Therefore, the first aim of the present study was to determine whether increased fructose or glucose oxidation efficiency was responsible for the higher exogenous-carbohydrate oxidation rate with the 0.8 ratio fructose:maltodextrin solution during exercise. As a consequence of this objective, solutions were designed to be isocaloric, equi-osmotic and ingested at the upper end of the spectrum likely to be most favourable to endurance performance ( $1.5 \text{ g}\cdot\text{min}^{-1}$ ) (for review, see Jeukendrup 2010).

Noteworthy, in an isocaloric comparison study, O'Brien and Rowlands (2011) observed a very-large increase in peak power (3.6%; 99% CL  $\pm 3.5\%$ ) in a slow-ramp incremental test with the ingestion of a 0.8 ratio fructose:maltodextrin solution relative to a 0.5 ratio solution, and a possible small increase relative to a 1.25 ratio solution. A mechanisms (co-variate) analysis indicated that the performance outcome was unrelated to exogenous-carbohydrate oxidation rate, but was influenced by nausea. Since nausea can be regarded as an integrated central perception of gut comfort, any elevation in its perception during exercise performance tests may distract subjects sufficiently to force a reduction in effort outweighing the only small metabolic benefit gained from increased exogenous-carbohydrate oxidation. Furthermore, lower gastrointestinal distress may indicate more rapid carbohydrate and fluid absorption. Faster gastric emptying and fluid absorption (Jeukendrup and Moseley 2010), and lower gastrointestinal discomfort (Jentjens, Achten *et al.* 2004) were reported with ingestion of 0.5 ratio fructose:glucose solutions compared to isocaloric glucose only solutions. These suggestions of faster fluid absorption with  $\sim 0.5$ -0.8 ratio fructose:glucose solutions (Jeukendrup and Moseley 2010; O'Brien and Rowlands 2011) support the findings of Shi *et al.* (1995) who found fluid absorption to be greatest with solutions of effective fructose:glucose ratios between  $\sim 0.5$  and 0.7. Hence, enhanced unilateral water absorption by solvent drag may be secondary to a greater rate of net carbohydrate co-transport. Given the synergism between carbohydrate and fluid absorption (Shi, Summers *et al.* 1995) and the role of circulatory-fluid homeostasis on high-intensity endurance performance (Gisolfi and Duchman 1992), determination of the effect of fructose:maltodextrin ingestion ratio on net fluid absorption rate was warranted because it might provide evidence of a physiological mechanism to explain the effect of the 0.8 ratio.

A final objective was to confirm the effect of the 0.8 fructose:maltodextrin ratio on endurance performance. The slow-ramp incremental test recently used by O'Brien and Rowlands (2011), while reliable and sensitive, may not well simulate the demands of competition. Therefore, the preload intensity was increased and the validated repeated-sprint endurance performance protocol used previously was employed (Thorburn, Vistisen *et al.* 2006; Rowlands, Thorp *et al.* 2007; Nelson, Phillips *et al.* 2011; Rowlands, Swift *et al.* 2011).

It was hypothesised that the 0.8 ratio would result in a) the highest exogenous-carbohydrate oxidation rate through highest exogenous-fructose oxidation efficiency and peak exogenous-glucose oxidation rate; b) faster carbohydrate absorption (unmeasured) would result in more rapid fluid absorption as measured by unilateral blood deuterium oxide appearance, and that this would be associated with better gut comfort and a substantial enhancement in performance.

## 2 Review of the Literature

### 2.1 Introduction

Ingestion of carbohydrate (e.g., fructose or glucose) solutions during prolonged exercise is associated with maintenance of blood glucose homeostasis, increased exogenous-carbohydrate oxidation rate, and faster intestinal fluid absorption (Coyle, Coggan *et al.* 1986). Carbohydrate ingestion has also been found to improve endurance performance and delay fatigue (Jeukendrup 2004).

The ingestion of multiple-transportable carbohydrates can further enhance performance in laboratory models (Currell and Jeukendrup 2008; Rowlands, Thorburn *et al.* 2008; Triplett, Doyle *et al.* 2010; O'Brien and Rowlands 2011) relative to single-transportable carbohydrates, with the location of the mechanism possibly being at the intestinal epithelia. The absorption of some monosaccharide combinations (e.g., glucose and fructose) occurs at a faster overall rate than singularly ingested carbohydrates (Shi, Summers *et al.* 1995) associated with the monosaccharide transporter specificity and an apparent sodium independent glucose transporter (SGLT1) saturation property (Wright, Martin *et al.* 2003).

Circulatory fluid homeostasis is also known to affect high-intensity endurance performance (Gisolfi, Summers *et al.* 1998). Similar to carbohydrate absorption, fluid absorption appears affected by the type and number of carbohydrates included in the ingested solution (Shi, Summers *et al.* 1995). Additionally, gastric emptying rate, carbohydrate concentration and solution osmolality play a role in fluid absorption rate (Shi and Passe 2010). A strong correlation exists between carbohydrate and fluid absorption (Shi and Passe 2010), and this synergism could be related to enhanced unilateral water absorption by solvent drag and/or cotransport of water with solutes (Loo, Wright *et al.* 2002).

The combined effects of carbohydrate and fluid absorption during exercise appear to influence gastrointestinal comfort. Distractions and effort reduction caused by decreased gut comfort might outweigh the potential ergogenic effects of ingested carbohydrate solutions, resulting in impaired performance (Shi, Horn *et al.* 2004; Thorburn, Vistisen *et al.* 2006; O'Brien and Rowlands 2011).

## 2.2 Carbohydrate Ingestion

### 2.2.1 The Significance of Carbohydrate Ingestion during Exercise

Investigations into the effects of carbohydrate supplementation during exercise began in the 1920s, with Levine and colleagues' (Levine, Gordon *et al.* 1924) observation of runners at the finish of the Boston Marathon. These authors attributed the runners' poor physical condition and fatigue to low blood glucose levels. A close correlation between the physical condition of runners finishing the race and their blood glucose concentrations was identified by the same group at the race the following year (Gordon, Kohn *et al.* 1925). These researchers also noted that those finishers who had consumed confectionary during the race were not only in better physical condition than those who had not, but also had faster finishing times. During the 1930s, the notion that maintaining carbohydrate status during exercise might benefit performance prompted further examination of the effect of carbohydrate consumption on exercise blood glucose concentrations (Dill, Edwards *et al.* 1932). Indeed, by determining respiratory exchange ratio, Christensen *et al.* (1939) concluded that the proportion of carbohydrate utilised during exercise rose with increasing exercise intensity.

Muscle biopsy studies during the late 1960s collectively highlighted a relationship between carbohydrate feeding, muscle glycogen status and exercise performance. For example, Bergstrom *et al.* (1967) observed ~20% reduction in muscle glycogen degradation with intravenous glucose infusion during 60 min of cycling. The same authors also reported a good correlation between initial glycogen concentration and time to exhaustion following a 3-day high-carbohydrate versus a protein and fat diet (Bergstrom, Hermansen *et al.* 1967). Later, Ahlborg and Felig (1976) observed two-fold greater glucose oxidation during a 4-h bout of exercise by glucose fed subjects relative to unfed controls. Following on from this, Coggan, Coyle and colleagues conducted a series of investigations during the 1980s examining the relationship between exogenous-carbohydrate delivery and performance. They concluded that carbohydrate ingestion or infusion during prolonged exercise could prevent a decline in plasma glucose concentration, maintain relatively high rates of carbohydrate oxidation, and delay fatigue during the later stages of prolonged intense exercise (Coyle, Hagberg *et al.* 1983; Coggan and Coyle 1987; Coggan and Coyle 1988). By the mid-late 1980s so-called *sports drinks* had become commercially available and in the early 1990s, athlete recommendations were for ingestion of 30–60 g·h<sup>-1</sup> of glucose, sucrose or maltodextrin in 4–8% rehydration solutions (Coyle 1991; Coyle and Montain 1992).

The next focus of carbohydrate research was to investigate whether carbohydrate absorption and/or oxidation rates varied among the different carbohydrate types (e.g., glucose, fructose, glucose polymer). Massicotte *et al.* (1986; 1989) conducted early studies comparing the oxidation rates of different carbohydrates. Ingested as individual isocaloric solutions, oxidation rates of exogenous fructose were 17–34% lower relative to exogenous glucose, while glucose polymer oxidation was 10% lower than glucose. However, despite differences in rates of exogenous-carbohydrate oxidation, total-carbohydrate oxidation rates were, on average similar, regardless of the specific carbohydrate under investigation. The lower efficiency and rate of fructose oxidation was attributed to its necessary conversion to glucose in the liver before being oxidised in peripheral tissue (Massicotte, Peronnet *et al.* 1986; Massicotte, Peronnet *et al.* 1989).

In 1994, Adopo *et al.* were the first to report the phenomena that oxidation of coingested glucose and fructose was significantly higher (21% and 38%, respectively) than when the same quantity of glucose or fructose was ingested individually. Numerous subsequent coingestion studies followed (Jentjens, Moseley *et al.* 2004; Jentjens and Jeukendrup 2005; Wallis, Rowlands *et al.* 2005; Hulston, Wallis *et al.* 2009) and evidence of an association between ingestion of carbohydrate-containing solutions and enhanced endurance performance has been provided (Currell and Jeukendrup 2008; Rowlands, Thorburn *et al.* 2008; Triplett, Doyle *et al.* 2010; O'Brien and Rowlands 2011). Specific sections on the benefits of multiple-carbohydrate ingestion follow later in this review.

## 2.2.2 Intestinal Absorption and Transport of Exogenous Carbohydrate

Exogenous carbohydrate is absorbed primarily in the small intestine and transported across the intestinal epithelia via both active and facilitated transport. Of the three most common dietary monosaccharides, all have low affinity for the high-capacity facilitative transporter, GLUT2. As well as being rapidly trafficked at the brush border membrane for hexose transport (Kellett, Brot-Laroche *et al.* 2008), GLUT2 is also thought responsible for exit of monosaccharides from the enterocyte across the basolateral membrane. The majority of glucose and galactose is transported via the sodium-dependent glucose-cotransporter (SGLT1) which also acts as a water channel and water co-transporter (Loo, Wright *et al.* 2002; Wright, Martin *et al.* 2003). Fructose on the other hand, diffuses passively down its gradient via the exclusive sodium-independent facilitative-transporter, GLUT5 (Wright, Martin *et al.* 2003), and there is some evidence for GLUT5-mediated fructose exit from the cell (Blakemore, Aledo *et al.* 1995). A further potential transporter of both glucose and fructose is GLUT7, however, this transporter is located more distally in the ileum (Li



2004), so is unlikely to play a major role in carbohydrate uptake from ingested carbohydrate-electrolyte solutions designed for sports use, which are absorbed mainly in the duodenojejunum.

Glucose absorption occurs predominantly in the SGLT1 dense jejunum (Shi and Passe 2010), with transporter saturation rates estimated at 0.81 (Rolston and Mathan 1990) to 1.7 g·min<sup>-1</sup> (Duchman, Ryan *et al.* 1997). Meanwhile, the saturation rate for fructose transport remains unknown. Absorption of exogenous fructose is via GLUT5 (Wright, Martin *et al.* 2003), but may also be supported synergistically by glucose-stimulated dose-dependent fructose absorption (Rumessen and Gudmand-Høyer 1986) - a potential candidate of which is GLUT2 (Jones, Butler *et al.* 2011). Both glucose and fructose utilise GLUT2 for facilitated basolateral exit from the enterocyte, with the possibility of competition for this transporter, and the potential of limiting absorption rate (Shi, Summers *et al.* 1995). However, perfusion of a 1:1 glucose and fructose solution (8%) produced significantly greater carbohydrate absorption than an 8% glucose-only solution ( $249 \pm 25$  v  $152 \pm 21$  mmol·hr<sup>-1</sup>·40cm<sup>-1</sup>, respectively) (Shi, Summers *et al.* 1995). Had the basolateral membrane been the rate-limiting step in carbohydrate absorption, similar absorption rates for both solutions would have been expected. With carbohydrate transporter proteins not uniformly distributed throughout the length of the small intestine, transporter-mediated absorption rates are differentially affected by transporter density as solutions move through the various intestinal segments (Shi and Passe 2010). Paracellular pathways offer an alternative route for carbohydrate transport, with solvent drag and the opening of tight junctions for water absorption enabling small solutes to move passively through the mucosal layer with water. Shi *et al.* (1997) attributed 19–27% of fructose transport to the paracellular pathway and observed that fructose absorption is enhanced in the presence of glucose. Furthermore, greater fluid absorption might increase the extent to which solutes are absorbed via solvent drag (Shi, Summers *et al.* 1994). Collectively, these synergisms may further augment carbohydrate absorption depending on the types and ratios of ingested carbohydrate.

### 2.2.3 Differences in Multiple- vs. Single-Transportable Carbohydrates

Given the transport mechanisms already described, it is not surprising that total carbohydrate absorption (Shi, Summers *et al.* 1995) and probably oxidation (Massicotte, Peronnet *et al.* 1989; Adopo, Peronnet *et al.* 1994; Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011) are greater with the ingestion of multiple- vs. single-carbohydrates. The extent to which the synergistic effects of solvent drag and glucose-enhanced fructose absorption contribute to total carbohydrate absorption is yet to be defined, but non-competitive glucose and fructose transport via SGLT1 and GLUT5, results in a cumulative absorption/oxidation response to carbohydrate

coingestion. The discovery by Adopo *et al.* (1994) that carbohydrate oxidation was 21% greater with coingestion compared to single-carbohydrate ingestion, has prompted repeated confirmation of the phenomenon for greater absorption and/or oxidation from multiple-transportable carbohydrate solutions. Using a triple-lumen technique at rest, Shi *et al.* (1995) found that, regardless of concentration or osmolality, solutions containing multiple-transportable carbohydrates were absorbed at a faster rate than any single-carbohydrate solution. In fact, carbohydrate absorption of an 8% glucose and fructose solution was ~60% faster than an 8% glucose only solution.

In a series of studies over the past decade, Jeukendrup and colleagues attempted to establish the maximum exogenous-carbohydrate oxidation rate attainable from ingested carbohydrate solutions. The highest exogenous-carbohydrate oxidation rate of  $\sim 1.75 \text{ g}\cdot\text{min}^{-1}$  was reported in a 2005 study (Jentjens and Jeukendrup 2005) in which  $1.2 \text{ g}\cdot\text{min}^{-1}$  of both glucose and fructose were ingested. Unsurprisingly, mean exogenous-carbohydrate oxidation rate was 50% higher with this 1:1 ratio fructose:glucose solution than with  $1.2 \text{ g}\cdot\text{min}^{-1}$  of glucose only. Even compared to  $1.8 \text{ g}\cdot\text{min}^{-1}$  ingestion of maltodextrin (Wallis, Rowlands *et al.* 2005) or glucose (Jentjens, Moseley *et al.* 2004), mean exogenous-carbohydrate oxidation rates of isocaloric 0.5 ratio fructose:maltodextrin (or glucose) solutions were 43% and 55% higher, respectively. Interestingly though, at a more moderate ingestion rate ( $0.8 \text{ g}\cdot\text{min}^{-1}$ ), no significant difference in exogenous-carbohydrate oxidation rate was observed between a 0.5 ratio fructose:glucose solution and an isocaloric glucose-only solution (Hulston 2009). These findings suggest that an ingestion rate of  $0.8 \text{ g}\cdot\text{min}^{-1}$  is below the saturation rate of SGLT1, so only minimal benefit would be gained by ingesting a second-transportable carbohydrate in an isocaloric solution. In contrast, SGLT1 was probably at maximal capacity with solutions ingested at higher rates ( $1.8\text{--}2.4 \text{ g}\cdot\text{min}^{-1}$ ).

## 2.2.4 Multiple-Transportable Carbohydrate Ratio

The ratio of ingested carbohydrates and the different intestinal transporters they utilise might influence carbohydrate absorption and oxidation, and even performance. From an absorption perspective, Rumessen and Gudmand-Høyer (1986) reported that fructose uptake occurred in a dose-dependent manner stimulated by glucose, and that regardless of whether fructose and glucose were ingested in free form or as sucrose, a 1:1 ratio resulted in greater fructose absorption than other glucose-fructose combinations. Furthermore, when Shi *et al.* (1995) infused six different sucrose or glucose and fructose solutions at the duodenojejenum, faster net-carbohydrate absorption occurred with effective 0.5–0.7 fructose:glucose ratio solutions than with effective 0.5

ratio solutions. Much of the work by the Jeukendrup group has focused on 0.5 fructose:glucose ratio solutions at varying concentrations and ingestion rates (Wallis, Rowlands *et al.* 2005; Jentjens, Underwood *et al.* 2006; Hulston, Wallis *et al.* 2009; Jeukendrup and Moseley 2010), however, the highest peak exogenous-carbohydrate oxidation rate of  $\sim 1.75 \text{ g}\cdot\text{min}^{-1}$  occurred with a fructose:glucose ratio of 1:1 (Jentjens and Jeukendrup 2005).

In the first study to specifically investigate fructose:glucose ratio and its effect on carbohydrate oxidation rate and performance, Rowlands *et al.* (2008), clamped maltodextrin ingestion at  $0.6 \text{ g}\cdot\text{min}^{-1}$  whilst manipulating fructose ingestion quantity to produce ratios of between 0.5 and 1.2 with total ingestion rate ranging from  $0.9\text{--}1.3 \text{ g}\cdot\text{min}^{-1}$ . A 0.83 ratio fructose:maltodextrin solution resulted in highest exogenous-carbohydrate oxidation rate, and attenuated fatigue during a repeated-sprint performance test compared to 0.5 and 1.17 ratio solutions. In a confirmatory followup study (O'Brien and Rowlands 2011), ingestion of a 0.8 ratio fructose:maltodextrin solution at  $1.8 \text{ g}\cdot\text{min}^{-1}$  resulted in 3.6% (99% CL;  $\pm 3.5\%$ ) higher peak power in an incremental performance test and an almost certain small increase in exogenous-carbohydrate oxidation rate, relative to an isocaloric 0.5 ratio solution. Most investigators attribute faster carbohydrate absorption and greater exogenous-carbohydrate oxidation rate of coingested solutions to the utilisation of multiple transporters (e.g., SGLT1, GLUT5, GLUT2) and the synergistic mechanisms of solvent drag and glucose-stimulated fructose absorption (Rumessen and Gudmand-Hoyer 1986). In summary, the weight of evidence suggests that exogenous-carbohydrate absorption and oxidation rates seem to be highest with solutions of fructose:glucose at ratios of around 0.7–1.0.

### 2.2.5 Measurement of Exogenous Carbohydrate Oxidation

Oxidation rates of ingested carbohydrates are commonly determined by way of isotopic tracers combined with indirect calorimetry of expired breath, enabling the exogenous component of expired breath to be distinguished from total carbohydrate oxidation. The stable isotope  $^{13}\text{C}$ , is the most widely used for calculating oxidation rates of ingested carbohydrate via mass spectrometry of  $^{13}\text{C}$  in expired breath (Jeukendrup and Jentjens 2000).  $^{13}\text{C}$ -enriched glucose or fructose is often added to carbohydrate solutions (Massicotte, Peronnet *et al.* 1986; Adopo, Peronnet *et al.* 1994; Burelle, Lamoureux *et al.* 2006; Smith, Zachwieja *et al.* 2010; Rowlands and Clarke 2011), ensuring a considerably higher tracer:tracee ratio  $^{13}\text{C}$  signal than can be achieved from carbohydrate with a naturally high  $^{13}\text{C}$ -abundance (i.e., carbohydrate from plants with a  $\text{C}_4$  photosynthetic cycle; namely maize/corn and sugar cane) (Lefebvre 1985). Due to the extensive

presence of  $^{13}\text{C}$  in everyday foods, its major limitation as a tracer is the requirement for subjects to avoid foods naturally high in  $^{13}\text{C}$  prior to, and for the duration of, the experimental period (Wagenmakers, Rehrer *et al.* 1993; Ruzzin, Peronnet *et al.* 2003; Wallis, Rowlands *et al.* 2005; Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011). Additional to dietary controls, most researchers incorporate background and baseline breath enrichment measurements during representative control trials using the experimental protocol, but with unenriched carbohydrate solutions. Inclusion of the radioactive isotope  $^{14}\text{C}$  in carbohydrate solutions is the oldest method for tracing the metabolism of ingested carbohydrate, and the use of both  $^{14}\text{C}$  and  $^{13}\text{C}$  allows for the metabolism of two coingested carbohydrates to be simultaneously evaluated (Hawley, Dennis *et al.* 1992; Wallis, Rowlands *et al.* 2005; Rowlands, Thorburn *et al.* 2008). The advantages of  $^{14}\text{C}$  as a tracer are its relative inexpensiveness, simple scintillation counter analysis of breath samples (Jeukendrup and Jentjens 2000; Rowlands, Thorburn *et al.* 2008), and virtually no naturally occurring background  $^{14}\text{C}$  to account for (Hawley, Dennis *et al.* 1992). However,  $^{14}\text{C}$  does carry the inherent chance of increased cancer risk associated with any radioactive substance, even at the medical diagnostic doses applied in the clinical research setting (International Commission on Radiological Protection (ICRP) 1991). Since carbohydrate delivered to the systemic circulation following intestinal absorption is rapidly oxidised by the contracting muscle, end-point oxidation of exogenous-carbohydrate as determined by isotopic tracers is likely to be a reliable indicator of the net metabolic efficacy of an ingested carbohydrate solution

### 2.3 Fluid Absorption

Regardless of the effects of carbohydrate in solutions ingested during endurance exercise, delivery of fluid is vital to the efficacy of carbohydrate-electrolyte beverages. The consequences of dehydration may be attenuated by absorption of ingested fluids and although the dehydration level at which performance is adversely affected has been questioned and debated (Sawka and Noakes 2007), it is generally acknowledged that effective fluid replacement will at least reduce or delay performance decrement (Sawka, Montain *et al.* 2001).

Three commonly used methods of investigating intestinal fluid absorption are deuterium oxide ( $\text{D}_2\text{O}$ ) ingestion,  $^{13}\text{C}$ -octanoic acid breath testing, and various intubation techniques.  $\text{D}_2\text{O}$  ingestion can be used to quantify fluid absorption rate by determining relative differences in  $\text{D}_2\text{O}$  accumulation in plasma or blood (Lambert, Ball *et al.* 1999). Despite bi-directional water flux, this method is considered a robust and reliable measure of fluid absorption provided the quantity of  $\text{D}_2\text{O}$  ingested is kept constant among trials (Lambert, Ball *et al.* 1999; Jeukendrup and Moseley

2010). Ingestion of  $^{13}\text{C}$ -octanoic acid enables quantification of fluid absorption by the appearance of  $^{13}\text{C}$  in expired breath (Perri, Pastore *et al.* 2005). Although reliable and non-invasive, this method is not feasible if  $^{13}\text{C}$  is also being used to measure exogenous-carbohydrate oxidation. Intubation techniques enable measurement of intestinal fluid absorption by aspiration of perfused or ingested solutions from various sections of the small intestine. The educated reader will interpret some intubation studies with caution, as marked differences in fluid absorption have been reported depending on the specific intestinal segment sampled (Lambert, Chang *et al.* 1997; Shi and Passe 2010).

### 2.3.1 Physiological Influences on Fluid Absorption

Exogenous fluid delivery to the circulatory system is the integration of gastric emptying and intestinal fluid absorption (Maughan 1991). Gastric emptying rate is regulated by feedback from the small intestine and controlled by stretch-, osmo- and nutrient-sensitive receptor-mediated opening of the pyloric sphincter (Thomson, Keelan *et al.* 2001). Fluid absorption, which is a passive process driven by the osmotic gradient across the intestinal mucosa, occurs primarily in the “leaky” duodenum (Lambert, Chang *et al.* 1997), and is highly correlated with total solute absorption (Shi 1995, Shi 2010). Permeability of the intestine reduces throughout its length (Fordtran and Saltin 1967), and in contrast to the duodenum, the jejunum is less permeable, but with substantially higher transporter density it is the site of greater solute stimulated fluid absorption (Shi and Passe 2010). In addition to the structural characteristics of the small intestine, fluid absorption is also influenced by other paracellular and transcellular pathways via osmosis, water channels, the opening of tight junctions and cotransport with solutes (Shi, Summers *et al.* 1995; Loo, Zeuthen *et al.* 1996; Ma, Verkman *et al.* 1999; Shi and Passe 2010).

### 2.3.2 Fluid/Carbohydrate Interaction

Recent analysis by Shi and Passe (2010) of 30 carbohydrate/fluid absorption studies revealed a large correlation between total solute- and water-transport in the jejunum. Furthermore, the authors recognised the critical role of multiple-transportable carbohydrates in water absorption, suggesting that opening of tight junctions for solvent drag, and activation of a variety of transporters can facilitate additional water transport. Loo *et al.* (1996) first proposed water cotransport with sugars when they reported that, in *Xenopus* oocytes, 260 molecules of water were coupled to each sugar molecule when transported, and estimated the effect could account for approximately 5 litres of water absorption daily in the human small intestine. Lambert *et al.* (1997) reported water flux in the duodenum was twice as high from ingestion of distilled water as

with a 6% sucrose-glucose-electrolyte solution ( $30.7 \text{ v } 15.0 \text{ mL}\cdot\text{cm}^{-1}\cdot\text{hr}^{-1}$ , respectively). However, in the transporter dense proximal jejunum, the carbohydrate-electrolyte solution yielded three-fold greater water flux than the distilled water ( $11.9 \text{ v } 3.8 \text{ mL}\cdot\text{cm}^{-1}\cdot\text{hr}^{-1}$ , respectively), probably the result of cotransport (Lambert, Chang *et al.* 1997). Furthermore, regardless of carbohydrate concentration (6%, 8% or 10%), ingestion of 1:1 fructose:glucose solutions resulted in greater fluid absorption than either a 6% maltodextrin solution or water (Davis, Burgess *et al.* 1990). Surprisingly though, these authors did not propose additional carbohydrate transporter utilisation as a mechanism for the increased fluid absorption. Similar results were observed when Jeukendrup and Moseley (2010) used serial dye dilutions and  $^{13}\text{C}$ -acetate and  $\text{D}_2\text{O}$  tracers to simultaneously measure gastric emptying and fluid absorption rates of 8.6% fructose-glucose and glucose-only solutions, and water. Although no differences were observed between water and the fructose-glucose solution, faster gastric emptying and plasma  $\text{D}_2\text{O}$  accumulation occurred with the fructose-glucose solution relative to the glucose-only solution. These findings support those from the classic triple-lumen study by Shi *et al.* (1995), in which all solutions containing multiple-transportable carbohydrates, even those hypertonic to plasma, yielded greater water absorption than any single-carbohydrate solution. The effect has been attributed by Shi *et al.* and others (Davis, Burgess *et al.* 1990; Jeukendrup and Moseley 2010), to the greater number of transport mechanisms stimulated by the additional carbohydrate, highlighting the facilitative role of multiple carbohydrates in fluid absorption and the variety of paracellular and transcellular pathways utilised.

### **2.3.3 Effect of Solution Osmolality and Concentration on Gastric Emptying and Fluid Absorption**

Solution osmolality and carbohydrate concentration are inversely related to gastric emptying rate (Vist and Maughan 1995; Murray, Bartoli *et al.* 1999). Early results from Costill and colleagues (Costill and Saltin 1974; Coyle, Costill *et al.* 1978) showed slower gastric emptying of even low concentration (<4.5%) carbohydrate solutions compared to water, whilst Jeukendrup *et al.* (2009) observed faster and greater fluid absorption with a 3% glucose solution relative to more concentrated glucose beverages. Conversely though, in a somewhat underpowered ( $n = 5$ ) triple-lumen study comparing hypo- (3%) and iso-tonic (6%) solutions of unspecified carbohydrates, and water, Rogers *et al.* (2005) observed no difference in net water flux, irrespective of whether intestinal segments were analysed individually or collectively. Also using triple-lumen perfusion, Rehrer *et al.* (1992) examined the effect of solution osmolality on fluid absorption and gastric emptying, and reported net water secretion at the jejunum from a 17% glucose solution (1223



mosmol·kg<sup>-1</sup>), whereas net water absorption resulted from an isocaloric maltodextrin solution (301 mosmol·kg<sup>-1</sup>) despite no apparent differences in gastric emptying rate. Vist and Maughan (1995) concluded that gastric emptying was more influenced by carbohydrate concentration than osmolality, with two-fold faster gastric emptying of a high-concentration hypotonic solution (~19%, 237 mosmol·kg<sup>-1</sup>) compared to an iso-caloric hypertonic solution (1300 mosmol·kg<sup>-1</sup>), whereas absorption of a low-concentration hypotonic solution (4%, 230 mosmol·kg<sup>-1</sup>) was approximately four-fold faster than the iso-osmotic 19% solution. These findings suggest that although concentration was the most influential factor in gastric emptying, the effect of osmolality became more apparent at high carbohydrate concentrations. Furthermore, no differences in total-fluid absorption in the duodenojejunum were evident between isocaloric hypo-, iso- and hypertonic multiple-carbohydrate solutions (Gisolfi, Summers *et al.* 1998), nor were differences in gastric emptying and fluid absorption rates observed among five 6% multiple-carbohydrate solutions (Gisolfi, Lambert *et al.* 2001). These results further indicated that concentration, not osmolality, was the most important factor influencing fluid absorption. In the latter study, not only osmolality, but also sodium content and carbohydrate type varied among the test solutions, so it is conceivable that the effect of these variables counteracted each other to yield neutral results. These multiple-carbohydrate studies provided further strength to the early findings of Shi *et al.* (1994; 1995), that when multiple-transportable carbohydrates are infused or ingested, solute transport, not osmolality, is the most important factor determining water absorption (Shi, Summers *et al.* 1995). However, in a recent review, Shi and Passe (2010) stated that osmolality is the driving force of water movement in the small intestine and is inversely related to water absorption.

## 2.4 Gastrointestinal Comfort

### 2.4.1 What is Gastrointestinal Distress?

Gastrointestinal distress covers a variety of symptoms including: burping, stomach bloating and fullness, stomach upset, nausea and abdominal cramping (Shi, Horn *et al.* 2004; O'Brien and Rowlands 2011); and has been attributed to factors such as: type, composition and amount of ingested food and fluid, carbohydrate malabsorption, gastric emptying and fluid absorption rates, and exercise mode, duration and intensity (Shi, Horn *et al.* 2004). In many of the studies reviewed, perceptual differences in gastrointestinal comfort were confined to variations in ingested solution, since exercise and environmental factors were standardised for all experimental conditions within each study.

## 2.4.2 The Importance of Gastrointestinal Comfort in the Context of Exercise Performance and Sports Drinks

Gastrointestinal distress tends to increase with exercise duration (Murray, Paul *et al.* 1989; Shi, Horn *et al.* 2004; Thorburn, Vistisen *et al.* 2006; Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011) and has been associated with reduced endurance performance. O'Brien and Rowlands (2011) recently linked increased gastrointestinal comfort (including lower perceptions of nausea, stomach fullness and abdominal cramping) with higher peak power, confirming the work of Thorburn *et al.* (2006), who observed that increased gastrointestinal distress (specifically, nausea) was associated with reduced sprint mean power. A mechanisms analysis of the O'Brien and Rowlands study revealed a mild-moderate statistical relationship between nausea and performance peak power. These authors suggested that, since nausea forms part of the central perception of gut comfort, the distraction and reduced effort resulting from elevated perception of nausea might have outweighed any ergogenic effect gained from increased carbohydrate availability. In a much publicised study, Murray *et al.* (1989) reported significantly greater gastrointestinal distress and a ~15% reduction in performance with ingestion of a 6% fructose-only solution compared to the same quantity of glucose or sucrose. Notwithstanding contradictory findings from numerous subsequent studies, the dogma that fructose ingestion causes gastrointestinal discomfort persisted for some time. Interestingly though, despite performance decrement and high gastrointestinal distress, none of the subjects in this study considered gastrointestinal distress as a major distraction or limitation to performance (Murray, Paul *et al.* 1989). In a recent meta-analysis, Vandenberg and Hopkins (2011) rated glucose polymer as the best single-source carbohydrate when consumed at high rates, not because of its lower osmolality, but because of the lower gastrointestinal distress associated with this hexose.

## 2.4.3 Factors Contributing to Gastrointestinal Comfort

Although no direct evidence was found in the literature, it is generally considered that gastrointestinal comfort is associated with factors similar to those influencing gastric emptying and intestinal fluid absorption, i.e., osmolality, concentration, carbohydrate type and ingestion volume. Shi *et al.* (2004) linked increased rates of gastrointestinal discomfort during intermittent, high-intensity exercise with higher carbohydrate concentration (8% vs 6%) and solution osmolality (434 vs 305 mOsm·kg<sup>-1</sup>). The higher concentration hypertonic solution, might have resulted in intestinal secretion or slowed fluid absorption and large residual gastric volume from slower gastric emptying, exacerbating gastrointestinal discomfort. In the much cited fructose ingestion study by Murray *et al.* (1989), high incidents of stomach upset may have been related



more to slow gastric emptying and/or intestinal absorption of large quantities of a single carbohydrate, than with fructose ingestion *per se*. A repeated and consistent finding in carbohydrate oxidation studies is the increased occurrence of gastrointestinal distress with single-carbohydrate solutions compared to solutions containing multiple-transportable carbohydrates (Jentjens, Achten *et al.* 2004; Jentjens, Moseley *et al.* 2004; Jentjens, Venables *et al.* 2004; Wallis, Rowlands *et al.* 2005; Jentjens, Underwood *et al.* 2006; Triplett, Doyle *et al.* 2010). Lower oxidation efficiency of single-carbohydrate solutions has been proposed as a probable cause of elevated gastrointestinal discomfort (Jeukendrup 2010), with symptoms arising from those factors already noted as being associated with fluid absorption and gastric emptying (i.e., osmolality, concentration, carbohydrate type, ingestion volume). Furthermore, highest oxidation efficiency of ~0.8 ratio fructose:maltodextrin solutions has also been associated with the lowest gastrointestinal distress (Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011). Given the strong correlation between carbohydrate and fluid absorption, increased oxidation efficiency might reduce the amount of unabsorbed carbohydrate and possibly fluid, accumulating in the gastrointestinal tract, thereby minimising the degree of gastrointestinal discomfort experienced with ingestion of carbohydrate solutions.

## 2.5 Performance Benefits of Multiple-Carbohydrate Ingestion

Bearing in mind that exercise rapidly elevates total carbohydrate utilisation, it is intuitive that consumption of carbohydrate during exercise must improve performance by maintaining blood glucose homeostasis and increasing exogenous-carbohydrate oxidation rate. However, despite a myriad of studies conducted over the past ~90 years, the carbohydrate administration regime (carbohydrate type, quantity, ingestion rate) that evokes the greatest performance benefit still remains unresolved (Karelis, Smith *et al.* 2010). Two reviews have been recently published in which the effect of carbohydrate administration on endurance performance was investigated from quite distinct angles; the potential mechanisms linking carbohydrate administration with performance (Karelis, Smith *et al.* 2010); and a statistical meta-analysis converting performance outcomes to a common metric (Vandenbogaerde and Hopkins 2011). According to Karelis *et al.* (2010) the weight of evidence indicates that carbohydrate ingested during exercise has a significant positive effect on endurance performance, but that the mechanisms involved are complex and likely to be multifactorial. From the meta-analysis of over 70 studies reviewed by Vandenbogaerde and Hopkins (2011), the supplement to infer the greatest performance benefit was ~3-10% of carbohydrate-plus-protein (glucose polymers ~0.7, fructose ~0.2, protein ~0.2 g·kg<sup>-1</sup>·h<sup>-1</sup>). However, in many of the studies reviewed, only single carbohydrates were provided, and

osmolality and gastrointestinal comfort were not accounted for. As already mentioned in the current review, osmolality and gastrointestinal comfort can impact exogenous-carbohydrate absorption and oxidation rates, fluid absorption and/or performance. In addition, some recent multiple-carbohydrate performance studies were excluded by Vandenbogaerde and Hopkins due to complexity of the performance tests employed.

Over 100 publications describe endurance performance with respect to carbohydrate vs. non-caloric placebo feeding, yet despite the evidence for greater carbohydrate oxidation with multiple-transportable carbohydrate solutions, only four have been investigations into the effect of carbohydrate type, concentration and/or ratio on performance outcomes. When isocaloric multiple- (fructose/glucose) and single-transportable (glucose-only) carbohydrate solutions were compared, 8% improvements in performance were reported following ingestion of the fructose/glucose solution (Currell and Jeukendrup 2008; Triplett, Doyle *et al.* 2010). These performance enhancements occurred with high carbohydrate doses (14.5%), and were consistent at ingestion rates of 1.8 and 2.4 g·min<sup>-1</sup>, with both 1.0 and 0.5 fructose:glucose ratio solutions. The first performance study to investigate the effect of fructose dose in multiple-carbohydrate solutions on performance, was conducted by Rowlands *et al.* (2008). Fructose:maltodextrin solutions at ratios of 0.5, 0.83 and 1.17 were ingested, and although differences in a repeated-sprint performance test were unclear, attenuated fatigue with the 0.8 ratio solution was reported. In a followup study examining the impact of carbohydrate ratio on performance, O'Brien and Rowlands (2011) manipulated fructose and maltodextrin quantity, providing fructose:maltodextrin solutions at ratios of 0.5, 0.8 and 1.25 at a fixed carbohydrate ingestion rate (1.8 g·min<sup>-1</sup>) and concentration (13.5%). Peak power in an incremental performance test was 3.6% (±3.5%) and 3.0% (±3.7%) higher with the 0.8 and 1.25 ratios respectively, relative to the 0.5 ratio solution.

Results from these multiple-carbohydrate studies provide evidence across a range of carbohydrate concentrations, ingestion rates and ratios, that multiple-transportable as opposed to single-carbohydrate solutions will probably enhance endurance performance, and that the fructose:maltodextrin ratio most beneficial to endurance performance might be ~0.8.

## 2.6 Summary

The body of evidence suggests that ingestion of multiple-transportable carbohydrates is superior to single-carbohydrate ingestion, especially in terms of exogenous-carbohydrate oxidation rate, and probably fluid absorption. While carbohydrate type (multiple vs. single) is the key determinant of carbohydrate absorption from the small intestine, the concentration of ingested carbohydrates

appears to have the most impact on fluid absorption, and a large correlation exists between carbohydrate and fluid absorption. Multiple-carbohydrate solutions have been associated with lower gastrointestinal distress, and this might also impact endurance performance outcomes. Solutions of fructose and glucose or maltodextrin at ratios of ~0.8 might be most beneficial to endurance sport performance with respect to maximising exogenous-carbohydrate oxidation and gastrointestinal comfort, and ultimately endurance performance.

### 3 Methods

#### 3.1 Subjects

Twelve trained male cyclists, mountain bikers and triathletes aged (SD) 36.2 (8.0) y and with a body mass of 79.4 (9.5) kg participated in the study. Maximal oxygen uptake ( $\text{VO}_{2\text{max}}$ ) and power ( $\text{W}_{\text{max}}$ ) were 59.1 (5.2)  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and 367.0 (31.6) W, respectively. All participants had been cycling >8 h per week for at least the previous 12 months and had been cycle racing regularly or been exposed to regular high-intensity training. Subjects were recruited from local Wellington cycle and triathlon clubs via club emailing lists; participants from previous studies were emailed or phoned directly; flyers were displayed at local cycle and sports shops, and on staff noticeboards at Wellington hospital and other organisations; and via word of mouth. Recruitment flyers are presented in Appendix A. Before participation, each subject read the study information sheet (Appendix B), was screened for contraindications to exercise by completing a General Health Questionnaire (Appendix C), and was fully informed of the purpose and risks of the procedure. All cyclists signed a written, informed consent form prior to commencing the study (Appendix D). This study was approved by the New Zealand Health and Disability Ethics Committee: Central Regional, MEC/10/05/049 (Appendix E).

#### 3.2 Experimental Design

The study design was a randomised, double-blind, 4-way crossover, in which the effects on outcomes of ingesting isosmotic, isocaloric solutions each containing one of three different ratios of fructose and maltodextrin/glucose, or artificially sweetened water, were compared. Each cyclist visited the laboratory a total of nine times during the 5-week study ( $\text{VO}_{2\text{max}}$  test and familiarisation ride, 4 weekly standardised training rides, 4 weekly experimental trials). The four experimental trials consisted of 2-h cycling at 57.5%  $\text{W}_{\text{max}}$ , followed shortly afterwards by a repeated-sprint performance test, both whilst ingesting the test solutions. Maize-derived naturally  $^{13}\text{C}$ -enriched maltodextrin and glucose and  $^{14}\text{C}$ -tracer enriched fructose were used to quantify oxidation rates of exogenous glucose and fructose, along with endogenous carbohydrate and fat oxidation rates using indirect calorimetry. The experimental trials were separated by 7 d, and for each subject, trials were conducted at the same time of day (starting between 0500 and 0630 h) in order to control for circadian variance.

### 3.3 Protocols

#### 3.3.1 Preliminary testing and familiarisation

At least 10 days prior to the start of the experimental trials, a progressive exercise protocol to volitional exhaustion was performed on an electronically-braked cycle ergometer (VeloTron Racer Mate, Seattle, WA) to determine  $\text{VO}_{2\text{max}}$  and  $W_{\text{max}}$ . After a warm-up period, the test commenced at a workload of  $3 \text{ W}\cdot\text{kg}(\text{body mass})^{-1}$ , and increased at a rate of 25 W every 150 s thereafter. Exhaustion was defined as when the subject could no longer maintain a pedal cadence of 70 rpm following 3 warnings to do so.  $\text{VO}_{2\text{max}}$  was measured on-line with a calibrated Moxus MaxII Metabolic System (AEI Technologies Inc, Naperville, IL) and taken as the highest attained 20 s average oxygen uptake.  $W_{\text{max}}$  was defined as the last completed work rate plus the fraction of time spent in the final non-completed work rate multiplied by the 25 W work rate increment. The results were used to determine the 50%  $W_{\text{max}}$  workload applied during the laboratory training sessions and the 57.5%  $W_{\text{max}}$  workload for experimental trials. Following the incremental test, participants rested for 10 min then completed a full familiarisation of the experimental trial including the repeated-sprint performance test. During all rides, environmental conditions were maintained at 20°C (1.0) and 43.8% (6.1) relative humidity by air conditioning and outside opening windows, with a standardised air flow maintained over the cyclist by a fan.

#### 3.3.2 Training and diet

Cyclists modified and recorded their training (Appendix F) and repeated this on a weekly basis as follows: *Day 1*, long duration ride (3-4 h); *Days 2 and 3*, medium-duration ride (2-3 h); *Day 4*, lab based training (2 h at 50%  $W_{\text{max}}$ ); *Day 5*, rest day; *Day 6*, experimental trial; *Day 7* recovery ride (1-2 h). Subjects were asked to record their food intake (Appendix F) the day prior to the first experimental trial and were instructed to repeat this intake the day before each of the 3 subsequent trials. To assist in standardising energy intake and hydration, subjects were also provided with a pre-packaged pasta meal (55 kJ, 1.85 g carbohydrate, 0.63 g protein, 0.62 g fat; per kg body mass) and a 600-mL drink bottle full of water, all to be consumed the evening before each experimental trial. In order to reduce the background  $^{13}\text{C}$ -enrichment, an extensive list of foods with a high natural abundance of  $^{13}\text{C}$  (i.e., from plants with a  $\text{C}_4$  photosynthetic cycle: maize, corn and sugar cane) was provided (included as part of Appendix B) and subjects were instructed not to consume such foods for at least 10-d before the first experimental trial and for the duration of the study.

### 3.3.3 Experimental trial

Subjects reported to the laboratory in the morning (between 0500 and 0630 h) following an overnight fast on *Day 6* of each weekly block. On arrival, a 20 GA cannula (Becton Dickinson Medical Pte Ltd, Singapore) was inserted into an antecubital vein. A 2-way stopcock valve (Becton Dickinson Medical Pte Ltd, Singapore) was connected to the cannula to allow for blood sampling at this point and during exercise, and was maintained patent with regular saline flushes. Following a resting blood sample, cyclists toileted and had their body mass recorded, then were seated next to the cycle ergometer to complete resting psychometric scales and resting expired breath sampling. Cyclists then mounted the cycle ergometer and cycled for 2 h at 57.5%  $W_{\max}$ . Experimental solutions were ingested and the following outcome variables collected, at rest and every 15 min during exercise, in the order of: exertional psychometric variable ratings; expired breath samples into a Douglas bag from a mixing chamber and into an anaesthetic bag; solution ingestion; and solution psychometric variable ratings. Blood samples were drawn at rest and at 28, 40, 50, 60, 70, 80 and 90 min during exercise. At the completion of the 2-h cycle, cyclists dismounted, had a final blood sample taken and the cannula removed from their arm. Subjects then toileted and had their body mass recorded, before remounting the cycle ergometer to complete the performance test.

### 3.3.4 Performance test

No breath or blood samples were collected during the performance test but the experimental solutions continued to be ingested. The performance test consisted of 10 maximal sprint efforts, interspersed and beginning with a recovery interval at 40%  $W_{\max}$ . The internal work to be done (kilocalories (kcal)) during the sprint (2-3 min) and recovery (5-6 min) periods was determined by individual  $W_{\max}$  ( $\text{kcal} = 0.125 \times W_{\max}$ ). Fixed linear workloads approximately equivalent to the load created by riding a 28, 39 or 48 front chain ring and a 10-sprocket, 21 to 11 tooth rear-cluster were selected from the Velotron software. An up-or-down gear switch was positioned on the end of the right handlebar brake hood to provide convenient changing of the gearing. Cyclists self-selected cadence and gearing, but were instructed to sprint as fast as possible until the required kcal were achieved. No verbal encouragement was provided to the participants; the only information given during the sprints was elapsed work completed (kcal) shown on a computer screen. Participants were given a verbal count down in preparation for the start of each sprint and at 20, 10, 5 and 2 kcal remaining in preparation for the end of each sprint. During the performance

test, psychometric data was collected immediately following sprints 1, 4, 7 and 10. An example of the data collection sheet is provided in Appendix G.

### 3.3.5 Breath sampling

Cyclists breathed through a mouthpiece and two-way valve (Hans Rudolph, Shawnee, KS) directed into a 5 L mixing chamber connected in series to a 6 L anaesthetic bag and Douglas bag. To stabilise respiration, cyclists breathed through the mouthpiece for ~1 min prior to 90 s collection of expired breath into the Douglas bag for calculation of oxygen consumption and carbon dioxide production rates. Expired breath samples were drawn into 2 x 10 mL evacuated tubes (Exetainer, Labco Ltd., High Wycombe, UK) for 5 s before and after each 15-min sampling point from a 20 GA needle positioned at the distal end of the mixing chamber. These were later used for quantification of  $^{13}\text{C}$  enrichment and subsequent calculation of exogenous-glucose oxidation. Further expired breath was then collected into the anaesthetic bag and used for the analysis of  $^{14}\text{CO}_2$  activity and subsequent calculation of exogenous-fructose oxidation.

## 3.4 Carbohydrate Solutions

Immediately prior to exercise, participants ingested a 400 mL bolus of experimental solution, followed by a further 200 mL at 15 min intervals throughout the 2-h ride. Excluding the initial double bolus, solutions were ingested at a rate of  $800 \text{ mL}\cdot\text{h}^{-1}$  during the 2-h ride. During the performance test, experimental solutions continued to be ingested on a per serving basis (200 mL) every 2 recoveries and sprints (~16 min) and ingested ad libitum. Four different solutions were prepared for ingestion during exercise. The three experimental solutions comprised fructose and maltodextrin and/or glucose, at ratios of 0.5:1, 0.8:1 and 1.25:1 (fructose:maltodextrin/glucose). Further details of solution composition are presented in Table 1. The quantity of maltodextrin in all 3 experimental solutions was fixed at  $0.67 \text{ g}\cdot\text{min}^{-1}$ , with glucose added to the 0.5- and 0.8-Ratio solutions to balance solution osmolality against fructose content, whilst maintaining isocaloricity and carbohydrate concentration. The control solution was water containing  $2.1 \text{ g}\cdot\text{L}^{-1}$  artificial sweetener (Sucaryl, Hansells, Masterton, NZ). Included in each solution was NaCl ( $1.17 \text{ g}\cdot\text{L}^{-1}$ ,  $20 \text{ mmol}\cdot\text{L}^{-1} \text{ Na}^+$ ), citric acid ( $2.11 \text{ g}\cdot\text{L}^{-1}$ ) and lime juice ( $16 \text{ g}\cdot\text{L}^{-1}$ ). Both the maltodextrin (Star-Dri 100, Tate & Lyle, Decatur, IL) and glucose (National Starch, Auckland, NZ) were maize-derived with  $^{13}\text{C}$ -enrichment of  $-10.4 \text{ ‰}$  and  $-10.78 \text{ ‰}$  (respectively) vs Pee Dee Bellemnitella (PDB). The fructose (Fructofin C, Danisco, Manukau, NZ) was sourced from beetroot ( $-15.7 \text{ ‰}$ ). The solution consumed between 0 and 105 min (1.8 L) was labelled with a total of 6.75 kilobecquerel



(0.3915 milliseverts) of U- $^{14}\text{C}_6$ -fructose (American Radiolabeled Chemicals, St Louis, MO). Justification for inclusion of U- $^{14}\text{C}_6$ -fructose in the experimental solutions, and calculations of activity are included within Appendix H. The U- $^{14}\text{C}_6$ -fructose was omitted from the solutions ingested during the performance test to minimise unnecessary exposure. Solutions consumed during the 2-h training rides contained the beetroot-derived fructose, maltodextrin-derived from tapioca (Briess Malt & Ingredients, Chilton, WI), and NaCl, citric acid and lime juice at the same rate as in the experimental solutions.

**Table 1.** Composition and carbohydrate concentration of test solutions.

Solution and Ratio (Fructose:Maltodextrin/Glucose)	Type	Ingestion rate (g·min <sup>-1</sup> )	Concentration (%)
0.5-Ratio (0.5:1)	Fructose	0.50	3.75
	Maltodextrin	0.67	7.50
	Glucose	0.33	
0.8-Ratio (0.8:1)	Fructose	0.67	5.02
	Maltodextrin	0.67	6.23
	Glucose	0.16	
1.25-Ratio (1.25:1)	Fructose	0.83	6.23
	Maltodextrin	0.67	5.02
	Glucose	0.00	

### 3.5 Fluid Absorption

At exactly 30 min into the 2-h cycle, 5 g 99.8% deuterium oxide (Cambridge Isotope Laboratories, Andover, MA) was ingested along with approximately half a serve of the experimental solution. The remaining solution serving was then poured into the drink container, and subjects used this as a mouth rinse before swallowing. This procedure was adopted to minimise residual D<sub>2</sub>O in the drink container and mouth.

### 3.6 Psychometric Scales

Perceptual ratings were recorded at rest, every 15 min during the 2-h ride and after sprints 1, 4, 7 and 10 of the performance test to rate the effect of solution carbohydrate ratio on physical exertion and gastrointestinal comfort. Perceived exertion (leg muscle strength and tiredness, and perceived effort) and gastrointestinal comfort (nausea, stomach fullness, abdominal cramping) and drink favourability (sweetness, palatability) markers were measured using linear scales. The exact wording of verbal anchors differed depending on the particular perceptual scale, but on each scale



the following levels of perception were represented: 0 (nothing), 2 (very mild), 4 (moderate), 6 (very high), 8 (maximal). Psychometric scales are presented in Appendix I. Participants were instructed to make a pen mark on a continuous scale, rating the strength of their perception of the measure. The numerical value for each verbal anchor was not displayed on the scale charts so as not to distract the participant from their rating. Responses for nausea, abdominal cramping and stomach fullness were chosen to determine the magnitude and temporal effects of solution carbohydrate ratios and exercise duration, and whether the consequence of these factors influenced exercise performance.

### **3.7 Plasma Biochemistry**

Blood samples taken at 28, 40, 50, 60, 70, 80 and 90 min were deproteinised and used for analysis of D<sub>2</sub>O accumulation. Whole blood (100 µL) was transferred from the syringe into 3 x 2 mL Eppendorf tubes each containing 500 µL perchloric acid, and were rested on ice for 10 min. Tubes were then centrifuged at 16000 G for 10 min at 4°C. Bicarbonate (250 µL) was added to each tube and the uncapped tubes were rested on ice for a further 10 min. Tubes were centrifuged at 4000 G for 2 min and the supernatant aspirated into 3 x 1.5 mL Eppendorf tubes and frozen at -80°C until analysis. Blood D<sub>2</sub>O enrichment was determined by continuous-flow isotope-ratio mass spectrometry (Finnigan DeltaV, Thermo Electron Corporation, Bremen, Germany). A detailed method for D<sub>2</sub>O analysis is presented in Appendix J.

### **3.8 Expired Breath**

Fractions of oxygen and carbon dioxide in expired gas were measured through the gas sampling function of the Moxus system. Expired gas volume from the Douglas bag was measured using PowerLab 4/20 spirometer and software (ADInstruments, Bella Vista, NSW). Volume calibration was carried out prior to sampling using a known volume (90 L) and verified again at the end of each testing session. Any drift was assumed to be linear, and raw volumes adjusted accordingly.

#### **3.8.1 <sup>13</sup>C analysis**

Breath samples were analysed for <sup>13</sup>C/<sup>12</sup>C by gas chromatography continuous flow isotope-ratio mass spectrometry (Finnigan Delta XP, Bremen, Germany).

### 3.8.2 $^{14}\text{C}$ analysis

Following gas collection into the anesthetic bag, a length of plastic tubing was fixed to the 3-way stop-cock valve on the bottom of the anesthetic bag, and the gas was bubbled through a  $\text{CO}_2$  trapping solution until the pink-coloured solution became clear, at which point exactly 1 mmol of  $\text{CO}_2$  was trapped. The trapping solution was contained in a 20 mL scintillation vial and comprised: 1 mL hyamine hydroxide in 1 M methanol (Fisher Scientific, Fair Lawn, NJ), 2 mL of 96% ethanol (VWR International Ltd, Poole, England) and 1-2 drops of phenolphthalein (Ajax Finechem, Auckland, NZ). Once 1 mmol  $\text{CO}_2$  was trapped, 17 mL scintillation cocktail (Ultima Gold XR, Perkin Elmer, Waltham, MA) was added to the trapping solution.  $^{14}\text{CO}_2$  radioactivity (disintegrations $\cdot\text{min}^{-1}$ , dpm; later converted to dpm $\cdot\text{mmol}^{-1}$ ) was determined by 10-min triplicate counts in a liquid scintillation counter (Wallac 1409 LS, Turku, Finland). Swabs of the equipment (drink bottles, mixing chamber, anesthetic bags and tubing) were taken at the completion of the experimental period and tested for  $^{14}\text{C}$ . All tests revealed no residual  $^{14}\text{C}$  build-up on the equipment.

### 3.8.3 Substrate oxidation

Total fat and carbohydrate oxidation rates ( $\text{g}\cdot\text{min}^{-1}$ ) were calculated using the non-protein respiratory quotient (Jeukendrup and Wallis 2005): carbohydrate oxidation ( $\text{g}\cdot\text{min}^{-1}$ ) =  $4.210\cdot\text{VCO}_2 - 2.962\cdot\text{VO}_2$ ; fat oxidation ( $\text{g}\cdot\text{min}^{-1}$ ) =  $1.695\cdot\text{VO}_2 - 1.701\cdot\text{VCO}_2$ . Energy potentials of 15.64  $\text{kJ}\cdot\text{g}^{-1}$  for carbohydrate and 40.81  $\text{kJ}\cdot\text{g}^{-1}$  for fat oxidation were used to estimate the contribution to energy expenditure. Endogenous carbohydrate oxidation was assumed to be 100% from muscle glycogen, therefore energy potential of 17.36  $\text{kJ}\cdot\text{g}^{-1}$  for endogenous carbohydrate (glycogen) oxidation was used to estimate the contribution to energy expenditure.

Isotopic enrichment of expired  $\text{CO}_2$  was expressed as the delta per thousand difference ( $\delta\text{‰}$ ) between  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample and a known laboratory reference standard (Pee Dee Belemnite; PDB) according to the formula:  $\delta^{13}\text{C} = [(^{13}\text{C}/^{12}\text{C} \text{ ratio sample} / ^{13}\text{C}/^{12}\text{C} \text{ ratio standard}) - 1] \cdot 10^3 \text{‰}$ , where,  $^{13}\text{C}/^{12}\text{C}$  standard = 0.0112372 (Craig 1957). Enrichment for glucose was  $-10.78 \text{‰}$  and for maltodextrin  $-10.40 \text{‰}$ . The amount of glucose oxidised was then calculated according to the formula: exogenous-carbohydrate oxidation ( $\text{g}\cdot\text{min}^{-1}$ ) =  $\text{VCO}_2 \cdot [(\delta_{\text{Exp}} - \delta_{\text{bkg}}) / (\delta_{\text{Ing}} - \delta_{\text{bkg}})] / k$ , in which  $\delta_{\text{bkg}}$  is the  $^{13}\text{C}$ -enrichment of expired air in the control condition,  $\delta_{\text{Exp}}$  is the  $^{13}\text{C}$ -enrichment of expired  $\text{CO}_2$  during the 2-h ride with  $^{13}\text{C}$ -enriched carbohydrate ingestion,  $\delta_{\text{Ing}}$  is the  $^{13}\text{C}$ -enrichment of the carbohydrate, and  $k$  is the volume of  $\text{CO}_2$  (L) produced via oxidation of 1 g

glucose ( $k = 0.7467$ ). The oxidation rate of ingested maltodextrin is given as grams of glucose equivalents oxidised, assuming that 1.00 g of maltodextrin provides 1.11 g of glucose, owing to the property of dehydration of the maltodextrin (Rowlands and Clarke 2011). Therefore, with a dextrose equivalent of 10.4, this conversion was applied to only 89.6% of the maltodextrin in solutions, while the remaining 10.4% was calculated gram for gram as glucose.

The rate of exogenous-fructose oxidation (EFO) was calculated according to the formula:  $EFO = VCO_2 \cdot [(^{14}CO_2 \cdot 6) / (SA \text{ Fruc})] \cdot (1/k)$ , where  $^{14}CO_2$  is the radioactivity of 1 mmol of expired  $CO_2$  ( $dpm \cdot mmol^{-1}$ ) multiplied by 6, because there are 6 carbon atoms per molecule of  $[U-^{14}C]$ fructose; SA Fruc is the specific activity of the ingested fructose ( $dpm \cdot mmol^{-1}$ ); and  $k$  is the volume of  $CO_2$  (L) produced by the oxidation of 1 g of fructose ( $k = 0.7467$ ).

The percentage efficiency of exogenous-glucose and -fructose metabolism was: oxidation/ingestion rate  $\cdot 100$ .

Calculation of exogenous-substrate oxidation is affected by the delayed equilibration of  $^{13}CO_2$  and  $^{14}CO_2$  with the large endogenous  $HCO_3^-$  pool. However, a physiological steady-state condition occurs relatively rapidly during exercise, and  $^{13}CO_2$  and  $^{14}CO_2$  in the expired air will be equilibrated with the  $^{13}CO_2/H^{13}CO_2$  and  $^{14}CO_2/H^{14}CO_2$  pools, respectively, from around 60 min of steady-state exercise (Robert, Koziol *et al.* 1987). As a consequence, the main outcome measures for substrate oxidation were from 60 to 120 min of exercise.

### 3.9 Sample Size

Most previous multiple cross-over studies of this type have generated sufficient data with 8-12 participants to make reasonable inferences from the sample to the true population effect. The primary outcome measures in this experiment were high-intensity performance and the oxidation of the ingested fructose and glucose (maltodextrin), and blood  $D_2O$  appearance. The reliability of the first was estimated at 3.1% (Rowlands, Thorp *et al.* 2007). Using the clinical likelihood sample size method of Hopkins *et al.* (2009) and assuming 0.93% as the smallest important effect magnitude (O'Brien and Rowlands 2011), a sample size of 10 was calculated. Similarly, the reliability of the oxidation rates was aligned to the reliability of the flow measurement, which is approximately 3%. Oxidation rate is a physiological mechanism to help explain the phenotypical response, and the smallest important effect is not currently known.

A Latin square (Williams design) was used, where, for four conditions, there are four non-sequential orders of application. Therefore, to balance the design, the sample size was increased from the 10 estimated above, to 12.

### 3.10 Statistical Analysis

#### 3.10.1 General method

The effects of fructose:maltodextrin ingestion ratio on outcomes were estimated with appropriate mixed models (Proc Mixed, SAS Version 9.1, SAS Institute, Cary, NC). Most dependent variables, except psychometric parameters and raw data expressed as a percent, were analysed after natural log-transformation to reduce effects of non-uniformity of error and to express changes as percents. For all datasets, fixed effects were treatment and the order term, which accounts for familiarisation, adaptation, or fatigue effects between consecutive trials. For the time-series data, the x-axis variable was grand-mean centered for linear modeling (as in regression analysis). Subject was the random effect. The within-subject coefficient of variation (CV) was estimated from the residual variance.

#### 3.10.2 Presentation of data

Subject descriptive and some outcome data are raw means and standard deviations. Means derived from the analysis of log-transformed variables are back-transformed least-squares means, with the associated between-subject spread represented by the coefficient of variation, which can be converted to a unit value by conversion to a factor. The size of the treatment effect on metabolic and psychometric outcomes was qualified using modified Cohen effect size (standardised difference) classification: trivial 0.0–0.2, small 0.2–0.6, moderate 0.6–1.2, large 1.2–2.0, very large 2.0–4.0, enormous >4.0 (Hopkins, Marshall *et al.* 2009). Sample size was adjusted for small sample bias where the standardised difference was applied ( $1-3/(4v-1)$ ), where  $v$  is the degrees of freedom for the SD (Hopkins, Marshall *et al.* 2009).

#### 3.10.3 Estimate precision and statistical inference

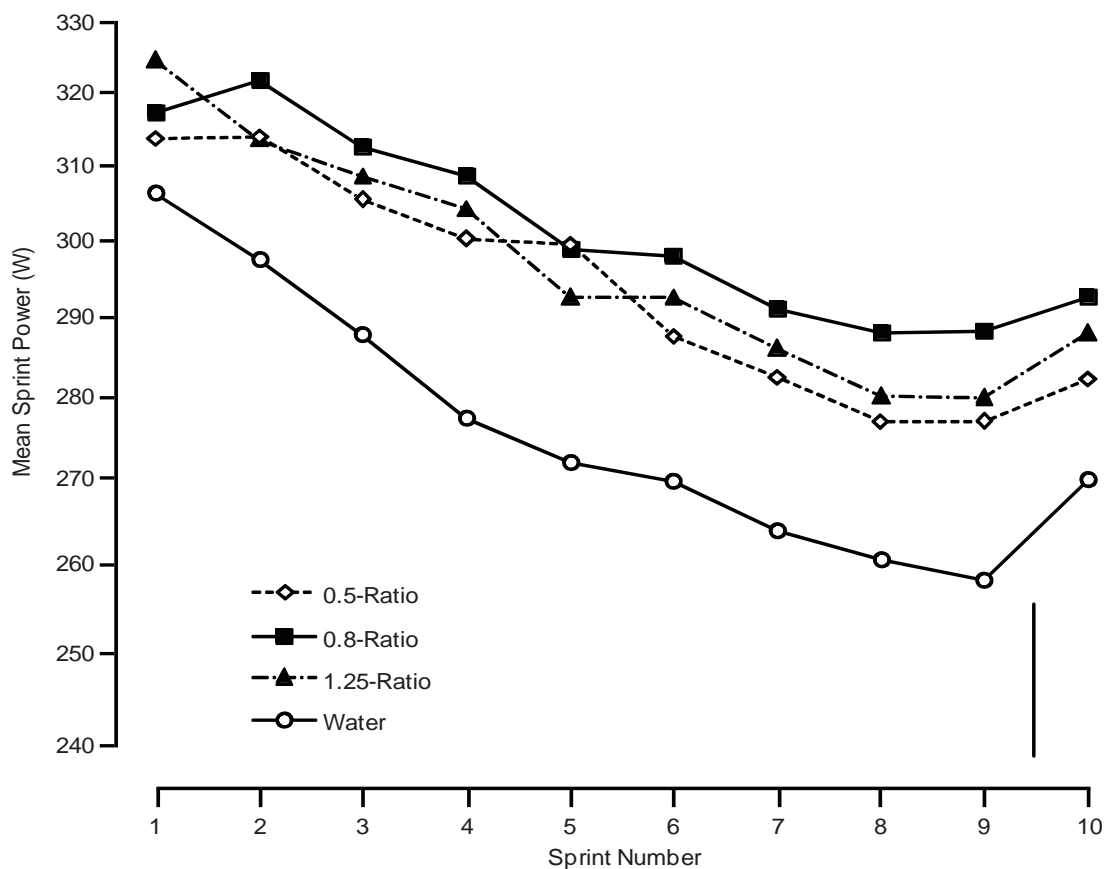
In light of limitations associated with traditional null hypothesis testing (Cohen 1994; Sterne and Smith 2001) and recent trends in inferential statistics, the magnitude-based inference approach was utilised: 90% confidence intervals for uncertainty in mechanistic variables, 99% confidence intervals on the harm side of uncertainty of performance, and interpretation of uncertainty in relation to effect-size magnitude thresholds rather than the null of traditional hypothesis testing

(Rowlands, Thorburn *et al.* 2008; Hopkins, Marshall *et al.* 2009). The threshold for a *substantial* change for mechanism outcomes was the conventional smallest standardised difference (0.2); for performance  $0.3 \times$  the within-subject CV in the performance test was used. Additionally the thresholds for moderate ( $0.9 \times$  within-subject CV), large ( $1.6 \times$  within-subject CV), very large ( $2.5 \times$  within-subject CV) and extremely large ( $4.0 \times$  within-subject CV) were used (Hopkins, Marshall *et al.* 2009). The within-subject SD was a surrogate for the variability in performance of well-trained cyclists in competition. The variability in performance in the repeated-sprint test was assumed to simulate the physical and physiological demands of a race (Bonetti and Hopkins 2010), and any difference in variability between laboratory and field assumed to also change in proportion to the magnitude of the response to treatment retaining a proportionate ratio of the sensitivity in response to treatment to the measurement error (Hopkins, Hawley *et al.* 1999). The smallest worthwhile effect of treatment on performance is between 0.3-0.7 times estimate for the CV (Hopkins, Hawley *et al.* 1999). A close relationship between the effect-size for intervention performance in the repeat-sprint test and in competition performance has been established recently (Rowlands, Swift *et al.* 2011). For mechanistic outcomes an effect was described as *unclear* if its confidence interval included both substantial positive *and* negative values (i.e.,  $>5\%$  probability that the true value is both substantially positive and negative). Otherwise, the probability of a substantial increase or decrease was calculated from the two-tailed t-distribution summarised as:  $<0.5\%$ , almost certainly not; 0.5-5%, very unlikely; 5-25%, unlikely; 25-75%, possible; 75-95%, likely; 95-99.5%, very likely;  $>99.5\%$ , almost certain. In the case where the majority ( $>50\%$ ) of the confidence interval lies between the threshold for substantiveness the effect was qualified *trivial* (negligible). For inference to the performance outcome, the clinical decision thresholds of Hopkins *et al.* (2009) was referred to, where an intervention is considered for adoption if the probability of *substantial* harm is  $<0.5\%$  and benefit  $>25\%$  (possible) or  $>75\%$  (likely); otherwise outcomes are inferred as for mechanism.

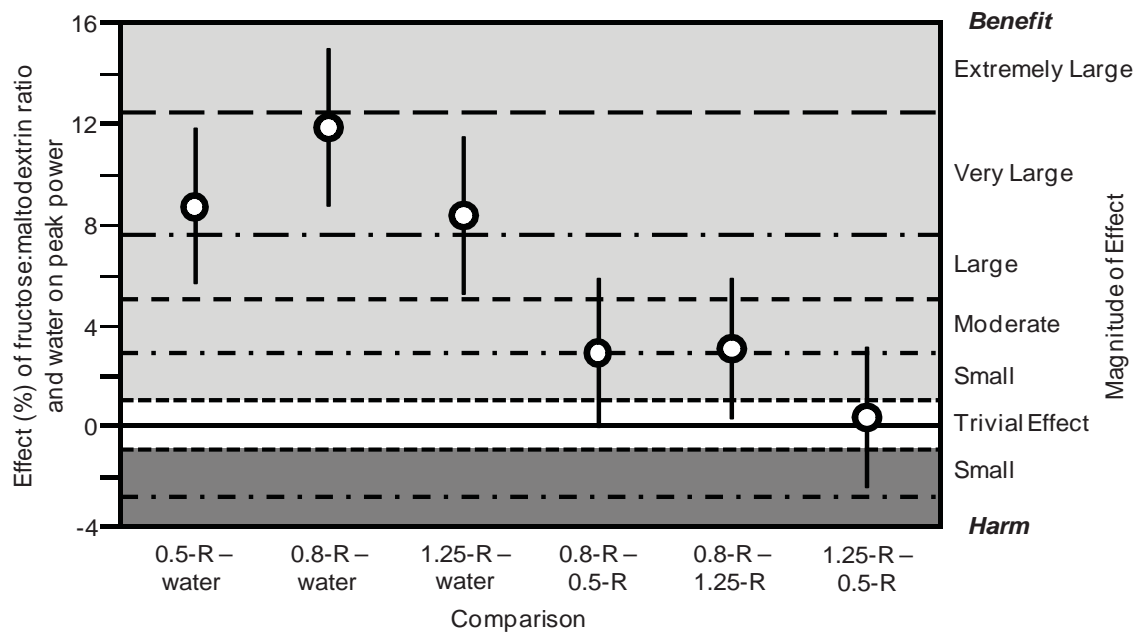
## 4 Results

### 4.1 Performance

Mean sprint-power over the 10-repeated-sprint performance test is shown in Fig 1. Overall mean sprint-power was 294, 303, 296 and 275 W (between-subject CV 18%) for 0.5-, 0.8-, 1.25-Ratio and Water, respectively. The differential comparison for the performance outcome is presented in Fig. 2. Relative to 0.5-Ratio, substantially higher mean power output was very likely in the 0.8-Ratio (likelihoods harm/trivial/benefit: 0.0/3.3/96.7), but very likely trivial in the 1.25-Ratio condition. Mean power with 0.8-Ratio was possibly higher (0.0/1.7/98.3) relative to 1.25-Ratio. Relative to Water, mean peak-power in all carbohydrate conditions was almost certainly higher (0.0/0.0/100). Reduction in mean power output (slope effect) over the 10 repeated-sprints was  $13.2\% \pm 3.4\%$ ,  $11.4\% \pm 3.5\%$ ,  $13.0\% \pm 3.5\%$  and  $14.5\% \pm 3.4\%$  for 0.5-, 0.8-, 1.25-Ratio and Water, respectively (Fig. 1.), while the difference in the decline of mean peak-power was possibly attenuated with 0.8-Ratio compared to 0.5- and 1.25-Ratio (Fig. 2.).



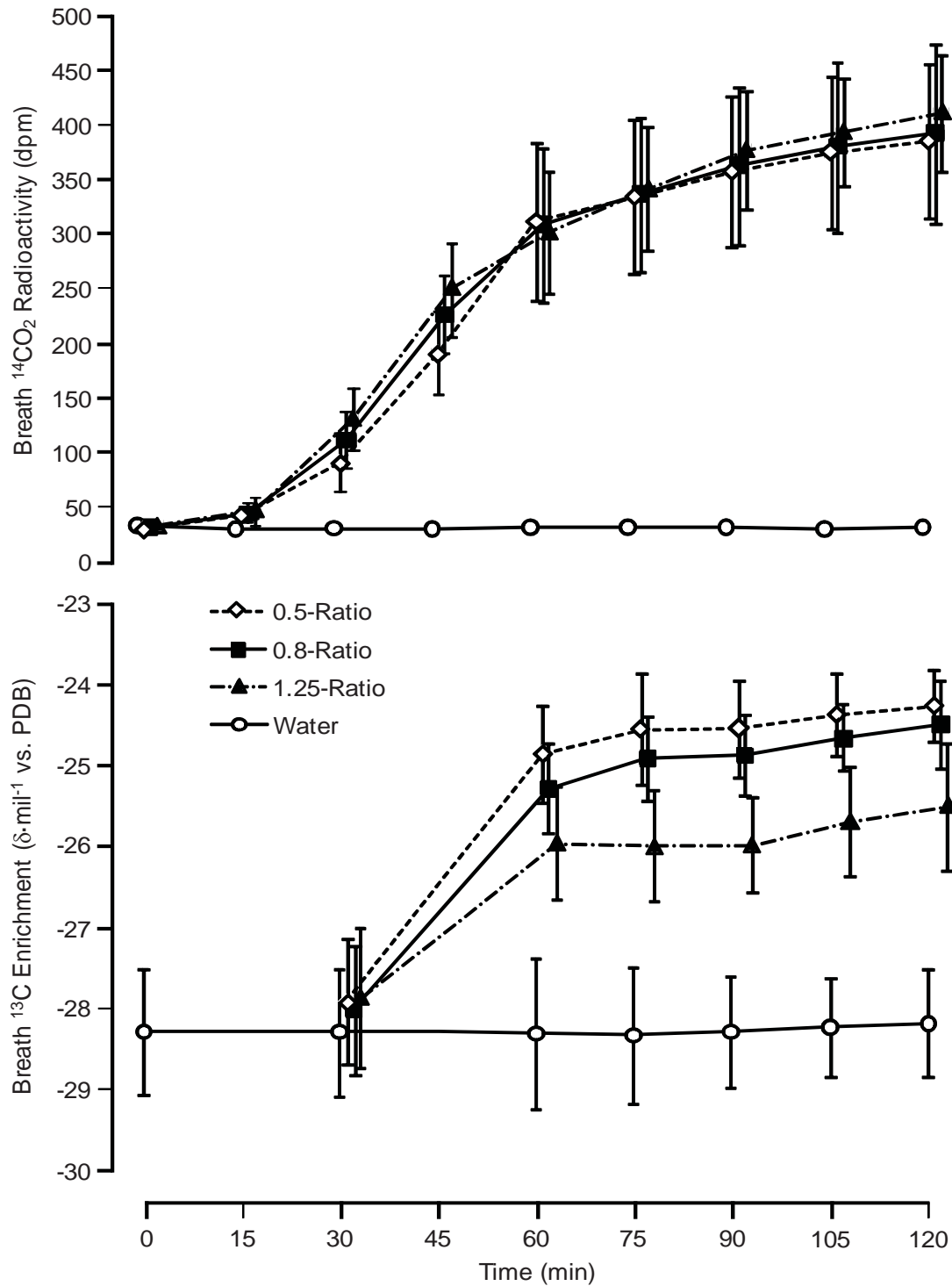
**Figure 1.** Pattern of mean sprint-power during the repeated-sprint performance test. Data are back log-transformed least-squares means. Bar represents the back-transformed composite between-subject coefficient of variation.



**Figure 2.** The effect of solution composition on performance test mean power. Point data are the back log-transformed least-squares means. Bars are the 99% confidence interval. Thresholds for small (0.3%), moderate (2.79%), large (4.96%), very large (7.5%) and extremely large (12.4%) effects are shown as dashed lines within the shaded zones. R, Ratio.

## 4.2 Stable and Radioactive Isotope Measurements

Breath  $^{14}\text{CO}_2$  radioactivity and  $^{13}\text{C}$  enrichment during the 2-h ride are shown in Fig. 3.



**Figure 3.** Breath  $^{14}\text{CO}_2$  activity and  $^{13}\text{C}$  enrichment during the 2-h ride. Data are raw means with the between-subject standard deviation, offset from the sampling point for visual clarity. The Water trial provided the  $^{14}\text{C}$  and  $^{13}\text{C}$  background, and standard deviations for  $^{14}\text{C}$  are obscured.



### 4.3 Substrate Oxidation

Oxidation rates are shown in Fig. 4, and exogenous-carbohydrate oxidation efficiency is presented in Fig. 5. Average substrate oxidation rates and exogenous-carbohydrate oxidation efficiencies for the 60 to 120 min period of the 2-h ride are summarised in Table 2, with the corresponding statistical comparisons in Table 3.

#### 4.3.1 Exogenous fructose oxidation

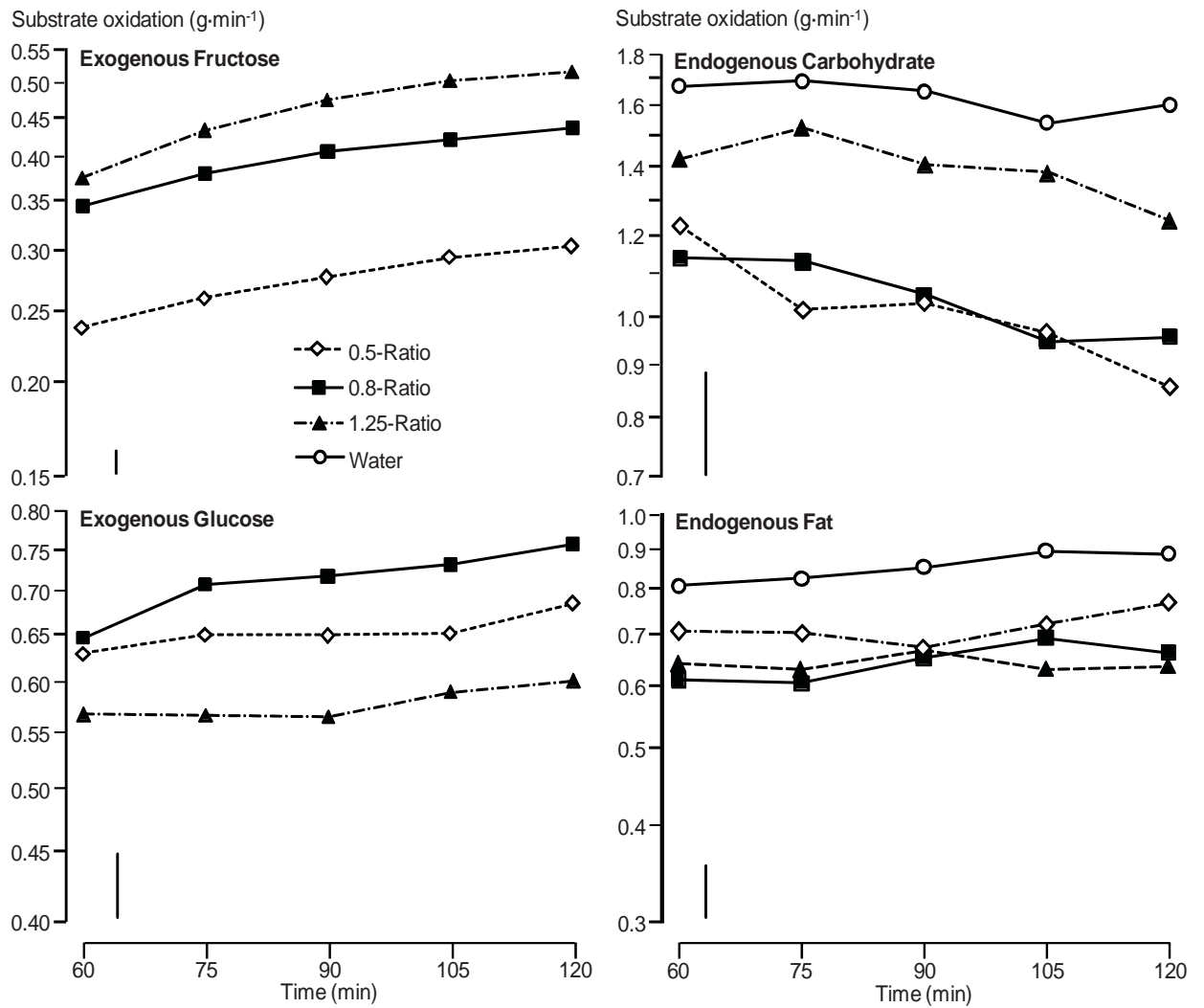
During the 60<sup>th</sup> to 120<sup>th</sup> min of the 2-h ride the oxidation rate of exogenous fructose was highest with the 1.25-Ratio, and increased with increasing dose (Table 2). Average exogenous-fructose oxidation efficiency was likely substantially higher with the 0.8-Ratio, than either 0.5- and 1.25-Ratio (Table 3).

#### 4.3.2 Exogenous glucose oxidation

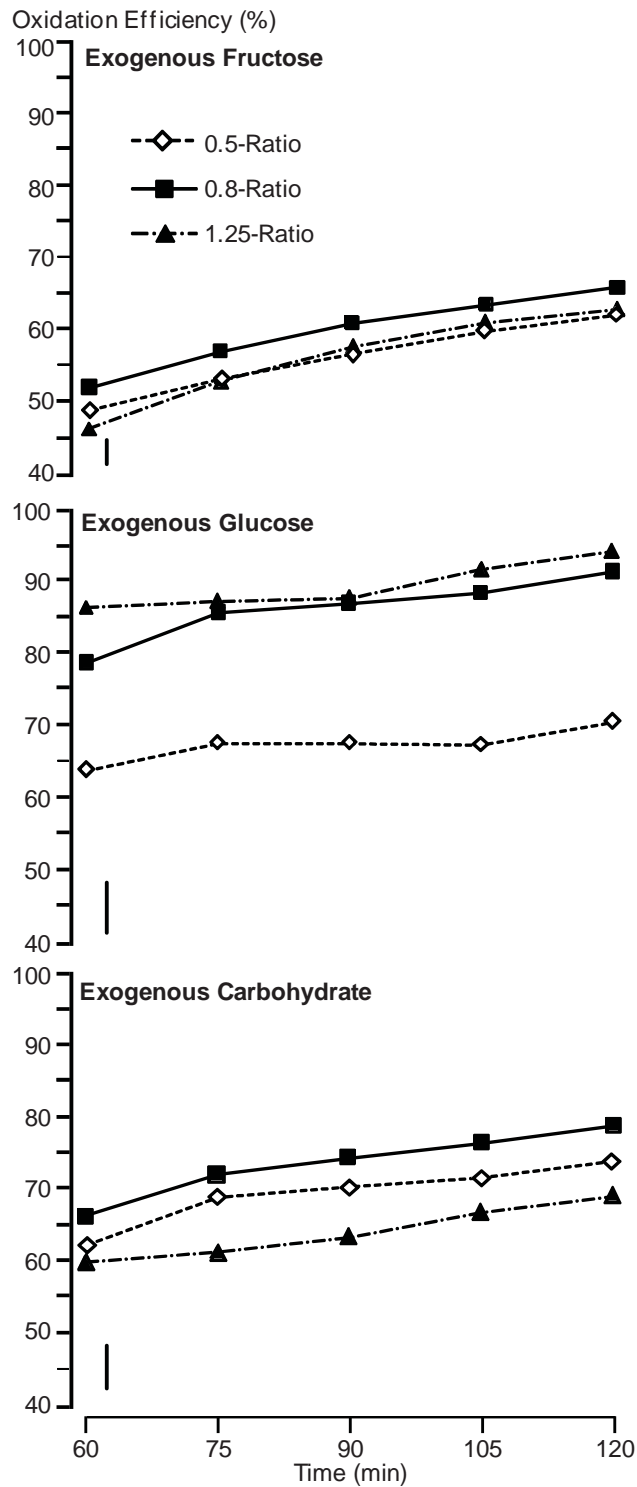
During the final 60 min of the 2-h ride, exogenous-glucose oxidation rate was possibly 8.8% ( $\pm 7.2\%$ ) and almost certainly 17.0% ( $\pm 5.7\%$ ) higher with 0.8- compared to 0.5- and 1.25-Ratio solutions, respectively. Exogenous-glucose oxidation efficiency was almost certainly lowest with 0.5-Ratio compared to the other carbohydrate conditions (Table 3).

#### 4.3.3 Total exogenous carbohydrate oxidation

The total exogenous-carbohydrate oxidation rate was higher in the 0.8-Ratio condition during the 2-h ride relative to the 0.5- and 1.25-Ratio conditions (Table 3); respective mean oxidation efficiencies are presented in Table 2. From the 60<sup>th</sup> to 120<sup>th</sup> min of the 2-h ride (slope effect), increases in the exogenous-carbohydrate oxidation rate of 16.6% ( $\pm 6.9\%$ ), 18.2% ( $\pm 6.8\%$ ) and 17.0% ( $\pm 7.2\%$ ) were observed in the 0.5-, 0.8- and 1.25-Ratio conditions respectively, without clear differences between the ratios (comparisons not shown) (Fig. 4).



**Figure 4.** The pattern of substrate oxidation during the 60<sup>th</sup>-120<sup>th</sup> min of the 2-h ride. Data are back log-transformed least-squares means. Bars represent the back-transformed composite between-subject coefficient of variation.



**Figure 5.** The pattern of efficiency of oxidation for exogenous fructose, exogenous glucose and total exogenous carbohydrate during the 60<sup>th</sup>-120<sup>th</sup> min of the 2-h ride. Data are back log-transformed least-squares means. Bars represent the back-transformed composite between-subject coefficient of variation.

#### 4.3.4 Endogenous and total carbohydrate oxidation

During the 2-h ride the endogenous-carbohydrate oxidation rate of Water was almost certainly higher than with the 0.5- and 0.8-Ratio conditions, while there were very likely moderate/large increases with the 1.25-Ratio condition relative to the 0.5- and 0.8-Ratio conditions (Fig. 4). Although no clear differences in the rate of total-carbohydrate oxidation were observed between any of the carbohydrate ingestion conditions, all were likely/very likely higher than Water by moderate/large amounts (Table 3). Differences in slope between the carbohydrate conditions were unclear (comparison not shown).

#### 4.3.5 Endogenous fat oxidation

Carbohydrate ratio had no clear effect on endogenous-fat oxidation rate (Fig. 4, Table 3); and as expected, fat oxidation was substantially higher in the Water condition relative to all carbohydrate ingestion conditions. Slope effects between conditions were either likely or possibly trivial.

**Table 2.** Oxidation rate of endogenous and exogenous substrates during the 60<sup>th</sup> to 120<sup>th</sup> min of the 2-h ride.

Substrate	Condition			
	Water	0.5-Ratio	0.8-Ratio	1.25-Ratio
<i>Oxidation Rate (g·min<sup>-1</sup>)</i>				
Exogenous Fructose	-	0.27 (46)	0.39 (56)	0.46 (53)
Exogenous Glucose	-	0.65 (30)	0.71 (14)	0.58 (28)
Total Exogenous Carbohydrate	-	1.03 (13)	1.10 (9)	0.95 (17)
Endogenous Carbohydrate	1.64 (30)	1.01 (28)	1.04 (39)	1.39 (27)
Total Carbohydrate	1.64 (30)	2.06 (21)	2.19 (27)	2.27 (19)
Endogenous Fat	0.85 (35)	0.71 (41)	0.64 (60)	0.64 (36)
<i>Oxidation Efficiency (%)</i>				
Exogenous Fructose	-	56 (12)	60 (7)	56 (10)
Exogenous Glucose	-	67 (16)	86 (11)	89 (21)
Total Exogenous Carbohydrate	-	70 (9)	74 (6)	64 (9)

Oxidation rate data are the back log-transformed least-squares mean values for 60 to 120 min sampling points inclusive. The between-subject standard deviation (SD, %) was derived from the analysis. Oxidation efficiency is the rate of carbohydrate oxidation/ingestion\*100.

**Table 3.** Summary of the effect of solution composition on substrate oxidation rate and efficiency from the 60<sup>th</sup> to 120<sup>th</sup> min period of the 2-h ride.

Substrate	Mean Effect Comparisons <sup>a</sup> (%) with $\pm 90\%CL^b$ and Qualitative Inference <sup>c</sup>					
	0.5-Ratio - Water	0.8-Ratio - Water	1.25-Ratio - Water	0.8-Ratio - 0.5-Ratio	0.8-Ratio - 1.25-Ratio	1.25-Ratio - 0.5-Ratio
<i>Oxidation Rate</i>						
Exogenous Fructose	-	-	-	44.7 $\pm$ 26.5 mod. $\hat{\uparrow}$ almost certain	-15.6 $\pm$ 21.2 unclear	67.2 $\pm$ 30.7 large $\hat{\uparrow}$ almost certain
Exogenous Glucose	-	-	-	8.8 $\pm$ 7.2 small $\hat{\uparrow}$ possible	17.0 $\pm$ 5.7 small $\hat{\uparrow}$ almost certain	-9.7 $\pm$ 6.2 small $\hat{\downarrow}$ likely
Total Exogenous Carbohydrate	-	-	-	6.4 $\pm$ 3.1 small $\hat{\uparrow}$ very likely	12.7 $\pm$ 2.6 mod. $\hat{\uparrow}$ almost certain	-7.2 $\pm$ 2.8 small $\hat{\downarrow}$ very likely
Endogenous Carbohydrate	-35.6 $\pm$ 13.1 mod. $\hat{\downarrow}$ almost certain	-32.6 $\pm$ 13.6 mod. $\hat{\downarrow}$ almost certain	-11.5 $\pm$ 17.9 unclear	4.6 $\pm$ 21.2 unclear	-31.3 $\pm$ 26.6 mod. $\hat{\downarrow}$ very likely	37.3 $\pm$ 27.8 large $\hat{\uparrow}$ very likely
Total Carbohydrate	24.6 $\pm$ 23.1 mod. $\hat{\uparrow}$ likely	32.5 $\pm$ 24.6 mod. $\hat{\uparrow}$ very likely	38.2 $\pm$ 25.7 large $\hat{\uparrow}$ very likely	6.4 $\pm$ 19.8 unclear	-4.3 $\pm$ 19.4 unclear	11.0 $\pm$ 20.6 unclear
Endogenous Fat	-14.3 $\pm$ 15.6 likely $\hat{\downarrow}$ small	-23.9 $\pm$ 13.8 mod. $\hat{\downarrow}$ very likely	-24.2 $\pm$ 13.8 mod. $\hat{\downarrow}$ very likely	-11.2 $\pm$ 16.2 unclear	0.4 $\pm$ 18.1 unclear	-11.6 $\pm$ 16.1 unclear
<i>Oxidation Efficiency</i>						
Exogenous Fructose	-	-	-	3.9 $\pm$ 2.2 small $\hat{\uparrow}$ likely	3.9 $\pm$ 2.2 small $\hat{\uparrow}$ likely	0.0 $\pm$ 2.2 unclear
Exogenous Glucose	-	-	-	18.5 $\pm$ 4.7 mod. $\hat{\uparrow}$ almost certain	-4.8 $\pm$ 4.9 small $\hat{\downarrow}$ possible	23.2 $\pm$ 4.9 large $\hat{\uparrow}$ almost certain
Total Exogenous Carbohydrate	-	-	-	4.1 $\pm$ 1.8 small $\hat{\uparrow}$ very likely	8.8 $\pm$ 1.9 small $\hat{\uparrow}$ almost certain	-4.7 $\pm$ 1.9 small $\hat{\downarrow}$ very likely

<sup>a</sup> Data are the percent effect of treatment relative to the reference condition on substrate oxidation rate (g·min<sup>-1</sup>).<sup>b</sup>  $\pm 90\%CL$ : add and subtract this number to the mean to obtain the 90% confidence limits for the true difference.<sup>c</sup> Qualified thresholds for standardised change: 0-0.2 trivial, 0.2-0.6 small, 0.6-1.2 moderate (mod.), 1.2-2.0 large, 2.0-4.0 very large. Threshold for probability of a substantial effect: <0.5% almost certainly not, 0.5-5% very unlikely, 5-25% unlikely, 25-75% possible, 75-95% likely, 95-99% very likely, >99% almost certain; where an effect is unclear if its confidence interval includes both substantial increases and decreases. Arrows indicate an increase ( $\hat{\uparrow}$ ) or decrease ( $\hat{\downarrow}$ ).

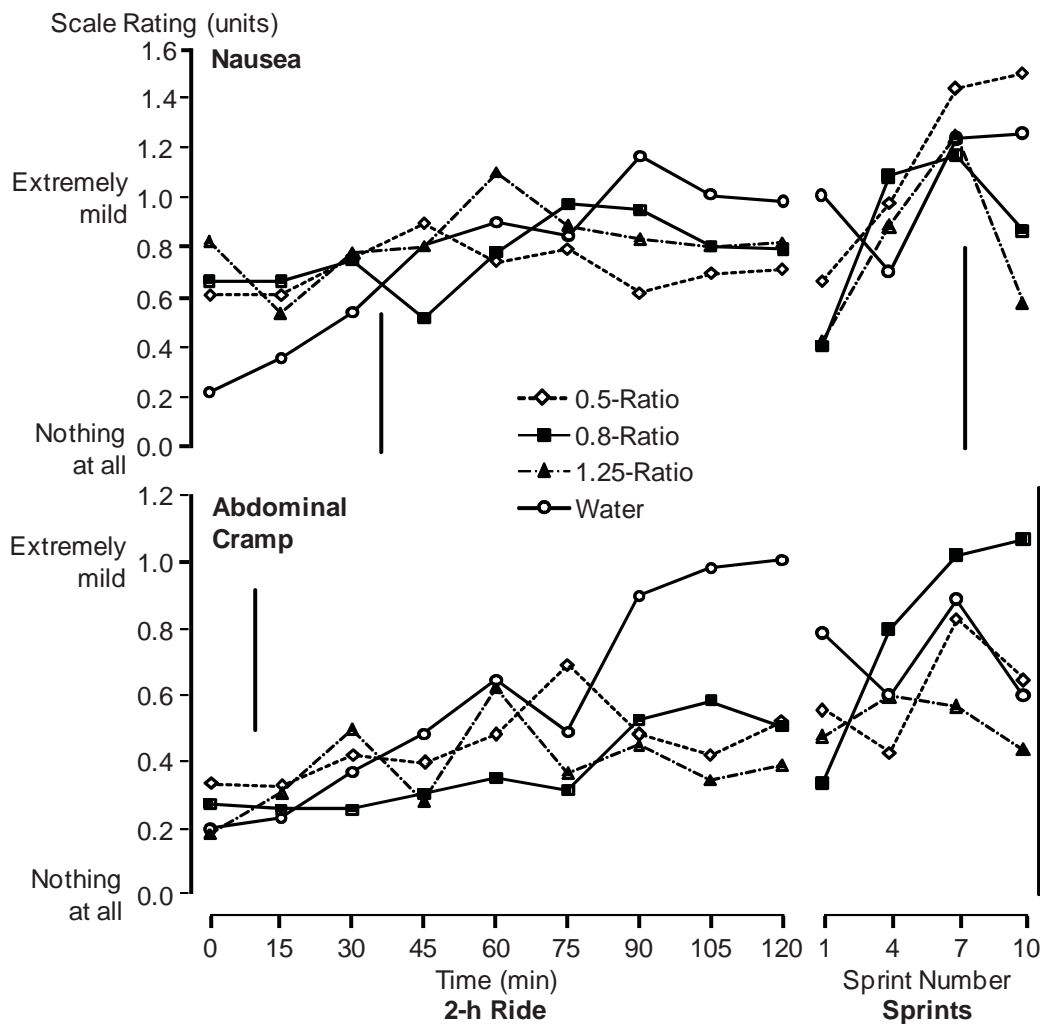
## 4.4 Gastrointestinal Comfort and Exertion

### 4.4.1 Gastrointestinal comfort

Perceptions of nausea during both the 2-h ride and the repeated-sprint performance test were less than *mild* (i.e., <2 scale unit) for all conditions, with trivial overall differences observed. The rate at which nausea perception increased (slope effect) during the 2-h ride was very likely moderately faster in the Water condition compared to all carbohydrate conditions, and possibly faster with 0.5-Ratio relative to the 0.8- and 1.25-Ratio conditions during the repeated-sprint performance test (Fig. 6).

Perceptions of abdominal cramping were approximately *extremely mild* (i.e., <1 scale unit) or lower for all conditions during both the 2-h ride and the repeated-sprint performance test. During the 2-h ride differences in abdominal cramping perception were likely higher with Water compared to 0.8- and 1.25-Ratio conditions (both  $0.2 \pm 0.1$  scale units); all other comparisons were trivial. There was a very likely faster rise (slope effect) in abdominal cramping perception during the 2-h ride with Water relative to 0.5-, 0.8- and 1.25-Ratio conditions ( $0.6 \pm 0.3$ ;  $0.4 \pm 0.3$ ; and  $0.7 \pm 0.3$  scale units, respectively). The increase in perception (slope effect) during the 10 repeated-sprints was likely faster with 0.8-Ratio relative to all other conditions, however, absolute differences remained <1 scale unit (Fig. 6).

Perception of stomach fullness (slope effect) rose in all conditions during the 2-h ride, although overall differences were trivial. There was a small increase in fullness perception (slope effect) with Water, while comparisons between carbohydrate conditions were all unclear.



**Figure 6.** Ratings of gastrointestinal comfort during the 2-h ride and the repeated-sprint performance test. Data are least-squares means and bars the composite between-subject standard deviation.

#### 4.4.2 Perceived exertion and muscle tiredness

Tiredness perception during the 10 repeated-sprints was very likely lower with 0.5-Ratio compared to 0.8- and 1.25-Ratio ( $0.7 \pm 0.5$  and  $0.6 \pm 0.5$  scale units, respectively), and almost certainly lower relative to Water ( $0.9 \pm 0.5$  scale units). Perceptions of tiredness increased in all conditions (slope effect) between the 1<sup>st</sup> and 10<sup>th</sup> sprint of the performance test. Relative to 0.8-Ratio the rate of increase in perception of tiredness (slope effect) was likely moderate with 0.5- and 1.25-Ratio solutions.

Strength perception during the performance test was likely small higher with the 0.5-Ratio compared to 0.8- and 1.25-Ratio solutions ( $0.6 \pm 0.5$  and  $0.4 \pm 0.5$  scale units, respectively).

Perception of strength decreased (slope effect) over the 10 repeated-sprints. Strength decline was slower (slope effect) in the 0.8-Ratio condition with differences possibly/likely small relative to Water and 0.5- and 1.25-Ratio ( $0.6 \pm 1.2$ ;  $0.6 \pm 1.2$ ;  $0.5 \pm 1.2$  scale units, respectively). All other comparisons were trivial or unclear.

Differences in perceptions of muscle soreness were likely/possibly trivial among all conditions during both the 2-h ride and the 10-repeated-sprint performance test. Perceptions of muscle soreness rose (slope effect) throughout the 2-h ride from *extremely mild* at time 0 to *moderate-severe* at time 120 min, and continued to rise (slope effect) during the 10 repeated-sprints, finishing between *severe* and *very severe* after the 10<sup>th</sup> sprint.

Differences in the perception of exertion were possibly trivial between all conditions in both the 2-h ride and the repeated-sprint performance test. Increases in perceived exertion (slope effect) with Water relative to 0.5- and 1.25-Ratio were possibly/likely small during both the 2-h ride and the performance test.

## 4.5 Drink Taste

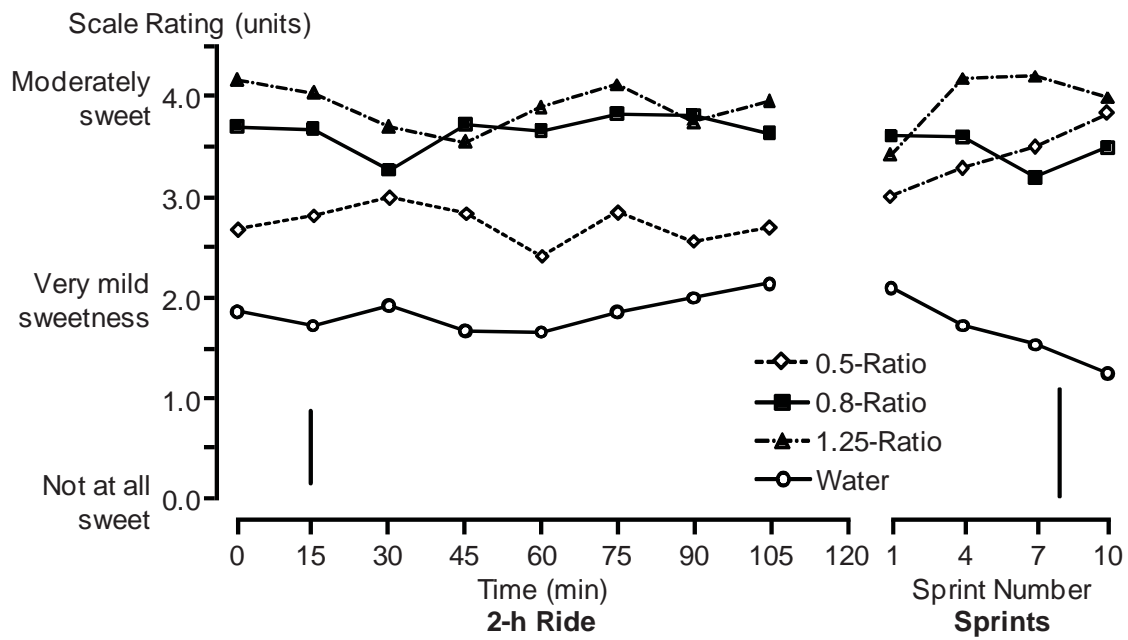
### 4.5.1 Palatability

Drink palatability during the 2-h ride was lowest in the Water condition, with small differences relative to 0.5-, 0.8- and 1.25-Ratio conditions ( $0.5 \pm 0.2$ ;  $0.3 \pm 0.2$ ; and  $0.3 \pm 0.2$  scale units $\cdot 120 \text{ min}^{-1}$ ) respectively; all other comparisons were likely trivial. During the 10 repeated-sprints drink palatability differences were possibly trivial among all conditions with only a  $\sim 0.2$  scale unit difference evident between all conditions at the completion of sprint-10.

### 4.5.2 Sweetness

The perception of sweetness during both the 2-h ride and the 10-repeated-sprint performance test was moderate/large almost certainly lower with Water than with any of the carbohydrate solutions. Sweetness perception during the 2-h ride was almost certainly lower with the 0.5-Ratio relative to 0.8- and 1.25-Ratio conditions ( $0.8 \pm 0.3$  and  $1.0 \pm 0.3$  scale units $\cdot 120\text{-min}^{-1}$ , respectively) (Fig. 7). Over the course of the 10 repeated-sprints, perception of sweetness declined (slope effect) with Water but increased with 0.5-Ratio ( $-0.7 \pm 1.3$  and  $0.8 \pm 1.4$  scale units $\cdot 10\text{-sprints}^{-1}$ , respectively). Most other comparisons were unclear or trivial.

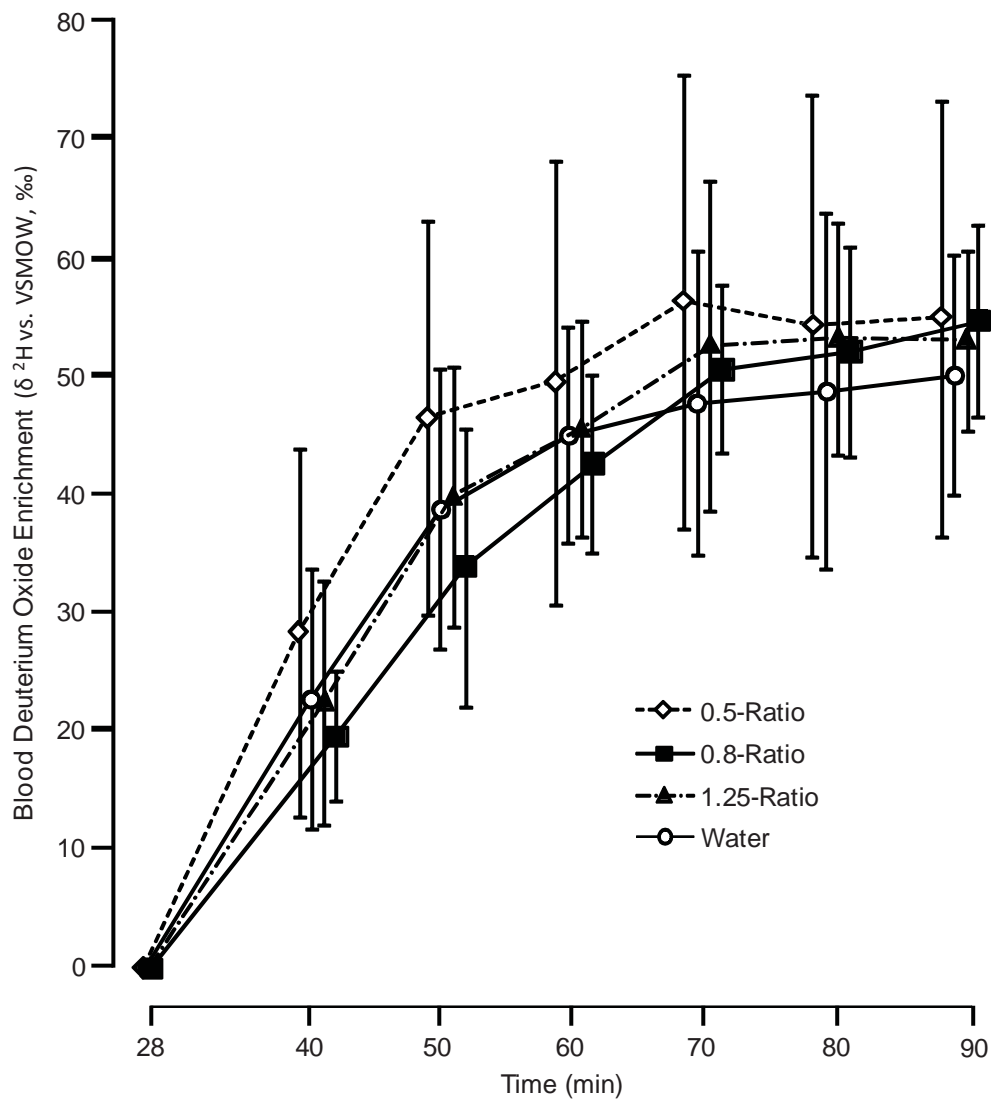




**Figure 7.** Ratings of drink sweetness during the 2-h ride and the repeated-sprint performance test. Data are least-squares means and bars the composite between-subject standard deviation.

#### 4.6 Fluid Absorption.

Blood deuterium enrichments rose over the 28- to 90-min period of the 2-h ride following ingestion of D<sub>2</sub>O at 30-min (Fig. 8). The initial rise in blood deuterium enrichment was faster with Water compared to the other solutions, but by 90-min, was only possible small lower enrichment with 0.8-Ratio relative to the other solutions ( $-19 \pm 40$ ,  $-15 \pm 56$  and  $-26 \pm 37\%$  for 0.8- vs. 0.5-, 1.25-Ratio, and Water, respectively), and with Water relative to 1.25-Ratio ( $-15 \pm 42\%$ ). All other comparisons were unclear (Fig. 8).



**Figure 8.** Blood D<sub>2</sub>O enrichment ( $\delta^2\text{H}$  vs. VSMOW, ‰) following ingestion of 5.00 g D<sub>2</sub>O at 30-min of exercise (VSMOW – Vienna Standard Mean Ocean Water). Data are raw means and bars are standard deviations, offset from the sampling point for visual clarity.

## 5 Discussion

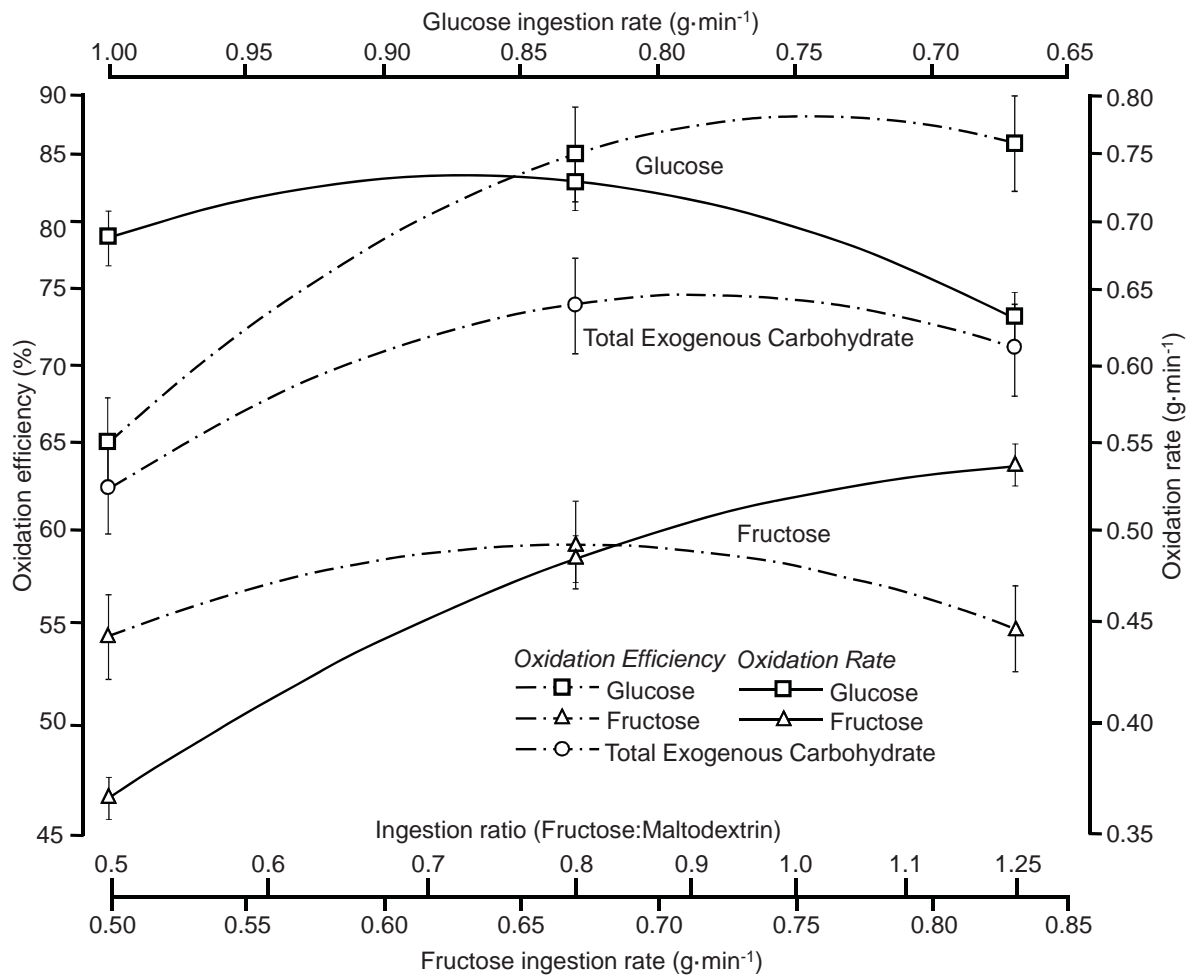
In this study the effect of ingesting solutions containing fructose and maltodextrin/glucose, at respective ratios of 0.5, 0.8 and 1.25 on exogenous-carbohydrate oxidation, fluid absorption, gastrointestinal comfort and high-intensity endurance performance was re-examined, but at a lower ingestion rate, equal osmolality and a more strenuous exercise test than previously (O'Brien and Rowlands 2011). The data provided further evidence that total exogenous-carbohydrate oxidation rate and high-intensity performance are substantially enhanced with a 0.8-Ratio solution vs. 0.5- and 1.25-Ratio solutions. New evidence from this dual  $^{14}\text{C}/^{13}\text{C}$  isotope evaluation of exogenous-fructose and -glucose oxidation rates revealed that the oxidation efficiency of both exogenous-fructose and exogenous-glucose was optimised in the 0.8-Ratio solution condition, with the effect driven by higher fructose oxidation efficiency. Despite these fructose and glucose oxidation outcomes, the effect of fructose:glucose ratio on fluid absorption rates was inconclusive.

The novel and most mechanistically informative outcomes of the present study were obtained from the dual tracer isotope ( $^{13}\text{C}$ -maltodextrin and  $^{14}\text{C}$ -fructose) investigation. By design, any meaningful confounding influences of osmolality or carbohydrate concentration on the rate of intestinal carbohydrate absorption and end-point oxidation were removed by clamping the osmolality of isocaloric solutions. These conditions allowed for inferences to be made with respect to the synergism associated with coingestion, leading to substantial increases both in exogenous-fructose oxidation efficiency and exogenous-glucose oxidation rate with the 0.8-Ratio solution, and resulted in 6–13% higher total exogenous-carbohydrate oxidation rates compared to the other carbohydrate solutions. Notwithstanding the 17% ( $\pm 5.7\%$ ) higher absolute exogenous-glucose oxidation rate in the 0.8- relative to the 1.25-Ratio condition, comparative exogenous-glucose oxidation efficiency was almost the same at 86% vs. 89%, respectively (Fig. 5.). Conversely, higher absolute oxidation rate ( $8.8\% \pm 7.2$ ) and efficiency ( $18.5\% \pm 4.7\%$ ) of exogenous glucose occurred with the 0.8-Ratio solution (Fig. 5.) despite the 0.5-Ratio glucose ingestion rate being 20% higher. These data suggest that the mean glucose ingestion rate ( $1.0 \text{ g}\cdot\text{min}^{-1}$ ) in the 0.5-Ratio solution was within the range of the active SGLT1 glucose transporter saturation, estimated from intubation studies to fall, on average, between 0.81 (Rolston and Mathan 1990) and  $1.7 \text{ g}\cdot\text{min}^{-1}$  (Duchman, Ryan *et al.* 1997). Regarding fructose absorption, the saturation point for facilitative diffusion of fructose by

GLUT5 is undefined. The present oxidation data are indicative of the dose-dependent glucose-stimulated fructose uptake reported by Rumessen and Gudmand-Høyer (1986) from sucrose or free fructose and glucose solutions. Shi *et al.* (1997) attributed 19-27% of fructose transport to the paracellular pathway through increased intestinal permeability in the presence of luminal glucose, and suggested that the opening of tight junctions for passive glucose absorption might enable further fructose to be transported by solvent drag (Shi, Summers *et al.* 1995; Shi, Schedl *et al.* 1997). Fructose absorption and oxidation rates, therefore, are probably related to factors other than transporter capacity.

The average maximal oxidation rate of a single ingested carbohydrate is no more than 1.0-1.1 g·min<sup>-1</sup> (Jentjens, Moseley *et al.* 2004), yet only in the 0.5-Ratio condition did glucose ingestion (1.0 g·min<sup>-1</sup>) come close to this maximum. It seems more plausible, therefore, that at the upper end of recommended mean glucose or glucose polymer ingestion rates (Jeukendrup 2010), factors more influential than intestinal transporter saturation determine the effect of ratio on rates of oxidation. Fig. 9 illustrates a levelling off of mean exogenous-glucose oxidation efficiency at ~0.75 g·min<sup>-1</sup> ingestion, suggesting that with further glucose ingestion, no further increase in oxidation efficiency occurred. The ratio-related maxima for fructose oxidation efficiency occurring at 0.67 g·min<sup>-1</sup> (0.8-Ratio), suggests a fructose absorption or metabolic synergism associated with reaching the exogenous-glucose oxidation maxima. Therefore, the current data suggests that the optima for fructose:maltodextrin ingestion ratio might lie within the ratio range ~0.81-0.98 (Fig. 9). This optima is consistent whether interpreted in absolute oxidation rate or in oxidation efficiency terms, and importantly, is physiologically robust, and is supported by previous findings for greater total exogenous-carbohydrate oxidation efficiency with a ratio of ~0.8 (Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011), and by the classic triple-lumen experiments of Shi *et al.* (1995).

In addition to intestinal mechanisms of fructose absorption, events at the liver might play a considerable role in fructose metabolism, and could at least partially explain the higher oxidation efficiency with the 0.8-Ratio solution. Although not quantified in the present investigation, oxidation of lactate cleaved in the liver from ingested fructose, might reveal some of the variations in overall exogenous-carbohydrate oxidation rates observed with the different ratio solutions. Lecoultre *et al.* (2010) examined the metabolic fate of fructose



**Figure 9.** Integrated model of oxidation rate and oxidation efficiency of exogenous fructose, exogenous glucose and total carbohydrate ingested in the three experimental fructose:glucose/maltodextrin ratios during endurance exercise. Point data are back log-transformed means and standard deviations. Curves are quadratics derived from a within-subject mixed model of the back log-transformed data.

coingested with glucose at a ratio of  $\sim 0.7$  during exercise, and found that  $\sim 29\%$  of the ingested fructose was released into the systemic circulation as glucose, and presumably subsequently oxidised in active skeletal muscle. Furthermore, enhanced oxidation of lactate derived from the ingested fructose provided an additional oxidative fuel for contracting muscle, and accounted for approximately half of the fructose oxidation (Lecoultré, Benoit *et al.* 2010). Similarly, Ahlborg and Björkman (1990) bypassed the intestinal absorption process during exercise by intravenously infusing fructose, and reported 78% of the fructose uptake was converted to lactate, pyruvate and glucose, while the remaining  $\sim 20\%$  of fructose was metabolised directly in working or resting muscle. Assuming intestinal absorption of fructose

is not limited by alterations in luminal fructose concentration, the effect of fructose:maltodextrin ratio on intestinal absorption will differentially affect hepatic metabolism of fructose by controlling concentration of this hexose in the portal vein. In the liver, following rapid and efficient extraction from portal blood, fructose is quickly phosphorylated to fructose 1-phosphate under the action of fructokinase (Tappy and Lê 2010). Subsequent conversion of fructose to glycogen, glucose, lactate and pyruvate for oxidation should determine the magnitude of ingested fructose stored (hepatic glycogen) or later released for metabolism in other tissues (Tappy and Lê 2010). Since it remains unknown if the rate of exogenous fructose oxidation is governed by intestinal absorption or by hepatic metabolism, specific investigation is warranted to better understand the effects of carbohydrate ingestion ratio on both the intestinal and hepatic metabolism as candidate sites for limitation of exogenous-fructose derived carbon. Ultimately, however, the site of metabolic limitation is of mainly academic interest in the face of a substantial performance benefit arising from the 0.8 fructose:maltodextrin ratio solution.

In the current study, gastrointestinal comfort was largely unaffected by solution composition during the 2-h ride. The absence of any clear treatment differences could be the result of less residual carbohydrate remaining in the gut compared with the previous study where carbohydrate was ingested at  $1.8 \text{ g}\cdot\text{min}^{-1}$  (O'Brien and Rowlands 2011), therefore removing gut comfort as a likely mechanism to explain the performance outcome. Similarly, severe gastrointestinal distress reported with ingestion of large quantities ( $2.4 \text{ g}\cdot\text{min}^{-1}$ ) of single-carbohydrate solutions (Jentjens, Achten *et al.* 2004; Triplett, Doyle *et al.* 2010) was likely also due to reduced gastric emptying and increased distension from high fluid secretion (Rehrer, Wagenmakers *et al.* 1992). Thorburn *et al.* (2006) reported a moderate negative statistical relationship between gastrointestinal discomfort and performance, and O'Brien and Rowlands (2011) reported a positive relationship between gut comfort and performance. However, in the present repeated maximal-sprint performance test, the 0.8-Ratio condition resulted in the highest increase in abdominal cramping, albeit extremely mild, but was also most beneficial to performance. It is suggested that, rather than being causal to reduced performance, larger increases in gastrointestinal discomfort with the 0.8-Ratio solution may have been the product of the substantially greater physical exertion afforded via metabolic or other mechanisms associated with the higher exogenous-carbohydrate oxidation rate. Indeed, Rowlands *et al.* (2011) recently proposed that the association between increased sprint power

and reduced gastrointestinal comfort in a laboratory mountain-bike race simulation was secondary to the apparent advantageous physiological consequences of multiple-transportable carbohydrate supplementation. Therefore, the metabolic or other effects of the 0.8-Ratio solution enabled higher work output in order to achieve increased power at maximal effort (repeated-sprint test), thus increasing the occurrence of gastrointestinal discomfort.

A brief comment is warranted regarding the unclear differences in fluid absorption, especially in light of the 6-13% variation in exogenous-carbohydrate oxidation rates between the carbohydrate conditions. Given the strong correlation between duodenojejunal fluid and carbohydrate absorption with multiple carbohydrate solutions (Shi, Summers *et al.* 1995; Shi and Passe 2010), faster fluid absorption will likely be promoted with more rapid carbohydrate transport. Assuming relative variances in measured oxidation rates are indicative of comparative differences in glucose transport, it was hypothesised that higher exogenous-carbohydrate flux would increase osmotic fluid absorption. However, the inconclusive results might be explained simply as excessive noise introduced by multiple pipetting steps in D<sub>2</sub>O blood accumulation analysis, or insufficient sensitivity (too low tracer:trace ratio), thus clouding any actual difference. Alternatively, the effect of carbohydrate ratio on gut absorption could in fact be trivial, and therefore the site for the mechanism of action to explain the increased exogenous-carbohydrate oxidation rate with 0.8-Ratio ingestion could be due to undefined metabolic events in the liver.

A major aim of the current study was to confirm the effect of the 0.8 fructose:maltodextrin ratio on endurance performance. Importantly, an average 3% moderate enhancement of mean power output and an accompanying attenuation in fatigue were observed during the repeated-sprint performance test and resulted from ingestion of the 0.8-Ratio solution. Notwithstanding that uncertainty in the mean power outcome allowed for trivial to very large effects, the 0.8-vs. 0.5-Ratio comparison was in line with earlier findings (O'Brien and Rowlands 2011) in which performance peak-power in a slow-ramp incremental test was 3.6% ( $\pm 3.5\%$ ) higher with a 0.8 ratio fructose:maltodextrin solution relative to a 0.5 ratio solution. In addition, the fatigue attenuation outcome (i.e., slope in the sprint test) with the 0.8-Ratio condition was consistent with the first study investigating carbohydrate ratios (Rowlands, Thorburn *et al.* 2008). Although the current 3% performance enhancement was substantially lower than the ~8% improvement in ~1-h and 100-k time trial performance reported in other coingestion

studies (Currell and Jeukendrup 2008; Triplett, Doyle *et al.* 2010), these latter results might rather be evidence of performance decrements caused by slower intestinal absorption and increased gastrointestinal distress from ingestion of large quantities of the impractical 14.5% glucose-only control solutions, rather than representative of the true magnitude of the performance improvements *per se*.

In the current study, with the influences of osmolality and carbohydrate concentration removed, previous carbohydrate ingestion ratios (i.e., 0.5, 0.8 and 1.25) were maintained, and at a fixed carbohydrate ingestion rate ( $1.5 \text{ g}\cdot\text{min}^{-1}$ ) the effect of ratio on absorption at the gut and consequent oxidation was determined. Previously (Rowlands, Thorburn *et al.* 2008), maltodextrin ingestion rate was clamped at  $0.6 \text{ g}\cdot\text{min}^{-1}$ , while manipulation of fructose ingestion quantity and total carbohydrate ingestion rate ( $0.9\text{-}1.3 \text{ g}\cdot\text{min}^{-1}$ ) achieved ratios similar to those in the present study. In a follow up study (O'Brien and Rowlands 2011) using the current ratios, osmolality was variable, but ingestion rate was fixed at  $1.8 \text{ g}\cdot\text{min}^{-1}$ . Consistent with the current findings, the highest exogenous-carbohydrate oxidation rate and greatest performance enhancement were reported with the 0.8-ratio solutions. Therefore, the present and previous data (Rowlands, Thorburn *et al.* 2008; O'Brien and Rowlands 2011) suggest that the fructose content of a carbohydrate-electrolyte solution for sports use might play a key role in performance enhancement, and that a ratio of around 0.8 fructose co-ingested with maltodextrin/glucose may improve performance compared to a carbohydrate solution with lower fructose content, or none at all. Additionally, the robustness of fructose and maltodextrin co-ingested at a ratio of 0.8 is confirmed across a wide range of carbohydrate ingestion rates, both above and below the average exogenous-carbohydrate ingestion-oxidation maxima. However, contrary to this confirmation for a 0.8 ratio, Vandenberg and Hopkins (2011) suggested in a recent meta-analysis of carbohydrate supplementation and endurance performance, that a ratio of only  $\sim 0.28$  fructose:glucose polymer plus protein inferred the greatest benefit to endurance performance. This meta-analytical model, however, included only one (Currell and Jeukendrup 2008) of the four performance studies already cited to specifically investigate carbohydrate type, concentration and/or ratio on performance outcomes with coingested carbohydrates (Currell and Jeukendrup 2008; Rowlands, Thorburn *et al.* 2008; Triplett, Doyle *et al.* 2010; O'Brien and Rowlands 2011).



The validated repeated-sprint test was chosen to measure performance outcomes as it is considered a reliable (Rowlands, Thorp *et al.* 2007) and valid (Rowlands, Swift *et al.* 2011) simulation of competition cycling performance. Furthermore, the intensity of the 2-h preload (57.5%  $W_{\max}$ ) was increased from previous studies in order to better reflect competition intensity. In a well designed two-way crossover study utilising the repeated-sprint test, Rowlands *et al.* (2011) determined that performance enhancements observed in lab-based performance tests translated to competitive mountain-bike racing. The design ensured athlete exposure to all the competitive and environmental pressures usually encountered during racing, but with well controlled preparation. Similar magnitude and direction of drink differential during the mountain-bike race, and differences in race time and sprint mean power in the laboratory test, were observed. Therefore it is likely that the 3% enhancement in performance with the current 0.8-Ratio solution, might also apply to competitive cycling performance.

Previously, Rowlands *et al.* (2008) observed an effect of drink sweetness and palatability, which might positively affect performance via the activation of brain mechanisms and brain centres associated with reward and motivation (Chambers, Bridge *et al.* 2009). In the present study though, there was no sweetness difference due to equal fructose/glucose:maltodextrin ratio between solutions. Therefore, the current study outcomes suggest performance might not be substantially influenced by drink sweetness, although a carefully designed study is required to verify any impact of drink taste on performance.

## 5.1 Future Research

Since fructose absorption and the subsequent rate of oxidation seem to be governed by factors other than intestinal transporter capabilities, specific investigation is warranted to better understand the effects of carbohydrate ingestion ratio on hepatic metabolism of fructose. Additionally, uncovering the exact mechanisms regulating fructose absorption might clarify the effects of carbohydrate ratio on fluid absorption rates, and whether glucose and fructose are equally influential when coingested.

Despite no apparent impact of drink sweetness on measured outcomes, sweetness and other taste perceptions have been linked to brain centres associated with reward and motivation. Therefore, further investigation into the effect of drink taste on performance is warranted.

## **6 Conclusion**

To conclude, we report substantially higher exogenous-fructose oxidation efficiency, exogenous-glucose oxidation rate and total exogenous-carbohydrate oxidation rate and efficiency with the ingestion of a solution comprising fructose and maltodextrin/glucose at a ratio of 0.8:1, relative to isocaloric, isosmotic 0.5- and 1.25-ratio solutions and water. Furthermore, we report a very likely moderate enhancement in mean sprint power during high-intensity endurance exercise. Therefore, oral energy-hydration formulations comprising fructose:maltodextrin at a ratio of around 0.8 may provide the most beneficial practical implications to endurance athletes.

## 7 References

- Adopo, E., F. Peronnet, et al. (1994). "Respective oxidation of exogenous glucose and fructose given in the same drink during exercise." Journal of Applied Physiology **76**(3): 1014-1019.
- Ahlborg, G. and O. Björkman (1990). "Splanchnic and muscle fructose metabolism during and after exercise." Journal of Applied Physiology **69**(4): 1244-51.
- Ahlborg, G. and P. Felig (1976). "Influence of glucose ingestion on fuel-hormone response during prolonged exercise." Journal of Applied Physiology **41**(5): 683-688.
- Bergstrom, J., L. Hermansen, et al. (1967). "Diet, muscle glycogen and physical performance." Acta Physiologica Scandinavica **71**(2): 140 - 50.
- Bergstrom, J. and E. Hultman (1967). "A study of the glycogen metabolism during exercise in man." Scandinavian Journal of Clinical & Laboratory Investigation **19**(3): 218-228.
- Blakemore, S., J. Aledo, et al. (1995). "The GLUT5 hexose transporter is also localized to the basolateral membrane of the human jejunum." Journal of Biochemistry **309**: 7-12.
- Bonetti, D. L. and W. G. Hopkins (2010). "Effects of hypotonic and isotonic sports drinks on endurance performance and physiology." Sportscience **14**: 63-70.
- Burelle, Y., M. C. Lamoureux, et al. (2006). "Comparison of exogenous glucose, fructose and galactose oxidation during exercise using <sup>13</sup>C-labelling." British Journal of Nutrition **96**(1): 56-61.
- Chambers, E. S., M. W. Bridge, et al. (2009). "Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity." The Journal of Physiology **587**(8): 1779-1794.
- Christensen, E. H. and O. Hansen (1939). "Zur methodik der respiratorischen quotientbestimmung in ruhe und bei arbeit. III: Arbeitsfähigkeit und ernahrung." Scand Arch Physiol **81**: 160-71.
- Coggan, A. R. and E. Coyle (1988). "Effect of carbohydrate feedings during high-intensity exercise." Journal of Applied Physiology **65**: 1703-1709.
- Coggan, A. R. and E. F. Coyle (1987). "Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion." Journal of Applied Physiology **63**: 2388-2395.
- Cohen, J. (1994). "The Earth is round ( $p < .05$ )." American Psychologist **49**(12): 997-1003.
- Costill, D. L. and B. Saltin (1974). "Factors limiting gastric-emptying during rest and exercise." Journal of Applied Physiology **37**(5): 679-683.

- Coyle, E. F. (1991). "Timing and method of increased carbohydrate intake to cope with heavy training, competition and recovery. [Review] [76 refs]." Journal of Sports Sciences: 29-51.
- Coyle, E. F., A. R. Coggan, et al. (1986). "Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate." Journal of Applied Physiology **61**(1): 165-72.
- Coyle, E. F., D. L. Costill, et al. (1978). "Gastric emptying rates for selected athletic drinks." Research Quarterly **49**(2): 119-24.
- Coyle, E. F., J. M. Hagberg, et al. (1983). "Carbohydrate feeding during prolonged strenuous exercise can delay fatigue." Journal of Applied Physiology **55**(1): 230-235.
- Coyle, E. F. and S. J. Montain (1992). "Benefits of fluid replacement with carbohydrate during exercise." Medicine & Science in Sports & Exercise **24**(9): S324-S330.
- Craig, H. (1957). "Isotopic standards for carbon and oxygen and correction factors." Geochimica et Cosmochimica Acta **12**: 133-149.
- Currell, K. and A. E. Jeukendrup (2008). "Superior endurance performance with ingestion of multiple transportable carbohydrates." Medicine and Science in Sports and Exercise **40**(2): 275-281.
- Davis, J. M., W. A. Burgess, et al. (1990). "Fluid availability of sports drinks differing in carbohydrate type and concentration." American Journal of Clinical Nutrition **51**(6): 1054-1057.
- Dill, D. B., H. T. Edwards, et al. (1932). "Studies in muscular activity: VII. Factors limiting the capacity for work." The Journal of Physiology **77**(1): 49-62.
- Duchman, S. M., A. J. Ryan, et al. (1997). "Upper limit for intestinal absorption of a dilute glucose solution in men at rest." Medicine & Science in Sports & Exercise **29**(4 (Apr)): 482-488.
- Fordtran, J. S. and B. Saltin (1967). "Gastric emptying and intestinal absorption during prolonged severe exercise." Journal of Applied Physiology **23**(3): 331-335.
- Gisolfi, C. V. and S. M. Duchman (1992). "Guidelines for optimal replacement beverages for different athletic events." Medicine & Science in Sports & Exercise **24**(6): 679-687.
- Gisolfi, C. V., G. P. Lambert, et al. (2001). "Intestinal fluid absorption during exercise: role of sport drink osmolality and [Na<sup>+</sup>]." Medicine & Science in Sports & Exercise **33**(6): 907-915.

- Gisolfi, C. V., R. W. Summers, et al. (1998). "Effect of beverage osmolality on intestinal fluid absorption during exercise." Journal of Applied Physiology **85**(5): 1941-8.
- Gordon, B., L. A. Kohn, et al. (1925). "Sugar content of the blood in runners following a marathon race." Journal of the American Medical Association **85**(7): 508-509.
- Hawley, J. A., S. C. Dennis, et al. (1992). "Oxidation of carbohydrate ingested during prolonged endurance exercise." Sports Medicine **14**(1): 27-42.
- Hawley, J. A., S. C. Dennis, et al. (1992). "Exogenous carbohydrate oxidation from maltose and glucose ingested during prolonged exercise." European Journal of Applied Physiology **64**(6): 523-7.
- Hopkins, W. G., J. A. Hawley, et al. (1999). "Design and analysis of research on sport performance enhancement." Medicine & Science in Sports & Exercise **31**(3): 472-485.
- Hopkins, W. G., S. W. Marshall, et al. (2009). "Progressive statistics for studies in sports medicine and exercise science." Medicine & Science in Sports & Exercise **41**(1): 3-13.
- Hulston, C. J., G. A. Wallis, et al. (2009). "Exogenous CHO oxidation with glucose plus fructose intake during exercise." Medicine & Science in Sports & Exercise **41**(2): 357-363 10.1249/MSS.0b013e3181857ee6.
- International Commission on Radiological Protection (ICRP) (1991). "Radiological Protection in Biomedical Research." Annals of the ICRP **22**(3).
- Jentjens, R. L., J. Achten, et al. (2004). "High oxidation rates from combined carbohydrates ingested during exercise." Medicine & Science in Sports & Exercise **36**(9): 1551-8.
- Jentjens, R. L. and A. E. Jeukendrup (2005). "High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise." British Journal of Nutrition **93**(4): 485-92.
- Jentjens, R. L., L. Moseley, et al. (2004). "Oxidation of combined ingestion of glucose and fructose during exercise." Journal of Applied Physiology **96**(4): 1277-1284.
- Jentjens, R. L., K. Underwood, et al. (2006). "Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat." Journal of Applied Physiology **100**(3): 807-816.
- Jentjens, R. L., M. C. Venables, et al. (2004). "Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise." Journal of Applied Physiology **96**(4): 1285-1291.
- Jeukendrup, A. E. (2004). "Carbohydrate intake during exercise and performance." Nutrition **20**(7-8): 669-77.

- Jeukendrup, A. E. (2010). "Carbohydrate and exercise performance: the role of multiple transportable carbohydrates." Current Opinion in Clinical Nutrition & Metabolic Care **13**(4): 452-457 10.1097/MCO.0b013e328339de9f.
- Jeukendrup, A. E., K. Currell, et al. (2009). "Effect of beverage glucose and sodium content on fluid delivery." Nutrition & Metabolism **6**(9).
- Jeukendrup, A. E. and R. L. Jentjens (2000). "Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research." Sports Medicine **29**(6): 407-24.
- Jeukendrup, A. E. and L. Moseley (2010). "Multiple transportable carbohydrates enhance gastric emptying and fluid delivery." Scandinavian Journal of Medicine & Science in Sports **20**(1): 112-121.
- Jeukendrup, A. E. and G. A. Wallis (2005). "Measurement of substrate oxidation during exercise by means of gas exchange measurements." International Journal of Sports Medicine **26**(Suppl. 1): S1-S10.
- Jones, H. F., R. N. Butler, et al. (2011). "Intestinal fructose transport and malabsorption in humans." American Journal of Physiology - Gastrointestinal and Liver Physiology **300**(2): G202-G206.
- Karelis, A. D., J. W. Smith, et al. (2010). "Carbohydrate administration and exercise performance: What are the potential mechanisms involved?" Sports Medicine **40**(9): 747-763.
- Kellett, G. L., E. Brot-Laroche, et al. (2008). "Sugar absorption in the intestine: The role of GLUT2." Annual Review of Nutrition **28**: 35-54.
- Lambert, C. P., D. Ball, et al. (1999). "The use of a deuterium tracer technique to follow the fate of fluids ingested by human subjects: Effects of drink volume and tracer concentration and content." Experimental Physiology **84**(2): 391-399.
- Lambert, G. P., R. T. Chang, et al. (1997). "Absorption from different intestinal segments during exercise." Journal of Applied Physiology **83**(1): 204-212.
- Lecoultre, V., R. Benoit, et al. (2010). "Fructose and glucose co-ingestion during prolonged exercise increases lactate and glucose fluxes and oxidation compared with an equimolar intake of glucose." The American Journal of Clinical Nutrition **92**(5): 1071-1079.
- Lefebvre, P. (1985). "From plant physiology to human metabolic investigations." Diabetologia **28**(5): 255-263.

- Levine, S. A., B. Gordon, et al. (1924). "Some changes in the chemical constituents of the blood following a marathon race." Journal of the American Medical Association **82**(22): 1778-1779.
- Loo, D. D., T. Zeuthen, et al. (1996). "Cotransport of water by the Na<sup>+</sup>/glucose cotransporter." Proceedings of the National Academy of Sciences **93**(23): 13367-13370.
- Loo, D. D. F., E. M. Wright, et al. (2002). "Water pumps." The Journal of Physiology **542**(1): 53-60.
- Ma, T., A. S. Verkman, et al. (1999). "Aquaporin water channels in gastrointestinal physiology." Journal of Physiology **517**(Pt 2): 317-26.
- Massicotte, D., F. Peronnet, et al. (1986). "Metabolic response to [13C]glucose and [13C]fructose ingestion during exercise." Journal of Applied Physiology **61**(3): 1180-4.
- Massicotte, D., F. Peronnet, et al. (1989). "Oxidation of a glucose polymer during exercise: comparison with glucose and fructose." Journal of Applied Physiology **66**: 179-183.
- Maughan, R. J. (1991). "Fluid and electrolyte loss and replacement in exercise." Journal of Sports Sciences **9**: 117-142.
- Murray, R., W. Bartoli, et al. (1999). "A comparison of the gastric emptying characteristics of selected sports drinks." International Journal of Sport Nutrition **9**(3): 263-74.
- Murray, R., G. L. Paul, et al. (1989). "The effects of glucose, fructose, and sucrose ingestion during exercise." Medicine & Science in Sports & Exercise **21**(3): 275-282.
- Nelson, A. R., S. M. Phillips, et al. (2011). "A protein-leucine supplement increases BCAA and nitrogen turnover but not performance." Medicine & Science in Sports & Exercise **Publish Ahead of Print**: 10.1249/MSS.0b013e3182290371.
- O'Brien, W. J. and D. S. Rowlands (2011). "Fructose-maltodextrin ratio in a carbohydrate-electrolyte solution differentially affects exogenous carbohydrate oxidation rate, gut comfort, and performance." American Journal of Physiology - Gastrointestinal and Liver Physiology **300**(1): G181-G189.
- Perri, F., M. R. Pastore, et al. (2005). "13C-octanoic acid breath test for measuring gastric emptying of solids." European Review for Medical and Pharmacological Sciences **9**: 3-8.



- Rehrer, N. J., A. J. M. Wagenmakers, et al. (1992). "Gastric emptying, absorption and carbohydrate oxidation during prolonged exercise." Journal of Applied Physiology **72**(2): 468-475.
- Richardson, R. B., D. W. Dunford, et al. (2001). "Influence of gender differences in the carbon pool on dose factors for intakes of tritium and <sup>14</sup>C-labeled compounds." Health Physics **81**(3): 302-12.
- Robert, J. J., J. Koziol, et al. (1987). "Use of <sup>13</sup>C-labeled glucose for estimating glucose oxidation: some design considerations." Journal of Applied Physiology **63**(5): 1725-1732.
- Rogers, J., R. W. Summers, et al. (2005). "Gastric emptying and intestinal absorption of a low-carbohydrate sport drink during exercise." International Journal of Sport Nutrition and Exercise Metabolism **15**(3): 220-35.
- Rolston, D. D. and V. I. Mathan (1990). "Jejunal and ileal glucose-stimulated water and sodium absorption in tropical enteropathy: Implications for oral rehydration therapy." Digestion **46**(1): 55-60.
- Rowlands, D. S. and J. Clarke (2011). "Lower oxidation of a high molecular weight glucose polymer vs. glucose during cycling." Applied Physiology, Nutrition, and Metabolism **36**(2): 298-306.
- Rowlands, D. S., M. Swift, et al. (2011). "The magnitude of enhancement of a composite versus single transportable carbohydrate solution on race and laboratory cycling performance is influenced by gut comfort." Applied Physiology, Nutrition, and Metabolism **in review**.
- Rowlands, D. S., M. S. Thorburn, et al. (2008). "Effect of graded fructose coingestion with maltodextrin on exogenous <sup>14</sup>C-fructose and <sup>13</sup>C-glucose oxidation efficiency and high-intensity cycling performance." Journal of Applied Physiology **104**: 1709-1719.
- Rowlands, D. S., R. M. Thorp, et al. (2007). "Effect of protein-rich feeding on recovery following intense exercise." International Journal of Sport Nutrition and Exercise Metabolism **17**: 521-543.
- Rumessen, J. J. and E. Gudmand-Høyer (1986). "Absorption capacity of fructose in healthy adults: Comparison with sucrose and its constituent monosaccharides." Gut **27**(10): 1161-1168.



- Ruzzin, J., F. Peronnet, et al. (2003). "Breath [ $^{13}\text{CO}_2$ ] recovery from an oral glucose load during exercise: comparison between [U- $^{13}\text{C}$ ] and [1,2- $^{13}\text{C}$ ]glucose." Journal of Applied Physiology **95**(2): 477-482.
- Sawka, M. N., S. J. Montain, et al. (2001). "Hydration effects on thermoregulation and performance in the heat." Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology **128**(4): 679-690.
- Sawka, M. N. and T. D. Noakes (2007). "Does dehydration impair exercise performance?" Medicine and Science in Sports and Exercise **39**(8): 1209-1217.
- Shi, X., M. K. Horn, et al. (2004). "Gastrointestinal discomfort during intermittent high-intensity exercise: effect of carbohydrate-electrolyte beverage." International Journal of Sport Nutrition & Exercise Metabolism **14**(6): 673-83.
- Shi, X. and D. H. Passe (2010). "Water and solute absorption from carbohydrate-electrolyte solutions in the human proximal small intestine: A review and statistical analysis." International Journal of Sport Nutrition and Exercise Metabolism **20**(5): 427-442.
- Shi, X., H. P. Schedl, et al. (1997). "Fructose transport mechanisms in humans." Gastroenterology **113**(4): 1171-1179.
- Shi, X., R. W. Summers, et al. (1994). "Effects of solution osmolality on absorption of select fluid replacement solutions in human duodenojejunum." Journal of Applied Physiology **77**(3): 1178-1184.
- Shi, X., R. W. Summers, et al. (1995). "Effects of carbohydrate type and concentration and solution osmolality on water absorption." Medicine & Science in Sports & Exercise **27**: 1607-1615.
- Smith, J. W., J. J. Zachwieja, et al. (2010). "Fuel selection and cycling endurance performance with ingestion of [ $^{13}\text{C}$ ]glucose: evidence for a carbohydrate dose response." Journal of Applied Physiology **108**(6): 1520-1529.
- Sterne, J. A. C. and G. D. Smith (2001). "Sifting the evidence-what's wrong with significance tests?" British Medical Journal **322**: 226-261.
- Tappy, L. and K. Lê (2010). "Metabolic Effects of Fructose and the Worldwide Increase in Obesity." Physiological Reviews **90**(1): 23-46.
- Thomson, A. B. R., M. Keelan, et al. (2001). "Small bowel review: Normal physiology part 1." Digestive Diseases and Sciences **46**(12): 2567-2587.

- Thorburn, M. S., B. Vistisen, et al. (2006). "Attenuated gastric tolerance but no benefit to performance with adaptation to octanoate-rich esterified oils in well-trained male cyclists." Journal of Applied Physiology **101**: 1733-1743.
- Triplett, D., J. A. Doyle, et al. (2010). "An isocaloric glucose-fructose beverage's effect on simulated 100-km cycling performance compared with a glucose-only beverage." International Journal of Sport Nutrition and Exercise Metabolism **20**(2): 122-131.
- Vandenbogaerde, T. J. and W. G. Hopkins (2011). "Effects of acute carbohydrate supplementation on endurance performance: A meta-analysis." Sports Medicine **41**(9): 773-92.
- Vist, G. E. and R. J. Maughan (1995). "The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man." Journal of Physiology(486): 523-531.
- Wagenmakers, A. J., N. J. Rehrer, et al. (1993). "Breath <sup>13</sup>CO<sub>2</sub> background enrichment during exercise: diet-related differences between Europe and America." Journal of Applied Physiology **74**(5): 2353-7.
- Wallis, G. A., D. S. Rowlands, et al. (2005). "Oxidation of combined ingestion of maltodextrins and fructose during exercise." Medicine & Science in Sports & Exercise **37**(3): 426-432.
- Wright, E. M., G. M. Martin, et al. (2003). "Intestinal absorption in health and disease - sugars." Best Practice and Research in Clinical Gastroenterology **17**: 943-956.

## Appendix A – Recruitment Email and Flyer

---



**Massey University**

Institute of  
Food Nutrition & Human Health



Te Kunenga  
ki Pūrehuroa

### **Massey researchers seek cyclists for carbohydrate oxidation and absorption study**

Road cyclists, mountain bikers, and triathletes are invited to participate in a research project investigating the ratio of carbohydrates (fructose:maltodextrin) in sports drink formulations that provides optimal intestinal absorption of fluids and carbohydrates.

Recent studies have found that sports drink containing a combination of fructose and maltodextrin have performance benefits over single carbohydrate drinks. We now wish to determine what ratio of these carbohydrates provides the best absorption in the intestine.

The study will be run in the Sport Science Lab at Massey University in Wellington over the next 3 months and consists of several rides (VO<sub>2</sub>max test, familiarisation trial, four testing rides) over 4 consecutive weeks. Testing rides will be in the early morning, but most other rides can be completed at a time that suits.

To participate in the study you should be male, aged 18 to 50 years, and in regular training.

You will learn your VO<sub>2</sub>max and peak power output.

If you are interested in participating or would like some more information, please contact:

Wendy O'Brien

Email: [wendy.obrien01@gmail.com](mailto:wendy.obrien01@gmail.com)

Phone: 027 276 7796

Or

Dr David Rowlands

Email: [d.s.rowlands@massey.ac.nz](mailto:d.s.rowlands@massey.ac.nz)

Phone: 801 5799 extension 6940 (wk)  
021 2099 383



## Appendix B – Information Sheet



**Massey University**

Institute of  
Food Nutrition & Human Health



Te Kunenga  
ki Pūrehuroa

### ***What ratio of fructose:maltodextrin co-ingestion results in the highest exogenous-carbohydrate oxidation rates and fluid absorption during endurance exercise?***

#### **INFORMATION SHEET**

##### **The Institute of Food, Nutrition and Human Health**

The Institute of Food, Nutrition and Human Health is part of Massey University. It includes a large team of lecturers and scientists that are interested in human nutrition, sport and exercise science, physiology, and health. The researchers in this project are Wendy O'Brien and Dr David Rowlands (head researcher). David is a senior lecturer in sport and exercise science at the Wellington campus and has research interests in the role of diet and training interventions on physiological and performance responses in athletes. Wendy is conducting this research for her Masters in Science thesis.

##### **Why Are We Doing This Study?**

Carbohydrate (e.g. sucrose – table sugar, glucose, maltodextrins – long chains of glucose) ingestion during exercise can enhance race-like performance tasks of ~45 min or longer. Sport scientists think benefit to performance occurs by increasing energy supply to the muscle, maintaining blood sugar supply to the brain, or by some yet-to-be defined psycho-biological effect. By avoiding dehydration during endurance exercise, performance can be maintained, as even low (~2%) levels of dehydration can result in performance decrement. Such findings have contributed to the widespread consumption of carbohydrate-rich drinks during competition, active physical recreation, and by others.

Increasing the carbohydrate intake during exercise will increase the amount of energy supplied to the muscle. However, it is now clear that the maximal rate that glucose or maltodextrins can be burnt at is about  $1.0 \text{ g} \cdot \text{min}^{-1}$ . The rate at which carbohydrate can be absorbed by the intestine is thought to be the major limitation to exogenous-carbohydrate oxidation during exercise. Therefore, ingesting carbohydrate at much more than this rate is unlikely to supply more energy to the muscles, with the extra carbohydrate being left in the stomach or intestine. In recent studies though, we have found that adding the fruit sugar fructose to maltodextrin in an exercise drink can increase the amount of energy supplied to the muscle 1.5 to 1.9-fold. Fructose is absorbed by the intestine differently to glucose, so by adding fructose to maltodextrin we think we can increase energy supply to the muscle. What we don't know yet however is what the optimal ingestion rate of fructose to maltodextrin is to maximise energy supply and fluid absorption, whilst minimising any gastric upsets, nor do we know what happens to your stored energy (glycogen). Consequently, the main purpose of this study is to determine the optimal rate of fructose and maltodextrin to provide maximum intestinal absorption of both fluid and carbohydrate, and what the associated effects are on the gastro-intestinal comfort, perceived effort, and prolonged high-intensity endurance performance.

##### ***Aims in Scientific Terms***

In this research we aim to determine the effect of the ingestion of three rates of fructose and maltodextrin on:

1. *total exogenous-carbohydrate and glycogen oxidation rate*
2. *fluid absorption rate*
3. *gastro-intestinal discomfort and perceived exertion*
4. *high-intensity cycling performance*

### Participant Recruitment

We would like to recruit 12 male cyclists, mountain bikers or triathletes. To participate in the study you should:

- be in regular training and be aged 18 to 50 years. Regular being defined as 8 or more hours of cycling per week for the last year or greater.
- have been cycle racing regularly or been exposed to regular high-intensity training. This is important because of the nature of the performance rides.
- have a measured  $\text{VO}_{2\text{max}}$  of at least  $55 \text{ ml} \cdot (\text{kg min})^{-1}$ .

Additional cyclists may be recruited to boost the statistical power if required.

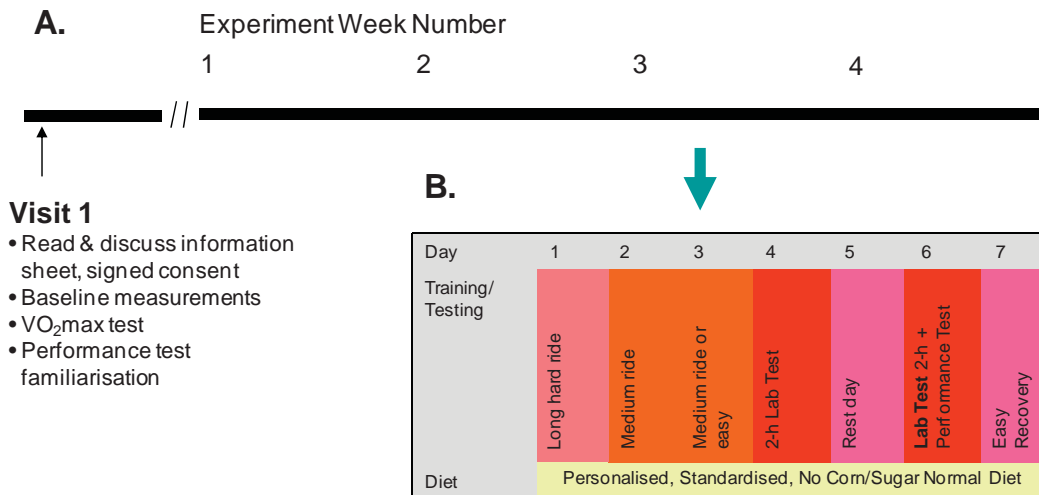
### What is involved?

If you decide to participate in our study, you will make a total of nine visits to the laboratory over about five weeks. The study design is termed a *randomized double-blind four-way cross-over*. That is, there are four experimental conditions (see below), presented to you in randomized (randomly assigned order of drink conditions) and blind fashion (you don't know what drink condition you are on) conducted over four weekly blocks. In addition, there is an initial trial for baseline fitness ( $\text{VO}_{2\text{max}}$  and peak power) and familiarisation. The study design is illustrated in Figure 1.

### Experimental Conditions

During the four experimental tests on Day 6, the following carbohydrate solutions will be ingested

1. 800 ml water per h
2. 30g ( $0.5 \text{ g} \cdot \text{min}^{-1}$ ) fructose + 60g ( $1.0 \text{ g} \cdot \text{min}^{-1}$ ) maltodextrin in 800 ml solution per h
3. 40g ( $0.67 \text{ g} \cdot \text{min}^{-1}$ ) fructose + 50g ( $0.83 \text{ g} \cdot \text{min}^{-1}$ ) maltodextrin in 800 ml solution per h
4. 50g ( $0.83 \text{ g} \cdot \text{min}^{-1}$ ) fructose + 40g ( $0.67 \text{ g} \cdot \text{min}^{-1}$ ) maltodextrin in 800 ml solution per h



**Figure 1.** Study design. **A.** Time course showing Visit 1 and the subsequent 4-week experimental block. **B.** Experimental components and tests per one weekly experimental block.

### Details

- **Visit 1.** On the first visit to the lab we will explain the study, answer any questions, and sign forms. If you are happy to continue at this time, we will then take some baseline measurements of body composition (weight, height), and conduct a test for  $\text{VO}_{2\text{max}}$  and peak power to establish baseline fitness. This ride will be followed with a practice ride to get familiar with the testing procedures and the performance test. Bring your riding shorts, top, shoes, and your bike or bike set-up measurements. We will set up the lab bikes to replicate your normal position on your bike. This visit will take about 3.5 h.

**Weekly Experimental Block.** The experiment is divided into four weekly blocks. Days 1-3 are standardised training sessions, Day 4 a 2 h medium-intensity (50% peak power) lab ride,



- Day 5 a rest day, Day 6 the main testing day, and Day 7 an easy training ride for recovery (Figure 1).
- **Day 4.** A 2 h moderate-intensity steady ride in the lab in warm conditions to simulate usual summer conditions. This ride can be completed at a prearranged time during the afternoon or evening that suits you. This visit will last about 2.25 h total.
- **Day 6.** This is the main testing day consisting of a 2 h moderate-intensity steady ride followed by the performance test (10 x maximal efforts and recovery) in warm conditions to simulate usual summer conditions. Tests will be conducted in the morning after an overnight fast, ie. no breakfast before you come in to the lab. A cannula will be placed in a suitable vein in your forearm for blood collection. Every 10-15 min during exercise, we will collect samples of breath and/or blood, and ask you to rate your perceived exertion, fatigue in the legs, and indicators of gastric distress, followed by the ingestion of a serve of the experimental carbohydrate solution. The breath and blood sampling will cease at the end of the 2 h ride. After this ride, you will get off the bike, we will remove the cannula, record your weight after you toilet, and then the performance test will begin. We will collect effort ratings at certain points during the tests and you will continue to ingest the experimental solutions at specified times. These visits will last about 4 h each.
- **Standardisation of Training.** In preparation for the study we would like you to *standardize your training program* from the week before the first weekly experimental block. What you have to do is simply maintain the same training sessions in terms of time and degree of effort on Days 1-3 and 7 of each proceeding week. A training log will be provided to help you. Training standardisation is to normalise the physical readiness before each performance test on Day 6.
- **Diet.** The day before the first experimental session (Day 5) we would like you to consume your normal diet and record it for replication preceding the testing in the following three weekly blocks. We will provide sheets for you to do this. The only change that you will need to make to your diet is the omission of certain foods that contain a small amount of the marker which we will add to the exercise drink to measure the amount of sugar being burnt. The foods that you may not eat from 1-week before the study are detailed in Appendix 1. Like replication of training, a standard diet is important to ensure similar muscle fuel (glycogen and fat) stores are present before testing performance. To help in standardising diet and pre-exercise fuel stores, a packaged meal and snack will be provided as the last meal the evening before the performance test.

### Data Management

All data and samples we collect during the course of the study will be stored in a lockable filing cabinet, or in cupboards or freezers only accessible to the research staff and lab technicians. Your name will not be recorded on any data sheets and sample containers, instead a subject code will be used to identify you. The list of allocated codes and names will be kept in a locked filing cabinet, and only the research staff will know your subject code. Data will be kept securely for a period of 5 years after the completion of the project, after which time it will be destroyed.

### Are any of the Procedures Harmful or Painful?

#### Blood Sampling

Cannula are small plastic tubes that are routinely placed into veins of participants in clinical research studies and in hospital patients. There is likely to be mild to moderate discomfort and small risk of bruising associated with the removal of a cannula. There are a total of four placements for the study, each on the morning before exercise on Day 6. The researchers are trained in cannula placement and the taking of blood samples via cannula. Approximately 50 ml of blood will be collected during each testing of the four sessions. The blood will be stored in a freezer for up to 36 months during which time biochemical analysis will be conducted on it.

#### D<sub>2</sub>O Labelled Water

The drink at one of the time points will contain a small amount of a marker which enables us to calculate how quickly the fluid from that drink is absorbed into the blood stream. The water will be labelled with a small amount of deuterium oxide (D<sub>2</sub>O), a stable isotope of hydrogen. The hydrogen and deuterium atoms mix, enabling movement of the water to be traced as it is absorbed by the body. We measure the rate of fluid absorption by how quickly D<sub>2</sub>O appears in blood samples.

#### <sup>13</sup>C and <sup>14</sup>C Markers

The maltodextrin and fructose in the drinks will contain very small amounts of markers which enable us to calculate, using highly sensitive laboratory equipment, how much of the ingested carbohydrates

are utilised for energy provision. The carbon (C) in the glucose chains in the maltodextrin will be labelled with a small amount of  $^{13}\text{C}$ , a stable isotope of carbon. The fructose will contain a small quantity of  $^{14}\text{C}$ -fructose.  $^{14}\text{C}$  is an isotope of carbon that lets go of some of its electrons and energy, hence it is termed radioactive. We measure the amount of glucose and fructose being metabolised (oxidised or burnt) during cycling by measuring the amount of  $^{13}\text{C}$  and  $^{14}\text{C}$  produced. This is done by collecting a sample of the carbon dioxide ( $\text{CO}_2$ ) produced by the muscles during exercise; the  $\text{CO}_2$  is expired in your breath, which we will collect during the experiment.

A unit used to measure the degree of radioactivity employed for medical, geological, or research purposes is the sievert. The maximum level of radioactivity to which you will be exposed is 0.39 millisieverts (mSv), which is similar to the range normally used in medical diagnostics and is classed as having a minor to intermediate level of risk. To place in perspective, other normal common exposures to radioactivity are shown in Table 1.

**Table 1.** Approximate effective doses from exposure to common environmental and medical radiation

Radiation Source	Effective dose (mSv)
Medical Diagnostic <sup>1</sup>	
Dental x-rays	0.01
Chest x-ray	0.02-0.08
Mammogram	0.25
Barium-enema x-ray	3.0
Chest CT scan	6-20
Cosmic rays	
Long-haul flight from Auckland to London <sup>2</sup>	0.05

Sources. <sup>1</sup> Cameron, J. A radiation unit for the public, University of Wisconsin – Madison. [jrcamero@facstaff.wisc.edu](mailto:jrcamero@facstaff.wisc.edu). <sup>2</sup> NRL, The exposure of NZ aircrew to cosmic radiation. IS19, 1998.

The average annual radiation dose to the public of New Zealand is about 2.5 mSv per year which mostly occurs from natural (2.0 mSv per year) and medical (0.5 mSv per year) sources. All doses of radiation, including the low doses from x-rays, have the potential to initiate cancer. 50 mSv is, conservatively, the lowest dose at which there is any evidence of cancer being caused in adults. It is also the highest dose which is allowed by regulation in any one year of occupational exposure. Dose rates greater than 50 mSv/yr arise from natural background levels in several parts of the world but do not cause any discernible harm to local populations. The risks associated with the exposure to a low dose of radiation are generally extremely low, giving only a very slight increase in the probability of cancer occurring many years or decades after the radiation exposure, which is impossible to discern from the known probable causes of cancer including cigarette smoking, dietary factors, and sunlight, which contribute to the death rate by cancer of around 1:4 (Table 2). For example, the lifetime risk for an adult with normal life expectancy from an effective dose of 0.5 mSv in terms of the International Commission on Radiological Protection (ICRP 62) risk categorisations is classified as minor (in the order of 1:30,000).

The storage and preparation of the  $^{14}\text{C}$ -fructose will be carried out in accordance with the procedures of the Radiation Safety Manual, and under the supervision of Dr Kevin Smidt.

**Table 2.** Approximate lifetime risks from various causes in New Zealand

All cancers	230 per 1000
Motor vehicle accidents	16 per 1000
Accidental falls	5 per 1000
Homicide	2 per 1000
Drowning	2 per 1000
Fire	6 per 10,000
Accidental poisoning	2 per 10,000
One year's natural background radiation	2 per 10,000
Cycling (per 10 miles, UK) <sup>1</sup>	1 per 1,000,000

Source. National Radiation Lab, MOH document C3, P29. 1994;  
<sup>1</sup><http://iopscience.iop.org/0952-4746/22/1/308>



### Gastric Distress

In similar experiments of this type, riders have reported bloating-like symptoms, some belching, some stomach cramping and nausea, and the urge to urinate. In some individuals high doses of fructose can cause some diarrhoea. Typically about one in 8-10 cyclists may suffer from one or more of these symptoms during one or more of the trials. At any time we can pause the ride to allow you to toilet.

### Exercise

There is often some physical and psychological discomfort associated with heavy exercise.

Recent evidence has indicated that even among healthy populations of athletes who exercise strenuously and regularly, there is some risk of sudden death due to heart failure. Though rare, such cases can occur in people who may have an undiagnosed condition. If you have any reason to suspect that you may have a cardiovascular problem, we suggest that you see your physician and get an ECG before you agree to participate.

If you have any additional medical concerns associated with this project, please contact your GP, or discuss with the researcher.

### Time Commitment

Experiment Component	Time Commitment (h)
VO <sub>2</sub> max peak power test & lab familiarization	3.5 h
Diet diary	0.25 h
Day 4: 2 h ride in lab	4 x 2.25 h
Day 6: Main testing rides in lab	4 x 4 h
<b>Total</b>	<b>28.75 h</b>

### Benefits

You will learn your VO<sub>2</sub>max and peak power output. A follow up laboratory test (e.g. VO<sub>2</sub>max, lactate threshold) will be provided free of charge if desired, which is normally worth \$225. You will partake in some challenging performance tests and likely increase your fitness. During the lab sessions you will be free to discuss any nutrition and training queries with those conducting the research. You will receive a summary of the results once the final results are available.

### What if I Suffer a Personal Injury?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Injury Prevention, Rehabilitation and Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

### Participant's Rights

You are under no obligation to accept this invitation. If you decide to participate, at any time, you will have the right to:

- decline to answer any particular question;
- withdraw from the study at any time;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded;
- have any blood samples returned to you after they have been analysed or request that they are disposed of through the Hospital mortuary.

**If you are interested in taking part or have questions, then contact:**

Wendy O'Brien  
Institute of Food, Nutrition, and Human Health  
Massey University Wellington Campus  
Pvt Bag 756, 63 Wallace St  
Wellington, New Zealand  
+64 27 276 7796  
e-mail: [wendy.obrien01@gmail.com](mailto:wendy.obrien01@gmail.com)

Dr David Rowlands  
Institute of Food, Nutrition, and Human Health  
Massey University Wellington Campus  
Pvt Bag 756, 63 Wallace St  
Wellington, New Zealand  
+64 4 801 5799 ext 6940  
e-mail: [d.s.rowlands@massey.ac.nz](mailto:d.s.rowlands@massey.ac.nz)

This project has been reviewed and approved by the Central Regional Ethics Committee, Wellington Application MEC/10/05/049. If you have any concerns about the ethics of this research, please contact Claire Lindsay, telephone 04 496 2405.

The  $^{14}\text{C}$  isotope procedures were reviewed and ratified by the National Radiation Laboratory. Contact Tony Cotterill, [tony\\_cotterill@nrl.moh.govt.nz](mailto:tony_cotterill@nrl.moh.govt.nz) or Glenn Stirling, [glenn\\_stirling@nrl.govt.nz](mailto:glenn_stirling@nrl.govt.nz)

## Guidelines for avoiding maize and sugar in diet in order to lower background $^{13}\text{C}$ enrichment in body-carbohydrate pool

*Contamination of the Tracer Signal.* We will be measuring the oxidation of the ingested glucose by tracing the appearance of  $^{13}\text{C}$  atoms in the expired air. The  $^{13}\text{C}$  comes from  $^{13}\text{C}$ -labelled glucose you consume in the lab. The tracers are stable isotopes and completely safe (i.e. non-radioactive). Since some carbohydrate food products (maize and sugar) contain a high natural abundance of  $^{13}\text{C}$  in their glucose or starch, you will be asked to avoid eating these products from the week before the experiment. If you fail to do this, our results will be invalid and we will not be able to use your data.

The depletion ride 7 days before the exercise test will empty your body of any residual  $^{13}\text{C}$ -enriched carbohydrate that might be stored in your muscles after ingesting  $^{13}\text{C}$ -enriched carbohydrates, such as corn and sugar. When you follow the low  $^{13}\text{C}$ -carbohydrate diet (see below) your body-glycogen stores should be replaced with carbohydrate that contains only the low natural background level of  $^{13}\text{C}$ .

### *What can I eat?*

You **can eat** all **bread/pasta/rice/potato**, all **vegetables (except sweetcorn)**, all **meats**, **some sauces** that come in jars (but check for corn flour and cane sugar), many **milk and dairy** products, **honey**, some **non-sugar jams** (check the labels). In fact you'll be amazed how much food you can eat (and you'll eat a healthier diet too!).

### *What foods do I have to avoid?*

Because  $^{13}\text{C}$  occurs in high levels in maize (sweet corn) and sugar cane we need to ask you to avoid all products that may include these ingredients. Most processed and many convenience foods will contain these sugars.

The list includes (more extensive list below)

- Any type of commercial sports drink, bar or gel
- Coke and Pepsi, etc (but diet drinks are OK)
- Many fruit juices (no-sugar added, pure fruit juice is OK - check label)
- Cereals made with maize or added sugar (Cornflakes, sweetened muesli or any other cereals containing corn flour or sugar, ie. most breakfast cereals. Natural muesli sweetened with honey, or with no added sugar is OK)
- Sweetened breads (hot cross buns, teacakes, cakes etc)
- Confectionery (lollies, chocolate etc)
- Cane sugar (honey is OK; or we can provide you with a cane sugar alternative)
- Jelly
- Any refined products that you think may contain cane sugar, corn flour (often used in soups and sauces as a thickener) or maize!
- Most processed foods contain maize or sugar, and are therefore unsuitable.
- Fructose is usually no good either because most comes from the cheap source – high-fructose corn syrup
- Most maltodextrin comes from corn and is therefore not suitable

## Foods to Avoid

- \* Cornflour, corn starch, sugar
- \* Cereals - Cornflakes, Weetbix, instant porridge packets, Ricies, most muesli, sweet cereals, etc... they contain sugar.
- \* Breakfast shakes/replacements e.g. 'Up and Go'
- \* Tinned fruit in syrup
- \* Golden syrup, most maple syrup
- \* Spreads:
  - Marmite/Vegemite
  - some peanut butter (check the label, it may or may not contain sugar)
  - Jam, marmalade (unless sugar free)
  - Relish, chutney, pickle, etc...
- \* Drinks:
  - Coke, Sprite, Tonic water, Soda water with a twist, other soft drinks, etc ...except Diet
  - Milo, bournvita, 'Pams' choca, ovaltine, drinking chocolate
  - Some sports water e.g. 'Mizone', 'H<sub>2</sub>Go'
  - Artificially flavoured drinks e.g. 'e<sup>2</sup>', 'G force'
  - Juice concentrates, Ribena, tomato juice, powdered fruit drinks
  - Flavoured milk, milkshakes
- \* Herbs and spices
  - 'Masterfoods' jars of fresh garlic, chilli ...etc...
  - 'Pams' chilli, ginger
  - 'Greggs' chilli, curries, coriander
  - 'Continental' curries ...etc...
- \* All mustards
- \* Tinned beans:
  - Chilli, Mexican, chick peas, kidney beans, etc (although there are some that don't contain sugar)
- \* Tinned spaghetti and baked beans
- \* Dips (salsa, guacamole, French onion, sundried tomato, sour cream-based ...etc...)
- \* Corn chips (including grain waves!), tortillas, most flavoured potato chips
- \* Tinned/jar/packet sauces
- \* All instant, flavoured pasta, rice and noodle snacks/meals
- \* Tomato puree, sauce, ketchup, chutney
- \* All tinned and packet (dried or fresh) soups
- \* All mayonnaise and salad dressings
- \* Most stocks (wet or dry) i.e. chicken, beef, vegetable ...
- \* Most soy sauces, worcestershire sauce
- \* Some frozen chips and wedges (corn starch/flour in the seasoning)
- \* Crumbed or battered products
  - Fish cakes, fingers, burgers, nuggets
  - Chicken fingers, burgers, nuggets
  - Lasagne toppas...etc
- \* Frozen dinners, including pizzas and pies!
- \* Sweet pastry
- \* Muesli bars, chocolate bars, yoghurt bars, cereal bars
- \* Crackers:
  - 'Real foods' corn thins
  - 'Le Snacks'
  - Rice crackers (e.g. 'Pams', 'Trident')
  - Meal mates
  - 'Huntley and Palmers' litebread
  - 'Arnotts' corn or original cruskits
  - 'Weight Watchers' crispbread
- \* 'Eta' roasted peanut items
- \* Flavoured milk, custard, ice cream (including lite), creamed rice, jelly, instant/packet desserts
- \* All yoghurts except unsweetened acidophilus and some honey sweetened (but check label), EasiYo natural yoghurt is OK and can be sweetened with honey
- \* Specialty breads e.g. bagels, sweet breads. Check the label of all breads

To be sure, simply check the "INGREDIENTS" list on the packet and avoid any products containing the words **sugar, maize, corn, sucrose, maltodextrin, fructose, dextrose, glucose**

## Appendix C – General Health Questionnaire

### Massey University Sport and Exercise Science

#### General Health Questionnaire

**Name:** .....

**Address:** .....

.....

.....

**Phone:** .....

**Emergency Contact:** .....

**Name of the investigator responsible for the study:**

.....

Please answer the following questions. This questionnaire has been designed to identify the small number of persons (15-69 years of age) for whom physical activity might be inappropriate and to provide the researchers with descriptive information about the participant. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed exercise is appropriate for you. Your answers will be kept strictly confidential.

1.	What is your date of birth? ..... (DD/MM/YY)  So your age is..... Years		
2.	Are you currently taking any medication?	YES	NO
3.	Has your GP ever advised you not to take vigorous exercise?	YES	NO
4.	Has your GP ever said you have "heart trouble"?	YES	NO
5.	Has your GP ever said you have high blood pressure?	YES	NO
6.	Have you ever taken medication for blood pressure or your heart?	YES	NO
7.	Do you feel pain in your chest when you undertake physical activity?	YES	NO

8.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
9.	Has your GP (or anyone else) said that you have raised blood cholesterol?	YES	NO
10.	Have you had a cold or feverish illness in the last month?	YES	NO
11.	Do you have any disorder of bleeding or clotting of the blood?	YES	NO
12.	Do you have any family history of any bleeding disorder or disorder of blood clotting?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14.	a) Do you suffer from back pain	YES	NO
	b) If yes, does it ever prevent you from exercising?	YES	NO
15.	Do you have moderate-severe liver or kidney disease?	YES	NO
16.	Do you suffer from asthma?	YES	NO
	If yes, do you control it with medication?	YES	NO
17.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
18.	a) Has your doctor ever said you have diabetes?	YES	NO
	b) Do you think you have diabetes?	YES	NO
19.	Do you suffer from any blood borne contagious diseases?	YES	NO
20.	a) Do you have any allergies?	YES	NO
	b) If yes, which ones? .....		
21.	Are you accustomed to vigorous exercise ( ~8h / week)?	YES	NO

**Diet Related Questions:**

22.	a) Do you have any food allergies? b) If yes, which ones? .....	YES	NO
23.	Are you Vegetarian?	YES	NO
24.	a) Do you eat milk, milk products and eggs? b) If no, what exactly do you not eat? .....	YES	NO
25.	Are you lactose-intolerant?	YES	NO
26.	Are there any fruits or vegetables you can't eat? If yes, which ones? .....	YES	NO
27.	Do you suffer from celiac disease?	YES	NO
28.	Are there any other foods you can't eat at all? If yes, what? .....	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

**Signed:** .....

**Date:** .....

## Appendix D – Consent Form

---



**Massey University**

Division of Exercise and Sport Sciences  
Institute of Food, Nutrition and Human Health  
Massey University Wellington

***What ratio of fructose:maltodextrin co-ingestion  
results in the highest exogenous-carbohydrate  
oxidation rates and greatest fluid absorption during  
endurance exercise?***

### PARTICIPANT CONSENT FORM - INDIVIDUAL

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

**Signature:** ..... **Date:** .....

**Full Name - printed** .....

This project has been reviewed and approved by the Central Regional Ethics Committee, Wellington Application MEC/10/05/049. If you have any concerns about the ethics of this research, please contact Claire Lindsay, telephone 04 496 2405.

The <sup>14</sup>C isotope procedures were reviewed and ratified by the National Radiation Laboratory. Contact Tony Cotterill, [tony\\_cotterill@nrl.moh.govt.nz](mailto:tony_cotterill@nrl.moh.govt.nz) or Glenn Stirling, [glenn\\_stirling@nrl.govt.nz](mailto:glenn_stirling@nrl.govt.nz)



## Appendix E – Ethics Committee Approval



### Multi-region Ethics Committee

Ministry of Health  
133 Molesworth Street  
PO Box 5013  
Wellington 6145  
Phone (04) 470 0655  
(04) 470 0646  
Fax (04) 496 2340

Email: [multiregion\\_ethicscommittee@moh.govt.nz](mailto:multiregion_ethicscommittee@moh.govt.nz)

31 May 2010

Mr David Rowlands  
Massey University  
Massey University Wellington  
63 Wallace Street  
P.O. Box 756  
Wellington

Dear David -

Re: Ethics ref: **MEC/10/05/049** (please quote in all correspondence)  
Study title: What ratio of fructose: maltodextrin co-ingestion results in the highest exogenous-carbohydrate oxidation rates and greatest fluid absorption during endurance exercise?  
Investigators: Mr David Rowlands, Ms Wendy O'Brien, Mr Andy Hollings, Mr Andre Nelson, Associate Professor Stephen Stannard  
Approved localities: Massey University and Victoria University – Wellington

This study was given ethical approval by the Multi-region Ethics Committee on 31<sup>st</sup> of May 2010.

#### Approved Documents

- Protocol number [...]
- Participant Information sheet version 1, dated 21<sup>st</sup> of April 2010
- Consent form version 1, dated 21<sup>st</sup> of April 2010
- Recruitment email, version 1, dated 21<sup>st</sup> of April 2010
- Health Screening Questionnaire,

This approval is valid until 31<sup>st</sup> of May 2012, provided that Annual Progress Reports are submitted (see below).

#### Access to ACC

For the purposes of section 32 of the Accident Compensation Act 2001, the Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out. Participants injured as a result of treatment received in this trial will therefore be eligible to be considered for compensation in respect of those injuries under the ACC scheme.

#### Amendments and Protocol Deviations

All significant amendments to this proposal must receive prior approval from the Committee. Significant amendments include (but are not limited to) changes to:

- the researcher responsible for the conduct of the study at a study site
- the addition of an extra study site
- the design or duration of the study
- the method of recruitment
- information sheets and informed consent procedures.

Significant deviations from the approved protocol must be reported to the Committee as soon as possible.

Annual Progress Reports and Final Reports

The first Annual Progress Report for this study is due to the Committee by 31 May 2011. The Annual Report Form that should be used is available at [www.ethicscommittees.health.govt.nz](http://www.ethicscommittees.health.govt.nz). Please note that if you do not provide a progress report by this date, ethical approval may be withdrawn.

A Final Report is also required at the conclusion of the study. The Final Report Form is also available at [www.ethicscommittees.health.govt.nz](http://www.ethicscommittees.health.govt.nz).

Requirements for the Reporting of Serious Adverse Events (SAEs)

For the purposes of the individual reporting of SAEs occurring in this study, the Committee is satisfied that the study's monitoring arrangements are appropriate.

SAEs occurring in this study must be individually reported to the Committee within 7-15 days only where they:

- are *unexpected* because they are not outlined in the investigator's brochure, and
- are not defined study end-points (e.g. death or hospitalisation), and
- occur in patients located in New Zealand, and
- if the study involves blinding, result in a decision to break the study code.

There is no requirement for the individual reporting to ethics committees of SAEs that do not meet all of these criteria. However, if your study is overseen by a data monitoring committee, copies of its letters of recommendation to the Principal Investigator should be forwarded to the Committee as soon as possible.

Please see [www.ethicscommittees.health.govt.nz](http://www.ethicscommittees.health.govt.nz) for more information on the reporting of SAEs, and to download the SAE Report Form.

We wish you all the best with your study.

Yours sincerely



Claire Lindsay  
Administrator  
Multi-region Ethics Committee  
Email: [claire\\_lindsay@moh.govt.nz](mailto:claire_lindsay@moh.govt.nz)

## Appendix F – Food and Training Diary

---

### INSTRUCTIONS FOR THE DIET RECORD

- Please eat foods that you would commonly eat on a regular basis, ensuring you don't eat any of the foods from the "Can't eat" list
- Try to avoid exotic foods that are likely to be unavailable in subsequent weeks – remember, you must repeat what you eat during week 1 in weeks 2 – 5.
- If you're eating out, try to select dishes that could be prepared similarly at home or at another eating place
- List foods soon after they are eaten
- Be as specific as possible when describing the food item eaten e.g. brown/wholegrain/white bread
- Describe the way it was cooked or prepared (e.g., *fresh, frozen, stewed, fried, baked, tinned*)
- Record the amount that was eaten in household measures – *example.g., g, ml, tablespoon (tbsp = 15ml), teaspoon (tsp = 5ml), cup (=250ml), slice (thick, thin, 1cm etc) or units (e.g., one medium apple)*
- Report only the food portion that was actually eaten, not what you left on the plate
- For tinned foods include the liquid in which it was tinned – *e.g., sliced peaches in heavy syrup, fruit cocktail in light syrup, tuna in water*
- Remember to record the amounts of visible fats (oils, butter, salad dressings, margarine, ...) you eat (e.g., spread on bread or dressing on salads) or use in cooking (e.g., oil to fry in etc)

### INSTRUCTIONS FOR THE TRAINING DIARY

- Please record your training with the level of detail implied in the boxes
- Record when (date, time), how long, at which intensity, and which type of exercise it was (cycling, running, kayaking, weights ...)
- If you have races or competitions record these as well

All of this is necessary, because you need to repeat the recorded diet and training patterns each week during the study

[illegible]

TRAINING DIARY – Week 1					
Day 1		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
Day 2		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
Day 3		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
Day 4		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
1	Cycle (in lab)	2:00		2:00	
Day 5		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
REST					
Day 6		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
1	Cycle (in lab)	2:00	2:00hr @ 57.5% W <sub>max</sub>		
2	Performance Test		Repeated sprint test		
Day 7		Day.....		Date.....	
Session #	Exercise	Duration	Intensity		
	Type	hh:mm	Low/Time	Med/Time	High/Time
Easy recovery session					

## Appendix G – Performance Test Data Sheet

Date:													
<b>Subject:</b>				<b>Condition:</b>				<b>Performance Test</b>					
<b>PPO</b>		<b>395 W</b>		<b>40% 158 W</b>		<b>Leg</b>	<b>Leg</b>	<b>Overall</b>	<b>RPE</b>	<b>Nausea</b>	<b>Cramp</b>	<b>Palatab</b>	<b>Sweetn</b>
		<b>Calories</b>	<b>Time</b>	<b>CHO DRINK</b>	<b>Strengt</b>	<b>Sore</b>	<b>Tired</b>						
<b>Sprints</b>													
rec 1		48.4	49.4		1st drink								
<b>spr 1</b>	78.8	88.8											
	93.8	96.8	98.8										
rec 2		147.1	148.1										
<b>spr 2</b>	177.5	187.5			2nd drink								
	192.5	195.5	197.5										
rec 3		245.9	246.9										
<b>spr 3</b>	276.3	286.3											
	291.3	294.3	296.3										
rec 4		344.6	345.6										
<b>spr 4</b>	375.0	385.0			3rd drink								
	390.0	393.0	395.0										
rec 5		443.4	444.4										
<b>spr 5</b>	473.8	483.8											
	488.8	491.8	493.8										
rec 6		542.1	543.1										
<b>spr 6</b>	572.5	582.5			4th drink								
	587.5	590.5	592.5										
rec 7		640.9	641.9										
<b>spr 7</b>	671.3	681.3											
	686.3	689.3	691.3										
rec 8		739.6	740.6										
<b>spr 8</b>	770.0	780.0			5th drink								
	785.0	788.0	790.0										
rec 9		838.4	839.4										
<b>spr 9</b>	868.8	878.8											
	883.8	886.8	888.8										
rec 10		937.1	938.1										
<b>spr 10</b>	967.5	977.5											
	982.5	985.5	987.5										
<b>Body Weight</b>				<b>Pre:</b>		<b>Post:</b>							

## Appendix H – $^{14}\text{C}$ -Fructose Dose

### Calculation of the $^{14}\text{C}$ -fructose dose

U- $^{14}\text{C}_6$ -fructose was used as the fructose tracer for this experiment to provide a complete metabolic trace for the monosaccharide. The minimum necessary activity of U- $^{14}\text{C}_6$ -fructose required for sufficient breath  $^{14}\text{CO}_2$  detection has been estimated at  $0.0020 \text{ MBq}\cdot\text{g}^{-1}$  of total fructose ingested. This estimate is based on the quantity of U- $^{14}\text{C}_6$ -fructose used in our previous study [1],  $0.0042 \text{ MBq}\cdot\text{g}^{-1}$  of total fructose ingested yielded a clear outcome above background. In our first study, calculations were based on that used by Jentjens *et al.* [2] to trace 108 g glucose ingested per hour in 600 mL solution (“High-Glu” solution), which provided clear breath  $^{14}\text{C}$  signal of  $\sim 100\text{--}1300 \text{ Bq}$ . D-[U- $^{14}\text{C}$ ]Fructose was obtained from Amersham Radiochemicals product number CFB47-50UCL. The product is in aqueous solution containing 3% ethanol, sterilised. Activity is  $11.1 \text{ GBq}/\text{mmol}$ ,  $299 \text{ mCi}/\text{mmol}$ , or  $7.4 \text{ MBq}/\text{mL}$ .

Using this tracer/tracee ratio the  $^{14}\text{C}$ -fructose dose rates during the 2-h ride will be:

Condition; carbohydrate ingestion rate	Pre-exercise double bolus	per 15 min (x 8)	Total fructose (g)	Total dose (MBq)	Total volume ingested (mL)	Total tracer to add ( $\mu\text{L}$ )
0.5 Ratio;  0.5 F + 1.0 M $\text{g}\cdot\text{min}^{-1}$	13.5 g fruct x $0.0033 \text{ MBq}\cdot\text{g}^{-1}$	6.75 g x $0.0033 \text{ MBq}\cdot\text{g}^{-1}$	67.5	0.225	1800	30.41
0.8 Ratio;  0.67 F + 0.83 M $\text{g}\cdot\text{min}^{-1}$	18.0 g fruct x $0.0025 \text{ MBq}\cdot\text{g}^{-1}$	9.00 g x $0.0025 \text{ MBq}\cdot\text{g}^{-1}$	90.0	0.225	1800	30.41
1.25 Ratio;  0.83 F + 0.67 M $\text{g}\cdot\text{min}^{-1}$	22.5 g fruct x $0.0020 \text{ MBq}\cdot\text{g}^{-1}$	11.25 g x $0.0020 \text{ MBq}\cdot\text{g}^{-1}$	112.5	0.225	1800	30.41



Assuming a peak fructose oxidation rate at 120 min of exercise of 0.7 g/min, the expected breath activities are approximately 50 dpm at t=15 min to 600 dpm. At the time of writing we are unsure if these activities are detectable within the range for measurement error in the beta counter we will use (A. Prof John Millar, Biological Sciences, Victoria University, Wellington). A pilot trial will be conducted to test procedures.

The total exposure per subject for the experiment is:

$$3 \times 0.225 \text{ MBq} = 0.675 \text{ MBq total ingested.}$$

### Effective radiation dose

The ICRP-72 model committed effective dose from ingestion for organic  $^{14}\text{C}$  compounds is  $5.8 \times 10^{-10} \text{ Sv Bq}^{-1}$  (Richardson, Dunford *et al.* 2001).

Therefore,  $0.675 \times 10^6 \text{ Bq}$  total ingested over 4-5 weeks

$$5.8 \times 10^{-10} \cdot 0.675 \times 10^6$$

$$= 3.915 \times 10^{-4}$$

$$= 0.39 \text{ mSv}$$

I was unable to find a biokinetic model for  $^{14}\text{C}$  fructose, which may have minor differences to glucose due to the slightly different metabolism of this monosaccharide, namely tendency for fatty-acid synthesis in the liver. However, fructose transporters (GLUT 5) are present in both the small intestine epithelia and skeletal muscle, suggesting rapid metabolism at rates similar to glucose, but limited by intestinal transport mechanisms.

### Comments on the Validity of ICRP-72 Model for the Present Experimental Application

1. *High percentage of ingested fructose oxidised rapidly during the present exercise model.*  
The ICRP-72 model for organic  $^{14}\text{C}$ , appears to assume conditions of a normal resting metabolic rate and carbon turnover and immediate whole-body distribution of the  $^{14}\text{C}$  after administration. This model may not be particularly accurate for the present experimental situation. Ingested carbohydrate (exogenous) is rapidly oxidised during exercise. In solution, Jentjens *et al* (2004) gave cyclists 0.6 and 1.2  $\text{g} \cdot \text{min}^{-1}$  fructose and glucose, respectively, during 2-h of exercise (same as present proposed study design). The percentage of ingested fructose and glucose oxidised – appearing as breath  $^{13}\text{CO}_2$  and  $^{14}\text{CO}_2$ , respectively – during the final 60 min of exercise was 63% to 64%. We reported previously (Rowlands, Thorburn *et al.* 2008) 52-62% of ingested fructose to be oxidised during the exercise, leaving the remaining substrate to be oxidised in the performance test or to enter the carbon pool for turnover at the reported C half life in humans of 10 days.
2. *Use of performance test to further accentuate oxidation rate and therefore metabolic clearance of  $^{14}\text{C}$  and  $^{13}\text{C}$  isotopes to bring back down the natural enrichment to baseline levels.* The 80 min exercise following the  $^{14}\text{C}$ -fructose exposure should result in the immediate oxidation of most of the ingested isotope.



3. *Carbon turnover is higher in athletes* in training because of higher caloric intake and daily utilisation. Also, the size of one of the slow turnover compartments adipose tissue is generally less than in normal population.
4. Given these two above factors relating to the nature of the experiment, the true effective dose is likely to be less than that estimated in the ICRP 72 model.

### **Laboratory Set up for Storage and Preparation of $^{14}\text{C}$**

Biochemistry space was set aside in the lab for this work. Procedures were implemented following the recommendation of NRL and the licensee Dr Kevin Smidt, Palmerston North Hospital.

### **References**

1. Wallis GA, Rowlands DS, Shaw C, Jentjens RL, and Jeukendrup AE. Oxidation of combined ingestion of maltodextrins and fructose during exercise. *Med Sci Sports Exerc* 37: 426-432, 2005.
2. Jentjens RL, and Jeukendrup AE. High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *Brit J Nutr* 93: 485-492, 2005.

## Appendix I – Psychometric Scales

### Preload Psychometric Scales

Do you feel any nausea?	How full does your stomach feel?	Do you feel any abdominal cramp?	How hard are you working?	How tired are your legs?
Unbearable	Completely full	Unbearable	Maximally	Exhausted
Extremely severe	Extremely full	Extremely severe	Extremely hard	Extremely weak
Very severe	Quite full	Very severe	Very hard	Very weak
Severe	Moderately full	Severe	Hard	Weak
Moderate	Half full	Moderate	Moderate	Moderate
Mild	Less than half full	Mild	Light	Strong
Very mild	Little fullness	Very mild	Very light	Very strong
Extremely mild	Very little fullness	Extremely mild	Extremely light	Extremely strong
Nothing at all	Not full at all	Nothing at all	Not at all	Completely fresh

How sweet is the drink?	How palatable is the drink?
Unbearably sweet	Totally unpalatable
Extremely sweet	Extremely unpalatable
Very sweet	Very unpalatable
Sweet	Mildly unpalatable
Moderately sweet	Neither palatable nor unpalatable
Mildly sweet	Mildly palatable
Very mild sweetness	Palatable
Very little sweetness	Very palatable
Not sweet at all	Extremely palatable

## Performance Test Psychometric Scales

How strong do your legs feel?	How sore are your legs?	How tired do you feel overall?	How hard are you working?
Maximally Strong	Unbearable	Completely fresh	Maximally
Extremely strong	Extreme	Extremely strong	Extremely hard
Very strong	Very severe	Very strong	Very hard
Strong	Severe	Strong	Hard
Moderate	Moderate	Moderate	Moderate
Weak	Mild	Weak	Light
Very weak	Very mild	Very weak	Very light
Extremely weak	Extremely mild	Extremely weak	Extremely light
Nothing at all	Nothing at all	Exhausted	Nothing at all

Do you feel any nausea?	Do you feel any abdominal cramp?	How palatable is the drink?	How sweet is the drink?
Unbearable	Unbearable	Totally unpalatable	Unbearably sweet
Extremely severe	Extremely severe	Extremely unpalatable	Extremely sweet
Very severe	Very severe	Very unpalatable	Very sweet
Severe	Severe	Mildly unpalatable	Sweet
Moderate	Moderate	Neither palatable nor unpalatable	Moderately sweet
Mild	Mild	Mildly palatable	Mildly sweet
Very mild	Very mild	Palatable	Very mild sweetness
Extremely mild	Extremely mild	Very palatable	Very little sweetness
Nothing at all	Nothing at all	Extremely palatable	Not sweet at all

## Appendix J – Blood D<sub>2</sub>O Analysis Method

---

### *Extended Method for Deproteinised Blood D<sub>2</sub>O Analysis*

Deproteinised blood samples were thawed, and 500  $\mu$ L transferred into glass vials for determination of hydrogen isotope ratios of water by thermal conversion to hydrogen and oxygen gas. Eight repeat water samples of 100 nL were injected via an autosampler (PAL Systems, CTC analytics, Switzerland) into the glassy carbon-lined ceramic reactor of a thermal conversion elemental analyser (Finnigan TC/EA, Thermo Electron Corporation, Bremen, Germany) held at 1400°C. The resulting hydrogen and oxygen gases, borne in a helium carrier gas stream, were separated on a packed gas chromatography column (molecular sieves, 5A, 100 mesh; dimensions 600 mm x 4 mm i.d.) and the hydrogen measured on an isotope ratio mass spectrometer (IRMS) in continuous flow mode. Results were processed using Isodat 2.5 software (Gas Isotope Ratio MS Software v2.5, Thermo Electron Corporation, Bremen, Germany) and each set of eight results was filtered by removal of values more than 1 standard deviation from the average. The average was corrected to international isotope scales using a three point calibration provided by three laboratory standards analysed before and after every batch of 96 samples. The three laboratory standards used were calibrated with International Atomic Energy Agency (IAEA) standard materials by a 5-member interlaboratory comparison exercise and range between -262.7 and -1.5‰ (i.e., ICE -262.7‰, TAP -81.0‰, SEA -1.5‰). The precision of analysis was typically 0.9 ‰ (1 SD). A single control sample (ITH1 50.1‰) was measure every 12 samples. The isotopic enrichment is expressed as  $\delta$ ‰ against the international water standard Vienna Standard Mean Ocean Water (VSMOW).