

Please cite the Published Version

Hannon, Sophie C , McStravick, James , Henthorn, Libby, Bawden, Stephen J , Tang, Jonathan CY , Dunn, Rachel, Makino, Ryosuke , Smith, Kenneth , Gonzalez, Javier T, Hodson, Nathan , Morton, James P , Kennerley, Aneurin J , and Hearnis, Mark A  (2025) Co-ingesting whey protein with dual-source carbohydrate enhances amino acid availability without compromising post-exercise liver glycogen resynthesis. *The Journal of Physiology*. JP288473 ISSN 0022-3751

DOI: <https://doi.org/10.1113/jp288473>

Publisher: Wiley

Version: Published Version

Downloaded from: <https://e-space.mmu.ac.uk/640988/>

Usage rights:  [Creative Commons: Attribution 4.0](https://creativecommons.org/licenses/by/4.0/)

Additional Information: This is an open access article published in *The Journal of Physiology*, by Wiley.

Data Access Statement: The data sets from the present study are available from the corresponding author upon reasonable request.

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

Co-ingesting whey protein with dual-source carbohydrate enhances amino acid availability without compromising post-exercise liver glycogen resynthesis

Sophie C. Hannon¹ , James McStravick¹ , Libby Henthorn¹, Stephen J. Bawden^{2,3,4} , Jonathan C. Y. Tang^{5,6} , Rachel Dunn^{5,6}, Ryosuke Makino⁷ , Kenneth Smith⁸ , Javier T. Gonzalez⁹, Nathan Hodson¹, James P. Morton¹⁰ , Aneurin J. Kennerley¹  and Mark A. Hearris¹ 

¹Institute of Sport, Department of Sports & Exercise Science, Manchester Metropolitan University, Manchester, UK

²NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust & The University of Nottingham, Nottingham, UK

³MRI Physics Group, Department of Medical Physics & Clinical Engineering, Singleton Hospital, Swansea, UK

⁴Institute of Life Sciences, Medical School, Swansea University, Swansea, UK

⁵Bioanalytical Facility, Norwich Medical School, University of East Anglia, Norwich, UK

⁶Department of Clinical Biochemistry, Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich, UK

⁷Department of Animal Science, Faculty of Agriculture, Iwate University, Iwate, Japan

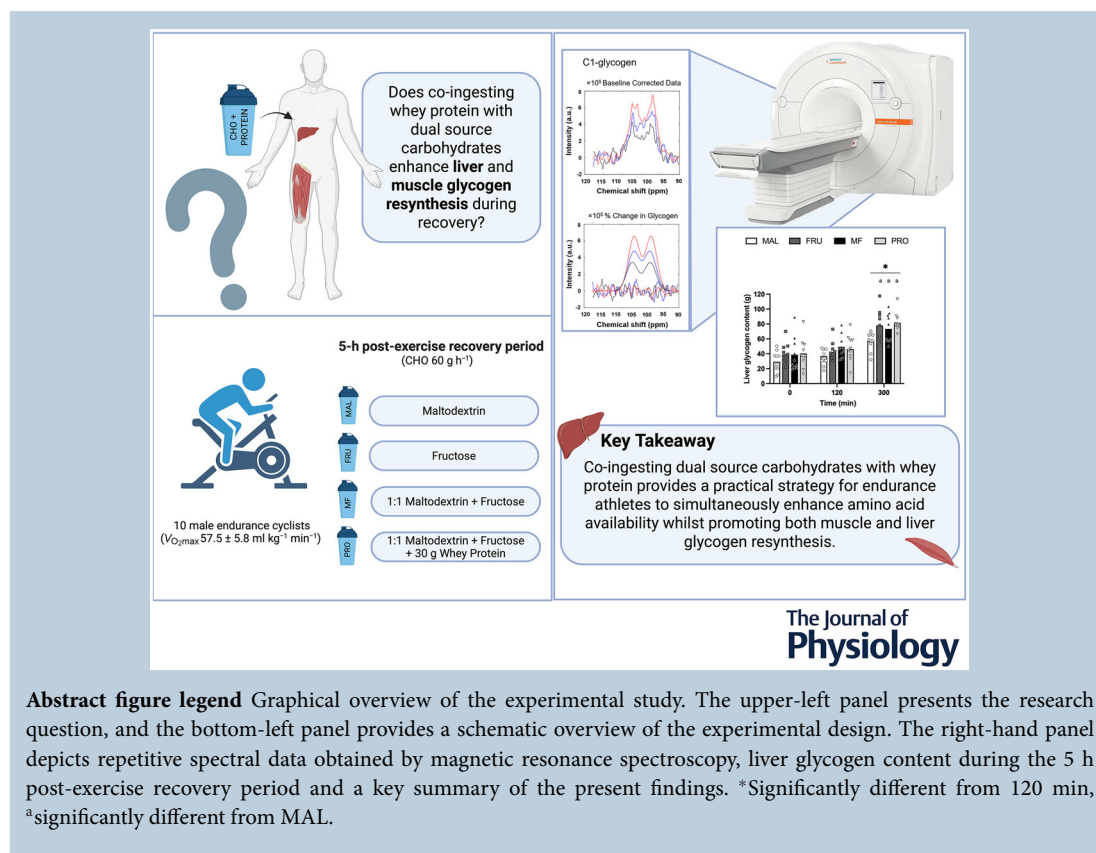
⁸Centre of Metabolism, Ageing & Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research and National Institute of Health Research, University of Nottingham, Derby, UK

⁹Centre for Nutrition & Exercise Metabolism, Department for Health, University of Bath, Bath, UK

¹⁰Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

Handling Editors: Paul Greenhaff & Bettina Mittendorfer

The peer review history is available in the Supporting Information section of this article (<https://doi.org/10.1113/JP288473#support-information-section>).



Abstract We examined the effects of ingesting maltodextrin and/or fructose with protein co-ingestion on post-exercise liver and muscle glycogen resynthesis. Following glycogen-depleting exercise, 10 well-trained male cyclists ingested 60 g h⁻¹ carbohydrate from either maltodextrin (MAL), fructose (FRU), 1:1 ratio of maltodextrin + fructose (MF) or 1:1 ratio of maltodextrin + fructose plus 30 g whey protein at 0 and 180 min (PRO) during a 5 h recovery period. ¹³C magnetic resonance spectroscopy and imaging were performed at 0, 120 and 300 min following exercise to determine liver and muscle glycogen concentrations and liver volume. Protein co-ingestion resulted in elevated serum insulin and plasma glucagon compared with FRU and MF ($P < 0.001$ for all). Similarly, serum insulin and plasma glucagon concentrations were markedly higher with MAL when compared with both FRU and MF ($P < 0.05$ for all), although plasma glucagon was also higher when compared with PRO ($P < 0.001$). Liver glycogen concentrations were significantly higher with FRU (275 ± 49 mmol L⁻¹), MF (255 ± 50 mmol L⁻¹) and PRO (283 ± 50 mmol L⁻¹) compared with MAL (204 ± 51 mmol L⁻¹) ($P < 0.05$ for all) following 5 h of recovery. However, muscle glycogen concentrations (mmol L⁻¹: MAL, 168 ± 33 ; FRU, 145 ± 32 ; MF, 151 ± 33 ; PRO 153 ± 33) were not different between trials ($P > 0.05$). We conclude that, despite enhancing glucagonaemia, co-ingestion of whey protein (to a 1:1 combination of maltodextrin and fructose) does not compromise post-exercise liver glycogen resynthesis, allowing for increased aminoacidaemia alongside rapid glycogen resynthesis.

(Received 24 January 2025; accepted after revision 19 June 2025; first published online 9 July 2025)

Corresponding author Mark Hearris: Institute of Sport, Department of Sports & Exercise Science, Manchester Metropolitan University, Manchester, M1 7EL, UK. Email: M.Hearris@mmu.ac.uk

Key points

- Endurance athletes commonly co-ingest carbohydrate and protein within the post-exercise recovery period to facilitate rapid glycogen repletion and muscle remodelling.
- Here we report that the ingestion of dual-source carbohydrate (a 1:1 ratio of maltodextrin and fructose) enhances liver glycogen repletion when compared with maltodextrin alone.
- Co-ingesting whey protein alongside this dual-source carbohydrate enhanced amino acid availability without compromising liver glycogen resynthesis, despite enhanced glucagonaemia.
- These data demonstrate that the co-ingestion of whey protein with dual-source carbohydrate provides a practical strategy to enhance amino acid availability (which provides an important substrate for post-exercise muscle remodelling) and rapid glycogen resynthesis.

Introduction

Endogenous and exogenous carbohydrate (CHO) provides an important substrate for energy production during prolonged moderate- to high-intensity (endurance) exercise (Bergström et al., 1967; Romijn et al.,

1993; van Loon et al., 2001). Despite this, the endogenous storage pool of CHO within the liver and skeletal muscle are relatively limited (Gonzalez et al., 2016). Whilst these stores only represent <5% of total energy stored, muscle glycogen contributes >50% towards total energy requirements during moderate- to high-intensity

Sophie Hannon is currently completing her PhD in carbohydrate metabolism at Manchester Metropolitan University, where she employs the use of non-invasive magnetic resonance spectroscopy to conduct her research. In addition to her academic research, Sophie is an accredited nutritionist with both the Sport and Exercise Nutrition Register (SENr) and the Association for Nutrition (AfN). She has applied experience providing nutritional support to athletes in both road cycling and team sports.



exercise (Romijn et al., 1993; van Loon et al., 2001) whereas liver-derived plasma glucose accounts for ~18% (van Loon et al., 2001). Considering this, endogenous CHO stores become significantly depleted following prolonged exercise (Casey et al., 2000; Fell et al., 2021) in an intensity- and duration-dependent manner, with the onset of fatigue strongly associated with glycogen depletion (Alghannam et al., 2016; Bergström et al., 1967; Casey et al., 2000). As such, the restoration of muscle and liver glycogen is regarded as a primary determinant of recovery between successive exercise bouts and subsequent exercise capacity (Casey et al., 2000; Grey et al., 2020; Williams et al., 2003). In particular, for athletes who have limited recovery time between exercise bouts, such as those competing in Grand Tours (cycling) or multi-day tournaments, the rapid repletion of endogenous CHO stores is recognized as a key performance priority that requires specific nutritional intervention.

In relation to muscle glycogen resynthesis, previous studies have demonstrated that the ingestion of $1.2 \text{ g kg}^{-1} \text{ h}^{-1}$ of glucose (polymers) immediately after exercise elicits maximal rates of glycogen synthesis, with no additional benefit of higher ingestion rates (Howarth et al., 2009). Furthermore, the use of dual-source CHOs (e.g. a combination of glucose and fructose or sucrose), which are considered to enhance intestinal CHO absorption, does not provide any additional benefit to muscle glycogen repletion (Casey et al., 2000; Fuchs et al., 2016; Wallis et al., 2008). However, the utility of dual-source CHOs is probably more relevant to liver glycogen resynthesis given that fructose is preferentially metabolized and retained in the liver (McGuinness & Cherrington, 2003). Indeed, two previous studies have reported significant increases in liver glycogen repletion following endurance exercise with the co-ingestion of glucose and fructose (or sucrose) when compared with glucose (polymers) alone, largely independent of the total amount of CHO ingested (Décombaz et al., 2011; Fuchs et al., 2016).

In addition to CHO, athletes are recommended to consume 20–30 g of high-quality protein at regular intervals throughout the recovery period (Thomas et al., 2016). Whilst protein co-ingestion provides essential amino acids to promote muscle protein synthesis (MPS) and subsequent muscle reconditioning (Churchward-Venne et al., 2020; Moore et al., 2009), protein also augments the insulin response to carbohydrate feeding (Jentjens et al., 2001; Van Loon, Saris, Kruijshoop et al., 2000; Van Loon, Saris, Verhagen et al., 2000) and can enhance muscle glycogen storage in response to suboptimal CHO intake ($0.8 \text{ g kg}^{-1} \text{ h}^{-1}$) (Van Loon, Saris, Kruijshoop et al., 2000). The increased insulin response to protein co-ingestion

may also play an important role in liver glycogen repletion by promoting hepatic glucose storage (Roden et al., 1996) and providing glucogenic amino acids that can be used as precursors for glycogen synthesis (Kurland & Pilkis, 1989). However, the co-ingestion of protein also enhances glucagon secretion (Van Hall et al., 2000), which is known to negatively regulate liver glycogen resynthesis (Roden et al., 1996). Indeed, inhibiting glucagon secretion during a hyperinsulinaemic clamp results in greater liver glycogen repletion than under basal glucagon conditions (Roden et al., 1996). Accordingly, it is possible that protein-induced glucagonaemia may impair liver glycogen synthesis rates, raising potential trade-offs for athletes wishing to optimize glycogen resynthesis alongside muscle reconditioning via amino acid availability.

To date, only one study has investigated the effect of post-exercise protein ingestion, demonstrating comparable increases in liver glycogen resynthesis in response to co-ingestion with suboptimal CHO intake ($0.8 \text{ g kg}^{-1} \text{ h}^{-1}$) containing a combination of maltodextrin and galactose when compared to CHO alone ($1.2 \text{ g kg}^{-1} \text{ h}^{-1}$) (Detko et al., 2013). It is noteworthy, however, that the post-exercise feeding strategies used in their study, which provide repeated small boluses of protein (enriched with high concentrations of insulinotropic amino acids) at 30 min intervals, are not reflective of the real-world feeding strategies adopted by elite endurance athletes. Furthermore, given that a fraction of the ingested CHO was replaced with protein to ensure treatments were energy matched, the effect of protein co-ingestion on liver glycogen synthesis under conditions of matched CHO intake, which more accurately reflect athlete practices, is still unknown. As such, an examination of post-exercise liver glycogen synthesis in response to protein co-ingestion using post-exercise feeding strategies that are reflective of real-world athlete practices is now warranted.

Accordingly, the primary aim of the present study was to quantify differences in both muscle and liver glycogen in response to dual-source carbohydrate when co-ingested with 30 g of whey protein following a 5 h post-exercise recovery period, using non-invasive ^{13}C magnetic resonance spectroscopy (MRS). To achieve our aim, trained male cyclists completed a bout of glycogen-depleting cycling and, during a 5 h recovery period, ingested 60 g h^{-1} of CHO from either: (1) maltodextrin, (2) fructose, (3) maltodextrin + fructose or (4) maltodextrin + fructose + whey protein. We hypothesized that the co-ingestion of whey protein would enhance plasma glucagonaemia and subsequently impair liver glycogen repletion.

Methodology

Ethical approval

All participants were fully informed of the experimental procedures and potential risks associated with the study before providing written informed consent prior to participation. All trials were conducted at Manchester Metropolitan University (MMU) Institute of Sport (Manchester, UK) following approval from MMU Faculty of Science and Engineering Research Ethics and Governance Committee (EthOS ID: 48959) in accordance with the latest revision of the *Declaration of Helsinki* (apart from registration in a publicly accessible database).

Participants

Ten endurance-trained amateur male cyclists (mean $\pm$ SD: age 29 ± 7 years; body mass 78.5 ± 6.4 kg; stature 181.9 ± 4.7 cm) volunteered to participate in the study. Mean maximal oxygen consumption ($\dot{V}_{O_{2\max}}$), peak power output (PPO) and power output at lactate threshold were 57.5 ± 5.8 mL kg⁻¹ min⁻¹, 389 ± 27 W and 172 ± 18 W, respectively. Subjects were defined as trained (Tier 2) or highly trained (Tier 3) in accordance with the criteria specified by McKay et al. (2022). Sample size was determined *a priori* and utilized previous liver glycogen data by Décombaz et al. (2013). Based on these data, the expected effect size was calculated from the difference in post-exercise liver glycogen content after the ingestion of a mixture of maltodextrin and fructose (52 ± 23 g) versus glucose polymer (23 ± 9 g). These data provide an effect size of $F = 0.725$ (converted from Cohen's $d_z = 1.45$), deeming a sample size of 10 participants sufficient to provide statistical power above 0.80 with an α -level of 0.05. This calculation is based on an F-test for repeated-measures ANOVA (within factors) with one group (reflecting the repeated-measures design), four measurements (corresponding to the four experimental treatments assessed at 300 min), a correlation among repeated measures of 0.5 and a non-sphericity correction (ϵ) of 0.75 (G*Power, Version 3.1, Düsseldorf, Germany). Exclusion criteria included a history of metabolic disease or pharmacological treatment during the testing period. All exclusion criteria were based on self-reporting.

Experimental overview

In a repeated-measures, counterbalanced, randomized crossover design, participants completed an exhaustive bout of glycogen-depleting exercise and subsequently ingested 60 g h⁻¹ CHO from either maltodextrin (MAL), fructose (FRU), a 1:1 ratio of maltodextrin + fructose (MF) or a 1:1 ratio of maltodextrin + fructose + whey protein (PRO; 30 g protein consumed at $t = 0$

and $t = 180$ min only) during a 5 h recovery period ($t = 0$ to $t = 300$ min). During this period, magnetic resonance imaging (MRI) and spectroscopy (MRS) were acquired immediately following exercise ($t = 0$ min) and at 2 h ($t = 120$ min) and 5 h ($t = 300$ min) following exercise, to establish liver volume and both muscle and liver glycogen concentrations. Blood samples and gastrointestinal symptoms (GIS) were also obtained throughout this period. Each experimental trial commenced after 24 h of standardized dietary intake. Figure 1 provides an overview of the experimental design.

Preliminary testing

At least 1 week prior to the first experimental trial, participants completed a two-stage incremental test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine lactate threshold (LT), maximal oxygen uptake ($\dot{V}_{O_{2\max}}$) and PPO as previously described (Fell et al., 2021). Briefly, participants commenced the first part of the incremental cycling test at 100 W, which increased by 25 W every 4 min. Capillary fingertip blood samples were obtained during the final ~ 30 s of each stage to assess blood lactate concentrations (Biosen C-Line, EKF Diagnostics, Cardiff, UK), and LT and lactate turnpoint (LTP) were defined as the first and second sustained increase in blood lactate concentrations above the baseline concentration respectively (Hofmann & Tschakert, 2017). After a 10 min rest, the second part of the incremental test commenced at the intensity of the penultimate 4 min stage from part one which increased by 25 W every minute until volitional exhaustion. $\dot{V}_{O_{2\max}}$ was established from the highest average 30 s period of oxygen consumption captured. The final power output and time at exhaustion enabled PPO to be calculated with the following equation (Kuipers et al., 1985):

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

where W_{final} is the final power output, t is the time spent in the final uncompleted stage (s), 60 is the duration of each stage (s) and PI is the increase in power output (W) between stages.

Pre-experimental controls

Participants were provided with a pre-packaged standardized diet (three meals, snacks and drinks), consisting of 6 g kg⁻¹ carbohydrate, 2 g kg⁻¹ protein and 0.7 g kg⁻¹ fat to consume 24 h prior to each experimental trial. Participants were also asked to refrain from physical activity, alcohol and caffeine consumption during this 24 h period to ensure appropriate standardization between trials.

Experimental protocol

Participants reported to the laboratory at ~08.00 h following a 12 h overnight fast and completed a bout of intermittent glycogen-depleting exercise (described below) to deplete both muscle and liver glycogen. Upon cessation of exercise, participants underwent baseline MRI and MRS measurements (Fig. 1) to establish liver volume and both muscle and liver glycogen concentrations. Participants took a 10 min shower and then had an indwelling cannula (BD Nexiva closed i.v. catheter system, Becton Dickinson Infusion Therapy Systems Inc, UT, USA) inserted into the antecubital vein before the recovery period commenced. Following the resting blood sample, participants completed GIS ratings before consuming the first experimental drink ($t = 0$ min). During the 5 h recovery period, drinks were provided every 60 min until $t = 240$ min. Blood samples were collected at 15 min intervals for the first 90 min and at 30 min intervals thereafter whilst GIS were taken

every 30 min until $t = 300$ min. Further MR scans were completed at $t = 120$ and $t = 300$ min, but no blood sample or ratings of GIS were acquired during the MR scan at $t = 150$ min.

Glycogen depletion protocol

Following a 10 min warm up at 50% PPO, participants completed a bout of intermittent glycogen-depleting cycling as previously described (Hearris et al., 2019). Briefly, participants cycled for 2 min at 90% PPO, followed immediately by a 2 min recovery period at 50% PPO, with this pattern of exercise being repeated until the desired intensity could no longer be sustained for 2 min. At this point, the duration of the work interval was reduced to 1 min interspersed with a 2 min recovery period at 50% PPO. Once participants were no longer able to complete 1 min cycling at 90% PPO, the exercise intensity was reduced to 80% PPO. This pattern of exercise continued

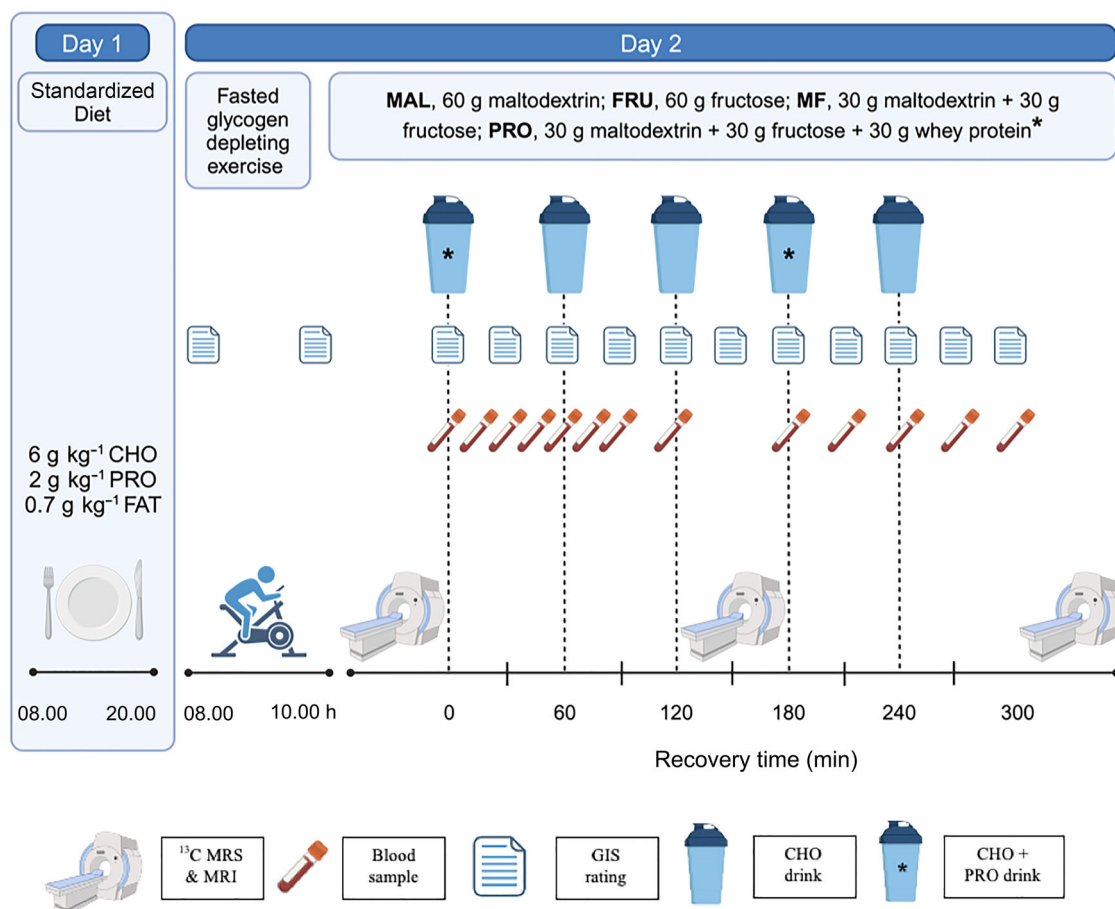


Figure 1. Schematic overview of the experimental design and nutritional protocols for each trial Following 24 h of dietary standardization, participants completed a bout of glycogen-depleting exercise in the fasted state. Upon completion, participants ingested 60 g h⁻¹ CHO from either maltodextrin (MAL), fructose (FRU), a 1:1 ratio of maltodextrin and fructose (MF) or a 1:1 ratio of maltodextrin and fructose plus 30 g whey protein at 0 and 180 min (PRO). ¹³C MRS and MRI were performed at $t = 0$, $t = 120$ and $t = 300$ min after depletion.

through to 70% and finally 60% PPO until subjects were unable to complete 1 min cycling at 60% PPO, at which point the exercise protocol was terminated. The pattern of exercise, total time to exhaustion and *ad libitum* water intake in the initial trial were recorded and replicated in all subsequent trials. All exercise trials were performed at the same time of day and under normal laboratory conditions ($20.1 \pm 1.3^\circ\text{C}$; $45 \pm 9\%$ humidity).

Carbohydrate beverages

Following the initial MR scan ($t = 0$ min), participants were provided with a carbohydrate-based recovery drink containing either 60 g maltodextrin (MAL), 60 g fructose (FRU), 30 g maltodextrin + 30 g fructose (MF) or 30 g maltodextrin + 30 g fructose + 30 g whey protein (PRO) (Science in Sport PLC, Blackburn, UK), mixed with 500 mL of water and 550 mg of sodium chloride (Science in Sport) to ingest at 60 min intervals during the 5 h recovery period ($t = 0$ to $t = 240$ min). Whey protein was only included in the PRO trial drink at $t = 0$ and $t = 180$ min to mimic the real-world feeding practices of endurance cyclists (Fig. 1).

Acquisition and measurement of liver and muscle glycogen concentrations

Glycogen concentrations were determined from the natural abundance signal of the C-1 carbon of glycogen at a frequency of ~ 100 ppm using a Siemens MAGNETOM Vida 3T MR system (Siemens Healthcare GmbH, Erlangen, Germany). A dual-channel $^1\text{H}/^{13}\text{C}$ transmit–receive flexible surface coil (01365; Rapid Biomedical GmbH, Rimpfing, Germany) was placed over the right lobe of the liver for liver glycogen measurements and the widest part of the vastus lateralis muscle for muscle glycogen assessment. The coil was secured with fabric straps to minimize movement, and automated 3D shimming ensured magnetic field uniformity over the active coil volume. Scout images were obtained at the start of each scan to confirm optimal coil position relative to the target tissue. The B_1 profile of the ^{13}C surface coil provided tissue localization and ^{13}C MRS was acquired using a standard pulse-acquired sequence with no decoupling (TR 200 ms, bandwidth 16 kHz, 4096 averages and 2048 spectral points). This simple pulse acquired sequence was used to ensure compatibility across a range of MR systems. The spectral window was centred on the glycogen C-1 peak (~ 100 ppm). Data acquisition lasted 15 min for each tissue site. Automated frequency, power adjustments and shim optimization ensured high spectral quality. Variability in ^{13}C glycogen was minimized by repositioning the coil in the same position for each acquisition in accordance with specific anatomical

landmarks and confirmed by obtaining scout images prior to each measurement. The distance between the coil and the target tissue (i.e. liver or muscle) within each participant was assumed to remain constant throughout the study (e.g. 4–6 weeks) as no changes in adipose tissue would be expected within our population of well-trained cyclists.

Data were processed offline using MATLAB 2024a (The MathWorks, Natick, MA, USA) with in-house software scripts. After applying 5 Hz line broadening, spectra were manually phase corrected and fitted using non-linear least squares with a Levenberg–Marquardt algorithm, employing Gaussian functions to model the coupled glycogen C-1 peak (Fig. 2). A 2 L glycogen phantom (100 mmol glycogen) was created to scale the measured glycogen signals and quantify *in vivo* glycogen concentrations. Due to the potential for variability in glycogen particle size, the molar mass of a single glycosyl unit (162 g mol^{-1}) was used to determine the mass of glycogen required to produce a 100 mmol concentration. As such, glycogen concentrations throughout this paper are presented as mmol of glycosyl units. Signal quantification was performed by comparing the area under the curve (AUC) to both a 50 mmol acetate fiducial marker (secured on the opposite coil surface for reference) and the 100 mmol glycogen phantom. Total liver glycogen content was calculated by multiplying liver glycogen concentration by individual liver volume (see below).

Measurement of liver volume

Liver volume was assessed using ^1H MRI scans. A Spine 32 and Body 18 array coil was used to acquire high-resolution 3D T2-weighted images of the liver. Volumetric segmentation of the liver was performed using 3D Slicer software with an artificial intelligence-based tool (Total segmentator) for precise volume extraction. The intra-individual coefficient of variation (CV) of duplicate liver volume measurements using Total segmentator was 1.5%.

Calculation of liver glycogen content

Total liver glycogen content (LGC) was calculated from the following equation:

$$\text{LGC (g)} = \frac{162 \times V_L \times [\text{Glyc}]}{1000}$$

where 162 represents the molar mass of a glycosyl unit (g mol^{-1}), V_L is liver volume (L) and *Glyc* is glycogen concentration (mM). Whilst glycogen concentrations are presented as mmol of glycosyl units (described above), LGC represents the total mass of glycogen in grams and is irrespective of changes in glycogen particle size.

Blood sampling and analysis

Venous blood samples were collected into K₂ EDTA and serum separation Vacutainers (BD Biosciences, Plymouth, UK) and either stored on ice or left to clot at room temperature for 30 min before centrifugation at 1500 g for 10 min at 4°C. Plasma and serum were aliquoted and stored at −80°C for subsequent analysis. Plasma samples were analysed for glucose, lactate and non-esterified fatty acids (NEFAs) using commercially available enzymatic spectrophotometric assays (Randox Laboratories Ltd, Crumlin, UK) and amino acids using GC-MS (TRACETM 1300 ISQ GC MS, Thermo Fisher Scientific, Waltham, MA, USA) using the internal standard method (Bukhari et al., 2015). Intra-assay CVs were 1.1, 1.2 and 0.8% for glucose, lactate and NEFAs, respectively. Serum insulin was measured using enzyme-linked immunoassay (Mercodia Insulin ELISA, Mercodia AB, Uppsala, Sweden). The inter-assay CVs were between 2.6 and 6.6% (over the assay range of 3–200 mU L^{−1}). Plasma glucagon was analysed using the Glucagon Quantikine ELISA Kit (DGCG0, Bio-Techne, Minneapolis, MN, USA) and processed by the DYNEX Agility automated ELISA system (DYNEX Technologies, Chantilly, VA, USA) according to the manufacturer's instructions. The inter-assay CVs were between 6.3 and 8.7% (over the assay range 31.3–2000 pg mL^{−1}).

Gastrointestinal symptoms

The severity of GIS (nausea, regurgitation, fullness, cramps, gas and urge to defecate) were recorded after the initial MR scan ($t = 0$ min) and at 30 min intervals during the 5 h recovery period using a 0–10 visual analogue scale (0 = no discomfort, 10 = unbearable discomfort) (Wilson, 2017). The sum of scores at each time point was collated for each gastrointestinal symptom.

Statistical analysis

All statistical analyses were performed using SPSS Statistics Version 29 (IBM, USA). A linear mixed model with random intercepts (to account for repeated measurements within subjects) and fixed effects for baseline, treatment, time and treatment $\times$ time interaction was used to analyse differences in muscle and liver glycogen concentrations, liver glycogen content and liver volume over time (e.g. at 120 and 300 min) and between treatments. A separate linear mixed model with random intercepts and fixed effects for treatment, time and treatment $\times$ time interaction was used to analyse differences over time (e.g. 0–300 min) and between treatments for plasma metabolites, amino acids and hormone concentrations. AUC was calculated using the trapezoid method and differences between treatments

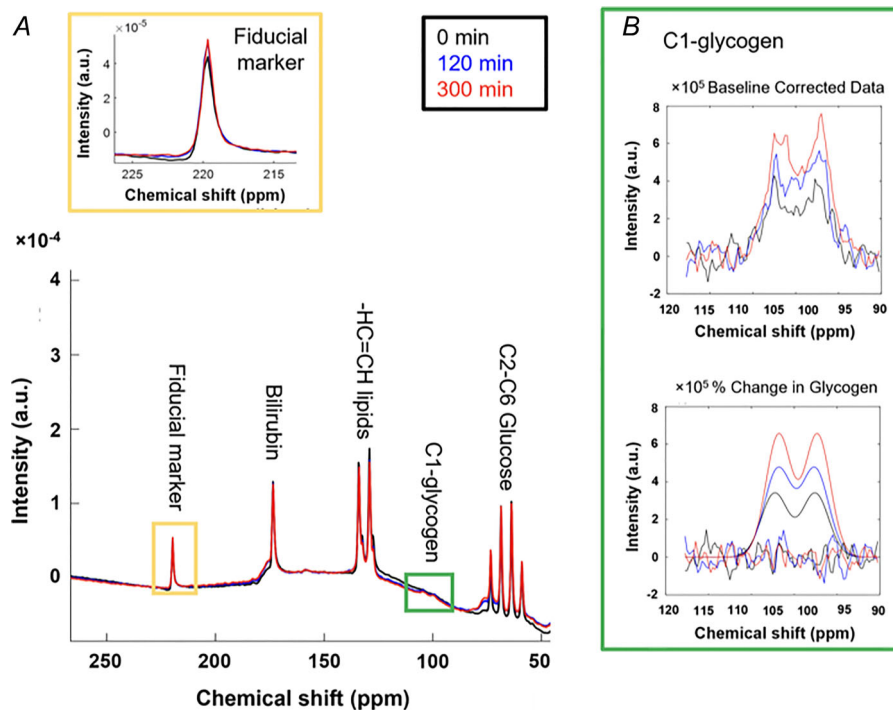


Figure 2. Labeled raw spectral data (A; baseline uncorrected) obtained by magnetic resonance spectroscopy (MRS; 3.0 T) at 0 (black), 120 (blue) and 300 (red) min of post-exercise recovery. Insert B shows the resultant coupled glycogen peaks at ~100 ppm. The baseline signal has been removed (fitted to a simple polynomial). Model data are displayed with the residual fit.

were assessed using a one-way repeated-measures ANOVA. For AUC analysis, data were imputed using linear regression modelling for the data points that were missing for the two incomplete FRU trials ($n = 8$). Pairwise comparisons were analysed using *post hoc* least significant difference (LSD) tests to locate specific differences (Parker & Weir, 2020) where a significant main effect or interaction was observed. In accordance with our primary outcome measure (i.e. glycogen concentrations and content at 300 min), *post hoc* pairwise comparisons between treatments at 300 min were analysed using LSD tests in the absence of a statistically significant treatment $\times$ time interaction. Ratings of gastrointestinal symptoms were analysed using the Friedman rank test with *post hoc* Wilcoxon's signed rank test. Due to technical issues with blood sampling (failed cannulae) all blood-related outcome measures are presented as $n = 9$. For outcome measures that include adjustments for baseline (i.e. liver volume, liver glycogen content, and liver and muscle glycogen concentrations) data in the text, figures and tables are presented as estimated marginal means $\pm$ SD. For all other outcomes, data in the text, figures and tables are presented as means $\pm$ SD with P values ≤ 0.05 indicating statistical significance.

Results

Glycogen depletion protocol

Mean PPO measured during preliminary testing was 390 ± 29 W (5.0 ± 0.4 W kg⁻¹). Consequently, mean power output during the depletion protocol was 351 ± 26 W (90% PPO), 312 ± 23 W (80% PPO), 273 ± 20 W (70% PPO), 234 ± 17 W (60% PPO) and 195 ± 14 W (50% PPO). Total mean cycling time was 82 ± 25 min (plus a 10 min warmup at 50% PPO), with high-intensity stages between 90 and 60% PPO averaging 36 ± 10 min.

Plasma metabolites

Plasma glucose concentrations rose to a greater extent in response to MAL and MF (treatment $\times$ time interaction, $P < 0.001$) when compared with both FRU and PRO trials ($P < 0.001$ for all) (Fig. 3A). Furthermore, the rise in plasma glucose was significantly higher in response to PRO when compared with FRU ($P = 0.028$). Accordingly, plasma glucose AUC was significantly higher in response to MAL and MF when compared with both FRU ($P = 0.010$ and 0.009 , respectively) and PRO ($P = 0.023$ and 0.011 , respectively) (Fig. 3B) yet no differences between PRO and FRU trials were present ($P = 0.210$).

The rise in plasma lactate concentrations was significantly highest in response to FRU (treatment $\times$ time interaction, $P < 0.001$) when compared with MAL, MF and PRO trials ($P < 0.001$ for all) (Fig. 3C). Plasma lactate concentrations were also higher following MF when compared with MAL ($P < 0.001$) and PRO ($P = 0.002$) trials and higher in response to PRO when compared with MAL ($P < 0.001$). As such, lactate AUC was significantly higher in response to FRU ($P < 0.001$), MF ($P = 0.004$) and PRO ($P = 0.013$) when compared with MAL. Moreover, lactate AUC was significantly higher following FRU and MF when compared with PRO ($P = 0.010$ and 0.044 , respectively) (Fig. 3D).

Plasma NEFA concentrations were significantly elevated throughout the recovery period in response to FRU (treatment $\times$ time interaction, $P < 0.001$) when compared to all other treatments ($P < 0.001$ for all) with no significant differences between the MAL, MF or PRO trials ($P > 0.05$ for all; Fig. 3E). Plasma NEFA AUC was significantly higher following FRU when compared with MAL ($P = 0.001$), MF ($P = 0.004$) and PRO ($P = 0.001$) trials, respectively (Fig. 3F).

Serum insulin

The rise in serum insulin concentrations was significantly higher following both MAL and PRO (treatment $\times$ time interaction, $P = 0.022$) when compared with FRU ($P < 0.001$ for both) and MF ($P = 0.004$ and $P < 0.001$, respectively; Fig. 4A). The rise in serum insulin was also higher following MF when compared with FRU ($P < 0.001$). Accordingly, serum insulin AUC was significantly higher in response to both MAL and PRO when compared with FRU ($P < 0.001$ and $P = 0.011$, respectively) and MF ($P = 0.018$ and $P = 0.049$, respectively; Fig. 4B). Serum insulin AUC was also significantly higher in response to MF compared with FRU ($P = 0.003$).

Plasma glucagon

The rise in plasma glucagon concentrations was significantly higher following MAL (treatment $\times$ time interaction, $P < 0.001$) when compared with FRU, MF and PRO ($P < 0.001$ for all) (Fig. 4C). Plasma glucagon concentrations were also higher following PRO when compared with both FRU and MF ($P < 0.001$ for all) and following FRU when compared with MF ($P < 0.001$). Plasma glucagon AUC was significantly higher in response to MAL and PRO trials when compared with both FRU ($P = 0.002$ and $P = 0.011$) and MF trials ($P < 0.001$ for both) (Fig. 4D). Plasma glucagon AUC was also higher in response to FRU when compared with MF ($P = 0.005$).

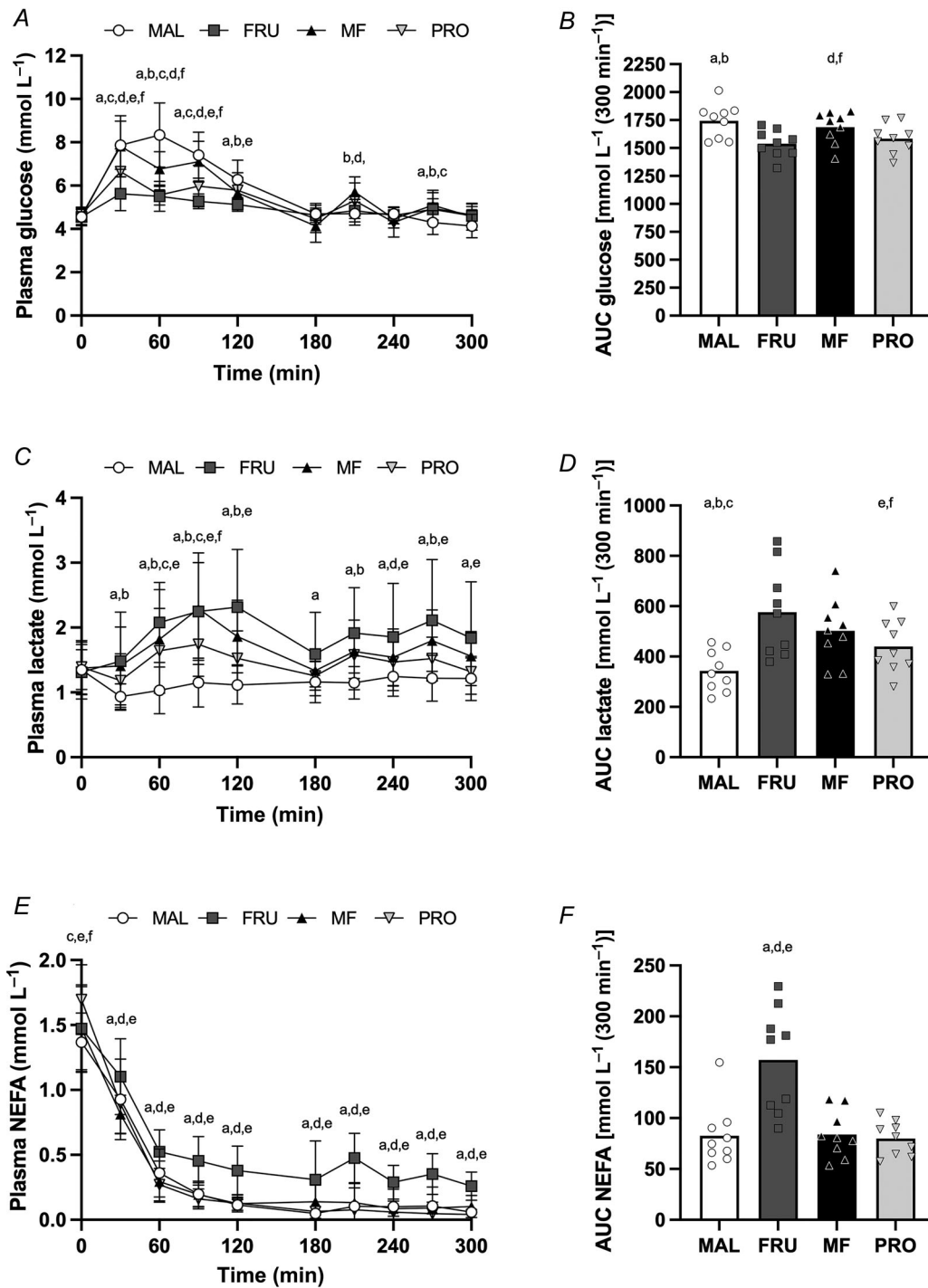


Figure 3. Plasma glucose (A), lactate (C) and non-esterified fatty acids (NEFA) (E) during the 5 h post-exercise recovery period and total area under the curve (AUC) for plasma glucose (B), lactate (D) and NEFA (F) in response to either maltodextrin (MAL), fructose (FRU), maltodextrin + fructose (MF) or maltodextrin + fructose + protein (PRO); $n = 9$ for all blood metabolites

^aMAL and FRU significantly different, ^bMAL and MF significantly different, ^cMAL and PRO significantly different, ^dFRU and MF significantly different, ^eFRU and PRO significantly different, ^fMF and PRO significantly different.

Plasma amino acid concentrations

Plasma essential amino acid (EAA) and branched-chain amino acid (BCAA) concentrations are presented in Fig. 5. Due to technical difficulties, quantifiable histidine peaks were not obtained and therefore EAAs are presented with the exception of histidine. Total AUC for BCAAs was significantly higher in response to the PRO trial when compared with MAL, FRU and MF trials, respectively ($P < 0.001$ for all) (Fig. 5B). Total AUC for BCAAs was also significantly higher in response to the FRU trial when compared with the MF trial ($P = 0.004$). Similarly, total AUC for EAAs was significantly higher in response to the PRO trial when compared with MAL, FRU and MF trials, respectively ($P < 0.001$ for all) (Fig. 5D). Furthermore, total AUC for EAAs was significantly higher in response to the FRU trial when compared with both MAL ($P = 0.002$) and MF trials ($P = 0.015$).

Liver glycogen concentrations

Significant time and treatment effects (both $P < 0.001$) were observed for liver glycogen concentrations, although no significant time $\times$ treatment interaction was observed ($P = 0.132$) (Fig. 6A). Liver glycogen concentrations increased significantly across the recovery period and were significantly higher at 300 min when compared with 120 min ($P < 0.001$). Furthermore, liver glycogen concentrations were also significantly higher in response to FRU, MF and PRO when compared with MAL across the recovery period ($P < 0.001$ for all). Following 300 min of recovery, liver glycogen concentrations were significantly higher in response to FRU ($275 \pm 49 \text{ mmol L}^{-1}$; $P < 0.001$), MF ($255 \pm 50 \text{ mmol L}^{-1}$; $P = 0.004$) and PRO ($283 \pm 50 \text{ mmol L}^{-1}$; $P < 0.001$) when compared with MAL ($204 \pm 51 \text{ mmol L}^{-1}$) where liver glycogen concentrations were 72 mmol L^{-1} [95%

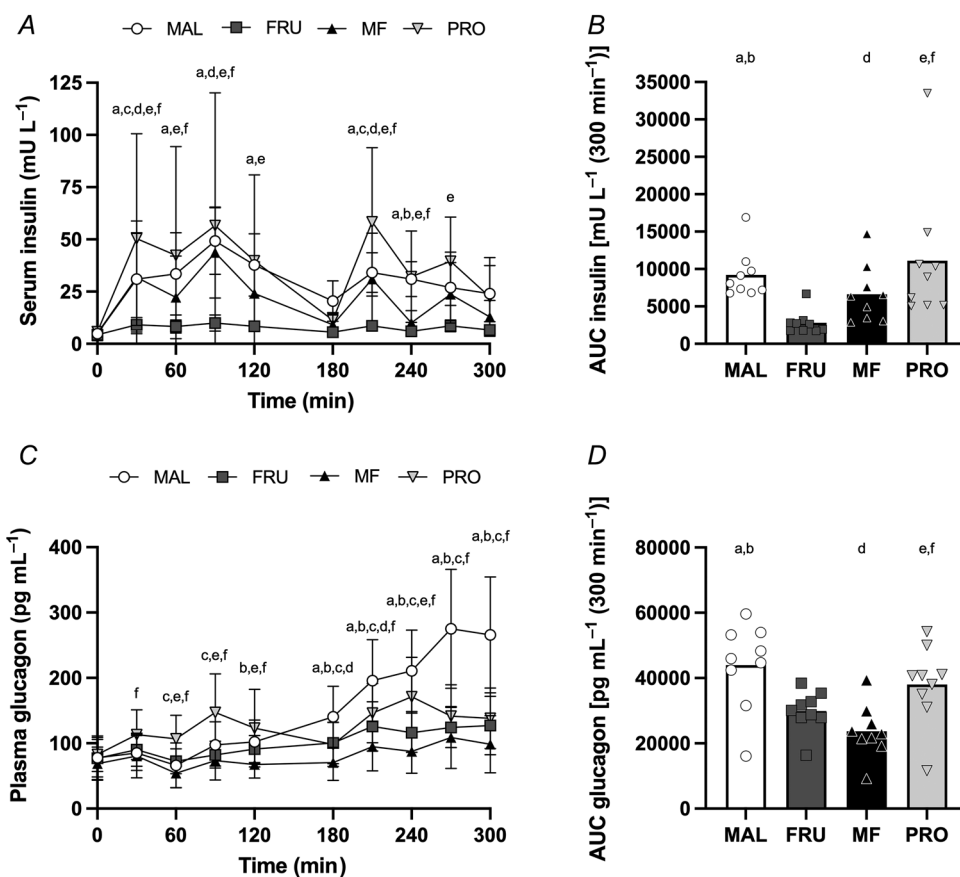


Figure 4. Serum insulin (A) and plasma glucagon (C) during the 5 h post-exercise recovery period and total area under the curve (AUC) for insulin (B) and glucagon (D) in response to either maltodextrin (MAL), fructose (FRU), maltodextrin + fructose (MF) or maltodextrin + fructose + protein (PRO); $n = 9$ for both hormones

^aMAL and FRU significantly different, ^bMAL and MF significantly different, ^cMAL and PRO significantly different, ^dFRU and MF significantly different, ^eFRU and PRO significantly different, ^fMF and PRO significantly different.

confidence interval (CI): 34–109 mmol L⁻¹], 52 mmol L⁻¹ (95% CI: 17–87 mmol L⁻¹) and 79 mmol L⁻¹ (95% CI: 44–115 mmol L⁻¹) higher in response to FRU, MF and PRO trials, respectively. Accordingly, the changes in liver glycogen concentrations across the 5 h recovery period were significantly higher (treatment effect, $P < 0.001$) in response to FRU (141 ± 49 mmol L⁻¹; $P < 0.001$), MF (122 ± 50 mmol L⁻¹; $P = 0.004$) and PRO (149 ± 50 mmol L⁻¹; $P < 0.001$) when compared with MAL (69 ± 51 mmol L⁻¹).

Liver volume

Significant time ($P < 0.001$) and treatment effects ($P = 0.010$) were observed for liver volume, but no significant time $\times$ treatment interaction was observed ($P = 0.453$) (Table 1). Liver volume increased significantly across the recovery period and was significantly higher at 300 min when compared with 120 min ($P < 0.001$). Furthermore, average liver volume across the recovery period was significantly higher in response to PRO

(1.77 ± 0.08 L) when compared with MAL (1.73 ± 0.08 L; $P = 0.17$) and FRU (1.71 ± 0.07 L; $P = 0.003$).

Liver glycogen content

Significant time and treatment effects (both $P < 0.001$) were observed for liver glycogen content whilst a trend towards significance was observed for the time $\times$ treatment interaction ($P = 0.071$) (Fig. 6B). Liver glycogen content increased significantly across the recovery period and was significantly higher at 300 min when compared with 120 min ($P < 0.001$). Furthermore, liver glycogen content was also significantly higher in response to FRU, MF and PRO when compared with MAL across the recovery period ($P < 0.001$ for all). Following 300 min of recovery, liver glycogen content was significantly higher in response to FRU (78 ± 13 g; $P < 0.001$), MF (74 ± 14 g; $P = 0.002$) and PRO (82 ± 13 g; $P < 0.001$) when compared with MAL (57 ± 14 g) where liver glycogen content was 21 g (95% CI: 10–31 g), 16 g (95% CI: 6–26 g) and 24 g (95% CI: 15–34 g) higher in response to FRU, MF and PRO trials,

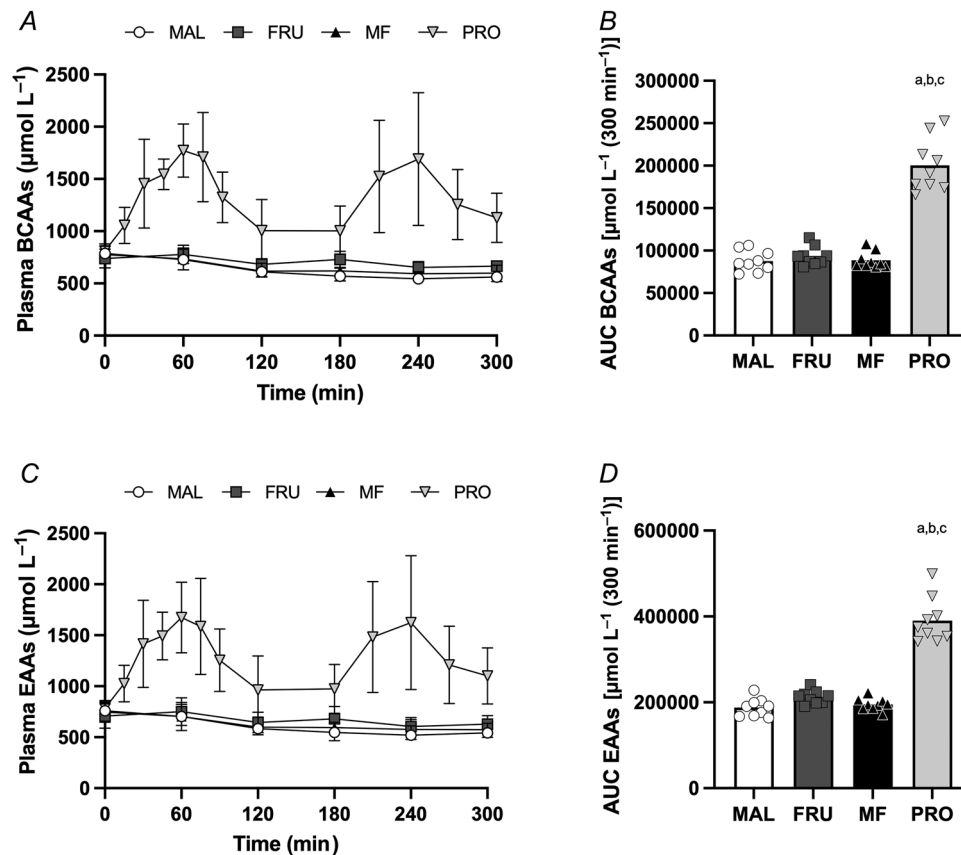


Figure 5. Plasma branched chain amino acids (BCAAs; A) and essential amino acids (EAAs; C) during the 5 h post-exercise recovery period

Area under the curve (AUC) values for BCAAs (B) and EAAs (D) when ingesting either maltodextrin (MAL), fructose (FRU), maltodextrin + fructose (MF) or maltodextrin + fructose + protein (PRO); $n = 9$ for all amino acids.

^aSignificantly different from MAL, ^bsignificantly different from FRU, ^csignificantly different from MF.

Table 1. Liver volume (L) during the 5 h post-exercise recovery period

	Time (min)		
	0	120	300 ^a
Maltodextrin (MAL) ^b	1.65 ± 0.30	1.67 ± 0.09	1.78 ± 0.09
Fructose (FRU) ^b	1.65 ± 0.27	1.68 ± 0.08	1.74 ± 0.08
Maltodextrin + fructose (MF)	1.67 ± 0.27	1.72 ± 0.09	1.79 ± 0.09
MF + protein (PRO)	1.68 ± 0.30	1.72 ± 0.09	1.81 ± 0.09

n = 10 for MAL, MF and PRO trials. Liver volumes at baseline are presented as raw means ± SD whereas estimated marginal means ± SD are presented at 120 and 300 min; *n* = 10 for MAL, MF and PRO trials; *n* = 9 for FRU trial at 120 min; and *n* = 8 for FRU trial at 300 min.

^aSignificantly different from 120 min.

^bSignificantly different from PRO.

respectively. Accordingly, the change in liver glycogen content across the 5 h recovery period was significantly higher (treatment effect, $P < 0.001$) in response to FRU (40 ± 13 g; $P < 0.001$), MF (36 ± 13 g; $P = 0.002$) and PRO (44 ± 13 g; $P < 0.001$) when compared with MAL (20 ± 14 g).

Muscle glycogen concentrations

Muscle glycogen concentrations significantly increased across the recovery period (time effect, $P < 0.001$) and were significantly higher at 300 min when compared with 120 min ($P < 0.001$). However, muscle glycogen concentrations were not significantly different between treatments (treatment effect, $P = 0.306$; treatment × time interaction, $P = 0.236$) (Fig. 7). Accordingly, the changes in muscle glycogen concentrations across the 5 h recovery period were not significantly different between treatments (treatment effect, $P = 0.306$).

Gastrointestinal symptoms

There were no significant differences between conditions for nausea, regurgitation, gas and urge to defecate ($P > 0.05$). There was a significant main effect for stomach fullness ($P = 0.014$), with *post hoc* comparisons revealing that FRU was significantly higher when compared to MAL ($P = 0.009$). Furthermore, a significant main effect was observed for abdominal cramps ($P = 0.016$), yet *post hoc* comparisons failed to determine significance between conditions. Two participants terminated the fructose trial at 110 and 180 min, respectively, due to self-reported severe gastrointestinal discomfort.

Discussion

Using non-invasive ^{13}C MRS, we examined the effects of co-ingesting whey protein in combination with dual-source carbohydrates on both muscle and liver

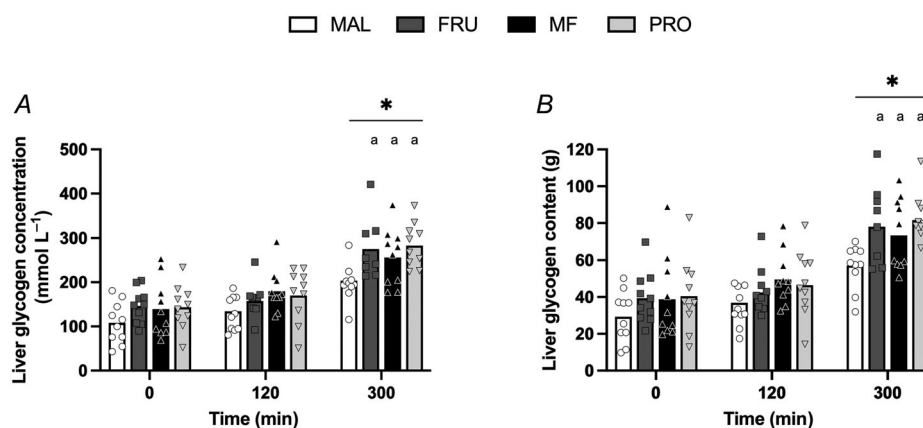


Figure 6. Liver glycogen concentrations (A) and liver glycogen content (B) during the 5 h post-exercise recovery period in response to either maltodextrin (MAL), fructose (FRU), maltodextrin + fructose (MF) or maltodextrin + fructose + protein (PRO).

Glycogen concentrations and content at baseline are presented as raw means ± SD whereas estimated marginal means ± SD are presented at 120 and 300 min. Individual raw data points are overlaid; *n* = 10 for MAL, MF and PRO trials; *n* = 9 for FRU trial at 120 min; and *n* = 8 for FRU trial at 300 min. ^aSignificantly different from MAL, *significantly different from 120 min.

glycogen resynthesis during a 5 h recovery period. We provide novel data by demonstrating that, following a prolonged bout of endurance exercise, (1) the ingestion of dual-source carbohydrates (e.g. maltodextrin and fructose) are superior in enhancing liver glycogen repletion when compared with maltodextrin alone and (2) the addition of 30 g whey protein enhances amino acid availability without compromising liver glycogen resynthesis. In relation to the goal of promoting optimal recovery from endurance exercise, our data therefore suggest that a combination of dual-source carbohydrates and whey protein enhance the repletion of endogenous carbohydrate stores whilst providing essential amino acids for the purpose of muscle remodelling.

To achieve our intended model of post-exercise glycogen resynthesis, we adopted a feeding strategy that accurately reflects the real-world CHO and protein intakes of endurance athletes. Indeed, whilst the repletion of glycogen is considered one of the key nutritional priorities for recovery, in practice, athletes commonly fail to meet current CHO intake guidelines of $1.2 \text{ g kg}^{-1} \text{ h}^{-1}$ (Doering et al., 2016; Heikura et al., 2017; McLeman et al., 2019). Furthermore, given the reported use of commercially available recovery products amongst endurance athletes in recovery (Doering et al., 2016), we adopted an absolute dose of both CHO and protein to reflect the doses commonly found in such formulations. Nonetheless, our chosen feeding strategy was successful in achieving glycogen repletion within both the liver and

skeletal muscle and the results are in accordance with previously reported rates of resynthesis in response to higher CHO intakes (Décombaz et al., 2011; Fuchs et al., 2016).

The augmented resynthesis of liver glycogen following dual-source carbohydrate ingestion (e.g. maltodextrin and fructose) is probably explained by the distinct metabolic fates of glucose (polymers) and fructose, in addition to the effects of fructose on hepatic handling of glucose. While glucose is predominantly released into systemic circulation for oxidation or storage as muscle glycogen, fructose is primarily metabolized by the liver, where it is either converted to glycogen or metabolized into lactate and glucose (Bergström & Hultman, 1967; Tappy & Rosset, 2017; Zierath et al., 1995). Furthermore, fructose stimulates hepatic glucose uptake and glycogen synthesis (Shiota et al., 2005). The present data are consistent with previous reports that demonstrate the co-ingestion of glucose (polymers) and fructose enhances liver glycogen repletion compared with glucose (polymers) alone when ingested at rates of $\sim 0.9\text{--}1.5 \text{ g kg}^{-1} \text{ h}^{-1}$ (Décombaz et al., 2011; Fuchs et al., 2016). Interestingly, rates of liver glycogen repletion in the present study ($7.0 \pm 4.4 \text{ g h}^{-1}$) are comparable to those previously reported by Décombaz et al. (2011) ($8.1 \pm 0.6 \text{ g h}^{-1}$) and Fuchs et al. (2016) ($6.6 \pm 3.3 \text{ g h}^{-1}$), despite the lower rate of carbohydrate ingestion. Taken together, these data suggest that liver glycogen resynthesis rates may be independent of the total amount of CHO ingested (within the ranges tested). However, despite the apparent importance of fructose in enhancing liver glycogen repletion, increasing the rate of fructose ingestion from 30 g h^{-1} (150 g total combined with 150 g maltodextrin) to 60 g h^{-1} (300 g total consumed in isolation) did not further augment liver glycogen resynthesis, as evidenced by the similar liver glycogen content observed after 5 h in the MF and FRU trials. Since low-dose fructose can potentially stimulate liver glycogen synthesis during hyperinsulinaemic, euglycaemic clamps (Petersen et al., 2001), it is possible that the effect of fructose on hepatic glycogen resynthesis is maximal at very low doses. Furthermore, given that the co-ingestion of maltodextrin can enhance fructose absorption (Truswell et al., 1988), further studies are required to examine the dose-dependent effects of fructose on liver glycogen resynthesis. Nonetheless, despite its failure to further augment liver glycogen synthesis, the ingestion of pure fructose resulted in enhanced gastrointestinal (GI) complaints throughout the recovery period, with two participants terminating the trial due to severe self-reported GI complaints. We also observed marked distension of the bowel in both participants' MRI images shortly before trial termination, which is probably explained by the fermentation of fructose in the intestine resulting in enhanced gas

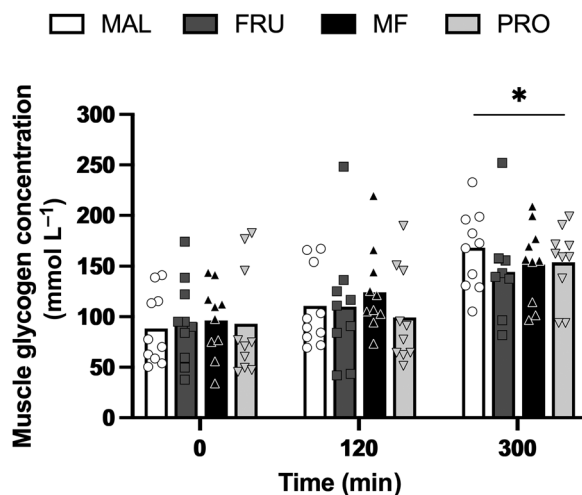


Figure 7. Muscle glycogen concentrations during the 5 h post-exercise recovery period when ingesting either maltodextrin (MAL), fructose (FRU), maltodextrin + fructose (MF) or maltodextrin + fructose + protein (PRO)

Glycogen concentrations at baseline are presented as raw means $\pm$ SD whereas estimated marginal means $\pm$ SD are presented at 120 and 300 min. Individual raw data points are overlaid; $n = 10$ for MAL, MF and PRO trials; $n = 9$ for FRU trial at 120 min; and $n = 8$ for FRU trial at 300 min. *Significantly different from 120 min.

production (DiNicolantonio & Lucan, 2015; Gibson et al., 2007).

Hepatic glycogen synthesis is regulated by circulating concentrations of both insulin and glucagon, yet, despite enhanced aminoacidaemia and insulinaemia, the ingestion of 30 g whey protein (combined with dual-source carbohydrate) immediately and 3 h following exercise did not further enhance liver glycogen repletion. However, given that liver glycogen synthesis rates appear to plateau at a portal vein insulin concentration of $\sim 200 \text{ pmol L}^{-1}$ (Roden et al., 1996), portal vein insulin concentrations in response to dual-source carbohydrate ingestion may have already been sufficient to maximize this response. Considering that portal vein insulin concentrations are reported to be 2–3-fold higher than peripheral concentrations (Horwitz et al., 1975), we anticipate that our reported peripheral insulin concentrations of 138 pmol L^{-1} ($1 \text{ mU} = 6 \text{ pmol}$) in response to combined maltodextrin and fructose ingestion would have been sufficient to maximize rates of liver glycogen synthesis. In relation to glucagon, the co-ingestion of whey protein with dual-source carbohydrate enhanced plasma glucagon concentrations when compared with dual-source carbohydrate alone. These findings are consistent with previous data which demonstrate increased arterial glucagon concentrations when whey protein is co-ingested with sucrose compared with sucrose ingestion alone (90 vs. 50 ng L^{-1}) (van Hall et al., 2000). However, despite the reported elevations in glucagon, liver glycogen repletion appeared to be unaffected by the co-ingestion of whey protein in the present study. Interestingly, plasma glucagon concentrations were further enhanced in response to the ingestion of maltodextrin. Although the present data are unable to provide insight into liver glycogen flux (i.e. synthesis and breakdown), it could be speculated that the lower liver glycogen content following maltodextrin ingestion may be explained by a reduction in synthesis (given that maltodextrin is not a direct substrate for liver glycogen synthesis) alongside an increase in breakdown (i.e. endogenous glucose production) given the marked elevation in plasma glucagon.

In relation to muscle, our data are consistent with previous studies which demonstrate that the ingestion of dual-source carbohydrates do not further accelerate muscle glycogen resynthesis when compared to glucose (polymers) alone (Casey et al., 2000; Fuchs et al., 2016; Moriarty et al., 1994; Trommelen et al., 2016; Wallis et al., 2008). Interestingly, muscle glycogen resynthesis over the 5 h recovery period following the ingestion of pure fructose was similar when compared with both maltodextrin and the combined maltodextrin and fructose trials. Although lower rates of post-exercise muscle glycogen resynthesis have been previously observed with pure fructose ingestion when compared

with glucose (Blom et al., 1987), these differences are probably attributed to the lower rates of ingestion ($0.35 \text{ g kg}^{-1} \text{ h}^{-1}$) when compared with the present study where fructose intake was more than double ($0.76 \text{ g kg}^{-1} \text{ h}^{-1}$). In support of this, comparable rates of muscle glycogen repletion have been previously observed in response to similarly large quantities of fructose ($\sim 300 \text{ g}$) when compared with glucose over a 24 h period (Rosset et al., 2017). Although ingested fructose does not appear to be a major precursor for glycogen synthesis, previous isotope tracer studies have demonstrated that conversion to glucose and lactate are the two major metabolic fates of fructose, accounting for $\sim 60\%$ of that ingested (Sun & Empie, 2012). Considering this, the observed 2-fold increase in plasma lactate concentrations throughout recovery following fructose ingestion is likely to explain the comparable muscle glycogen synthesis observed when compared with the ingestion of maltodextrin. Indeed, previous data have demonstrated a strong positive correlation between plasma lactate concentrations and muscle glycogen resynthesis following fructose ingestion ($r = 0.75$) (Rosset et al., 2017).

Despite augmented insulinaemia, muscle glycogen resynthesis was not enhanced in response to protein co-ingestion. These findings are in contrast to previous studies that demonstrate enhanced muscle glycogen resynthesis when protein is co-ingested with suboptimal CHO intake (Van Loon, Saris, Kruijshoop et al., 2000). Although CHO intake in the present study is comparable to that of previous studies (i.e. $\sim 0.8 \text{ g kg}^{-1} \text{ BM h}^{-1}$), both average ($\sim 40 \text{ mU L}^{-1}$) and peak ($\sim 60 \text{ mU L}^{-1}$) insulin concentrations in the present study are markedly lower than those reported in previous studies in response to CHO–protein co-ingestion (Jentjens et al., 2001; Van Loon, Saris, Verhagen et al., 2000). Such differences in insulinaemia are unsurprising given that previous studies have used repeated doses (e.g. $0.4 \text{ g kg}^{-1} \text{ BM h}^{-1}$) fed at 30 min intervals in combination with highly insulinotropic amino acids when compared with the 30 g whey protein fed at $t = 0$ and $t = 180 \text{ min}$ in the present study. Given that the pattern of protein ingestion in the present study is more representative of real-world athlete practices, these data question the practical relevance of protein co-ingestion to enhance muscle glycogen resynthesis.

From a practical perspective, the augmented liver glycogen content observed in response to FRU, MF and PRO trials when compared with MAL are likely to be physiologically relevant to performance during prolonged endurance events. For example, following 5 h of recovery, liver glycogen content was 21 g (95% CI: $10\text{--}31 \text{ g}$), 16 g (95% CI: $6\text{--}26 \text{ g}$) and 24 g (95% CI: $15\text{--}34 \text{ g}$) higher in response to FRU, MF and PRO trials, respectively. Such differences in glycogen

content equate to ~ 68 – 105 kJ (assuming an efficiency of 22%) and would provide sufficient energy to sustain an additional ~ 2 min of exercise at $\sim 70\%$ $\dot{V}_{O_{2\max}}$ in well-trained male cyclists, representing a meaningful performance improvement. Nonetheless, we recognize that individual responses should be considered when providing nutritional recommendations to promote post-exercise liver glycogen repletion. Furthermore, whilst we observed no statistical differences in muscle glycogen concentrations following 5 h of recovery, small differences in absolute glycogen concentrations may be relevant to exercise performance. Indeed, muscle glycogen concentrations following MAL were 23 mmol L^{-1} (95% CI: -2 to 49 mmol L^{-1}), 16 mmol L^{-1} (95% CI: -8 to 41 mmol L^{-1}) and 14 mmol L^{-1} higher (95% CI: -10 to 39 mmol L^{-1}) when compared with FRU, MF and PRO trials. Assuming an average muscle volume of 5 L (Casey et al., 2000) this difference would equate to ~ 11 – 18 g muscle glycogen although the physiological relevance of such differences remains unclear, given that the magnitude of short-term muscle glycogen resynthesis does not always translate to improved endurance performance (Casey et al., 2000). Finally, we also recognize that the observed effect sizes are smaller than those used for our *a priori* sample size calculation, which may have limited our ability to detect statistically significant differences despite potentially meaningful physiological effects.

In summary, we demonstrate that the ingestion of dual-source CHO enhances liver glycogen repletion whilst maintaining comparable rates of muscle glycogen resynthesis when compared with maltodextrin alone. Furthermore, despite elevations in circulating glucagon, the enhanced liver glycogen repletion observed with dual-source CHO is not impaired by the co-ingestion of whey protein. When considering the importance of post-exercise protein ingestion for muscle reconditioning and the habitual protein intake patterns of elite endurance athletes, our data demonstrate that combining dual-source carbohydrate (achieved via a 1:1 combination of maltodextrin and fructose) with high-quality whey protein provides a practical strategy to simultaneously enhance amino acid availability and endogenous glycogen resynthesis.

References

- Alghannam, A. F., Jedrzejewski, D., Bilzon, J., Thompson, D., Tsintzas, K., & Betts, J. A. (2016). Influence of post-exercise carbohydrate-protein ingestion on muscle glycogen metabolism in recovery and subsequent running exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, **26**(6), 572–580.
- Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica*, **71**(2–3), 140–150.
- Bergström, J., & Hultman, E. (1967). A study of the glycogen metabolism during exercise in man. *Scandinavian Journal of Clinical and Laboratory Investigation*, **19**(3), 218–228.
- Blom, P. C. S., Høstmark, A. T., Vaage, O., Kardel, K. R., & Mæhlum, S. (1987). 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.
- Bukhari, S. S. I., Phillips, B. E., Wilkinson, D. J., Limb, M. C., Rankin, D., Mitchell, W. K., Kobayashi, H., Greenhaff, P. L., Smith, K., & Atherton, P. J. (2015). Intake of low-dose leucine-rich essential amino acids stimulates muscle anabolism equivalently to bolus whey protein in older women at rest and after exercise. *American Journal of Physiology-Endocrinology and Metabolism*, **308**(12), E1056–E1065.
- Casey, A., Mann, R., Banister, K., Fox, J., Morris, P. G., Macdonald, I. A., & Greenhaff, P. L. (2000). Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by ^{13}C MRS. *American Journal of Physiology-Endocrinology and Metabolism*, **278**(1), E65–E75.
- Churchward-Venne, T. A., Pinckaers, P. J. M., Smeets, J. S. J., Betz, M. W., Senden, J. M., Goossens, J. P. B., Gijsen, A. P., Rollo, L., Verdijk, L. B., & van Loon, L. J. C. (2020). Dose-response effects of dietary protein on muscle protein synthesis during recovery from endurance exercise in young men: A double-blind randomized trial. *American Journal of Clinical Nutrition*, **112**(2), 303–317.
- 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.
- Detko, E., O'Hara, J. P., Thelwall, P. E., Smith, F. E., Jakovljevic, D. G., King, R. F. G. J., & Trenell, M. I. (2013). Liver and muscle glycogen repletion using ^{13}C magnetic resonance spectroscopy following ingestion of maltodextrin, galactose, protein and amino acids. *British Journal of Nutrition*, **110**(5), 848–855.
- DiNicolantonio, J. J., & Lucan, S. C. (2015). Is fructose malabsorption a cause of irritable bowel syndrome? *Medical Hypotheses*, **85**(3), 295–297.
- Doering, T. M., Reaburn, P. R., Cox, G., & Jenkins, D. G. (2016). Comparison of postexercise nutrition knowledge and postexercise carbohydrate and protein intake between Australian masters and younger triathletes. *International Journal of Sport Nutrition and Exercise Metabolism*, **26**(4), 338–346.
- 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.

- Fuchs, C. J., Gonzalez, J. T., Beelen, M., Cermak, N. M., Smith, F. E., Thelwall, P. E., Taylor, R., Trenell, M. I., Stevenson, E. J., & Van Loon, L. J. C. (2016). Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion compared with glucose ingestion in trained athletes. *Journal of Applied Physiology*, **120**(11), 1328–1334.
- Gibson, P. R., Newnham, E., Barrett, J. S., Shepherd, S. J., & Muir, J. G. (2007). Review article: Fructose malabsorption and the bigger picture. *Alimentary Pharmacology & Therapeutics*, **25**(4), 349–363.
- Gonzalez, J. T., Fuchs, C. J., Betts, J. A., & van Loon, L. J. C. (2016). Liver glycogen metabolism during and after prolonged endurance-type exercise. *American Journal of Physiology-Endocrinology and Metabolism*, **311**(3), E543–E553.
- Gray, E. A., Green, T. A., Betts, J. A., & Gonzalez, J. T. (2020). Postexercise glucose–fructose coingestion augments cycling capacity during short-term and overnight recovery from exhaustive exercise, compared with isocaloric glucose. *International Journal of Sport Nutrition and Exercise Metabolism*, **30**(1), 54–61.
- Harris, M. A., Hammond, K. M., Seaborne, R. A., Stocks, B., Shepherd, S. O., Philp, A., Sharples, A. P., Morton, J. P., & Louis, J. B. (2019). Graded reductions in preexercise muscle glycogen impair exercise capacity but do not augment skeletal muscle cell signaling: Implications for CHO periodization. *Journal of Applied Physiology*, **126**(6), 1587–1597.
- Heikura, I. A., Stellingwerff, T., Mero, A. A., Uusitalo, A. L. T., & Burke, L. M. (2017). A mismatch between athlete practice and current sports nutrition guidelines among elite female and male middle- and long-distance athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, **27**(4), 351–360.
- Hofmann, P., & Tschakert, G. (2017). Intensity- and duration-based options to regulate endurance training. *Frontiers in Physiology*, **8**, 337.
- Horwitz, D. L., Starr, J. I., Mako, M. E., Blackard, W. G., & Rubenstein, A. H. (1975). Proinsulin, insulin and C-peptide concentrations in human portal and peripheral blood. *Journal of Clinical Investigation*, **55**(6), 1278–1283.
- Howarth, K. R., Moreau, N. A., Phillips, S. M., & Gibala, M. J. (2009). Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology*, **106**(4), 1394–1402.
- Jentjens, R., Van Loon, L. J. C., Mann, C. H., Wagenmakers, A. J. M., & Jeukendrup, A. E. (2001). Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. *Journal of Applied Physiology*, **91**(2), 839–846.
- Kuipers, H., Verstappen, F. T. J., Keizer, H. A., Geurten, P., & van Kranenburg, G. (1985). Variability of aerobic performance in the laboratory and its physiologic correlates. *International Journal of Sports Medicine*, **06**(04), 197–201.
- Kurland, I. J., & Pilkis, S. J. (1989). Indirect versus direct routes of hepatic glycogen synthesis. *Federation of American Societies for Experimental Biology Journal*, **3**(11), 2277–2281.
- McGuinness, O. P., & Cherrington, A. D. (2003). Effects of fructose on hepatic glucose metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care*, **6**(4), 441–448.
- 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.
- McLeman, L. A., Ratcliffe, K., & Clifford, T. (2019). Pre- and post-exercise nutritional practices of amateur runners in the UK: Are they meeting the guidelines for optimal carbohydrate and protein intakes? *Sport Sciences for Health*, **15**(3), 511–517.
- Moore, D. R., Tang, J. E., Burd, N. A., Rerечich, T., Tarnopolsky, M. A., & Phillips, S. M. (2009). Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *The Journal of Physiology*, **587**(4), 897–904.
- Moriarty, K. T., McIntyre, D. G. O., Bingham, K., Coxon, R., Glover, P. M., Greenhaff, P. L., Macdonald, I. A., Bachelard, H. S., & Morris, P. G. (1994). Glycogen resynthesis in liver and muscle after exercise: Measurement of the rate of resynthesis by ^{13}C magnetic resonance spectroscopy. *Magma: Magnetic Resonance Materials in Physics, Biology, and Medicine*, **2**(3), 429–432.
- Parker, R. A., & Weir, C. J. (2020). Non-adjustment for multiple testing in multi-arm trials of distinct treatments: Rationale and justification. *Clinical Trials (London, England)*, **17**(5), 562–566.
- Petersen, K. F., Laurent, D., Yu, C., Cline, G. W., & Shulman, G. I. (2001). Stimulating effects of low-dose fructose on insulin-stimulated hepatic glycogen synthesis in humans. *Diabetes*, **50**(6), 1263–1268.
- Roden, M., Perseghin, G., Petersen, K. F., Hwang, J.-H., Cline, G. W., Gerow, K., Rothman, D. L., & Shulman, G. I. (1996). The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. *Journal of Clinical Investigation*, **97**(3), 642–648.
- 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. *American Journal of Physiology*, **265**, E380–E391.
- Rosset, R., Lecoultre, V., Egli, L., Cros, J., Dokumaci, A. S., Zwygart, K., Boesch, C., Kreis, R., Schneiter, P., & Tappy, L. (2017). Postexercise repletion of muscle energy stores with fructose or glucose in mixed meals. *American Journal of Clinical Nutrition*, **105**(3), 609–617.
- Shiota, M., Galassetti, P., Igawa, K., Neal, D. W., & Cherrington, A. D. (2005). Inclusion of low amounts of fructose with an intraportal glucose load increases net hepatic glucose uptake in the presence of relative insulin deficiency in dog. *American Journal of Physiology-Endocrinology and Metabolism*, **288**(6), E1160–E1167.
- Sun, S. Z., & Empie, M. W. (2012). Fructose metabolism in humans – What isotopic tracer studies tell us. *Nutrition and Metabolism*, **9**(1), 89.

- Tappy, L., & Rosset, R. (2017). Fructose metabolism from a functional perspective: Implications for athletes. *Sports Medicine*, **47**(S1), 23–32.
- Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the Academy of Nutrition and Dietetics*, **116**(3), 501–528.
- Trommelen, J., Beelen, M., Pinckaers, P. J. M., Senden, J. M., Cermak, N. M., & Van Loon, L. J. C. (2016). Fructose coingestion does not accelerate postexercise muscle glycogen repletion. *Medicine and Science in Sports and Exercise*, **48**(5), 907–912.
- Truswell, A. S., Seach, J. M., & Thorburn, A. W. (1988). Incomplete absorption of pure fructose in healthy subjects and the facilitating effect of glucose. *American Journal of Clinical Nutrition*, **48**(6), 1424–1430.
- Van Hall, G., Shirreffs, S. M., & Calbet, J. A. L. (2000). Muscle glycogen resynthesis during recovery from cycle exercise: No effect of additional protein ingestion. *Journal of Applied Physiology*, **88**(5), 1631–1636.
- Van Loon, L. J. C., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. M., & Wagenmakers, A. J. M. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, **536**(1), 295–304.
- Van Loon, L. J. C., Saris, W. H. M., Kruijshoop, M., & Wagenmakers, A. J. M. (2000). Maximizing postexercise muscle glycogen synthesis: Carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *American Journal of Clinical Nutrition*, **72**(1), 106–111.
- Van Loon, L. J. C., Saris, W. H. M., Verhagen, H., & Wagenmakers, A. J. M. (2000). Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *American Journal of Clinical Nutrition*, **72**(1), 96–105.
- Wallis, G. A., Hulston, C. J., Mann, C. H., Roper, H. P., Tipton, K. D., & Jeukendrup, A. E. (2008). Postexercise muscle glycogen synthesis with combined glucose and fructose ingestion. *Medicine and Science in Sports and Exercise*, **40**(10), 1789–1794.
- Williams, M. B., Raven, P. B., Fogt, D. L., & Ivy, J. L. (2003). Effects of recovery beverages on glycogen restoration and endurance exercise performance. *Journal of Strength and Conditioning Research*, **17**, 12–19.
- 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.
- Zierath, J. R., Nolte, L. A., Wahlström, E., Galuska, D., Shepherd, P. R., Kahn, B. B., & Wallberg-Henriksson, H. (1995). Carrier-mediated fructose uptake significantly contributes to carbohydrate metabolism in human skeletal muscle. *Biochemical Journal*, **311**(2), 517–521.

Additional information

Data availability statement

The data sets from the present study are available from the corresponding author upon reasonable request.

Competing interests

J.T.G. is an investigator on research grants funded by Biotechnology and Biological Sciences Research Council (BBSRC), Medical Research Council (MRC), British Heart Foundation, The Rank Prize Funds, The European Society for Clinical Nutrition and Metabolism (ESPEN), LRS, ARLA Foods Ingredients, and Kenniscentrum Suiker and Voeding and has completed paid consultancy for PepsiCo and SVGC. J.P.M. is a consultant for SiS and has previously received research funding from SiS, Glaxosmithkline (GSK) and Lucozade Ribena Suntory (LRS) on glycogen metabolism and exercise. M.A.H. has previously received research funding from SiS on glycogen metabolism and exercise.

Author contributions

S.H., J.T.G., J.P.M., A.J.K. and M.A.H. designed the study. S.H., J.M., L.H., J.C.Y.T., R.D., R.M., K.S. and A.J.K. performed experiments; S.H., S.B., J.T.G., N.H., J.P.M., A.J.K. and M.A.H. analysed the data and interpreted the results. S.H., J.P.M. and M.A.H. drafted the manuscript and J.M., L.H., S.B., J.C.Y.T., R.D., R.M., K.S., J.T.G., N.H. and A.J.K. edited and revised the manuscript. All authors approved the final version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This study was funded by a research grant from Science in Sport (SiS) PLC.

Keywords

¹³C magnetic resonance spectroscopy, glycogen, liver, protein, recovery

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History