

# **Optimisation of carbohydrate intake for skeletal muscle glycogen synthesis and endurance cycling performance**

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the degree of Doctor of Philosophy.

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## **Declaration**

I declare that the work in this thesis was carried out in accordance with regulations of Liverpool John Moores University and apart from help and advice of my supervisors, the work presented is entirely my own.

This thesis has not been presented to any other institutions for examination. Some of this work has been submitted for publication or has been published in academic journals. I declare I have not used any Artificial Intelligence tools or technologies in the preparation of this thesis.

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## Abstract

The importance of a high pre-exercise CHO intake for increased skeletal muscle glycogen concentrations and improved endurance capacity has long been understood. However, there is high variability in reported muscle glycogen concentrations following similar CHO intakes, and increased glycogen stores have not always translated into improved endurance performance. Therefore, this thesis aimed to investigate the relationship between CHO feeding, CHO availability, whole muscle glycogen, and endurance cycling performance.

Accordingly, Chapter 4 aimed to describe and quantify the relationship between dietary CHO intake and muscle glycogen in previous research with a systematic review and meta-analysis. Studies were sourced from 5 electronic databases and were assessed using tally markings (56 trials,  $n = 571$  participants), linear regression and a generic inverse-variance random effects meta-analysis with subgroups used to investigate heterogeneity (17 trials). Linear regression indicated a significant linear relationship between relative CHO intake and whole muscle glycogen for endurance trained individuals ( $r^2 = 0.493$ ,  $P < 0.001$ ). Meta-analyses revealed increased dietary CHO intake from a low-moderate to high-very high quantity ( $<$  and  $> 6.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , respectively) significantly increased muscle glycogen concentrations ( $198.8 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ , 95% CI from  $148.0$  to  $249.7 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ,  $P < 0.0001$ ) but displayed significant heterogeneity ( $P < 0.0001$ ,  $I^2 = 94\%$ ). Glycogen concentration primarily depends on quantity of CHO ingested, with period of increased CHO intake, participant training status, and exercise all being potent effectors. Based on current findings to optimise pre-competition muscle glycogen stores athletes should consume  $> 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 36-72 h pre-competition, with the use of an exercise stimulus combined with consumption of high glycaemic CHO

allowing more rapid enhancement of stores (24-36 h). However, significant heterogeneity in meta-analysis results raises uncertainty regarding recommendations.

As such, Chapter 5 aimed to determine the dose-response between dietary CHO intake and muscle glycogen in endurance trained individuals, whilst controlling the previously determined key effectors of glycogen storage and replicating real world pre-competition training and nutrition practices of athletes. Following two days of standardised dietary intake and exercise prescription, 11 endurance trained participants (8 males, 3 females; age,  $24 \pm 5$  years; body mass,  $71.2 \pm 12.0$  kg;  $\dot{V}O_{2\max}$ ,  $56 \pm 6$  mL·kg<sup>-1</sup>·min<sup>-1</sup>; PPO,  $306 \pm 54$  W; LT1,  $164 \pm 49$  W) consumed either 6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO for the following 48 h, before returning to the laboratory (day 5) for a high CHO breakfast and muscle biopsy 2 h post-prandial. Muscle glycogen was significantly higher following 10 compared to 6 and 8 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO ( $635.5 \pm 78.0$ ,  $460.9 \pm 100.7$  and  $506.1 \pm 124.0$  mmol·kg<sup>-1</sup> DM, respectively,  $P < 0.03$ ), with no difference between 6 and 8 g·kg<sup>-1</sup>·day<sup>-1</sup> ( $P = 1.00$ ). There was a significant strong positive correlation between relative ( $r = 0.71$ ,  $P < 0.001$ ), absolute CHO intake ( $r = 0.64$ ,  $P < 0.001$ ) and whole muscle glycogen concentration. In agreement with Chapter 4, there was a strong linear dose-response between dietary CHO intake and muscle glycogen concentrations, however study data suggest intakes  $\geq 10$  g·kg<sup>-1</sup>·day<sup>-1</sup> are necessary to maximise muscle glycogen stores in real world training conditions.

Whether maximisation of muscle glycogen stores is required to optimise endurance performance remains unclear, as almost all CHO loading studies have failed to adequately blind study participants to CHO intake. As such Chapter 6 aimed to determine whether increased glycogen stores translated into improved endurance performance. In a repeated measures

double blind design, 9 endurance trained males ( $\dot{V}O_{2\max}$ ,  $63.4 \pm 5.2 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; PPO,  $367 \pm 37 \text{ W}$ ) completed 3 x 4 days of dietary control and prescribed exercise designed to mimic pre-competition practices of endurance cyclists, consuming 6, 8 or  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 48 h, before completing a performance test the following morning (2 h steady state pre-load and ~30 min cycling time trial). There was no significant difference in TT completion time ( $36 \text{ min } 46 \text{ s} \pm 4 \text{ min } 18 \text{ s}$ ,  $34 \text{ min } 55 \text{ s} \pm 5 \text{ min } 12 \text{ s}$  and  $35 \text{ min } 46 \text{ s} \pm 5 \text{ min } 56 \text{ s}$ ;  $P = 0.16$ ) or mean power output ( $226 \pm 22$ ,  $239 \pm 29$  and  $234 \pm 29 \text{ W}$ ;  $P = 0.10$ ) between 6 vs 8 and  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , respectively. However, there was a large effect size ( $\eta^2_p = 0.21$  and  $\eta^2_p = 0.26$ , respectively) possibly biased by the placebo effect, as 3 participants who correctly identified the placebo condition had the greatest improvements in cycling performance. In conclusion, current data suggests CHO loading with a high or very high CHO intake ( $8$  or  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) provides no benefit to endurance cycling performances lasting ~2.5 h under real world conditions of high exogenous CHO availability.

Lastly, fuelling during endurance exercise has evolved towards greater amounts of CHO ingested per hour, however the effects of different CHO ingestion patterns during exercise have scarcely been investigated in cycling (Chapter 7). In a randomised counterbalanced order, 20 recreationally active males ( $\dot{V}O_{2\max}$ ,  $50.4 \pm 3.8 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; LT1,  $139 \pm 29 \text{ W}$ ) cycled for 180 min at LT1 and consumed  $90 \text{ g}\cdot\text{h}^{-1}$  of CHO, either as  $22.5\text{g}$  every 15 min or  $45\text{g}$  every 30 min. Physiological responses showed no difference between conditions ( $P > 0.20$ ) or significant interactions ( $P > 0.30$ ), except for blood glucose which saw a transient difference during the first 30 min (interaction;  $P = 0.03$ ). Whole body CHO and fat oxidation were not different between conditions ( $2.38 \pm 0.37$  and  $2.33 \pm 0.39 \text{ g}\cdot\text{min}^{-1}$ ,  $P = 0.25$ , and  $0.19 \pm 0.07$  vs  $0.22 \pm 0.08 \text{ g}\cdot\text{min}^{-1}$ ,  $P = 0.10$ , respectively). Ingesting a larger CHO amount at less regular intervals during prolonged cycling had minimal impact on physiological responses to exercise,

whole-body substrate oxidation and gut discomfort, allowing athletes to freely select their preferred strategy.

In conclusion, this thesis provides novel data describing the linear dose-response relationship between CHO intake and muscle glycogen, indicating that higher CHO intakes may be better to maximise muscle glycogen concentrations under real world training conditions. However, under conditions studied in this thesis (2.5 h endurance cycling), it appears unnecessary for endurance trained cyclists to CHO load with  $\geq 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO prior to  $\sim 2.5 \text{ h}$  of endurance cycling, provided exogenous CHO provision pre and during exercise is optimal. However, greater exercise demands (increased intensity and duration), compared to the current exercise protocol, may require adjustments to CHO availability, possibly achieved with CHO loading to maximise glycogen stores.

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## List of abbreviations

|                                            |                                         |
|--------------------------------------------|-----------------------------------------|
| <b>AMP:</b>                                | Adenosine monophosphate                 |
| <b>AMPK:</b>                               | AMP-activated protein kinase            |
| <b>ATP:</b>                                | Adenosine triphosphate                  |
| <b>cm:</b>                                 | Centimetre                              |
| <b>CO<sub>2</sub>:</b>                     | Carbon dioxide                          |
| <b>CV:</b>                                 | Coefficient of variation                |
| <b>ECW:</b>                                | Extracellular water                     |
| <b>g:</b>                                  | Gram                                    |
| <b>GI:</b>                                 | Gastrointestinal                        |
| <b>g·day<sup>-1</sup>:</b>                 | Grams per day                           |
| <b>g·h<sup>-1</sup>:</b>                   | Grams per hour                          |
| <b>g·kg<sup>-1</sup>:</b>                  | Grams per kilogram of body mass         |
| <b>g·kg<sup>-1</sup>·day<sup>-1</sup>:</b> | Grams per kilogram of body mass per day |
| <b>g·min<sup>-1</sup>:</b>                 | Grams per minute                        |
| <b>h:</b>                                  | Hours                                   |
| <b>HCl:</b>                                | Hydrochloric acid                       |
| <b>HR:</b>                                 | Heart rate                              |
| <b>h·day<sup>-1</sup>:</b>                 | Hours per day                           |
| <b>ICW:</b>                                | Intracellular water                     |
| <b>IQR:</b>                                | Interquartile range                     |
| <b>K:</b>                                  | Potassium                               |
| <b>KCl:</b>                                | Potassium Chloride                      |
| <b>kHz:</b>                                | Kilohertz                               |

|                                             |                                            |
|---------------------------------------------|--------------------------------------------|
| <b>kJ:</b>                                  | Kilojoules                                 |
| <b>km:</b>                                  | Kilometre                                  |
| <b>KOH</b>                                  | Potassium hydroxide                        |
| <b>kJ·min<sup>-1</sup>:</b>                 | Kilojoules per minute                      |
| <b>LT:</b>                                  | Lactate threshold                          |
| <b>mg:</b>                                  | Milligrams                                 |
| <b>min:</b>                                 | Minutes                                    |
| <b>mL:</b>                                  | Millilitres                                |
| <b>mL·kg<sup>-1</sup>·min<sup>-1</sup>:</b> | Millilitre per kilogram per minute         |
| <b>mM:</b>                                  | Millimolar                                 |
| <b>mmol·kg<sup>-1</sup> DM:</b>             | Millimolar per kilogram of dry muscle mass |
| <b>mmol·kg<sup>-1</sup> WM:</b>             | Millimolar per kilogram of wet muscle mass |
| <b>mmol·L<sup>-1</sup>:</b>                 | Millimolar per litre                       |
| <b>n:</b>                                   | number                                     |
| <b>nM:</b>                                  | Nanometre                                  |
| <b>O<sub>2</sub>:</b>                       | Oxygen                                     |
| <b>RER:</b>                                 | Respiratory exchange ration                |
| <b>RoB:</b>                                 | Risk of bias                               |
| <b>RPE:</b>                                 | Rating of perceived exertion               |
| <b>rpm:</b>                                 | revolutions per minute                     |
| <b>s:</b>                                   | Seconds                                    |
| <b>SD:</b>                                  | Standard deviation                         |
| <b>SE:</b>                                  | Standard error                             |
| <b>SGLT1:</b>                               | Sodium dependent glucose transporter 1     |
| <b>SS:</b>                                  | Steady state                               |

|                                                    |                                 |
|----------------------------------------------------|---------------------------------|
| <b>TBW:</b>                                        | Total body water                |
| <b>UDP:</b>                                        | Uridine diphosphate             |
| <b>VAS:</b>                                        | Visual analogue scale           |
| <b><math>\dot{V}\text{CO}_2</math>:</b>            | Ventilatory carbon dioxide      |
| <b><math>\dot{V}\text{O}_2</math>:</b>             | Ventilatory oxygen              |
| <b><math>\dot{V}\text{O}_{2\text{max}}</math>:</b> | Maximal oxygen uptake           |
| <b>vs:</b>                                         | Versus                          |
| <b>W:</b>                                          | Watts                           |
| <b><math>\text{W}\cdot\text{kg}^{-1}</math>:</b>   | Watts per kilogram of body mass |
| <b><math>^{\circ}\text{C}</math>:</b>              | Degrees Celsius                 |

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## **Chapter One:**

### **General Introduction**

*This chapter introduces the role of pre-exercise dietary CHO intake for muscle glycogen synthesis and concentration, whilst exploring the implications for endurance cycling performance.*

## 1.1 Background

The importance of a high dietary CHO intake days pre-exercise was recognised as early as the 1930s, as Christensen and Hansen (1939) reported improved exercise capacity following three days of a high CHO diet compared to a high fat diet (90 vs 5% total energy intake per day from CHO, respectively). The introduction of the muscle biopsy technique by Scandinavian researchers ~30 years later, discovered a very high CHO intake post-exhaustive exercise resulted in a supra-physiological enhancement of muscle glycogen stores (termed glycogen ‘supercompensation’), which improved exercise capacity through a delayed onset of fatigue, suggested to be from greater muscle glycogen availability during exercise, allowing prolonged maintenance of blood glucose due to liver glycogen sparing (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967).

These studies signified the birth of the ‘classic’ CHO loading protocol, where six days of diet and training manipulation (72 h low CHO and 72 h high CHO, with an exhaustive cycling session on day 1 and 3) increased glycogen stores to  $\sim 800 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$  (Bergström et al., 1967). Over the last five decades CHO loading has developed and evolved, with the incorporation of shorter periods of high-very high CHO intakes ( $\geq 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 24 - 48 h) and less intense exercise pre- or during- loading seemingly achieving a similar enhancement of muscle glycogen stores (Sherman et al., 1981; Bussau et al., 2002). These discoveries formed the basis of contemporary nutrition guidelines pre-endurance exercise, where dependent on exercise intensity and duration, athletes are recommended to consume moderate to very high CHO intakes of  $6\text{-}12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 36-48 h pre-competition (Thomas et al., 2016).

Despite the relationship between pre-exercise dietary CHO intake and muscle glycogen appearing linear, with a saturation point (‘ceiling’ effect) suggested at  $7\text{-}10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO (Burke et al., 2017; Burke et al., 1995; Costill et al., 1981), many studies feeding identical CHO

quantities across similar loading durations ( $\sim 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 48-72 h) have reported considerable variability in muscle glycogen concentrations ( $450 - 800 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ; Adamo et al., 1998; Bergström et al., 1967; Kavouras et al., 2004; McLay et al., 2007; Roberts et al., 2016), even when well-established effectors of glycogen synthesis, such as participant training status (Greiwe et al., 1999; Hickner et al., 1997) or exercise conditions (Blom et al., 1987) were matched. Whereas others have reported no changes in muscle glycogen, despite  $\geq 3 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  difference in CHO intake between conditions (Bradley et al., 2016; Burke et al., 1995). Such discrepancies raise questions regarding the nature of the relationship between dietary CHO intake and muscle glycogen, suggesting additional considerations are required when implementing CHO loading strategies in the real world. Particularly as almost all previous CHO loading studies have rested participants during the loading period, whereas endurance athletes continue training pre-competition to maintain training adaptations, albeit at a lighter relative exercise intensity (Coyle et al., 1984; Mujika and Padilla, 2000). Surprisingly, this has rarely been considered (Sherman et al., 1981; Doering et al., 2019), and no physiological or metabolic data has been collected during such exercise sessions.

Despite a saturation point of muscle glycogen previously being suggested (Burke et al., 1995), there are a limited number of dose-response studies comparing multiple levels of CHO intakes (Gollnick et al., 1972; Costill et al., 1981; Maunder et al., 2021), however the CHO quantities consumed were low ( $\sim 2.3$ ,  $\sim 4.8$  and  $\sim 6.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) considering contemporary recommendations (Thomas et al., 2016). Combined with the small study sample sizes in two of the aforementioned studies ( $n = 4$ ), this raises concerns regarding generalisability and statistical strength of outcomes (Gollnick et al., 1972; Costill et al., 1981). Ultimately, there is a lack of certainty regarding the relationship between dietary CHO intake and muscle glycogen, particularly at higher CHO intakes ( $\geq 7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ), as most modern-day endurance athletes adhere to in the lead up to competition (anecdotal evidence from the field; Thomas et al., 2016).

However, higher CHO intakes can result in negative implications such as GI discomfort symptoms (Lamb et al., 1991; Rehrer et al., 1992) and/or increased body mass due to water retention (Olsson and Saltin, 1970), which could hinder endurance performance through discomfort/pain or impaired exercise efficiency through decreased  $W \cdot kg^{-1}$  ratio, respectively.

Nonetheless, CHO loading is considered essential to optimise endurance exercise performance and endurance capacity in events lasting >90 min (Hawley et al., 1997a; Thomas et al., 2016), as almost all previous research has shown improved endurance performance (Rauch et al., 1995; Karlsson and Saltin, 1971; Widrick et al., 1993) or capacity (Ahlborg et al., 1967; Bergström and Hultman, 1966; Bergström et al., 1967; Bosch et al., 1993; Galbo et al., 1979; Jensen et al., 2020; Lamb et al., 1991). Interestingly, none of the aforementioned studies incorporated participant blinding. In fact, only two CHO loading studies have incorporated double blinding, and both reported no improvement in endurance cycling performance, despite significant enhancement of muscle glycogen stores (Burke et al., 2000; Tomcik et al., 2018). This highlights a potential placebo effect which could have biased previous CHO loading research, which has previously been suggested (Burke et al., 2000), but never truly investigated, raising questions regarding the ‘well-established’ nature of the relationship between CHO loading, muscle glycogen and endurance cycling performance.

## **1.2 Aims and objectives**

The overall aim of this thesis was to optimise CHO intake recommendations in the days before endurance competition to maximise skeletal muscle glycogen stores and endurance performance, with practical considerations for real-world conditions, as well as optimise CHO delivery during exercise. These aims will be achieved through the following objectives;

- 1) A systematic review of the literature and meta-analysis to determine the relationship between CHO intake and pre-exercise skeletal muscle glycogen concentration, and to characterise other important covariates that determine glycogen concentrations.
- 2) Determine the dose-response relationship between dietary CHO intake and skeletal muscle glycogen under real world training conditions, whilst considering potential negative practical implications.
- 3) Investigate the potential placebo effect of CHO loading on endurance cycling performance.
- 4) Determine the effects of different CHO feeding frequencies during endurance cycling exercise on physiological responses, substrate utilisation, gut comfort and exercise capacity.

## **Chapter Two:**

### **Literature review**

*This chapter provides a brief historical review of the literature regarding CHO loading and the evolution of contemporary CHO intake guidelines with considerations for glycogen structure, storage and metabolism.*

## 2.1 Historical background

In the early 20<sup>th</sup> century, it was first suggested that manipulation of dietary CHO and fat intake in the days pre-exercise affected RER between O<sub>2</sub> and CO<sub>2</sub> during submaximal cycling (Zuntz, 1901; Frenzel and Reach, 1901). This was later confirmed by a series of experiments by Krogh and Lindhard (1920) using the first cycle ergometer to accurately control power output and a chemical analyser with the ability to determine O<sub>2</sub> and CO<sub>2</sub> of expired air within 0.001%. Authors highlighted participants felt more fatigued during and following 2 h of submaximal cycling when a high fat vs a high CHO diet was consumed. This seminal work also discovered CHO utilisation, to generate energy in the form of ATP, was 11% more oxygen efficient during exercise compared to fat. This research area was continued by Christensen and Hansen (1939), with a seminal study which showed 3 days of a high CHO diet resulted in enhanced cycling exercise capacity at a fixed workload, compared to 3 days of a high fat diet. The proposed mechanisms being high CHO availability allowing a continued maintenance of blood glucose concentrations in comparison to the high fat diet, where CHO availability was limited. Despite often not being recognised as CHO loading literature *per se* (likely due to the inability to measure muscle glycogen at the time), it was these early studies which laid the foundations for future work within the area, as authors highlighted for the first time, a key role of dietary CHO intake in the days pre-endurance exercise.

In the 1960s, the introduction of the percutaneous muscle biopsy technique allowed direct measurement of muscle glycogen concentrations within skeletal muscle. Bergström and Hultman (1966), using themselves as participants, completed exhaustive cycling exercise in one leg only (sitting either side of a cycle ergometer), before consuming a high CHO diet for the following 3 days ( $\sim 8.7$  and  $8.2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  [or  $\sim 550$  and  $650 \text{ g}$ ] for J.B. and E.H., respectively). Muscle biopsies taken from both exercised and rested legs following 1 day revealed glycogen concentration had replenished from almost total depletion to above the

‘normal’ values of the rested legs. The second- and third-day muscle glycogen concentration rose ‘dramatically’, achieving a doubling of values reported under rested conditions. Authors concluded that localised glycogen depletion enhanced resynthesis within previously worked muscle, suggesting this could be due to increased glucose uptake at the cell membrane. Shortly after this pioneering study, Bergström et al. (1967) introduced what would later become recognised as the ‘classic’ CHO loading protocol, where 3 days of low CHO intake followed by 3 days of high CHO ( $\sim 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ), each preceded by exhaustive cycling exercise, resulted in a supercompensation of muscle glycogen stores to  $\sim 800 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$  (or 3.3 g per 100g of wet muscle mass). This enhancement of glycogen stores resulted in a doubling of cycling exercise capacity, supporting the findings of Christensen and Hansen (1939). Further work showed this protocol translated into increased 30 km running performance (Karlsson and Saltin, 1971), supporting the ergogenic effect of CHO loading and glycogen supercompensation on endurance performance.

A decade later Sherman et al. (1981) introduced a modified strategy, where an exhaustive glycogen depleting exercise and low CHO intake period was found to be unnecessary to achieve a supra-physiological enhancement of muscle glycogen stores, making CHO loading less burdensome for athletes to implement in the lead up to competition. Interestingly, in the previous study, higher glycogen stores did not improve 20.9 km running performance, likely due to stores being sufficient in the control condition (mixed/moderate CHO diet) within the endurance trained cohort, to complete  $\sim 80$  min of intense exercise. This finding, later supported by others (Hawley et al., 1997b; Madsen et al., 1990), led to CHO loading not being recommended for intense exercise lasting  $< 90$  min (Hawley et al., 1997a).

In the early 21st century CHO loading was ‘updated’ (Bussau et al., 2002), where authors claimed no exercise and 24 h of a very high dietary CHO intake ( $\sim 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) was sufficient to achieve a similar enhancement of glycogen stores ( $\sim 800 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ). In

reality, as participants completed their final training session ~12 h prior to the increase in CHO intake, which upregulated post-exercise glycogen synthesis, the true loading period was ~ 36 h, despite CHO intake remaining habitual during the first 12 h or so. Nonetheless, this study, and others (Fairchild et al., 2002) highlighted that CHO loading could achieve a near maximal enhancement of glycogen stores within shorter time periods (< 72 h) with a very high CHO intake of  $\sim 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ .

Thereafter, CHO loading literature has primarily investigated concepts surrounding the strategy, such as the effects of co-ingestion of other compounds such as protein or creatine (Hill et al., 2013; Roberts et al., 2016; Tomcik et al., 2018), the ability to repeatedly supercompensate glycogen stores (McInerney et al., 2005; Doering et al., 2019) or maintain elevated glycogen concentrations (Arnall et al., 2007), as well as consisting of narrative reviews summarising previous research and providing recommendations for CHO intakes pre-endurance exercise > 90 min (Hawley et al., 1997a; Hargreaves et al., 2004; Jeukendrup, 2011). This evolution of CHO loading strategies over the past century has shaped contemporary pre-exercise nutritional guidelines for endurance athletes, where dependent on the demands of competition/training (exercise intensity and duration) athletes are recommended to consume 8 to  $12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 36-48 h pre-exercise to achieve an enhancement of muscle glycogen stores (Thomas et al., 2016; Burke et al., 2017; Table 2.1).

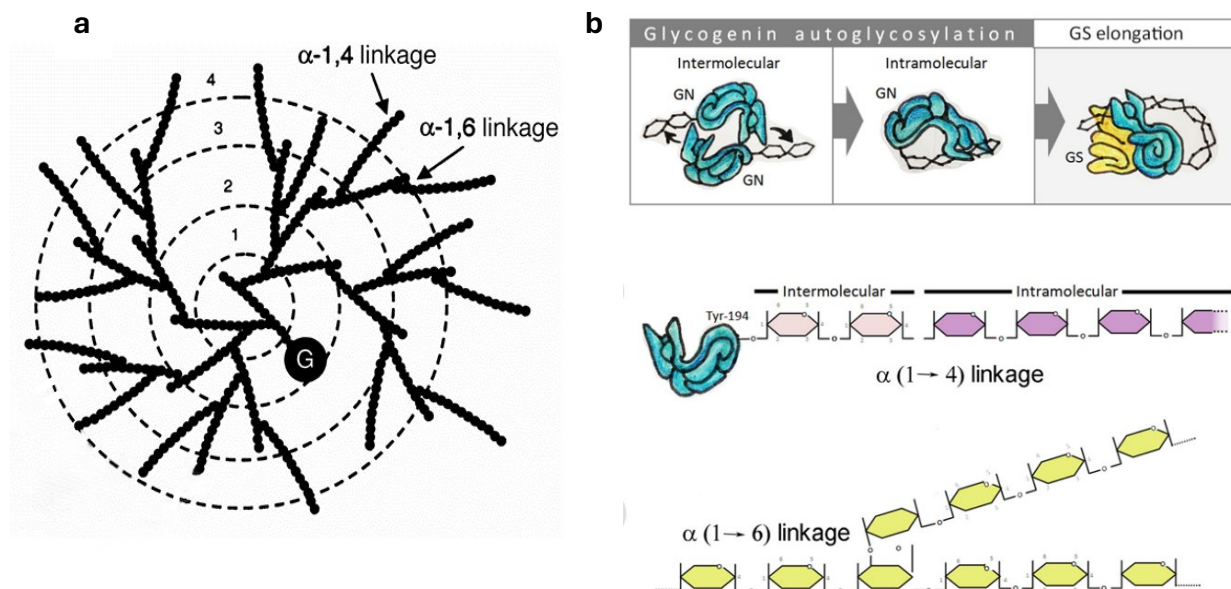
**Table 2.1.** Contemporary CHO intake recommendations adapted from Thomas et al. (2016)

| Exercise situation                                                                                 | CHO targets                                                        |
|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| Light or low intensity or skill-based                                                              | 3-5 g·kg <sup>-1</sup> ·day <sup>-1</sup>                          |
| Moderate exercise program (~1 h·day <sup>-1</sup> )                                                | 5-7 g·kg <sup>-1</sup> ·day <sup>-1</sup>                          |
| High endurance program (1-3 h·day <sup>-1</sup> )                                                  | 6-10 g·kg <sup>-1</sup> ·day <sup>-1</sup>                         |
| Very high extreme commitment<br>(>4-5 h·day <sup>-1</sup> moderate to high intensity)              | 8-12 g·kg <sup>-1</sup> ·day <sup>-1</sup>                         |
| Acute fuelling to promote high CHO availability and optimal performance in competition or training |                                                                    |
| General fuelling for events < 90 min                                                               | 7-12 g·kg <sup>-1</sup> ·day <sup>-1</sup> as for daily fuel needs |
| CHO loading for event >90 min<br>sustained/intermittent exercise                                   | 36-48 h of 10-12 g·kg <sup>-1</sup> ·day <sup>-1</sup>             |

## 2.2 Glycogen storage, structure and synthesis

CHO stored within the body as glycogen, primarily within the muscle and liver (~400 and 100-120 g, respectively, under ‘normal’ physiological conditions), are a rapidly available crucial energy source during prolonged endurance exercise (Hearris et al., 2018). Glycogen  $\beta$ -granules, the primary type found within skeletal muscle, are comprised of a protein core (glycogenin) and multiple branches of chained glucose residues (Marchand et al., 2002; Prats et al., 2018). *De novo* glycogen synthesis requires glycogenin auto-catalysation, where glucose transferred from UDP-glucose is bound at Tyrosine-194, with a further 7-10 glucose residues chained together by  $\alpha$ -1,4 glycosidic bonds. Thereafter, glycogen synthase, bound to glycogenin, catalyses the addition of further glucose residues, with new glucose chains connected at

branching points by glycogen branching enzyme with  $\alpha$ -1,6 glycosidic bonds, forming the tiered structure of the granule (Figure 2.1; Prats et al., 2018; Smythe and Cohen, 1991). Multiple glycogen granules, typically 20-30 nM in size (ranging from 10-44 nM), are heterogeneously stored across the muscle within specific subcellular pools (Nielsen and Ørtenblad, 2013). During periods of enhanced glycogen storage through increased dietary CHO intake, the numerical density of glycogen increases, as opposed to the size of existing granules (Jensen et al., 2021), which seems inefficient considering theoretical storage capacity of the granule and that average glycogen granule size is  $\sim$ 24 nM (Marchand et al., 2002). However, larger particles have higher steric hindrance, meaning larger glycogen granules could impede glycogenolysis during exercise due to spatial constraints within the musculature (Shearer and Graham, 2004; Jensen et al., 2021). Therefore, medium sized particles offer a metabolic advantage of faster glycogen breakdown during exercise.



**Figure 2.1.** The glycogen granule (**a**) and *de novo* glycogen synthesis (**b**) taken from Shearer and Graham (2004) and Prats et al. (2018), respectively.

### ***2.2.1 Regulation of glycogen synthesis***

Glycogen synthesis is regulated allosterically and covalently through substrate availability and activation or deactivation of specific rate limiting transport proteins such as GLUT4, which is responsible for glucose transport into the muscle. When inactive GLUT4 is intracellular, the action of insulin (binding to its muscular receptor) or the onset of exercise initiates GLUT4 translocation to the muscle membrane. GLUT4 regulation during exercise is multifaceted, with potential effectors including SR  $\text{Ca}^{2+}$  release, ATP turnover activation of AMPK, and contraction induced mechanical stretch activation of p38 MAPK (Jensen and Richter, 2012). Upon the cessation of exercise, GLUT4 activation only persists for 30-60 min (Murray and Rosenbloom, 2018), highlighting the importance of CHO ingestion early post-exercise, and subsequent insulin-dependent augmentation of GLUT4 activity, for maximal glucose uptake into the muscle and glycogen resynthesis.

Another key regulatory enzyme is glycogen synthase, which plays a key role in the attachment of glucose to the glycogen granule (as previously described). The regulation of glycogen synthase is complex due to multiple phosphorylation sites, where phosphorylation and dephosphorylation deactivates and activates the enzyme. Protein phosphatase 1 catalyses the phosphorylation of glycogen synthase, whereas glycogen synthase kinase dephosphorylates the enzyme to its active form. Allosterically, when glucose enters the muscle, it is phosphorylated by hexokinase to glucose-6-phosphate, which dependent on exercise status and energy demands, enters glycolysis or allosterically activates glycogen synthase. Glycogen concentration also regulates glycogen synthase, as high stores suppress and low/depleted stores augment activity, respectively (Katz, 2022; Roach 2012). Therefore, utilisation of glycogen stores act as a strong driver for resynthesis, which can increase rates by >2 fold in the 6 h immediately post-exercise (Zachwieja et al., 1991). Thereafter, ingestion of CHO, and insulin mediated activation of glycogen synthase kinase (through the phosphoinositide 3-kinase, Akt

pathway) activates glycogen synthase and augments glycogen synthesis (Jensen and Richter, 2012; Roach, 2012).

More recently the role of AMPK in the upregulation of glycogen synthesis and muscle glycogen supercompensation was suggested (Hingst et al., 2018). Primarily through activation of an AMPK isoform enhancing fat oxidation, inhibiting CHO oxidation and channelling more glucose for glycogen synthesis (Hingst et al., 2018; Katz, 2022).

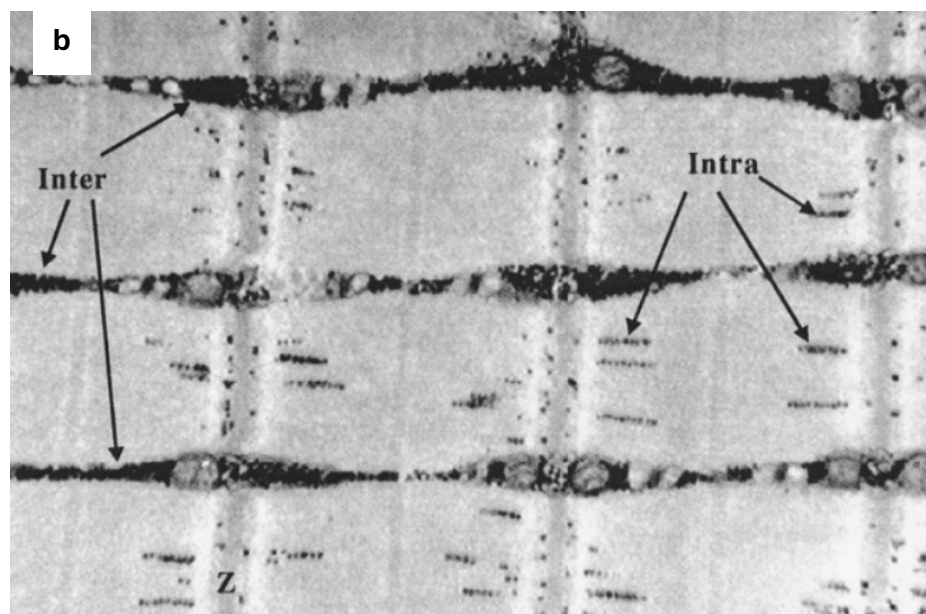
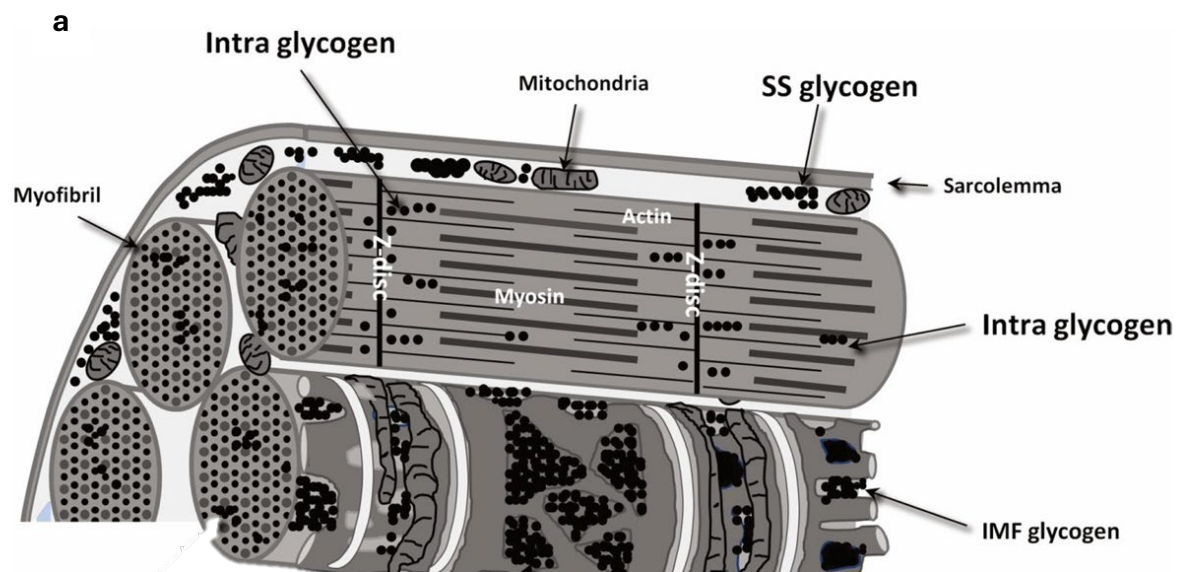
Additionally, suppression of glycogen phosphorylase is a determinant of glycogen synthesis. Maximal glycogen phosphorylase concentrations are 20-30-fold more abundant than glycogen synthase in vitro, suggesting that glycogen phosphorylase (and glycogen breakdown) predominates when enzyme activities are elevated during exercise (Katz, 2022). Therefore, glycogen phosphorylase inhibition is a requirement for net positive glycogen synthesis.

### ***2.2.2 Subcellular muscle glycogen storage***

Studies using transmission electron microscopy have allowed the identification of specific storage locations within muscle, as glycogen granules are stored across three specific pools: Between the myofibrils (intermyofibrillar glycogen; close to mitochondria and sarcoplasmic reticulum), within the myofibrils (intramyofibrillar glycogen; within contractile filaments and the I-band), or in the subsarcolemmal region beneath the plasma membrane near the mitochondria and cell nuclei (Nielsen and Ørtenblad, 2013). The majority of glycogen is stored at the intermyofibrillar level (75%), whilst the remaining ~5 and ~15% of total glycogen is stored at the intramyofibrillar and subsarcolemmal regions, respectively.

Glycogen storage locations are influenced by training status, as fibre type differences occur in endurance trained muscle (Nielsen et al., 2010a; Nielsen et al., 2010b), with 80 and 30% more intramyofibrillar and subsarcolemmal glycogen in the more oxidative type I muscle fibres,

whereas more glycolytic type II fibres contain 10 % more intermyofibrillar glycogen (Nielsen et al., 2011). The fact that differences do not occur in obese or recreationally active individuals (Nielsen et al., 2010a; Nielsen et al., 2010b) suggest distributions are an adaptation to endurance training and serve to benefit endurance capacity, likely through prolonged muscular function and associated fatigue resistance (Nielsen et al., 2009; Ørtenblad et al., 2011). CHO availability also regulates the distribution of glycogen across subcellular locations, as Jensen et al. (2020) recreated the ‘classic’ CHO loading protocol (Bergström et al., 1967) and reported significantly increased intermyofibrillar glycogen in type I fibres consuming high compared to a moderate and low CHO diet (8.0, 3.2 and 0.2 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO). Whereas intra and subsarcolemmal glycogen did not differ between high and moderate CHO conditions. Glycogen utilisation during endurance cycling exercise also has a subcellular component, as depletion of intramyofibrillar glycogen has been linked to fatigue development through impaired SR Ca<sup>2+</sup> release, effecting contractile function (Marchand et al., 2007; Nielsen et al., 2011; Jensen et al., 2020; Jensen et al., 2021).



**Figure 2.2.** Muscle glycogen subcellular storage locations presented as a graphic (a) taken from Nielsen and Ørtenblad (2013) and from transmission electron microscope imaging (b) taken from Marchand et al. (2002), respectively.

## **2.3 Glycogen utilisation during endurance exercise**

Glycogen breakdown (glycogenolysis) occurs through the combined action of the key enzymes glycogen phosphorylase and glycogen debranching enzyme, which free glucose residues from the chained branches of  $\alpha$ -1,4 and  $\alpha$ -1,6 glycosidic bonds, respectively. Glycogen phosphorylase also catalyses formation of glucose-1-phosphate from glucose, which is then phosphorylated to glucose-6-phosphate by phosphoglucomutase feeding into glycolysis and subsequent downstream utilisation. During prolonged endurance exercise at intensities  $\geq 70\%$   $\dot{V}O_{2\max}$ , glucose is derived primarily from muscle glycogen (Romijn et al., 1993; van Loon et al., 2001), with substrate utilisation during exercise also regulated by exercise duration (Watt et al., 2002) and glycogen availability (Arkinstall et al., 2004). Considering this CHO dependence during endurance exercise (Hawley and Leckey, 2015; Leckey et al., 2016), and the link between intramyofibrillar glycogen depletion and muscular fatigue (Nielsen et al., 2011; Jensen et al., 2020), optimisation of endogenous *and* exogenous CHO availability is essential for promoting endurance exercise capacity and performance, with CHO availability manipulated at three key stages surrounding competition and high intensity training sessions.

## **2.4 Carbohydrates and endurance exercise performance**

### **2.4.1 Pre-exercise**

As previously described, CHO loading with a high to very high CHO intake for 36-48 h pre-competition can lead to an enhancement or supercompensation of muscle glycogen stores (further discussed in Chapter 4), which has been associated with increased endurance capacity by 20% (Hawley et al., 1997a) and, in some cases, enhanced endurance exercise performance (Karlsson and Saltin, 1971; Rauch et al., 1995; Table 2.2). The suggested mechanisms being increased glycogen availability leading to a delayed onset of fatigue, through liver glycogen

and/or intramyofibrillar glycogen sparing during exercise (Gonzalez et al., 2015; Jensen et al., 2020). However, as previously stated interpretation of CHO loading literature can be challenging for athletes, coaches and practitioners, as glycogen synthesis and/or concentration can be affected by multiple variables such as training status or sex, quantity of CHO ingested, duration increased CHO intake, exercise (duration and intensity) and other potential study methodological factors (exercise mode, muscle biopsy location or biochemical analysis technique). The effects on endurance exercise performance are also less clear than originally believed, as previous results have a risk of bias due to poor control for the placebo effect. Whilst those who accounted for this effect through double blinding reported no endurance cycling performance enhancement post-CHO loading (Burke et al., 2000; Tomcik et al., 2018).

In the acute pre-exercise period contemporary nutritional guidelines recommend ingestion of 1-4 g·kg<sup>-1</sup> of CHO 1-4 h pre-exercise (Thomas et al., 2016) with the exact quantity fine-tuned based on upcoming exercise demands. Exogenous CHO provision pre-exercise increases glycogen synthesis (Coyle et al., 1985) and endurance cycling performance by ~15% (Sherman et al., 1989), at least in overnight fasted conditions, where these recommendations are of particular importance due to suboptimal liver glycogen stores (Iwayama et al., 2021; Hargreaves et al., 2004). In such circumstances, there is potential for enhanced exercise capacity consuming multiple CHO sources (e.g. glucose + fructose; Podlogar et al., 2022) due to superior liver glycogen synthesis vs consumption of glucose alone (Décombaz et al., 2011).

CHO consumption 30-60 min pre-exercise is generally not recommended due to risk of rebound hypoglycaemia, where blood glucose concentrations rapidly drop <3.5 mmol·L<sup>-1</sup> within 30 min of exercise, which can cause unfavourable symptoms or metabolic perturbations (blunted hepatic glucose production and insulin mediated lipolysis inhibition). However, typically symptoms only occur in susceptible individuals and metabolic effects are transient,

with little scientific evidence of impaired performance (Jeukendrup and Killer, 2010; Sherman et al., 1991).

**Table 2.2.** Previous studies that investigated CHO loading and endurance exercise capacity or performance (> 90 min), adapted from Hawley et al. (1997a).

| Study                  | Exercise test                                    | CHO loading conditions                                                                                                                                                         | $\Delta$ Muscle glycogen | Performance improvement?                                                                                  |
|------------------------|--------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------|
| Ahlborg et al., 1967   | Cycling TTE at 85% of HR 170 b·min <sup>-1</sup> | 72 h <0.1 g·kg·day <sup>-1</sup><br>Normal/mixed<br>72 h >9.5 g·kg·day <sup>-1</sup>                                                                                           | +70%<br><br>+79%         | Yes; 64 min<br>66 min<br>72 min<br><br><i>P</i> < 0.01 for all                                            |
| Bergström et al., 1967 | Cycling TTE at 75% $\dot{V}O_{2\max}$            | 72 h <0.1 g·kg·day <sup>-1</sup><br>Normal/mixed<br>72 h ~8.3 g·kg·day <sup>-1</sup>                                                                                           | + 64%<br><br>+ 89%       | Yes; 57 ± 2 min<br>114 ± 5 min<br>167 ± 18 min<br><br><i>P</i> < 0.01 for all                             |
| Bosch et al., 1993     | 180 min cycle at 70% $\dot{V}O_{2\max}$          | Normal/mixed<br>72 h ~8.1 g·kg·day <sup>-1</sup>                                                                                                                               | +56%                     | Yes; only 50% of participants completed exercise in mixed                                                 |
| Brewer et al., 1988    | Running TTE at 70% $\dot{V}O_{2\max}$            | Comp. MCHO (4.6 g·kg·day <sup>-1</sup> )<br>Comp. HCHO (7.7 g·kg·day <sup>-1</sup> )<br>Simple MCHO (4.0 g·kg·day <sup>-1</sup> )<br>Simple HCHO (7.0 g·kg·day <sup>-1</sup> ) | Not reported             | Yes; 106 ± 24 min<br>133 ± 46 min<br>114 ± 16 min<br>141 ± 27 min<br><br><i>P</i> < 0.01 for MCHO vs HCHO |

|                              |                                          |                                                                                      |                                         |                                                                                                                    |
|------------------------------|------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| Burke et al.,<br>2000        | 100 km cycling<br>TT                     | 72 h 5.8 g·kg·day <sup>-1</sup><br>72 h 9 g·kg·day <sup>-1</sup>                     | +18%                                    | No; 148 ± 10 min<br>149 ± 11 min<br><i>P</i> > 0.05                                                                |
| Galbo et al.,<br>1979        | Running TTE at<br>70% $\dot{V}O_{2\max}$ | 96 h <0.1 g·kg·day <sup>-1</sup><br>96 h ~7.3 g·kg·day <sup>-1</sup>                 | +67%                                    | Yes; 64 ± 16 min<br>106 ± 13 min                                                                                   |
| Jensen et al.,<br>2020       | Cycling TTE at<br>75% $\dot{V}O_{2\max}$ | 72 h <0.1 g·kg·day <sup>-1</sup><br>Normal/mixed<br>72 h ~8.3 g·kg·day <sup>-1</sup> | +89%<br>+94%                            | Yes; 69 ± 18 min<br>112 ± 22 min<br>150 ± 30 min<br><i>P</i> < 0.001                                               |
| Karlsson and<br>Saltin, 1971 | 30 km running<br>race                    | Normal/mixed<br>72 h ~9 g·kg·day <sup>-1</sup>                                       | +94%                                    | Yes; 143 ± 20 min<br>135 ± 18 min                                                                                  |
| Lamb et al.,<br>1991         | Running TTE at<br>75% $\dot{V}O_{2\max}$ | 50% CHO Pasta<br>50% CHO Supplement<br>90% CHO Pasta<br>90% CHO Supplement           | +33.9%<br>pasta<br>+44.9%<br>supplement | Yes; 153 ± 13 min<br>139 ± 7 min<br>169 ± 8 min<br>168 ± 7 min<br><i>P</i> < 0.05 for both 50 vs<br>90% CHO groups |
| Tomcik et<br>al., 2018       | 120 km cycling<br>TT + TTE               | 48 h 6 g·kg·day <sup>-1</sup><br>48 h 12 g·kg·day <sup>-1</sup>                      | +25%                                    | No difference in TT<br>finish time or TTE<br><i>P</i> > 0.05                                                       |

|                       |                                                                           |                                                                                                                                                                                            |                          |                                                                                                                                                                 |
|-----------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Widrick et al., 1993  | 70 km cycling<br>TT                                                       | 48 h 2.3 g·kg·day <sup>-1</sup><br>48 h 6.5 g·kg·day <sup>-1</sup><br>48 h 2.3 g·kg·day <sup>-1</sup> + CHO<br>during exercise<br>48 h 6.5 g·kg·day <sup>-1</sup> + CHO<br>during exercise | +55%<br><br><br><br>+81% | Yes; 123 ± 7 min<br>119 ± 5 min<br>121 ± 5 min<br><br>117 ± 4 min<br><i>P</i> < 0.05 for both 6.5 vs<br>2.3 g·kg <sup>-1</sup> ·day <sup>-1</sup><br>conditions |
| Williams et al., 1992 | 30 km treadmill<br>running TT                                             | Habitual 5.1 g·kg·day <sup>-1</sup><br>72 h 8.6 g·kg·day <sup>-1</sup> + 96 h 6.9<br>g·kg·day <sup>-1</sup>                                                                                | Not<br>reported          | 138 ± 17 min<br>135 ± 17 min<br>(increased speed in last 5<br>km; <i>P</i> < 0.001)                                                                             |
| Rauch et al., 1995    | 2 h cycle at 75%<br>VO <sub>2max</sub> , 5 x 60s<br>sprints, 60 min<br>TT | 72 h 6.1 g·kg·day <sup>-1</sup><br>72 h 10.5 g·kg·day <sup>-1</sup>                                                                                                                        | +45%                     | Yes;<br>36.7 ± 3.6 km<br>38.0 ± 3.6 km<br><i>P</i> < 0.05                                                                                                       |

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#### ***2.4.2 During exercise***

The importance of CHO feeding during endurance exercise has been accepted since the seminal studies of Coyle et al. (1992) showed improved cycling exercise capacity with ingestion of glucose and glucose + fructose compared to a zero CHO group. The mechanisms being provision of additional CHO for ATP formation, resulting in delayed onset of fatigue by maintenance of euglycemia, maintenance of increased CHO oxidation and sparing of limited endogenous glycogen stores (Gonzalez et al., 2015; for detailed review see Stellingwerff and Cox, 2014), as well as a stimulatory effect on the central nervous system through CHO sensing within the oral cavity (Chambers et al., 2009). As such endurance athletes are recommended to consume 60-90 g·h<sup>-1</sup> of combined dual source CHO such as glucose and fructose at a ratio of 2:1 or 1:08, to utilise multiple transporters within the gut (SGLT-1 and GLUT 5, respectively) and achieve peak exogenous CHO oxidation rates >1.0-1.1 g·min<sup>-1</sup> (the saturation point of consuming glucose alone). As always, recommendations are dependent on exercise duration and intensity (Thomas et al., 2016), with recent suggestions of higher intakes (120 g·h<sup>-1</sup>) being superior for maximisation of exogenous CHO oxidation rates (1.6-1.8 g·min<sup>-1</sup>; Hearn et al., 2022). In contrast, recent work in endurance trained cyclists (Podlogar et al., 2025) found a personalised approach to CHO consumption during exercise to be effective, as participants achieved individual peak exogenous CHO oxidation rates through ingesting glucose alone at 50-80 g·h<sup>-1</sup>, without risking GI discomfort symptoms through pushing extreme CHO intakes and tolerance through ingestion of  $\geq 90$  g·h<sup>-1</sup>.

The specific CHO product (gel, fluid, chew or a combination) has little impact on exogenous CHO oxidation during prolonged submaximal exercise or endurance capacity (Hearn et al.,

2022). The effects of different CHO feeding frequencies of the same absolute CHO intake has rarely been considered, particularly in endurance cycling.

### ***2.4.3 Post-exercise***

Depletion or utilisation of existing muscle glycogen stores through an exercise stimulus acts as a strong driver for glycogen resynthesis (Bergström and Hultman, 1966; Bergström et al., 1967; Fairchild et al., 2002), which occurs in two phases. The initial insulin-independent acute phase, 0-4 h post-exercise, driven by lowered glycogen stores and continued post-exercise glucose uptake, is often considered a window of opportunity due to markedly increased glycogen synthesis rates, which can be further enhanced by CHO provision and associated insulin secretion (Prats et al., 2009; Price et al., 1994). The second more prolonged phase is insulin dependent and can last up to 48 h post-exercise (Mikines et al., 1988). Typically, provided CHO ingestion is adequate, glycogen stores are repleted to near baseline values within ~24 h post exercise (Bergström and Hultman, 1966). If time is limited (< 24 h) between endurance competitions (e.g., grand tour cycling) or high intensity training sessions and the aim is to maximise muscle and liver glycogen resynthesis to ensure optimal stores, athletes should consume  $1\text{--}1.2\text{ g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  for 4 h immediately post-exercise (Thomas et al., 2016) as a series of small meals (Burke et al., 2017) with the use of high glycaemic index foods (Burke et al., 1993) containing multiple transportable CHOs (e.g. glucose + fructose; Décombaz et al., 2011). However, in endurance sports this is not always the case, and in some circumstances, there is no requirement to optimise immediate post-competition resynthesis (e.g. next training session is low intensity). In such situations, consuming an appropriate CHO intake throughout the day ( $8\text{--}12\text{ g}\cdot\text{kg}^{-1}$  of CHO, based on competition exercise energy expenditure), irrespective of timing and type of CHO would likely be adequate (Burke et al., 2017).

## **2.5 Potential negative practical implications of carbohydrate loading**

### ***2.5.1 Gastrointestinal discomfort***

GI discomfort symptoms are commonly associated with high CHO intakes during exercise (Rehrer et al., 1992; Pfeiffer et al., 2012), as ingestion of  $> 60 \text{ g}\cdot\text{h}^{-1}$  of glucose can surpass absorptive capabilities of intestinal transporters (Jentjens et al., 2004), which in combination with restricted gut blood flow during moderate-high intensity exercise (Brouns and Beckers, 1993), can cause GI discomfort symptoms (particularly in individuals who are unfamiliar with high CHO intakes during exercise). Symptoms range from mild nausea, gas/flatulence, cramps or bloating to more severe such as diarrhoea or vomiting (Rehrer et al., 1992). However, in CHO loading research the potential effects of high dietary CHO intakes on GI comfort have seldom been considered (Lamb et al., 1991). Theoretically, symptoms would likely differ compared to CHO consumption during exercise, due to CHO loading being over a longer period (36-48 h), as appose to reaching upper limits of intestinal absorption during shorter exercise periods of restricted GI blood flow (2-6 h). However, this is of practical relevance, as persistent symptoms could carry over into competition and negatively impact performance (further discussed in Chapters 5 and 6).

### ***2.5.2 Increased body mass***

It is commonly accepted that CHO loading is associated with  $\sim 2\%$  increase in body mass, due to increased total body water (TBW) resulting from each 1 g of glycogen being bound to 3-4 g of water (Olsson and Saltin, 1970). Such a response could be unfavourable in endurance cycling, where a high power:weight ratio is important for performance. However, arguably the importance of higher glycogen availability for endurance exercise capacity, could outweigh the potential negative implications of a 2% increase in body mass, particularly considering the

increase would be transient, only occurring early during exercise as sweat losses and glycogen utilisation would decrease body mass throughout (Podlogar and Wallis, 2022). Furthermore, recent studies using BIA methodologies have shown mixed results, with TBW unaffected by CHO loading and increased glycogen concentrations (Schytz et al., 2023; Shiose et al., 2016). Ultimately, further work is required to determine the magnitude of the relationship between glycogen and body water (further discussed in Chapters 5 and 6).

## **2.6 Challenges of endurance performance testing**

Measuring endurance exercise performance is an integral part of sport science research, however producing accurate and reliable results can be challenging in laboratory conditions, as the level of control required to determine small worthwhile performance improvements can easily be lost through equipment error, lack of statistical power (sample size), experimental procedures or techniques implemented by researchers. For example, providing inconsistent warm-up periods or different levels of encouragement to participants could influence performance (Hopkins et al., 1999; Hopkins et al., 2001). Furthermore, control of potential confounding variables within study designs introduce variability and/or bias (e.g. nutritional status, fatigue state, alcohol or caffeine ingestion, laboratory temperature and humidity, participant and researcher blinding, control/placebo condition etc). Nonetheless, even when experimental controls are adequate, accurately and reliably measuring endurance cycling performance improvements can be difficult. Necessary considerations are discussed within the following sections and outlined in Table 2.3.

### ***2.6.1 Performance testing equipment***

Laboratory performance testing in cycling models typically use cycle ergometers, which measure power against a resistance (air, electromagnetic or friction). Ergometry is associated with systematic and random measurement error which can lead to differences of 0.5-2.0% (Maxwell et al., 1998) or even as high as 6-12% (Cumming et al., 1968) in actual vs displayed cycling power. Considering the finite differences between winning and losing at the elite level, where a 0.5-2% increase in power could be the difference between winning or losing (Paton and Hopkins, 2001; Hopkin et al., 1999), such error rates would disguise meaningful performance improvements leading to false study conclusions. As such, the importance of choosing an accurate type and model of ergometer, which has been appropriately calibrated is paramount (Paton and Hopkins, 2001).

### ***2.6.2 Performance exercise testing protocol***

Despite keen interest in endurance cycling performance, and the importance of accurate and reliable measures, there is no agreed upon optimal endurance cycling performance test. Over the last century, endurance exercise capacity tests have commonly been used (Christensen and Hansen, 1939; Bergström et al., 1967; Coyle et al., 1992; Hearris et al., 2022), where participants continuously cycle at a fixed submaximal workload until exhaustion, with total time used as an indirect performance marker. Results of such tests can be highly variable due to psychological factors (e.g. motivation or boredom), as within participant CVs reported in previous studies in cycling range from 17 to 40% (Jeukendrup et al., 1996; Krebs and Powers, 1989). Furthermore, generalisability of results is debatable, as in practice, athletes would never cycle at a single submaximal intensity to exhaustion. Nonetheless, it is a marker of endurance capacity, which is associated with endurance exercise performance.

To ensure accuracy and reliability of results, and study conclusions, researchers should choose validated endurance exercise performance protocols that have similarities to real world competition (Hopkins et al., 2001), unfortunately this is commonly not the case. However, there is an extensive body of work focussed on validating commonly used performance tests (Coggan and Costill, 1984; Doyle and Martinez, 1998; Hickey et al., 1992; Hopkins et al., 1999), whilst others have introduced and validated reliable cycling performance testing protocols (Jeukendrup et al., 1996; Schabert et al., 1998). For example, Jeukendrup et al. (1996) proposed a simulated cycling TT based on completing a set amount of mechanical work as quickly as possible (commonly referred to as work done TT), following a pre-load of steady state submaximal cycling, which reported strong repeatability of 3.5% (further details in Chapter 6). Further, the use of ‘real-world’ cycling performance tests, which incorporate 1-4 km sprint periods throughout a distance-based TT have also emerged (Burke et al., 2000; Tomcik et al., 2018), mimicking the stochastic nature of endurance competition, whilst reporting reproducible results (within participant CV of 1.7%; Schabert et al., 1998).

Despite this, choosing the most appropriate performance testing protocol can be challenging and is often determined by available resources and equipment. One of the most important considerations is choosing a performance protocol which is transferrable to the demands of competition (Paton and Hopkins, 2001). Not only to allow theoretical comparisons between laboratory and competitive performance, but also to incorporate familiarity within endurance trained participants who will understand appropriate pacing, promoting reliability of outcomes.

### ***2.6.3 Other potential sources of bias***

*Participants.* When measuring endurance cycling performance, as expected, more trained individuals produce greater reliability. Particularly if the chosen protocol requires individual

pacing or self-selection of exercise intensity. This is primarily due to familiarity, as trained individuals are accustomed to high intensity efforts, and pacing during training and competition, minimising variability (Hopkins et al., 2001).

*Learning effects.* It is well established that a prior familiarisation session is a crucial component of performance testing (Hibbert et al., 2017; Hopker et al., 2009; Borg et al., 2018). Familiarising participants with a specific performance testing protocol allows an opportunity to learn (or confirm) the appropriate pacing and feel the required effort (Corbett et al., 2009). Thereafter, the learning effect associated with completing the same task/test on multiple occasions is minimised during experimental conditions (Hibbert et al., 2017). Interestingly, Hopkins et al. (2001) reported a mean performance improvement of 1.2% from trial 1 to 2 across 30 studies of exercise performance. Whereas the increase from trial 2 to 3 was only 0.3%. Highlighting perhaps there is a need to familiarise participants on multiple occasions, to truly minimise learning effects. However, this is also likely influenced by participant familiarity with the type of exercise conducted in the performance test.

*Placebo effect.* Originally discovered in medical research in the mid-1900s (Beecher, 1955) the placebo effect is defined as a desirable outcome resulting from an expected response from a treatment (Beedie et al., 2018). It is also well-established to influence sports performance outcomes across numerous disciplines, including cycling (Clark et al., 2000; Beedie et al., 2006; Beedie and Foad, 2009; Foad et al., 2008). The exact mechanisms are currently unknown, however they are believed to be related to multiple neurobiological pathways, as different types of responses can occur through individual expectations and different inductions of the effect (Beedie et al., 2018). The well-established potency of this effect makes adequate control,

through experimental blinding of conditions, a fundamental requirement for research investigating sports performance. However, interestingly, almost all prior CHO loading research studies have failed to control for this (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967; Lamb et al., 1991; Bosch et al., 1993; Galbo et al., 1979; Jensen et al., 2020), which is of particular relevance considering high CHO diets have been associated with enhanced endurance cycling capacity for almost a century (Krogh and Lindhard, 1920; Christensen and Hansen, 1939; Bergström and Hultman, 1966; Bergström et al., 1967).

**Table 2.3.** List of desirable features for studies investigating physical performance mechanisms and quantifying athletic performance enhancement, adapted from Hopkins et al. (1999).

|                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Studies investigating mechanisms of physical performance | <p>Repeated measures design and analysis</p> <p>Sample size adequate to delimit effects that are substantial for the average subject</p> <p>Reliable performance test</p> <p>Familiarisation trials for the performance test</p> <p>Random sampling of participants from a defined population (e.g. endurance trained)</p> <p>Random assignment of subjects to treatment groups, balanced for subject characteristics that could interact with treatment</p> <p>Double blind presentation of treatments</p> <p>Balanced order of treatments and adequate washout period in crossover trials</p> <p>Control of conditions and behaviours (e.g. temperature, diet, training) that could affect performance/interact with treatment between subjects and trials</p> <p>Changes in appropriate variables assayed to explore mechanisms (e.g. muscle enzymes)</p> |
| Studies quantifying athletic performance enhancement     | <p>Sample size adequate to delimit effects that are substantial for the average subject</p> <p>Reliable performance test</p> <p>Selection of best possible athletes as subjects</p> <p>Special care with replication of training and diet between trials</p> <p>Conditions and behaviours mimic real life</p> <p>Design and analysis for individual differences</p> <p>Changes in appropriate variable assayed to exclude placebo effects in unblinded studies</p> <p>Magnitude of treatment effect and confidence limits stated and interpreted for athletes in competitive events</p>                                                                                                                                                                                                                                                                      |

## **2.7 Summary**

It has long been recognised that CHO play a crucial role in ATP production during endurance training and competition. The evolution of contemporary nutritional guidelines, and in particular CHO loading, have led to a wealth of knowledge regarding different strategies to promote CHO availability through manipulation of quantity, timing and type. However, guidelines could be considered generalised and vague, with variable glycogen concentrations reported in research making guidelines challenging to comprehend and apply in practice, particularly as study methodological differences introduce heterogeneity in results. As such, the following chapters within this thesis will attempt to clarify some of the questions surrounding this topic whilst assessing the importance of CHO loading for glycogen concentrations and endurance cycling performance.

## **Chapter Three:**

### **General methodology**

*This chapter provides general and conceptual information regarding methodologies used during data collection of upcoming experimental chapters.*

### **3.1 Location and ethics statement**

All experimental exercise testing sessions and biochemical analyses were conducted at Liverpool John Moores University, Byrom Street campus, Tom Reilly Building physiology and biochemistry laboratories. Ethical approval for all studies was granted by the Liverpool John Moores University Ethics committee in line with the Declaration of Helsinki.

### **3.2 Study participant characteristics**

General participant characteristics are displayed in Table 3.1. Participants for Study 1 and 2 (Chapters 5 and 6, respectively) were endurance trained cyclists, in line with characteristics outlined by De Pauw et al. (2013) and Decroix et al. (2016) for males and females, respectively. Study 3 participants were recreationally active but were not undertaking regular structured endurance training. All participants were healthy and not suffering with any musculoskeletal injuries or illnesses during experimental periods, which was confirmed using a physical activity and readiness to exercise questionnaire (PAR-Q). The general exclusion criteria across all studies included individuals aged <18 or >50 years old, smokers or chronic history of smoking, individuals with food allergies or intolerances and individuals following restrictive diets habitually (e.g. keto or low CHO diets). Following verbal and written information regarding study procedures and risks associated with participation, written informed consent was obtained from all study participants.

**Table 3.1.** General physiological characteristics for participants in experimental studies, presented as mean  $\pm$  SD.

|                                                              | Study 1 (n =11) | Study 2 (n = 9) | Study 3 (n = 20) |
|--------------------------------------------------------------|-----------------|-----------------|------------------|
| Age (years)                                                  | 24 $\pm$ 5      | 31 $\pm$ 10     | 24 $\pm$ 3       |
| Height (cm)                                                  | 174 $\pm$ 10    | 179 $\pm$ 8     | 181 $\pm$ 6      |
| Body mass (kg)                                               | 71.2 $\pm$ 12.0 | 78.3 $\pm$ 6.7  | 76.2 $\pm$ 7.5   |
| $\dot{V}O_{2\max}$ (mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) | 56 $\pm$ 6      | 63 $\pm$ 5      | 50 $\pm$ 4       |
| PPO (W)                                                      | 306 $\pm$ 54    | 367 $\pm$ 37    | 314 $\pm$ 31     |

### 3.3 Cardiovascular and respiratory measures

#### 3.3.1 Heart rate

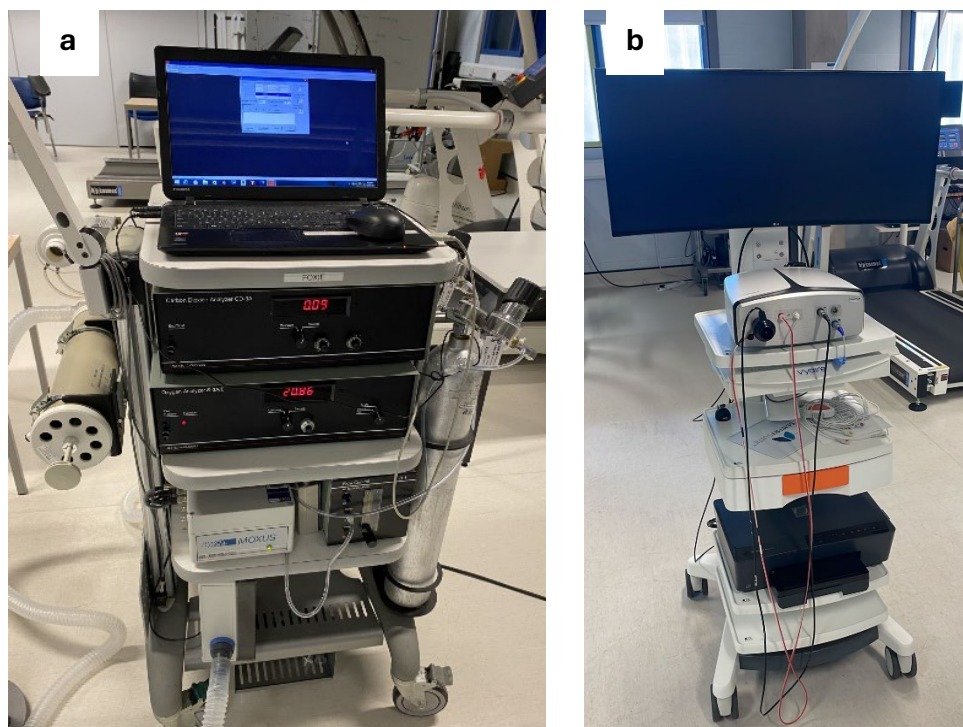
HR was collected during exercise in all studies using Bluetooth, wearable chest-strap Polar H10 monitors (Kempele, Finland), connected through the Polar mobile app.

#### 3.3.2 Collection of respiratory gases

Respiratory gases during exercise were collected using either a Moxus modular metabolic system (AEI Technologies, Illinois, USA) during Study 1 and 2, previously validated against gold standard Douglas bags (Rosdahl et al., 2013) or the Vyntus CPX (Vyaire Medical, Illinois, USA) in Study 3, which has previously shown acceptable error ( $< 5\%$ ) in measurement of respiratory variables (Van Hooren et al., 2024).

Metabolic carts were calibrated with a known concentration of  $O_2$  and  $CO_2$ , as well as a separate volume calibration prior to every testing session, with the Moxus calibrated to 0.0% volume error. When collecting expired air during experimental trials, a two-way Hans Rudolph valve and mouthpiece was used for the Moxus, whereas for the Vyntus a turbine and sample

line was attached to a mouthpiece. A mouthpiece was deemed more practical for repeated short 2-5 min collection periods. Whereas for determination of  $\dot{V}O_{2\max}$  in preliminary testing, a face mask was used to allow continuous collection of respiratory gases whilst participants cycled to volitional exhaustion. This ensured minimal disruption and allowed participants to breathe freely, without having to consciously hold the mouthpiece within the oral cavity.



**Figure 3.1.** Moxus (a) and Vyntus CPX (b) metabolic carts used for collection of respiratory gases during exercise sessions.

### ***3.3.3 Determination of substrate oxidation***

Whole-body CHO and fat oxidation during exercise was determined using stoichiometric equations outlined by Jeukendrup and Wallis (2005) for moderate to high intensity exercise, assuming negligible protein oxidation, as shown below:

$$\text{CHO oxidation (g} \cdot \text{min}^{-1}) = 4.210 \cdot \text{VCO}_2 - 2.962 \cdot \text{VO}_2$$

$$\text{Fat oxidation (g} \cdot \text{min}^{-1}) = 1.695 \cdot \text{VO}_2 - 1.701 \cdot \text{VCO}_2$$

## **3.4 Subjective and visual analogue scales**

Throughout experimental procedures multiple subjective scales were used pre-, post-, and during exercise sessions. Participants were familiarised with each scale prior to data collection to ensure understanding and promote meaningful subjective scoring.

### ***3.4.1 Rating of perceived exertion***

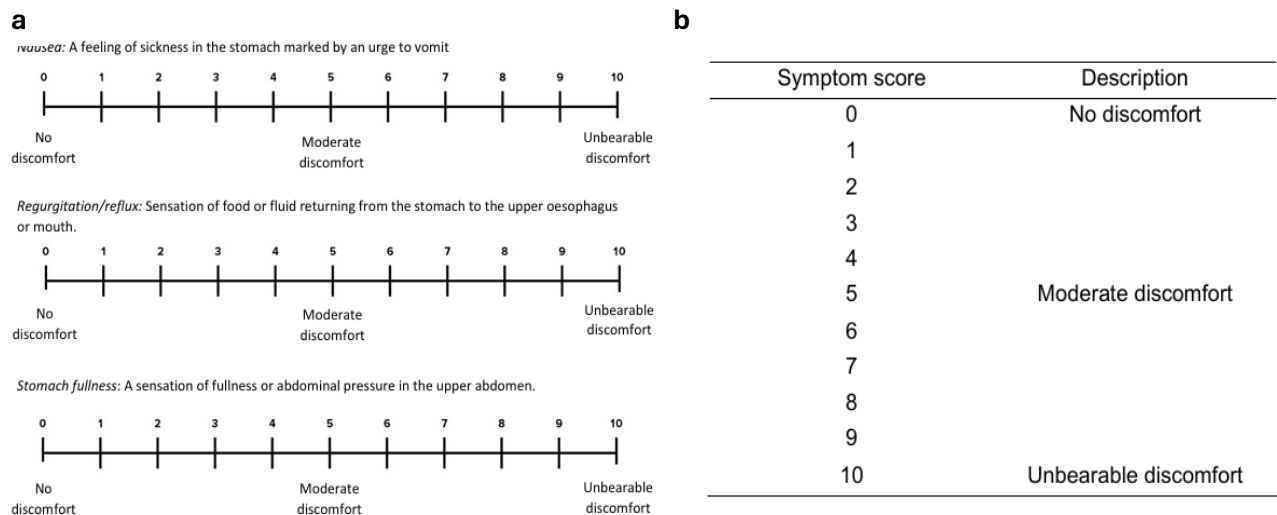
Subjective feelings of perceived exertion during exercise were scored on the Borg 6-20 RPE scale (Borg, 1982: Figure 3.2).

| Rating | Perceived Exertion |
|--------|--------------------|
| 6      | No exertion        |
| 7      | Extremely light    |
| 8      |                    |
| 9      | Very light         |
| 10     |                    |
| 11     | Light              |
| 12     |                    |
| 13     | Somewhat hard      |
| 14     |                    |
| 15     | Hard               |
| 16     |                    |
| 17     | Very hard          |
| 18     |                    |
| 19     | Extremely hard     |
| 20     | Maximal exertion   |

**Figure 3.2.** Borg 6-20 rating of perceived exertion scale used during exercise sessions.

### ***3.4.2 Gastrointestinal discomfort symptoms***

Subjective gut comfort was recorded on a 0-10 scale for symptoms of nausea, reflux, stomach fullness, abdominal cramps, flatulence and urge to defecate. Scores of 0, 5 and 10 indicated no discomfort at all, moderate and unbearable discomfort, respectively (Wilson, 2017). Symptoms were briefly described to participants to ensure comprehension (e.g. nausea; a feeling of sickness in the stomach marked by an urge to vomit). In Study 1 this scale was provided as a questionnaire (Figure 3.3a), whereas in Study 2 and 3 a 0-10 visual scale was used, as these scores were collected during exercise (Figure 3.3b)



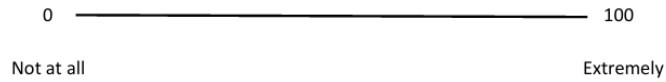
**Figure 3.3.** Gastrointestinal discomfort scales used during data collection in a questionnaire format (**a**) or as a visual scale used for scores collected during exercise (**b**).

### 3.4.3 Hunger and satiety

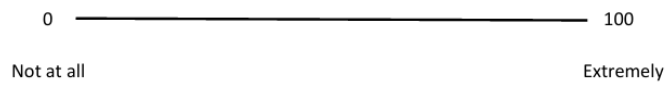
Hunger and satiety visual analogue scales were completed on a series of 0-100 mm lines which corresponded to ‘not at all’ and ‘extremely’, respectively. Participants were asked to draw a vertical line, based on how they felt corresponding to statements of; ‘I feel hungry’, ‘My stomach feels full’, ‘Desire to eat something savoury’, ‘Desire to eat something sweet’, ‘I feel physically tired’, ‘I feel sleepy/drowsy/half-awake’, ‘I feel energetic/active/lively’, ‘I feel lethargic/sluggish’ (Hengist et al., 2020; Figure 3.4).

Draw a straight vertical line along the 100 mm line, which corresponds to how you feel in relation to the statements below.

1. *'I feel hungry'*



2. *'My stomach feels full'*



**Figure 3.4.** Hunger visual analogue scale examples for statements of ‘I feel hungry’ and ‘My stomach feels full’.

### 3.5 Preliminary testing procedures

All preliminary testing sessions for determination of lactate thresholds, maximal oxygen uptake and PPO were identical across experimental studies. Upon arrival at the laboratory height and body mass were measured semi-nude (Seca, Hamburg, Germany), before participants were seated for resting measures of HR (Polar H10, Kempele, Finland), blood glucose and lactate using capillary fingertip blood samples, which were immediately analysed (Biosen C-Line, EKF Diagnostics, Cardiff, UK). Participants then completed incremental lactate threshold and  $\dot{V}O_{2\max}$  tests on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Briefly, participants began cycling at 75 or 100 W (females and males, respectively) and exercise intensity increased 25 W following each 4 min stage. In the final 30 s of each stage, a fingertip blood sample was collected and immediately analysed for blood glucose and lactate, with RPE

(Borg, 1982) and HR also collected in the final 10 s of each stage. The test was terminated when participants reached the onset of blood lactate accumulation  $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$  (Heck et al., 1985).

Following 10 min rest, participants returned to the ergometer to complete a  $\dot{V}\text{O}_{2\text{max}}$  test. The test began at 75 or 100 W and exercise intensity increased 25 W every 1 min until volitional exhaustion. Gas exchange was measured continuously throughout using a metabolic cart (Moxus [Study 1 and 2] or Vyntus CPX [Study 3]) with  $\dot{V}\text{O}_{2\text{max}}$  defined as the highest  $\dot{V}\text{O}_2$  sustained over a 30-s average. HR and RPE were collected in the final 10 s of each stage and were used as indirect markers of volitional exhaustion. PPO was determined to describe participant characteristics using the equation outlined by Kuipers et al. (1985):

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

Where  $W_{\text{final}}$  refers to the power output of the last complete stage, time refers to the time spent in the final incomplete stage (s), 60 is the duration of each stage (s) and PI refers to the power increase with each stage (25 W).

### **3.6 Muscle and blood sample collection and analysis**

#### ***3.6.1 Muscle sample collection and storage***

Muscle samples were collected from the *vastus lateralis* muscle in the upper lateral portion of the thigh using the Weil Blakesley Conchotome muscle biopsy technique (Henriksson, 1979; Figure 3.5). Prior to sample collection participants were supine and the collection site was sterilised. Local anaesthetic (2 mL, 0.5% Marcaine) was applied to the area, before a 5-10 mm incision was made through the skin and fascia to allow biopsy needles to reach muscle tissue.

The needle was inserted, the tip was opened and closed before being rotated through 90-180° to cut the muscle. Once collected, muscle samples were dissected of visible fat and connective tissue before being snap frozen in liquid nitrogen and stored at -80 °C for later analysis.



**Figure 3.5.** Weil Blakesley Conchotome muscle biopsy needle (**a**) taken from Patel et al. (2011) and image taken during muscle biopsy procedures during Study 1 data collection (**b**).

### ***3.6.2 Determination of muscle glycogen concentrations***

Whole muscle glycogen concentration was determined with acid hydrolysis (van Loon et al., 2000). Stored samples were freeze dried and dissected of visible blood, fat or connective tissue under a microscope (Zeiss, Germany). The remaining muscle mass (~ 5 mg) was weighed and split into duplicates for each timepoint. 500  $\mu$ L of 1 M hydrochloric acid was added to each sample, which was heated for 3 h at 95 °C in a water bath to hydrolyse glycogen to glycosyl units through the breakdown of glycosidic bonds which link glucose molecules together to form the glycogen granule. Samples were cooled at room temperature, before being neutralised with 250  $\mu$ L of Tris/KOH saturated with KCl. The remaining homogenate was separated through centrifugation, and the supernatant was analysed in duplicate for glucose using a commercially available kit (GLUC-HK; Randox Laboratories, Antrim, UK).

### ***3.6.3 Glycogen assay methodological improvements***

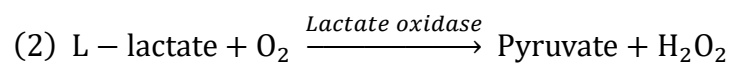
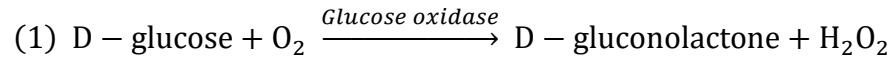
The first practice of the glycogen assay using pork muscle samples had unacceptably high coefficient of variation between duplicates of the same sample (20-30%), which continued when the assay was tested on human muscle which could not be used for the study (due to ergometer technical error). As such, key determinants of variability within the assay were investigated to improve laboratory procedures and confidence in study results. To determine where these issues occurred the assay was conducted on numerous occasions, systematically manipulating specific elements (Table 3.2), using standards derived from glycogen powder and/or directly from a glucose solution, as well as cuts of store-bought beef where glycogen concentration was previously described (Immonen et al., 2000).

Standards from glycogen powder were prepared based on the molecular weight of glycogen ( $162 \text{ g}\cdot\text{mol}^{-1}$ ), where 40.5 mg of bovine liver glycogen (Sigma-Aldrich, Merck, Germany) was mixed in 5 mL of distilled  $\text{H}_2\text{O}$  to achieve a concentration of 50 mM. In line with this, standards were created at a concentration of 800 mM and serial dilutions were used to achieve concentrations of 400, 200, 100 and 50 mM. Standards made directly from glucose solution ( $1 \text{ mg}\cdot\text{mL}^{-1}$  or 5.55 mM) were diluted 50%, with distilled  $\text{H}_2\text{O}$  or a 2:1 ratio of HCl:Tris KOH (as per assay procedures). Beef samples were freeze dried and treated with identical procedures to human muscle, to practice and fine tune the methodology before using precious human muscle samples.

### ***3.6.4 Blood samples collection and storage***

Capillary blood samples were collected using the finger prick method, where a lancet was used to pierce the skin on the fingertip to collect 20  $\mu\text{L}$  of blood in a capillary tube. Samples were then mixed with a known volume of haemolysing solution within a pre-filled micro-tube before

being analysed for glucose and lactate through enzymatic amperometric methods using a dual-channel Biosen C-line analyser (EKF Diagnostic, Cardiff, UK). Briefly, the analyser measures the following reactions of glucose oxidase (1) and lactate oxidase (2) which are immobilised on a microchip:



The resultant H<sub>2</sub>O<sub>2</sub> is detected by the electrode, and the current allows indirect measurements of whole blood glucose and lactate, as the analyser was calibrated every hour using a known concentration for both metabolites.

During Study 2, venous blood samples were collected using an indwelling cannula inserted into a superficial antecubital vein. Samples were collected in vacutainers containing lithium heparin (BD Biosciences, UK), which were immediately stored on ice, until completion of the exercise session, before being centrifuged (1500 g for 10 min at 4 °C) and the remaining plasma stored at -80 °C until analysis later.

### ***3.6.5 Analysis of muscle biopsy and blood plasma samples metabolites***

Randox Daytona<sup>+</sup> spectrophotometer (Randox Laboratories, Antrim, UK) was used to determine values of glucose from muscle supernatant (Study 1), and glucose, lactate and NEFA from plasma of blood samples collected during exercise (Study 2) using commercially available kits analysed in duplicate. The CV between duplicate samples were < 5%.

The spectrophotometer determined metabolite concentrations through measuring the intensity of light (photons) after it passed through the analysed samples within a cuvette. Briefly, a halogen lamp transmits a beam of light through a lens and prism creating spectrum. Dependent on the analysed metabolite, a wavelength was selected, and the photometer measured the photons absorbed within enzyme catalysed reactions for each metabolite. The analyser was calibrated in the morning prior to any measurement according to manufacturer instructions.

**Table 3.2.** Investigating variability within muscle glycogen assay procedures.

| Question                                                                                                                        | Method                                                                                                                                                                                                                                     | Outcome                                                                                                                                                                                                                                                                                                     |
|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Equipment issue or user error? (pipetting or weighing scales)                                                                   | <p>Pipetting checked with H<sub>2</sub>O at 500 <math>\mu</math>l (n = 10)</p> <p>Weighing scales accuracy assessed vs other scales within the laboratory across two days.</p>                                                             | <p>Pipettes were recently calibrated and accurately used (mean difference 1.7%).</p> <p>Both weighing scales were accurate within <math>0.0001 \pm 0.0002</math>g across repeated measurements.</p>                                                                                                         |
| Was there an issue with the Randox Daytona <sup>+</sup> ? Could the chemicals interfere with the enzymatic glucose measurement? | Glucose solution standards were diluted with distilled H <sub>2</sub> O and HCl: Tris/KOH mixture (concentration and volume as standard procedures) and analysed on the Randox Daytona <sup>+</sup> at concentrations of 2.73 mM (n = 20). | <p>Mean glucose was <math>2.70 \pm 0.09</math> mM, achieving a mean difference of 1.39%.</p> <p>Indicating no issue with the analyser for glucose measurements.</p>                                                                                                                                         |
| How robust was the assay to changes in HCl concentrations?                                                                      | Hydrolysis of liver glycogen powder standards with 0.5, 1 and 1.8 M HCl (n = 8 for each HCl concentration at glycogen concentrations of 800, 400, 200, 100 and 50 mM).                                                                     | <p>Mean difference of ~20% at each glycogen concentration with minimal effect of HCl concentrations. Discovered the formula converting glucose values to glycogen failed to account for additional volume (100 <math>\mu</math>L) of the standards themselves and glycogen powder purity was only ~85%.</p> |

|                                                                                   |                                                                                                                                                                                                                                                       |                                                                                                                                                            |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| How robust was the assay to changes in volumes of chemicals?                      | Conducted hydrolysis using beef, according to standard procedures (n = 10) and with -50% volume of HCl and Tris KOH (n = 10).                                                                                                                         | Glycogen values were comparable for both methods (mean CV $5.4 \pm 3.9$ and $4.7 \pm 2.6$ %, respectively).                                                |
| What extent did differences in sample treatment affect the CV between duplicates? | Conducted the assay as previously described using beef (n = 10). Two samples were manipulated to determine the effects of non-muscle material (connective tissue) and large weight difference between duplicates of the same sample (9.6 vs 15.4 mg). | CV between duplicates was substantially affected by both factors (34.2 and 34.6% respectively). Mean CV with removal of these factors was $5.9 \pm 4.2$ %. |

### 3.7 Dietary control, food provision and preparation

All calorie containing foods and fluids across experimental periods for Study 1 and 2 were provided precooked and weighed to the nearest 0.05 g, along with dietary instructions and a checklist. All meal plans were created using an online nutrition software (Nutritics, Dublin, Ireland), where online nutritional information was cross-checked against nutritional information provided on packaging for all food items. Participants were also asked to send photos of each meal at the time of consumption via online messenger to confirm adherence (WhatsApp, Meta, USA). The plans provided a healthy mixed diet in the form of usual meals (breakfast, lunch and dinner) with various snacks throughout each day. Meals and snacks were standardised as closely as possible between conditions and participants, with high CHO sources primarily manipulated to achieve different CHO loading conditions (e.g. orange juice, pasta, jam, or fruits). Example foods provided in Study 1 and 2 are presented in Figure 3.6.



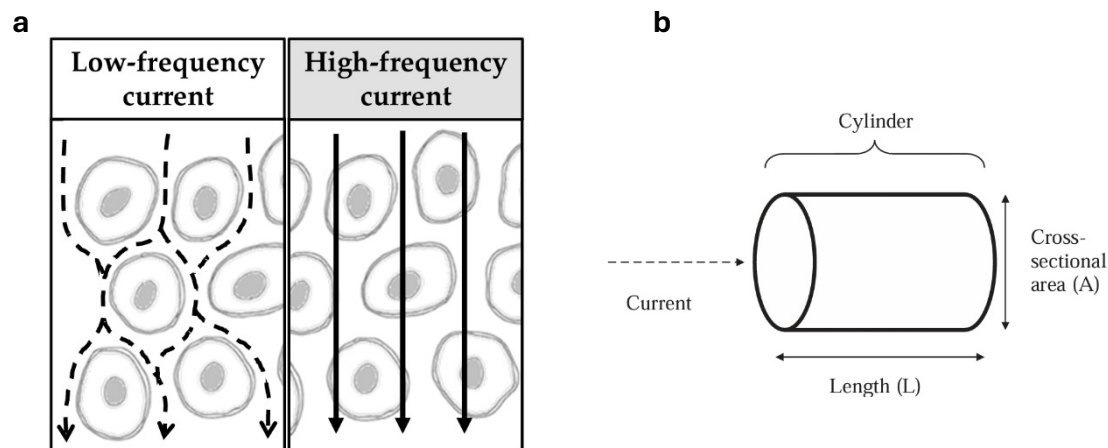
**Figure 3.6.** Example of foods provided during Study 1 and 2.

### **3.8 Measurements of body mass and water**

Body mass and water content post-CHO loading was measured using an eight electrode multifrequency bioelectrical impedance (BIA; Seca mBCA 515, Hamburg, Germany), previously validated in healthy males (Bosy-Westphal et al., 2013). Post CHO loading measurements were conducted semi-nude in an overnight fasted state, with participants asked not to consume any fluids in the morning, and to void their bladder pre-measurement.

Multifrequency BIA passes electrical currents through electrodes, in contact with the hands and feet, at different frequencies ranging from 0 to 1000 kHz with low frequencies flowing through extracellular space, whilst high frequencies flow through both intra and extracellular spaces (Shiose et al., 2022). The resistance to these currents combined with known length and cross-sectional area (Figure 3.7; height and lean body mass, respectively), create an impedance quotient which has a relationship with body water content, allowing determination of body composition and compartmental water distribution through empirical linear regression models

(Kyle et al., 2004a). All measurements were completed in line with recommendations for clinical applications outlined by Kyle et al. (2004b).



**Figure 3.7.** General principles of bioelectrical impedance indicating the effects of different electrical frequencies (a) and the general relationship used to determine body water content (b) taken from Shiose et al. (2022) and Kyle et al. (2004a), respectively.

### 3.9. Controlling the placebo effect

To measure endurance performance in Study 2 (Chapter 6), adequate control for the placebo effect through double blinding was considered a priority. To incorporate this, each experimental condition provided  $6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO as whole foods, with the remaining CHO for the 8 and  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  conditions provided as drinks (Maltodextrin, Bulk powders, UK), with composition determined by body mass. During the  $6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  condition, participants received zero calorie placebo drinks, consumed at the same timepoints. All drinks across conditions were colour and taste-matched using orange flavoured cordial and sucralose.

Pre-data collection drink compositions were extensively piloted using different groups of individuals who were ineligible to participate ( $n = 6$  per drink formula). Pilot participants were provided ~100 ml of a placebo, moderate and high CHO drink in a randomised order, each separated by ~5 min, before completing a questionnaire asking them to identify which group each drink belonged to, with options 'I don't know' and 'all drinks are the same' also available. The aim of the test was not explained to participants beforehand as they were simply asked to try the drinks and provide their opinion on CHO content. In the final test only 1 out of 6 participants correctly identified true CHO content of all drinks. As such this formula was chosen to conduct blinding (example for 70 kg participant presented in Chapter 6, Table 6.3).

To incorporate blinding of researchers conducting experimental procedures, a researcher outside the research team prepared drinks for all study participants and was the only individual aware of which trial code (A, B or C) corresponded to experimental conditions (6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup>). R.O.J led all data collection sessions and was the only individual aware of which trial code (A, B or C) was being completed at any given time. During data collection researchers were blinded to respiratory gas data and performance outcomes, which were blindly exported, to ensure double blinding was upheld.

To assess blinding efficacy following completion of all three trials, participants were asked to try and differentiate which trial corresponded to which CHO intake condition, with the option of 'I don't know' also available (Kolahi et al., 2009).

### **3.10. Statistical analysis**

Sample size estimations for experimental trials were conducted using G\*Power (v 3.1.9.7, Kiel, Germany). All statistical analyses were conducted using SPSS statistical software (v 29, IBM, Chicago USA). Analyses were checked for normal distribution using the Shapiro Wilks test,

combined with visual inspection of Q-Q plots. Non-normal data distributions were analysed with non-parametric tests (Wilcoxon signed rank or Friedman test). Normally distributed complete data sets were analysed using paired t-tests or repeated measures analysis of variance (ANOVA) with one or two independent variables of condition, or condition and time, respectively. Data sets with missing data points were analysed using linear mixed models, estimating missing data with either maximum likelihood or restricted maximum likelihood methods. Prior to analysis residuals were checked for normality as previously described. Figures were created using GraphPad Prism (v10, Massachusetts, USA). All values are presented as means  $\pm$  SD unless otherwise stated, with significance set at  $P < 0.05$ .

## **Chapter Four:**

# **Dietary CHO intake and skeletal muscle glycogen: A systematic review and meta-analysis**

## 4.1 Abstract

High dietary CHO intake in the days pre-competition (CHO loading) is recommended to optimise glycogen stores for endurance exercise performance. However, previous reports show high variability in muscle glycogen concentrations following similar CHO intakes, therefore the amount of CHO required to achieve a supraphysiological enhancement of glycogen stores remains unclear. As such, the aim of this systematic review and meta-analysis was to determine the relationship between dietary CHO availability and muscle glycogen, whilst exploring covariates and their impact on glycogen concentration. Studies were sourced from 5 electronic databases (December 2024) and reference lists of included articles. Included studies were published randomised or non-randomised trials that reported CHO intake and exercise for  $\geq 24$  h prior to collection of muscle samples using muscle biopsies to measure whole skeletal muscle glycogen concentration. The Cochrane RoB 2 tool was used to determine risk of bias. Included studies were assessed descriptively using tally markings and quantitatively using a generic inverse-variance random effects meta-analysis with subgroups used to investigate heterogeneity. Descriptive synthesis included 56 trials ( $n = 571$  participants), 17 were included within the meta-analysis. Linear regression indicated a significant linear relationship between relative CHO intake and whole muscle glycogen for recreationally active ( $r^2 = 0.351$ ,  $P < 0.001$ ) and endurance trained individuals ( $r^2 = 0.493$ ,  $P < 0.001$ ). Meta-analyses revealed increased dietary CHO intake from a low-moderate to high-very high quantity ( $<$  and  $> 6.5$  g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively) significantly increased muscle glycogen concentrations (198.8 mmol·kg<sup>-1</sup> DM, 95% CI from 148.0 to 249.7 mmol·kg<sup>-1</sup> DM,  $P < 0.0001$ ) but displayed significant heterogeneity ( $P < 0.0001$ ,  $I^2 = 94\%$ ). The delta between CHO intake conditions (expressed relative to body mass and as absolute values), duration of CHO loading interventions and participant training status all displayed a significant subgroup effect ( $P \leq 0.01$ ), but failed to explain significant heterogeneity, which likely resulted from the confounded

nature of these variables. Glycogen concentration primarily depends on quantity of CHO intake, with period of increased CHO intake, participant training status, and exercise all being potent effectors. Based on current findings to optimise pre-competition muscle glycogen stores athletes should consume  $> 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 36-72 h pre-competition, with the use of an exercise stimulus combined with consumption of high glycaemic CHO allowing more rapid enhancement of stores (24-36 h). However, significant heterogeneity and confounding in previous research introduce uncertainty in these recommendations, and further well-controlled studies are required to determine the true optimal pre-exercise CHO intake to maximise glycogen concentrations and endurance performance.

## 4.2 Introduction

Endogenous stores of dietary CHO, in the form of muscle glycogen, are the primary fuel source during moderate to high intensity endurance exercise (Romijn et al., 1993; Arkinstall et al., 2004). Limited stores within skeletal muscle and the liver (~400 and ~100-120 g, respectively, at rest under 'normal' physiological conditions) are rapidly depleted during endurance sports competitions, leading to impaired endurance exercise capacity due to fatigue and hypoglycaemia (Hearris et al., 2018; Bergström et al., 1967; Coyle et al., 1986). As such, endurance athletes increase dietary CHO intake and decrease training load in the days pre-competition to maximise glycogen stores within skeletal muscle, a strategy termed CHO loading (Bergström and Hultman, 1966; Bergström et al., 1967; Karlsson and Saltin, 1971).

The 'classic' CHO loading strategy was first introduced by Scandinavian researchers in the 1960s, where two exhaustive exercise sessions were separated by 72 h of low dietary CHO intake, followed by 72 h of rest and high CHO intake (~ 575 g·day<sup>-1</sup>). This resulted in supra-physiological muscle glycogen stores (~ 800 mmol·kg<sup>-1</sup> DM), a phenomenon termed muscle glycogen supercompensation (Bergström et al., 1967). CHO loading strategies have since been modified and updated, suggesting that shorter periods (24-48 h) of very high CHO intakes, without exhaustive exercise, can be equally effective in achieving greater muscle glycogen storage (Sherman et al., 1981; Bussau et al., 2002; Burke et al., 2017). As such contemporary nutritional guidelines recommend 8-12 g·kg<sup>-1</sup>·day<sup>-1</sup> for 36-48 h pre-endurance competition, to optimise muscle glycogen stores and subsequent endurance exercise performance (Thomas et al., 2016).

Despite over half a century of research, and the importance of a high CHO intake for supercompensation of muscle glycogen stores being well-established (Bergström et al., 1967; Sherman et al., 1981; Bussau et al., 2002), surprisingly a systematic review and meta-analysis that objectively quantifies the effectiveness of nutritional interventions to maximise muscle glycogen resynthesis has never been conducted. In a narrative review by Burke et al. (2017) CHO loading *per se* was not specifically addressed with only 5 studies included within the evolutionary explanation of CHO loading, all with relatively small sample sizes ( $n < 10$ ). Furthermore, interpretation of the literature can be challenging due to high variability in the magnitude of the relationship between dietary CHO intake and muscle glycogen, as studies that utilised similar CHO loading protocols ( $\sim 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 72 h) in endurance trained participants have reported glycogen values ranging from 450-800  $\text{mmol}\cdot\text{kg}^{-1}$  DM (Bergström et al., 1967; Blom et al., 1987; Kavouras et al., 2004; Roberts et al., 2016). The reason behind such high variability in the values reported are unclear, as well-established effectors of glycogen synthesis (CHO quantity, duration and training status) were matched (Costill et al., 1981; Hickner et al., 1997; Greiwe et al., 1999). Therefore, a better understanding of the effectors which influence glycogen stores is required. The aim of this systematic review and meta-analysis was to determine the relationship between dietary CHO intake and muscle glycogen concentration, and to characterise important covariates of muscle glycogen storage.

### 4.3 Methods

This systematic review and meta-analysis was registered on the Open Science Framework in December 2023 (<https://osf.io/bdasy/>). The Preferred Reporting Items for Systematic Reviews and Meta-analysis Statement (PRISMA) and Cochrane handbook were followed throughout the review protocol (Moher et al., 2015; Higgins et al., 2024). Peer reviewed, English written,

published journal articles were included, whilst conference proceedings were excluded due to insufficient information. The inclusion criteria were as follows:

- i) Randomised, counterbalanced or non-randomised control trials
- ii) Healthy human, male and/or female participants (aged 18-60 years)
- iii) Whole skeletal muscle glycogen reported as means  $\pm$  SD or SE, in mmol $\cdot$ kg<sup>-1</sup> DM or calculable as such, analysed from muscle samples obtained using muscle biopsies and biochemical analysis.
- iv) CHO intake (relative to body mass [g $\cdot$ kg<sup>-1</sup> $\cdot$ day<sup>-1</sup>] or calculable as such) and exercise status reported for  $\geq$  24 h pre-biopsy.

Any studies that failed to meet the above criteria were excluded. No exclusion criteria were specified for muscle biopsy location, yet only *vastus lateralis* and *gastrocnemius* biopsies surpassed the screening process. Non-randomised trials were included to incorporate all relevant information within qualitative and descriptive synthesis.

#### ***4.3.1 Search strategy and yield***

Five online databases (PubMed, Web of Science, Cochrane, SportDiscus and Scopus) were independently searched by two researchers, in February 2022 and December 2024, using the following combination of key words; (CHO OR carbohydrate OR maltodextrin OR glucose OR sucrose OR fructose) AND (Load\* OR supercompensat\*) AND (muscle OR glycogen OR stores OR storage) AND (concentration OR content OR synthesis OR resynthesis OR utilization OR accumulation). No database filters or limits were used to include all potential studies within the screening process. Full search records were exported to Endnote citation manager (v 20, Philadelphia, USA) for title and abstract screening.

#### **4.3.2 Data extraction**

Articles which surpassed title and abstract screening were read in full, with imperative study data extracted onto a custom Microsoft Excel spreadsheet (Microsoft, California, USA). In line with the PICOS terms (Population, Intervention, Comparison, Outcome), data surrounding participants (sample size, sex, age, training level,  $\dot{V}O_{2\max}$ ), CHO loading interventions (glycogen depletion protocol, relative and absolute CHO intake, CHO type [food vs supplements and glycaemic index], loading protocol duration, exercise conducted during loading days), study outcome (whole muscle glycogen) and study design were all extracted. Other potentially important variables, such as muscle biopsy method (Bergström, Conchotome or gun) and location, biochemical analysis technique, and study dietary control method were also extracted. Participant training status/performance level was categorised using criteria outlined by De Pauw et al. (2013) and Decroix et al. (2016) for males and females respectively. Decisions were made based on available information reported within published articles. If limited information was available, provided  $\geq 2$  of the performance level criteria were met, participants were categorised accordingly. If no information was available to provide a judgement, author description was accepted. It was noted whether studies reported  $\dot{V}O_{2\max}$  or  $\dot{V}O_{2\text{peak}}$ , however for training status classification, both were considered  $\dot{V}O_{2\max}$  (Day et al., 2003).

All muscle glycogen values were presented as means  $\pm$  SD or SE, either in text, tables or figures. To quantify data points presented exclusively in figures ( $n = 16$  studies), an online plot digitizer was used (Plot Digitizer, POrbital, Mumbai, India), which was tested for accuracy using studies containing data presented in text and figures, which generated a mean difference of  $2.2 \pm 5.5$  and  $5.5 \pm 7.5$  mmol $\cdot$ kg $^{-1}$  DM for studies originally presented in mmol $\cdot$ kg $^{-1}$  DM and those converted from mmol $\cdot$ kg $^{-1}$  wet mass to DM, respectively (Adamo et al., 1998; Bussau et al., 2002; James et al., 2001; Fairchild et al., 2002; Sherman et al., 1981; Bradley et al., 2016;

Fell et al., 2021; Hawley et al., 1997b; McLay et al., 2007). Data not presented as  $\text{mmol}\cdot\text{kg}^{-1}$  DM was converted using factors outlined by Areta and Hopkins (2018). Of studies included within descriptive synthesis, glycogen values were reported as  $\text{mmol}\cdot\text{kg}^{-1}$  wet mass ( $n = 25$ ), grams per 100g of wet mass (Bergström et al., 1967; Kochan et al., 1979), grams per kg of wet mass (Sasaki et al., 1991) or  $\mu\text{mol}$  per gram of wet mass (Nicklas et al., 1989).

#### ***4.3.3 Descriptive methods***

The literature search and descriptive synthesis aimed to collect robust evidence within the topic, whilst describing different participant and methodological characteristics between studies. As such descriptive analysis used tallies of extracted data, which was conducted in two stages by an individual researcher. The first stage focussed on study and participant characteristics whilst the second focussed on differences between study exercise interventions, CHO loading protocols and muscle biopsy measures. Tallies were totalled and repeated by the same researcher to ensure accuracy, with discrepancies repeated a third time. To compare differences in CHO intake a low, moderate, high and very high relative CHO intake was defined as  $< 3$ ,  $3\text{--}6.5$ ,  $>6.5\text{--}9$  and  $>9 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (adapted from Areta and Hopkins, 2018).

To visually describe the relationship between dietary CHO intake (relative and absolute) and skeletal muscle glycogen, simple linear and nonlinear 2<sup>nd</sup> degree polynomial regressions were conducted using information included within descriptive synthesis. Individual study groups/conditions were plotted based on CHO intake during the intervention and muscle glycogen, and separated by participant training status (recreationally active, endurance trained and well trained). Study conditions that received additional interventions that significantly influenced muscle glycogen concentrations, such as multiple biopsies (Costill et al., 1988), creatine ingestion (Tomcik et al., 2018), delayed CHO refeed (Bradley et al., 2017) or repeated

glycogen depletion (McInerney et al., 2005) were excluded, whilst control/placebo conditions were included provided inclusion criteria were satisfied. Sensitivity analyses were conducted to determine effects of including all types of study design within the same regression model and to determine the best fit nonlinear polynomial model (see Chapter 10 Appendices; supplementary material).

#### ***4.3.4 Quantitative meta-analysis methods.***

To quantify meaningful differences between dietary CHO intake and skeletal muscle glycogen a refined inclusion criteria was introduced to primarily include the most robust study protocols with the lowest risk of bias within the meta-analysis. As such studies were required to be randomised control trials where a low-moderate CHO intake control was compared against an intervention group which consumed a high-very high relative CHO intake (0-6.5 versus > 6.5 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively). CHO intake was required to be consistent throughout the loading period with total loading duration matched across conditions (e.g. 3 g·kg<sup>-1</sup>·day<sup>-1</sup> for 48 h vs 8 g·kg<sup>-1</sup>·day<sup>-1</sup> for 48 h).

The meta-analysis was conducted using a general inverse-variance random effects model on RevMan (v 5.4.1, Copenhagen, Denmark), where a mean difference and standard error of the mean difference was calculated for each pairwise comparison. As magnitude of treatment effect can be influenced by experimental design, parallel group and crossover trials were analysed separately (Morris and DeShon, 2002). For crossover trials (n = 11) dependency was accounted for using previously described methods (Elbourne et al., 2002). Two studies reported individual participant data in the original articles (Rauch et al., 1995; Walker et al., 2000), all other authors were contacted, and a further two authors provided individual participant data (Kavouras et al., 2004; Schytz et al., 2023). For the remaining seven crossover trials, a pooled correlation

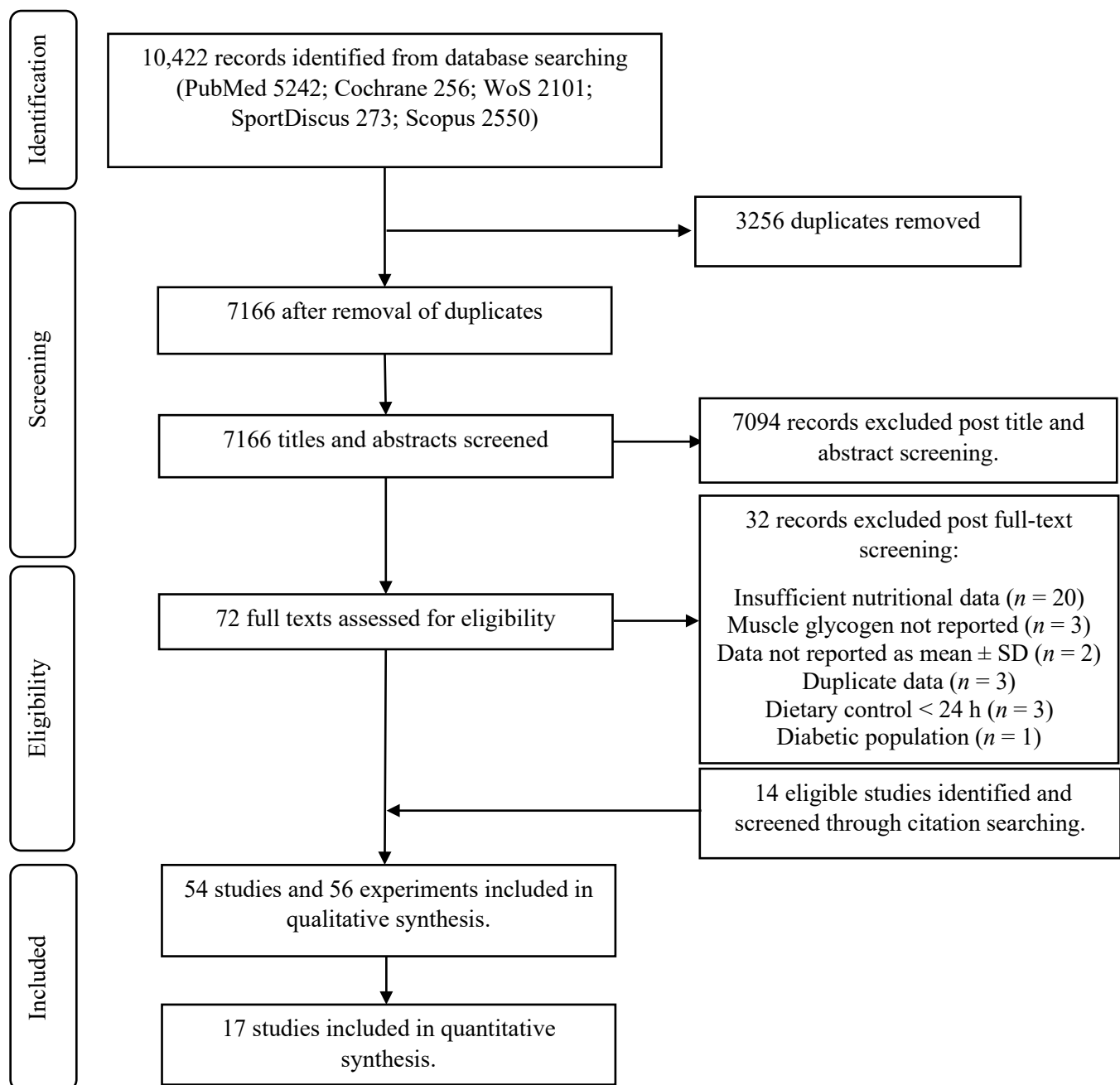
coefficient was used based on the available calculated correlation data (Kavouras et al 2004; Schytz et al., 2023; Rauch et al., 1995; Walker et al., 2000). Two mixed design trials were included within the quantitative synthesis, where two separate groups (males and females) were compared across repeated measures of manipulated CHO intake. Groups within the same defined category of CHO intake were combined using previously described methods (Chapter 10 supplementary material; Higgins et al., 2024) and pairwise comparisons were then treated as crossover (Tarnopolsky et al., 2001) or parallel group trials where appropriate (Tarnopolsky et al., 1995). Sensitivity analyses were conducted to determine the effects of choosing a fixed vs random effects model and ensure borrowed correlation coefficients did not significantly impact results (see Chapter 10; Table 4.6).

Authors pre-identified the differences in CHO intake between conditions (expressed as relative and absolute intake), duration of manipulated CHO intake (loading duration), participant training status and sex, and muscle biopsy location as variables that could explain predicted study heterogeneity, which was explored with subgroup analyses. Pre-analysis, subgroup comparisons between different defined levels of CHO intake (e.g. low vs high, moderate vs high, moderate vs very high etc) were planned, however this analysis was not deemed appropriate due to vastly uneven covariate distributions (2, 3, 5 and 11 comparisons included within each subgroup) and impaired sensitivity. Therefore, subgroups for the delta ( $\Delta$ ) in relative and absolute CHO intake between low-moderate vs high-very high CHO loading interventions were explored, with subgroups specified at 1 g·kg<sup>-1</sup>·day<sup>-1</sup> increments from 1.5 g·kg<sup>-1</sup>·day<sup>-1</sup> and 100 g increments from 100 g·day<sup>-1</sup> of CHO, respectively. These increments were pre-determined based on the possibility to result in a small but worthwhile change in glycogen. Number of subgroups were determined post hoc as this was dependent on study interventions. Significant heterogeneity was set at  $P < 0.1$  to account for multiple comparisons, and the variability in the effect estimate caused by heterogeneity was described using the  $I^2$

statistic, with scores of 0-40, 30-60, 50-90 and 75-100% corresponding to might be important, moderate, substantial and considerable heterogeneity respectively (Higgins et al., 2024).

#### ***4.3.5 Risk of bias assessment.***

All trials were assessed for risk of bias using the Cochrane risk of bias tools (RoB 2) for crossover and parallel group trials (Sterne et al., 2019). The bias assessment was conducted on a whole study level, where six domains were assessed on use of randomisation, carryover effects, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of reported results. Each domain contained a series of signalling questions answered ‘yes’, ‘probably yes’, ‘no’, ‘probably no’ or ‘no information’. The risk of bias categorised for each domain (‘low risk’, ‘some concern’ or ‘high risk’) was judged by researchers and later confirmed by a pre-determined algorithm. Overall risk of bias was based on the highest risk score across all domains. Assessments were completed by two researchers independently (R.O.J and J.B.L) with results compared, and any disagreements discussed until an agreement was reached.



**Figure 4.1.** PRISMA flowchart showing each stage of the literature search

## 4.4 Results

### 4.4.1 Descriptive synthesis

*Study selection and characteristics.* Figure 4.1 displays the search yield and each stage of the search in a PRISMA flow chart. The search discovered 10,422 potential articles across five electronic databases. Following removal of duplicates and title and abstract screening, 72 articles were read in full, with a further 32 excluded due to failure to meet the inclusion criteria. Fourteen additional studies were identified through citation searching of all included full texts and existing narrative reviews within the area (Burke et al., 2004; Hargreaves et al., 2004; Burke et al., 2017). Therefore, 54 published studies containing 56 experiments were included in the qualitative and descriptive analysis (henceforth terms studies, trials or experiments encompass all 56 included protocols; experiments from the same journal article are differentiated by <sup>[1]</sup> and <sup>[2]</sup> corresponding to the order presented within original articles [Blom et al., 1987; Costill et al., 1981]). Thirty-seven experiments were randomised, 22 of which used a repeated measures or crossover design, with the remaining 10 and 5 parallel groups or mixed study designs, respectively. The remaining 19 trials were not randomised (15 repeated measures, 2 parallel groups and 2 mixed design). Only seven experiments incorporated blinding, as 3 studies blinded participants only (Bradley et al., 2017; Flynn et al., 1987; Hill et al., 2013) and 4 were double-blinded (Burke et al., 2000; Foskett et al., 2008; Tarnopolsky et al., 2001; Tomcik et al., 2018). Study and participant characteristics are reported in Table 4.1.

*Participants.* The descriptive synthesis included 571 participants in total, primarily aged 18-30 years (80.4% of experiments), with only one study reporting participant mean age > 35 years old ( $37 \pm 7$  years; Doering et al., 2019). Only 5.4% of experiments included solely female participants (McLay et al., 2007; Nicklas et al., 1989; Walker et al., 2000) whilst 16.0%

recruited both males and females either as combined (Doering et al., 2019; Greiwe et al., 1999; Jansson and Kaijser, 1982; Vandenberghe et al., 1995) or separate groups (Impey et al., 2020; James et al., 2001; Tarnopolsky et al., 1990; Tarnopolsky et al., 1995; Tarnopolsky et al., 2001). The remaining 78.6% included males exclusively. Participants were primarily endurance trained or well-trained trained as 41.1 and 21.4% of trials recruited participants at performance level 3 and 4, respectively (De Pauw et al., 2013; Decroix et al., 2016). 25% recruited recreationally active individuals, whilst the remaining 12.5% had sedentary participants (Duhamel et al., 2006; Kochan et al., 1979), elite team sport athletes from rugby and ice hockey (Akermark et al., 1996; Bradley et al., 2016), or compared untrained vs recreationally active, trained or well-trained participants (Blom et al., 1987<sup>[1]</sup>; Hickner et al., 1997; Greiwe et al., 1999).

*CHO loading interventions (dose, duration and type).* Twenty-six studies compared experimental conditions of different defined levels of CHO intake (2 compared low vs moderate [Jansson and Kaijser, 1982; Widrick et al., 1993], 4 low vs high [Bergström et al., 1967; Duhamel et al., 2006; Galbo et al., 1979; Kavouras et al., 2004], 1 compared low vs very high [Arkinstall et al., 2004], 11 moderate vs high [Adamo et al., 1998; Akermark et al., 1996; Bosch et al., 1996; Burke et al., 2000; MacDougall et al., 1977; McLay et al., 2007; Sasaki et al., 1991; Sherman et al., 1981; Tarnopolsky et al., 1990; Vandenberghe et al., 1995; Walker et al., 2000], 7 compared moderate vs very high [Bussau et al., 2002; Doering et al., 2019; Hawley et al., 1997b; McInerney et al., 2005; Rauch et al., 1995; Schytz et al., 2023; Tomcik et al., 2018] and 1 compared high vs very high CHO [Burke et al 1995]). A further 6 studies (Blom et al., 1987<sup>[1]</sup>; Bradley et al., 2016; James et al, 2001; Lamb et al., 1991; Nickas et al., 1989; Lamb et al., 1991) compared different CHO intakes however expressed relative to body mass ( $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) conditions fell within the same defined category (i.e. moderate vs moderate).

Only three trials included multiple conditions of varied CHO intake in a dose-response type study design (Costill et al., 1981<sup>[2]</sup>; Tarnopolsky et al., 1995; Tarnopolsky et al., 2001). Nineteen studies compared the exact same relative CHO intake across conditions. Manipulation of CHO intake/CHO loading was not an aim of these studies, but rather a controlled factor. Trials were included within descriptive synthesis as all inclusion criteria were met and information was relevant for between study comparisons, provided the primary study intervention did not significantly impact glycogen concentrations (if this was the case, only the control conditions were considered).

The most common CHO loading/dietary control duration was 48 and 72 h (12 and 18 trials, respectively; Table 4.1). Twelve trials used 24 and 96 h loading periods (6 each), whilst others used less common durations of 36, 70 or 84 h. A further 4 experiments had periods  $\geq 5$  days (Jansson and Kaijser, 1982; Kirwan et al., 1988; Roberts et al., 2016; Walker et al., 2000), whilst 5 studies used different periods of dietary control between conditions, where a control/baseline period of moderate CHO intake and a muscle biopsy was followed by experimental conditions (Doering et al., 2019; McInerney et al., 2005; Sherman et al., 1983; Vandenberghe et al., 1995). Only 12 studies collected muscle biopsy samples across multiple consecutive loading days (Adamo et al., 1998; Blom et al., 1987<sup>[1]</sup>; Blom et al., 1987<sup>[2]</sup>; Bradley et al., 2017; Bussau et al., 2002; Costill et al., 1981<sup>[1]</sup>; Hickner et al., 1997; Kirwan et al., 1988; Kochan et al., 1979; Roberts et al., 2016; Sherman et al., 1981; Sherman et al., 1983).

CHO type was poorly described, as 23 out of 56 trials did not provide enough information regarding CHO consumed to judge glycaemic index. Twenty-six studies provided mixed diets containing both high and low glycaemic index foods, whilst 5 trials provided exclusively high glycaemic index CHO sources (Burke et al., 1995, Burke et al., 1996; Bussau et al., 2002; Fairchild et al., 2001; Galbo et al., 1979). Only two trials compared different types of CHO sources (Burke et al., 1993; Costill et al., 1981<sup>[1]</sup>).

Overall, dietary interventions were well controlled, as 83.9% of studies provided participants with food items and meals. However, only 10.6% of these studies measured and reported actual dietary compliance. The remaining 9 experiments (16.1%) either did not specifically state how dietary intake was controlled (Bosch et al., 1996; Costill et al., 1981<sup>[1]</sup>; Costill et al., 1981<sup>[2]</sup>; Jansson and Kaijser, 1982; Kochan et al., 1979; Sherman et al., 1983) or provided participants with meal plans (Flynn et al., 1987; Hawley et al., 1997b; Nicklas et al., 1989). Only 17 studies allowed exercise during the loading period (30.4%), these sessions were primarily light-moderate intensity steady state sessions, short duration sessions designed to mimic a pre-competition taper or described as ‘habitual’ training. In the remaining 69.6%, participants were rested throughout the loading period.

*Exercise interventions and muscle biopsy information.* Exercise pre-loading was very common (43 out of 56 studies), with some studies allowing participants to continue habitual training which was standardised between conditions (Akermark et al., 1996; Bradley et al., 2016; Burke et al., 2000; Kirwan et al., 1988), however most studies utilised specific glycogen depletion protocols (39 out of 43). Four studies had baseline glycogen measurements or control conditions that did not complete depleting exercise, and experimental conditions that did (Doering et al., 2019; Kochan et al., 1979; McInerney et al., 2005; Nelson et al., 2001). Cycling was the primary exercise mode as 72.3% of protocols were in cycling models. The remaining 21.3 and 6.4% of studies were in running or other mixed protocols, respectively. In line with this, 48 trials collected muscle biopsy samples from the *vastus lateralis*, whilst 7 out of 56 took samples from the *gastrocnemius*, all of which were running models (Blom et al., 1987<sup>[1]</sup>; Blom et al., 1987<sup>[2]</sup>; Costill et al., 1981<sup>[1]</sup>; Costill et al., 1981<sup>[2]</sup>; Kirwan et al., 1988; Sherman et al., 1981; Sherman et al., 1983). Impey et al. (2020) was the only study to collect samples from both locations.

*Muscle glycogen.* Participant baseline glycogen concentrations were reported by 9 trials (Arnall et al., 2007; Blom et al., 1987<sup>[1]</sup>; Bussau et al., 2002; Fairchild et al., 2002; Goforth et al., 1997; James et al., 2001; Kochan et al., 1979; Lamb et al., 1991; Nelson et al., 2001) with glycogen values ranging from 367.1 to 487.2 mmol·kg<sup>-1</sup> DM.

Recreationally active individuals demonstrated a significant linear relationship between relative CHO intake and whole muscle glycogen ( $F_{1,37} = 20.04$ ,  $r^2 = 0.351$ ,  $P < 0.001$ ; Figure 4.2a). This increased in endurance trained individuals, as relative CHO intake predicted 49.3% of the variation in muscle glycogen ( $F_{1,72} = 69.91$ ,  $P < 0.001$ ; Figure 4.2b), with results consistent across second-degree polynomial models ( $r^2 = 0.351$ ,  $P < 0.001$  and  $r^2 = 0.520$ ,  $P < 0.001$ , for recreationally active and endurance trained, respectively). Whereas simple linear regression for well-trained participants indicated that relative CHO intake only predicted 11.0% of the variation in muscle glycogen ( $F_{1,22} = 2.71$ ,  $P = 0.11$ ; Figure 4.2c), which was also consistent in a second-degree polynomial model ( $r^2 = 0.123$ ,  $P = 0.25$ ; Figure 4.2f).

Regression results were consistent for absolute CHO intake, as recreationally active ( $F_{1,35} = 13.35$ ,  $r^2 = 0.276$ ,  $P < 0.001$ ; Figure 4.3a) and endurance trained individuals ( $F_{1,72} = 62.30$ ,  $r^2 = 0.464$ ,  $P < 0.001$ ; Figure 4.3b) had a significant linear relationship between absolute CHO intake and muscle glycogen. Results were consistent across second-degree polynomial regression models ( $r^2 = 0.283$ ,  $P = 0.003$  and  $r^2 = 0.500$ ,  $P < 0.001$ , respectively). For well-trained participants absolute CHO intake only predicted 6.1% of the variance in glycogen concentration ( $F_{1,21} = 1.36$ ,  $P = 0.257$ ; Figure 4.3c), which was maintained in a second-degree polynomial model ( $F_{2,20} = 0.65$ ,  $r^2 = 0.061$ ,  $P = 0.53$ ; Figure 4.3f).

**Table 4.1.** Summary of studies included within descriptive synthesis, outlining experimental conditions, participant characteristics, CHO loading interventions (CHO intake [relative and absolute] and duration), muscle biopsy location and post-load muscle glycogen concentration. Data presented as means  $\pm$  SE.

| Study                      | Experimental condition                                      | Participant info |                    | Depletion<br>exercise | CHO intake                                           |                                    | Duration | Biopsy<br>location | Whole muscle<br>glycogen<br>(mmol·kg <sup>-1</sup> DM) | Significance                                                    |
|----------------------------|-------------------------------------------------------------|------------------|--------------------|-----------------------|------------------------------------------------------|------------------------------------|----------|--------------------|--------------------------------------------------------|-----------------------------------------------------------------|
|                            |                                                             | n and<br>sex     | Training<br>status |                       | Relative<br>(g·kg <sup>-1</sup> ·day <sup>-1</sup> ) | Absolute<br>(g·day <sup>-1</sup> ) |          |                    |                                                        |                                                                 |
| Adamo et al.,<br>1998      | LCHO                                                        | 9 M              | PL 3               | Yes                   | 3.4                                                  | 262                                | 48 h     | VL                 | 294.0                                                  | <i>P</i> < 0.05<br>between conditions                           |
|                            | vHCHO                                                       |                  |                    |                       | 8.2                                                  | 640                                |          |                    | 480.0                                                  |                                                                 |
| Akermark et al.,<br>1996   | MCHO                                                        | 16 M             | PL 5               | Yes                   | 6.2                                                  | 498                                | 72 h     | VL                 | 352.4 ± 30.5                                           | <i>P</i> < 0.05<br>between conditions                           |
|                            | HCHO                                                        | 16 M             | PL 5               |                       | 8.4                                                  | 675                                |          |                    | 430.7 ± 30.5                                           |                                                                 |
| Arkinstall et al.,<br>2004 | LCHO                                                        | 7 M              | PL 3               | Yes                   | 0.7                                                  | 56                                 | 48 h     | VL                 | 180.0 ± 17.0                                           | <i>P</i> < 0.001 between low<br>and very high CHO<br>conditions |
|                            | LCHO                                                        |                  |                    |                       | 0.7                                                  | 56                                 |          |                    | 223.0 ± 25.0                                           |                                                                 |
|                            | vHCHO                                                       |                  |                    |                       | 10                                                   | 803                                |          |                    | 591.0 ± 34.0                                           |                                                                 |
|                            | vHCHO                                                       |                  |                    |                       | 10                                                   | 803                                |          |                    | 601.0 ± 52.0                                           |                                                                 |
| Arnall et al.,<br>2007     | vHCHO + 3d                                                  | 6 M              | PL 3               | Yes                   | 9.1                                                  | 720                                | 72 h     | VL                 | 752.6 ± 130.5                                          | <i>P</i> < 0.05<br>vs baseline                                  |
|                            | vHCHO + 5d                                                  | 6 M              | PL 3               |                       | 9.1                                                  | 720                                |          |                    | 791.7 ± 60.9                                           |                                                                 |
|                            | vHCHO + 7d                                                  | 5 M              | PL 3               |                       | 9.1                                                  | 720                                |          |                    | 596 ± 43.5                                             |                                                                 |
|                            | (+ days investigated<br>maintenance of<br>increased stores) |                  |                    |                       |                                                      |                                    |          |                    |                                                        |                                                                 |

|                                     |                                                                             |            |              |     |            |            |      |    |                                  |                                                                                     |
|-------------------------------------|-----------------------------------------------------------------------------|------------|--------------|-----|------------|------------|------|----|----------------------------------|-------------------------------------------------------------------------------------|
| Bergström et al.,<br>1967           | High fat + PRO<br>HCHO                                                      | 9 M        | PL 3         | Yes | 0<br>8.3   | 0<br>575   | 72 h | VL | 152.2 ± 24.2<br>799.4 ± 72.5     | $P < 0.001$<br>between conditions                                                   |
| Blom et al.,<br>1987 <sup>[1]</sup> | HCHO untrained<br>HCHO well-trained                                         | 6 M<br>6 M | PL 1<br>PL 4 | Yes | 7.9<br>9   | 600<br>600 | 70 h | G  | 287.1 ± 37.8<br>583.3 ± 132.7    | $P < 0.05$<br>between conditions                                                    |
| Blom et al.,<br>1987 <sup>[2]</sup> | HCHO exercise<br>HCHO rest<br>(days pre-depletion)                          | 6 M        | PL 3         | Yes | 8.3<br>8.3 | 600<br>600 | 70 h | G  | 654.2 ± 94.8<br>711.7 ± 104.4    | $P > 0.05$<br>between conditions                                                    |
| Bosch et al.,<br>1996               | MCHO<br>HCHO                                                                | 7 M<br>9 M | PL 2<br>PL 2 | No  | 4<br>8.1   | 303<br>600 | 72 h | VL | 539.4 ± 30.5<br>843.9 ± 17.4     | NC                                                                                  |
| Bradley et al.,<br>2016             | MCHO<br>MCHO                                                                | 7 M<br>7 M | PL 5<br>PL 5 | Yes | 3<br>6     | 264<br>528 | 36 h | VL | 449.0 ± 19.3 †<br>444.0 ± 30.6 † | <i>Unclear</i> differences<br>between conditions<br>(magnitude-based<br>statistics) |
| Bradley et al.,<br>2017             | MCHO immediate<br>MCHO delayed<br>(acute post-depletion<br>refeed strategy) | 8 M<br>7 M | PL 2<br>PL 2 | Yes | 6<br>6     | 524<br>524 | 72 h | VL | 416 ± 56.9 †<br>362 ± 54.0 †     | NC                                                                                  |
| Burke et al.,<br>1993               | vHCHO low GCI<br>vHCHO high GCI                                             | 5 M        | PL 4         | Yes | 10<br>10   | 687<br>687 | 24 h | VL | 462.8 ± 33.5<br>575.9 ± 32.6     | $P < 0.05$<br>between conditions                                                    |

|                                        |                                    |       |      |     |                    |                    |                             |    |                              |                                                                |
|----------------------------------------|------------------------------------|-------|------|-----|--------------------|--------------------|-----------------------------|----|------------------------------|----------------------------------------------------------------|
| Burke et al.,<br>1995                  | HCHO                               | 8 M   | PL 3 | Yes | 7                  | 518                | 24 h                        | VL | 522.9 ± 41.8                 | <i>P</i> > 0.05 between<br>conditions                          |
|                                        | HCHO + fat +PRO                    |       |      |     | 7                  | 518                |                             |    | 452.4 ± 16.5                 |                                                                |
|                                        | vHCHO Energy match                 |       |      |     | 11.8               | 873                |                             |    | 519.4 ± 25.2                 |                                                                |
| Burke et al.,<br>1996                  | vHCHO Gorging                      | 8 M   | PL 3 | Yes | 10                 | 702                | 24 h                        | VL | 565.1 ± 44.4                 | <i>P</i> > 0.05 between<br>conditions                          |
|                                        | vHCHO Nibbling                     |       |      |     | 10                 | 702                |                             |    | 628.6 ± 53.1                 |                                                                |
| Burke et al.,<br>2000                  | MCHO                               | 7 M   | PL 4 | Yes | 5.8                | 419                | 72 h                        | VL | 485.0 ± 48.4 †               | <i>P</i> < 0.05 between<br>conditions                          |
|                                        | vHCHO                              |       |      |     | 9.0                | 646                |                             |    | 572 ± 40.4 †                 |                                                                |
| Bussau et al.,<br>2002                 | Baseline                           | 8 M   | PL 4 | No  | 5.8                | 447                | 24 h                        | VL | 417.6 ± 17.4                 | <i>P</i> < 0.05<br>vs baseline                                 |
|                                        | vHCHO                              |       |      |     | 10.2               | 785                | 72 h                        |    | 848.3 ± 74.0                 |                                                                |
| Costill et al.,<br>1981 <sup>[1]</sup> | HCHO complex<br>HCHO simple        | 6 M   | PL 3 | Yes | 9 + 5.8<br>9 + 5.8 | 648+415<br>648+415 | 24h +24h<br>(48 h<br>total) | G  | 725.1 ± 41.3<br>642.1 ± 32.6 | <i>P</i> < 0.05 between<br>conditions                          |
| Costill et al.,<br>1981 <sup>[2]</sup> | LCHO                               | 4 M   | PL 3 | Yes | 2.4                | 188                | 24 h                        | G  | 289.7 ± 33.9                 | <i>P</i> < 0.05 between high<br>conditions vs LCHO<br>and MCHO |
|                                        | MCHO                               |       |      |     | 4.7                | 375                |                             |    | 322.8 ± 17.0                 |                                                                |
|                                        | HCHO                               |       |      |     | 6.6                | 525                |                             |    | 440.2 ± 90.9                 |                                                                |
|                                        | HCHO                               |       |      |     | 6.6                | 525                |                             |    | 546.4 ± 47.4                 |                                                                |
| Costill et al.,<br>1988                | HCHO control                       | 8 M   | PL 3 | Yes | 6.6                | 500                | 48 h                        | VL | 604.2                        | <i>P</i> > 0.05<br>between conditions                          |
|                                        | HCHO distal<br>(multiple biopsies) | (4 M) |      |     | 6.6                | 500                |                             |    | 551.1                        |                                                                |

|                           |                                 |       |      |     |      |     |      |    |                |                                       |
|---------------------------|---------------------------------|-------|------|-----|------|-----|------|----|----------------|---------------------------------------|
| De Bock et al.,<br>2007   | MCHO                            | 8 M   | PL 2 | No  | 6.1  | 458 | 72 h | VL | 455.0 ± 40.0   | <i>P</i> > 0.05<br>between conditions |
|                           | MCHO                            |       |      |     | 6.1  | 458 |      |    | 466.0 ± 19.0   |                                       |
| Doering et al.,<br>2019   | Baseline                        | 6M 1F | PL 3 | No  | 5.2  | 396 | 24 h | VL | 583.6 ± 45.3 † | Conditions vs baseline                |
|                           | vHCHO                           |       |      | Yes | 10.6 | 807 | 96 h |    | 835.1 ± 46.1 † | <i>P</i> = 0.04                       |
|                           | vHCHO 2 <sup>nd</sup> depletion |       |      | Yes | 10.6 | 807 | 96 h |    | 848.3 ± 45.5 † | <i>P</i> = 0.01                       |
| Duhamel et al.,<br>2006   | LCHO                            | 10 M  | PL 1 | Yes | 1.1  | 94  | 96 h | VL | 365.7 ± 26.6   | <i>P</i> < 0.05<br>between conditions |
|                           | HCHO                            |       |      |     | 7.5  | 657 |      |    | 568.5 ± 44.9   |                                       |
| Fairchild et al.,<br>2002 | Baseline                        | 7 M   | PL 3 | No  | 5.6  | 430 | 96 h | VL | 474.6 ± 35.7   | <i>P</i> < 0.05<br>vs baseline        |
|                           | vHCHO                           |       |      | Yes | 10.3 | 793 | 24 h |    | 862.2 ± 57.0   |                                       |
| Fell et al., 2021         | vHCHO                           | 8 M   | PL 3 | Yes | 12   | 883 | 36 h | VL | 698.0 ± 34.6 † | <i>P</i> = 0.14<br>between conditions |
|                           | vHCHO                           |       |      |     | 12   | 883 |      |    | 742.0 ± 38.5 † |                                       |
|                           | vHCHO                           |       |      |     | 12   | 883 |      |    | 767.0 ± 30.8 † |                                       |
| Flynn et al.,<br>1987     | HCHO                            | 8 M   | PL 3 | Yes | 6.9  | 500 | 48 h | VL | 794.3 ± 28.7   | <i>P</i> > 0.05<br>between conditions |
|                           | HCHO                            |       |      |     | 6.9  | 500 |      |    | 778.7 ± 47.4   |                                       |
|                           | HCHO                            |       |      |     | 6.9  | 500 |      |    | 824.3 ± 41.8   |                                       |
|                           | HCHO                            |       |      |     | 6.9  | 500 |      |    | 836.1 ± 65.3   |                                       |
| Foskett et al.,<br>2008   | vHCHO                           | 6 M   | PL 2 | Yes | 10   | 750 | 48 h | VL | 512.0 ± 41.6 † | <i>P</i> > 0.05<br>between conditions |
|                           | vHCHO                           |       |      |     | 10   | 750 |      |    | 533.0 ± 31.4 † |                                       |

|                         |                                                                      |                          |                  |     |                      |                    |                              |    |                                                               |                                                                                                                              |
|-------------------------|----------------------------------------------------------------------|--------------------------|------------------|-----|----------------------|--------------------|------------------------------|----|---------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| Galbo et al.,<br>1979   | Fat + PRO<br>HCHO                                                    | 7 M                      | PL 3             | Yes | 0<br>7.3             | 0<br>559           | 96 h                         | VL | 195.5 ± 26.1<br>487.1 ± 65.2                                  | $P < 0.05$<br>between conditions                                                                                             |
| Goforth et al.,<br>1997 | vHCHO                                                                | 14 M                     | PL 2             | Yes | 9.3                  | 720                | 72 h                         | VL | 729.0 ± 83.9 †                                                | $P < 0.05$<br>vs baseline                                                                                                    |
| Greiwe et al.,<br>1999  | vHCHO untrained<br>vHCHO 10wk trained                                | 2M 4F                    | PL 1<br>PL 2     | Yes | 10<br>10             | 630<br>630         | 48 h                         | VL | 428.5 ± 85.7<br>795.6 ± 124.9                                 | $P < 0.05$<br>between conditions                                                                                             |
| Hawley et al.,<br>1997b | MCHO<br>vHCHO                                                        | 6 M                      | PL 4             | No  | 5.9<br>9.3           | 426<br>661         | 72 h                         | VL | 459.0 ± 33.9 †<br>565.0 ± 25.3 †                              | $P < 0.05$<br>between conditions                                                                                             |
| Hickner et al.,<br>1997 | vHCHO trained<br>vHCHO trained<br>vHCHO untrained<br>vHCHO untrained | 3 M<br>3 M<br>3 M<br>3 M | PL 3<br><br>PL 1 | Yes | 10<br>10<br>10<br>10 | 736<br><br>688     | 48 h<br>72 h<br>48 h<br>72 h | VL | 635.1 ± 167.5<br>792.1 ± 90.5<br>275.8 ± 31.3<br>584.6 ± 37.4 | $P < 0.05$ between<br>trained vs untrained<br>$P = 0.46$ for 48 vs 72 h<br>trained<br>$P < 0.01$ for 48 vs 72 h<br>untrained |
| Hill et al., 2013       | vHCHO + PRO<br>vHCHO                                                 | 6 M                      | PL 3             | No  | 8 + 10<br>8 + 10     | 592+740<br>592+740 | 14 d +<br>48h                | VL | 633.0 ± 82.0<br>695.0 ± 61.0                                  | $P > 0.05$<br>between conditions                                                                                             |

|                           |                                                      |       |      |     |           |         |          |    |                |                                                |                                               |
|---------------------------|------------------------------------------------------|-------|------|-----|-----------|---------|----------|----|----------------|------------------------------------------------|-----------------------------------------------|
| Impey et al.,<br>2020     | MCHO for all, post-load running exercise manipulated | 11 M  | PL 2 | Yes | 6         | 457     | 48 h     | VL | 446.0 ± 74.0   | <i>P</i> < 0.001 for males vs females in the G |                                               |
|                           |                                                      |       |      |     | 6         | 457     |          |    | 421.0 ± 74.0   |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 457     |          |    | 409.0 ± 91.0   |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 457     |          |    | G              |                                                | 504.0 ± 157.0                                 |
|                           |                                                      |       |      |     | 6         | 457     |          |    |                |                                                | 498.0 ± 207.0                                 |
|                           |                                                      |       |      |     | 6         | 457     |          |    | 542.0 ± 117.0  |                                                | <i>P</i> > 0.05 between all other conditions. |
|                           |                                                      | 10 F  | PL 2 |     | 6         | 369     |          | VL | 367.0 ± 148.0  |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 369     |          |    | 430.0 ± 128.0  |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 369     |          |    | 373.0 ± 95.0   |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 369     |          | G  | 433.0 ± 117.0  |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 369     |          |    | 296.0 ± 117.0  |                                                |                                               |
|                           |                                                      |       |      |     | 6         | 369     |          |    | 438.0 ± 140.0  |                                                |                                               |
| James et al.,<br>2001     | vHCHO                                                | 6 M   | PL 3 | Yes | 10.5      | 872     | 72 h     | VL | 796.1 ± 44.4 † | <i>P</i> > 0.05                                |                                               |
|                           | vHCHO pre-menses                                     | 6 F   | PL 3 |     | 9.9       | 684     |          |    | 878.7 ± 35.5 † | between conditions                             |                                               |
|                           | vHCHO post-menses                                    |       |      |     | 9.9       | 684     |          |    | 839.6 ± 24.9 † | <i>P</i> < 0.05 vs baseline for all            |                                               |
| Jansson and Kaijser, 1982 | LCHO                                                 | 4M 3F | PL 2 | No  | 0.3       | 21      | 120 h    | VL | 239.0 ± 25.7 † | <i>P</i> < 0.05                                |                                               |
|                           | MCHO                                                 |       |      |     | 5.9       | 383     |          |    | 445.0 ± 43.5 † | between conditions                             |                                               |
| Kavouras et al.,<br>2004  | LCHO                                                 | 12 M  | PL 3 | Yes | 1.4       | 100     | 72 h     | VL | 314.1 ± 24.4   | <i>P</i> < 0.05                                |                                               |
|                           | vHCHO                                                |       |      |     | 8.2       | 600     |          |    | 454.6 ± 40.9   | between conditions                             |                                               |
| Kirwan et al.,<br>1988    | MCHO + HCHO                                          | 10 M  | PL 4 | Yes | 6.2 + 8   | 413+533 | 72h +96h | G  | 524.6 ± 30.9   | <i>P</i> < 0.05                                |                                               |
|                           | MCHO + MCHO                                          |       |      |     | 6.2 + 3.9 | 413+260 | 72h +96h |    | 355.0 ± 41.8   | between conditions                             |                                               |

|                            |                                      |      |      |     |      |     |      |    |                |                                                                                  |
|----------------------------|--------------------------------------|------|------|-----|------|-----|------|----|----------------|----------------------------------------------------------------------------------|
| Kochan et al.,<br>1979     | vHCHO no depletion                   | 6 M  | PL 1 | No  | 11.3 | 823 | 96 h | VL | 502.3 ± 67.6   | <i>P</i> < 0.05<br>between conditions                                            |
|                            | vHCHO depletion<br>(one leg model)   |      |      | Yes | 11.3 | 823 |      |    | 912.9 ± 77.3   |                                                                                  |
| Lamb et al.,<br>1991       | vHCHO pasta                          | 7 M  | PL 4 | Yes | 11.3 | 809 | 84 h | VL | 566.4 ± 55.2   | <i>P</i> > 0.05<br>between conditions<br><i>P</i> < 0.05<br>vs baseline for both |
|                            | vHCHO supplement                     | 7 M  | PL 4 |     | 11.7 | 800 |      |    | 653.4 ± 51.3   |                                                                                  |
| MacDougall et<br>al., 1977 | MCHO                                 | 3 M  | PL 2 | Yes | 3.2  | 252 | 24 h | VL | 347.6 ± 10.0   | <i>P</i> > 0.05<br>between conditions                                            |
|                            | HCHO                                 | 3 M  | PL 2 |     | 7.7  | 617 |      |    | 367.6 ± 17.4   |                                                                                  |
| McInerney et<br>al., 2005  | Baseline                             | 6 M  | PL 3 | No  | 6    | 435 | 24 h | VL | 435.0 ± 57.0   | <i>P</i> < 0.01<br>between conditions and<br>vs baseline                         |
|                            | vHCHO                                |      |      | Yes | 12   | 870 | 48 h |    | 713.0 ± 60.0   |                                                                                  |
|                            | vHCHO 2 <sup>nd</sup> depletion      |      |      |     | 12   | 870 |      |    | 409.0 ± 40.0   |                                                                                  |
| McLay et al.,<br>2007      | MCHO midfollicular                   | 9 F  | PL 3 | Yes | 5.2  | 359 | 72 h | VL | 575.0 ± 47.7 † | <i>P</i> = 0.02 for moderate<br>CHO vs all other<br>conditions                   |
|                            | MCHO midluteal                       |      |      |     | 5.2  | 359 |      |    | 771.0 ± 45.7 † |                                                                                  |
|                            | HCHO midfollicular                   |      |      |     | 8.4  | 551 |      |    | 728.0 ± 47.0 † |                                                                                  |
|                            | HCHO midluteal                       |      |      |     | 8.4  | 551 |      |    | 756.0 ± 37.3 † |                                                                                  |
| Nelson et al.,<br>2001     | HCHO depletion                       | 12 M | PL 2 | Yes | 6.6  | NC  | 72 h | VL | 602.0 ± 39.5 † | <i>P</i> < 0.05 vs baseline<br><i>P</i> > 0.05 between<br>conditions             |
|                            | HCHO no depletion<br>(one leg model) |      |      | No  | 6.6  | NC  |      |    | 488.0 ± 29.4 † |                                                                                  |

|                             |                    |        |      |     |            |         |        |    |                           |                     |
|-----------------------------|--------------------|--------|------|-----|------------|---------|--------|----|---------------------------|---------------------|
| Nicklas et al.,<br>1989     | MCHO midluteal     | 6 F    | PL 2 | Yes | 3.9        | 237     | 72 h   | VL | 451.1 ± 23.9              | $P > 0.05$          |
|                             | MCHO midfollicular |        |      |     | 4.2        | 254     |        |    | 404.6 ± 25.7              | between conditions  |
| Rauch et al.,<br>1995       | MCHO               | 8 M    | PL 4 | No  | 6.2        | 639     | 72 h   | VL | 452.4 ± 34.8              | $P < 0.0001$        |
|                             | vHCHO              |        |      |     | 10.5       | 750     |        |    | 665.6 ± 39.2              | between conditions  |
| Roberts et al.,<br>2016     | HCHO               | 7 M    | PL 2 | Yes | 8.1        | 637     | 6 days | VL | 789.0 ± 38.0              | $P < 0.01$          |
|                             | HCHO + Creatine    | 7 M    |      |     | 8.2        | 645     |        |    | 948.0 ± 75.0              | between conditions  |
| Sasaki et al.,<br>1991      | MCHO               | 5 M    | PL 2 | Yes | 3.4        | 214     | 7 days | VL | 260.8 ± 45.9              | $P < 0.05$          |
|                             | HCHO               |        |      |     | 8          | 499     |        |    | 840.2 ± 94.4              | between conditions  |
| Schytz et al.,<br>2023      | MCHO               | 22 M   | PL 2 | Yes | 4          | 309     | 48 h   | VL | 367.0 ± 14.1 <sup>†</sup> | $P < 0.0001$        |
|                             | HCHO               |        |      |     | 10         | 773     |        |    | 525.0 ± 14.4 <sup>†</sup> | between conditions  |
| Sherman et al.,<br>1981     | MCHO               | 6 M    | PL 4 | Yes | 5.1        | 353     | 72 h   | G  | 693.4 ± 28.7              | $P < 0.05$          |
|                             | 3d LCHO 3d HCHO    |        |      |     | 7.8        | 542     |        |    | 903.9 ± 50.9              | between MCHO and    |
|                             | 3d MCHO 3d HCHO    |        |      |     | 7.8        | 542     |        |    | 884.4 ± 32.6              | HCHO conditions     |
| Sherman et al.,<br>1983     | HCHO exercise      | 5 M    | PL 4 | Yes | 12.7 + 7.1 | 800+450 | 24 h + | G  | 474.6 ± 30.5              | $P < 0.05$ for both |
|                             | HCHO rest          | 5M     |      |     | 11.6 + 6.5 | 800+450 | 6 days |    | 613.8 ± 26.1              | conditions vs pre-  |
|                             | Pre-marathon       | (10 M) |      |     | 8.2        | 542     | 72 h   |    | 852.6 ± 33.1              | marathon            |
| Tarnopolsky et<br>al., 1990 | MCHO               | 6 F    | PL 4 | No  | 5.4        | 317     | 48 h   | VL | 438.0 ± 105.6             | $P > 0.05$          |
|                             | HCHO               | 6 M    | PL 4 |     | 7.1        | 478     |        |    | 471.4 ± 28.3              | between conditions  |

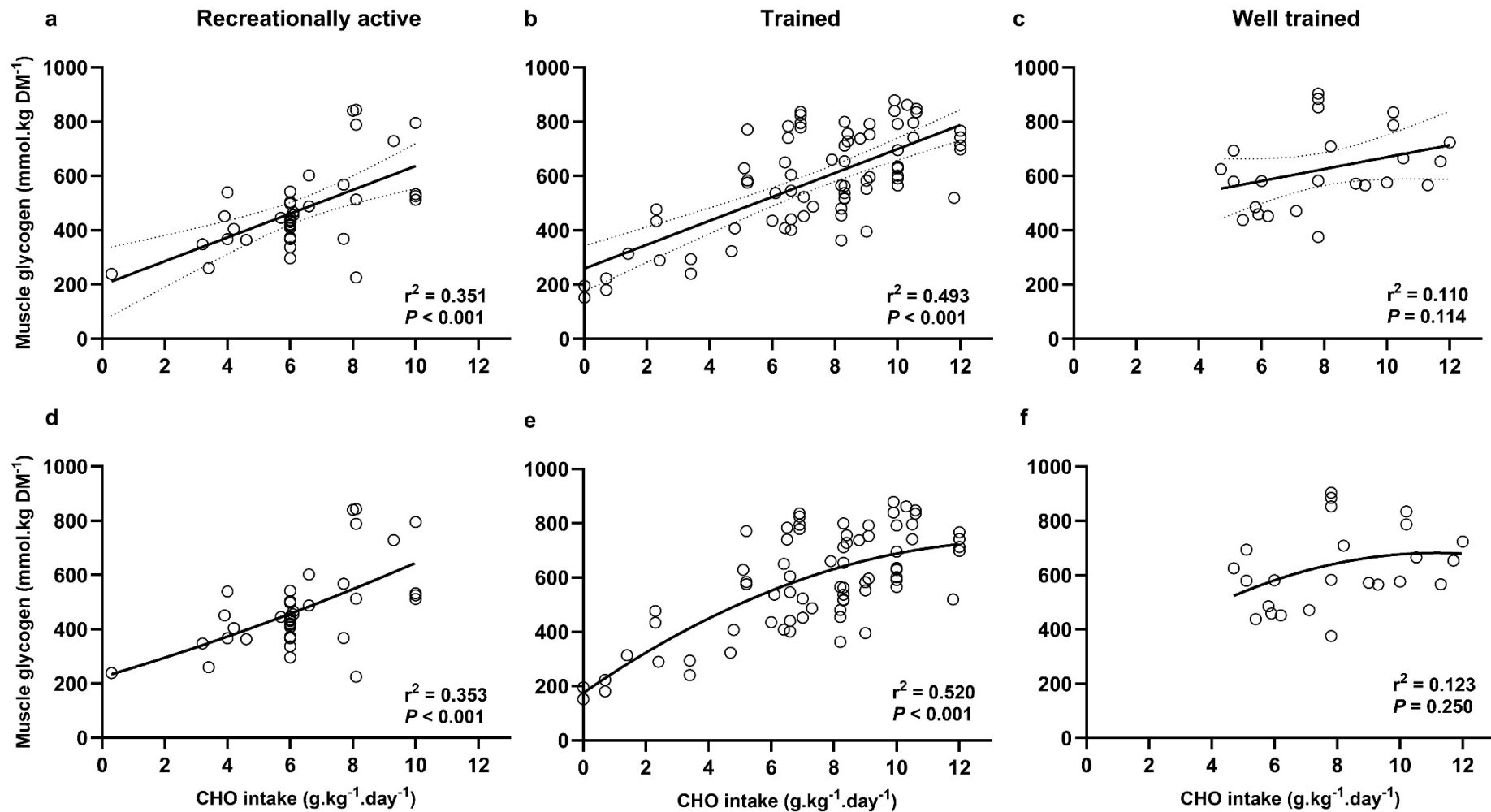
|                           |                  |       |      |     |      |     |        |    |                |                                                           |
|---------------------------|------------------|-------|------|-----|------|-----|--------|----|----------------|-----------------------------------------------------------|
| Tarnopolsky et al., 1995  | MCHO             | 8 F   | PL 3 | No  | 4.8  | 274 | 96 h   | VL | 407.3 ± 27.6 † | <i>P</i> < 0.01<br>between male HCHO<br>vs male LCHO      |
|                           | MCHO             |       |      |     | 6.4  | 370 |        |    | 407.8 ± 20.2 † |                                                           |
|                           | HCHO             | 7 M   | PL 3 |     | 6.6  | 492 |        |    | 401.5 ± 29.0 † |                                                           |
|                           | HCHO             |       |      |     | 8.2  | 614 |        |    | 565.4 ± 33.3 † |                                                           |
| Tarnopolsky et al., 2001  | HCHO             | 6 M   | PL 3 | Yes | 7.9  | 585 | 96 h   | VL | 660.2 ± 49.0†  | <i>P</i> < 0.05 for males<br>vHCHO and HCHO vs<br>MCHO    |
|                           | vHCHO            |       |      |     | 10.5 | 778 |        |    | 741.1 ± 76.6†  |                                                           |
|                           | MCHO (habitual)  |       |      |     | 6.1  | 452 |        |    | 537.2 ± 32.1†  | <i>P</i> < 0.05 for females<br>HCHO vs MCHO<br>(habitual) |
|                           | MCHO             | 7 F   | PL 3 |     | 6.4  | 380 |        |    | 650.6 ± 71.0†  |                                                           |
|                           | HCHO             |       |      |     | 8.8  | 523 |        |    | 737.7 ± 74.9†  |                                                           |
|                           | MCHO (habitual)  |       |      |     | 5.1  | 303 |        |    | 629.1 ± 87.3†  |                                                           |
| Tomcik et al., 2018       | MCHO + Creatine  | 9 M   | PL 4 | Yes | 6    | 469 | 48 h   | VL | 589.0 ± 31.3 † | <i>P</i> < 0.05<br>between MCHO and<br>vHCHO conditions   |
|                           | vHCHO + Creatine |       |      |     | 12   | 938 |        |    | 692.0 ± 32.3 † |                                                           |
|                           | MCHO             | 9 M   |      |     | 6    | 469 |        |    | 581.0 ± 42.3 † |                                                           |
|                           | vHCHO            |       |      |     | 12   | 938 |        |    | 724.0 ± 36.0 † |                                                           |
| Vandenberghe et al., 1995 | MCHO             | 10 NC | PL 2 | Yes | 4.6  | 313 | 5 days | VL | 364.0 ± 23.0   | <i>P</i> < 0.05<br>between conditions                     |
|                           | HCHO             |       |      |     | 7.7  | 525 | 72 h   |    | 568.0 ± 35.0   |                                                           |
| Walker et al., 2000       | MCHO             | 6 F   | PL 4 | Yes | 4.7  | 266 | 96 h   | VL | 625.2 ± 50.1   | <i>P</i> < 0.05<br>between conditions                     |
|                           | HCHO             |       |      |     | 8.2  | 464 |        |    | 709.0 ± 44.8   |                                                           |

|                         |      |     |      |     |     |     |      |    |              |                  |
|-------------------------|------|-----|------|-----|-----|-----|------|----|--------------|------------------|
| Widrick et al.,<br>1993 | LCHO | 8 M | PL 3 | Yes | 2.3 | 181 | 48 h | VL | 434.1 ± 26.1 | $P < 0.05$       |
|                         | LCHO |     |      |     | 2.3 | 181 |      |    | 477.2 ± 23.1 | Between LCHO and |
|                         | MCHO |     |      |     | 6.5 | 511 |      |    | 783.9 ± 42.2 | MCHO conditions  |
|                         | MCHO |     |      |     | 6.5 | 511 |      |    | 740.4 ± 45.2 |                  |

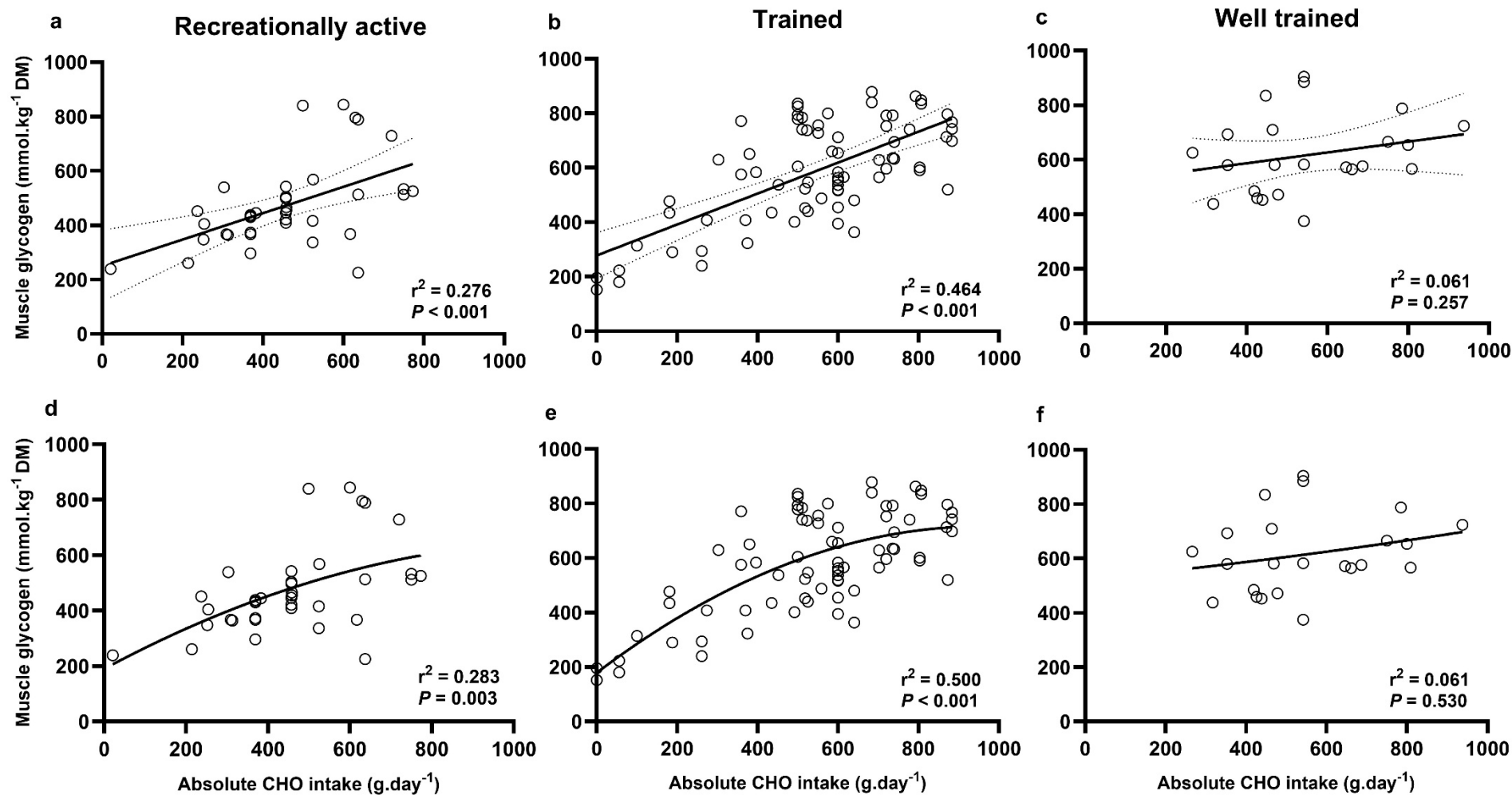
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<sup>†</sup> Indicates calculated SE from SD presented in original article. Abbreviations include LCHO, MCHO, HCHO and vHCHO for low, moderate, high and very high carbohydrate intake as previously defined (< 3, 3-6.5, >6.5-9 and > 9 g·kg<sup>-1</sup>·day<sup>-1</sup> respectively); F, females; G, *gastrocnemius*; GCI, glycaemic index; h, hours; M, males; NC, not confirmed; PL, performance level; PRO, protein; VL; *vastus lateralis*.

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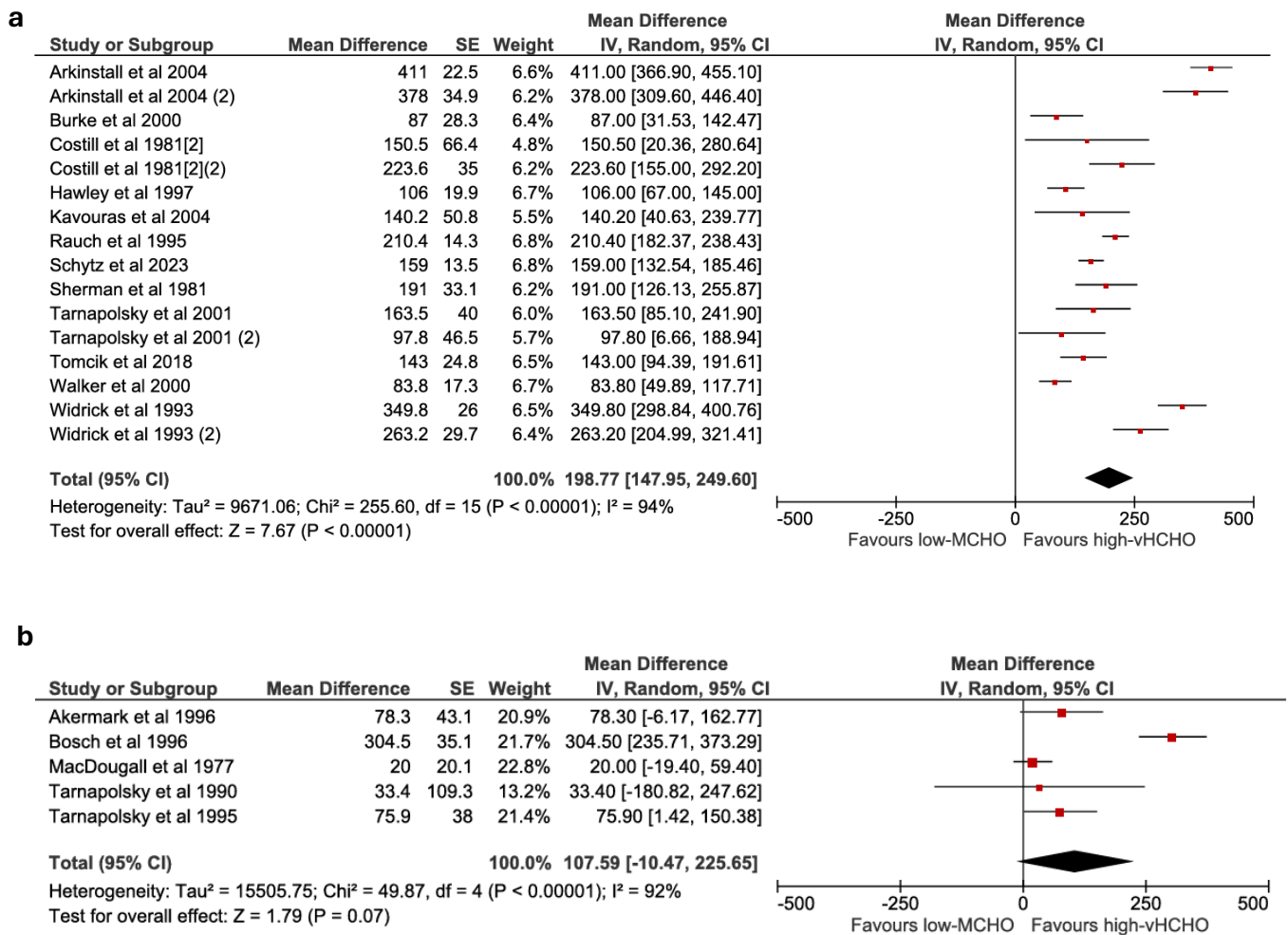
**Figure 4.2.** Simple linear (a, b, c) and nonlinear second-degree polynomial regression analyses (d, e, f) of relative CHO intake and whole muscle glycogen in recreationally active (a and d), endurance trained (b and e) and well-trained individuals (c and f).



**Figure 4.3.** Simple linear (a, b, c) and nonlinear second-degree polynomial regression analyses (d, e, f) of absolute CHO intake and whole muscle glycogen in recreationally active, endurance trained (b and e) and well-trained individuals (c and f).

#### ***4.4.2 Effectors of muscle glycogen concentration***

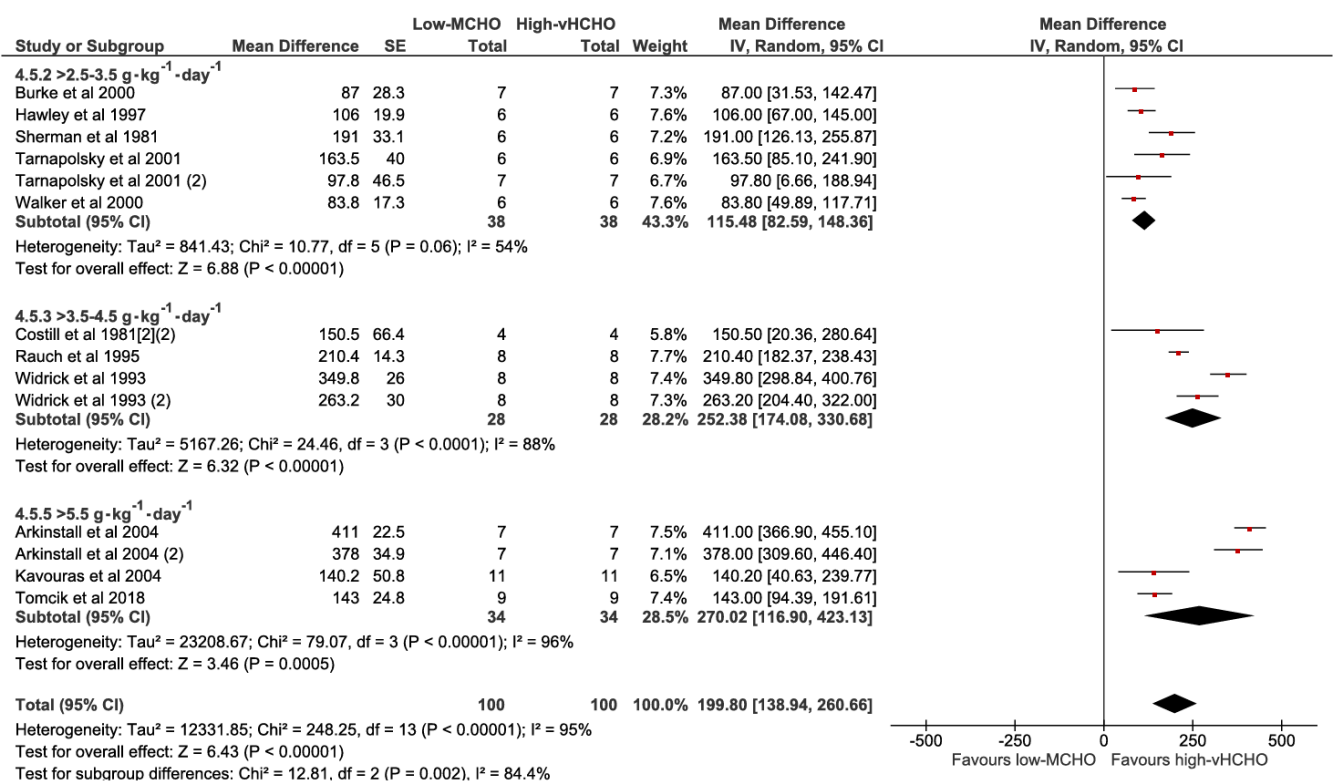
*CHO intake.* Seventeen studies were included within the meta-analyses (12 repeated measures and 5 parallel groups; Figure 4.4). Increasing relative CHO intake from a low-moderate to high-very high intake increased muscle glycogen 198.8 mmol·kg<sup>-1</sup> DM (95% CI = 148.0 to 249.6 mmol·kg<sup>-1</sup> DM, n = 252, Z = 7.67, *P* < 0.00001) and 107.6 mmol·kg<sup>-1</sup> DM (95% CI = -10.5 to 225.7 mmol·kg<sup>-1</sup> DM, n = 81, Z = 1.79, *P* = 0.07) for repeated measures and parallel group trials, respectively. There was considerable significant heterogeneity for both repeated measures (Chi<sup>2</sup> = 255.63, df = 15, *P* < 0.0001, I<sup>2</sup> = 94%) and parallel groups designs (Chi<sup>2</sup> = 49.87, df = 4, *P* < 0.0001, I<sup>2</sup> = 92%), which was expected at this stage of the analysis, as sources of heterogeneity were yet to be explored.



**Figure 4.4.** Forest plot of meta-analyses conducted for randomised crossover (a) and parallel groups (b) study designs that investigated the effects of a low-moderate versus high-very high CHO intake on whole skeletal muscle glycogen.

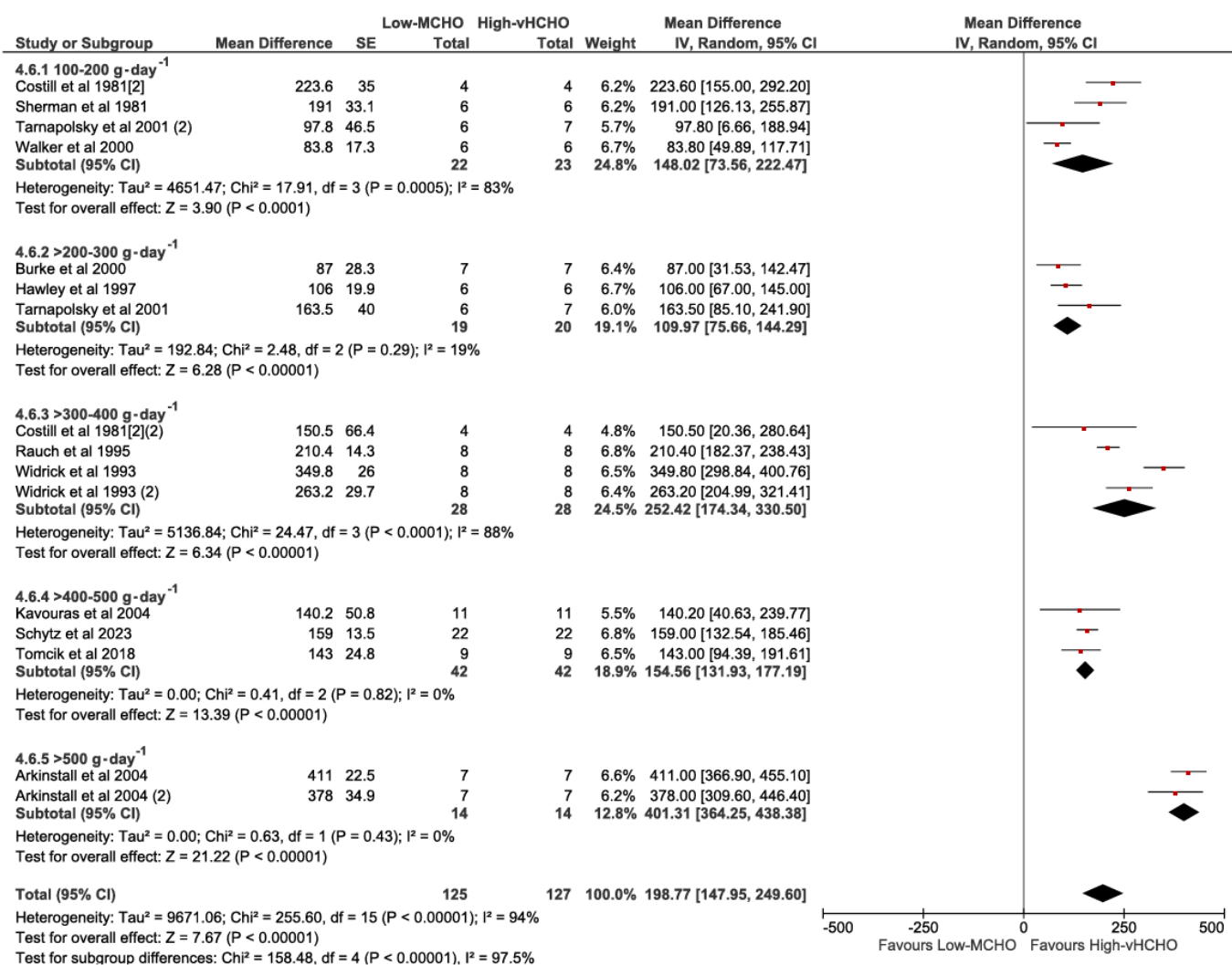
The  $\Delta$  relative CHO intake between low-moderate versus high-very high conditions were assessed as incremental  $1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  subgroups, however only  $>2.5\text{-}3.5$ ,  $>3.5\text{-}4.5$  and  $>5.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  subgroups were possible as the  $1.5\text{-}2.5$  and  $>4.5\text{-}5.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  subgroups only contained one pairwise comparison each (Costill et al., 1981<sup>[2]</sup>; Schytz et al., 2023). There was a significant quantitative subgroup effect ( $P = 0.002$ ), where increased  $\Delta$  between relative CHO intakes resulted in a stepwise increase in muscle glycogen across subgroups (Figure 4.5).

However, effect estimates should be interpreted with caution, as despite an improvement in heterogeneity, all subgroups still displayed significant heterogeneity ( $I^2 = 54\%$ ,  $P = 0.06$ ;  $I^2 = 88\%$ ,  $P < 0.0001$ ;  $I^2 = 96\%$ ,  $P < 0.0001$ , respectively). Covariance distribution was similar as 4-6 study pairwise comparisons contributed to each subgroup with a similar number of participants ( $n = 28$ -38 participants per condition, per subgroup; Figure 4.5).



**Figure 4.5.** Forest plot of meta-analysis conducted in crossover design studies with subgroups for the  $\Delta$  relative CHO intake between defined low-moderate and high-very high CHO conditions at subgroups of 2.5-3.5, 3.5-4.5 and  $> 5.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ .

Subgroups for  $\Delta$  absolute CHO intake between conditions were 100-200, >200-300, >300-400, >400-500 and >500 g, as the <100 g subgroup only contained one pairwise comparison (Costill et al., 1981<sup>[2]</sup>). There was a significant subgroup effect ( $P < 0.0001$ ), as the magnitude of the effect of CHO intake on muscle glycogen increased in line with increased  $\Delta$  (Figure 4.6). The >200-300, >400-500 and >500 g subgroups had no significant heterogeneity ( $I^2 = 19\%$ ,  $P = 0.29$ ;  $I^2 = 0\%$ ,  $P = 0.82$ ;  $I^2 = 0\%$ ,  $P < 0.43$ , respectively), whilst the >100-200g and >300-400 g groups also saw decreased heterogeneity compared to the overall effect estimate for crossover trials, however heterogeneity was still considerable and significant ( $I^2 = 83\%$ ,  $P < 0.001$  and  $I^2 = 88\%$ ,  $P < 0.0001$ , respectively). However, the number of studies contributing to each subgroup was low, particularly in those with low heterogeneity, as only 3, 3 and 2 pairwise comparisons contributed to the >200-300, >400-500 and >500 g subgroups, respectively.



**Figure 4.6.** Forest plot of meta-analysis conducted in crossover design studies with subgroups for the  $\Delta$  absolute CHO intake between defined low-moderate and high-very high CHO intake conditions in 100 g·day<sup>-1</sup> increments from 100 to >500 g·day<sup>-1</sup> of CHO.

*CHO loading duration.* There was a significant subgroup effect ( $P = 0.004$ ) as the 24 and 48-h loading periods had a greater impact on muscle glycogen compared to 72 and 96 h. The 48 and 72 h subgroups had considerable significant heterogeneity ( $P < 0.0001$  for both), whereas the 24 h and 96 h subgroups did not. However, effect estimates should be cautiously interpreted due to uneven covariance distribution across loading durations and vastly different numbers of participants contributing to each subgroup (Table 4.2). Bussau et al. (2002) reported no benefit in terms of glycogen concentration following a longer loading period (24 vs 72 h), whereas others have shown a continued increase of muscle glycogen stores following high CHO intakes over periods of 48 (Adamo et al., 1998), 72 (Roberts et al., 2016) and 96 h (Kochan et al., 1979) vs 24 h. In endurance trained individuals, enhanced glycogen concentrations ( $> 600 \text{ mmol} \cdot \text{kg}^{-1} \text{ DM}$ ) were consistently reported following a high/very high CHO intake for periods 48-72 h (Table 4.1), whereas only 2 study groups have achieved this with a 24 h loading protocol (Burke et al., 1996; Fairchild et al., 2002).

*Training status.* Subgroup analysis was attempted for participant training status, however only trained and well-trained subgroups contained more than one pairwise comparison. There was a significant subgroup difference ( $P = 0.01$ ) with higher glycogen for the trained versus well trained subgroup. However, both groups had significant heterogeneity ( $P < 0.0001$  and  $I^2 \geq 88\%$ , for both) and the covariance distribution was uneven with an additional 40 participants in the trained subgroup vs well trained (Table 4.2). Individual studies that investigated differences in glycogen concentration following 72 h of a very high CHO intake in trained versus untrained participants (Blom et al. 1987<sup>[1]</sup>; Hickner et al., 1997) showed significantly greater enhancement of glycogen stores in the trained group ( $+296.2$  and  $+207.5 \text{ mmol} \cdot \text{kg}^{-1} \text{ DM}$ , respectively). Further, Greiwe et al. (1999) reported significantly greater muscle glycogen

concentrations ( $+367.1 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ) from the same CHO loading protocol in participants post-10-week endurance cycling training programme.

*Exercise pre- and during loading.* Kochan et al. (1979) and Nelson et al. (2001) compared depletion vs no depletion specifically using one-legged models. Both studies reported significantly higher muscle glycogen stores in the pre-CHO loading exercised leg compared to the non-exercised control. Nevertheless, numerous others have reported similar levels of glycogen when a high or very high CHO intake was not preceded by depleting exercise (Bosch et al., 1996; Bussau et al., 2002; De Bock et al., 2007; Hawley et al., 1997b; Hill et al., 2013; Rauch et al., 1995; Tarnopolsky et al., 1990; Tarnopolsky et al., 1995). Between study comparisons suggest exercise during the loading period does not negatively impact glycogen concentrations, provided exercise is light/moderate intensity and relatively short duration ( $\leq 60$  min), replicating a pre-competition taper (Doering et al., 2019; Sherman et al., 1981; Tarnopolsky et al., 1995; Tarnopolsky et al., 2001) and CHO intake is high-very high. In agreement, Sherman et al. (1983) found challenging exercise in the days post-marathon to negatively impact glycogen concentrations compared to rest when dietary CHO intake was high ( $> 7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ).

**Table 4.2.** Summary statistics for subgroup analyses of pre-identified covariates investigated to explore heterogeneity.

| Variable             | Subgroup         | n of<br>comparisons<br>(participants) | Mean<br>difference<br>(mmol·kg <sup>-1</sup><br>DM) | Low<br>95%<br>CI | High<br>95%<br>CI | Overall<br>effect<br><i>P</i> | I <sup>2</sup><br>( <i>P</i> value) |
|----------------------|------------------|---------------------------------------|-----------------------------------------------------|------------------|-------------------|-------------------------------|-------------------------------------|
| Loading<br>duration  | 24 h             | 2 (8)                                 | 207.7                                               | 147.0            | 268.4             | <i>P</i> < 0.001              | 0%<br>( <i>P</i> = 0.33)            |
|                      | 48 h             | 7 (134)                               | 283.0                                               | 183.1            | 382.8             | <i>P</i> < 0.001              | 97%<br>( <i>P</i> < 0.001)          |
|                      | 72 h             | 5 (76)                                | 148.0                                               | 90.0             | 206.0             | <i>P</i> < 0.001              | 85%<br>( <i>P</i> < 0.001)          |
|                      | 96 h             | 3 (38)                                | 106.4                                               | 59.0             | 153.7             | <i>P</i> < 0.001              | 40%<br>( <i>P</i> = 0.19)           |
| Subgroup differences |                  |                                       |                                                     |                  |                   | <b><i>P</i> = 0.004</b>       |                                     |
| Training<br>status   | Trained          | 9 (124)                               | 248.4                                               | 173.9            | 322.9             | <i>P</i> < 0.001              | 91%<br>( <i>P</i> < 0.001)          |
|                      | Well-<br>trained | 6 (84)                                | 136.6                                               | 86.5             | 186.8             | <i>P</i> < 0.001              | 88%<br>( <i>P</i> < 0.001)          |
| Subgroup differences |                  |                                       |                                                     |                  |                   | <b><i>P</i> = 0.01</b>        |                                     |

CI, confidence interval; h, hours; n, number of pairwise comparisons per subgroup.

*Other considered covariates.* No subgroup analysis was attempted for participant sex or muscle biopsy location due to substantially uneven covariate distributions between categorised subgroups (1 study recruited females only, 13 recruited males only and 3 recruited both; 2 studies biopsied the *gastrocnemius* whilst the remaining 15 took muscle samples from the *vastus lateralis*). When CHO intake was matched, males and females appear to have the same capacity to increase muscle glycogen stores (Walker et al., 2000; Tarnopolsky et al., 2001). Impey et al., (2020) reported lower glycogen concentrations in the *gastrocnemius* of female participants compared to males, however no differences occurred in the *vastus lateralis*. When other covariates were similar (CHO intake, loading duration and participant training status) studies that collected biopsies from the *gastrocnemius* (Blom et al., 1987<sup>[1]</sup>; Blom et al., 1987<sup>[2]</sup>; Costill et al., 1981<sup>[1]</sup>; Costill et al., 1981<sup>[2]</sup>; Kirwan et al., 1988; Sherman et al., 1981; Sherman et al., 1983) have reported comparable glycogen concentrations to those collecting samples from the *vastus lateralis* (Arnall et al., 2007; Bergström et al., 1967; Burke et al., 1995).

#### **4.4.3 Risk of bias results**

Of the 39 experiments assessed as crossover trials, 5 studies were deemed high risk (Galbo et al., 1979; Greiwe et al., 1999; Jansson and Kaijser, 1982; McNerney et al., 2005; Nicklas et al., 1989), as trials were not randomised and participants were aware of trial allocation, which was dictated by other factors (e.g. menstrual cycle phase or pre- and post-10 weeks of endurance training). Similarly, 25 studies posed some concerns (Table 4.3) primarily due to lack of detail regarding randomisation and allocation concealment (domain 1), where randomisation or allocation concealment techniques were inadequately described. Of the 25, two studies also raised some concerns for domain 2 (period and carryover effects) as total number of participants were unequal across trial sequences (Bergström et al., 1967; Costill et

al., 1988a). The remaining 9 repeated measures studies were deemed low risk of bias. Of the 17 studies assessed as parallel grouped trials, 1 study was deemed high risk of bias (James et al., 2001), due to no information regarding randomisation and participants likely being aware of the upcoming interventions (domain 1; judgement made based on statement that the study protocol was described to participants and males only had 1 experimental condition). Similarly, a further 10 studies were deemed of some concern due to lack of detail regarding randomisation and/or allocation concealment techniques (Table 4.4), with the remaining 6 studies deemed low risk of bias (Arnall et al., 2007; Bradley et al 2017; Roberts et al., 2016; Tarnopolsky et al., 1995; Tarnopolsky et al., 2001).

**Table 4.3.** Cochrane risk of bias tool for repeated measures trials.

| Study                               | D1 | DS | D2 | D3 | D4 | D5 | Overall |
|-------------------------------------|----|----|----|----|----|----|---------|
| Adamo et al., 1998                  | !  | +  | +  | +  | +  | +  | !       |
| Arkinstall et al., 2004             | +  | +  | +  | +  | +  | +  | +       |
| Bergström et al., 1967              | !  | !  | +  | +  | +  | +  | !       |
| Blom et al., 1987 <sup>[2]</sup>    | !  | +  | +  | +  | +  | +  | !       |
| Burke et al., 1993                  | !  | +  | +  | +  | +  | +  | !       |
| Burke et al., 1995                  | !  | +  | +  | +  | +  | +  | !       |
| Burke et al., 1996                  | !  | +  | +  | +  | +  | +  | !       |
| Burke et al., 2000                  | +  | +  | +  | +  | +  | +  | +       |
| Bussau et al., 2002                 | !  | +  | +  | +  | +  | +  | !       |
| Costill et al., 1981 <sup>[1]</sup> | !  | +  | +  | +  | +  | +  | !       |
| Costill et al., 1981 <sup>[2]</sup> | !  | +  | +  | +  | +  | +  | !       |
| Costill et al., 1988                | !  | !  | +  | +  | +  | +  | !       |
| De Bock et al., 2007                | +  | +  | +  | +  | +  | +  | +       |
| Doering et al., 2019                | !  | +  | +  | +  | +  | +  | !       |
| Duhamel et al., 2006                | !  | +  | +  | +  | +  | +  | !       |
| Fairchild et al., 2002              | !  | +  | +  | +  | +  | +  | !       |
| Fell et al., 2021                   | !  | +  | +  | +  | +  | +  | !       |
| Flynn et al., 1987                  | !  | +  | +  | +  | +  | +  | !       |
| Foskett et al., 2008                | +  | +  | +  | +  | +  | +  | +       |
| Galbo et al., 1979                  | -  | +  | +  | +  | +  | +  | -       |
| Goforth et al., 1997                | !  | +  | +  | +  | +  | +  | !       |
| Greiwe et al., 1999                 | -  | +  | +  | +  | +  | +  | -       |

|                           |  |  |  |  |  |  |  |
|---------------------------|--|--|--|--|--|--|--|
| Hawley et al., 1997b      |  |  |  |  |  |  |  |
| Hill et al., 2013         |  |  |  |  |  |  |  |
| Jansson and Kaijser, 1982 |  |  |  |  |  |  |  |
| Kavouras et al., 2004     |  |  |  |  |  |  |  |
| Kochan et al., 1979       |  |  |  |  |  |  |  |
| Kirwan et al., 1988       |  |  |  |  |  |  |  |
| McInerney et al., 2005    |  |  |  |  |  |  |  |
| McLay et al., 2007        |  |  |  |  |  |  |  |
| Nelson et al., 2001       |  |  |  |  |  |  |  |
| Nicklas et al., 1989      |  |  |  |  |  |  |  |
| Rauch et al., 1995        |  |  |  |  |  |  |  |
| Sasaki et al., 1991       |  |  |  |  |  |  |  |
| Schytz et al., 2023       |  |  |  |  |  |  |  |
| Sherman et al., 1981      |  |  |  |  |  |  |  |
| Tomcik et al., 2018       |  |  |  |  |  |  |  |
| Walker et al., 2000       |  |  |  |  |  |  |  |
| Widrick et al., 1993      |  |  |  |  |  |  |  |

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**Table 4.4.** Cochrane risk of bias tool for parallel groups or mixed design trials.

| Study                            | D1 | DS | D2 | D3 | D4 | D5 | Overall |
|----------------------------------|----|----|----|----|----|----|---------|
| Akermark et al., 1996            | !  | +  | +  | +  | +  | !  | !       |
| Arnall et al., 2007              | +  | +  | +  | +  | +  | +  | +       |
| Blom et al., 1987 <sup>[1]</sup> | !  | +  | +  | +  | +  | +  | !       |
| Bosch et al., 1996               | !  | +  | +  | +  | +  | +  | !       |
| Bradley et al., 2016             | !  | +  | +  | +  | +  | !  | !       |
| Bradley et al., 2017             | +  | +  | +  | +  | +  | +  | +       |
| Hickner et al., 1997             | !  | +  | +  | +  | +  | +  | !       |
| Impey et al., 2020               | !  | +  | +  | +  | +  | +  | !       |
| James et al., 2001               | -  | +  | +  | +  | +  | +  | -       |
| Lamb et al., 1991                | !  | +  | +  | +  | +  | +  | !       |
| MacDougall et al., 1977          | !  | +  | +  | +  | +  | +  | !       |
| Roberts et al., 2016             | +  | +  | +  | +  | +  | +  | +       |
| Sherman et al., 1983             | +  | +  | +  | +  | +  | +  | +       |
| Tarnopolsky et al., 1990         | !  | +  | +  | +  | +  | +  | !       |
| Tarnopolsky et al., 1995         | +  | +  | +  | +  | +  | +  | +       |
| Tarnopolsky et al., 2001         | +  | +  | +  | +  | +  | +  | +       |
| Vandenberghe et al., 1995        | !  | +  | +  | +  | +  | +  | !       |

## 4.5 Discussion

Despite over half a century of research, surprisingly a systematic review and meta-analysis that objectively quantifies the effectiveness of CHO loading to maximise muscle glycogen concentrations has never been conducted. As a result, this review, aimed to describe and quantify the relationship between pre-exercise dietary CHO intake and whole skeletal muscle glycogen, whilst identifying key covariates of glycogen concentration. Meta-analyses did not allow an accurate quantification of the magnitude of the relationship due to considerable significant heterogeneity, which resulted from the confounding effect of important covariates across studies. Nonetheless, regression analyses indicated a linear dose-response relationship, and a hierarchy regarding covariates, with quantity of CHO ingested, training status, loading duration and exercise (pre- and during loading) all being important effectors of muscle glycogen synthesis and concentration.

Quantity of CHO ingested appears the most important factor to achieve an enhancement of muscle glycogen stores, as simple linear regression results indicated relative CHO intake predicted 49.3% of the variation in muscle glycogen in endurance trained individuals ( $P < 0.001$ ; Figure 4.2a), indicating a dose-response relationship. In agreement, subgroup analyses revealed a significant subgroup effect for  $\Delta$  CHO intake between low-moderate and high-very high conditions, as  $\Delta$  relative CHO intake subgroups ( $>2.5$ - $3.5$  to  $>5.5$   $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) increased muscle glycogen in a stepwise manner (Figure 4.5). This was somewhat expected considering heightened CHO availability through increased dietary CHO ingestion is a potent regulator of glycogen synthesis and adequate glucose within the muscle is a requirement to achieve increased glycogen storage (Bergström et al., 1967; Jentjens and Jeukendrup, 2003). However, meta-analysis results suggest a saturation point, as muscle glycogen only marginally increased

(+17.6 mmol·kg<sup>-1</sup> DM) from >3.5-4.5 to the >5.5 g·kg<sup>-1</sup>·day<sup>-1</sup> subgroups. Considering habitual CHO intakes of trained individuals (~3-6 g·kg<sup>-1</sup>·day<sup>-1</sup> dependent on training demands), an increase of 4.5 g·kg<sup>-1</sup>·day<sup>-1</sup> would achieve 7.5 to 10.5 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO, matching the previously suggested saturation point (7-10 g·kg<sup>-1</sup>·day<sup>-1</sup>; Burke et al., 1995; Burke et al., 2017). In contrast, subgroup analysis for  $\Delta$  absolute CHO intake suggested the opposite, as the highest subgroup (>500 g·day<sup>-1</sup>) showed the greatest enhancement of glycogen stores (+401.3 mmol·kg<sup>-1</sup> DM), suggesting higher CHO intakes are superior for maximisation of glycogen stores pre-competition. This was supported by regression analysis as second-degree polynomial regressions were consistent with simple linear models (Figure 4.2). The curve does appear somewhat hyperbolic, as the linearity of the relationship weakened as CHO intake increased  $\geq 10$  g·kg<sup>-1</sup>·day<sup>-1</sup>, suggesting the true saturation point may be higher than previously suggested (Burke et al., 2017), at least in endurance trained individuals. It should be considered only 5 study groups contributed to very high CHO intakes (~12 g·kg<sup>-1</sup>·day<sup>-1</sup>), one of which could be considered an outlier (Burke et al., 1995); therefore, this slight plateau could also be due to lack of information at higher CHO intakes (Figure 4.2b; Figure 4.3b).

The discrepancy between subgroup analyses of the  $\Delta$  CHO consumed highlights the challenging nature of interpreting CHO loading research, particularly as both analyses display considerable significant heterogeneity across multiple subgroups, uneven covariance distributions and relatively small numbers of study pairwise comparisons contributing to each subgroup (below the  $n = 10$  recommended by statisticians; Figures 4.5 and 4.6; Higgins et al., 2024). As such the magnitude of the relationship between dietary CHO intake and muscle glycogen remains unclear. Conversely, regression analyses indicate a clear dose-response relationship, and it remains to be determined whether the previously suggested ‘ceiling’ effect truly exists (Burke et al., 1995). Ultimately, irrespective of how CHO intake was expressed

(relative vs absolute), a marked increase in CHO ingestion appears the most important factor to attain a significant enhancement of muscle glycogen stores (Figure 4.2; Figure 4.3), supported by the consistency of linear regression results for muscle glycogen across both relative and absolute CHO intakes. The exact quantity required to achieve supercompensation is debatable, as other factors will also influence requirements, particularly the energy demands of upcoming exercise.

Meta-analysis results suggest 24 and 48 h were optimal durations to achieve a 207.7 (95% CI from 147.0 to 268.4 mmol·kg<sup>-1</sup> DM) and 283.0 mmol·kg<sup>-1</sup> DM (95% CI from 147.0 to 268.4 mmol·kg<sup>-1</sup> DM) increase in muscle glycogen concentration, whereas longer loading periods of 72 and 96 h did not increase muscle glycogen to the same extent (Table 4.2). Bussau et al. (2002) also claimed 24 h was sufficient to achieve significant enhancement of glycogen stores with a very high CHO intake (10.2 g·kg<sup>-1</sup>), and that 72 h provided no further benefit. However, the true loading period was ~36 h (as the final training session was ~12 h prior to initiation of high CHO intake). Furthermore, within the quantitative synthesis only 1 study (2 pairwise comparisons) contributed to the effect estimate at 24 h (Costill et al., 1981<sup>[2]</sup>), and the 48-h subgroup displayed considerable significant heterogeneity (Table 4.2), suggesting untrustworthy effect estimates. In agreement, studies included within the descriptive synthesis showed a continued increase of glycogen stores with 48-72 vs 24 h when CHO intake were >8 g·kg<sup>-1</sup>·day<sup>-1</sup> (Adamo et al., 1998; Costill et al., 1981<sup>[1]</sup>; Kochan et al., 1979; Roberts et al., 2016; Sherman et al., 1981). Further, others reported replenishment of glycogen to expected baseline values (~400 mmol·kg<sup>-1</sup> DM) following 24 h of increased CHO intake post-glycogen depletion (Bergström and Hultman, 1966; MacDougall et al., 1977; Kochan et al., 1979), however these participants were not endurance trained. In contrast, Fairchild et al. (2002) reported one of the highest glycogen values (862.2 ± 57.0 mmol·kg<sup>-1</sup> DM) following 10.3 g·kg<sup>-1</sup> of CHO for 24 h

in endurance trained participants, suggesting when all factors are optimal (very high CHO intake, high glycaemic index CHO, heightened post-exercise glycogen resynthesis, endurance trained participants), it is possible to achieve glycogen supercompensation within 24 h. However, in most cases ~1.5-fold enhancement of stores occurs within 24 h (Burke et al., 1993; Burke et al., 1995; Burke et al., 1996) with glycogen values commonly ~200 mmol·kg<sup>-1</sup> DM less than those reported in the previous study (Fairchild et al., 2002). Therefore, it seems 36 – 72 h is required to maximise pre-competition glycogen stores when CHO intake is high-very high (8-12 g·kg<sup>-1</sup>·day<sup>-1</sup>). As athletes aim to optimise stores as efficiently as possible, to minimise effects on training and competition preparations, 36-48 h may be a balance of what is practically achievable whilst allowing a sufficient window to optimise synthesis.

It is well-established high glycaemic index CHO sources are superior for achieving a greater enhancement of glycogen synthesis and concentrations (Blom et al., 1987b; Kiens et al., 1990), at least during shorter ≤ 24 h periods (Burke et al., 1993). High glycaemic index CHO sources provide readily available glucose molecules for absorption within the gut, resulting in higher blood glucose and insulin responses, greater glucose uptake by the muscle and increased glycogen synthesis compared to low glycaemic index CHO sources, which require longer to digest and do not incur the same blood glucose response (Blom et al., 1987b; Kiens et al., 1990; Burke et al., 1993). In contrast, Costill et al. (1981<sup>[1]</sup>) suggested there was no effect of consuming simple compared to complex CHO sources over a 24 h loading period. However, classification of complex and simple CHO sources do not correspond to high and low glycaemic index, as complex CHO can still be high glycaemic index (Jenkins et al., 1981). This likely contributed to the lack of differences between conditions reported in the previous study, and unfortunately foods consumed by participants were not adequately described to judge glycaemic index (Costill et al., 1981<sup>[1]</sup>; Burke et al., 2017). Based on existing evidence, athletes

implementing shorter suboptimal duration loading periods pre-competition ( $< 36$  h) should primarily consume high glycaemic index CHO sources and take advantage of the acute phase of increased post-exercise resynthesis. For athletes using longer loading periods ( $> 36$  h), a mixed healthy diet with a combination of low and high glycaemic index foods would likely be adequate, as longer recovery periods appear to minimise the importance of CHO type (Burke et al., 2017; Jentjens and Jeukendrup, 2003). However, as achieving high and very high CHO intakes can be challenging, due to potential GI discomfort (Lamb et al., 1991; Tarnopolsky et al., 2001), athletes should supplement nutritional intake with low fibre, high glycaemic index sources, making ingestion more tolerable.

Training status significantly impacted glycogen concentration ( $P = 0.01$ ); however, subgroup effect estimates are untrustworthy due to high subgroup heterogeneity and unbalanced covariance distribution (Table 4.3). A previous meta-analysis of CHO intake and muscle glycogen reported that under periods of normal and high CHO availability for every  $10 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  increase in  $\dot{V}\text{O}_{2\text{max}}$ , individuals have  $67 \pm 15$  and  $123 \pm 42 \text{ mmol} \cdot \text{kg}^{-1} \text{ DM}$  greater glycogen concentrations at rest (Areta and Hopkins, 2018). In agreement, previous studies have consistently shown endurance trained individuals have a greater ability to store muscle glycogen post-exercise with increased dietary CHO intake (Blom et al., 1987; Hickner et al., 1997; Greiwe et al., 1999). The mechanisms behind this being a 2-fold higher glycogen synthesis rate in the first 6 h post-exercise, primarily due to greater GLUT4 activity (and content) and resultant augmented glucose transport into the muscle (Blom et al., 1987; Hickner et al., 1997; Greiwe et al., 1999), as well as higher glycogen synthase activity immediately post-exercise (Hickner et al., 1997). Furthermore, trained individuals have a greater composition of type I muscle fibres, and considering glycogen is preferentially stored in the intermyofibrillar space of type I fibres following a high CHO intake for 72 h (Jensen et al.,

2021), theoretically trained individuals have greater capacity for glycogen storage. Interestingly, regression data showed no significant linear relationship between CHO intake and muscle glycogen in well trained participants (Figure 4.2c; Figure 4.3c). One potential reason behind this, could be as training status increases, required CHO intake to achieve maximisation of glycogen stores diminishes due to upregulation of post-exercise CHO storage via greater transport enzyme (GLUT 4) activity and content post-exercise. However, results were likely effected by the smaller number of study groups in the well-trained regression analysis, particularly at low and very high CHO intakes, where no studies fed  $< 4.7$  or  $> 12.0$   $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO (Figure 4.2c), limiting the ability to detect potential linearity through a smaller spread across CHO intakes compared to the other regression models.

Conducting exercise pre-CHO loading, either as a depletion or a challenging high intensity exercise stimulus increases muscle glycogen synthesis rates compared to rested conditions as glycogen utilisation drives localised glycogen resynthesis in the exercised muscle through increased glycogen synthase activity (Bergström and Hultman, 1966; Bergström et al., 1972; Kochan et al., 1979; Nelson et al., 2001) and suppression of glycogen phosphorylase (Katz, 2022). However, it should be noted previous studies were conducted in relatively untrained participants, as others have shown a very high CHO intake ( $9\text{-}10$   $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) without challenging exercise pre-CHO loading to increase glycogen concentrations in endurance trained individuals (Bussau et al., 2002; Hill et al., 2013; Rauch et al., 1995). However, considering the beneficial mechanisms of an exercise stimulus for glycogen synthesis, athletes should strategically implement CHO loading around pre-competition training schedules, where elite athletes typically complete their final high intensity training sessions 2-3 days prior (unpublished observations from the field) which would provide a strong stimulus to drive glycogen resynthesis. In combination with an aggressive acute post-exercise refeed of CHO

(1.2 g·kg<sup>-1</sup>·h<sup>-1</sup> for 4 h, in line with contemporary guidelines; Thomas et al., 2016) and a high-very high CHO intake for the subsequent 48 h, in theory, this would optimise glycogen synthesis and maximise pre-competition glycogen stores (provided no further moderate-high intensity exercise was conducted).

Exercise during the loading period itself also requires consideration, as training at a moderate-high intensity in the immediate days pre-competition would result in utilisation of glycogen stores, potentially leading to suboptimal glycogen concentrations for endurance performance. Between study comparisons suggest, provided CHO intake is very high and training intensity is low-moderate (e.g. pre-competition taper), there is no negative effect of training during the loading period on muscle glycogen concentration (Doering et al., 2019; Sherman et al., 1981; Tarnopolsky et al., 2001), which is common practice for endurance athletes to maintain training adaptations (Mujika and Padilla, 2000). Interestingly, 39 out of the 56 studies rested participants during periods of increased CHO intake, which does not represent the real-world pre-competition practices of athletes. Furthermore, those who included light-moderate intensity exercise sessions as a simulated pre-competition taper, collected no measurements during exercise to describe physiological or metabolic responses (Doering et al., 2019; Sherman et al., 1981; Tarnopolsky et al., 2001). Considering when glycogen stores are high, the contribution of glycogen to CHO oxidation and exercise energy expenditure at the same relative exercise intensity is increased (Arkinstall et al., 2004; Romijn et al., 1993), there would be greater glycogen utilisation in exercise sessions conducted during the loading period (provided intensity and duration were consistent). Alternatively, these sessions provide an exercise stimulus, with augmented post-exercise synthesis rates possibly replenishing glycogen stores (provided post-exercise CHO ingestion was adequately increased and other effectors [duration and type] were optimal), which could explain the high glycogen concentrations

reported in the previous studies (Doering et al., 2019; Sherman et al., 1981; Tarnopolsky et al., 2001). Nevertheless, these suggestions are speculative and require further investigation.

Participant biological sex has previously been suggested as a covariate of glycogen concentration, as Tarnopolsky et al. (1995) suggested females had an impaired ability to store muscle glycogen. However, differences were due to variable CHO intakes between conditions as opposed to biological sex (6.4 vs 8.2 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO for females and males, respectively) as a result of prescribed CHO intake expressed as % energy intake as opposed to relative to body mass. When relative or absolute CHO intake (g·kg<sup>-1</sup>·day<sup>-1</sup> or g·day<sup>-1</sup>, respectively) was similar between males and females, either within the same study (Tarnopolsky et al., 2001; Impey et al., 2020; James et al., 2001) or between different trials (McLay et al., 2007; Nicklas et al., 1989; Walker et al., 2000), females have the same capacity for muscle glycogen storage, with glycogen values similar to those reported in males (Table 4.1). Effects of female menstrual cycle phase on muscle glycogen storage are inconclusive, as some have reported greater glycogen stores from a moderate CHO intake (3-5.2 g·kg<sup>-1</sup>·day<sup>-1</sup>) for females in the mid-luteal phase compared to the mid-follicular (Nicklas et al., 1989; McLay et al., 2007). However, when CHO intake was high-very high (8-10 g·kg<sup>-1</sup>·day<sup>-1</sup>) no differences were apparent between menstrual cycle phase (James et al., 2001; McLay et al., 2007). These mixed results justify further investigation; however the practical relevance is debatable due to the alignment of competition days and menstrual cycle phase being beyond the control of female athletes.

A limitation of the quantitative analysis is the observational nature of subgroup analyses (Higgins et al., 2024); however, caution has been suggested throughout regarding interpretation of effect estimates, with transparency in terms of covariance distribution (number of pairwise

comparisons and participants) and heterogeneity. It could be argued meta-regression may have been more statistically effective in assessing effects of covariates on muscle glycogen, however due to the confounded nature of the covariates that contributed to heterogeneity associated with measurements of glycogen concentrations (loading duration, CHO intake and training status were all significant effectors of glycogen concentrations, and studies were not classifiable due to inconsistency across the different levels of these factors) a meta-regression of studies that met the inclusion criteria for quantitative synthesis was not possible. Furthermore, it should be acknowledged that potential publication bias could have impacted review results, where studies with significant positive findings were more likely to be published in comparison to those reporting no statistically significant effect.

#### ***4.5.1 Conclusions***

This systematic review and meta-analysis indicated a linear dose-response relationship between dietary CHO intake and muscle glycogen, as well as a multifaceted heterogeneity associated with the relationship, as quantity of CHO ingested (expressed relative to body mass or as an absolute value), loading duration, training status and exercise (pre- and during the loading period) were key determinants of glycogen concentrations. Achieving an optimisation of pre-competition glycogen stores is a balancing act of what is optimal for glycogen synthesis and what is practical for athletes to implement in the real world, which can be achieved with different combinations of these key effectors. Findings revealed a hierarchy, as dietary CHO intake ( $\geq 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 36-48 h) was the most important factor to maximise muscle glycogen stores. Despite specific depleting exercise pre-loading not being a pre-requisite for trained individuals, athletes should strategically implement loading strategies around their final challenging training session pre-competition to efficiently maximise glycogen stores, provided

this is necessary to meet the demands of upcoming exercise. However, significant heterogeneity and confounding in past research introduce uncertainty in these recommendations, and further well-controlled studies are required to determine the true optimal pre-exercise CHO intake to maximise glycogen concentrations and endurance performance.

#### ***4.5.2 Link to next chapter***

Despite being a well-researched area within sports nutrition, the magnitude of the relationship between dietary CHO intake and muscle glycogen remains unclear due to confounding of key effectors of glycogen concentration across previous studies (CHO quantity, loading duration, training status, exercise during/pre-loading). As such the true dose-response remains debatable, particularly as the previously suggested saturation point of CHO intake for muscle glycogen storage ( $7\text{-}10\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ; Costill et al., 1981; Burke et al., 1995; Burke et al., 2017) contrasts with current review regression data (Figure 4.2 and 4.3). Therefore, it is now necessary to determine the dose-response relationship of CHO loading for skeletal muscle glycogen, whilst adequately controlling determined effectors of glycogen storage (Chapter 4), and replicating the pre-competition training practices of endurance athletes.

## **Chapter Five:**

### **Dose-response of pre-exercise dietary CHO availability for skeletal muscle glycogen synthesis in endurance trained cyclists**

*This work has been submitted for publication in Sports Medicine and will be presented orally at The European College of Sports Science (ECSS) conference in Rimini, 2025.*

## 5.1 Abstract

High dietary CHO intake is important for competitive endurance sport due to increased muscle glycogen stores; however, the dose-response of this relationship is unclear. In a counterbalanced repeated measures design, 11 endurance trained participants (8 males, 3 females; age,  $24 \pm 5$  years; body mass,  $71.2 \pm 12.0$  kg;  $\dot{V}O_{2\max}$ ,  $56 \pm 6$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ; PPO,  $306 \pm 54$  W; LT1, 164 W) undertook 3 x 5-days of dietary control and prescribed exercise designed to mimic pre-competition practices of endurance cyclists. Following two days of standardised dietary intake and exercise prescription, participants consumed either 6, 8 or 10 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$  of CHO for the following 48 h, before returning to the laboratory (day 5) for body mass and water measurements, high CHO breakfast and muscle biopsy 2 h post-prandial. Muscle glycogen was significantly higher following 10 compared to 6 and 8 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$  of CHO ( $635.5 \pm 78.0$ ,  $460.9 \pm 100.7$  and  $506.1 \pm 124.0$  mmol $\cdot$ kg $^{-1}$  DM, respectively,  $P < 0.03$ ), however there was no difference between 6 and 8 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$  ( $P = 1.00$ ). There was a significant strong positive correlation between relative ( $r = 0.71$ ,  $P < 0.001$ ) and absolute CHO intake ( $r = 0.64$ ,  $P < 0.001$ ) and whole muscle glycogen concentration. In conclusion, there is a strong linear dose-response between dietary CHO intake and muscle glycogen concentration, with intakes  $\geq 10$  g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$  appearing necessary to maximise muscle glycogen storage in real world training conditions, with minimal impact on gut comfort and body mass.

## 5.2 Introduction

Increased dietary CHO intake and decreased training load in the days pre-competition is a well-established strategy used by endurance athletes (termed CHO loading) to enhance skeletal muscle glycogen stores (Karlsson and Saltin, 1971; Burke et al., 2000; Costill et al., 1981; Sherman et al., 1981; Lamb et al 1991). Historically, this strategy was first introduced in the 1960s, where it was discovered manipulation of CHO intake and exercise could ‘dramatically’ increase muscle glycogen stores (Bergström and Hultman, 1966). Shortly after, the ‘classic’ CHO loading regimen was introduced, where two exhaustive cycling sessions separated by 3 days low CHO intake were followed by 3 days of a high CHO intake (Bergström et al., 1967). This ‘supercompensation’, in some cases by almost twofold (Arnall et al., 2007; Bussau et al., 2002; Fairchild et al., 2002; James et al., 2001), of limited glycogen stores within skeletal muscle has been shown to improve endurance exercise capacity (Bergström et al., 1967; Lamb et al., 1991; Doering et al., 2019) and, in some cases, endurance exercise performance lasting  $\geq 90$  min (Rauch et al., 1995; Hawley et al., 1997a). CHO loading has since been modified (Sherman et al., 1981), updated (Bussau et al., 2002) and ultimately has evolved into contemporary nutrition guidelines, which advocate  $8\text{--}12\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 36–48 h prior to endurance competitions or prolonged high intensity endurance exercise  $\geq 2.5\text{h}$  (Burke et al., 2017; Thomas et al., 2016).

However, despite over half a century of research, the relationship between high (and very high) dietary CHO intakes and skeletal muscle glycogen is unclear. A systematic review of existing literature (Chapter 4) revealed significantly variable concentrations in muscle glycogen from similar CHO intakes ( $480\text{--}840\text{ g}\cdot\text{kg}^{-1}\text{ dry mass (DM)}$  from  $7.9\text{--}8.3\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO), even when well-established effectors of glycogen synthesis such as training status, and exercise (mode, duration, intensity) were similar (Greiwe et al 1999; Blom et al., 1987;

Sherman et al., 1981). Whereas others have reported no change in glycogen concentration, despite  $\geq 3 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  differences in CHO intake between conditions (Bradley et al., 2016; Burke et al., 1995). Further, during most CHO loading studies participants were rested during periods of increased CHO intake (Bergström et al., 1967; Bussau et al., 2002; Costill et al., 1981; Fairchild et al., 2002), with only a limited number of studies replicating real-world pre-competition training practices of endurance athletes (i.e. exercise taper), where despite reduced load a daily training stimulus is maintained (Doering et al., 2019; Sherman et al., 1981; Tarnopolsky et al., 2001).

Surprisingly, only one dose-response study has investigated multiple levels of dietary CHO intake within endurance trained individuals. Experimental conditions provided 2.3, 4.7 and 6.6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 24 h (Costill et al., 1981), which is low considering current guidelines (Thomas et al., 2016). Furthermore, this study had a small sample size ( $n = 4$ ), raising questions regarding statistical strength of results. Therefore, further research is warranted, particularly looking at the dose-response of higher CHO intakes (in line with current contemporary guidelines), as a ‘ceiling’ effect has previously been suggested at 7-10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO (Burke et al., 1995; Burke et al., 2017), but not consistently shown.

Finally, in addition to the uncertainty regarding CHO loading associated performance improvements, this strategy carries a risk of impairing performance through potential GI discomfort symptoms and increased body mass. Considerations for GI tolerability are required due to the well-established association between gut discomfort symptoms and high CHO consumption during exercise (Rehrer et al., 1992; de Oliveria et al., 2014). It has also previously been shown that muscle glycogen supercompensation increases body mass 1-2%

due to increased body water (Shiose et al., 2016), as 1 g of glycogen is bound to 3-4 g of water (Olsson and Saltin, 1970).

The primary aim of this study was to investigate the dose-response relationship of dietary CHO availability and whole muscle glycogen in endurance trained individuals under real-world conditions, whilst considering tolerability and gut comfort. A secondary aim was to investigate the relationship between TBW and skeletal muscle glycogen. We hypothesised that increased CHO intake would result in a stepwise increase in skeletal muscle glycogen, however the magnitude of the increase would be smaller when comparing high vs very high CHO intakes. Further, there would be mild symptoms of GI discomfort reported across conditions and increased skeletal muscle glycogen concentrations would increase body mass through increased total and intracellular body water bound within stored glycogen.

## **5.3 Methods**

### ***5.3.1 Participants***

Eleven endurance trained individuals participated in the study (8 males, 3 females; Table 5.1), in line with the classification of training status outlined by De Pauw et al. (2013) and Decroix et al. (2016) for male and female ‘trained’ cyclists, respectively. Participants were provided with all information regarding study procedures before written informed consent was obtained. The study was approved by the Liverpool John Moores University Research Ethics Committee (Ethics code: 22/SPS/041). Information regarding menstrual function was collected using an adapted LEAF-Q (Melin et al., 2014).

**Table 5.1.** Study 1 participant characteristics.

|                                                              | Males (n = 8) | Females (n = 3) | Combined (n = 11) |
|--------------------------------------------------------------|---------------|-----------------|-------------------|
| Age (years)                                                  | 24 ± 6        | 25 ± 3          | 24 ± 5            |
| Height (cm)                                                  | 178 ± 8       | 165 ± 10        | 174 ± 10          |
| Body mass (kg)                                               | 75.2 ± 10.9   | 60.6 ± 8.8      | 71.2 ± 12.0       |
| $\dot{V}O_{2\max}$ (L·min <sup>-1</sup> )                    | 4.81 ± 0.48   | 2.94 ± 0.30     | 4.3 ± 0.98        |
| $\dot{V}O_{2\max}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) | 59 ± 3        | 48 ± 4          | 56 ± 6            |
| PPO (W)                                                      | 331 ± 40      | 241 ± 15        | 306 ± 54          |
| PPO (W·kg <sup>-1</sup> )                                    | 4.4 ± 0.4     | 4.0 ± 0.4       | 4.3 ± 0.4         |
| Lactate threshold (W)                                        | 177 ± 52      | 128 ± 9         | 164 ± 49          |
| Training history (years)                                     | 4 ± 3         | 4 ± 2           | 4 ± 3             |
| Training load (h·week <sup>-1</sup> )                        | 7 ± 3         | 6 ± 2           | 7 ± 3             |

### 5.3.2 Study overview

A schematic representation of the protocol can be seen in Figure 5.1. In a randomised repeated measures design (block randomisation), participants completed 3 x 5-day periods of dietary control and prescribed exercise designed to mimic pre-competition practices of competitive endurance cyclists. Participants were unaware of upcoming treatments until the first day of each condition. Days 1 and 2 standardised physiological condition and muscle glycogen concentrations through ~36 h of a low-moderate CHO intake (4 g·kg<sup>-1</sup>·day<sup>-1</sup>), followed by very challenging cycling exercise session. Days 3 and 4 participants consumed a moderate, high or very high CHO intake (6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively), before returning to the laboratory on the morning of day 5 for a high CHO breakfast, 2 h rest period and muscle biopsy. Experimental conditions were separated by ≥ 7 days.

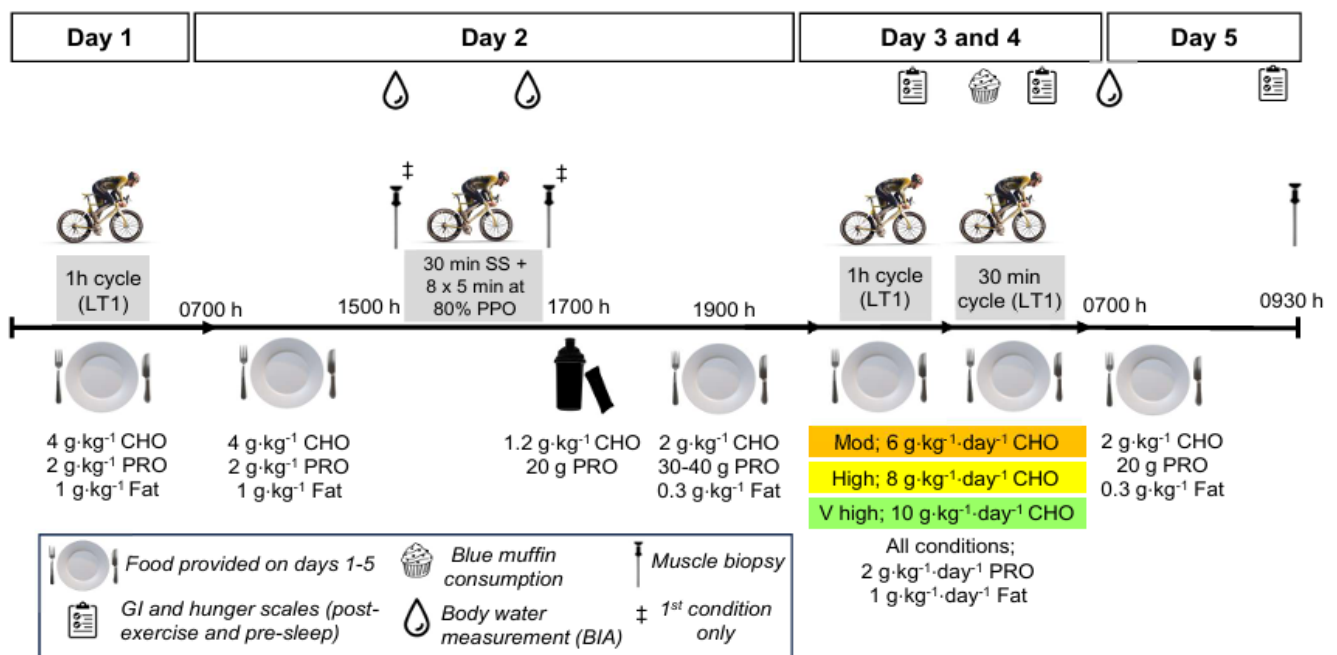
### **5.3.3 Preliminary testing**

Height and body mass were measured semi-nude (Seca, Hamburg, Germany), before participants were seated for resting measures of HR (Polar H10, Kempele, Finland), blood glucose and lactate using capillary fingertip blood samples, which were immediately analysed (Biosen C-Line, EKF Diagnostics, Cardiff, UK). Participants then completed incremental lactate threshold and maximal oxygen consumption ( $\dot{V}O_{2\max}$ ) tests on a cycle ergometer (Lode Excalibur sport, Groningen, Netherlands). Briefly, participants began cycling at 100 W (75 W for females) and exercise intensity increased 25 W following each 4 min stage. In the final 30 s of each stage, a fingertip blood sample was collected and immediately analysed for blood glucose and lactate, with HR collected in the final 10 s of each stage. The test was terminated when participants reached the onset of blood lactate accumulation  $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$  (Heck et al., 1985).

Following 10 min rest, participants completed a  $\dot{V}O_{2\max}$  test on the same ergometer. The test began at 100 W and exercise intensity increased 25 W every 1 min until volitional exhaustion. Gas exchange was measured continuously throughout using a metabolic cart (Moxus, AEI Technologies, Illinois, USA), with  $\dot{V}O_{2\max}$  defined as the highest  $\dot{V}O_2$  sustained over a 30-s average. HR and RPE were collected in the final 10 s of each stage and were used as indirect markers of volitional exhaustion. PPO was determined using the equation outlined by Kuipers et al. (1985). Prior to the next laboratory visit participants were asked to record their habitual dietary intake for 48 h, during weekdays, using the remote food photography method (Martin et al., 2009; Martin et al., 2012).

### 5.3.4 Experimental trials

*Prescribed exercise.* On days 1, 3 and 4, participants visited the laboratory at 1500 h and completed 60-, 60- and 30-min steady state light-moderate intensity cycling exercise, respectively (LT1;  $164 \pm 49$  W). HR was measured at rest and every 5 min with RPE collected at the end of each session. Exercise sessions were designed to mimic a pre-competition taper, where even during a CHO loading period athletes would still complete light-moderate intensity training to maintain training adaptations (Mujika and Padilla, 2000). Participants completed no additional exercise outside what was prescribed during trials.



**Figure 5.1.** Study 1 schematic overview of study experimental conditions.

*Hard cycling session.* On day 2, participants arrived at the laboratory at 1500 h to complete a glycogen depletion protocol adapted from Stepto et al. (2001), previously shown to lower muscle glycogen ~50% (Yeo et al., 2008). The session consisted of a 5 min warm-up at 100 W, 30 min cycle at an intensity ~65%  $\dot{V}O_{2\max}$ , followed by 2 min active recovery at 100 W. Following this, participants completed 8 x 5 min intervals at 80% PPO, separated by 1 min active recovery at 100 W. If unable to complete a 5-min interval, the active recovery was initiated at task failure (inability to maintain a cadence > 60 rpm for 10 s consecutively) and exercise intensity decreased (-5% PPO) for the remaining intervals. Exact work and recovery times were replicated for all conditions to standardise absolute work done and level of glycogen depletion.

*Dietary manipulation.* Days 1 and 2 (pre-hard exercise) participants consumed a low-moderate CHO intake ( $4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) as well as 2, 1 and  $0.5\text{-}0.6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of protein, fat and dietary fibre respectively. Immediately post-depletion, participants were provided with  $1.2 \text{ g}\cdot\text{kg}^{-1}$  CHO and 20 g protein in the form of a sports drink and protein bar (Science in Sport, UK). Two hours later, participants consumed an evening meal consisting of  $2 \text{ g}\cdot\text{kg}^{-1}$  CHO, 30-40 g protein and  $0.3 \text{ g}\cdot\text{kg}^{-1}$  of fat. This was deemed a more accurate representation of post-exercise practices of elite athletes (anecdotal evidence from the field), where consuming  $1.2 \text{ g}\cdot\text{kg}^{-1}$  of CHO per hour for 4 h, as contemporary guidelines recommend (Thomas et al., 2016), is rarely achieved. Days 3 and 4, participants consumed either 6, 8 or  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO as well as 2, 1 and  $0.5\text{-}0.6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of protein, fat and dietary fibre respectively (Table 5.2). On the morning of day 5 (0715-0730 h) participants consumed a high CHO breakfast providing  $2 \text{ g}\cdot\text{kg}^{-1}$  CHO, 20 g protein and  $0.3 \text{ g}\cdot\text{kg}^{-1}$  of fat. A day 3 example meal plan for a 74 kg participant is displayed in Table 5.3.

All foodstuff for each 5-day experimental period was provided pre-packaged and weighed (nearest 0.05 g), along with instructions and a checklist. Participants were asked to send photos of each meal at the time of consumption via online messenger to confirm adherence (WhatsApp, Meta, USA). Plans provided a standardised healthy mixed diet in the form of usual meals (breakfast, lunch and dinner) with various snacks throughout each day. High and very high CHO conditions were supplemented with high CHO, zero fibre products such as jelly sweets, orange juice and sports drinks. Food containers were weighed pre and post provision to account for any leftovers ( $97.1 \pm 1.0$  % compliance), with participants instructed to only consume what was provided by researchers. Water consumption was *ad libitum*, with total fluid intake monitored across conditions. No caffeine or alcohol consumption was permitted throughout each 5-day period due to potential impacts on glycogen synthesis (Pedersen et al., 2008; Burke et al., 2003).

*Subjective assessment of gut comfort, stool characteristics and gut transit time.* Days 3 and 4 participants were asked to record frequency and time of day of bowel movements and stool type using the Bristol stool chart (Lewis and Heaton, 1997). Post-exercise and pre-sleep on days 3 and 4, and pre-biopsy day 5, participants completed subjective scales of gut discomfort symptoms and hunger as previously described (see Chapter 3 General methods). Gut comfort was recorded on a 0-10 scale for symptoms of nausea, reflux, stomach fullness, abdominal cramps, flatulence and urge to defecate (Wilson, 2017), whilst hunger scales were completed on a series of 0-100 mm lines (Hengist et al., 2020). Post-lunch on day 4 participants consumed two muffins coloured with a blue dye (~60 g each, containing 0.75 g of royal blue food colouring), and were asked to note down the exact time of consumption as well as the day and time of the first excretion event with visible blue colour within stool (Asnicar et al., 2020).

*Body water measurements.* TBW and ECW were measured in standardised conditions on 3 occasions per condition (pre- and post-depletion on day 2 and the morning of day 5 in an overnight fasted state) using an eight-electrode multifrequency bioelectrical impedance (BIA; Seca mBCA 515, Hamburg, Germany), previously validated in healthy adults (Bosy-Westphal et al., 2013). ICW was calculated as the difference between TBW and ECW.

*Muscle biopsies.* Five biopsies were obtained in total, pre- and post-exercise day 2 in the first experimental condition (to determine baseline muscle glycogen concentrations following ~36 h of  $4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  CHO and confirm level of glycogen depletion, respectively) and 2 h post-breakfast day 5 in each condition, to simulate ‘pre-race’ muscle glycogen concentrations. Biopsies were obtained from the *vastus lateralis* under local anaesthesia (Marcaine 0.5 %), as previously described (Chapter 3 General methods). To avoid impact of multiple biopsies (Costill et al., 1988a), pre- and post-depletion biopsies were taken from the same leg with subsequent biopsies taken from alternate legs. Upon collection, samples were dissected from visible connective tissue and fat before being frozen in liquid nitrogen and stored at -80 °C for later analysis.

*Biochemical analysis of muscle glycogen.* Muscle glycogen concentration was determined with acid hydrolysis as previously detailed (Doering et al., 2019). Prior to hydrolysis, samples were freeze dried and dissected of visible blood, fat or connective tissue under a microscope (Zeiss, Germany). The remaining muscle mass (3-5 mg) was weighed and split into duplicates for each timepoint. 500  $\mu\text{L}$  of 1 M of hydrochloric acid was added to each sample, which was heated for 3 h at 95 °C in a water bath to hydrolyse glycogen to glycosyl units. Samples were cooled at room temperature, before being neutralised with 250  $\mu\text{L}$  of Tris/KOH saturated with KCl.

Samples were centrifuged and glucose concentrations of the remaining supernatant were quantified using a commercially available kit (GLUC-HK, Randox Laboratories, Antrim, UK). Post-depletion values below analyser sensitivity range were associated with 40% measurement error (Randox Laboratories, Antrim, UK), as such for these data points (8 out of 11) the lowest detectable value ( $0.35 \text{ mmol}\cdot\text{L}^{-1}$ ) was used to convert glucose values to glycogen. Coefficient of variation between duplicates of the same muscle sample was  $10.5 \pm 9.3 \%$ .

### ***5.3.5 Statistical analysis***

All data is presented as means  $\pm$  SD unless otherwise stated. Linear mixed models were used to analyse whole muscle glycogen concentration due to 3 missing samples, where CHO intake and participants were fixed and random effects, respectively. Residuals were checked for normality using the Shapiro-Wilks test accompanied by visual inspection of Q-Q plots. Missing data points were estimated using the maximum likelihood method. Pearson's correlation was used to assess the strengths of the linear relationship between absolute CHO intake (g) and whole muscle glycogen concentration. Ordinal data (stool type and gut discomfort symptoms) were analysed using the Friedman's test, where median scores across each loading period (day 3, 4 and 5) were compared between conditions. Significant differences were explored with Wilcoxon signed-rank tests with Bonferroni adjustment to account for multiple comparisons. The remaining continuous variables were checked for normality (as previously described) and where appropriate analysed using a one- or two-way repeated measures ANOVA, with Bonferroni post hoc tests used to explore significant pairwise comparisons. All data was analysed using SPSS (v 29, IBM, USA) with significance set at  $P < 0.05$ . Figures were created using GraphPad Prism (v 10, Massachusetts, USA).

## 5.4 Results

### 5.4.1 Nutrition, fluid intake, hunger and satiety

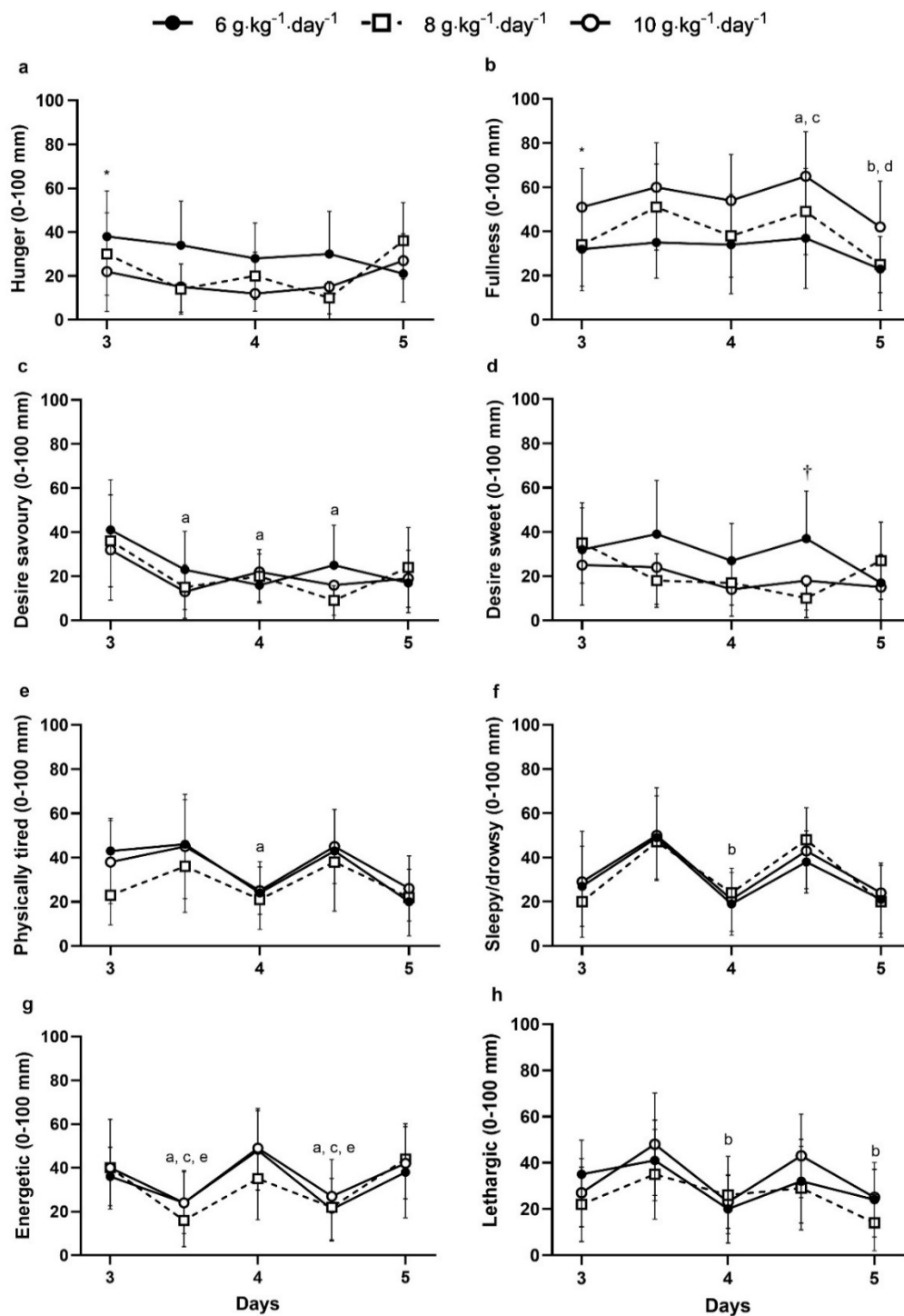
Nutritional intake across each experimental period was successfully controlled, with prescribed and actual nutritional intake closely matched (Table 5.2). As a result, CHO consumption on days 1 and 2 were similar across all conditions ( $P = 1.00$ ), whilst being significantly different across loading days for 6, 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  ( $P < 0.001$ ). There was no main effect of condition ( $P = 0.11$ ) or time ( $P = 0.14$ ) for fluid intake, as participants consumed  $2.1 \pm 0.5$ ,  $2.5 \pm 1.0$  and  $2.6 \pm 0.8$  L across day 3 and 4 for 6, 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , respectively. Participants were significantly hungrier (condition effect,  $P = 0.03$ ; Figure 5.2a) when consuming 6 compared to 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO ( $P = 0.05$ ), and significantly fuller (condition effect,  $P = 0.01$ ) consuming 10 vs 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO ( $P = 0.02$ ; Figure 5.2b). Desire for savoury had a significant main effect for time ( $P = 0.02$ ; Figure 5.2c), post hoc tests revealed greater desire post-exercise day 3 compared to all other timepoints ( $P < 0.05$ ). Desire for sweet saw an interaction effect ( $P = 0.05$ ) as scores were higher on day 4 whilst consuming 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO (Figure 5.2d). There was no main effect for condition on subjective scores of tiredness ( $P = 0.38$ , Figure 5.2e), sleepiness ( $P = 0.72$ , Figure 5.2f), energy ( $P = 0.42$ , Figure 5.2g) or lethargy/sluggishness ( $P = 0.36$ , Figure 5.2h).

**Table 5.2.** Participant prescribed and actual nutritional intake (based on % daily compliance) during habitual free living and experimental conditions of CHO intake (4, 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>).

|                   |                       | Habitual   | 4          | 6          | 8          | 10         |
|-------------------|-----------------------|------------|------------|------------|------------|------------|
| <i>Prescribed</i> |                       |            |            |            |            |            |
| CHO               | (g)                   | -          | 285 ± 48   | 427 ± 72   | 570 ± 96   | 712 ± 120  |
|                   | (g·kg <sup>-1</sup> ) | -          | 4.0        | 6.0        | 8.0        | 10.0       |
| Protein           | (g)                   | -          | 143 ± 24   | 143 ± 24   | 143 ± 24   | 143 ± 24   |
|                   | (g·kg <sup>-1</sup> ) | -          | 2.0        | 2.0        | 2.0        | 2.0        |
| Fat               | (g)                   | -          | 71 ± 12    | 71 ± 12    | 71 ± 12    | 71 ± 12    |
|                   | (g·kg <sup>-1</sup> ) | -          | 1.0        | 1.0        | 1.0        | 1.0        |
| Energy            | (kcal)                | -          | 2351 ± 397 | 2921 ± 493 | 3490 ± 590 | 4060 ± 686 |
| <i>Actual</i>     |                       |            |            |            |            |            |
| CHO               | (g)                   | 273 ± 70   | 277 ± 49   | 419 ± 74   | 560 ± 100  | 691 ± 120  |
|                   | (g·kg <sup>-1</sup> ) | 3.9 ± 1.0  | 3.9 ± 0.1  | 5.9 ± 0.1  | 7.8 ± 0.2  | 9.7 ± 0.3  |
| Protein           | (g)                   | 132 ± 43   | 138 ± 24   | 140 ± 25   | 140 ± 25   | 138 ± 24   |
|                   | (g·kg <sup>-1</sup> ) | 1.9 ± 0.6  | 1.9 ± 0.1  | 2.0 ± 0.0  | 2.0 ± 0.0  | 1.9 ± 0.1  |
| Fat               | (g)                   | 69 ± 38    | 69 ± 12    | 70 ± 12    | 70 ± 13    | 69 ± 12    |
|                   | (g·kg <sup>-1</sup> ) | 1.4 ± 0.5  | 1.0 ± 0.0  | 1.0 ± 0.0  | 1.0 ± 0.0  | 1.0 ± 0.0  |
| Energy            | (kcal)                | 2539 ± 679 | 2283 ± 402 | 2863 ± 507 | 3427 ± 610 | 3940 ± 682 |

**Table 5.3.** 24 h meal plan example (day 3) during the loading period for a 74 kg participant.

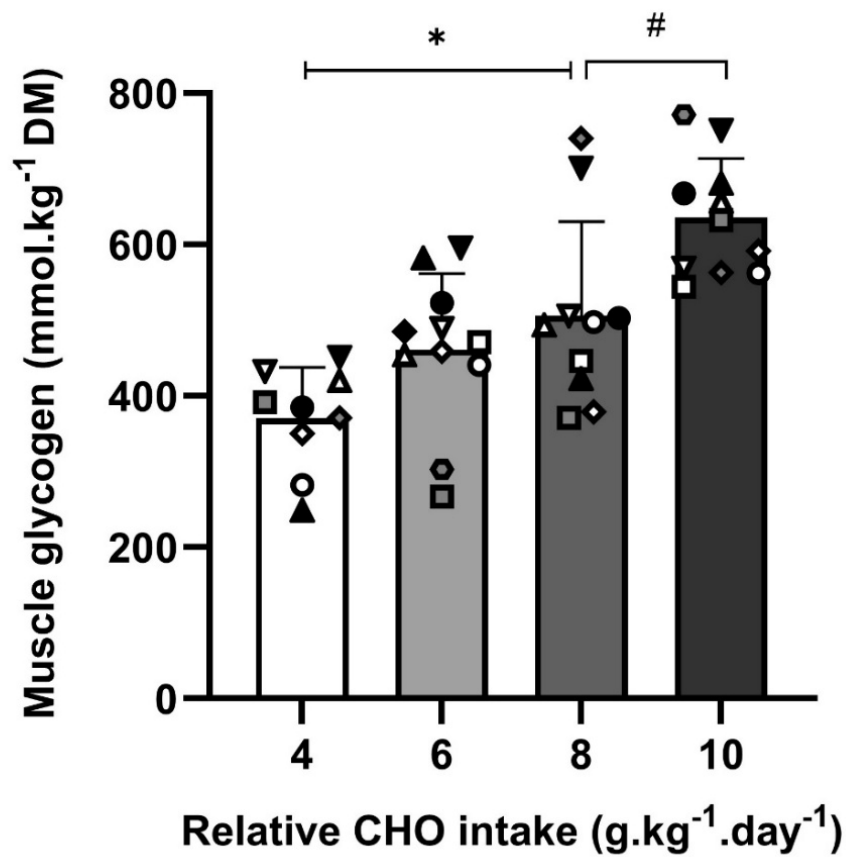
| Meal                          | 6 g·kg <sup>-1</sup> ·day <sup>-1</sup>                                                                                        | 8 g·kg <sup>-1</sup> ·day <sup>-1</sup>                                                                          | 10 g·kg <sup>-1</sup> ·day <sup>-1</sup>                                                                                       |
|-------------------------------|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Breakfast                     | 115 g granola, 180 mL semi-skimmed milk, 60 g blueberries, 100 g grapes, 222 mL orange juice                                   | 140 g granola, 160 mL semi-skimmed milk, 60 g blueberries, 100 g grapes, 412 mL orange juice                     | 150 g granola, 180 mL semi-skimmed milk, 60 g blueberries, 100 g grapes, 544 g orange juice                                    |
| Morning snack                 | 20 g Strawberry jam, 1 bagel                                                                                                   | 40 g Strawberry jam, 1 bagel                                                                                     | 40 g Strawberry jam, 1 bagel, 2 banana soren (60 g)                                                                            |
| Lunch                         | 195 g chicken, 110 g sweetcorn, 100 g green beans, 60 g sweet chilli sauce, 250 g golden vegetable rice, 500 mL Lucozade sport | 144 g chicken, 100 g sweetcorn, 100 g green beans, 55 g sweet chilli sauce, 250 g golden vegetable rice          | 129 g chicken, 110 g sweetcorn, 100 g green beans, 60 g sweet chilli sauce, 250 g golden vegetable rice, 500 mL Lucozade sport |
| Afternoon snack               | N/A                                                                                                                            | 500 mL Lucozade sport, 75 g Haribo Tangfastics                                                                   | 50 g SiS Go Energy, 90 g Haribo Tangfastics                                                                                    |
| Dinner                        | 250 g boiled pasta, 180 g Bolognese sauce, 15 g coconut oil and 138 g turkey mince                                             | 300 g boiled pasta, 180 g Bolognese sauce, 100 g onion, 2 g coconut oil and 130 g turkey mince                   | 335 g boiled pasta, 180 g Bolognese sauce, 8 g coconut oil and 115 g turkey mince, 50 g SiS Go energy                          |
| Eve snack                     | 34 g cashew nuts                                                                                                               | 42 g cashew nuts and 2 wholemeal biscuits (28 g)                                                                 | 34 g cashew nuts                                                                                                               |
| Absolute macronutrient intake | 3041 kcal, 444.0 g CHO, 148.0 g PRO, 74.0 g fat, 34.0 g fibre                                                                  | 3631 kcal, 592.0 g CHO, 148.0 g PRO, 74.0 g fat, 38.2 g fibre                                                    | 4224 kcal, 740.0 g CHO, 148.0 g PRO, 74.0 g fat, 41.0 g fibre                                                                  |
| Relative macronutrient intake | 6.0 g·kg <sup>-1</sup> CHO, 2.0 g·kg <sup>-1</sup> PRO, 1.0 g·kg <sup>-1</sup> fat, 0.5 g·kg <sup>-1</sup> fibre               | 8.0 g·kg <sup>-1</sup> CHO, 2.0 g·kg <sup>-1</sup> PRO, 1.0 g·kg <sup>-1</sup> fat, 0.5 g·kg <sup>-1</sup> fibre | 10.0 g·kg <sup>-1</sup> CHO, 2.0 g·kg <sup>-1</sup> PRO, 1.0 g·kg <sup>-1</sup> fat, 0.6 g·kg <sup>-1</sup> fibre              |



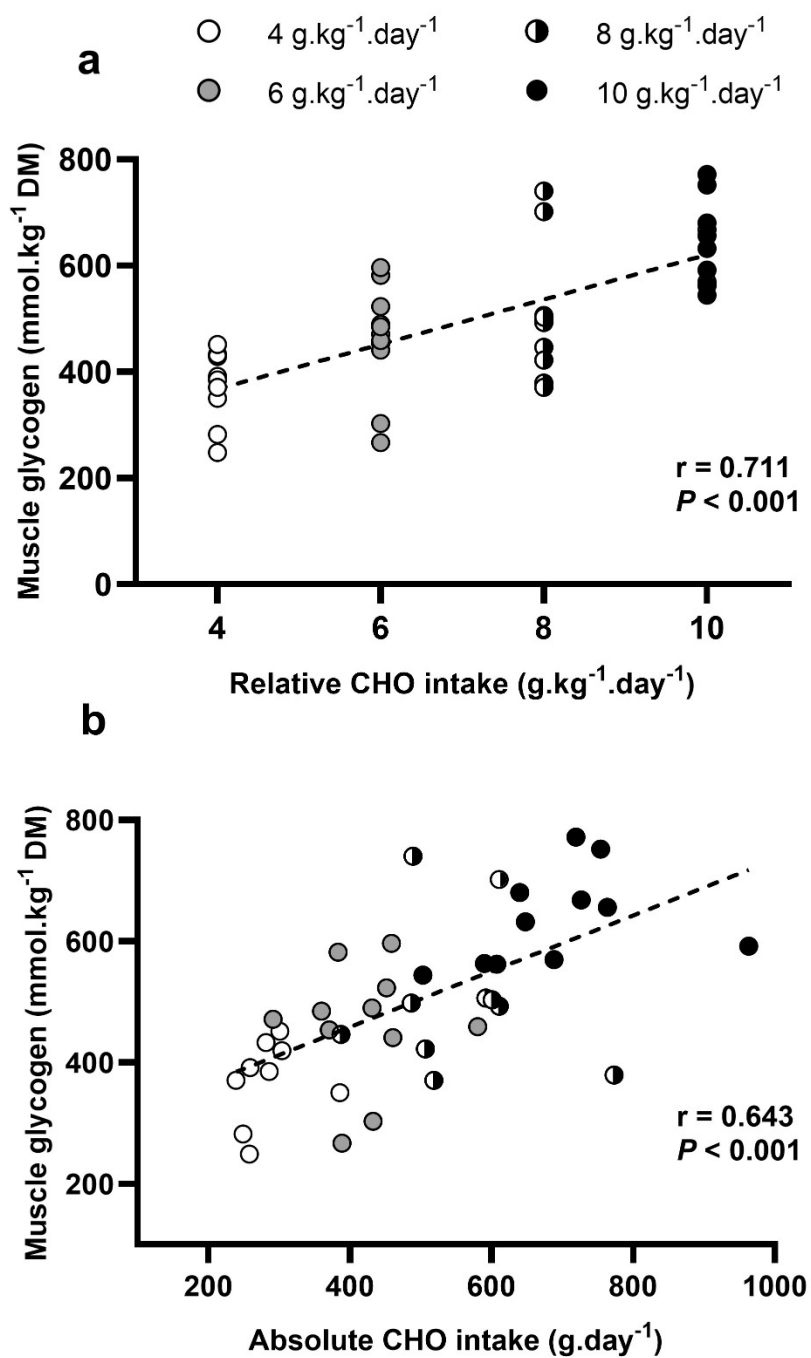
**Figure 5.2.** Subjective VAS for hunger (a), stomach fullness (b), desire for savoury (c) or sweet (d), physical tiredness (e), sleepiness (f), energy/liveliness (g) and lethargy/sluggishness (h) post-exercise, pre-sleep on days 3 and 4, and morning of day 5, under conditions of high CHO availability (6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup>). \* Significant main effect of condition (6 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup>). † Significant interaction effect. <sup>a</sup> significant time effect vs day 3 post exercise. <sup>b</sup> significant time effect vs day 3 pre-sleep. <sup>c</sup> significant time effect vs day 4 post exercise. <sup>d</sup> significant time effect vs day 4 pre-sleep. <sup>e</sup> significant time effect vs day 5.

#### **5.4.2 Muscle glycogen concentrations**

The hard cycling session decreased glycogen concentrations by 71% from baseline ( $-266.9 \pm 79.4 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ,  $P < 0.001$ ). There was a significant main effect of CHO intake on muscle glycogen concentration ( $P < 0.001$ ), where consuming a high or very high relative CHO intake (8 or  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) for 48 h resulted in significantly greater glycogen concentrations compared to baseline (Figure 5.3). There was no significant difference following consumption of 4 vs 6 ( $P = 0.20$ ) or 6 vs 8  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO ( $P = 1.00$ , respectively), whilst  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  resulted in significantly higher glycogen concentrations compared to all other conditions ( $P < 0.05$ ). Delta between conditions were similar, as  $2 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  increases in CHO intake increased muscle glycogen  $107.1 \pm 123.2$ ,  $35.3 \pm 118.8$  and  $117.8 \pm 137.3 \text{ mmol kg}^{-1} \text{ DM}$  from 4 to 6, 6 to 8 and 8 to  $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  respectively ( $P = 0.47$ ,  $\eta^2_p = 0.09$ ). There was a significant strong positive correlation between relative ( $r = 0.71$ ,  $P < 0.001$ ; Figure 5.4a) and absolute CHO intake ( $r = 0.64$ ,  $P < 0.001$ ; Figure 5.4b) compared with whole muscle glycogen.



**Figure 5.3.** Whole skeletal muscle glycogen concentrations following consumption of a diet containing either 4, 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO for 36-48 h. \* Significantly different from 10 g.kg<sup>-1</sup>.day<sup>-1</sup>. # Significantly different from 4 g.kg<sup>-1</sup>.day<sup>-1</sup>. Symbols represent individual data points.



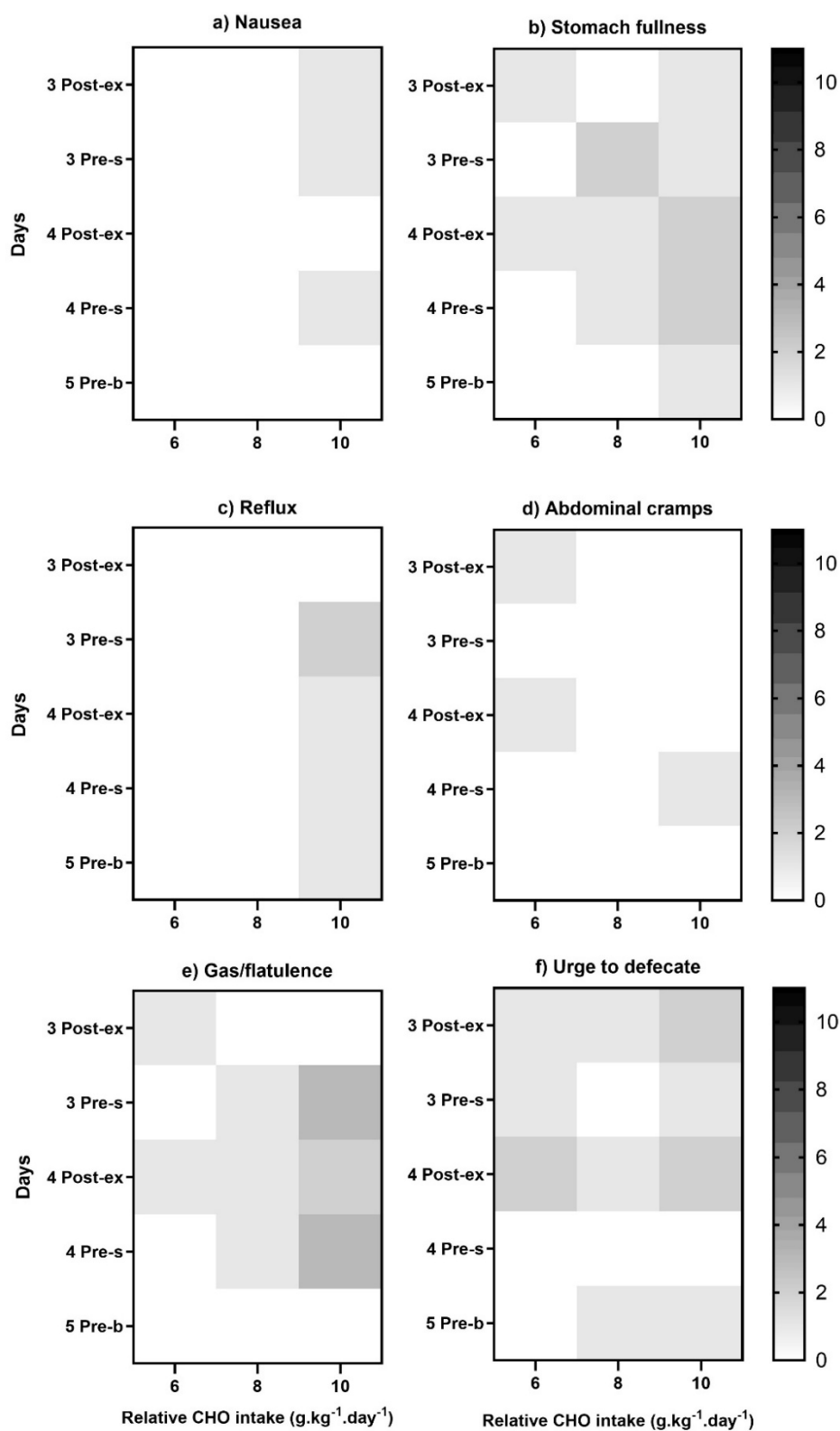
**Figure 5.4.** Correlation of whole skeletal muscle glycogen and relative (a) or absolute CHO intake (b) following consumption of a diet containing either 4, 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO for 36-48 h.

### 5.4.3 Gastrointestinal measures

Median (IQR) stool type did not differ ( $\chi^2 [2] = 0.29$ ,  $P = 0.87$ ) whilst consuming 6, 8 or 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO (4.0 [3.3 to 4.5], 4.0 [4.0 to 4.0] and 4.0 [4.0 to 4.0] respectively). Bowel movement frequency saw a significant main effect for time ( $P < 0.001$ ), showing a decrease the morning of day 5 compared to days 3 and 4. There was no significant main effect for condition ( $P = 0.50$ ) or interaction ( $P = 0.50$ ). Gut transit time saw no significant main effect for condition ( $P = 0.27$ ) as mean gut transit time was  $20.2 \pm 5.0$ ,  $19.0 \pm 4.0$  and  $18.7 \pm 5.8$  h following 6, 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO respectively. Gut comfort symptoms were similar between conditions ( $P > 0.05$ ), apart from stomach fullness, where participants reported significantly greater feelings of fullness when consuming 10 compared to 6 or 8  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  CHO (Table 5.4). However, there were a higher number of moderate-severe symptoms (scores  $\geq 5$ ) reported during the 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  condition for nausea, reflux, stomach fullness and gas/flatulence. Severe scores were spread out across time, with no specific timepoint during the loading period having  $>3$  moderate-severe scores (Figure 5.5). One female participant accounted for 55% of scores  $\geq 5$  across symptoms of nausea, reflux, stomach fullness and flatulence.

**Table 5.4.** Median (IQR) gut comfort symptoms (0-10) across 48 h loading period when participants consumed 6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO. Wilcoxon signed rank test significance (\*) set at  $P \leq 0.017$  (Bonferroni correction).

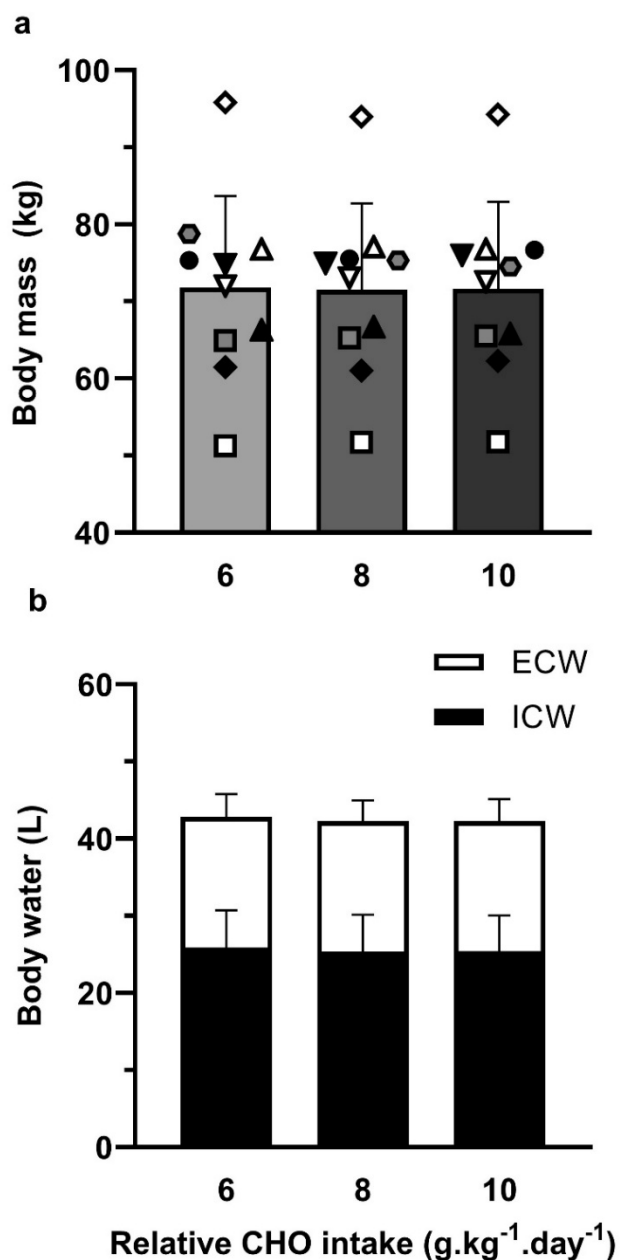
| GI symptom       | 6 g·kg <sup>-1</sup><br>·day <sup>-1</sup> | 8 g·kg <sup>-1</sup><br>·day <sup>-1</sup> | 10 g·kg <sup>-1</sup><br>·day <sup>-1</sup> | Friedman test                   | Wilcoxon signed rank                                                                                    |
|------------------|--------------------------------------------|--------------------------------------------|---------------------------------------------|---------------------------------|---------------------------------------------------------------------------------------------------------|
| Nausea           | 0 (0-0)                                    | 0 (0-0)                                    | 0 (0-1)                                     | $P = 0.097$                     | -                                                                                                       |
| Reflux           | 0 (0-0)                                    | 0 (0-0)                                    | 0 (0-2)                                     | $P = 0.150$                     | -                                                                                                       |
| Stomach fullness | 1 (0-1)                                    | 0 (0-2)                                    | 2 (0-4)                                     | <b><math>P = 0.006^*</math></b> | 6 vs 8 $P = 0.53$<br><b>6 vs 10 <math>P = 0.017^*</math></b><br><b>8 vs 10 <math>P = 0.014^*</math></b> |
| Cramps           | 0 (0-1)                                    | 0 (0-0)                                    | 0 (0-0)                                     | $P = 0.779$                     | -                                                                                                       |
| Gas/flatulence   | 0 (0-1)                                    | 0 (0-3)                                    | 0 (0-2)                                     | $P = 0.355$                     | -                                                                                                       |
| Urge to defecate | 0 (0-2)                                    | 0 (0-0)                                    | 0 (0-2)                                     | $P = 0.156$                     | -                                                                                                       |



**Figure 5.5.** Total number of GI discomfort symptoms scored >4 during 48 h CHO loading period where participants consumed 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO (Post-ex, post-exercise; Pre-s, pre-sleep; Pre-b, pre-biopsy).

#### 5.4.4 Body mass and body water

Body mass measured on the morning of day 5 did not differ between conditions ( $P = 0.522$ , Figure 5.6a). This was also the case for TBW, ECW and ICW ( $P = 1.00$ ,  $P = 0.25$  and  $P = 0.58$ ) as there was no difference between conditions (Figure 5.6b).



**Figure 5.6.** Body mass (a) and TBW (b) expressed as ICW and ECW of endurance trained males and females following consumption of a diet containing 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO for 48 h.

## 5.5 Discussion

This study reports for the first time the dose-response of dietary CHO intake and skeletal muscle glycogen in endurance trained males and females with CHO intakes from 6 to 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, whilst replicating a real-world pre-competition exercise taper. Results show a linear increase in muscle glycogen concentrations with increasing CHO intake, however 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO resulted in greater muscle glycogen concentrations compared to all other conditions, with no visible saturation point or ‘ceiling’ effect. All conditions were well tolerated in terms of gut comfort, with minimal impact on markers of gut function and no changes in body mass, TBW, ECW or ICW between conditions.

In line with history of this strategy (Bergström et al., 1967; Bussau et al., 2002; Fairchild et al., 2002; Sherman et al., 1981) a high and very high relative CHO intake (8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>) for 48 h resulted in significantly increased muscle glycogen compared to baseline (day 2 pre-exercise), where participants consumed a moderate CHO intake (4 g·kg<sup>-1</sup>·day<sup>-1</sup>) for ~36 h (Figure 5.3). When expressed as a % of baseline, increases in glycogen concentration (137 and 172% for 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively), were smaller than the 200% increase suggested by Burke et al. (2017). Despite previous reports showing a doubling of glycogen stores (Arnall et al., 2007; Bergström et al., 1967; Bussau et al., 2002; Fairchild et al., 2002; James et al., 2001), it seems achieving this level of ‘supercompensation’ is less common, as current study values are in line with reported glycogen concentrations outlined by Areta and Hopkins (2018). Interestingly, many previous reports of doubling glycogen stores (Bergström et al., 1967; Bussau et al., 2002; Fairchild et al., 2002; James et al., 2001), rested participants throughout the loading period and were provided a high-very high CHO intake (8.3-10.5 g·kg<sup>-1</sup>·day<sup>-1</sup> for 24-72 h). Considering when endogenous stores within muscle are abundant, glycogen is

preferentially used at the same relative exercise intensity (Arkinstall et al., 2004), this suggests the contribution of CHO (from endogenous muscle glycogen) to total energy expenditure would be greater during training sessions conducted in the loading period (provided intensity and duration are matched). This could explain why the current study did not reach the ~200% increase from baseline, previously reported (Bergström et al., 1967; Bussau et al., 2002; Fairchild et al., 2002; James et al., 2001) and suggested by Burke et al. (2017).

Unexpectedly, the 10 g·kg<sup>-1</sup>·day<sup>-1</sup> condition resulted in a significantly higher glycogen concentration compared to 8 g·kg<sup>-1</sup>·day<sup>-1</sup>, suggesting this level of CHO intake in the days leading up to competition may be optimal to ensure a ‘supercompensation’ of muscle glycogen stores. These findings contradict previous suggestions within the literature of a ‘ceiling’ effect for muscle glycogen synthesis at 7-10 g·kg<sup>-1</sup>·day<sup>-1</sup> (Burke et al. 1995; Burke et al., 2017). Burke et al. (1995) originally proposed this threshold after 24 h of 7 vs 11.8 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO resulted in no difference in glycogen (522.8 ± 41.8 and 519.4 ± 25.2 mmol·kg<sup>-1</sup> DM respectively). Values are comparable with the 8, but not the 10 g·kg<sup>-1</sup>·day<sup>-1</sup> condition in the current study, where glycogen concentration was 22.4% higher, despite a lower relative CHO intake (-1.8 g·kg<sup>-1</sup>·day<sup>-1</sup>). One possible reason behind this discrepancy was the ingestion pattern of CHO immediately post-depletion, as small regular feedings have repeatedly been shown to enhance glycogen synthesis post-exercise, where insulin sensitivity and glycogen synthase activity is augmented (Ivy et al., 1988; Wojtaszewski et al., 2001). Despite a similar absolute CHO intake 0-4 h post-depletion in both studies (~220 g), the current study provided two feedings (1.2 g·kg<sup>-1</sup> CHO immediately post-exercise and 2 g·kg<sup>-1</sup> CHO meal ~2 h later) as opposed to one large bolus (Burke et al. 1995), which may have optimised the resynthesis of glycogen in the immediate post-depletion period. However, previous literature has shown an adequate post-exercise recovery (≥ 24 h) to diminish the importance of CHO timing (Parkin et

al. 1997; Burke et al., 1996). Therefore, a more likely contributor is total time of increased CHO availability, as current study participants experienced ~60 h of increased dietary CHO intake (acute re-feed combined with 48-h loading period) compared to 24 h in the previous study (Burke et al., 1995). Bussau et al. (2002) suggested an 'improved' protocol only required 24-36 h to maximise glycogen stores, with additional time providing no further benefit. However, numerous others have shown a continued enhancement of muscle glycogen stores over multiple days of a high-very high CHO intake (Adamo et al. 1998; Blom et al., 1987; Hickner et al., 1997; Kochan et al., 1979; Roberts et al 2016), suggesting the 2.5-fold longer period of increased CHO availability in the current study was responsible for the higher glycogen concentrations reported, despite a smaller relative CHO intake. It is reasonable to expect that there is a physiological upper limit, where further increases in CHO intake or duration of enhanced CHO availability would not further augment glycogen synthesis (and content). However, it cannot be concluded where this threshold occurs from current study data. Interestingly, there was no difference between glycogen concentrations following 4 vs 6, or 6 vs 8 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO for 36-48 h (Figure 5.3). This was despite the delta of glycogen concentration from 4-6 and 6-8 being similar to 8-10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO ( $P = 0.47$ ) and being comparable to the expected increase in glycogen presented by Areta and Hopkins (2018) when CHO intake increased from normal to high ( $102 \pm 47$  mmol·kg<sup>-1</sup> DM). However, differences in glycogen between 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO were detected, again advocating that a very high CHO intake ( $\geq 10$  g·kg<sup>-1</sup>·day<sup>-1</sup>) may be required to ensure an optimisation of muscle glycogen stores. This was further supported by a significant large positive correlation between CHO intake (relative and absolute) and whole muscle glycogen ( $r = 0.71$ ,  $P < 0.001$  and  $r = 0.64$ ,  $P < 0.001$ , respectively), which was expected, as a 'loading' period of increased CHO intake should result in increased muscle glycogen concentration, irrespective of how it is described. Nevertheless, the strong dose-response relationship showed no indication of a plateau (Figure

5.4), suggesting higher CHO intakes could result in greater glycogen concentrations under current experimental conditions.

The discrepancy between delta glycogen data and significant differences between conditions could partly be explained by individual variability, as the larger standard deviations suggest a higher variability for 6 and 8 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO, supported by the wider spread of individual data points (Figure 5.3). A possible contributor to this variability could be biochemical determination of whole muscle glycogen from biopsy samples which, despite being the gold standard, can introduce variability within results. Muscle biopsies take a snapshot of muscle (20-200 mg) and assume this will be similar across the whole muscle; however, it is well-established that glycogen is selectively stored in three distinct subcellular locations (intra- and inter-myofibrillar, and subsarcolemmal), with CHO loading resulting in preferential storage in type I muscle fibres of trained individuals (Ørtenblad et al., 2013; Jensen et al 2020). Furthermore, glycogen particles can vary in size (10 – 44 nM; Marchand et al., 2002), and CHO loading leads to increased numerical density of glycogen particles as opposed to increased size of existing granules (Jensen et al., 2020). As such, theoretically, two biopsy samples separated by 3-5cm could have different whole muscle glycogen concentrations.

GI data showed high and very high dietary CHO intakes were well-tolerated in a cohort of endurance trained individuals, with a negligible effect on markers of gut transit time and function measured using the blue dye method and Bristol stool scale. To the authors knowledge, this is the first time the effects of CHO loading on gut comfort and function have been measured, with previous studies focussed on GI discomfort symptoms associated with high CHO intakes (90-120 g.h<sup>-1</sup>) during exercise (Rehrer et al., 1992; Peters et al., 1993; Hearn et

al., 2022). The high tolerance seen in the current study likely resulted from the control of known influencers of gut comfort and function during exercise, such as nutritional intake (specifically dietary fibre, fat and protein), intensity of exercise conducted during the loading period (light to moderate intensity; LT1) and participant training status, where most endurance trained individuals were familiar with and commonly used CHO loading strategies pre-competition (Brouns and Beckers, 1993; de Oliveira et al., 2014). Stomach fullness was greater consuming 10 vs 6 and 8 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO (Table 5.3), with a greater occurrence of severe scores in the high and very high CHO conditions (Figure 5.5). However, median stomach fullness in the 10 g·kg<sup>-1</sup>·day<sup>-1</sup> condition was below a moderate severity score of 5, total number of severe scores were low across all symptoms and severe scores were heterogeneously scattered across loading periods (Figure 5.3). Interestingly, one female accounted for 55% of all symptoms scored  $\geq 5$ . Further investigation revealed this participant had never competed in endurance sport, (despite training regularly for 5 years) and habitually consumed the lowest quantity of CHO (2.9 g·kg<sup>-1</sup>·day<sup>-1</sup>), suggesting the severity of her symptoms was due to a lack of familiarity with high CHO intakes.

Surprisingly, body mass, TBW, ECW and ICW did not differ between conditions (Figure 5.5). This is in contrast to current literature where body mass has been shown to increase following CHO loading (Bone et al., 2016; Schytz et al., 2023), due to the positive relationship between muscle glycogen and body water, with a ratio of 1:3 or 1:4 commonly accepted (Bergström and Hultman., 1972; Fernandez-Elias et al., 2015; Olsson and Saltin, 1970). Considering current study muscle glycogen and skeletal muscle mass data (BIA), if glycogen and water were bound at a 1:4 ratio, body mass (and TBW) should have increased approximately 0.12, 0.58 and 0.70 kg (or L) from 6 to 8, 8 to 10, and 6 to 10 g·kg<sup>-1</sup>·day<sup>-1</sup> CHO, respectively. The reasons behind this discrepancy are unclear, however, a narrative review by Shiose et al. (2022) summarised

the current literature regarding the glycogen: water relationship, and suggested the story was less clear than commonly believed with early studies in animal models (Bridge and Bridges, 1931; McBride et al., 1941; Richter et al., 1988; Sherman et al., 1982) and recent studies in humans showing no changes in TBW following manipulation of glycogen stores (Schytz et al., 2023; Shiose et al., 2016; Shiose et al., 2018).

Interestingly, all studies in humans that showed no effect of increased glycogen concentration on TBW (current study included) used multifrequency BIA or BIS (Schytz et al., 2023; Shiose et al., 2016; Shiose et al., 2018), which despite being validated against the gold standard isotope dilution techniques (Kerr et al., 2015; Matias et al., 2016; Bosy-Westphal et al., 2013), can be affected by other factors such as changes in electrolyte balance (O'Brien et al., 1993). Shiose et al. (2016) found a 0.9 L (2.4%) increase in TBW following 72 h of a very high CHO intake ( $12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) when measured using deuterium dilution, whereas BIS only showed a 0.4 L increase. This did not reach statistical significance, but was an increase of  $\sim 1\%$ , in line with the expected increases in the current study. Perhaps use of BIS/BIA, combined with the relatively small study sample size ( $n \leq 11$ ), were not sensitive enough to detect minor changes in TBW expected with  $2\text{-}4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  increments in CHO intake (1-2 % or 0.5-0.7 L). Particularly as current study measurements were made on a whole-body level, as opposed to segmental, which could have been more effective considering the focus on cycling exercise, where upper body changes in muscle glycogen were likely minimal (Shiose et al., 2016). In agreement, the studies in humans that have reported a positive relationship between glycogen storage and water (Olsson and Saltin, 1970; Bone et al., 2017; Fernandez-Elias et al., 2015) often compared extreme conditions of CHO availability, where participants underwent upper and lower body glycogen depletion or completed multiple day depletion periods, as well as

comparing vastly different CHO intakes ( $\geq 6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  differences between conditions), which resulted in larger differences in muscle glycogen and possibly TBW.

Fernandez-Elias et al. (2015) concluded their findings agreed with the 1:3 glycogen: water ratio, however this was following depletion in hot conditions ( $\sim 33 \text{ }^{\circ}\text{C}$ ), where fluid, but not CHO intake, was limited during recovery. When fluid intake was matched to fluid losses during the recovery period, a smaller ratio was reported (1:1.17) suggesting the glycogen: water relationship is affected by fluid intake (at least in the immediate recovery phase following dehydration and glycogen depletion). Nonetheless this highlights that TBW, ECW and ICW is regulated independently of glycogen, despite its highly hydrophilic nature. Current study fluid intake across the loading period (days 3 and 4), despite not reaching statistical significance, had a mean difference of 0.5 L between 6 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  conditions, which could have impacted measurements, and is a limitation of the current study. Future studies are warranted to clarify the mechanisms that regulate this relationship, especially as a higher water content in muscle, in previous research, has been assumed to be bound to glycogen despite many of the measures used to support this relationship being indirect (Nygren et al., 2001; Fernandez-Elias et al., 2015) or conducted at the whole-body level (Olsson and Saltin, 1970). Furthermore, another potential influencer of fluid retention, dietary fibre intake, has seldom been considered.

### ***5.5.1 Conclusions***

The current study, for the first time, reports the dose-response of moderate, high and very high dietary CHO intake on skeletal muscle glycogen storage in endurance trained males and females. Study results suggest a very high dietary CHO intake ( $10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) may be necessary to maximise muscle glycogen stores, at least under these conditions, which were

designed to mimic pre-competition training practices of endurance cyclists (light intensity exercise), whilst adhering to contemporary nutritional guidelines. The dose-response relationship between dietary CHO intake and glycogen concentration appears strongly linear, with no visible saturation point. All strategies were well tolerated in endurance trained individuals who had prior experience of CHO loading and high CHO diets. Despite a plethora of research within this topic, further research is warranted, with a particular focus on higher CHO intakes to identify the saturation point for glycogen concentration, and the relationship between muscle glycogen and body water.

### ***5.5.2 Link to next chapter***

Having determined a strong linear dose-response relationship between dietary CHO intake and muscle glycogen, with higher CHO intakes seemingly optimal for maximal glycogen storage under real-world training conditions (Chapters 4 and 5), the next step is to determine whether increased glycogen storage translates into improved endurance cycling performance. This should be tested using a double blinded repeated measures study design to adequately control for the placebo effect (Chapter 2, section 2.6) which could have biased previous CHO loading research performance outcomes.

## **Chapter Six:**

**Pre-exercise dietary CHO intake and endurance cycling  
performance: Pre-requisite or placebo?**

## 6.1 Abstract

High dietary CHO intake days pre-competition to enhance glycogen stores is considered a pre-requisite for optimal endurance exercise performance in competitions longer than 90 min. However, this conclusion has been reached based on previous CHO loading research, which has not adequately controlled for a possible performance enhancing placebo effect. To address the effect of CHO loading independent of the placebo effect 9 endurance trained males ( $\dot{V}O_{2\max}$   $63.4 \pm 5.2$  mL $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ; PPO,  $367 \pm 37$  W) completed 3 x 4 days of dietary control and prescribed exercise designed to mimic pre-competition practices of endurance cyclists in a placebo controlled, double-blind repeated measures design. Participants consumed 6, 8 or 10 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$  of CHO on days 2 and 3, before returning to the laboratory on the morning of day 4 to complete a performance test (2 h pre-load and ~30 min cycling time trial). There was no significant difference in TT completion time (36 min 46 s  $\pm$  4 min 18 s, 34 min 55 s  $\pm$  5 min 12 s and 35 min 46 s  $\pm$  5 min 56 s;  $P = 0.16$ ;  $\eta^2_p = 0.21$ ) or mean power output ( $226 \pm 22$ ,  $239 \pm 29$  and  $234 \pm 29$  W;  $P = 0.10$ ;  $\eta^2_p = 0.26$ ) between 6 vs 8 and 10 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$ , respectively. However, there was a large effect size ( $\eta^2_p = 0.21$  and  $\eta^2_p = 0.26$ , respectively), which was possibly biased by the placebo effect, as participants who correctly identified the placebo condition showed the greatest improvements in endurance cycling performance. In conclusion, current data suggests CHO loading with a high or very high CHO intake (8 or 10 g $\cdot$ kg $^{-1}\cdot$ day $^{-1}$ ) provides no benefit to endurance cycling performance lasting ~2.5 h under real world conditions of high exogenous CHO availability.

## 6.2 Introduction

The importance of high dietary CHO availability for endurance exercise performance is well established. As such endurance athletes use CHO loading strategies, of increased dietary CHO intake days pre-competition, to enhance muscle glycogen stores above ‘normal’ physiological values ( $\sim 400 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$ ; Bergström et al., 1967; Areta and Hopkins, 2018). It is commonly accepted that CHO loading delays fatigue by  $\sim 20\%$  and increases endurance performance by 2-3 % (Hawley et al., 1997a), with contemporary guidelines advocating  $8\text{-}12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO for 36-48 h prior to exercise lasting  $\geq 2.5 \text{ h}$ .

Despite the benefits of CHO loading being well-accepted, surprisingly only three studies within the area have used a placebo control group to blind participants and researchers to actual participant CHO intake prior to endurance performance measures (Hawley et al. 1997b; Burke et al., 2000; Tomcik et al., 2018). Interestingly all three studies showed no benefit of a high-very high CHO intake in the days pre-exercise, despite significantly increased muscle glycogen stores. These studies highlight a potential placebo effect associated with CHO loading research and endurance cycling performance, which has previously been suggested (Burke et al., 2000), but not fully investigated. Considering the well-established nature of the placebo effect and its ability to influence sports performance simply through the beliefs of athletes and study participants (Halsen and Martin, 2013; Beedie et al., 2018), it could be that the metabolic importance of CHO loading for endurance performance has been exaggerated by the placebo effect.

Furthermore, CHO loading studies commonly rest participants during loading periods, which may increase CHO availability, and therefore glycogen concentration – due to reduced CHO utilisation – but is not a true representation of what endurance athletes do in the real-world. Of

the limited studies that replicated real world training conditions (Tarnopolsky et al., 2001; Doering et al., 2019), no data was collected during these sessions to describe physiological responses to exercise and substrate metabolism. Conducting exercise during the loading period would result in athletes using muscle glycogen, possibly leading to reduced pre-exercise glycogen concentrations.

The current study aimed to investigate the potential placebo effect of CHO loading on endurance cycling performance whilst replicating real-world pre-competition habits of endurance cyclists. A secondary aim was to determine whether training sessions conducted during the loading period would result in increased CHO oxidation due to utilisation of glycogen stores. It was hypothesized that CHO loading would result in a small, but worthwhile performance improvement in very high CHO condition compared to a moderate CHO intake. A very high CHO intake and resultant increase in muscle glycogen would also increase CHO oxidation during exercise conducted in the loading period.

## **6.3 Methods**

### ***6.3.1 Participants***

Nine endurance trained tier 2 or 3 males participated in the study (McKay et al., 2022), categorised as endurance trained cyclists (Table 6.1) in line with the classification of De Pauw et al. (2013). Participants were provided written and verbal information regarding procedures before written informed consent was attained. The study was approved by the Liverpool John Moores University Research Ethics Committee (Ethics code; 23/SPS/040). Sample size estimation was determined using performance test data of Rauch et al. (1995), where a similar cycling time trial (TT) duration following a CHO loading protocol of 6 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup> for

72 h resulted in TT mean power output of  $219 \pm 17$  and  $233 \pm 15$  W, respectively. This provided an effect size of 0.87 and corresponded to an a-priori sample size of 10 to achieve an alpha of 0.05 and power of 0.80 (G\*Power, v 3.1.9.7, Kiel, Germany).

**Table 6.1.** Study 2 participant characteristics (n = 9)

|                                                              |                |
|--------------------------------------------------------------|----------------|
| Age (years)                                                  | $31 \pm 10$    |
| Height (cm)                                                  | $179 \pm 8$    |
| Body mass (kg)                                               | $78.3 \pm 6.7$ |
| $\dot{V}O_{2\max}$ (L·min <sup>-1</sup> )                    | $5.1 \pm 0.6$  |
| $\dot{V}O_{2\max}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) | $63.4 \pm 5.2$ |
| PPO (W)                                                      | $367 \pm 37$   |
| PPO (W·kg <sup>-1</sup> )                                    | $4.7 \pm 0.5$  |
| Lactate threshold 1 (W)                                      | $181 \pm 39$   |
| Lactate threshold Dmax (W)                                   | $188 \pm 24$   |
| Training history (years)                                     | $8 \pm 9$      |
| Training load (h·week <sup>-1</sup> )                        | $7 \pm 3$      |

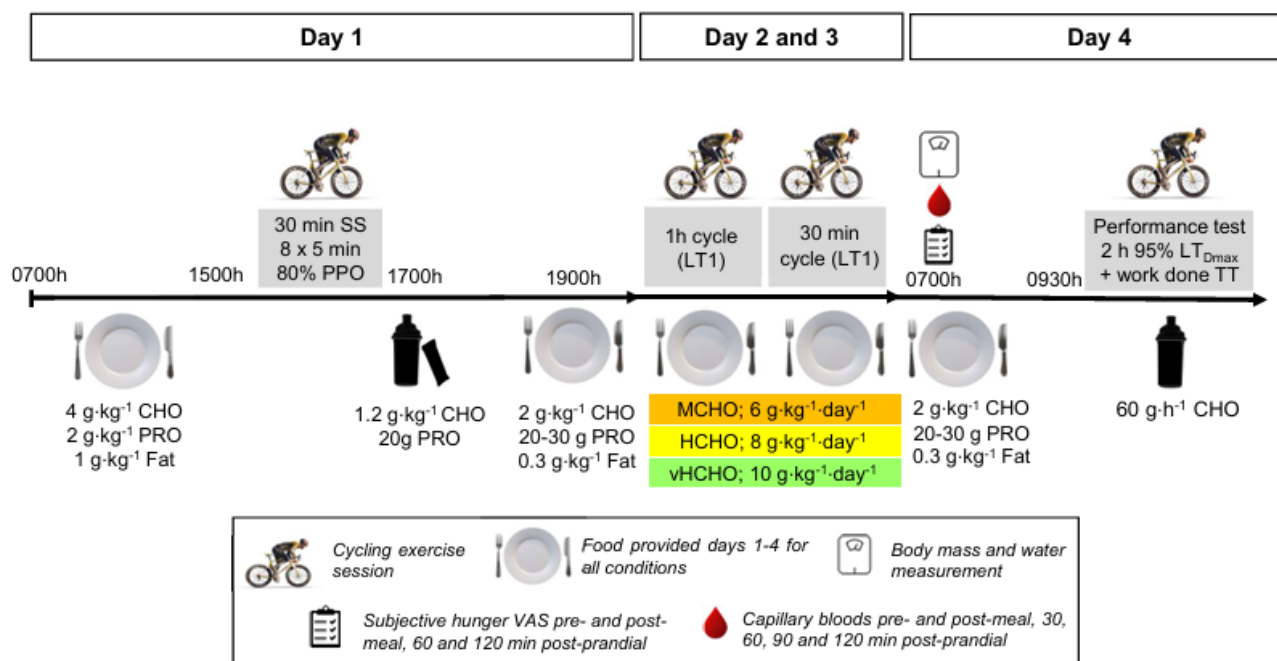
### 6.3.2 Study overview

In a randomised, double blind repeated measures design (block randomisation), participants completed 3 x 4-day experimental conditions, which involved prescribed cycling exercise on each day and controlled nutritional intake throughout (Figure 6.1). Day 1 participants consumed a moderate CHO intake ( $4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ), before completing a high intensity interval cycling session (see 6.3.5 Experimental conditions). Days 2 and 3 participants consumed either 6, 8 or  $10 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  of CHO, and completed a light-moderate intensity cycling session designed to replicate pre-competition practices of endurance cyclists. The morning of day 4, participants returned to the laboratory for a high CHO breakfast ( $2 \text{ g} \cdot \text{kg}^{-1}$ ) and 2 h rest period, before completing a cycling exercise performance test (Lode Excalibur sport, Groningen,

Netherlands). Experimental conditions were separated by  $\geq 5$  days, with exercise sessions conducted in controlled laboratory conditions (temperature  $21.1 \pm 1.0$  °C; humidity  $32 \pm 7\%$ )

### ***6.3.3 Preliminary testing***

All preliminary testing procedures were identical to those previously described within Chapter 3 (General methods). Briefly, height and body mass were measured semi-nude (Seca, Hamburg, Germany), before completing a standard incremental lactate threshold and maximal oxygen consumption ( $\dot{V}O_{2\max}$ ) tests on a cycle ergometer, where participants began cycling at 100 W and exercise intensity increased 25 W every 4 min. In the final 30 s of each 4 min stage a fingertip blood sample was collected and immediately analysed for blood glucose and lactate (Biosen C-Line, EKF Diagnostics, Cardiff, UK), with ratings of perceived exertion (RPE; Borg, 1982) and HR also collected in the final 10 s. The test was terminated when participants reached the onset of blood lactate accumulation  $\geq 4$  mmol·L<sup>-1</sup> (Heck et al., 1985). Following 10 min rest, participants returned to the ergometer to complete a  $\dot{V}O_{2\max}$  test, which began at 100 W and exercise intensity increased 25 W every 1 min until volitional exhaustion. Gas exchange was measured continuously using a metabolic cart (Moxus, AEI Technologies, USA), with  $\dot{V}O_{2\max}$  defined as the highest  $\dot{V}O_2$  sustained over a 30-s average. HR and RPE were collected in the final 10 s of each stage and were used as indirect markers of volitional exhaustion. PPO was determined using the equation outlined by Kuipers et al. (1985), as previously described (see Chapter 3; General methods).



**Figure 6.1.** Study 2 schematic overview of experimental conditions.

### 6.3.4 Baseline performance tests

Prior to experimental conditions participants completed the endurance cycling performance test on two occasions (separated by  $\geq 7$  days), to ensure familiarity with testing procedures and pacing strategy, confirm exercise intensities were appropriate and minimise learning effect between conditions. Twenty-four hours prior, and the morning of the first baseline test participants recorded habitual nutritional intake using a dietary recording sheet and food weighing scales, combined with remote food photography over online messenger (WhatsApp, Meta, USA). Methods were combined to more accurately determine nutritional intake, as the previously validated remote food photography method in isolation can produce  $\sim 10\%$  variability (Stables et al., 2021). Dietary intake was replicated 24 h prior, and the morning of the second baseline performance test. Participants arrived in the laboratory 2 h post-breakfast ( $\sim 0930$  h; standardised within participant) and provided a urine sample to confirm hydration

status using a pen refractometer (Atago, Tokyo, Japan), before semi-nude body mass was recorded (Seca, Hamburg, Germany). Participants set the cycle ergometer to their personal preferences (seat and handlebar height/position), which was replicated for all future sessions. HR and respiratory gases were measured at rest as previously described, before participants began a 5 min warm-up (100 W), followed by 120 min cycle at an intensity equivalent to 95% LT<sub>Dmax</sub> (179 ± 23 W; Cheng et al., 1992). HR and RPE were recorded every 20 min, with respiratory gases collected for the final 5 of every 20 min. Participants were provided 60 g·h<sup>-1</sup> of CHO (63.1g Maltodextrin in 600 mL water; Bulk powders, London, UK) with a feeding frequency of 200 mL every 20 min. CHO type and quantity was deemed appropriate as exercise intensity and duration, as well as the endurance trained nature of participants, suggested no benefit of multiple transportable CHO (Rowlands and Hourtham, 2017).

Following the 120 min SS cycle, and 2 min active recovery at 100 W, participants began a simulated ~30 min time-trial (TT), where completion of a set amount of mechanical work (496 ± 50 kJ) was required, with the cycle ergometer set to cadence-dependent linear mode. The alpha level was determined for each participant based on preferred cadence (determined from preliminary testing) and total work to be completed. Total work was calculated for each individual according to the following formula (Jeukendrup et al., 1996):

$$\text{Total work (J)} = 0.75 \cdot \text{PPO} \cdot 1800\text{s}$$

Participants cycling at their individual preferred cadence would result in an exercise intensity equivalent to 75% PPO. HR, elapsed time, cadence and power output were not visible to participants or researchers, and no music or verbal encouragement was provided. The only

feedback provided to participants was verbal confirmation of 25, 50, 75 and 90% of total work completed. Water consumption was allowed *ad libitum* throughout, and CHO was provided during the TT through 150 mL of Maltodextrin (10 g CHO) at 10 and 20 min. No CHO was provided thereafter to match absolute CHO intake across participants. Exercise tests of this nature have previously been validated with a repeatability CV of 3.5% (Jeukendrup et al., 1996).

### **6.3.5 Experimental conditions**

*Hard cycling exercise session.* Day 1 participants arrived at the laboratory at 1500 h to complete a challenging exercise protocol adapted from Stepto et al. (2001). The session consisted of a 5 min warmup at 100 W, 30 min cycle at an intensity equivalent to 65%  $\dot{V}O_{2\max}$ , followed by 2 min active recovery at 100 W. Following this, participants completed 8 x 5 min intervals at 80% PPO, separated by 1 min active recovery at 100 W. If unable to complete a 5-min interval, the active recovery was initiated at task failure (inability to maintain a cadence > 60 rpm for 10 s consecutively) and exercise intensity decreased (-5% PPO) for the remaining intervals. Exact work and recovery times were replicated for all conditions to standardise absolute work done and level of glycogen depletion. This protocol previously depleted muscle glycogen by  $\sim 260 \text{ mmol}\cdot\text{kg}^{-1} \text{ DM}$  (Study 1; Chapter 5). Participants were asked not to take part in any strenuous physical activity, and not to consume any alcohol at least 24 h prior to beginning experimental conditions.

*Dietary control.* Day 1 participants consumed a moderate CHO intake ( $4 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ), alongside 2, 1 and  $0.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of protein, fat and fibre respectively. Immediately post-depletion, participants consumed  $1.2 \text{ g}\cdot\text{kg}^{-1}$  of CHO and 20 g protein, in the form of a sports

drink (Science in Sport, UK) and protein bar (My Protein, UK), in line with acute post-exercise CHO guidelines (Thomas et al., 2016). This was followed by a high CHO dinner (2 g·kg<sup>-1</sup> CHO, 30-40 g protein and 0.3 g·kg<sup>-1</sup> of fat) ~ 2 h later that evening. This refeed strategy was considered more practical, and better matched post-training practices of endurance cyclists in the field, where 1-1.2 g·kg<sup>-1</sup> every hour for 4 h is rarely achieved (Thomas et al., 2016). Day 2 and 3 participants consumed either a moderate, high or very high CHO intake (6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup> respectively), as well as 2, 1 and 0.5 g·kg<sup>-1</sup>·day<sup>-1</sup> of protein, fat and dietary fibre respectively (Table 6.2). All conditions provided 6 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO, and all other macronutrients, through meals and snacks that were pre-cooked and weighed to the nearest 0.05 g, along with a checklist (as previously described; Chapter 3 General methods). Participants were asked to send photos of each meal at time of consumption via online messenger to confirm adherence (WhatsApp, Meta, USA). Plans provided a healthy mixed diet in the form of usual meals (breakfast, lunch and dinner) with various snacks throughout each day. Participants were instructed to only consume what was provided. Water consumption was *ad libitum*, with total fluid intake monitored across conditions. This dietary control method has previously shown 97.1 ± 1.0 % compliance (Study 1; Chapter 5).

*Experimental blinding.* The remaining CHO for the high and very high loading conditions (8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>) were provided as drinks, with drink composition based on body mass (example for 70 kg participant displayed in Table 6.3). In the 6 g·kg<sup>-1</sup>·day<sup>-1</sup> condition participants consumed zero-calorie placebo drinks at the same timepoints. All drinks were taste and colour-matched using calorie-free cordial and sucralose. Pre-data collection drink compositions were extensively piloted using different groups of individuals who were ineligible to participate (n = 6 per group). In the final test only 1 out of 6 participants correctly identified true CHO content of all drinks (Chapter 3 General methods).

To incorporate double blinding, a researcher outside the research team prepared all study drinks for all participants and was the only individual aware of which trial code (A, B or C) corresponded to actual experimental conditions (6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup>). The first author (R.O.J) led all data collection sessions and was the only individual aware of which trial code (A, B or C) was being completed at any given time. To assess blinding efficacy following completion of all three trials, participants were asked to try and differentiate which trial corresponded to which CHO intake condition, with the option of ‘I don’t know’ also available (Kolahi et al., 2009).

**Table 6.2.** Nutritional intake 24 h pre-baseline performance tests (habitual) and during experimental conditions (6 g·kg<sup>-1</sup>·day<sup>-1</sup>).

|                         | Habitual   | All conditions |
|-------------------------|------------|----------------|
| <i>24 h intake</i>      |            |                |
| CHO (g)                 | 284 ± 53   | 469 ± 37       |
| (g·kg <sup>-1</sup> )   | 3.7 ± 0.7  | 6.0            |
| Protein (g)             | 122 ± 47   | 156 ± 12       |
| (g·kg <sup>-1</sup> )   | 1.6 ± 0.6  | 2.0            |
| Fat (g)                 | 70 ± 19    | 78 ± 6         |
| (g·kg <sup>-1</sup> )   | 0.9 ± 0.2  | 1.0            |
| Energy (kcal)           | 2265 ± 383 | 3205 ± 255     |
| <i>Pre-PT breakfast</i> |            |                |
| CHO (g)                 | 79 ± 24    | 156 ± 12       |
| (g·kg <sup>-1</sup> )   | 1.0 ± 0.2  | 2.0            |
| Protein (g)             | 28 ± 15    | 23 ± 3         |
| (g·kg <sup>-1</sup> )   | 0.3 ± 0.2  | 0.3            |
| Fat (g)                 | 15 ± 7     | 19 ± 1         |
| (g·kg <sup>-1</sup> )   | 0.2 ± 0.1  | 0.2            |
| Energy (kcal)           | 566 ± 165  | 888 ± 70       |

**Table 6.3.** Experimental blinding drinks composition example for 70 kg participant.

|                                          | 6 g·kg <sup>-1</sup> ·day <sup>-1</sup> | 8 g·kg <sup>-1</sup> ·day <sup>-1</sup> | 10 g·kg <sup>-1</sup> ·day <sup>-1</sup> |
|------------------------------------------|-----------------------------------------|-----------------------------------------|------------------------------------------|
| Maltodextrin (g)                         | 0                                       | 21                                      | 42                                       |
| Water (mL)                               | 400                                     | 400                                     | 400                                      |
| Zero kcal cordial (mL)                   | 25                                      | 25                                      | 25                                       |
| Sucralose (g)                            | 0.1                                     | 0.1                                     | 0.1                                      |
| Drinks (per day)                         | 7                                       | 7                                       | 7                                        |
| CHO per drink (g)                        | 0                                       | 20                                      | 40                                       |
| Total daily CHO (g)                      | 0                                       | 140                                     | 280                                      |
| Total daily CHO<br>(g·kg <sup>-1</sup> ) | 0.0                                     | 2.0                                     | 4.0                                      |

*Prescribed exercise sessions.* At 1500 h on days 2 and 3 participants arrived at the laboratory to complete 60- and 30-min light-moderate intensity (LT1;  $181 \pm 39$  W) cycling exercise sessions. HR and RPE was collected at rest and every 10 min, with respiratory gases also collected in the final 2 of every 10 min of exercise (as previously described). To ensure blinding, gas measurements were not visible in real time and were exported blindly by researchers following each session. Gas data was viewed for the first time when analysed, once all participants had completed all experimental conditions.

*Day 4 Performance tests.* Participants arrived at the laboratory for a TBW measurement (0700 h, overnight fasted) using eight-electrode multifrequency bioelectrical impedance (BIA; Seca mBCA 515, Hamburg, Germany), before being provided a high CHO breakfast (2 g·kg<sup>-1</sup> of CHO) and 700 mL of water to be consumed slowly over the next 2 h (Sawka et al., 2007). Immediately pre-, post-meal and every 30 min thereafter for 120 min a capillary blood sample

was collected and analysed (finger prick method) to assess post-prandial glucose and lactate responses under different CHO loading conditions. Additionally, pre-, post-meal, 60- and 120-min post-prandial, visual analogue scales were completed to describe subjective feelings of hunger and satiety (Hengist et al., 2020), where participants were asked to draw a vertical line along a 0-100 mm scale ranging from ‘Not at all’ (0) to ‘Extremely’ (100) relating to how they felt.

Following the 120 min post-prandial assessment period, an indwelling cannula was fitted in the antecubital vein to allow collection of venous blood samples into lithium heparin vacutainers (BD Biosciences, UK) at rest and every 20 min of exercise. Following collection of each sample, the cannula was flushed with sterile saline (Midmeds, Hertford, UK). Vacutainers were stored on ice until centrifugation (1500g for 15 min at 4 °C) with aliquots stored at -80 °C until analysis with commercially available kits for glucose, lactate and non-esterified fatty acids (NEFA; Randox Laboratories, Antrim, Ireland). The remaining performance test procedures were as previously described (see 6.3.4 Baseline performance tests), with the addition of GI discomfort symptoms collected every 30 min. Gut discomfort symptoms were scored on a 0-10 scale (Wilson, 2017), with 0, 5 and 10 corresponding to ‘no discomfort’, ‘moderate’ and ‘unbearable discomfort’, respectively. Stoichiometric equations were used to determine CHO and fat oxidation during exercise across all exercise sessions (Jeukendrup and Wallis, 2005).

### ***6.3.6 Statistical analysis***

Data presented as means  $\pm$  SD unless otherwise stated and was checked for normality using the Shapiro-Wilks test and visual inspection of Q-Q plots (residuals checked for linear mixed

models). A two-way ANOVA was used to determine effects of condition, time and the condition x time interaction for variables measured over multiple timepoints (HR,  $\dot{V}O_2$ , RPE, RER, CHO and fat oxidation for 60- and 30-min cycling session during the loading period and the 120 min steady state stage of the performance test, post-prandial blood glucose and lactate responses and subjective scores of hunger/satiety), with significance investigated with Bonferroni post hoc tests. For plasma metabolites (glucose, lactate and NEFA) during the 120 min SS period of the performance test linear mixed models were used due to missing data (restricted maximum likelihood), where condition and time were fixed effects and participant was a random effect. Alternatively, continuous variables measured at one timepoint across 3 conditions (Total CHO and fat utilisation during exercise, day 4 body mass, TBW and urine specific gravity) and performance variables (completion time and mean power output) were analysed using one-way repeated measures ANOVA. Effect sizes were provided as partial eta squared ( $\eta^2_p$ ) where 0.01, 0.06 and 0.14 corresponded to a small, medium and large effect, respectively (Cohen, 1988). A paired t-test was used to determine differences between baseline performance tests ( $n = 7$  due to technical error, and one participant performing significantly worse in their second baseline due to recent illness). All statistical analyses were conducted on SPSS (v 29, IBM, Chicago, USA), with figures created using GraphPad Prism (v 10, Massachusetts, USA).

## **6.4 Results**

### ***6.4.1 Nutrition and fluid intake***

Information regarding participant macronutrient intake during habitual 24 h period (pre-baseline performance tests) and during experimental conditions are presented in Table 6.2. Participant fluid intake during each loading period was similar across conditions ( $P = 0.54$ ,  $\eta^2_p$

= 0.07) as participants consumed  $4.6 \pm 1.0$  and  $4.4 \pm 1.0$ ,  $4.4 \pm 0.7$  and  $4.4 \pm 0.6$ ,  $4.3 \pm 0.7$  and  $4.1 \pm 0.7$  across days 2 and 3 (including drinks provided for blinding) for 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively. There was a main effect for experimental days ( $P < 0.001$ ,  $\eta^2_p = 0.77$ ) as fluid intake was lower on day 1 ( $3.2 \pm 1.0$ ,  $3.1 \pm 0.7$  and  $3.2 \pm 0.9$  for 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively;  $P \leq 0.003$ ) compared to days 2 and 3 due to consumption of blinding drinks on days 2 and 3.

#### **6.4.2 Day 4 Performance test pre-load (120 min SS)**

Pre-exercise hydration status was comparable across conditions ( $P = 0.18$ ) as participant urine specific gravity was  $1.007 \pm 0.005$ ,  $1.008 \pm 0.006$  and  $1.005 \pm 0.005$  for 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively. HR ( $P = 0.34$ ,  $\eta^2_p = 0.13$ ), RPE ( $P = 0.51$ ,  $\eta^2_p = 0.07$ ) and  $\dot{V}O_2$  ( $P = 0.64$ ,  $\eta^2_p = 0.04$ ) during 120 min steady state portion of the performance test was comparable across conditions. There was a main effect of time as HR, RPE and  $\dot{V}O_2$  increased with exercise ( $P \leq 0.001$  for all; Table 6.4). Blood glucose at rest and during exercise did not differ between CHO loading conditions ( $P = 0.08$ ), but significantly changed over time ( $P < 0.001$ ) as values at rest and following 20 min of exercise were lower than all other timepoints ( $P < 0.001$ ; Figure 6.2a). Blood lactate was significantly greater following 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO ( $P = 0.02$ ) and was elevated during the first 60 min of exercise ( $P < 0.001$ ; Figure 6.2b) before returning to near baseline in the final 60 min of steady state cycling. NEFA significantly differed between conditions ( $P = 0.004$ ) as concentrations were greater in 6 and 8 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup> ( $P < 0.05$ ; Figure 6.2c) and increased similarly over time in all conditions (time effect;  $P < 0.001$ ).

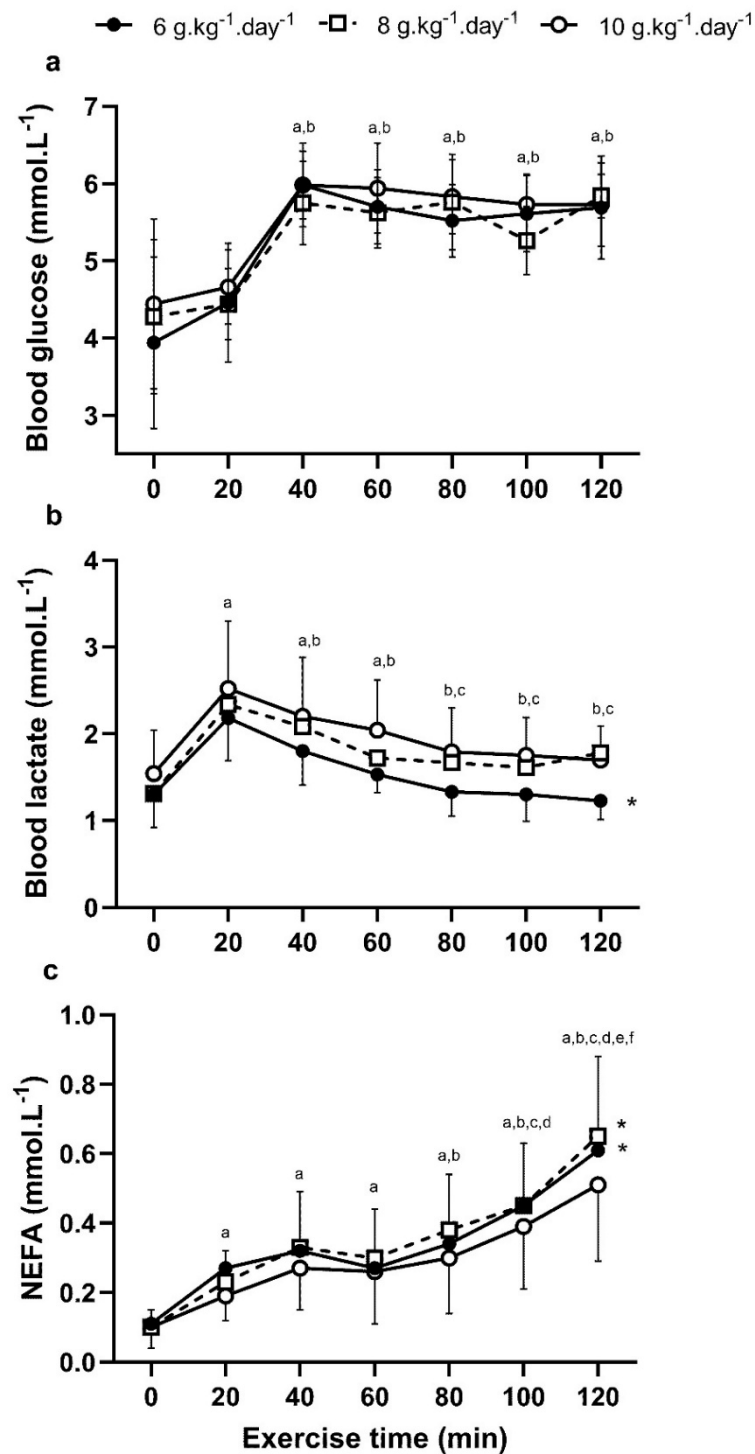
RER ( $P = 0.02$ ,  $\eta^2_p = 0.41$ ), CHO oxidation ( $P = 0.01$ ,  $\eta^2_p = 0.42$ ) and fat oxidation ( $P = 0.023$ ,  $\eta^2_p = 0.43$ ) had a significant main effect of condition as RER and CHO oxidation were higher

in 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> and fat oxidation was higher in 6 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup> (Figure 6.3). Substrate utilisation changed over time as RER ( $P < 0.001$ ,  $\eta^2_p = 0.80$ ) and CHO oxidation decreased ( $P < 0.001$ ,  $\eta^2_p = 0.76$ ), whilst fat oxidation increased ( $P < 0.001$ ,  $\eta^2_p = 0.82$ ). There was no interaction as pattern of the responses were similar across conditions ( $P = 0.59$ ,  $P = 0.07$ ,  $P = 0.50$  for RER, CHO and fat oxidation, respectively). Total CHO utilisation was significantly greater ( $P = 0.01$ ,  $\eta^2_p = 0.42$ ) in 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> ( $275 \pm 29$  vs  $216 \pm 45$  g, respectively;  $P = 0.02$ ). Accordingly, total fat utilisation was significantly greater ( $P = 0.02$ ,  $\eta^2_p = 0.43$ ) in 6 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup> ( $93 \pm 32$  vs  $67 \pm 20$  g, respectively;  $P = 0.03$ ). Total exercise energy expenditure over time did not differ between conditions ( $P = 0.91$ ,  $\eta^2_p = 0.11$ ), and there was no main effect for time ( $P = 0.21$ ,  $\eta^2_p = 0.19$ ) or interaction ( $P = 0.56$ ,  $\eta^2_p = 0.07$ ; Table 6.4). Subjective gut comfort was similar across conditions as no participants scored >3 during exercise, for any symptoms. In fact, all participants scored zero for symptoms of nausea, reflux, cramps and flatulence across all timepoints.

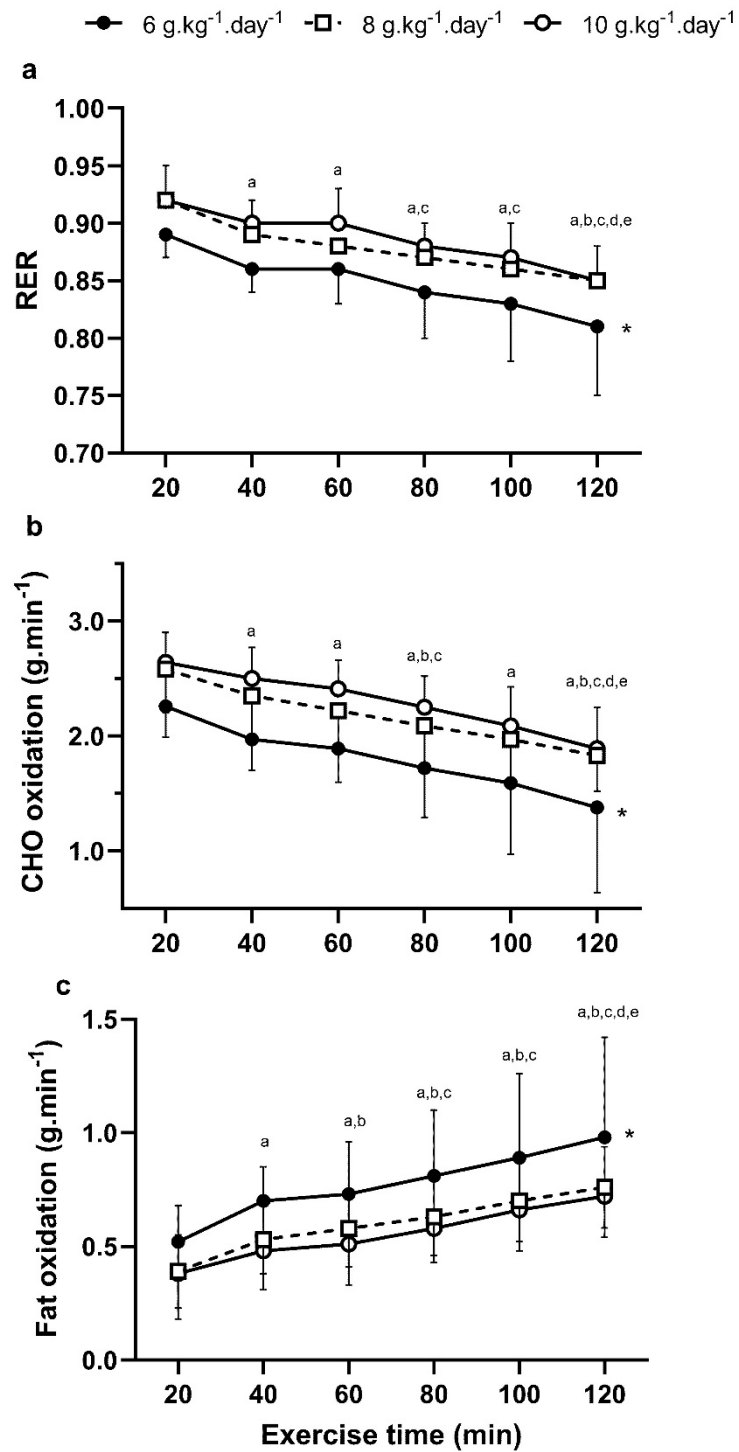
**Table 6.4.** Physiological responses during 120 min steady state cycle at intensity equivalent to 95% LT<sub>Dmax</sub> following 48 h of either 6, 8 or 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO.

| Condition                                | Exercise<br>time (min) | HR (b·min <sup>-1</sup> ) | RPE                     | $\dot{V}O_2$<br>(L·min <sup>-1</sup> ) | TEE<br>(kJ·min <sup>-1</sup> ) |
|------------------------------------------|------------------------|---------------------------|-------------------------|----------------------------------------|--------------------------------|
| 6 g·kg <sup>-1</sup> ·day <sup>-1</sup>  | 0                      | 58 ± 10                   | -                       | -                                      | -                              |
|                                          | 20                     | 133 ± 12 <sup>a</sup>     | 12 ± 1                  | 2.91 ± 0.39                            | 60.2 ± 8.5                     |
|                                          | 40                     | 138 ± 11 <sup>a,b</sup>   | 12 ± 1                  | 3.00 ± 0.39 <sup>b</sup>               | 62.0 ± 8.0                     |
|                                          | 60                     | 138 ± 9 <sup>a,b</sup>    | 12 ± 1                  | 3.00 ± 0.39                            | 61.9 ± 7.7                     |
|                                          | 80                     | 140 ± 11 <sup>a,b</sup>   | 13 ± 1 <sup>b</sup>     | 3.02 ± 0.40 <sup>b</sup>               | 62.2 ± 7.7                     |
|                                          | 100                    | 142 ± 9 <sup>a,b</sup>    | 13 ± 1 <sup>b,c,d</sup> | 3.07 ± 0.38 <sup>b,d</sup>             | 62.8 ± 7.0                     |
|                                          | 120                    | 144 ± 10 <sup>a,b</sup>   | 14 ± 1 <sup>b,c,d</sup> | 3.08 ± 0.41 <sup>b,d</sup>             | 62.9 ± 7.6                     |
| 8 g·kg <sup>-1</sup> ·day <sup>-1</sup>  | 0                      | 61 ± 6                    | -                       | -                                      | -                              |
|                                          | 20                     | 135 ± 11 <sup>a,b</sup>   | 11 ± 1                  | 2.88 ± 0.38                            | 60.7 ± 7.8                     |
|                                          | 40                     | 140 ± 13 <sup>a,b</sup>   | 12 ± 1                  | 2.96 ± 0.36 <sup>b</sup>               | 61.9 ± 7.5                     |
|                                          | 60                     | 140 ± 11 <sup>a,b</sup>   | 12 ± 1                  | 2.97 ± 0.36                            | 62.0 ± 7.4                     |
|                                          | 80                     | 140 ± 10 <sup>a,b</sup>   | 13 ± 1 <sup>b</sup>     | 3.00 ± 0.33 <sup>b</sup>               | 61.5 ± 7.5                     |
|                                          | 100                    | 140 ± 11 <sup>a,b</sup>   | 13 ± 2 <sup>b,c,d</sup> | 3.04 ± 0.33 <sup>b,d</sup>             | 62.2 ± 7.8                     |
|                                          | 120                    | 144 ± 11 <sup>a,b</sup>   | 13 ± 2 <sup>b,c,d</sup> | 3.05 ± 0.34 <sup>b,d</sup>             | 62.2 ± 7.8                     |
| 10 g·kg <sup>-1</sup> ·day <sup>-1</sup> | 0                      | 62 ± 9                    | -                       | -                                      | -                              |
|                                          | 20                     | 137 ± 13 <sup>a</sup>     | 12 ± 1                  | 2.93 ± 0.37                            | 61.3 ± 7.8                     |
|                                          | 40                     | 141 ± 10 <sup>a,b</sup>   | 12 ± 1                  | 3.01 ± 0.39 <sup>b</sup>               | 62.6 ± 8.1                     |
|                                          | 60                     | 141 ± 10 <sup>a,b</sup>   | 12 ± 1                  | 3.02 ± 0.37 <sup>b</sup>               | 62.6 ± 7.7                     |
|                                          | 80                     | 142 ± 10 <sup>a,b</sup>   | 13 ± 1 <sup>b</sup>     | 3.04 ± 0.40 <sup>b</sup>               | 62.4 ± 8.5                     |
|                                          | 100                    | 143 ± 10 <sup>a,b</sup>   | 13 ± 1 <sup>b,c,d</sup> | 3.05 ± 0.40 <sup>b,d</sup>             | 62.5 ± 9.3                     |
|                                          | 120                    | 144 ± 9 <sup>a,b</sup>    | 14 ± 2 <sup>b,c,d</sup> | 3.05 ± 0.41 <sup>b,d</sup>             | 61.3 ± 9.8                     |

<sup>a</sup> Significance vs 0 min, <sup>b</sup> Significance vs 20 min, <sup>c</sup> Significance vs 40 min, <sup>d</sup> Significance vs 60 min.



**Figure 6.2.** Plasma metabolite responses of glucose (a), lactate (b) and NEFA (c) during 120 min steady state cycle at 95% LT<sub>Dmax</sub> following consumption of a diet containing 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO for 48 h (n = 7). \* Indicates a significant difference vs 10 g.kg<sup>-1</sup>.day<sup>-1</sup>, <sup>a</sup> significant vs 0 min, <sup>b</sup> significant vs 20 min, <sup>c</sup> significant vs 40 min, <sup>d</sup> significant vs 60 min, <sup>e</sup> significant vs 80 min and <sup>f</sup> significant vs 100 min.



**Figure 6.3.** RER (a), CHO (b) and fat oxidation (c) during 120 min steady state cycle at 95% LT<sub>Dmax</sub> following consumption of a diet containing 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO for 48 h. \*Indicates a significant difference vs 10 g.kg<sup>-1</sup>.day<sup>-1</sup>, <sup>a</sup> significant vs 20 min, <sup>b</sup> significant vs 40 min, <sup>c</sup> significant vs 60 min, <sup>d</sup> significant vs 80 min and <sup>e</sup> significant vs 100 min.

### 6.4.3 TT Performance data

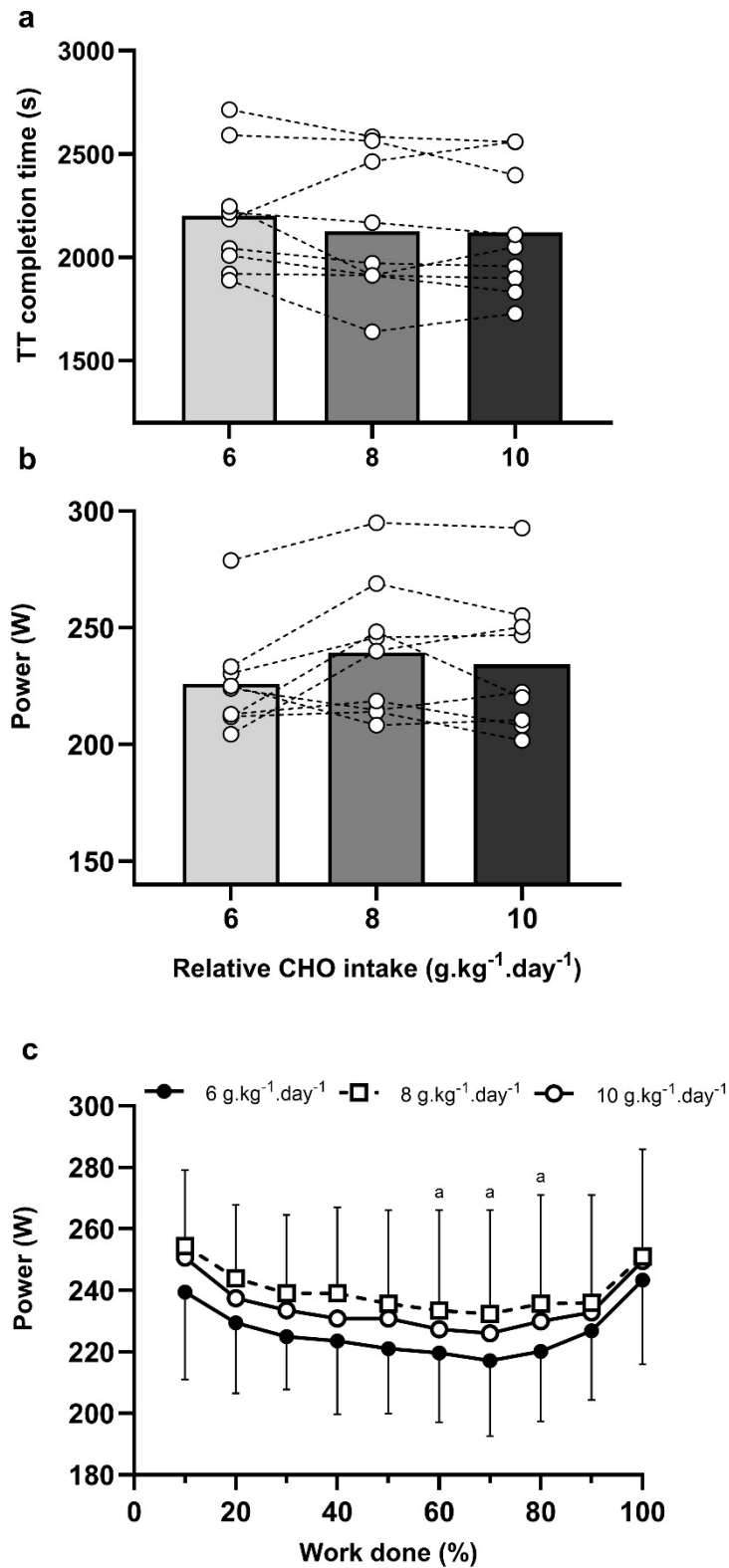
Baseline performance tests 1 and 2 did not significantly differ for mean power ( $t = 1.6$ ,  $P = 0.16$ ;  $231 \pm 36$  and  $221 \pm 36$  W, respectively), as the CV within subject repeatability from baseline 1 to 2 was  $5.3 \pm 3.7\%$  (removal of a significant outlier [CV >12%] lowered this to  $4.0 \pm 1.9\%$  for  $n = 6$  participants). For experimental conditions there was no significant trial order effect ( $P = 0.30$ ,  $\eta^2_p = 0.14$ ), as TT finish time in order of 1-3 was 36 min 42 s  $\pm$  4 min 46 s, 35 min 25 s  $\pm$  5 min 38 s and 35 min 21 s  $\pm$  5 min 12 s, respectively. There was no significant difference in TT completion time between conditions ( $P = 0.16$ ,  $\eta^2_p = 0.21$ ) as participants completed work done TTs in 36 min 46 s  $\pm$  4 min 18 s, 34 min 55 s  $\pm$  5 min 12 s and 35 min 46 s  $\pm$  5 min 56 s for 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup> (Figure 6.4a). There was also no significant difference in overall mean power output between conditions ( $P = 0.10$ ,  $\eta^2_p = 0.26$ ; Figure 6.4b) or power expressed over 10% increments of work done (condition effect;  $P = 0.09$ ,  $\eta^2_p = 0.26$ ; Figure 6.4c). There was a time main effect ( $P = 0.005$ ,  $\eta^2_p = 0.49$ ), as power steadily decreased, reaching significance at 60% work done before increasing toward earlier values in the final 20% (interaction effect;  $P = 0.66$ ,  $\eta^2_p = 0.07$ ). No participants successfully identified all 3 study conditions, however 4 out of 9 participants identified the placebo condition post-data collection.

### 6.4.4 Exercise during CHO loading on day 2 and 3

HR ( $P = 0.97$ ,  $\eta^2_p = 0.001$ ), RPE ( $P = 0.07$ ,  $\eta^2_p = 0.31$ ) and  $\dot{V}O_2$  ( $P = 0.23$ ,  $\eta^2_p = 0.17$ ) during 60 min cycling exercise session on day 2 were similar across conditions. There was a main effect of time, as HR ( $P < 0.001$ ,  $\eta^2_p = 0.97$ ), RPE ( $P = 0.01$ ,  $\eta^2_p = 0.57$ ) and  $\dot{V}O_2$  ( $P < 0.001$ ,  $\eta^2_p = 0.73$ ) all increased with exercise time (Table 6.5). However, substrate oxidation did differ between conditions (Figure 6.5), as RER ( $P = 0.003$ ,  $\eta^2_p = 0.61$ ) and CHO oxidation (condition

effect;  $P = 0.006$ ,  $\eta^2_p = 0.55$ ) were higher in 8 and 10 vs 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , whilst fat oxidation was higher in 6 vs 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (condition effect;  $P = 0.002$ ,  $\eta^2_p = 0.58$ ). There was also a main effect for time, as RER ( $P < 0.001$ ,  $\eta^2_p = 0.68$ ) and CHO oxidation decreased throughout exercise ( $P = 0.004$ ,  $\eta^2_p = 0.50$ ), whilst fat oxidation increased ( $P < 0.001$ ,  $\eta^2_p = 0.76$ ).

On day 3 during the 30 min cycling exercise at LT1 (Table 6.6), there was no main effect of condition for HR ( $P = 0.35$ ,  $\eta^2_p = 0.12$ ), RPE ( $P = 0.68$ ,  $\eta^2_p = 0.04$ ) or  $\dot{V}\text{O}_2$  ( $P = 0.44$ ,  $\eta^2_p = 0.10$ ). Similarly to the 60 min exercise sessions, substrate oxidation saw a significant main effect of condition as RER ( $P = 0.004$ ,  $\eta^2_p = 0.52$ ) and CHO oxidation ( $P = 0.004$ ,  $\eta^2_p = 0.49$ ) were higher and fat oxidation was lower ( $P = 0.007$ ,  $\eta^2_p = 0.46$ ) in the 10 compared to the 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  condition (Figure 6.5). RER and fat oxidation had a significant main effect for time ( $P = 0.024$ ,  $\eta^2_p = 0.38$  and  $P = 0.005$ ,  $\eta^2_p = 0.48$ , respectively) whilst CHO oxidation did not ( $P = 0.19$ ,  $\eta^2_p = 0.20$ ). However, there was a significant interaction ( $P = 0.04$ ,  $\eta^2_p = 0.26$ ) as CHO oxidation had decreased by 30 min of exercise, but only in the 8  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  condition.



**Figure 6.4.** Time trial completion time (a), mean power output (b) and mean power every 10% of work done (c) post-120 min steady state cycle at 95%  $\text{LT}_{\text{Dmax}}$ , following 48 h of CHO loading with 6, 8 or 10  $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  of CHO. <sup>a</sup> indicates a significant difference vs 100%.

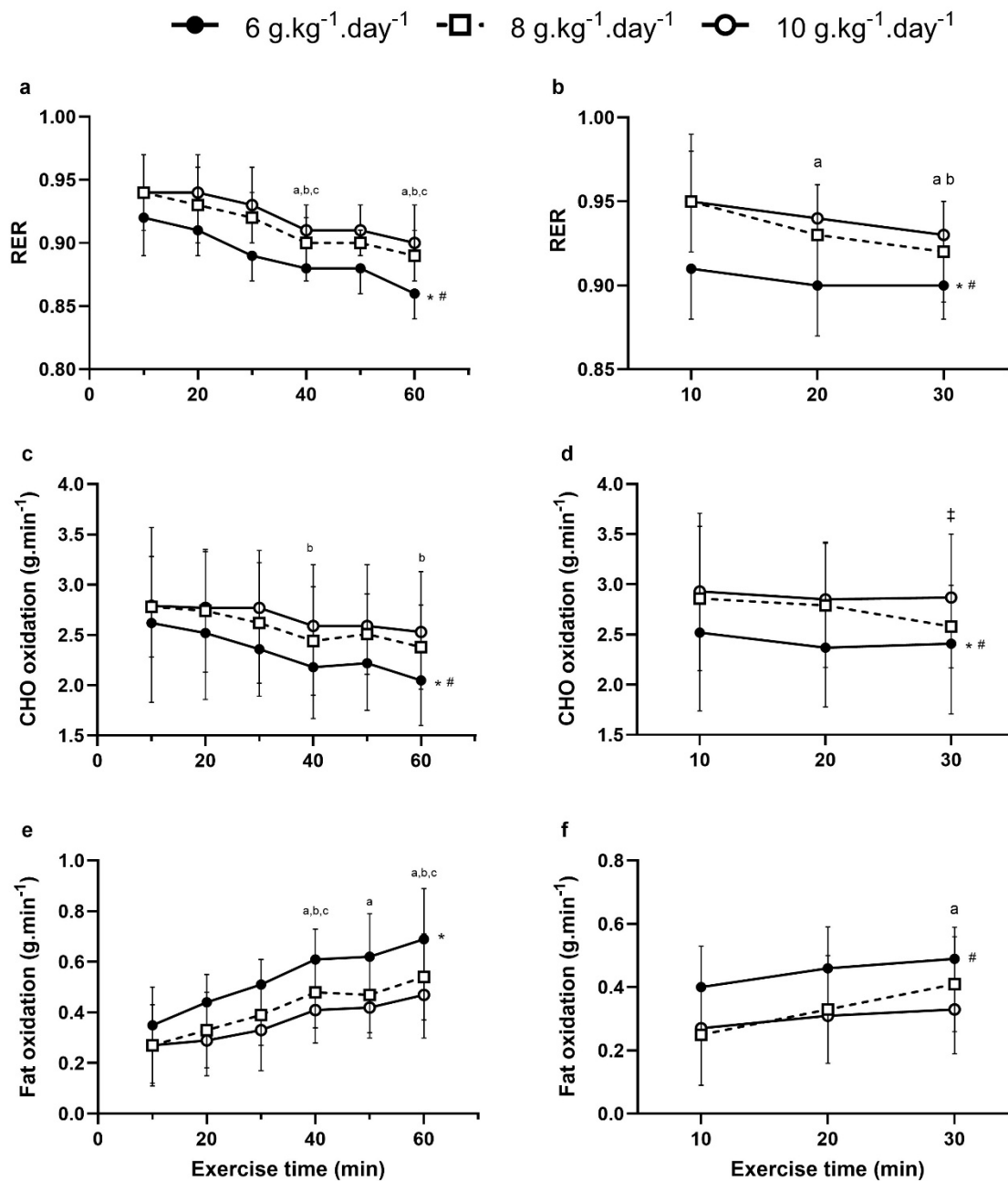
**Table 6.5.** Physiological responses throughout 60 min cycling session during CHO loading at intensity equivalent to LT1.

| Condition                                | Exercise<br>time (min) | HR (b·min <sup>-1</sup> ) | RPE    | $\dot{V}O_2$ (L·min <sup>-1</sup> ) |
|------------------------------------------|------------------------|---------------------------|--------|-------------------------------------|
| 6 g·kg <sup>-1</sup> ·day <sup>-1</sup>  | 0                      | 62 ± 8                    | -      | -                                   |
|                                          | 10                     | 129 ± 13 <sup>a</sup>     | 11 ± 2 | 2.82 ± 0.54                         |
|                                          | 20                     | 131 ± 12 <sup>a</sup>     | 11 ± 2 | 2.93 ± 0.53 <sup>b</sup>            |
|                                          | 30                     | 134 ± 13 <sup>a</sup>     | 12 ± 2 | 2.94 ± 0.49 <sup>b</sup>            |
|                                          | 40                     | 136 ± 13 <sup>a,b</sup>   | 12 ± 2 | 2.99 ± 0.56 <sup>b</sup>            |
|                                          | 50                     | 136 ± 15 <sup>a,b</sup>   | 12 ± 2 | 3.04 ± 0.59 <sup>b</sup>            |
|                                          | 60                     | 137 ± 15 <sup>a,b</sup>   | 12 ± 2 | 3.05 ± 0.59 <sup>b,c,e</sup>        |
| 8 g·kg <sup>-1</sup> ·day <sup>-1</sup>  | 0                      | 61 ± 6                    | -      | -                                   |
|                                          | 10                     | 129 ± 12 <sup>a</sup>     | 11 ± 2 | 2.79 ± 0.51                         |
|                                          | 20                     | 132 ± 12 <sup>a</sup>     | 11 ± 2 | 2.88 ± 0.58 <sup>b</sup>            |
|                                          | 30                     | 132 ± 12 <sup>a</sup>     | 12 ± 2 | 2.90 ± 0.55 <sup>b</sup>            |
|                                          | 40                     | 134 ± 13 <sup>a,b</sup>   | 12 ± 2 | 2.93 ± 0.59 <sup>b</sup>            |
|                                          | 50                     | 137 ± 12 <sup>a,b</sup>   | 12 ± 2 | 2.98 ± 0.59 <sup>b</sup>            |
|                                          | 60                     | 138 ± 11 <sup>a,b</sup>   | 12 ± 2 | 3.02 ± 0.57 <sup>b,c,e</sup>        |
| 10 g·kg <sup>-1</sup> ·day <sup>-1</sup> | 0                      | 62 ± 7                    | -      | -                                   |
|                                          | 10                     | 129 ± 12 <sup>a</sup>     | 11 ± 2 | 2.78 ± 0.55                         |
|                                          | 20                     | 132 ± 13 <sup>a</sup>     | 11 ± 2 | 2.84 ± 0.47 <sup>b</sup>            |
|                                          | 30                     | 135 ± 13 <sup>a</sup>     | 11 ± 2 | 2.92 ± 0.53 <sup>b</sup>            |
|                                          | 40                     | 135 ± 13 <sup>a,b</sup>   | 11 ± 2 | 2.92 ± 0.55 <sup>b</sup>            |
|                                          | 50                     | 136 ± 13 <sup>a,b</sup>   | 12 ± 2 | 2.95 ± 0.58 <sup>b</sup>            |
|                                          | 60                     | 137 ± 12 <sup>a,b</sup>   | 12 ± 2 | 2.99 ± 0.57 <sup>b,c,e</sup>        |

<sup>a</sup> Significant vs 0 min, <sup>b</sup> significant vs 20 min, <sup>c</sup> significant vs 30 min, <sup>d</sup> significant vs 40 min and <sup>e</sup> significant vs 50 min.

**Table 6.6.** Physiological responses throughout 30 min cycling session during CHO loading at intensity equivalent to LT1.

| Condition                                                                                                  | Exercise<br>time (min) | HR (b·min <sup>-1</sup> ) | RPE    | $\dot{V}O_2$ (L·min <sup>-1</sup> ) |
|------------------------------------------------------------------------------------------------------------|------------------------|---------------------------|--------|-------------------------------------|
| 6 g·kg <sup>-1</sup> ·day <sup>-1</sup><br>(30 min)                                                        | 0                      | 64 ± 7                    | -      | -                                   |
|                                                                                                            | 10                     | 130 ± 12 <sup>a</sup>     | 11 ± 1 | 2.85 ± 0.54                         |
|                                                                                                            | 20                     | 132 ± 13 <sup>a</sup>     | 11 ± 1 | 2.84 ± 0.49 <sup>b</sup>            |
|                                                                                                            | 30                     | 134 ± 13 <sup>a</sup>     | 12 ± 1 | 2.94 ± 0.58 <sup>b</sup>            |
| 8 g·kg <sup>-1</sup> ·day <sup>-1</sup><br>(30 min)                                                        | 0                      | 63 ± 7                    | -      | -                                   |
|                                                                                                            | 10                     | 133 ± 11 <sup>a</sup>     | 11 ± 2 | 2.81 ± 0.56                         |
|                                                                                                            | 20                     | 137 ± 14 <sup>a</sup>     | 12 ± 1 | 2.91 ± 0.57 <sup>b</sup>            |
|                                                                                                            | 30                     | 137 ± 12 <sup>a</sup>     | 12 ± 1 | 2.92 ± 0.58 <sup>b</sup>            |
| 10 g·kg <sup>-1</sup> ·day <sup>-1</sup><br>(30 min)                                                       | 0                      | 61 ± 8                    | -      | -                                   |
|                                                                                                            | 10                     | 129 ± 12 <sup>a</sup>     | 11 ± 1 | 2.89 ± 0.64                         |
|                                                                                                            | 20                     | 134 ± 13 <sup>a</sup>     | 12 ± 1 | 2.93 ± 0.61 <sup>b</sup>            |
|                                                                                                            | 30                     | 137 ± 12 <sup>a</sup>     | 12 ± 1 | 2.98 ± 0.63 <sup>b</sup>            |
| <sup>a</sup> Significant vs 0 min, <sup>b</sup> significant vs 20 min, <sup>c</sup> significant vs 30 min. |                        |                           |        |                                     |



**Figure 6.5.** RER, CHO and fat oxidation during 60- (a, c and e) and 30- min (b, d and f) cycling exercise at an intensity equivalent to LT1 during a CHO loading period (days 2 and 3), respectively, where participants consumed 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO. \* Indicates significant difference vs 10 g.kg<sup>-1</sup>.day<sup>-1</sup>. # Significant difference vs 8 g.kg<sup>-1</sup>.day<sup>-1</sup>. ‡Significant interaction. <sup>a</sup> Significant vs 10 min, <sup>b</sup> significant vs 20 min and <sup>c</sup> significant vs 30 min of exercise.

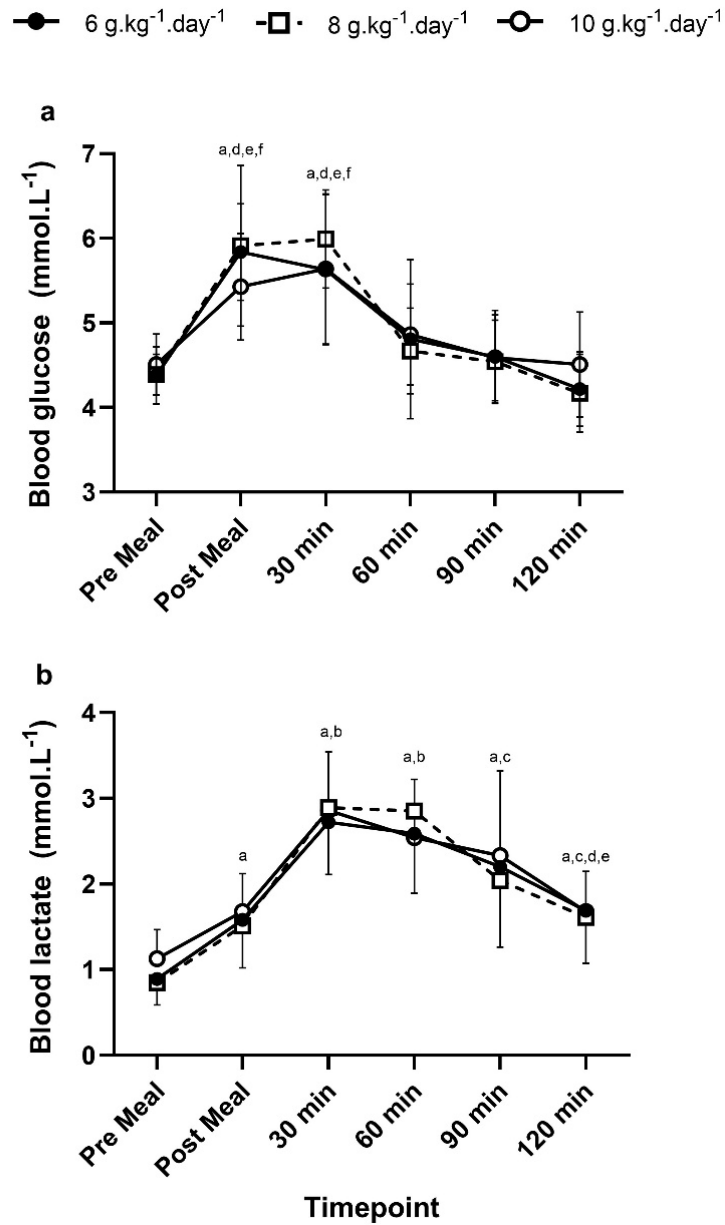
#### **6.4.5 Day 4 body mass and body water**

Post-load body mass did not differ ( $P = 0.56$ ,  $\eta^2_p = 0.06$ ) between 6, 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  ( $77.7 \pm 6.5$ ,  $77.6 \pm 6.3$  and  $77.9 \pm 6.0$  kg, respectively). This was also the case for TBW ( $P = 0.71$ ,  $\eta^2_p = 0.04$ ;  $48.3 \pm 3.2$ ,  $48.3 \pm 3.5$  and  $48.1 \pm 3.0$  L), ECW ( $P = 0.84$ ,  $\eta^2_p = 0.02$ ;  $19.2 \pm 1.4$ ,  $19.2 \pm 1.3$  and  $19.2 \pm 1.2$  L) and ICW ( $P = 0.58$ ,  $\eta^2_p = 0.06$ ;  $29.1 \pm 1.9$ ,  $29.0 \pm 2.2$  and  $29.0 \pm 1.9$  L) across 6, 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ , respectively.

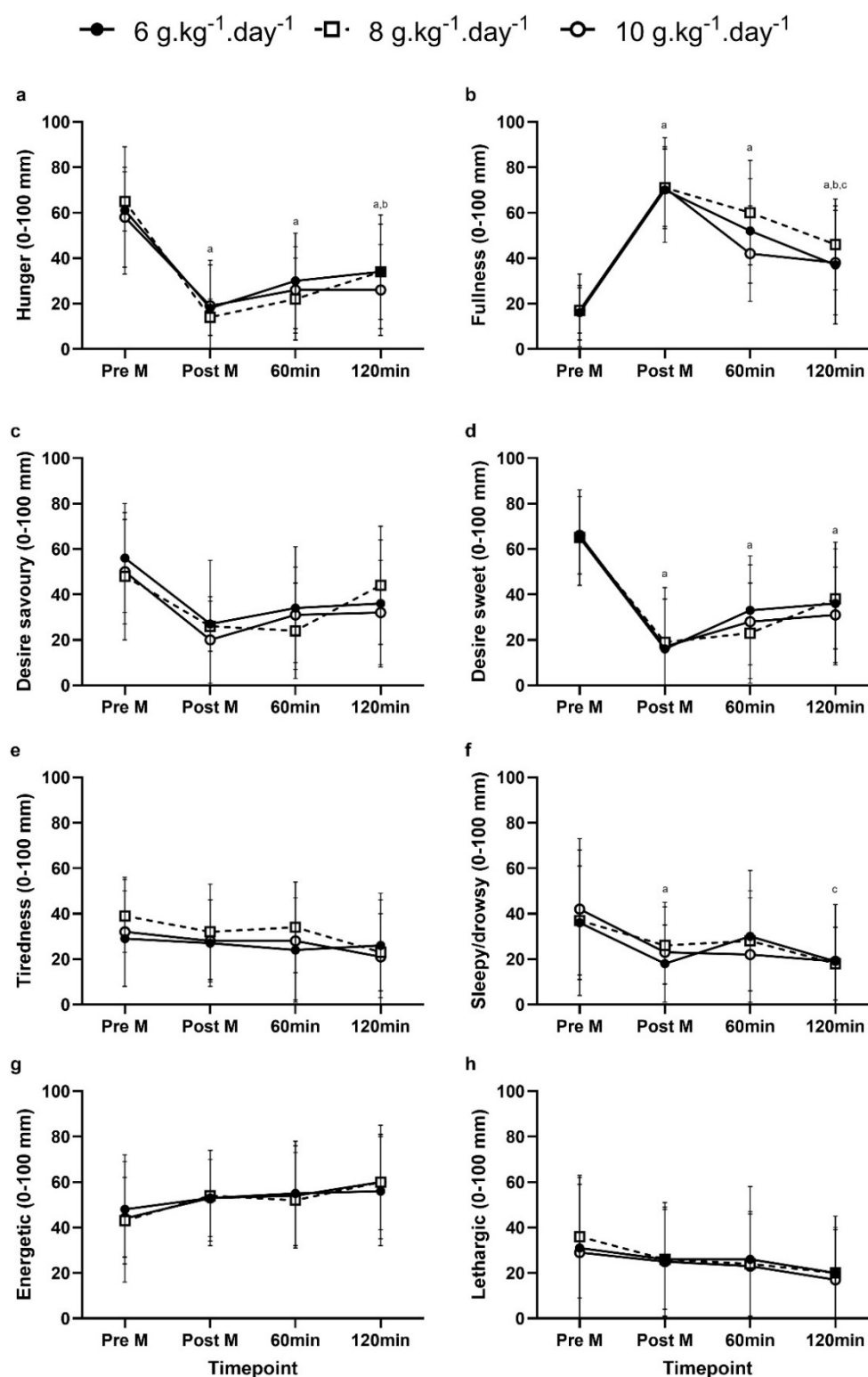
#### **6.4.6 Day 4 post-prandial response to high CHO breakfast**

There was no condition main effect for blood glucose ( $P = 0.85$ ,  $\eta^2_p = 0.02$ ) and lactate ( $P = 0.75$ ,  $\eta^2_p = 0.04$ ), or interaction as conditions responded similarly over time ( $P = 0.20$ ,  $\eta^2_p = 0.16$  and  $P = 0.53$ ,  $\eta^2_p = 0.09$ , respectively; Figure 6.6). There was a main effect for time ( $P < 0.001$ ,  $\eta^2_p = 0.75$  and  $P < 0.001$ ,  $\eta^2_p = 0.84$ , respectively) as glucose and lactate increased immediately and 30 min post-prandial ( $P < 0.05$ ) before decreasing to near baseline levels by 60 min and remained stable thereafter.

Participants scored comparably for subjective feeling of hunger ( $P = 0.47$ ), fullness ( $P = 0.10$ ), desire for savoury ( $P = 0.41$ ), desire for sweet ( $P = 0.77$ ), tiredness ( $P = 0.18$ ), sleepiness ( $P = 0.84$ ), energy ( $P = 0.95$ ) and lethargy ( $P = 0.83$ ) across conditions throughout the morning of day 4 pre-performance test (Figure 6.7).



**Figure 6.6.** Capillary blood glucose (a) and lactate (b) pre- and post- high CHO breakfast (2 g.kg<sup>-1</sup>) following 48 h of CHO loading with 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO. <sup>a</sup> Indicates a significant difference vs pre-meal, <sup>b</sup> significant vs post-meal, <sup>c</sup> significant vs 30 min, <sup>d</sup> significant vs 60 min, <sup>e</sup> significant vs 90 min and <sup>f</sup> significant vs 120 min.



**Figure 6.7.** Subjective scores for feeling of hunger (a), fullness (b), desire for savoury (c), desire for sweet (d), physically tired (e), sleepiness (f), energy (g) and lethargy (h) pre- and post- high CHO breakfast (2 g.kg<sup>-1</sup>) following 48 h of CHO loading where participants consumed 6, 8 or 10 g.kg<sup>-1</sup>.day<sup>-1</sup> of CHO. <sup>a</sup> Indicates a significant difference vs pre-meal, <sup>b</sup> significant vs post-meal, <sup>c</sup> significant vs 60 min, <sup>d</sup> significant vs 120 min.

## 6.5 Discussion

CHO loading with a high-very high CHO intake to maximise muscle glycogen stores pre-endurance competition is common practice for endurance athletes and is considered a prerequisite for optimal endurance exercise performance, in line with contemporary nutritional guidelines (Thomas et al., 2016). However, the main finding of the current study was that CHO loading with a moderate, high and very high CHO intake for 48 h (6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively) did not significantly impact endurance cycling performance under real-world conditions.

This is the first study replicating real-world conditions of exercise and nutrition preparation for racing followed by a performance test with a double-blind placebo-controlled experimental protocol. Similar to other studies which incorporated a double-blind design (Burke et al., 2000; Tomcik et al., 2018) comparing a very high vs moderate pre-exercise CHO intake (9-12 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> for 48-72 h, respectively), there was no significant performance effect of increased CHO intake during CHO loading. These findings contrast with previous research, where participants were not blinded to CHO loading conditions, which showed improvements in endurance performance (Karlsson and Saltin, 1971; Williams et al., 1992; Rauch et al., 1995; Widrick et al., 1993) and exercise capacity (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967; Lamb et al., 1991; Bosch et al., 1993; Galbo et al., 1979; Jensen et al., 2020).

One possible reason for this discrepancy is a placebo effect within previous CHO loading research (Burke et al., 2000), as increased dietary CHO intake for optimal muscle glycogen concentrations (and performance) pre-endurance exercise is well-accepted within scientific and

endurance sport communities (Bergström et al, 1967; Karlsson and Saltin, 1971; Widrick et al., 1993), as well as being advocated by nutritional guidelines for endurance sports performance (Thomas et al., 2016; Burke et al., 2017). Therefore, study participants would have associated higher CHO diets with improved performance leading to bias in study results. As a result, a mechanism of increased endurance performance/capacity in previous unblinded CHO loading research could be primarily psychological, as controlling for placebo effects are a fundamental requirement in research investigating sports performance (Halson and Martin, 2013; Beedie and Foad, 2009). Particularly considering the well-established potency of the placebo effect across numerous sporting contexts such as weightlifting (Ariel and Saville, 1972), sprint distance running (Beedie et al., 2007; McClung and Collins 2007) and endurance cycling (Foad et al., 2008; Beedie et al., 2006; Clark et al., 2000).

It should be noted, despite not reaching statistical significance ( $P > 0.10$ ), there was a large effect size of condition for performance outcomes in the current study ( $\eta^2_p = 0.21 - 0.26$ ), as increased mean TT completion time (5.0 and 2.9 %) and mean power (5.8 and 3.5 %) was improved in 8 and 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup>, respectively (Figure 6.4). Considering within participant repeatability (~4% from baseline 1 to 2) and that elite level endurance competitions are decided by the smallest margins (< 1%), this improvement would likely be a meaningful difference in practice (Burke et al., 2000; Hopkins et al., 1999). However, further investigation of current study data revealed that 3 individuals who successfully identified the placebo conditions had the greatest improvements in mean power (mean difference; +12.8 and 12.3% [n = 3] for 8 and 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO, respectively). Whereas participants who were successfully blinded only saw marginal or no improvement for 8 and 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> (mean difference +2.6% and -0.5%, respectively [n = 6]), suggesting the placebo effect could have biased these performances and skewed magnitude-based statistics in favour of higher CHO intakes. In

agreement, previous sports performance research showed the placebo effect to result in improvements ranging from 1-50% (Beedie and Foad, 2009), however in cycling models the magnitude was smaller (2-4 % improvements in ~60 min TT mean power; Clark et al., 2000; Beedie et al., 2006). The exact mechanisms behind the placebo effect are believed to be neurobiological (Halson and Martin, 2013; Beedie et al., 2018), and may result from increased motivation when receiving the treatment group due to an expectation of improvement (Beedie et al., 2007), a lack of motivation during the control condition where participants limit themselves through self-doubt and expected poorer performance (Foad et al., 2008; Beedie et al., 2007), or likely a combination of both dependent on individual perceptions and beliefs. In line with this, power output over time was marginally higher (Figure 6.4c) in the 8 and 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> conditions (non-significant;  $P > 0.05$ ), suggesting motivation/effort was the effecting factor (at least in those who believed they consumed high CHO), as early during the maximal effort glycogen levels should have been adequate to maintain an optimal power output across all conditions. If glycogen depletion and muscular fatigue were the true cause of this difference, it would be expected to occur later during the TT.

Another possible reason behind the nonsignificant difference between performance in the current study could be adequate CHO availability across all conditions, limiting previously reported ergogenic effects higher muscle glycogen stores in 10 vs 6 and 8 g·kg<sup>-1</sup>·day<sup>-1</sup> conditions (Study 1; Chapter 5). It is possible glycogen concentrations following 6 g·kg·day<sup>-1</sup> of CHO for 48 h in endurance trained individuals (~ 500 mmol·kg<sup>-1</sup> DM; Study 1, Chapter 5; Areta and Hopkins, 2018) combined with exogenous CHO feeding during exercise (60 g·h<sup>-1</sup>), and a high CHO breakfast 2 h pre-exercise (2 g·kg<sup>-1</sup>), was adequate to meet current study exercise performance demands, which admittedly was toward the lower end of CHO loading recommendations (~2.5 h; Thomas et al., 2016). This hypothesis is supported by maintenance

of euglycemia during 120 min SS exercise across conditions (Figure 6.2a), however, unfortunately to allow uninterrupted TT performance, no bloods were taken during the TT itself to confirm plasma glucose concentrations during maximal effort. In line with this, many previous reports of improved exercise capacity compared a high vs low CHO intake group and often used overnight fasted participants who did not ingest any CHO during exercise (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967). In such situations of restricted exogenous CHO availability there is greater reliance on endogenous glycogen stores, which would be suboptimal in the liver following an overnight fast (Nilsson and Hultman, 1973; Iwayama et al., 2021). Therefore, previous CHO loading studies would have seen an inflated ergogenic effect of muscle glycogen supercompensation in comparison to current study and real-world racing conditions (Bergström et al., 1967; Widrick et al., 1993). Perhaps the ergogenic effect of CHO loading and muscle glycogen supercompensation is only apparent under similar physiological conditions, where alternative sources of CHO availability fail to meet exercise demands, such as elite level endurance cycling races, which often last 4-6 h per day with exercise energy expenditure surpassing exogenous feeding capabilities (Saris et al., 1989). However, current study total CHO oxidation was higher in 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> ( $P < 0.05$ ), which considering exogenous CHO intake was identical between conditions, and that increased glycogen availability stimulates preferential utilisation at the same relative exercise intensity, likely resulted in greater glycogen utilisation in the 10 g·kg<sup>-1</sup>·day<sup>-1</sup> condition (Hargreaves et al., 1995; Burke et al., 2000; Arkinstall et al., 2004; Widrick et al., 1993). This did not result in any changes in physiological responses to exercise between conditions (Table 6.2), which is surprising considering CHO utilisation is more metabolically efficient (Krogh and Lindhard, 1920). Although it is likely the 120 min SS period was not long/intense enough to induce differences in substrate utilisation that would translate into changes in physiological

responses, or perhaps the modest study sample size limited the ability to detect such differences.

The current study performance test was chosen as an attempt to balance practical relevance, with laboratory data collection. Where physiological and metabolic demands simulated some racing conditions, as a cyclist sitting within the peloton could be riding at a SS intensity for a prolonged period, before an ‘attack’ or climb in the final stages of racing required a maximal effort. This exercise profile also allowed collection of venous bloods and respiratory gases during the first 120 min of exercise, where prolonged moderate-high exercise intensities ( $\geq 70\% \dot{V}O_{2\max}$ ) would harm validity of equations (Jeukendrup and Wallis, 2005). The maximal intensity 30 min work done TT has also previously been validated, with strong repeatability compared to variable exercise capacity tests at a fixed submaximal workload (CV 3.5 versus 26.6%, respectively; Jeukendrup et al., 1996) which have commonly been used in previous CHO loading research (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967; Lamb et al., 1991; Bosch et al., 1993; Galbo et al., 1979). Lastly, a post-hoc power calculation based on current study data ( $\eta^2_p = 0.21$  and  $0.26$  for mean TT completion time and mean power, respectively) along with the sample size of 9 achieved and an alpha of 0.05 achieved a power of 0.88 and 0.95, respectively.

Another important finding in the current study was increased whole-body CHO oxidation and RER in the 10 and 8 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> condition, during 60- and 30-min light-moderate intensity sessions conducted during the loading period. This is in line with the findings of Arkinstall et al. (2004) and previous suggestions (Study 1; Chapter 5) where increased glycogen availability associated with increased CHO intakes regulate substrate utilization in favour of CHO

metabolism during light-moderate intensity exercise. Furthermore, considering glucose uptake is not affected by glycogen concentrations at the same relative exercise intensity (Hargreaves et al., 1995) it is assumed whole body glycogen accounted for the increased CHO oxidation. Particularly as participant were not fed exogenous CHO during these exercise sessions. This increased utilisation may explain differences in glycogen concentrations in 8 vs 10 g·kg<sup>-1</sup>·day<sup>-1</sup>, reported in the previous study (Study 1; Chapter 5), where similar CHO utilisation between conditions meant that comparatively, post-exercise there was less CHO available in the 8 g·kg<sup>-1</sup>·day<sup>-1</sup> condition. This supports previous suggestions that a higher CHO intake of ≥10 g·kg<sup>-1</sup>·day<sup>-1</sup> may be necessary to account for these training sessions conducted during the loading period in real-world pre-competition conditions for prolonged duration or maximal intensity endurance exercise performance (> 3 h).

In support of previous findings (Study 1; Chapter 5), there was no change in body mass, TBW, ECW or ICW between conditions ( $P > 0.05$ ). This contrasts the common belief suggested by previous studies that every 1 g of glycogen is bound to 3-4 g of water (Ferandez-Elias et al., 2015; Olsson and Saltin, 1970), and that CHO loading and the resultant enhancement of muscle glycogen stores increases body mass by ~2% (Burke et al., 2017). The exact reason behind this discrepancy is currently unclear and could be related to multiple methodological factors such as measurement techniques used to determine body water (Olsson and Saltin, 1970; Shiose et al., 2016), different levels of manipulated CHO intake and glycogen concentrations (Bone et al., 2016), exercise conducted and level of glycogen depletion (full body or localised) pre CHO loading, as well as fluid and electrolyte balance (O'Brien et al., 1993). Further research is warranted to corroborate these findings and disentangle potential methodological considerations which may have impacted current or previous study results.

There were no differences in post-prandial blood glucose and lactate (Figure 6.6), or subjective hunger scores (Figure 6.7) pre- and post- high CHO 'pre-race' breakfast following CHO loading with 6, 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO (and the resultant differences in muscle glycogen). We therefore hypothesise that post-prandial hormonal responses to the same high CHO meal were not affected by differences in skeletal muscle glycogen stores. It is well-established that changes in CHO content of the meal itself (Raben et al., 1994) and participant training status (King et al., 1990) may affect metabolite and hormonal responses, but interestingly this does not seem to be the case for muscle glycogen concentrations. However, insulin response was not directly measured in the current study to confirm this suggestion.

One limitation of the current study was only collecting blinding efficacy questionnaires at the end of individual participant study completion. Collecting responses following each condition to gauge participant perception immediately post-performance test in each condition would have provided further insight into beliefs. Furthermore, despite rigorous pilot testing, 4 out of 9 participants successfully identified the placebo condition, which likely biased performance test results. However, this did provide further evidence to support the potential placebo effect associated with CHO loading and endurance cycling performance. Future research should investigate this with stronger indistinguishable study blinding and perhaps a longer duration/higher intensity performance test, more representative of physiological and metabolic demands of elite endurance cycling competitions, where athletes could be more reliant on endogenous glycogen stores.

### ***6.5.1 Conclusions***

Current study findings suggests that CHO loading with a high and very high CHO intake (8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup>) and the resultant increase in muscle glycogen stores (reported in Chapters 4 and 5), provided no benefit for endurance cycling performance outcomes (~2.5 h), under real-world pre-competition nutrition and training conditions. Study results suggest a placebo effect associated with CHO loading and endurance cycling performance. Despite over half a century of research, further work with rigorous double blind crossover designs are required to confirm whether the ergogenic effect of CHO loading exists in real-world racing conditions.

### ***6.5.2 Link to next chapter***

Previous chapters have determined a strong linear dose-response of CHO intake for muscle glycogen concentrations (Chapters 4 and 5), with larger CHO intakes superior for maximisation of glycogen stores, however this does not seem to translate into improved endurance cycling performance (~2.5 h), at least compared to conditions of moderate pre-exercise CHO intake with high exogenous CHO availability. A reoccurring concept throughout the thesis, is the effect of CHO availability (primarily increased glycogen stores) on substrate utilisation during exercise, with high CHO availability leading to increased CHO oxidation during exercise. However, a practical question from the field is whether CHO ingestion patterns during exercise, of the same absolute quantity of CHO, can affect substrate oxidation, physiological responses to exercise, GI comfort and exercise capacity.

## **Chapter Seven:**

### **Different CHO ingestion patterns do not affect physiological responses, whole-body substrate oxidation or gastrointestinal comfort in cycling**

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## 7.1 Abstract

Fuelling during endurance exercise has evolved towards greater amounts of CHO ingested per hour, which can prove challenging for athletes. However, the effects of different CHO ingestion patterns during exercise have scarcely been investigated in cycling. Twenty recreationally active males cycled for 180 min at LT1 on two occasions in a randomised counterbalanced order. Participants consumed 90 g·h<sup>-1</sup> of CHO, either as 22.5g every 15 min or 45g every 30 min. Respiratory gases, blood glucose, lactate, HR, RPE, and GI symptoms were assessed every 15 min. Physiological responses showed no difference between conditions ( $P > 0.20$ ) or significant interactions ( $P > 0.30$ ), except for blood glucose which saw a transient difference during the first 30 min (interaction;  $P = 0.03$ ). Whole body CHO and fat oxidation were not different between conditions ( $2.38 \pm 0.37$  and  $2.33 \pm 0.39$  g·min<sup>-1</sup>,  $P = 0.25$ , and  $0.19 \pm 0.07$  vs  $0.22 \pm 0.08$  g·min<sup>-1</sup>,  $P = 0.10$ , respectively). Subjective markers of GI symptoms did not differ between conditions ( $P > 0.05$ ) and only 1 participant scored >4 across any symptom. In conclusions, ingesting a larger CHO amount at less regular intervals during prolonged cycling had minimal impact on physiological responses to exercise, whole-body substrate oxidation and gut discomfort, allowing athletes to freely select their preferred strategy.

## 7.2 Introduction

The importance of high CHO availability during endurance cycling performance (> 90 min) is well established (Coyle et al., 1992; Currell and Jeukendrup, 2008; Burke et al., 2017). The primary reason being an ability to maintain moderate-high exercise intensities, through a delayed onset of fatigue by withstanding liver glycogen depletion, the maintenance of blood glucose concentration (euglycemia), increased CHO oxidation, and stimulating the central nervous system (Gonzalez et al., 2015; Stellingwerff and Cox, 2014). As a result, contemporary nutritional guidelines recommend a CHO intake of up to  $90 \text{ g}\cdot\text{h}^{-1}$  of multiple transportable CHO for prolonged exercise lasting  $\geq 2.5 \text{ h}$  (Thomas et al., 2016; Jeukendrup, 2004), with recent reports suggesting additional benefits from higher intakes of  $120 \text{ g}\cdot\text{h}^{-1}$  (Podlogar et al., 2022; Urdampilleta et al., 2020). These practices have allowed exogenous CHO oxidation to reach as high as  $1.6$  or  $1.8 \text{ g}\cdot\text{min}^{-1}$  in some reports (Hearris et al., 2022; Jentjens and Jeukendrup, 2005), allowing a sparing of limited endogenous liver glycogen stores (Stellingwerff et al., 2007; Gonzalez et al., 2015). However, ingesting such large quantities of CHO can be challenging for endurance cyclists under race conditions (Pfeiffer et al., 2012).

To achieve such high exogenous CHO oxidation, athletes are required to have regular CHO feedings, typically consuming sports supplements such as energy gels, bars and sports drinks 3-4 times per hour (every 15-20 min). Such strategies can become tedious or impractical (anecdotal evidence from the field), possibly impacting race ‘flow’ and focus, which could prove costly considering races at the elite level are often decided by key moments such as an unpredicted attack or breakaway from the peloton. A less frequent, larger CHO bolus could provide a more practical solution. However, maintaining a high CHO intake with less frequent feedings may pose an increased risk of developing GI discomfort (Stocks et al., 2016), with moderate-severe symptoms likely impairing exercise performance.

Despite decades of work focussed on CHO intake during cycling (Coyle et al., 1986; Coyle et al., 1997; Hearn et al., 2022), very little research has focused on different ingestion patterns of the same absolute quantity of CHO. To the authors' knowledge only one study has previously investigated this in cycling (Fielding et al., 1985), reporting no change in physiological responses or RER during exercise between CHO conditions, however the quantity of CHO ingested was extremely low considering current contemporary guidelines (21.5 vs 90 g·h<sup>-1</sup>). As CHO availability during exercise is a profound regulator of physiological responses and substrate utilisation (Coyle et al., 1997; Fell et al., 2021), these data are not applicable to athletes today, who can consume >4-fold the CHO quantity per hour compared to the previous study (Saris et al., 1989; Strobel et al., 2022). Several studies have investigated the effects of a glucose bolus pre-exercise (Krzentowski et al., 1984; Guzenec et al., 1989) and reported similar peak exogenous CHO oxidation rates (0.48 - 0.65 g·min<sup>-1</sup>) when compared to others who investigated more frequent feeding strategies (Massicotte et al., 1989, 1990 and 1994). More recently, Stocks et al. (2016) and Mears et al. (2020) specifically investigated the effects of CHO feeding strategies in well trained cross-country skiers and runners, reporting changes in lipid and exogenous CHO oxidation, respectively, due to manipulated CHO ingestion patterns. However, differences in the feeding strategies and exercise intensities utilised, as well as alterations in physiological demands across the sporting disciplines (walking, running and cross-country skiing) make previous study results not directly transferable to endurance cycling.

As such, there is no cycling-specific, up to date information available to inform endurance cyclists and practitioners regarding the effects of different CHO ingestion patterns (frequency and dose) during prolonged cycling under conditions of high CHO availability, as all modern-day endurance cyclists would find themselves during competition. Therefore, the aim of this

study was to investigate the effects of different CHO ingestion patterns on physiological responses to exercise, substrate oxidation, GI symptoms and exercise capacity. We hypothesized that there would be no effect of feeding frequencies on physiological responses to exercise, substrate oxidation or exercise capacity. We also hypothesised there would be moderate reports of GI symptoms across both conditions.

## **7.3 Methods**

### **7.3.1 *Participants***

Twenty tier 1 recreationally active males, as outlined by McKay and colleagues (2022), participated in the study (Table 7.1). Participants were healthy, training 3-4 times per week and not following CHO-restrictive diets. Written and verbal information regarding study procedures was provided before written informed consent was obtained. The study was approved by the Liverpool John Moores University Research Ethics Committee. Sample size estimation was determined based on lipid oxidation data of Stocks et al. (2016), where high CHO consumption at a high and low feeding frequency during exercise resulted in mean lipid oxidation values of  $0.29 \pm 0.12$  and  $0.24 \pm 0.13$  g·min<sup>-1</sup>. It provided an effect size of 0.4 and corresponded to an a-priori sample size of 8 to achieve an alpha of 0.05 and power of 0.95. However, as the low frequency ingestion strategy used by Stocks et al. (2016) was extreme compared to the current study, a more conservative approach was deemed appropriate, using a small-moderate effect size of 0.25 (Cohen, 1988) which required an a-priori sample size of 18 to achieve an alpha of 0.05 and power of 0.95 (G\*Power, v 3.1.9.7, Kiel, Germany).

### 7.3.2 Study design

In a randomised, counterbalanced crossover design, participants completed 2 experimental trials, consisting of 180 min cycling at an intensity equivalent to LT1 (defined as baseline blood lactate value +1 mmol·L<sup>-1</sup>; Zoladz et al., 1995). Trials were preceded by a 24 h dietary control period, designed to provide high CHO availability. During exercise, participants ingested 90 g·h<sup>-1</sup> of CHO in the form of CHO gels, with different ingestion patterns (frequency and dose) of either a 22.5g CHO gel every 15 min or a 45g CHO gel every 30 min (Figure 7.1). All visits were separated by  $\geq 7$  days.

**Table 7.1.** Study 3 participant characteristics (n = 20)

|                                                              |                 |
|--------------------------------------------------------------|-----------------|
| Age (years)                                                  | 24 $\pm$ 3      |
| Body mass (kg)                                               | 76.2 $\pm$ 7.5  |
| Height (cm)                                                  | 181.0 $\pm$ 5.5 |
| $\dot{V}O_{2\max}$ (L·min <sup>-1</sup> )                    | 3.82 $\pm$ 0.41 |
| $\dot{V}O_{2\max}$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> ) | 50.4 $\pm$ 3.8  |
| PO at LT1 (W)                                                | 139 $\pm$ 29    |
| PPO (W)                                                      | 314 $\pm$ 31    |
| PPO (W·kg <sup>-1</sup> )                                    | 4.1 $\pm$ 0.4   |

### 7.3.3 Preliminary testing

Height and body mass were measured semi-nude (Seca, Hamburg, Germany), before participants were seated for resting measures of HR (Polar H10, Kempele, Finland), blood glucose and lactate using capillary fingertip blood samples, which were immediately analysed

(Biosen C-Line, EKF Diagnostics, Cardiff, UK). Participants then completed incremental lactate threshold and maximal oxygen consumption ( $\dot{V}O_{2\max}$ ) tests on a cycle ergometer (Lode, Groningen, Netherlands) as previously described (Chapter 3). Briefly, participants began cycling at 100 W and exercise intensity increased 25 W following each 4 min stage. In the final 30 s of each stage, a fingertip blood sample was collected and immediately analysed for blood glucose and lactate, with RPE (Borg, 1982) and HR also collected in the final 10 s of each stage. The test was terminated when participants reached the onset of blood lactate accumulation  $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$  (Heck et al., 1985).

Following 10 min rest, participants returned to the ergometer to complete a  $\dot{V}O_{2\max}$  test. The test began at 100 W and exercise intensity increased 25 W every 1 min until volitional exhaustion. Gas exchange was measured continuously throughout using a metabolic cart (Vyntus CPX, Vyaire Medical, Chicago, USA), with  $\dot{V}O_{2\max}$  defined as the highest  $\dot{V}O_2$  sustained over a 30-s average. HR and RPE were collected in the final 10 s of each stage and were used as indirect markers of volitional exhaustion. PPO was determined to describe participant characteristics using the equation outlined by Kuipers et al. (1985). Following a further 10 min of rest, participants began a familiarisation period where the experimental procedures (subsequently described) were replicated identically for 60 min, followed by an exercise capacity test. No CHO was ingested during the familiarisation, with participants provided with 150 mL of water every 15 min.

#### ***7.3.4 Pre-experimental controls***

Twenty-four hours prior to both experimental trials, participants were asked to follow a meal plan designed to replicate pre-competition practices of endurance athletes. In line with contemporary nutritional guidelines, the meal plan provided 8, 2 and 1  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO,

protein, and fat, respectively. The plan provided ~1 L of fluids, with participants also instructed to consume a further 1-1.5 L of water throughout the day. On the morning of the experimental trial, participants were asked to consume a high CHO breakfast, containing 2 g·kg<sup>-1</sup> of CHO, 25 g and 5 g of protein and fat, respectively, as well as ~500 mL of fluids. Meal plans instructed participants to consume foods and beverages at usual mealtimes in the form of breakfast, lunch, and dinner, with various snacks between meals. To make the quantity of food more tolerable, the meal plans provided a healthy mixed diet, supplemented with additional high glycaemic index foods and high CHO beverages. Plans contained fruits, fruit juice, oats, bread, jam, rice, chicken, sweet chilli sauce and small portions of vegetables (to minimise dietary fibre intake) and were created using an online nutrition software (Nutritics, Dublin, Ireland). To confirm adherence, participants were asked to send photographs of meals prior to consumption using online messenger (WhatsApp, Meta, California, USA). Participants were free to consume water as desired but were asked not to consume any calorie containing beverages or foodstuffs outside what was outlined within the plans. Additionally, no exercise, consumption of caffeine or alcohol was permitted 24 h prior to each experimental trial.

### ***7.3.5 Experimental trials***

On the morning of each trial (9:00 am  $\pm$  40 min; standardised within participant), at least 60 min post-breakfast consumption (between 0600 and 0800 h, with time replicated across conditions), participants were seated for collection of resting values of HR, blood glucose and lactate, completed a standardised cycling warm up (10 min at 100 W) and then cycled for 180 min at an intensity corresponding to LT1 (139  $\pm$  29 W). In the final 30 s of every 15 min, capillary blood samples were collected for determination of blood glucose and lactate, with HR and RPE also collected. Expired gas was collected and averaged for the final 2 of every 15 min.

Following this, subjective GI symptoms were recorded on a 0-10 scale as previously described (Chapter 3 General methods; Wilson, 2017).

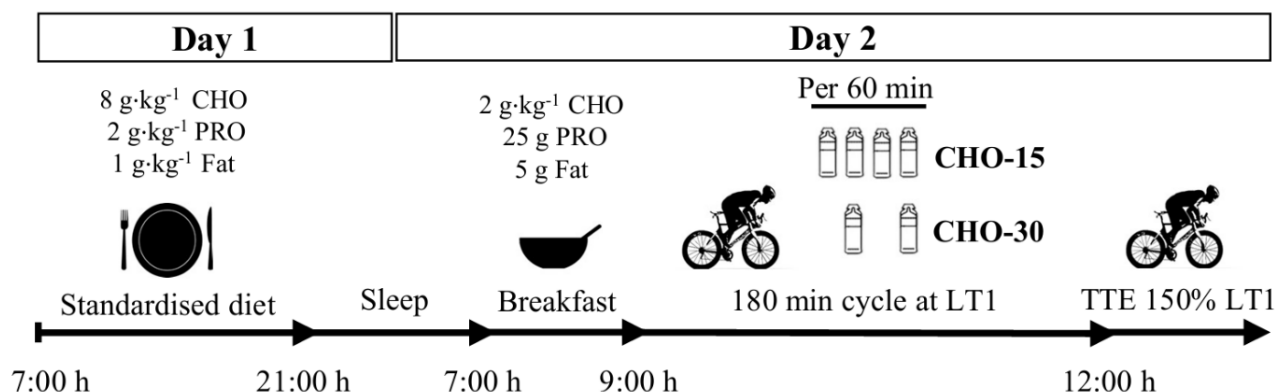
Dependent on experimental condition, participants were provided with 6 or 12 CHO gels in total, consuming either 22.5 g CHO every 15 min or 45 g CHO every 30 min (CHO-15 and CHO-30, respectively). These specific CHO doses were based on anecdotal evidence from the field, typical feeding strategies used at the elite level and CHO doses of commercially available gels (e.g. Science in sport CHO gels; 22 g or 40 g CHO). Gels were specifically formulated for the purpose of the study with a combination of glucose and fructose at a 2:1 ratio (Keto Life, Lancashire, UK), in line with CHO intake recommendations during exercise lasting >2.5 h (Thomas et al., 2016). 150 mL of water was also provided every 15 minutes to maintain and standardise hydration status. Every 30 min following the consumption of a gel and prior to the ingestion of water, participants were asked to provide a 0-10 score for the perception of sweetness and the desire to consume CHO, where 0, 5 and 10 indicated no sweetness, perfectly sweet and unbearably sweet or no desire, moderate desire, and strong desire, respectively.

Following the 180 min, once all measures were collected and the final 150 mL of water was consumed, participants began a time to exhaustion cycling test at an intensity corresponding to 150% LT1 ( $209 \pm 43$  W). Where participants cycled until volitional exhaustion, defined as an inability to maintain a cadence > 60 rpm for 10 s consecutively. Participants were allowed to consume water *ad libitum* during this period. Participants were not directly told their performance time; however, total exercise time, cadence and fixed power output were visible.

Respiratory gases were used to determine mean whole-body CHO and fat oxidation ( $\text{g}\cdot\text{min}^{-1}$ ) for every 15 min of the 180 min cycle at LT1 using stoichiometric equations (Jeukendrup and Wallis, 2005). Total exercise energy expenditure was estimated assuming 1g of CHO and fat corresponded to 17.57 and 39.33 kJ, respectively (Ferannini, 1988).

### **7.3.6 Statistical analysis**

All statistical analyses were conducted using SPSS (v 29, IBM, Chicago, United States). All data were checked for normality using the Shapiro-Wilks test. Two-way repeated measures ANOVA were used to determine interactions and main effects for condition and time for physiological responses to exercise (HR, RPE,  $\dot{V}\text{O}_2$ , blood glucose and lactate) and substrate oxidation (RER, CHO and fat oxidation [ $\text{g}\cdot\text{min}^{-1}$ ], exercise energy expenditure and % contribution of CHO and fat to exercise energy expenditure). Significant main effects were further analysed using Bonferroni post hoc tests to explore where significant differences occurred. ANOVA effect sizes are  $\eta^2_p$  with values of 0.01, 0.06 and 0.14 corresponding to a small, medium and large effect respectively (Cohen, 1988). Total CHO and fat oxidation (g), and exercise capacity were analysed using the Wilcoxon ranked test (nonparametric equivalent to paired t-test) as data were not normally distributed. Cohen's  $d$  effect sizes were calculated by dividing the standardised test statistic (Z score) by the square root of the number of observations, with an effect size of 0.2, 0.5 and 0.8 equating to a small, moderate and large effect, respectively (Cohen, 1988). One participant was excluded from the analysis for stomach fullness as they reported a severe score of 7 throughout exercise for both conditions, which based on his physical appearance, suggested lack of understanding of the severity of symptom scores. All values are presented as means  $\pm$  SD unless otherwise stated, with significance set at  $P < 0.05$ .



**Figure 7.1.** Study 3 schematic overview of experimental protocol for each condition.

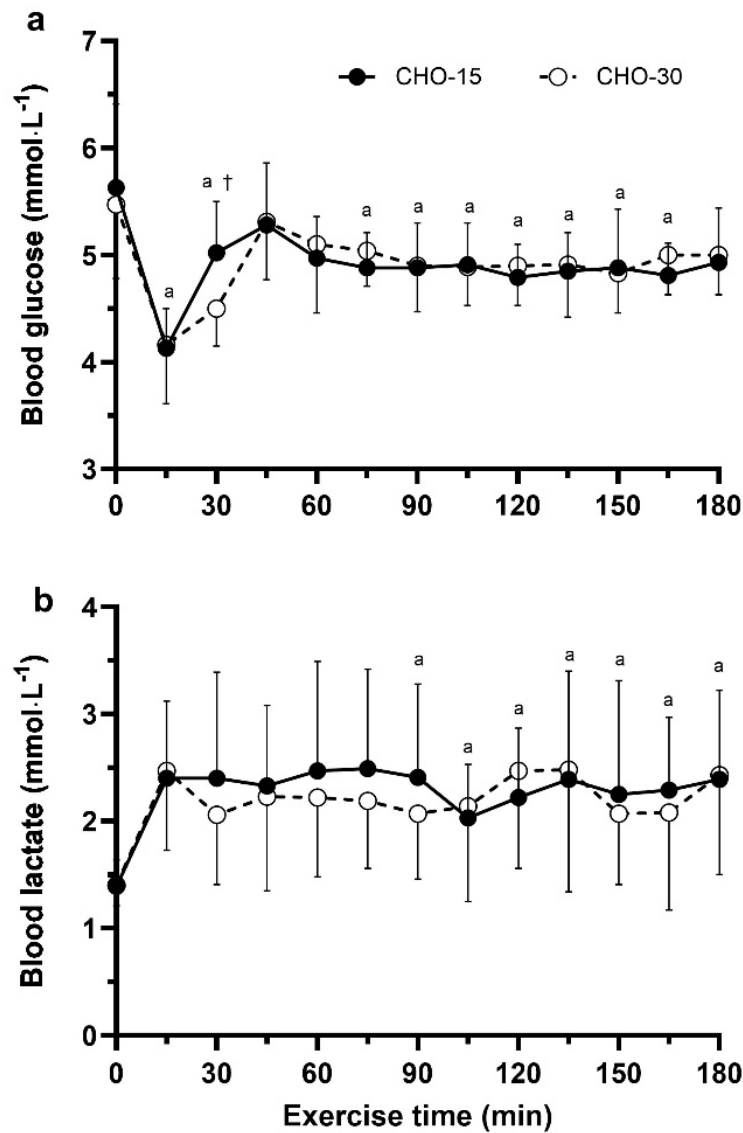
## 7.4 Results

### 7.4.1 Physiological responses

HR, RPE and absolute  $\dot{V}O_2$  responded similarly in both trials, with no main effect for condition ( $P = 0.22$ ;  $P = 0.46$ ;  $P = 0.82$ , respectively) and no significant interaction ( $P = 0.37$ ;  $P = 0.49$ ;  $P = 0.32$ , respectively). HR, RPE and absolute  $\dot{V}O_2$  increased throughout exercise time ( $P < 0.001$ , for all variables), reaching significance vs the first timepoint following 15, 30 and 150 min, respectively (Table 7.2).

Blood glucose and lactate were similar in both CHO-15 and CHO-30 ( $P = 0.96$ ,  $\eta^2_p < 0.001$ ;  $P = 0.34$ ,  $\eta^2_p = 0.05$ , respectively). Blood glucose decreased from resting concentrations following 15 min of exercise in both conditions (main effect for time:  $P < 0.001$ ,  $\eta^2_p = 0.56$ ), before increasing at 30 min. This increase was greater in CHO-15 versus CHO-30 (+0.93 and 0.34 mmol·L<sup>-1</sup>, respectively; interaction effect:  $P = 0.03$ ,  $\eta^2_p = 0.11$ ). At 45 min of exercise

glucose concentrations were again comparable, which was maintained thereafter (Figure 7.2a). Blood lactate saw no significant interaction ( $P = 0.42$ ), however there was a significant main effect for time ( $P < 0.001$ ,  $\eta^2_p = 0.27$ ) as lactate increased similarly from rest (+42.2 and +43.1 % for CHO-15 and CHO-30, respectively) and remained elevated in both conditions throughout exercise (Figure 7.2b;  $P < 0.01$ ).



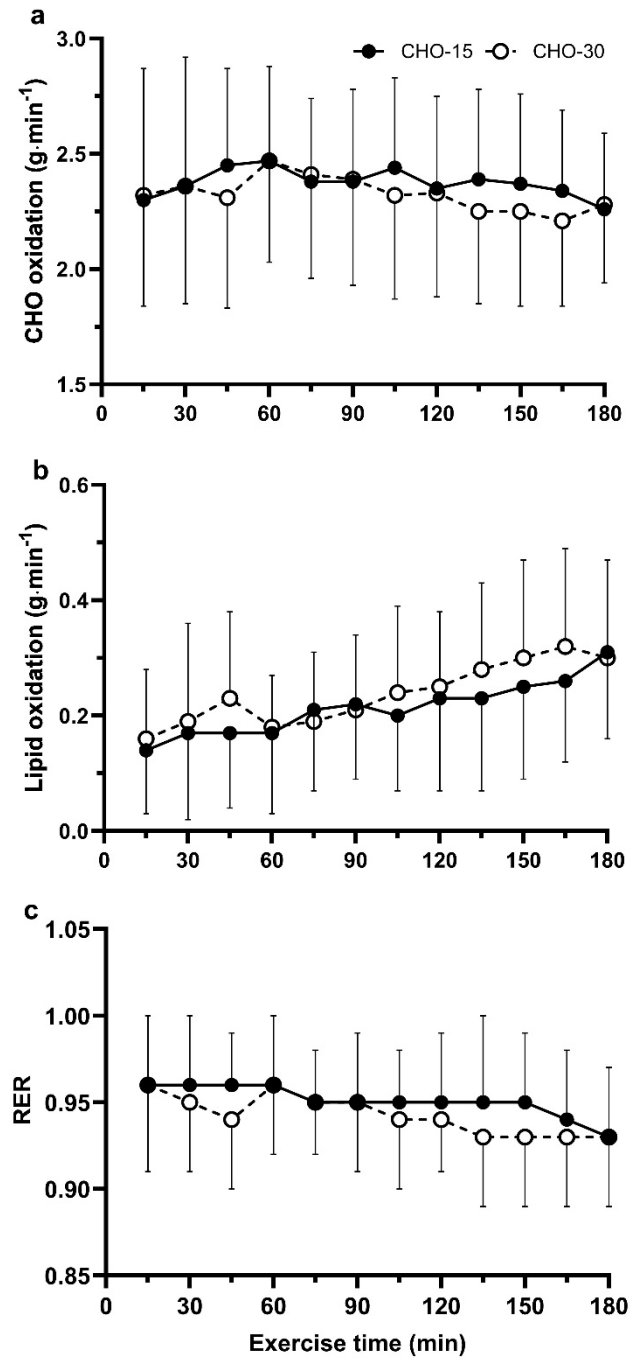
**Figure 7.2.** Plasma glucose (**a**) and lactate (**b**) concentrations during 180 min steady state cycling with high CHO availability through different CHO ingestion patterns of 22.5 g CHO every 15 min (CHO-15) or 45 g CHO every 30 min (CHO-30). <sup>a</sup> Significantly different from first timepoint. <sup>†</sup>Significant interaction.

#### 7.4.2 Substrate utilisation

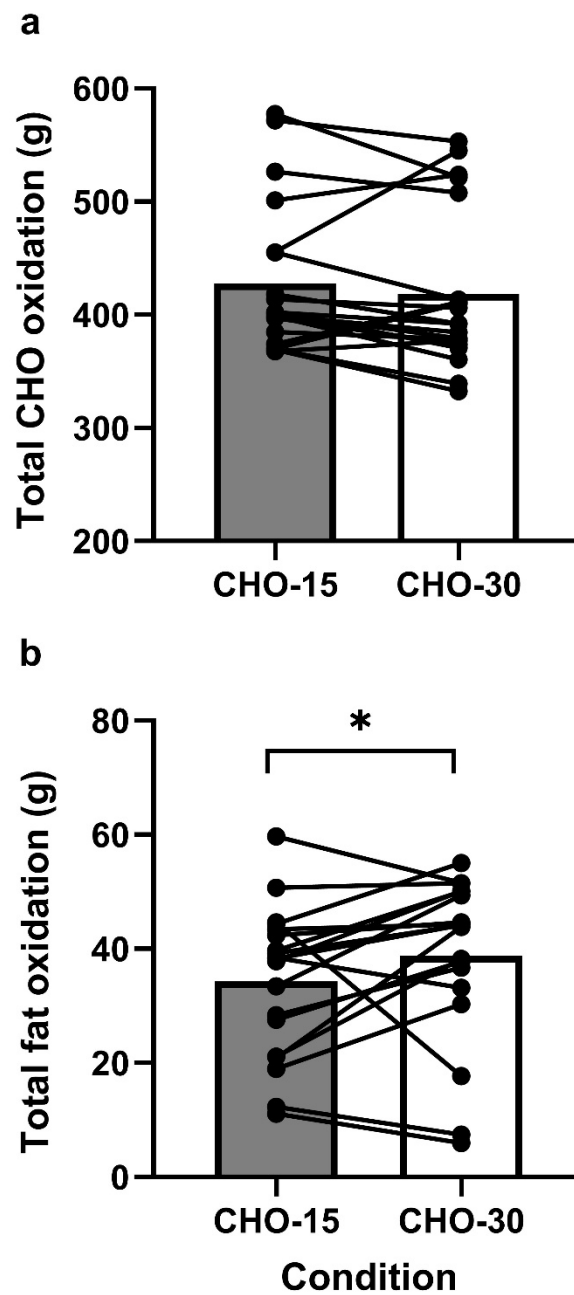
RER showed no main effect for condition ( $P = 0.16$ , Figure 7.4c) or interaction ( $P = 0.18$ ) and decreased steadily throughout the exercise period for both conditions ( $-0.03$  from 15 to 180 min, time effect  $P < 0.001$ ,  $\eta^2_p = 0.30$ ). Mean whole body CHO oxidation ( $2.38 \pm 0.37$  and  $2.31 \pm 0.39$  g·min<sup>-1</sup> for CHO-15 and CHO-30, respectively) was similar in both conditions ( $P = 0.25$ ,  $\eta^2_p = 0.07$ ; Figure 7.4), with no main effect for time or interaction ( $P = 0.09$ ,  $\eta^2_p = 0.10$  and  $P = 0.11$ ,  $\eta^2_p = 0.09$  respectively; Figure 7.3). Fat oxidation was not significantly affected by condition ( $P = 0.10$ ;  $\eta^2_p = 0.14$ ; Figure 7.3) and increased ~2-fold by 180 min of exercise ( $P < 0.001$ ,  $\eta^2_p = 0.44$ ), which reached significance compared to the first timepoint at 120 min and thereafter ( $P < 0.05$ ). There was no significant interaction effect for fat oxidation ( $P = 0.09$ ).

Exercise energy expenditure increased over time ( $P < 0.001$ ) reaching significance compared to the first time point following 60 min of exercise ( $P = 0.01$ ). There was no main effect for condition ( $P = 0.92$ ) or significant interaction ( $P = 0.51$ ) as exercise energy expenditure was comparable for both conditions (Table 7.2). Mean contribution of CHO to total exercise energy expenditure was  $84 \pm 9$  % and  $82 \pm 10$  % for CHO-15 versus CHO-30 respectively, with the remaining 16 and 18% being accounted for by fat utilisation ( $P = 0.07$ ). Throughout exercise time, % CHO decreased in line with the % increase in fat utilisation ( $P < 0.001$ ) reaching statistical significance at 165 min of exercise. There was no time-condition interaction ( $P = 0.08$ ).

There was no significant difference between total CHO oxidation in CHO-15 vs CHO-30 ( $P = 0.13$ ; Figure 7.4). However, there was a moderate effect size ( $ES = 0.48$ ), as median CHO oxidation was 401.8 and 392.3g for CHO-15 and CHO-30 respectively. There was a significant difference between CHO-15 and CHO-30 in total fat oxidation ( $P = 0.04$ ,  $ES = 0.65$ ) as median fat utilisation was 38.3 and 44.2 g for CHO-15 and CHO-30, respectively (Figure 7.4).



**Figure 7.3.** CHO oxidation (a), fat oxidation (b), and RER (c) throughout 180 min steady state cycling with high CHO availability through different CHO ingestion patterns.



**Figure 7.4.** Total CHO (a) and fat oxidation (b) with individual data points, during 180 min steady state cycling with high CHO availability through different CHO ingestion patterns.

\*Indicates significant difference between conditions.

### **7.4.3 Subjective responses**

Mean subjective scores for GI symptoms did not differ between conditions (Table 7.3 and 7.4), except for urge to defecate, however this was not considered meaningful as the highest reported score was  $\leq 3$ . There was no time main effect for symptoms of stomach fullness, abdominal cramps or urge to defecate. Nausea and gas/flatulence showed a trend, as scores increased with exercise time and CHO ingestion. Reflux showed a significant main effect for time, with increased symptoms during the final 1 h of exercise, however, no individual participant scored  $>4$  for nausea, gas/flatulence or reflux across any timepoint. There was no interaction effect across any GI discomfort symptoms. No participant scored  $>4$  for any variable in either condition, except for stomach fullness, which saw a trend, due to one participant who reported a peak score of 7 for CHO-15 at 165 and 180 min.

Perceptions of sweetness saw a significant interaction ( $P = 0.04$ ) as scores increased with exercise time ( $P = 0.06$ ), the increase was greater in CHO-30 versus CHO-15 ( $+0.9$  and  $+0.4$ , respectively). Mean perceptions of sweetness scores were  $5.6 \pm 1.9$  and  $6.1 \pm 1.6$  for CHO-15 and CHO-30 respectively (no main effect for condition:  $P = 0.14$ ). Mean desire to consume CHO were  $2.0 \pm 2.5$  and  $2.1 \pm 2.0$  (no main effect for condition:  $P = 0.69$ , interaction effect:  $P = 0.62$ ) which was consistent over time (time effect:  $P = 0.35$ ).

### **7.4.4 Exercise capacity**

There was no difference ( $P = 0.79$ , ES = 0.08) in exercise capacity between CHO-15 and CHO-30 as median capacity time was 9 min and 8 min 25 sec, respectively.

**Table 7.2.** HR, RPE,  $\dot{V}O_2$  and total energy expenditure (TEE) during 180 min steady state cycle with high CHO availability consuming either 22.5 g every 15 min (CHO-15) or 45 g every 30 min (CHO-30).

| Condition | Exercise<br>time (min) | HR<br>(b·min <sup>-1</sup> ) | RPE                 | $\dot{V}O_2$<br>(L·min <sup>-1</sup> ) | TEE<br>(kJ·min <sup>-1</sup> ) |
|-----------|------------------------|------------------------------|---------------------|----------------------------------------|--------------------------------|
| CHO-15    | 15                     | 132 ± 14                     | 10 ± 2              | 2.15 ± 0.48                            | 46.2 ± 10.0                    |
|           | 30                     | 132 ± 13                     | 10 ± 2 <sup>a</sup> | 2.23 ± 0.47                            | 48.0 ± 10.1                    |
|           | 45                     | 133 ± 15                     | 10 ± 2 <sup>a</sup> | 2.31 ± 0.39                            | 49.8 ± 8.0                     |
|           | 60                     | 135 ± 14                     | 11 ± 1 <sup>a</sup> | 2.32 ± 0.40                            | 50.1 ± 7.9                     |
|           | 75                     | 136 ± 14                     | 11 ± 2 <sup>a</sup> | 2.35 ± 0.40                            | 50.2 ± 8.2                     |
|           | 90                     | 136 ± 14                     | 12 ± 2 <sup>a</sup> | 2.35 ± 0.42                            | 50.4 ± 8.5                     |
|           | 105                    | 136 ± 14                     | 12 ± 2 <sup>a</sup> | 2.38 ± 0.42                            | 51.0 ± 8.5                     |
|           | 120                    | 138 ± 14 <sup>a</sup>        | 12 ± 2 <sup>a</sup> | 2.37 ± 0.42                            | 50.5 ± 8.6                     |
|           | 135                    | 140 ± 17 <sup>a</sup>        | 12 ± 2 <sup>a</sup> | 2.39 ± 0.44                            | 51.3 ± 8.5                     |
|           | 150                    | 140 ± 16 <sup>a</sup>        | 13 ± 2 <sup>a</sup> | 2.40 ± 0.44 <sup>a</sup>               | 51.4 ± 9.0                     |
|           | 165                    | 142 ± 13 <sup>a</sup>        | 13 ± 2 <sup>a</sup> | 2.41 ± 0.42 <sup>a</sup>               | 51.4 ± 8.5                     |
|           | 180                    | 144 ± 16 <sup>a</sup>        | 13 ± 2 <sup>a</sup> | 2.44 ± 0.40 <sup>a</sup>               | 51.8 ± 7.9                     |
| CHO-30    | 15                     | 132 ± 12                     | 10 ± 2              | 2.20 ± 0.39                            | 47.1 ± 8.4                     |
|           | 30                     | 131 ± 14                     | 10 ± 2 <sup>a</sup> | 2.28 ± 0.42                            | 49.1 ± 8.7                     |
|           | 45                     | 137 ± 14                     | 11 ± 2 <sup>a</sup> | 2.30 ± 0.42                            | 49.4 ± 8.4                     |
|           | 60                     | 136 ± 13                     | 11 ± 1 <sup>a</sup> | 2.32 ± 0.41                            | 50.3 ± 8.0                     |
|           | 75                     | 136 ± 14                     | 11 ± 2 <sup>a</sup> | 2.34 ± 0.43                            | 50.1 ± 8.7                     |
|           | 90                     | 137 ± 15                     | 11 ± 2 <sup>a</sup> | 2.34 ± 0.41                            | 50.3 ± 8.1                     |
|           | 105                    | 139 ± 14                     | 11 ± 1 <sup>a</sup> | 2.35 ± 0.44                            | 50.4 ± 8.4                     |
|           | 120                    | 140 ± 16 <sup>a</sup>        | 12 ± 2 <sup>a</sup> | 2.38 ± 0.42                            | 50.9 ± 8.4                     |
|           | 135                    | 143 ± 15 <sup>a</sup>        | 12 ± 2 <sup>a</sup> | 2.38 ± 0.43                            | 50.6 ± 8.3                     |
|           | 150                    | 144 ± 14 <sup>a</sup>        | 12 ± 2 <sup>a</sup> | 2.41 ± 0.43 <sup>a</sup>               | 51.3 ± 8.6                     |
|           | 165                    | 144 ± 15 <sup>a</sup>        | 13 ± 2 <sup>a</sup> | 2.41 ± 0.40 <sup>a</sup>               | 51.3 ± 7.8                     |
|           | 180                    | 147 ± 16 <sup>a</sup>        | 13 ± 2 <sup>a</sup> | 2.44 ± 0.41 <sup>a</sup>               | 52.0 ± 7.8                     |

**Table 7.3.** GI symptom scores during 180 min steady state exercise with high CHO availability through consumption of either 22.5 g every 15 min (CHO-15) or 45 g every 30 min (CHO-30).

| Condition | Exercise<br>time<br>(min) | Nausea<br>(0-10) | Reflux<br>(0-10) | Stomach<br>fullness<br>(0-10) | Cramps<br>(0-10) | Flatulence/<br>gas (0-10) | Urge to<br>defecate<br>(0-10) |
|-----------|---------------------------|------------------|------------------|-------------------------------|------------------|---------------------------|-------------------------------|
| CHO-15    | 15                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 30                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 45                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 60                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 1                         |
|           | 75                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 1                         |
|           | 90                        | 0 ± 1            | 0 ± 1            | 1 ± 2                         | 0 ± 1            | 0 ± 0                     | 0 ± 1                         |
|           | 105                       | 0 ± 0            | 0 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 1                         |
|           | 120                       | 0 ± 1            | 0 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 1                         |
|           | 135                       | 0 ± 0            | 1 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 150                       | 0 ± 1            | 1 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 165                       | 0 ± 1            | 1 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 180                       | 0 ± 1            | 0 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 1                         |
| CHO-30    | 15                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 30                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 1            | 0 ± 0                     | 0 ± 0                         |
|           | 45                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 1                         |
|           | 60                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 75                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 90                        | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 105                       | 0 ± 0            | 0 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 0                     | 0 ± 0                         |
|           | 120                       | 0 ± 0            | 0 ± 0            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 135                       | 0 ± 1            | 0 ± 1            | 1 ± 2                         | 0 ± 1            | 0 ± 1                     | 0 ± 0                         |
|           | 150                       | 0 ± 1            | 0 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 165                       | 1 ± 1            | 1 ± 1            | 1 ± 2                         | 0 ± 0            | 0 ± 1                     | 0 ± 0                         |
|           | 180                       | 0 ± 1            | 1 ± 1            | 1 ± 2                         | 0 ± 0            | 1 ± 1                     | 0 ± 0                         |

**Table 7.4.** Two-way ANOVA test statistics for GI symptom scores during 180 min steady state exercise with high CHO availability through consumption of either 22.5 g every 15 min (CHO-15) or 45 g every 30 min (CHO-30).

| GI Symptom       | Condition effect             | Time effect                  | Interaction effect |
|------------------|------------------------------|------------------------------|--------------------|
| Nausea           | $P = 0.56$                   | $P = 0.07$                   | $P = 0.23$         |
| Reflux           | $P = 0.24$                   | <b><math>P = 0.04</math></b> | $P = 0.19$         |
| Stomach fullness | $P = 0.36$                   | $P = 0.76$                   | $P = 0.07$         |
| Cramps           | $P = 0.67$                   | $P = 0.44$                   | $P = 0.37$         |
| Flatulence/gas   | $P = 0.95$                   | $P = 0.06$                   | $P = 0.57$         |
| Urge to defecate | <b><math>P = 0.05</math></b> | $P = 0.30$                   | $P = 0.18$         |

## 7.5 Discussion

The primary aim of the study was to investigate the effects of different CHO ingestion patterns (22.5 g every 15 min versus 45 g every 30 min) on physiological responses to exercise, whole-body substrate oxidation, and GI symptoms during endurance cycling with high CHO availability. As hypothesised, there was no meaningful difference in physiological responses to exercise or whole-body substrate oxidation between conditions. Furthermore, both strategies were well tolerated, with minimal GI symptoms reported. Overall, current study data suggests the larger less frequent CHO feeding frequency used in the current study is a feasible, practical nutritional strategy for endurance cycling.

Physiological responses to exercise were not significantly different between conditions with no significant interactions for HR, RPE, absolute  $\dot{V}O_2$  or blood lactate, respectively (Table 7.2). The less frequent, higher CHO dose (45g CHO every 30 min) showed no exacerbated exercise induced stress, perceived exertion or oxygen demand compared to the frequent lower dose CHO ingestion pattern (22.5g every 15-20 min) more commonly utilised during competition and laboratory-based studies (Coyle et al., 1986; Jentjens et al., 2004; Fell et al., 2021; Hearn et al., 2022), thus providing an alternative feeding strategy. Despite feeding being less disruptive compared to sports such as running, or skiing, where athletes are required to slow down to ingest CHO, endurance cycling under race conditions with regular feedings every 15-20 min (as required to achieve the recommended CHO intake of 90-120 g·h<sup>-1</sup>), can be tedious or impractical. A larger less frequent dose, or perhaps a mixed, flexible approach throughout competition could better suit athletes.

As hypothesised, whole body CHO oxidation rates were similar in both conditions (Figure 7.3). These values are comparable to Mears et al. (2020), where ingestion of a sports drink (60 g·h<sup>-1</sup>) at a feeding frequency of every 5 or 20 min resulted in whole body CHO oxidation rates of  $2.23 \pm 0.45$  and  $2.15 \pm 0.47$  g·min<sup>-1</sup>, respectively. Mears et al. (2020) reported 23% higher exogenous CHO oxidation in the larger less frequent ingestion pattern (200 mL every 20 min), despite previous literature suggesting a similar pattern (increased oxidation during initial 75-90 min, followed by plateau) and peak rate of exogenous CHO oxidation during exercise when ingesting a 100 g glucose bolus (Krzentowski et al., 1984; Guezennec et al., 1989) compared to more frequent feedings every 20 min (Massicotte et al., 1989, 1990, 1994). Unfortunately, it cannot be confirmed which response would occur in the current study, as exogenous CHO was not specifically measured. However, Mears et al. (2020) attributed differences in exogenous CHO oxidation to gastric emptying, where the larger bolus provided greater total

volume of fluid per feeding, increasing gastric pressure, and emptying, which allowed earlier absorption and subsequent utilisation of CHO (Rehrer et al., 1992; Noakes et al., 1991; Costill and Saltin, 1974). Current study conditions were not perfectly matched for total volume of fluid ingested (840 and 720 mL·h<sup>-1</sup> for CHO-15 and CHO-30 respectively) as both CHO gels provided 60 mL of fluid per feeding as well as 150 mL of water every 15 min, suggesting that gastric emptying, and perhaps exogenous CHO oxidation, could have differed between conditions. However, differences in absolute CHO content of ingested solutions have previously been shown to decrease gastric emptying (Rehrer et al., 1990), but increase exogenous CHO oxidation (Rehrer et al., 1992), as a reduction in gastric emptying does not necessarily mean a reduction in CHO absorption (Noakes et al., 1991). Therefore, it is possible, that both these regulatory factors counteracted one another to maintain a similar exogenous CHO oxidation rate between current study conditions. The use of stable isotope tracers in future cycling studies is required to confirm the exact effects of different CHO ingestion patterns, as utilised in the current study, on endogenous and exogenous oxidation rates.

In agreement with Mears et al (2020), fat oxidation (g·min<sup>-1</sup>) was not significantly different between CHO ingestion patterns utilised in the current study. In contrast, Stocks et al. (2016) showed high CHO intake over low frequency of ingestion during cross country skiing to decrease fat oxidation, which elicited a greater reliance on CHO from endogenous glycogen stores. Such a response has potential to negatively impact exercise performance due to earlier onset of fatigue (Coyle et al., 1992; Stellingwerff and Cox, 2014). There may be a threshold whereby a large bolus, too infrequently, will lead to metabolic perturbations that are unfavourable for endurance performance due to highly elevated insulin concentrations during exercise, which blunt lipid mobilisation, decreasing fat oxidation (Jeukendrup et al., 1999). Nonetheless, in the current study, substrate oxidation data (Figure 7.3) suggest the lower

frequency ingestion strategy utilised (45g CHO every 30 min) was below this threshold, supporting use as a practical feeding strategy. In fact, total fat oxidation was greater in CHO-30 compared to CHO-15 ( $P = 0.04$ ,  $ES = 0.39$ ), with a large effect size expressed over exercise time ( $\eta^2_p = 0.14$ ). Whether this corresponded to a sparing of endogenous glycogen stores through increased fat utilisation or would be a meaningful difference in practice remains unclear.

Blood glucose was comparable between conditions, with euglycemia maintained throughout the 180 min of exercise in both conditions (Figure 7.2a). However, there was an initial decrease observed following 15 min of cycling, likely due to the high CHO breakfast ~60 min pre-exercise. This is a well-established phenomenon, where the combined effects of post-prandial insulin-mediated glucose uptake into the muscle and blunted hepatic glucose production, as well as further augmented glucose uptake via exercise induced activation of calcium dependent GLUT 4 transporters, results in a mismatch between glucose uptake into the muscle and rate of appearance in the blood (Ahlborg and Felig., 1977; Costill et al., 1977; Coyle et al., 1997; Jeukendrup and Killer, 2010). Following 30 min of exercise, glucose increased for both conditions, with the greater increase in CHO-15 caused by exogenous CHO gel ingestion. Any early increased reliance on endogenous stores (pre-exogenous CHO provision) was transient and only occurred during the initial 30 min of exercise, minimally impacting the rate of glycogen depletion or the onset of fatigue (Gleeson et al., 1986; Sherman et al., 1991), as supported by current study RPE and exercise capacity data where responses between conditions were matched. It should be noted, earlier provision of exogenous CHO during exercise (as in CHO-15), would be more beneficial in restoring blood glucose concentrations and avoid potential negative symptoms of rebound hypoglycaemia (blood glucose  $< 3.5 \text{ mmol}\cdot\text{L}^{-1}$ ). However, appropriate timing of the pre-exercise meal (at least 1-4 h pre-exercise), or

consumption of CHO immediately pre-exercise would minimise this risk (Jeukendrup and Killer, 2010).

Mean GI discomfort symptoms did not differ between conditions for any symptom other than the urge to defecate, where no participants recorded a score >3, confirming that any discomfort was minimal (Wilson, 2017). This is in line with Mears et al. (2020), where sports drink ingestion every 5 min, or 20 min ( $60 \text{ g}\cdot\text{h}^{-1}$  in both conditions), showed minimal discomfort symptoms. In contrast, in Stocks et al. (2016) well-trained cross-country skiers reported higher GI discomfort symptoms when consuming higher quantities of CHO at less frequent intervals, likely a result of the specific ingestion pattern utilised, where participants were provided two large boluses ( $686 \pm 83 \text{ mL}$ ) of a 24% CHO solution 5 min prior to, and during a high intensity performance test. Both the higher CHO dose and exercise intensity (potentially through restricted GI blood flow [Brouns and Beckers, 1993]) contributed to differences compared to the current study, where participants consumed much smaller more tolerable quantities of CHO (22.5 and 45g as 60 mL CHO gels every 15 and 30 min respectively) during a light-moderate intensity steady state cycling bout ( $\sim 60\% \dot{V}\text{O}_{2\text{max}}$ ). Again, this highlights a threshold where CHO ingestion patterns (a dose too large, too infrequently) may negatively impact gut comfort, and result in a suboptimal physiological condition for endurance cycling performance. However other confounding methodological differences (participant training status, type of CHO consumed, CHO form [gel vs drink] and exercise mode) makes identifying exactly where this threshold occurs impossible currently in endurance cycling. That being said, both strategies utilised in the current study were effective with minimal impact of GI discomfort.

Exercise capacity at 150% of LT1 following 180 min steady state cycling did not differ between CHO-15 and CHO-30, which was expected, as participants in both conditions consumed the

same absolute quantity of CHO during exercise ( $90 \text{ g}\cdot\text{h}^{-1}$ ), and an almost identical study protocol from our lab (prescribed nutritional intake and exercise [type, intensity and duration]) showed similar results in an endurance trained population whilst feeding  $120 \text{ g}\cdot\text{h}^{-1}$  (Hearris et al., 2022). The variable nature of such tests due to psychological factors (motivation/boredom) may have limited the ability to determine small differences between conditions (Jeukendrup et al., 1996). However, all participants seemingly provided a maximal effort, which, combined with the large study sample size, should have minimised this effect.

To the authors knowledge this is the first-time participant perceptions of sweetness and desire to consume CHO during endurance exercise was measured. Perceptions of sweetness increased in both conditions; however, the increase was greater during CHO-30 as participants found the larger CHO gels to be sweeter, presumably due to the greater absolute quantity of glucose (30g) and fructose (15g) per feeding. Of note, mean values between conditions were similar, both being close to the optimal value of 5, suggesting the strategies were well tolerated. However, participant desire to consume CHO throughout exercise was consistently low across both conditions. Despite near optimal sweetness and minimal GI discomfort symptoms, participants had very little desire to consume CHO. This may be attributed to a lack of understanding of the importance of CHO for endurance exercise, or simply a lack of experience consuming nutritional products during exercise, as a high rate of CHO ingestion during exercise ( $90 \text{ g}\cdot\text{h}^{-1}$ ) was a new experience for all but two study participants.

One limitation of the study was participant training status, despite being healthy and recreationally active, participants were not endurance trained and were unfamiliar with consuming high CHO quantities during exercise, which impacted study results. Therefore, care

should be taken not to generalise findings to endurance trained individuals, without considering the metabolic differences between populations. Another limitation was poor blinding of participants to exercise capacity time. Despite not being directly told by researchers, an estimate was visible through total exercise time on display, which could have impacted participant motivation, hence the little emphasis placed on this data throughout.

### ***7.5.1 Conclusions***

The use of a larger CHO dose at less frequent feeding intervals in endurance cycling, as utilised in the current study (45 g every 30 min), is a feasible and perhaps more practical nutritional strategy, with no meaningful negative impact on physiological responses to exercise, whole-body substrate oxidation or GI discomfort compared to the more commonly used feeding frequency (22.5 g every 15 min). However, different CHO ingestion patterns are an area with little research focus, particularly in cycling models. Further work is needed, in well trained cyclists, with the use of stable isotope tracers to confirm the impact of different strategies on the contribution of exogenous and endogenous CHO to overall exercise energy expenditure, and ultimately, performance.

## **Chapter Eight:**

### **Synthesis of findings**

*This chapter provides an overview of thesis findings and conclusions, whilst considering aims and objectives, as well as practical applications and directions for future research.*

## 8.1 Achievement of aims and objectives

The overall aim of this thesis was to optimise CHO intake recommendations in the days before endurance competition to maximise skeletal muscle glycogen stores and endurance performance, with practical considerations for real-world conditions and optimisation of CHO delivery during exercise. This aim was achieved through the completion of separate, but inter-related research projects, each focused on a specific objective.

- 1) *A systematic review of the literature and meta-analysis to determine the relationship between CHO intake and pre-exercise skeletal muscle glycogen concentration, and to characterise other important covariates determining glycogen concentrations (Chapter 4).*

Using a systematic search of existing literature and meta-analytic models, considerable significant heterogeneity ( $I^2 > 90\%$ ) was identified between glycogen values reported across CHO loading studies due to confounding of key effectors of glycogen storage. This made drawing strong conclusions regarding the CHO intake required to maximally promote glycogen concentrations difficult, limiting the ability to meaningfully quantify the magnitude of the relationship between dietary CHO intake and whole muscle glycogen. However, this chapter indicated a strong linear dose-response relationship between CHO intake and muscle glycogen ( $r^2 = 0.49$ ,  $P < 0.001$ ), and identified level of CHO intake, loading duration, training status and exercise as key effectors of glycogen storage, which can be manipulated to maximise glycogen concentrations, with many different combinations capable of achieving this goal. Findings suggested  $> 8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 36-48 h pre-competition, in most cases, would result in increased muscle glycogen stores.

- 2) *Determine the dose-response relationship between dietary CHO intake and skeletal muscle glycogen concentration mimicking real world pre-competition conditions (Chapter 5).*

In agreement with Chapter 4, a strong linear dose-response relationship ( $r = 0.71$  and  $P < 0.001$ ) between dietary CHO intake and muscle glycogen was determined, suggesting no saturation point or ‘ceiling’ effect at 7-10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO under real world training conditions. In fact, ingestion of  $\geq 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  during CHO loading appears necessary for a maximisation of glycogen stores (Figure 5.2), possibly to account for increased CHO utilisation during exercise throughout the loading period. There were no negative implications associated with high and very high CHO intakes, as gut comfort and function ( $P > 0.05$ ), body mass and TBW ( $P > 0.50$ ) were similar across conditions, showing high CHO intakes are well tolerated in an endurance trained cohort.

- 3) *Investigate the potential placebo effect of CHO loading on endurance cycling performance (Chapter 6).*

CHO loading with 8 and 10 vs 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  of CHO improved cycling TT mean power by 5.8 and 3.5%, respectively, however this was not statistically significant ( $P = 0.10$ ,  $\eta^2_p = 0.26$ ). Further, study blinding data suggested the placebo effect influenced these results, as three participants who performed best consuming 8 and 10  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  correctly identified the 6  $\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  condition. Considering the potency of this effect in previous sports performance studies (Beedie and Foad, 2009; Beedie et al., 2007; McClung and Collins 2007; Foad et al., 2008; Beedie et al., 2006; Clark et al., 2000), combined with improved performance primarily

apparent in those who correctly identified the placebo condition (5-20 % improvement in TT power and completion time), suggests a contribution by the placebo effect. Therefore, in some situations, it seems CHO loading with 8 and 10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO provides no benefit for endurance cycling performance lasting ~2.5 h, under real-world conditions of high exogenous CHO availability.

*4) Determine the effects of different CHO feeding frequencies during endurance cycling exercise on physiological responses, substrate utilisation, gut comfort and exercise capacity (Chapter 7).*

Manipulation of ingestion patterns of the same quantity of CHO has the ability to result in unfavourable metabolic perturbations during endurance exercise (suppressed fat oxidation and greater reliance on endogenous CHO stores; Stocks et al., 2016). However, the two CHO ingestion patterns utilised in the current study of 90 g·h<sup>-1</sup> fed as 22.5 g every 15 or 45 g every 30 min (chosen to replicate the commercially available choices for endurance athletes in practice), did not result in any meaningful changes in metabolic and physiological responses to exercise, gut comfort or exercise capacity. Which suggests an ability to freely choose a strategy which best suits practical preferences during exercise.

## **8.2 General discussion and practical applications**

The novel data presented within this thesis provides new insights into CHO loading and CHO ingestion during exercise, a heavily published research area which has informed contemporary nutrition guidelines for endurance sports performance. Thesis findings challenge multiple common beliefs in the field, such as a saturation point of dietary CHO intake (7-10 g·kg<sup>-1</sup>·day<sup>-1</sup>

<sup>1</sup>) for maximal glycogen storage, the importance of CHO loading for optimal endurance performance in events lasting >90 min, or the 1:3 relationship between glycogen and body water. Thesis findings highlight a need for further research to clarify suggestions and conclusions, using highly controlled conditions representative of real-world training and nutrition practices of athletes, with moderate-large sample sizes of endurance trained participants.

### ***8.2.1 Linear dose-response relationship between pre-exercise CHO intake and muscle glycogen***

The relationship between dietary CHO intake and muscle glycogen has previously been suggested to be linear, with a plateau and saturation point at 7-10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO (Costill et al., 1981; Burke et al., 1995; Burke et al., 2017). In contrast, Chapter 4 regression data (Figure 4.2; Figure 4.3) indicated a linear dose-response relationship, whereby increased CHO intake accounted for ~50% of the variability in muscle glycogen. This was confirmed by Study 1 (Chapter 5), which for the first time, reported a strong linear dose-response between CHO intake and muscle glycogen concentration under real-world training conditions using a highly controlled repeated measures design (Chapter 5; Figure 5.4), controlling identified effectors of glycogen synthesis and concentration (Chapter 4). In fact, this relationship suggested that ≥10 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO was necessary to maximally increase pre-exercise glycogen stores, as glycogen concentrations were significantly increased in the 10 vs 6 and 8 g·kg<sup>-1</sup>·day<sup>-1</sup> conditions (Figure 5.3), possibly to account for increased CHO utilisation during exercise conducting during the loading period (Chapter 6). Therefore, to maximise glycogen stores with CHO loading during a pre-competition exercise taper, Chapter 4 and 5 suggest athletes should consume ≥10 g·kg<sup>-1</sup>·day<sup>-1</sup> of primarily high glycaemic index CHO for 36-60 h, with the loading

period strategically implemented following the last challenging exercise session pre-competition, to take advantage of heightened post-exercise glycogen synthesis rates.

### ***8.2.2 Negative implications of CHO loading?***

The purported negative implications of CHO loading (GI discomfort and increased body mass) appear minimal in endurance trained cyclists under the experimental conditions outlined in Chapter 5 and 6. GI discomfort symptoms have previously been associated with a very high CHO intake over multiple days ( $9\text{--}12\text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ; Lamb et al., 1991; Tarnopolsky et al., 2001). However, findings highlight that nutritional strategies can be implemented to make CHO loading with high-very high CHO intakes more tolerable. Primarily the control of dietary fibre, fat and protein intake, and in agreement with Lamb et al. (1991), the use of sports supplements and fluids (which also aid in minimising dietary fibre intake) renders very high CHO intakes more tolerable compared to ingestion of solid whole foods (Brouns and Beckers, 1993; de Oliveira et al., 2014). Another likely reason for high tolerability was the endurance trained nature of participants, where most study participants were familiar with and commonly used CHO loading strategies pre-competition (Chapter 5 and 6). Highlighting that familiarity with high CHO intakes makes achieving contemporary recommendations more manageable, as indicated in Chapter 5, where one female participant who was unfamiliar with CHO loading, accounted for 55% of moderate-severe GI discomfort symptom scores.

Interestingly, body mass, TBW and ICW were not affected by increased CHO intake (Chapter 5 and 6), which contradicts the common idea that each gram of glycogen is bound to 3-4 g of water (Olsson and Saltin, 1970; Fernandez-Elias et al., 2015). The reason behind this discrepancy remains elusive and could be related to the methods used to measure body water

(in current or previous research), as recent studies using BIA or BIS technology also showed no changes in TBW following manipulation of glycogen stores (Schytz et al., 2023; Shiose et al., 2016; Shiose et al., 2018). Perhaps BIA was not sensitive enough to detect minor changes (1-2 % or 0.5-0.7 L) in body mass and TBW expected with 2-4 g·kg<sup>-1</sup>·day<sup>-1</sup> increments in CHO intake, or measures could have been affected by other factors such as changes in fluid intake or electrolyte balance during the loading period (O'Brien et al., 1993). Another possibility is that this relationship does not exist under current study conditions (Chapter 5 and 6), which is possible considering studies that support this relationship (Olsson and Saltin, 1970; Bone et al., 2017; Fernandez-Elias et al., 2015) compared extremes of CHO availability, where participants underwent upper and lower body glycogen depletions or completed multiple day depletion periods, as well as ingested vastly different CHO intakes ( $\geq 6$  g·kg<sup>-1</sup>·day<sup>-1</sup> difference) between conditions, resulting in larger differences in muscle glycogen and potentially body mass. Unfortunately, these suggestions cannot be confirmed from the current body of work and further research is required.

Nonetheless it seems CHO loading strategies used during Study 1 and 2 (Chapter 5 and 6) can enhance muscle glycogen stores compared to baseline values, without incurring the commonly accepted negative implications. However, whether a very high CHO intake ( $\geq 10$  g·kg<sup>-1</sup>·day<sup>-1</sup>) and maximisation of muscle glycogen stores is practically needed to optimise endurance cycling performance remains debatable (Chapter 6).

### ***8.2.3 Does the placebo effect exist in CHO loading research?***

The placebo effect in CHO loading research had previously been suggested, but not fully investigated. Of note, all previous reports of improved endurance performance and/or capacity,

did not incorporate blinding of participants to CHO intake days pre-test (Karlsson and Saltin, 1971; Williams et al., 1992; Rauch et al., 1995; Widrick et al., 1993; Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967; Lamb et al., 1991; Bosch et al., 1993; Galbo et al., 1979; Jensen et al., 2020). Whilst the only studies which have assessed CHO loading using double-blind repeated measures designs, in endurance trained individuals, reported no significant improvements in cycling TT completion time or mean power output, despite significantly increased muscle glycogen stores (Burke et al., 2000; Tomcik et al., 2018; Chapter 5 and 6). Controlling the placebo effect is a fundamental requirement in research investigating sports performance (Beedie and Foad, 2009). Particularly considering the well-established prevalence of this effect across numerous sporting contexts (Ariel and Saville, 1972; Beedie et al., 2007; McClung and Collins 2007; Beedie et al., 2018), including endurance cycling (Foad et al., 2008; Beedie et al., 2006; Clark et al., 2000). In agreement, in Chapter 6, three individuals who successfully identified the placebo conditions, providing justification for their beliefs, also saw the largest improvements in TT completion time and mean power for 8 and 10 vs 6 g·kg<sup>-1</sup>·day<sup>-1</sup> of CHO, respectively (~ 12%; Figure 6.4), suggesting the placebo effect could have biased these performances.

It cannot be confirmed that the placebo effect was the primary mechanism for increased performance in previous studies, however, Chapter 6 results suggest this effect has biased previous CHO loading results in favour of high CHO conditions. Particularly considering increased dietary CHO intakes have been linked to improved endurance exercise capacity for over a century (Krogh and Lindhard, 1920; Christensen and Hansen, 1939), as well as being advocated by contemporary nutritional guidelines for endurance sports performance (Thomas et al., 2016; Burke et al., 2017).

#### ***8.2.4 Pre-exercise CHO intake, muscle glycogen and endurance cycling performance.***

To ensure CHO loading results in maximisation of muscle glycogen stores pre-competition under real world training conditions, athletes should consume a very high dietary CHO intake  $\geq 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  (Chapters 4 and 5). However, whether this translates to optimal endurance cycling performance remains to be determined. Contemporary recommendations advocate CHO loading with a very high CHO intake ( $10\text{-}12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) to optimise performance in endurance exercise  $>90 \text{ min}$  (Hawley et al., 1997a; Thomas et al., 2016). However, Chapter 6 indicates that, in some situations, endurance exercise performance lasting  $\sim 2.5 \text{ h}$  may not benefit from CHO loading, and the resultant increase in muscle glycogen stores. This may only be the case when exogenous CHO intake is high during- and immediately pre-exercise (Widrick et al., 1993; Chapter 6), as in real-world racing conditions. In agreement, many previous reports of improved exercise capacity following CHO loading have compared high vs low CHO groups of overnight fasted participants who did not ingest CHO during exercise (Bergström and Hultman, 1966; Bergström et al., 1967; Ahlborg et al., 1967; Jensen et al., 2020). In such situations of restricted exogenous CHO availability there is greater reliance on endogenous glycogen stores, which would be suboptimal in the liver following an overnight fast (Nilsson and Hultman, 1973; Iwayama et al., 2021). Therefore, it is possible that previous CHO loading study conditions may have inflated the ergogenic effect of muscle glycogen supercompensation in comparison to nutritional status in real-world racing conditions (Bergström et al., 1967; Widrick et al., 1993), which questions whether CHO loading (i.e. ingesting CHO above normal) truly benefits endurance cycling performance in practice or has the ergogenic effect been exaggerated in previous research by the placebo effect, low exogenous CHO availability and limited liver glycogen stores.

### 8.3 Future research recommendations

The findings presented within this thesis have raised more questions regarding pre-exercise dietary CHO intake, skeletal muscle glycogen and endurance cycling performance, while challenging common beliefs existing in this field. Recommendations for future research to advance knowledge within this area are presented below:

- *CHO loading with higher CHO intakes.* No studies have determined whole muscle glycogen concentrations using muscle biopsies from CHO intakes  $>12 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ . Can this lead to even further increases in muscle glycogen storage or is this where the saturation point/ceiling effect occurs? If glycogen stores do increase further, what does this mean for endurance performance? Is this quantity of CHO tolerable in terms of GI comfort through low fibre intake, high glycaemic index CHO, high CHO sports supplements and fluids? Would this lead to detectable differences in body mass and TBW?
- *CHO loading strategies in an elite population.* Training status was confirmed as a key effector of glycogen storage and time efficiency of supercompensation/storage enhancement. What does this mean for elite populations? Can they synthesise more glycogen from lower CHO intakes due to increased GLUT 4 and glycogen synthase content and activity? Would this mean elite level athletes require smaller CHO intakes or less time (e.g.  $8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$  for 24 h) to maximise glycogen concentrations? Hopefully, increased use of non-invasive measures of muscle glycogen (MRI technology) will increase participation of elite athletes in research, to answer these questions and better inform practice and contemporary guidelines.

- *What about the liver?* Despite the focus of this thesis being glycogen storage within muscle, the importance of liver glycogen stores (due to depletion being associated with fatigue, inability to maintain euglycemia, and impaired endurance performance/capacity) should not be overlooked. It remains to be determined whether it is possible to raise liver glycogen concentrations to supra-physiological levels through manipulation of dietary CHO intake (CHO type and/or quantity).
- *Relationship between glycogen and TBW.* Clarification is needed regarding the relationship between increased glycogen storage, body mass and TBW. As the previously reported ~2% increase in body mass associated with CHO loading and glycogen supercompensation has been discredited under real world conditions of moderate, high and very high pre-exercise CHO intake (Chapter 5 and 6). The reason behind this discrepancy remains unclear.
- *CHO loading for endurance cycling performance.* Assessment of endurance cycling performance with better indistinguishable study blinding is required to confirm suggestions the placebo effect impacted study results (Chapter 6). If this is the case, further double-blind repeated measures studies in endurance trained individuals are required to re-evaluate contemporary CHO guidelines and determine where the threshold of exercise intensity and duration occurs, to require CHO loading with a high and very high CHO intake for optimal endurance cycling performance (if there is one).
- *CHO feeding frequencies.* Chapter 7 identified an under-researched area and lay the groundwork for future studies, which should be conducted in endurance trained or elite level participants to ensure generalisable findings. Stable isotope tracers should be used

to determine whether CHO ingestion patterns used in practice affect endogenous and exogenous substrate oxidation rates.

## **8.4 Summary**

The novel data presented within this thesis highlights that despite CHO loading being a heavily researched area within performance nutrition, there are still many unanswered questions regarding pre-exercise dietary CHO intake, muscle glycogen and endurance performance. The strong linear dose-response relationship between pre-exercise dietary CHO intake and skeletal muscle glycogen suggests very high CHO intakes ( $\geq 10 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) are recommended to maximise muscle glycogen stores in real world conditions. That being said, this may not always be a requirement for optimal endurance performance, where exercise intensity and duration permit, a moderate CHO intake ( $\sim 6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) and exogenous CHO provision can be sufficient for optimal performance in endurance exercise lasting up to 2.5 h (Chapter 6). The demands of previous and upcoming exercise sessions should dictate level of CHO availability required and whether CHO loading for maximisation of glycogen stores is truly necessary to optimise performance.

## **Chapter Nine:**

### **References**

Adamo, K. B., Tarnopolsky, M. A., & Graham, T. E. (1998). Dietary carbohydrate and postexercise synthesis of proglycogen and macroglycogen in human skeletal muscle. *The American journal of physiology*, 275(2), E229–E234. <https://doi.org/10.1152/ajpendo.1998.275.2.E229>

Ahlborg, B., Bergström, J., Brohult, J., Ekelund, L., Hultman, E. & Maschio, G. (1967) Human muscle glycogen content and capacity for prolonged exercise after different diets. *Foersvarsmedicin* 1967 (3), 85-99.

Ahlborg, G., & Felig, P. (1977). Substrate utilization during prolonged exercise preceded by ingestion of glucose. *The American journal of physiology*, 233(3), E188–E194. <https://doi.org/10.1152/ajpendo.1977.233.3.E188>

Akermark, C., Jacobs, I., Rasmusson, M., & Karlsson, J. (1996). Diet and muscle glycogen concentration in relation to physical performance in Swedish elite ice hockey players. *International journal of sport nutrition*, 6(3), 272–284. <https://doi.org/10.1123/ijasn.6.3.272>

Areta, J. L., & Hopkins, W. G. Skeletal Muscle Glycogen Content at Rest and During Endurance Exercise in Humans: A Meta-Analysis. *Sports medicine (Auckland, N.Z.)*. 2018; 48 (9), 2091–2102. <https://doi.org/10.1007/s40279-018-0941-1>

Arkinstall, M. J., Bruce, C. R., Clark, S. A., Rickards, C. A., Burke, L. M., & Hawley, J. A. (2004). Regulation of fuel metabolism by preexercise muscle glycogen content and exercise intensity. *Journal of applied physiology (Bethesda, Md. : 1985)*, 97(6), 2275–2283. <https://doi.org/10.1152/japplphysiol.00421.2004>

Ariel, G., and Saville, W. (1972) Anabolic steroids: the physiological effects of placebos. *Med. Sci. Sports Exerc.* 4, 124 - 126.

Arnall, D. A., Nelson, A. G., Quigley, J., Lex, S., Dehart, T., & Fortune, P. (2007). Supercompensated glycogen loads persist 5 days in resting trained cyclists. *European journal of applied physiology*, 99(3), 251–256. <https://doi.org/10.1007/s00421-006-0340-4>

Asnicar, F., Leeming, E. R., Dimidi, E., Mazidi, M., Franks, P. W., Al Khatib, H., Valdes, A. M., Davies, R., Bakker, E., Francis, L., Chan, A., Gibson, R., Hadjigeorgiou, G., Wolf, J., Spector, T. D., Segata, N., & Berry, S. E. (2021). Blue poo: impact of gut transit time on the gut microbiome using a novel marker. *Gut*, 70 (9), 1665–1674. <https://doi.org/10.1136/gutjnl-2020-323877>

Beecher, H. K. (1955). The powerful placebo. *Journal of the American Medical Association*, 159(17), 1602–1606.

Beedie, C., Benedetti, F., Barbiani, D., Camerone, E., Cohen, E., Coleman, D., Davis, A., Elsworth-Edelsten, C., Flowers, E., Foad, A., Harvey, S., Hettinga, F., Hurst, P., Lane, A., Lindheimer, J., Raglin, J., Roelands, B., Schiphof-Godart, L., & Szabo, A. (2018). Consensus statement on placebo effects in sports and exercise: The need for conceptual clarity, methodological rigour, and the elucidation of neurobiological mechanisms. *European journal of sport science*, 18(10), 1383–1389. <https://doi.org/10.1080/17461391.2018.1496144>

Beedie, C. J., Coleman, D. A., & Foad, A. J. (2007). Positive and negative placebo effects resulting from the deceptive administration of an ergogenic aid. *International journal of sport nutrition and exercise metabolism*, 17(3), 259–269. <https://doi.org/10.1123/ijsnem.17.3.259>

Beedie, C. J., & Foad, A. J. (2009). The placebo effect in sports performance: a brief review. *Sports medicine (Auckland, N.Z.)*, 39(4), 313–329. <https://doi.org/10.2165/00007256-200939040-00004>

Beedie, C. J., Stuart, E. M., Coleman, D. A., & Foad, A. J. (2006). Placebo effects of caffeine on cycling performance. *Medicine and science in sports and exercise*, 38(12), 2159–2164. <https://doi.org/10.1249/01.mss.0000233805.56315.a9>

Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta physiologica Scandinavica*, 71(2), 140–150. <https://doi.org/10.1111/j.1748-1716.1967.tb03720.x>

Bergström, J., & Hultman, E. (1966). Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature*, 210(5033), 309–310. <https://doi.org/10.1038/210309a0>

Bie, C., Ma, Y., van Zijl, P. C. M., Yadav, N. N., Xu, X., Zheng, H., Liang, D., Zou, C., Areta, J. L., Chen, L., & Zhou, Y. (2024). In vivo imaging of glycogen in human muscle. *Nature communications*, 15(1), 10826. <https://doi.org/10.1038/s41467-024-55132-x>

Billat, V., Renoux, J. C., Pinoteau, J., Petit, B., & Koralsztein, J. P. (1994). Reproducibility of running time to exhaustion at VO<sub>2</sub>max in subelite runners. *Medicine and science in sports and exercise*, 26(2), 254–257. <https://doi.org/10.1249/00005768-199402000-00018>

Blom, P. C., Costill, D. L., & Vøllestad, N. K. (1987). Exhaustive running: inappropriate as a stimulus of muscle glycogen super-compensation. *Medicine and science in sports and exercise*, 19(4), 398–403.

Blom PC, Høstmark AT, Vaage O, Kardel KR, Maehlum S. (1987b) Effect of different post-exercise sugar diets on the rate of muscle glycogen synthesis. *Medicine and Science in Sports and Exercise*. 19(5), 491-496.

Bone, J. L., Ross, M. L., Tomcik, K. A., Jeacocke, N. A., Hopkins, W. G., & Burke, L. M. (2017). Manipulation of Muscle Creatine and Glycogen Changes Dual X-ray Absorptiometry Estimates of Body Composition. *Medicine and science in sports and exercise*, 49(5), 1029–1035. <https://doi.org/10.1249/MSS.0000000000001174>

Borg GA. (1982) Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 14: 377–381

Borg, D. N., Osborne, J. O., Stewart, I. B., Costello, J. T., Sims, J. N. L., & Minett, G. M. (2018). The reproducibility of 10 and 20km time trial cycling performance in recreational cyclists, runners and team sport athletes. *Journal of science and medicine in sport*, 21(8), 858–863. <https://doi.org/10.1016/j.jsams.2018.01.004>

Bosch, A. N., Dennis, S. C., & Noakes, T. D. (1993). Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, 74(4), 1921–1927. <https://doi.org/10.1152/jappl.1993.74.4.1921>

Bosch, A. N., Weltan, S. M., Dennis, S. C., & Noakes, T. D. (1996). Fuel substrate kinetics of carbohydrate loading differs from that of carbohydrate ingestion during prolonged exercise. *Metabolism: clinical and experimental*, 45(4), 415–423. [https://doi.org/10.1016/s0026-0495\(96\)90213-9](https://doi.org/10.1016/s0026-0495(96)90213-9)

Bosy-Westphal A, Schautz B, Later W, Kehayias JJ, Gallagher D, Müller MJ. What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. *Eur J Clin Nutr.* 2013 Jan;67 Suppl 1:S14-21. <https://doi.org/10.1038/ejcn.2012.160>

Bradley, W. J., Hannon, M. P., Benford, V., Morehen, J. C., Twist, C., Shepherd, S., Cocks, M., Impey, S. G., Cooper, R. G., Morton, J. P., & Close, G. L. (2017). Metabolic demands and replenishment of muscle glycogen after a rugby league match simulation protocol. *Journal of science and medicine in sport*, 20(9), 878–883. <https://doi.org/10.1016/j.jsams.2017.02.005>

Bradley, W. J., Morehen, J. C., Haigh, J., Clarke, J., Donovan, T. F., Twist, C., Cotton, C., Shepherd, S., Cocks, M., Sharma, A., Impey, S. G., Cooper, R. G., Maclaren, D. P., Morton, J. P., & Close, G. L. (2016). Muscle glycogen utilisation during Rugby match play: Effects of pre-game carbohydrate. *Journal of science and medicine in sport*, 19 (12), 1033–1038. <https://doi.org/10.1016/j.jsams.2016.03.008>

Brewer, J., Williams, C., & Patton, A. (1988). The influence of high carbohydrate diets on endurance running performance. *European journal of applied physiology and occupational physiology*, 57(6), 698–706. <https://doi.org/10.1007/BF01075991>

Bridge, E.M.; Bridges, E. (1931) The relation of glycogen to water storage in the liver. *J. Biol. Chem*, 93, 181–187.

Brouns, F., & Beckers, E. (1993). Is the gut an athletic organ? Digestion, absorption and exercise. *Sports medicine (Auckland, N.Z.)*, 15(4), 242–257. <https://doi.org/10.2165/00007256-199315040-00003>

Burke, L. M., Collier, G. R., Broad, E. M., Davis, P. G., Martin, D. T., Sanigorski, A. J., & Hargreaves, M. (2003). Effect of alcohol intake on muscle glycogen storage after prolonged exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, 95(3), 983–990. <https://doi.org/10.1152/jappphysiol.00115.2003>

Burke, L. M., Collier, G. R., Beasley, S. K., Davis, P. G., Fricker, P. A., Heeley, P., Walder, K., & Hargreaves, M. (1995). Effect of coingestion of fat and protein with carbohydrate feedings on muscle glycogen storage. *Journal of applied physiology (Bethesda, Md. : 1985)*, 78(6), 2187–2192. <https://doi.org/10.1152/jappl.1995.78.6.2187>

Burke, L. M., Collier, G. R., Davis, P. G., Fricker, P. A., Sanigorski, A. J., & Hargreaves, M. (1996). Muscle glycogen storage after prolonged exercise: effect of the frequency of carbohydrate feedings. *The American journal of clinical nutrition*, 64(1), 115–119. <https://doi.org/10.1093/ajcn/64.1.115>

Burke, L. M., Collier, G. R., & Hargreaves, M. (1993). Muscle glycogen storage after prolonged exercise: effect of the glycemic index of carbohydrate feedings. *Journal of applied physiology (Bethesda, Md. : 1985)*, 75(2), 1019–1023. <https://doi.org/10.1152/jappl.1993.75.2.1019>

Burke, L. M., Hawley, J. A., Schabort, E. J., St Clair Gibson, A., Mujika, I., & Noakes, T. D. (2000). Carbohydrate loading failed to improve 100-km cycling performance in a placebo-controlled trial. *Journal of applied physiology (Bethesda, Md. : 1985)*, 88(4), 1284–1290. <https://doi.org/10.1152/jappl.2000.88.4.1284>

Burke, L. M., van Loon, L. J. C., & Hawley, J. A. (2017). Postexercise muscle glycogen resynthesis in humans. *Journal of applied physiology (Bethesda, Md.: 1985)*, 122(5), 1055–1067. <https://doi.org/10.1152/jappphysiol.00860.2016>

Bur, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Medicine and science in sports and exercise*, 48(3), 543–568. <https://doi.org/10.1249/MSS.0000000000000852>

Bussau, V. A., Fairchild, T. J., Rao, A., Steele, P., & Fournier, P. A. (2002). Carbohydrate loading in human muscle: an improved 1 day protocol. *European journal of applied physiology*, 87(3), 290–295. <https://doi.org/10.1007/s00421-002-0621-5>

Chambers, E. S., Bridge, M. W., & Jones, D. A. (2009). Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *The Journal of physiology*, 587(Pt 8), 1779–1794. <https://doi.org/10.1113/jphysiol.2008.164285>

Cheng, B., Kuipers, H., Snyder, A. C., Keizer, H. A., Jeukendrup, A., & Hesselink, M. (1992). A new approach for the determination of ventilatory and lactate thresholds. *International journal of sports medicine*, 13(7), 518–522. <https://doi.org/10.1055/s-2007-1021309>

Christensen, E.H. & Hansen, O. (1939) Arbeitsfähigkeit und Ernährung. *Skand. Arch. Physiol.* 81, 160-171. <https://doi.org/10.1111/j.1748-1716.1939.tb01320.x>

Clark, V. R., Hopkins, W. G., Hawley, J. A., & Burke, L. M. (2000). Placebo effect of carbohydrate feedings during a 40-km cycling time trial. *Medicine and science in sports and exercise*, 32(9), 1642–1647. <https://doi.org/10.1097/00005768-200009000-00019>

Coggan, A. R., & Costill, D. L. (1984). Biological and technological variability of three anaerobic ergometer tests. *International journal of sports medicine*, 5(3), 142–145. <https://doi.org/10.1055/s-2008-1025896>

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Routledge.  
<https://doi.org/10.4324/9780203771587>

Corbett, J., Barwood, M. J., & Parkhouse, K. (2009). Effect of task familiarisation on distribution of energy during a 2000 m cycling time trial. *British journal of sports medicine*, 43(10), 770–774. <https://doi.org/10.1136/bjsm.2008.056416>

Costill, D. L., Coyle, E., Dalsky, G., Evans, W., Fink, W., & Hoopes, D. (1977). Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *Journal of applied physiology: respiratory, environmental and exercise physiology*, 43(4), 695–699.  
<https://doi.org/10.1152/jappl.1977.43.4.695>

Costill, D. L., Pearson, D. R., & Fink, W. J. (1988). Impaired muscle glycogen storage after muscle biopsy. *Journal of applied physiology (Bethesda, Md. : 1985)*, 64(5), 2245–2248.  
<https://doi.org/10.1152/jappl.1988.64.5.2245>

Costill, D. L., & Saltin, B. (1974). Factors limiting gastric emptying during rest and exercise. *Journal of applied physiology*, 37(5), 679–683.  
<https://doi.org/10.1152/jappl.1974.37.5.679>

Costill, D. L., Sherman, W. M., Fink, W. J., Maresh, C., Witten, M., & Miller, J. M. (1981). The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. *The American journal of clinical nutrition*, 34(9), 1831–1836.  
<https://doi.org/10.1093/ajcn/34.9.1831>

Coyle, E. F., Coggan, A. R., Hemmert, M. K., & Ivy, J. L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of applied physiology (Bethesda, Md. : 1985)*, 61(1), 165–172. <https://doi.org/10.1152/jappl.1986.61.1.165>

Coyle E. F. (1992). Carbohydrate feeding during exercise. *International journal of sports medicine*, 13 Suppl 1, S126–S128. <https://doi.org/10.1055/s-2007-1024615>

Coyle, E. F., Jeukendrup, A. E., Wagenmakers, A. J., & Saris, W. H. (1997). Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *The American journal of physiology*, 273(2 Pt 1), E268–E275. <https://doi.org/10.1152/ajpendo.1997.273.2.E268>

Coyle, E. F., Martin, W. H., 3rd, Sinacore, D. R., Joyner, M. J., Hagberg, J. M., & Holloszy, J. O. (1984). Time course of loss of adaptations after stopping prolonged intense endurance training. *Journal of applied physiology: respiratory, environmental and exercise physiology*, 57 (6), 1857–1864. <https://doi.org/10.1152/jappl.1984.57.6.1857>

Cumming, G. R., & Alexander, W. D. (1968). The calibration of bicycle ergometers. *Canadian journal of physiology and pharmacology*, 46(6), 917–919. <https://doi.org/10.1139/y68-145>

Currell, K., & Jeukendrup, A. E. (2008). Superior endurance performance with ingestion of multiple transportable carbohydrates. *Medicine and science in sports and exercise*, 40(2), 275–281. <https://doi.org/10.1249/mss.0b013e31815adf19>

Day, J. R., Rossiter, H. B., Coats, E. M., Skasick, A., & Whipp, B. J. (2003). The maximally attainable VO<sub>2</sub> during exercise in humans: the peak vs. maximum issue. *Journal of applied physiology (Bethesda, Md. : 1985)*, 95(5), 1901–1907. <https://doi.org/10.1152/japplphysiol.00024.2003>

De Bock, K., Derave, W., Ramaekers, M., Richter, E. A., & Hespel, P. (2007). Fiber type-specific muscle glycogen sparing due to carbohydrate intake before and during exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, 102(1), 183–188. <https://doi.org/10.1152/japplphysiol.00799.2006>

Décombaz, J., Jentjens, R., Ith, M., Scheurer, E., Buehler, T., Jeukendrup, A., & Boesch, C. (2011). Fructose and galactose enhance postexercise human liver glycogen synthesis. *Medicine and science in sports and exercise*, 43(10), 1964–1971. <https://doi.org/10.1249/MSS.0b013e318218ca5a>

Decroix, L., De Pauw, K., Foster, C., & Meeusen, R. (2016). Guidelines to Classify Female Subject Groups in Sport-Science Research. *International journal of sports physiology and performance*, 11(2), 204–213. <https://doi.org/10.1123/ijsp.2015-0153>

de Oliveira, E. P., Burini, R. C., & Jeukendrup, A. (2014). Gastrointestinal complaints during exercise: prevalence, etiology, and nutritional recommendations. *Sports medicine (Auckland, N.Z.)*, 44 Suppl 1(Suppl 1), S79–S85. <https://doi.org/10.1007/s40279-014-0153-2>

De Pauw, K., Roelands, B., Cheung, S. S., de Geus, B., Rietjens, G., & Meeusen, R. (2013). Guidelines to classify subject groups in sport-science research. *International journal of sports physiology and performance*, 8(2), 111–122. <https://doi.org/10.1123/ijsp.8.2.111>

Doering, T. M., Cox, G. R., Areta, J. L., & Coffey, V. G. (2019). Repeated muscle glycogen supercompensation with four days' recovery between exhaustive exercise. *Journal of science and medicine in sport*, 22(8), 907–911. <https://doi.org/10.1016/j.jsams.2019.03.009>

Doyle, J. A., & Martinez, A. L. (1998). Reliability of a protocol for testing endurance performance in runners and cyclists. *Research quarterly for exercise and sport*, 69(3), 304–307. <https://doi.org/10.1080/02701367.1998.10607698>

Duhamel, T. A., Perco, J. G., & Green, H. J. (2006). Manipulation of dietary carbohydrates after prolonged effort modifies muscle sarcoplasmic reticulum responses in exercising males. *American journal of physiology. Regulatory, integrative and comparative physiology*, 291(4), R1100–R1110. <https://doi.org/10.1152/ajpregu.00858.2005>

Elbourne, D. R., Altman, D. G., Higgins, J. P., Curtin, F., Worthington, H. V., & Vail, A. (2002). Meta-analyses involving cross-over trials: methodological issues. *International journal of epidemiology*, 31(1), 140–149. <https://doi.org/10.1093/ije/31.1.140>

Elliott-Sale, K. J., Minahan, C. L., de Jonge, X. A. K. J., Ackerman, K. E., Sipilä, S., Constantini, N. W., Lebrun, C. M., & Hackney, A. C. (2021). Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. *Sports medicine (Auckland, N.Z.)*, 51(5), 843–861. <https://doi.org/10.1007/s40279-021-01435-8>

Fairchild, T. J., Fletcher, S., Steele, P., Goodman, C., Dawson, B., & Fournier, P. A. (2002). Rapid carbohydrate loading after a short bout of near maximal-intensity exercise. *Medicine and science in sports and exercise*, 34(6), 980–986. <https://doi.org/10.1097/00005768-200206000-00012>

Febbraio, M. A., Chiu, A., Angus, D. J., Arkinstall, M. J., & Hawley, J. A. (2000). Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance. *Journal of applied physiology (Bethesda, Md. : 1985)*, 89(6), 2220–2226. <https://doi.org/10.1152/jappl.2000.89.6.2220>

Fell, J. M., Hearn, M. A., Ellis, D. G., Moran, J. E. P., Jevons, E. F. P., Owens, D. J., Strauss, J. A., Cocks, M., Louis, J. B., Shepherd, S. O., & Morton, J. P. (2021). Carbohydrate improves exercise capacity but does not affect subcellular lipid droplet morphology, AMPK and p53 signalling in human skeletal muscle. *The Journal of physiology*, 599(11), 2823–2849. <https://doi.org/10.1113/JP281127>

Ferrannini E. (1988). The theoretical bases of indirect calorimetry: a review. *Metabolism: clinical and experimental*, 37(3), 287–301. [https://doi.org/10.1016/0026-0495\(88\)90110-2](https://doi.org/10.1016/0026-0495(88)90110-2)

Fernández-Elías, V. E., Ortega, J. F., Nelson, R. K., & Mora-Rodriguez, R. (2015). Relationship between muscle water and glycogen recovery after prolonged exercise in the heat in humans. *European journal of applied physiology*, 115(9), 1919–1926. <https://doi.org/10.1007/s00421-015-3175-z>

Fielding, R. A., Costill, D. L., Fink, W. J., King, D. S., Hargreaves, M., & Kovaleski, J. E. (1985). Effect of carbohydrate feeding frequencies and dosage on muscle glycogen use during exercise. *Medicine and science in sports and exercise*, 17(4), 472–476. <https://doi.org/10.1249/00005768-198508000-00012>

Flynn, M. G., Costill, D. L., Hawley, J. A., Fink, W. J., Neufer, P. D., Fielding, R. A., & Sleeper, M. D. (1987). Influence of selected carbohydrate drinks on cycling performance and glycogen use. *Medicine and science in sports and exercise*, 19(1), 37–40.

Foad, A. J., Beedie, C. J., & Coleman, D. A. (2008). Pharmacological and psychological effects of caffeine ingestion in 40-km cycling performance. *Medicine and science in sports and exercise*, 40(1), 158–165. <https://doi.org/10.1249/mss.0b013e3181593e02>

Foster, C., Costill, D. L., & Fink, W. J. (1979). Effects of preexercise feedings on endurance performance. *Medicine and science in sports*, 11(1), 1–5.

Foskett, A., Williams, C., Boobis, L., & Tsintzas, K. (2008). Carbohydrate availability and muscle energy metabolism during intermittent running. *Medicine and science in sports and exercise*, 40(1), 96–103. <https://doi.org/10.1249/mss.0b013e3181586b2c>

Frentzel, J., & Reach, F. (1901) Untersuchungen zur Frage nach der Quelle der Muskelkraft. *Pflüger's Arch* 83:447

Galbo, H., Holst, J. J., & Christensen, N. J. (1979). The effect of different diets and of insulin on the hormonal response to prolonged exercise. *Acta physiologica Scandinavica*, 107(1), 19–32. <https://doi.org/10.1111/j.1748-1716.1979.tb06438.x>

Gleeson, M., Maughan, R. J., & Greenhaff, P. L. (1986). Comparison of the effects of pre-exercise feeding of glucose, glycerol and placebo on endurance and fuel homeostasis in man. *European journal of applied physiology and occupational physiology*, 55(6), 645–653. <https://doi.org/10.1007/BF00423211>

Goforth, H. W., Jr, Arnall, D. A., Bennett, B. L., & Law, P. G. (1997). Persistence of supercompensated muscle glycogen in trained subjects after carbohydrate loading. *Journal of applied physiology (Bethesda, Md. : 1985)*, 82(1), 342–347. <https://doi.org/10.1152/jappl.1997.82.1.342>

Gollnick, P. D., Piehl, K., Saubert, C. W., 4th, Armstrong, R. B., & Saltin, B. (1972). Diet, exercise, and glycogen changes in human muscle fibers. *Journal of applied physiology*, 33(4), 421–425. <https://doi.org/10.1152/jappl.1972.33.4.421>

Gonzalez, J. T., Fuchs, C. J., Smith, F. E., Thelwall, P. E., Taylor, R., Stevenson, E. J., Trenell, M. I., Cermak, N. M., & van Loon, L. J. (2015). Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *American journal of physiology. Endocrinology and metabolism*, 309(12), E1032–E1039. <https://doi.org/10.1152/ajpendo.00376.2015>

Greiwe, J. S., Hickner, R. C., Hansen, P. A., Racette, S. B., Chen, M. M., & Holloszy, J. O. (1999). Effects of endurance exercise training on muscle glycogen accumulation in humans. *Journal of applied physiology (Bethesda, Md. : 1985)*, 87(1), 222–226. <https://doi.org/10.1152/jappl.1999.87.1.222>

Guezennec, C. Y., Satabin, P., Duforez, F., Merino, D., Peronnet, F., & Koziat, J. (1989). Oxidation of corn starch, glucose, and fructose ingested before exercise. *Medicine and science in sports and exercise*, 21(1), 45–50. <https://doi.org/10.1249/00005768-198902000-00009>

Halsen, S. L., & Martin, D. T. (2013). Lying to win-placebos and sport science. *International journal of sports physiology and performance*, 8(6), 597–599. <https://doi.org/10.1123/ijsp.8.6.597>

Hargreaves, M., Hawley, J. A., & Jeukendrup, A. (2004). Pre-exercise carbohydrate and fat ingestion: effects on metabolism and performance. *Journal of sports sciences*, 22(1), 31–38. <https://doi.org/10.1080/0264041031000140536>

Hargreaves, M., McConell, G., & Proietto, J. (1995). Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *Journal of applied physiology (Bethesda, Md. : 1985)*, 78(1), 288–292. <https://doi.org/10.1152/jappl.1995.78.1.288>

Hawley, J. A., & Leckey, J. J. (2015). Carbohydrate Dependence During Prolonged, Intense Endurance Exercise. *Sports medicine (Auckland, N.Z.)*, 45 Suppl 1(Suppl 1), S5–S12. <https://doi.org/10.1007/s40279-015-0400-1>

Hawley, J. A., Schabort, E. J., Noakes, T. D., & Dennis, S. C. (1997a). Carbohydrate-loading and exercise performance. An update. *Sports medicine (Auckland, N.Z.)*, 24(2), 73–81. <https://doi.org/10.2165/00007256-199724020-00001>

Hawley, J. A., Palmer, G. S., & Noakes, T. D. (1997b). Effects of 3 days of carbohydrate supplementation on muscle glycogen content and utilisation during a 1-h cycling performance. *European journal of applied physiology and occupational physiology*, 75(5), 407–412. <https://doi.org/10.1007/s004210050180>

Hearris, M. A., Hammond, K. M., Fell, J. M., & Morton, J. P. (2018). Regulation of Muscle Glycogen Metabolism during Exercise: Implications for Endurance Performance and Training Adaptations. *Nutrients*, 10(3), 298. <https://doi.org/10.3390/nu10030298>

Hearris, M. A., Pugh, J. N., Langan-Evans, C., Mann, S. J., Burke, L., Stellingwerff, T., Gonzalez, J. T., & Morton, J. P. (2022). <sup>13</sup>C-glucose-fructose labeling reveals comparable exogenous CHO oxidation during exercise when consuming 120 g/h in fluid, gel, jelly chew, or coingestion. *Journal of applied physiology (Bethesda, Md. : 1985)*, 132(6), 1394–1406. <https://doi.org/10.1152/jappphysiol.00091.2022>

Heck, H., Mader, A., Hess, G., Mücke, S., Müller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l lactate threshold. *International journal of sports medicine*, 6(3), 117–130. <https://doi.org/10.1055/s-2008-1025824>

Hengist, A., Edinburgh, R. M., Davies, R. G., Walhin, J. P., Buniam, J., James, L. J., Rogers, P. J., Gonzalez, J. T., & Betts, J. A. (2020). Physiological responses to maximal eating in men. *The British journal of nutrition*, 124(4), 407–417. <https://doi.org/10.1017/S0007114520001270>

Henriksson K. G. (1979). "Semi-open" muscle biopsy technique. A simple outpatient procedure. *Acta neurologica Scandinavica*, 59(6), 317–323.

Hibbert, A. W., Billaut, F., Varley, M. C., & Polman, R. C. J. (2017). Familiarization Protocol Influences Reproducibility of 20-km Cycling Time-Trial Performance in Novice Participants. *Frontiers in physiology*, 8, 488. <https://doi.org/10.3389/fphys.2017.00488>

Hickey, M. S., Costill, D. L., McConell, G. K., Widrick, J. J., & Tanaka, H. (1992). Day to day variation in time trial cycling performance. *International journal of sports medicine*, 13(6), 467–470. <https://doi.org/10.1055/s-2007-1021299>

Hickner, R. C., Fisher, J. S., Hansen, P. A., Racette, S. B., Mier, C. M., Turner, M. J., & Holloszy, J. O. (1997). Muscle glycogen accumulation after endurance exercise in trained and untrained individuals. *Journal of applied physiology (Bethesda, Md. : 1985)*, 83(3), 897–903. <https://doi.org/10.1152/jappl.1997.83.3.897>

Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions* version 6.5 (updated August 2024). Cochrane, 2024. Available from [www.training.cochrane.org/handbook](http://www.training.cochrane.org/handbook).

Hill, K. M., Stathis, C. G., Grinfeld, E., Hayes, A., & McAinch, A. J. (2013). Co-ingestion of carbohydrate and whey protein isolates enhance PGC-1 $\alpha$  mRNA expression: a randomised, single blind, cross over study. *Journal of the International Society of Sports Nutrition*, 10(1), 8. <https://doi.org/10.1186/1550-2783-10-8>

Hingst, J. R., Bruhn, L., Hansen, M. B., Rosschou, M. F., Birk, J. B., Fentz, J., Foretz, M., Viollet, B., Sakamoto, K., Færgeman, N. J., Havelund, J. F., Parker, B. L., James, D. E., Kiens, B., Richter, E. A., Jensen, J., & Wojtaszewski, J. F. P. (2018). Exercise-induced molecular mechanisms promoting glycogen supercompensation in human skeletal muscle. *Molecular metabolism*, 16, 24–34. <https://doi.org/10.1016/j.molmet.2018.07.001>

Hopker, J. G., Coleman, D. A., Wiles, J. D., & Galbraith, A. (2009). Familiarisation and reliability of sprint test indices during laboratory and field assessment. *Journal of sports science & medicine*, 8(4), 528–532.

Hopkins, W. G., Hawley, J. A., & Burke, L. M. (1999). Design and analysis of research on sport performance enhancement. *Medicine and science in sports and exercise*, 31(3), 472–485. <https://doi.org/10.1097/00005768-199903000-00018>

Hopkins, W. G., Schabert, E. J., & Hawley, J. A. (2001). Reliability of power in physical performance tests. *Sports medicine (Auckland, N.Z.)*, 31(3), 211–234. <https://doi.org/10.2165/00007256-200131030-00005>

Immonen, K., Ruusunen, M., Hissa, K., & Puolanne, E. (2000). Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. *Meat science*, 55(1), 25–31. [https://doi.org/10.1016/s0309-1740\(99\)00121-7](https://doi.org/10.1016/s0309-1740(99)00121-7)

Impey, S. G., Jevons, E., Mees, G., Cocks, M., Strauss, J., Chester, N., Laurie, I., Target, D., Hodgson, A., Shepherd, S. O., & Morton, J. P. (2020). Glycogen Utilization during Running: Intensity, Sex, and Muscle-Specific Responses. *Medicine and science in sports and exercise*, 52(9), 1966–1975. <https://doi.org/10.1249/MSS.0000000000002332>

Ivy, J. L., Katz, A. L., Cutler, C. L., Sherman, W. M., & Coyle, E. F. (1988). Muscle glycogen synthesis after exercise: effect of time of carbohydrate ingestion. *Journal of applied physiology (Bethesda, Md. : 1985)*, 64(4), 1480–1485. <https://doi.org/10.1152/jappl.1988.64.4.1480>

Iwayama K, Tanabe Y, Tanji F, Ohnishi T, Takahashi H. (2021) Diurnal variations in muscle and liver glycogen differ depending on the timing of exercise. *J Physiol Sci.*; 71:35. <https://doi.org/10.1186/s12576-021-00821-1>.

Jansson, E., & Kaijser, L. (1982). Effect of diet on muscle glycogen and blood glucose utilization during a short-term exercise in man. *Acta physiologica Scandinavica*, 115(3), 341–347. <https://doi.org/10.1111/j.1748-1716.1982.tb07087.x>

James, A. P., Lorraine, M., Cullen, D., Goodman, C., Dawson, B., Palmer, T. N., & Fournier, P. A. (2001). Muscle glycogen supercompensation: absence of a gender-related difference. *European journal of applied physiology*, 85(6), 533–538. <https://doi.org/10.1007/s004210100499>

Jensen, R., Ørtenblad, N., Stausholm, M. H., Skjaerbaek, M. C., Larsen, D. N., Hansen, M., Holmberg, H. C., Plomgaard, P., & Nielsen, J. (2021). Glycogen supercompensation is due to increased number, not size, of glycogen particles in human skeletal muscle. *Experimental physiology*, 106(5), 1272–1284. <https://doi.org/10.1113/EP089317>

Jensen, R., Ørtenblad, N., Stausholm, M. H., Skjaerbaek, M. C., Larsen, D. N., Hansen, M., Holmberg, H. C., Plomgaard, P., & Nielsen, J. (2020). Heterogeneity in subcellular muscle glycogen utilisation during exercise impacts endurance capacity in men. *The Journal of physiology*, 598(19), 4271–4292. <https://doi.org/10.1113/JP280247>

Jensen, T. E., & Richter, E. A. (2012). Regulation of glucose and glycogen metabolism during and after exercise. *The Journal of physiology*, 590(5), 1069–1076. <https://doi.org/10.1113/jphysiol.2011.224972>

Jentjens, R. L., Achten, J., & Jeukendrup, A. E. (2004). High oxidation rates from combined carbohydrates ingested during exercise. *Medicine and science in sports and exercise*, 36(9), 1551–1558. <https://doi.org/10.1249/01.mss.0000139796.07843.1d>

Jentjens, R., & Jeukendrup, A. (2003). Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports medicine (Auckland, N.Z.)*, 33(2), 117–144. <https://doi.org/10.2165/00007256-200333020-00004>

Jentjens, R. L., & Jeukendrup, A. E. (2005). High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise. *The British journal of nutrition*, 93(4), 485–492. <https://doi.org/10.1079/bjn20041368>

Jeukendrup A. E. (2004). Carbohydrate intake during exercise and performance. *Nutrition (Burbank, Los Angeles County, Calif.)*, 20(7-8), 669–677. <https://doi.org/10.1016/j.nut.2004.04.017>

Jeukendrup, A. E., & Jentjens, R. (2000). Oxidation of carbohydrate feedings during prolonged exercise: current thoughts, guidelines and directions for future research. *Sports medicine (Auckland, N.Z.)*, 29(6), 407–424. <https://doi.org/10.2165/00007256-200029060-00004>

Jeukendrup, A. E., & Killer, S. C. (2010). The myths surrounding pre-exercise carbohydrate feeding. *Annals of nutrition & metabolism*, 57 Suppl 2, 18–25. <https://doi.org/10.1159/000322698>

Jeukendrup, A., Saris, W. H., Brouns, F., & Kester, A. D. (1996). A new validated endurance performance test. *Medicine and science in sports and exercise*, 28(2), 266–270. <https://doi.org/10.1097/00005768-199602000-00017>

Jeukendrup, A. E., Wagenmakers, A. J., Stegen, J. H., Gijsen, A. P., Brouns, F., & Saris, W. H. (1999). Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. *The American journal of physiology*, 276(4), E672–E683. <https://doi.org/10.1152/ajpendo.1999.276.4.E672>

Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International journal of sports medicine*, 26 Suppl 1, S28–S37. <https://doi.org/10.1055/s-2004-830512>

Karlsson, J., & Saltin, B. (1971). Diet, muscle glycogen, and endurance performance. *Journal of applied physiology*, 31(2), 203–206. <https://doi.org/10.1152/jappl.1971.31.2.203>

Katz A. (2022). A century of exercise physiology: key concepts in regulation of glycogen metabolism in skeletal muscle. *European journal of applied physiology*, 122(8), 1751–1772. <https://doi.org/10.1007/s00421-022-04935-1>

Kavouras, S. A., Troup, J. P., & Berning, J. R. (2004). The influence of low versus high carbohydrate diet on a 45-min strenuous cycling exercise. *International journal of sport nutrition and exercise metabolism*, 14(1), 62–72. <https://doi.org/10.1123/ijsnem.14.1.62>

Keeffe, E. B., Lowe, D. K., Goss, J. R., & Wayne, R. (1984). Gastrointestinal symptoms of marathon runners. *The Western journal of medicine*, 141(4), 481–484

Kerr, A., Slater, G., Byrne, N., & Chaseling, J. (2015). Validation of Bioelectrical Impedance Spectroscopy to Measure Total Body Water in Resistance-Trained Males. *International journal of sport nutrition and exercise metabolism*, 25(5), 494–503. <https://doi.org/10.1123/ijsnem.2014-0188>

Kiens, B., Raben, A. B., Valeur, A. K., & Richter, E. A. (1990) Benefit of dietary simple carbohydrates on the early post-exercise muscle glycogen repletion in male athletes. *Med Sci Sports Exerc.* 22(4): S88

Kirwan, J. P., Costill, D. L., Mitchell, J. B., Houmard, J. A., Flynn, M. G., Fink, W. J., & Beltz, J. D. (1988). Carbohydrate balance in competitive runners during successive days of intense training. *Journal of applied physiology (Bethesda, Md. : 1985)*, 65(6), 2601–2606. <https://doi.org/10.1152/jappl.1988.65.6.2601>

King, D. S., Staten, M. A., Kohrt, W. M., Dalsky, G. P., Elahi, D., & Holloszy, J. O. (1990). Insulin secretory capacity in endurance-trained and untrained young men. *The American journal of physiology*, 259(2 Pt 1), E155–E161. <https://doi.org/10.1152/ajpendo.1990.259.2.E155>

Kochan, R. G., Lamb, D. R., Lutz, S. A., Perrill, C. V., Reimann, E. M., & Schlender, K. K. (1979). Glycogen synthase activation in human skeletal muscle: effects of diet and exercise. *The American journal of physiology*, 236(6), E660–E666. <https://doi.org/10.1152/ajpendo.1979.236.6.E660>

Kolahi, J., Bang, H., & Park, J. (2009). Towards a proposal for assessment of blinding success in clinical trials: up-to-date review. *Community dentistry and oral epidemiology*, 37(6), 477–484. <https://doi.org/10.1111/j.1600-0528.2009.00494.x>

Krebs, P. S. & Powers, S. K. (1989). Reliability of laboratory endurance tests. *Med. Sci. Sports Exerc.* 31.

Krogh, A., & Lindhard, J. (1920). The Relative Value of Fat and Carbohydrate as Sources of Muscular Energy: With Appendices on the Correlation between Standard Metabolism and the Respiratory Quotient during Rest and Work. *The Biochemical journal*, 14 (3-4), 290–363. <https://doi.org/10.1042/bj0140290>

Krzentowski, G., Jandrain, B., Pirnay, F., Mosora, F., Lacroix, M., Luyckx, A. S., & Lefebvre, P. J. (1984). Availability of glucose given orally during exercise. *Journal of applied physiology: respiratory, environmental and exercise physiology*, 56(2), 315–320. <https://doi.org/10.1152/jappl.1984.56.2.315>

Kuipers H, Verstappen FTJ, Keizer HA, Geurten P & Van Kranenburg g (1985). Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 06, 197–201

Kyle, U. G., Bosaeus, I., De Lorenzo, A. D., Deurenberg, P., Elia, M., Gómez, J. M., Heitmann, B. L., Kent-Smith, L., Melchior, J. C., Pirlich, M., Scharfetter, H., Schols, A. M., Pichard, C., & Composition of the ESPEN Working Group (2004a). Bioelectrical impedance analysis--part I: review of principles and methods. *Clinical nutrition (Edinburgh, Scotland)*, 23(5), 1226–1243. <https://doi.org/10.1016/j.clnu.2004.06.004>

Kyle, U. G., Bosaeus, I., De Lorenzo, A. D., Deurenberg, P., Elia, M., Manuel Gómez, J., Heitmann, B. L., Kent-Smith, L., Melchior, J. C., Pirlich, M., Scharfetter, H., Schols, A.M., Pichard, C., & ESPEN (2004b). Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clinical nutrition (Edinburgh, Scotland)*, 23(6), 1430–1453. <https://doi.org/10.1016/j.clnu.2004.09.012>

Lamb, D. R., Snyder, A. C., & Baur, T. S. (1991). Muscle glycogen loading with a liquid carbohydrate supplement. *International journal of sport nutrition*, 1(1), 52–60. <https://doi.org/10.1123/ijasn.1.1.52>

Leckey, J. J., Burke, L. M., Morton, J. P., & Hawley, J. A. (2016). Altering fatty acid availability does not impair prolonged, continuous running to fatigue: evidence for carbohydrate dependence. *Journal of applied physiology (Bethesda, Md. : 1985)*, 120(2), 107–113. <https://doi.org/10.1152/japplphysiol.00855.2015>

Lewis, S. J., & Heaton, K. W. (1997). Stool form scale as a useful guide to intestinal transit time. *Scandinavian journal of gastroenterology*, 32(9), 920–924. <https://doi.org/10.3109/00365529709011203>

MacDougall, J. D., Ward, G. R., & Sutton, J. R. (1977). Muscle glycogen repletion after high-intensity intermittent exercise. *Journal of applied physiology: respiratory, environmental and exercise physiology*, 42(2), 129–132. <https://doi.org/10.1152/jappl.1977.42.2.129>

Madsen, K., Pedersen, P. K., Rose, P., & Richter, E. A. (1990). Carbohydrate supercompensation and muscle glycogen utilization during exhaustive running in highly trained athletes. *European journal of applied physiology and occupational physiology*, 61(5-6), 467–472. <https://doi.org/10.1007/BF00236069>

Marchand, I., Chorneyko, K., Tarnopolsky, M., Hamilton, S., Shearer, J., Potvin, J., and Graham, T.E. (2002) Quantification of subcellular glycogen in resting human muscle: granule size, number, and location. *J. Appl. Physiol.* 93, 1598–1607.

Marchand, I., Tarnopolsky, M., Adamo, K. B., Bourgeois, J. M., Chorneyko, K., & Graham, T. E. (2007). Quantitative assessment of human muscle glycogen granules size and number in subcellular locations during recovery from prolonged exercise. *The Journal of physiology*, 580(Pt. 2), 617–628. <https://doi.org/10.1113/jphysiol.2006.122457>

Martin, C. K., Han, H., Coulon, S. M. et al. (2009) A novel method to remotely measure food intake of free-living individuals in real time: the remote food photography method. *Br J Nutr* 2009; 101:446–456.

Martin, C. K., Correa, J. B., Han, H., Allen, H. R., Rood, J. C., Champagne, C. M., Gunturk, B. K., & Bray, G. A. (2012). Validity of the Remote Food Photography Method (RFPM) for estimating energy and nutrient intake in near real-time. *Obesity (Silver Spring, Md.)*, 20(4), 891–899. <https://doi.org/10.1038/oby.2011.344>

Matias, C. N., Santos, D. A., Júdice, P. B., Magalhães, J. P., Minderico, C. S., Fields, D. A., Lukaski, H. C., Sardinha, L. B., & Silva, A. M. (2016). Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models. *Clinical nutrition (Edinburgh, Scotland)*, 35(2), 468–474. <https://doi.org/10.1016/j.clnu.2015.03.013>

Massicotte, D., Péronnet, F., Adopo, E., Brisson, G. R., & Hillaire-Marcel, C. (1994). Effect of metabolic rate on the oxidation of ingested glucose and fructose during exercise. *International journal of sports medicine*, 15(4), 177–180. <https://doi.org/10.1055/s-2007-1021043>

Massicotte, D., Péronnet, F., Brisson, G., Bakkouch, K., & Hillaire-Marcel, C. (1989). Oxidation of a glucose polymer during exercise: comparison with glucose and fructose. *Journal of applied physiology (Bethesda, Md. : 1985)*, 66(1), 179–183. <https://doi.org/10.1152/jappl.1989.66.1.179>

Massicotte, D., Péronnet, F., Brisson, G., Boivin, L., & Hillaire-Marcel, C. (1990). Oxidation of exogenous carbohydrate during prolonged exercise in fed and fasted conditions. *International journal of sports medicine*, 11(4), 253–258. <https://doi.org/10.1055/s-2007-1024802>

Maunder, E., Bradley, H. E., Deane, C. S., Hodgson, A. B., Jones, M., Joannis, S., Turner, A. M., Breen, L., Philp, A., & Wallis, G. A. (2021). Effects of short-term graded dietary carbohydrate intake on intramuscular and whole body metabolism during moderate-intensity exercise. *Journal of applied physiology (Bethesda, Md. : 1985)*, 131(1), 376–387. <https://doi.org/10.1152/japplphysiol.00811.2020>

Maxwell, B. F., Withers, R. T., Ilsley, A. H., Wakim, M. J., Woods, G. F., & Day, L. (1998). Dynamic calibration of mechanically, air- and electromagnetically braked cycle ergometers. *European journal of applied physiology and occupational physiology*, 78(4), 346–352. <https://doi.org/10.1007/s004210050430>

McBride, J.; Guest, M.M.; Scott, E. (1941) The storage of the major liver components; emphasizing the relationship of glycogen to water in the liver and the hydration of glycogen. *J. Biol. Chem.*, 139, 943–952.

McClung, M., & Collins, D. (2007). "Because I know it will!": placebo effects of an ergogenic aid on athletic performance. *Journal of sport & exercise psychology*, 29(3), 382–394. <https://doi.org/10.1123/jsep.29.3.382>

McInerney, P., Lessard, S. J., Burke, L. M., Coffey, V. G., Lo Giudice, S. L., Southgate, R. J., & Hawley, J. A. (2005). Failure to repeatedly supercompensate muscle glycogen stores in highly trained men. *Medicine and science in sports and exercise*, 37(3), 404–411. <https://doi.org/10.1249/01.mss.0000155699.51360.2f>

McKay, A. K. A., Stellingwerff, T., Smith, E. S., Martin, D. T., Mujika, I., Goosey-Tolfrey, V. L., Sheppard, J., Burke, L.M. (2022) Defining Training and Performance Caliber: A Participant Classification Framework. *International journal of sports physiology and performance*, 17(2), 317–331. <https://doi.org/10.1123/ijsp.2021-0451>

McLay, R. T., Thomson, C. D., Williams, S. M., & Rehrer, N. J. (2007). Carbohydrate loading and female endurance athletes: effect of menstrual-cycle phase. *International journal of sport nutrition and exercise metabolism*, 17(2), 189–205. <https://doi.org/10.1123/ijsnem.17.2.189>

Mears, S. A., Boxer, B., Sheldon, D., Wardley, H., Tarnowski, C. A., James, L. J., & Hulston, C. J. (2020). Sports Drink Intake Pattern Affects Exogenous Carbohydrate Oxidation during Running. *Medicine and science in sports and exercise*, 52(9), 1976–1982. <https://doi.org/10.1249/MSS.0000000000002334>

Melin, A., Tornberg, A. B., Skouby, S., Faber, J., Ritz, C., Sjödén, A., & Sundgot-Borgen, J. (2014). The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad. *British journal of sports medicine*, 48(7), 540–545. <https://doi.org/10.1136/bjsports-2013-093240>

Mikines, K. J., Farrell, P. A., Sonne, B., Tronier, B., & Galbo, H. (1988). Postexercise dose-response relationship between plasma glucose and insulin secretion. *Journal of applied physiology* (Bethesda, Md. : 1985), 64(3), 988–999. <https://doi.org/10.1152/jappl.1988.64.3.988>

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L. A., & PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews*, 4(1), 1. <https://doi.org/10.1186/2046-4053-4-1>

Morris, S. B., & DeShon, R. P. (2002). Combining effect size estimates in meta-analysis with repeated measures and independent-groups designs. *Psychological methods*, 7(1), 105–125. <https://doi.org/10.1037/1082-989x.7.1.105>

Mujika, I., & Padilla, S. (2000) Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. *Sports medicine* (Auckland, N.Z.), 30(2), 79–87. <https://doi.org/10.2165/00007256-200030020-00002>

Muros, J. J., Sánchez-Muñoz, C., Hoyos, J., & Zabala, M. (2019). Nutritional intake and body composition changes in a UCI World Tour cycling team during the Tour of Spain. *European journal of sport science*, 19(1), 86–94. <https://doi.org/10.1080/17461391.2018.1497088>

Murray, B., & Rosenbloom, C. (2018). Fundamentals of glycogen metabolism for coaches and athletes. *Nutrition reviews*, 76(4), 243–259. <https://doi.org/10.1093/nutrit/nuy001>

Nelson, A. G., Arnall, D. A., Kokkonen, J., Day, R., & Evans, J. (2001). Muscle glycogen supercompensation is enhanced by prior creatine supplementation. *Medicine and science in sports and exercise*, 33(7), 1096–1100. <https://doi.org/10.1097/00005768-200107000-00005>

Nicklas, B. J., Hackney, A. C., & Sharp, R. L. (1989). The menstrual cycle and exercise: performance, muscle glycogen, and substrate responses. *International journal of sports medicine*, 10(4), 264–269. <https://doi.org/10.1055/s-2007-1024913>

Nielsen, J., Holmberg, H. C., Schröder, H. D., Saltin, B., & Ørtenblad, N. (2011). Human skeletal muscle glycogen utilization in exhaustive exercise: role of subcellular localization and fibre type. *The Journal of physiology*, 589(Pt 11), 2871–2885. <https://doi.org/10.1113/jphysiol.2010.204487>

Nielsen, J., Mogensen, M., Vind, B. F., Sahlin, K., Højlund, K., Schröder, H. D., & Ortenblad, N. (2010a). Increased subsarcolemmal lipids in type 2 diabetes: effect of training on localization of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. *American journal of physiology. Endocrinology and metabolism*, 298(3), E706–E713. <https://doi.org/10.1152/ajpendo.00692.2009>

Nielsen, J., & Ørtenblad, N. (2013). Physiological aspects of the subcellular localization of glycogen in skeletal muscle. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, 38(2), 91–99. <https://doi.org/10.1139/apnm-2012-0184>

Nielsen, J., Suetta, C., Hvid, L. G., Schröder, H. D., Aagaard, P., & Ortenblad, N. (2010b). Subcellular localization-dependent decrements in skeletal muscle glycogen and mitochondria content following short-term disuse in young and old men. *American journal of physiology. Endocrinology and metabolism*, 299(6), E1053–E1060. <https://doi.org/10.1152/ajpendo.00324.2010>

Nilsson, L. H., Hultman, E. (1973) Liver glycogen in man—the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scand J Clin Lab Investig.*;32:325–30. <https://doi.org/10.3109/00365517309084355>.

Noakes, T. D., Rehrer, N. J., & Maughan, R. J. (1991). The importance of volume in regulating gastric emptying. *Medicine and science in sports and exercise*, 23(3), 307–313.

Nygren, A. T., Karlsson, M., Norman, B., & Kaijser, L. (2001). Effect of glycogen loading on skeletal muscle cross-sectional area and T2 relaxation time. *Acta physiologica Scandinavica*, 173(4), 385–390. <https://doi.org/10.1046/j.1365-201X.2001.00913.x>

Olsson, K. E., & Saltin, B. (1970). Variation in total body water with muscle glycogen changes in man. *Acta physiologica Scandinavica*, 80(1), 11–18. <https://doi.org/10.1111/j.1748-1716.1970.tb04764.x>

Ørtenblad, N., Westerblad, H., & Nielsen, J. (2013). Muscle glycogen stores and fatigue. *The Journal of physiology*, 591(18), 4405–4413. <https://doi.org/10.1113/jphysiol.2013.251629>

Parkin, J. A. M., Carey, M. F., Martin, I. K., Stojanovska, L. & Febbraio, M. A. (1997) Muscle glycogen storage following prolonged exercise: effect of timing of ingestion of high glycemic index food. *Med Sci Sports Exerc* 29: 220–224. doi:10.1097/00005768-199702000-00009.

Patel, H., Syddall, H. E., Martin, H. J., Cooper, C., Stewart, C., & Sayer, A. A. (2011). The feasibility and acceptability of muscle biopsy in epidemiological studies: findings from the Hertfordshire Sarcopenia Study (HSS). *The journal of nutrition, health & aging*, 15(1), 10–15. <https://doi.org/10.1007/s12603-011-0006-8>

Paton, C. D., & Hopkins, W. G. (2001). Tests of cycling performance. *Sports medicine (Auckland, N.Z.)*, 31(7), 489–496. <https://doi.org/10.2165/00007256-200131070-00004>

Pedersen, D.J., Lessard, S.J., Coffey, V.G., Churchley, E.G., Wootton, A.M., Ng, T., Watt, M.J., Hawley, J.A. (2008) High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine. *J Appl Physiol* (1985) 105: 7–13.

Peters, H. P., van Schelven, F. W., Verstappen, P. A., de Boer, R. W., Bol, E., Erich, W. B., van der Togt, C. R., & de Vries, W. R. (1993). Gastrointestinal problems as a function of carbohydrate supplements and mode of exercise. *Medicine and science in sports and exercise*, 25(11), 1211–1224.

Pfeiffer, B., Stellingwerff, T., Hodgson, A. B., Randell, R., Pöttgen, K., Res, P., & Jeukendrup, A. E. (2012). Nutritional intake and gastrointestinal problems during competitive endurance events. *Medicine and science in sports and exercise*, 44(2), 344–351.  
<https://doi.org/10.1249/MSS.0b013e31822dc809>

Phillips, S. M., Green, H. J., Tarnopolsky, M. A., Heigenhauser, G. J., & Grant, S. M. (1996). Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *The American journal of physiology*, 270(2 Pt 1), E265–E272.  
<https://doi.org/10.1152/ajpendo.1996.270.2.E265>

Podlogar, T., Cirnski, S., Bokal, Š., Verdel, N., & Gonzalez, J. T. (2022). Addition of Fructose to a Carbohydrate-Rich Breakfast Improves Cycling Endurance Capacity in Trained Cyclists. *International journal of sport nutrition and exercise metabolism*, 32(6), 439–445.  
<https://doi.org/10.1123/ijsnem.2022-0067>

Podlogar, T., Cooper-Smith, N., Gonzalez, J.T. *et al* (2025) Personalised carbohydrate feeding during exercise based on exogenous glucose oxidation: a proof-of-concept study. *Perform. Nutr.* 1, 2. <https://doi.org/10.1186/s44410-025-00003-9>

Podlogar, T., & Wallis, G. A. (2022). New Horizons in Carbohydrate Research and Application for Endurance Athletes. *Sports medicine (Auckland, N.Z.)*, 52(Suppl 1), 5–23. <https://doi.org/10.1007/s40279-022-01757-1>

Prats, C., Graham, T. E., & Shearer, J. (2018). The dynamic life of the glycogen granule. *The Journal of biological chemistry*, 293(19), 7089–7098. <https://doi.org/10.1074/jbc.R117.802843>

Prats, C., Helge, J. W., Nordby, P., Qvortrup, K., Ploug, T., Dela, F., & Wojtaszewski, J. F. (2009). Dual regulation of muscle glycogen synthase during exercise by activation and compartmentalization. *The Journal of biological chemistry*, 284(23), 15692–15700. <https://doi.org/10.1074/jbc.M900845200>

Price, T. B., Rothman, D. L., Taylor, R., Avison, M. J., Shulman, G. I., & Shulman, R. G. (1994). Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *Journal of applied physiology (Bethesda, Md. : 1985)*, 76(1), 104–111. <https://doi.org/10.1152/jappl.1994.76.1.104>

Raben, A., Kiens, B., & Richter, E. A. (1994). Differences in glycaemia, hormonal response and energy expenditure after a meal rich in mono- and disaccharides compared to a meal rich in polysaccharides in physically fit and sedentary subjects. *Clinical physiology (Oxford, England)*, 14(3), 267–280. <https://doi.org/10.1111/j.1475-097x.1994.tb00384.x>

Rauch, L. H., Rodger, I., Wilson, G. R., Belonje, J. D., Dennis, S. C., Noakes, T. D., & Hawley, J. A. (1995). The effects of carbohydrate loading on muscle glycogen content and cycling performance. *International journal of sport nutrition*, 5(1), 25–36. <https://doi.org/10.1123/ijns.5.1.25>

Rehrer, N. J., Brouns, F., Beckers, E. J., ten Hoor, F., & Saris, W. H. (1990). Gastric emptying with repeated drinking during running and bicycling. *International journal of sports medicine*, 11(3), 238–243. <https://doi.org/10.1055/s-2007-1024799>

Rehrer, N. J., van Kemenade, M., Meester, W., Brouns, F., & Saris, W. H. (1992). Gastrointestinal complaints in relation to dietary intake in triathletes. *International journal of sport nutrition*, 2(1), 48–59. <https://doi.org/10.1123/ijasn.2.1.48>

Richter, E. A., Hansen, S. A., & Hansen, B. F. (1988). Mechanisms limiting glycogen storage in muscle during prolonged insulin stimulation. *The American journal of physiology*, 255(5 Pt 1), E621–E628. <https://doi.org/10.1152/ajpendo.1988.255.5.E621>

Roach, P. J., Depaoli-Roach, A. A., Hurley, T. D., & Tagliabracci, V. S. (2012). Glycogen and its metabolism: some new developments and old themes. *The Biochemical journal*, 441(3), 763–787. <https://doi.org/10.1042/BJ20111416>

Roberts, P. A., Fox, J., Peirce, N., Jones, S. W., Casey, A., & Greenhaff, P. L. (2016). Creatine ingestion augments dietary carbohydrate mediated muscle glycogen supercompensation during the initial 24 h of recovery following prolonged exhaustive exercise in humans. *Amino acids*, 48(8), 1831–1842. <https://doi.org/10.1007/s00726-016-2252-x>

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *The American journal of physiology*, 265(3 Pt 1), E380–E391. <https://doi.org/10.1152/ajpendo.1993.265.3.E380>

Rosdahl, H., Lindberg, T., Edin, F., & Nilsson, J. (2013). The Moxus Modular metabolic system evaluated with two sensors for ventilation against the Douglas bag method. *European journal of applied physiology*, 113(5), 1353–1367. <https://doi.org/10.1007/s00421-012-2551-1>

Rowlands, D. S., & Houltham, S. D. (2017). Multiple-Transportable Carbohydrate Effect on Long-Distance Triathlon Performance. *Medicine and science in sports and exercise*, 49(8), 1734–1744. <https://doi.org/10.1249/MSS.0000000000001278>

Saris, W.H.M., Van Erp-Baart, M.A., Brouns, F., Westerterp, K.R. and ten Hoor, F. (1989). Study on food intake and energy expenditure during extreme sustained exercise: the Tour de France. *International Journal of Sports Medicine*, 10(suppl. 1), S26–S31

Sasaki, H., Hotta, N., & Ishiko, T. (1991). Comparison of sympatho-adrenal activity during endurance exercise performed under high- and low-carbohydrate diet conditions. *The Journal of sports medicine and physical fitness*, 31(3), 407–412.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and science in sports and exercise*, 39(2), 377–390. <https://doi.org/10.1249/mss.0b013e31802ca597>

Schytz, C. T., Ørtenblad, N., Birkholm, T. A., Plomgaard, P., Nybo, L., Kolnes, K. J., Andersen, O. E., Lundby, C., Nielsen, J., & Gejl, K. D. (2023). Lowered muscle glycogen reduces body mass with no effect on short-term exercise performance in men. *Scandinavian journal of medicine & science in sports*, 33(7), 1054–1071. <https://doi.org/10.1111/sms.14354>

Schabort, E. J., Hawley, J. A., Hopkins, W. G., Mujika, I., & Noakes, T. D. (1998). A new reliable laboratory test of endurance performance for road cyclists. *Medicine and science in sports and exercise*, 30(12), 1744–1750. <https://doi.org/10.1097/00005768-199812000-00014>

Shearer, J., & Graham, T. E. (2004). Novel aspects of skeletal muscle glycogen and its regulation during rest and exercise. *Exercise and sport sciences reviews*, 32(3), 120–126. <https://doi.org/10.1097/00003677-200407000-00008>

Sherman, W. M., Brodowicz, G., Wright, D. A., Allen, W. K., Simonsen, J., & Dernbach, A. (1989). Effects of 4 h pre exercise carbohydrate feedings on cycling performance. *Medicine and science in sports and exercise*, 21(5), 598–604.

Sherman, W. M., Costill, D. L., Fink, W. J., Hagerman, F. C., Armstrong, L. E., & Murray, T. F. (1983). Effect of a 42.2-km footrace and subsequent rest or exercise on muscle glycogen and enzymes. *Journal of applied physiology: respiratory, environmental and exercise physiology*, 55(4), 1219–1224. <https://doi.org/10.1152/jappl.1983.55.4.1219>

Sherman, W. M., Costill, D. L., Fink, W. J., & Miller, J. M. (1981). Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. *International journal of sports medicine*, 2(2), 114–118. <https://doi.org/10.1055/s-2008-1034594>

Sherman, W. M., Peden, M. C., & Wright, D. A. (1991). Carbohydrate feedings 1 h before exercise improves cycling performance. *The American journal of clinical nutrition*, 54(5), 866–870. <https://doi.org/10.1093/ajcn/54.5.866>

Shiose, K., Takahashi, H., & Yamada, Y. (2022). Muscle Glycogen Assessment and Relationship with Body Hydration Status: A Narrative Review. *Nutrients*, 15(1), 155. <https://doi.org/10.3390/nu15010155>

Shiose, K., Yamada, Y., Motonaga, K., & Takahashi, H. (2018). Muscle glycogen depletion does not alter segmental extracellular and intracellular water distribution measured using bioimpedance spectroscopy. *Journal of applied physiology (Bethesda, Md. : 1985)*, 124(6), 1420–1425. <https://doi.org/10.1152/japplphysiol.00666.2017>

Shiose, K., Yamada, Y., Motonaga, K., Sagayama, H., Higaki, Y., Tanaka, H., & Takahashi, H. (2016). Segmental extracellular and intracellular water distribution and muscle glycogen after 72-h carbohydrate loading using spectroscopic techniques. *Journal of applied physiology (Bethesda, Md. : 1985)*, 121(1), 205–211. <https://doi.org/10.1152/japplphysiol.00126.2016>

Shiose, K., Yamada, Y., Motonaga, K., & Takahashi, H. (2018). Muscle glycogen depletion does not alter segmental extracellular and intracellular water distribution measured using bioimpedance spectroscopy. *Journal of applied physiology (Bethesda, Md. : 1985)*, 124(6), 1420–1425. <https://doi.org/10.1152/japplphysiol.00666.2017>

Smythe, C., & Cohen, P. (1991). The discovery of glycogenin and the priming mechanism for glycogen biogenesis. *European journal of biochemistry*, 200(3), 625–631. <https://doi.org/10.1111/j.1432-1033.1991.tb16225.x>

Stables, R. G., Kasper, A. M., Sparks, S. A., Morton, J. P., & Close, G. L. (2021). An Assessment of the Validity of the Remote Food Photography Method (Termed Snap-N-Send) in Experienced and Inexperienced Sport Nutritionists. *International journal of sport nutrition and exercise metabolism*, 31(2), 125–134. <https://doi.org/10.1123/ijsnem.2020-0216>

Stellingwerff, T., Boon, H., Gijzen, A. P., Stegen, J. H., Kuipers, H., & van Loon, L. J. (2007). Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use. *Pflugers Archiv : European journal of physiology*, 454(4), 635–647. <https://doi.org/10.1007/s00424-007-0236-0>

Stellingwerff, T., & Cox, G. R. (2014). Systematic review: Carbohydrate supplementation on exercise performance or capacity of varying durations. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*, 39(9), 998–1011. <https://doi.org/10.1139/apnm-2014-0027>

Stepto, N. K., Martin, D. T., Fallon, K. E., & Hawley, J. A. (2001). Metabolic demands of intense aerobic interval training in competitive cyclists. *Medicine and science in sports and exercise*, 33(2), 303–310. <https://doi.org/10.1097/00005768-200102000-00021>

Sterne, J.A.C., Savović, J., Page, M.J., Elbers, R.G., Blencowe, N.S., Boutron, I., Cates, C.J., Cheng, H-Y, Corbett, M.S., Eldridge, S.M., Hernán, M.A., Hopewell, S., Hróbjartsson, A., Junqueira, D.R., Jüni, P., Kirkham, J.J., Lasserson, T., Li, T., McAleenan, A., Reeves, B.C., Shepperd, S., Shrier, I., Stewart, L.A., Tilling, K., White, I.R., Whiting, P.F., Higgins, J.P.T. (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*, 366: 14898.

Stocks, B., Betts, J. A., & McGawley, K. (2016). Effects of carbohydrate dose and frequency on metabolism, gastrointestinal discomfort, and cross-country skiing performance. *Scandinavian journal of medicine & science in sports*, 26(9), 1100–1108. <https://doi.org/10.1111/sms.12544>

Tarnopolsky, L. J., MacDougall, J. D., Atkinson, S. A., Tarnopolsky, M. A., & Sutton, J. R. (1990). Gender differences in substrate for endurance exercise. *Journal of applied physiology* (Bethesda, Md. : 1985), 68(1), 302–308. <https://doi.org/10.1152/jappl.1990.68.1.302>

Tarnopolsky, M. A., Atkinson, S. A., Phillips, S. M., & MacDougall, J. D. (1995). Carbohydrate loading and metabolism during exercise in men and women. *Journal of applied physiology* (Bethesda, Md. : 1985), 78(4), 1360–1368. <https://doi.org/10.1152/jappl.1995.78.4.1360>

Tarnopolsky, M. A., & Ruby, B. C. (2001). Sex differences in carbohydrate metabolism. *Current opinion in clinical nutrition and metabolic care*, 4(6), 521–526. <https://doi.org/10.1097/00075197-200111000-00010>

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Medicine and science in sports and exercise*, 48(3), 543–568. <https://doi.org/10.1249/MSS.0000000000000852>

Tomcik, K. A., Camera, D. M., Bone, J. L., Ross, M. L., Jeacocke, N. A., Tachtsis, B., Senden, J., VAN Loon, L. J. C., Hawley, J. A., & Burke, L. M. (2018). Effects of Creatine and Carbohydrate Loading on Cycling Time Trial Performance. *Medicine and science in sports and exercise*, 50(1), 141–150. <https://doi.org/10.1249/MSS.0000000000001401>

Urdampilleta, A., Arribalzaga, S., Viribay, A., Castañeda-Babarro, A., Seco-Calvo, J., & Mielgo-Ayuso, J. (2020). Effects of 120 vs. 60 and 90 g/h Carbohydrate Intake during a Trail Marathon on Neuromuscular Function and High Intensity Run Capacity Recovery. *Nutrients*, 12(7), 2094. <https://doi.org/10.3390/nu12072094>

Vandenberghe, K., Hespel, P., Vanden Eynde, B., Lysens, R., & Richter, E. A. (1995). No effect of glycogen level on glycogen metabolism during high intensity exercise. *Medicine and science in sports and exercise*, 27(9), 1278–1283.

Van Hooren, B., Souren, T., & Bongers, B. C. (2024). Accuracy of respiratory gas variables, substrate, and energy use from 15 CPET systems during simulated and human exercise. *Scandinavian journal of medicine & science in sports*, 34 (1), e14490. <https://doi.org/10.1111/sms.14490>

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H., & Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of physiology*, 536(Pt 1), 295–304. <https://doi.org/10.1111/j.1469-7793.2001.00295.x>

van Loon, L. J., Saris, W. H., Kruijshoop, M., & Wagenmakers, A. J. (2000). Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *The American journal of clinical nutrition*, 72(1), 106–111. <https://doi.org/10.1093/ajcn/72.1.106>

Walker, J. L., Heigenhauser, G. J., Hultman, E., & Spriet, L. L. (2000). Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. *Journal of applied physiology* (Bethesda, Md. : 1985), 88(6), 2151–2158. <https://doi.org/10.1152/jappl.2000.88.6.2151>

Watt, M. J., Heigenhauser, G. J., & Spriet, L. L. (2002). Intramuscular triacylglycerol utilization in human skeletal muscle during exercise: is there a controversy?. *Journal of applied physiology* (Bethesda, Md. : 1985), 93(4), 1185–1195. <https://doi.org/10.1152/japplphysiol.00197.2002>

Westerterp, K. (2017) Control of energy expenditure in humans. *Eur J Clin Nutr*, 71, 340–344. <https://doi.org/10.1038/ejcn.2016.237>

Widrick, J. J., Costill, D. L., Fink, W. J., Hickey, M. S., McConell, G. K., & Tanaka, H. (1993). Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration. *Journal of applied physiology* (Bethesda, Md. : 1985), 74(6), 2998–3005. <https://doi.org/10.1152/jappl.1993.74.6.2998>

Williams, C., Brewer, J., & Walker, M. (1992). The effect of a high carbohydrate diet on running performance during a 30-km treadmill time trial. *European journal of applied physiology and occupational physiology*, 65(1), 18–24. <https://doi.org/10.1007/BF01466269>

Wilson P. B. (2017). Frequency of Chronic Gastrointestinal Distress in Runners: Validity and Reliability of a Retrospective Questionnaire. *International journal of sport nutrition and exercise metabolism*, 27(4), 370–376. <https://doi.org/10.1123/ijsnem.2016-0305>

Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L., & Hawley, J. A. (2008). Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *Journal of applied physiology (Bethesda, Md. : 1985)*, 105(5), 1462–1470. <https://doi.org/10.1152/jappphysiol.90882.2008>

Zachwieja, J. J., Costill, D. L., Pascoe, D. D., Robergs, R. A., & Fink, W. J. (1991). Influence of muscle glycogen depletion on the rate of resynthesis. *Medicine and science in sports and exercise*, 23(1), 44–48.

Zoladz, J. A., Rademaker, A. C., & Sargeant, A. J. (1995). Non-linear relationship between O<sub>2</sub> uptake and power output at high intensities of exercise in humans. *The Journal of physiology*, 488 (Pt 1) (Pt 1), 211–217. <https://doi.org/10.1113/jphysiol.1995.sp020959>

Zuntz, N. (1901) Ueber die Bedeutung der verschiedenen Nährstoffe als Erzeuger der Muskelkraft. *Pflüger, Arch.* 83, 557–571. <https://doi.org/10.1007/BF01746509>

**Chapter Ten:**  
**Appendices**

## **A) Systematic review and meta-analysis supplementary material**

### ***A.1. Regression models sensitivity analysis***

To determine whether inclusion of all study designs within the same regression models effected results, regression models were run for relative CHO intake and muscle glycogen with the inclusion of only repeated measures designs compared with results when all study designs were included. Repeated measures simple linear regression for endurance trained ( $F_{1,68} = 63.52$ ,  $r^2 = 0.483$ ,  $P < 0.0001$ ) and well trained ( $F_{1,22} = 2.26$ ,  $r^2 = 0.093$ ,  $P = 0.147$ ) were similar to results reported with inclusion of all study designs ( $F_{1,72} = 69.91$ ,  $r^2 = 0.493$ ,  $P < 0.0001$  and  $F_{1,22} = 2.71$ ,  $r^2 = 0.110$ ,  $P = 0.114$ , respectively). Indicating results were robust to the inclusion of different study designs within analysis. This was deemed more appropriate than excluding information from studies that utilised between subjects or mixed designs, as all other inclusion criteria were met and there was no other rationale for exclusion (e.g. measurement error).

To determine the most appropriate nonlinear regression model, additional analyses were conducted with third degree polynomial models for relative and absolute CHO intake. Assessment of the Akaike Information Criterion (AIC) and adjusted  $r^2$  indicated 2<sup>nd</sup> degree polynomial models best fitted the pooled data (Table 4.5). Third degree Polynomial models were unstable for absolute CHO intake, which was indicated by error messages in GraphPad.

**Table 4.5.** Sensitivity analysis to assess goodness of fit of nonlinear regression models used to describe the relationship between dietary CHO intake and muscle glycogen using studies included within systematic review descriptive synthesis.

| Polynomial Model       | CHO intake | Training status | df | $r^2$ | Adjusted $r^2$ | AIC   |
|------------------------|------------|-----------------|----|-------|----------------|-------|
| 2 <sup>nd</sup> degree | Relative   | Trained         | 71 | 0.520 | 0.507          | 726.1 |
| 3 <sup>rd</sup> degree |            |                 | 70 | 0.521 | 0.500          | 728.4 |
| 2 <sup>nd</sup> degree | Relative   | Well trained    | 21 | 0.123 | 0.039          | 246.0 |
| 3 <sup>rd</sup> degree |            |                 | 20 | 0.147 | 0.019          | 248.5 |
| 2 <sup>nd</sup> degree | Absolute   | Trained         | 71 | 0.500 | 0.486          | 729.2 |
| 3 <sup>rd</sup> degree |            |                 | 70 | 0.503 | 0.482          | 731.0 |
| 2 <sup>nd</sup> degree | Absolute   | Well trained    | 20 | 0.061 | -0.033         | 236.2 |
| 3 <sup>rd</sup> degree |            |                 | 19 | 0.062 | -0.086         | 239.5 |

## ***A.2. Meta-analysis imputations***

To quantify effect measures, mean difference and standard error of the mean difference was calculated using the equations below (Elbourne et al. 2002):

$$1) \ MD = M_E - M_C$$

$$2) \ SE \ (MD) = \frac{SD_{diff}}{\sqrt{n}}$$

Where  $MD$  is mean difference,  $M_E$  is the mean of experimental group and  $M_C$  is the mean of control group.  $SE$  is the standard error of the mean difference;  $SD_{diff}$  is the standard deviation of the differences and  $n$  is the number of participants. For parallel group trials, this was done using the calculator function in RevMan where between subject group means,  $n$  of participants and standard deviations were entered, and effect estimates were provided.

For crossover trials dependency (use of the same participants across experimental conditions) was accounted for to minimise unit of analysis error. Only two studies included within the analysis reported individual participant data (Rauch et al., 1995; Walker et al., 2000). Authors from the 11 remaining crossover design experiments were contacted, and individual participant data was requested. Two groups of authors provided individual participant data (Kavouras et al., 2004; Schytz et al., 2023), others stated that individual data was not available, or a response was not received. As the required statistics are rarely reported in published articles, imputations were required using the following equations based on the information available in published reports (Elbourne et al., 2002):

$$SD_{diff} = \sqrt{SD_E^2 + SD_C^2 - (2 \times Corr \times SD_E \times SD_C)}$$

Where  $SD_E$  and  $SD_C$  are the standard deviations of experimental and control group, respectively.  $Corr$  refers to a correlation coefficient which was borrowed from the studies which provided adequate statistical information (Rauch et al., 1995; Walker et al., 2000; Kavouras et al., 2004; Schytz et al., 2023). Correlation coefficient was calculated as shown below:

$$Corr = \frac{SD_E^2 + SD_C^2 - SD_{diff}^2}{2 \times SD_E \times SD_C}$$

A pooled borrowed correlation coefficient, taken from studies that provided individual participant information (Kavouras et al., 2004; Rauch et al., 1995; Schytz et al., 2023; Walker et al., 2000) was used for any studies where the necessary data was not available (Arkinstall et al., 2004; Burke et al., 2000; Costill et al., 1981; Hawley et al., 1997b; Sherman et al., 1981; Tomcik et al., 2018).

### A.3. Combining groups

If multiple groups within the mixed design studies used CHO loading interventions that fell within the same defined category of CHO intake (i.e. low-moderate or high-very high) these groups were combined to create a single pairwise comparison which was included within the meta-analysis. Groups were combined using the following equations:

$$M_{combined} = \frac{n_1 M_1 + n_2 M_2}{n_1 + n_2}$$

$$SD_{combined} = \sqrt{\frac{(n_1 - 1) SD_1^2 + (n_2 - 1) SD_2^2 + \frac{n_1 n_2}{n_1 + n_2} (M_1^2 + M_2^2 - 2M_1 M_2)}{n_1 + n_2 - 1}}$$

Where  $n$ ,  $M$ , and  $SD$  are number of participants, mean and standard deviation, respectively. Experimental conditions of Tarnopolsky et al. (1995) compared males versus females across two conditions where CHO intake for male and female conditions were defined as high and moderate, respectively. As such conditions within the same sex were combined (as above) to allow a single pairwise comparison of a moderate versus high CHO intake, which was a parallel groups comparison and was treated as such within the meta-analysis. Tarnopolsky et al. (2001) each compared males and females across 3 separate conditions of varied CHO intake. For males, conditions were defined as moderate, high and very high, with the high and very high conditions combined. For females conditions were moderate, moderate and high, with both moderate conditions combined. These groups were treated as independent crossover trials for the meta-analysis with dependency accounted for as previously described.

#### ***A.4. Sensitivity analysis: Fixed vs Random effects models and imputed correlation coefficients***

A sensitivity analysis was performed to ensure decisions regarding the type of model (fixed versus random effects) and the chosen correlation coefficient from imputations had minimal impact on overall results of the meta-analysis. This was done by running the analysis on two more occasions using lower and higher imputed correlation coefficients of 0.5499 and 0.9460 borrowed from data presented by Schytz et al. (2023) and Rauch et al. (1995), respectively. The results of this analysis are presented in Table 4.6.

**Table 4.6.** Sensitivity analysis summary of fixed versus random effects meta-analysis models with imputations using different borrowed correlation coefficients.

| Model                                                                   | Study design    | Correlation coefficient | n   | Mean diff (mmol·kg <sup>-1</sup> DM) | Low 95% CI | High 95% CI | Overall effect   | I <sup>2</sup> |
|-------------------------------------------------------------------------|-----------------|-------------------------|-----|--------------------------------------|------------|-------------|------------------|----------------|
| Fixed                                                                   |                 |                         |     |                                      |            |             |                  |                |
| effects                                                                 | Within groups   | 0.55                    | 252 | 185.6                                | 172.3      | 198.9       | <i>P</i> < 0.001 | 91%            |
|                                                                         |                 | 0.81                    | 252 | 190.3                                | 178.6      | 202.0       | <i>P</i> < 0.001 | 94%            |
|                                                                         |                 | 0.95                    | 252 | 182.6                                | 173.1      | 192.2       | <i>P</i> < 0.001 | 96%            |
|                                                                         | Parallel groups | N/A                     | 81  | 85.7                                 | 56.8       | 114.6       | <i>P</i> < 0.001 | 92%            |
|                                                                         |                 |                         |     |                                      |            |             |                  |                |
| Random                                                                  |                 |                         |     |                                      |            |             |                  |                |
| effects                                                                 | Within group    | 0.55                    | 252 | 200.0                                | 151.1      | 248.9       | <i>P</i> < 0.001 | 91%            |
|                                                                         |                 | 0.81                    | 252 | 198.8                                | 148.0      | 249.6       | <i>P</i> < 0.001 | 94%            |
|                                                                         |                 | 0.95                    | 252 | 198.0                                | 146.6      | 249.4       | <i>P</i> < 0.001 | 96%            |
|                                                                         | Parallel groups | N/A                     | 81  | 107.6                                | -10.5      | 225.7       | <i>P</i> = 0.07  | 92%            |
|                                                                         |                 |                         |     |                                      |            |             |                  |                |
| n, number of participants; CI, confidence interval; SE, standard error. |                 |                         |     |                                      |            |             |                  |                |