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Dose–Response of Dietary Carbohydrate Intake on Skeletal Muscle Glycogen, Gastrointestinal Comfort and Body Composition in Endurance-Trained Individuals in Simulated Preparation for Competition

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ABSTRACT

High dietary carbohydrate (CHO) intake and reduced exercise training are recommended to optimize muscle glycogen stores pre-endurance sports competition. However, the optimal CHO intake to support muscle glycogen synthesis and the dose–response of this relationship are still unknown in athletes who continue training pre-competition. This study investigated the effects of different CHO intakes on muscle glycogen concentration. In a counterbalanced repeated measures design, 11 endurance-trained participants (3 females, 8 males; age, 24 ± 5 years; body mass, 71.2 ± 12.0 kg; $\dot{V}O_{2max}$, 56 ± 6 mL kg^{-1} min^{-1}) undertook 3 × 5 days of exercise and dietary control. During the final 48 h, participants ingested 6, 8 or 10 g kg^{-1} day^{-1} CHO prior to the assessment of muscle glycogen, gastrointestinal (GI) comfort and body composition. Muscle glycogen concentration was significantly higher following 10 vs. 6 and 8 g kg^{-1} day^{-1} (635.5 ± 78.0 vs. 460.9 ± 100.7 and 506.1 ± 124.0 mmol kg^{-1} dry mass, respectively, $p < 0.03$), with no difference between 6 and 8 g kg^{-1} day^{-1} ($p = 1.00$). There was a strong positive correlation between relative CHO intake ($r = 0.71$, $p < 0.001$) and skeletal muscle glycogen concentration. There was no effect of CHO intake on body mass ($p = 0.70$) or GI symptoms ($p > 0.05$), except fullness. In conclusion, there was a linear dose–response between dietary CHO intake and muscle glycogen in a protocol mimicking real-world training and nutrition practices, as 10 g kg^{-1} day^{-1} achieved the highest muscle glycogen concentrations, with no detectable effect on body mass or GI symptoms, except increased fullness.

1 | Introduction

Increased dietary carbohydrate (CHO) intake and decreased training load in the days pre-competition ('CHO loading') is a well-established strategy used by endurance athletes to enhance skeletal muscle glycogen stores [1–4]. This strategy was first introduced in the 1960s, where it was recognized that glycogen-depleting exercise followed by a high CHO diet could lead to a two-fold increase in muscle glycogen concentrations [5], a phenomenon now termed 'glycogen supercompensation'. Thereafter, Bergström et al. [6] introduced what is now recognized as the 'classic' CHO loading protocol, where two exhaustive glycogen-depleting cycling sessions were separated by 72 h low CHO intake, followed by 72 h of a high CHO intake. CHO loading has since been modified [3], updated [7] and ultimately evolved into contemporary nutrition guidelines, which advocate 10–12 g of CHO per kg of body mass per day (g kg^{-1} day^{-1}) for 36–48 h prior to endurance competitions or prolonged high intensity endurance exercise ≥ 1.5 h [8, 9]. Regardless of the protocol used, increases of glycogen stores within skeletal muscle have been related to improvements in time to fatigue [4, 6], work capacity [10] and endurance

exercise performance lasting ≥ 90 min [11, 12]. However, despite over half a century of research, the relationship between high/very high dietary CHO intakes (≥ 8 g kg^{-1} day^{-1}) and skeletal muscle glycogen is not completely clear.

A recently conducted systematic review and meta-analysis of existing literature on CHO loading [Jones et al. unpublished data, *in review*] revealed significant heterogeneity in reported muscle glycogen concentrations from similar CHO intakes (480–840 mmol kg^{-1} dry mass [DM] from 7.9–8.3 g kg^{-1} day^{-1} of CHO), even when well-established effectors of glycogen synthesis such as training status, CHO loading duration and exercise (mode, duration, intensity) were similar [3, 13, 14]. Conversely, Burke et al. [15] reported no change in glycogen concentration, despite a 5 g kg^{-1} day^{-1} difference in CHO intake between study conditions (7 vs. 12 g kg^{-1} day^{-1}). Furthermore, only a limited number of studies have replicated real-world pre-competition training practices (i.e., exercise taper) of endurance athletes [3, 10, 16, 17], as the majority of CHO loading studies rested participants during periods of increased CHO intake [1, 2, 6, 7, 12, 18], limiting the ecological validity of these findings for a competitive endurance-sport setting. As such, the dose–response of CHO loading with moderate-very high CHO intakes (6–10 g kg^{-1} day^{-1}) on muscle glycogen concentration in these practical endurance sport scenarios remains unclear.

Finally, high and very high dietary CHO intakes carry a risk of harming performance due to potential gastrointestinal (GI) distress and increased body mass. Considerations for GI tolerability are required because research shows an association between gut discomfort symptoms pre- and during exercise resulting from pre-exercise high CHO ingestion [4, 16, 19]. Additionally, given increased glycogen in muscle is associated with increased osmotic pressure in the sarcoplasm, it has been reported that 3–4 g of water are bound to every 1 g of muscle glycogen [20]. Consequently, muscle glycogen supercompensation has been shown to increase body mass by around 1%–2% [21, 22], representing a potential challenge for weight-sensitive sports.

The primary aim of the study was to investigate the dose–response relationship of dietary CHO intake (from moderate–very high CHO) and muscle glycogen concentration, for the first time, in endurance trained individuals mimicking real-world race preparation whilst considering tolerability and gut comfort. A secondary aim was to investigate the relationship between total body water and skeletal muscle glycogen. We hypothesized 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 versus very high CHO availability (8 vs. 10 g kg^{-1} day^{-1}) due to a saturation point ('ceiling' effect [8, 15]). We also hypothesized that there would be increased prevalence of moderate–severe GI discomfort symptoms reported following a very high CHO intake (10 g kg^{-1} day^{-1}), and higher muscle glycogen concentrations would increase body mass through increased total body and intracellular water bound within stored glycogen.

2 | Methods

2.1 | Participants

Eleven endurance trained individuals participated in the study (8 males, 3 females; Table 1), in line with the classification of training status outlined by De Pauw et al. [23] and Decroix et al. [24] for male and female cyclists, respectively. Information regarding female menstrual cycle and hormonal contraceptive use is presented in Tables S1 and S2. Participants were provided 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).

2.2 | Study Overview

A schematic representation of the protocol can be seen in Figure 1. In a randomized counterbalanced cross-over design

TABLE 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 [BM] (kg)	75.2 ± 10.9	60.6 ± 8.8	71.2 ± 12.0
Fat free mass [FFM] (kg)	63.8 ± 6.0	46.2 ± 7.4	58.5 ± 10.4
$\dot{V}O_{2max}$ (L min^{-1})	4.81 ± 0.48	2.94 ± 0.30	4.30 ± 0.98
$\dot{V}O_{2max}$ (mL kg^{-1} BM min^{-1})	59 ± 3	48 ± 4	56 ± 6
$\dot{V}O_{2max}$ (mL kg^{-1} FFM min^{-1})	76.2 ± 7.7	64.1 ± 6.6	72.6 ± 9.1
PPO (W)	331 ± 40	241 ± 15	306 ± 54
PPO (W kg^{-1})	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^{-1})	7 ± 3	6 ± 2	7 ± 3

Note: Presented as mean ± SD.

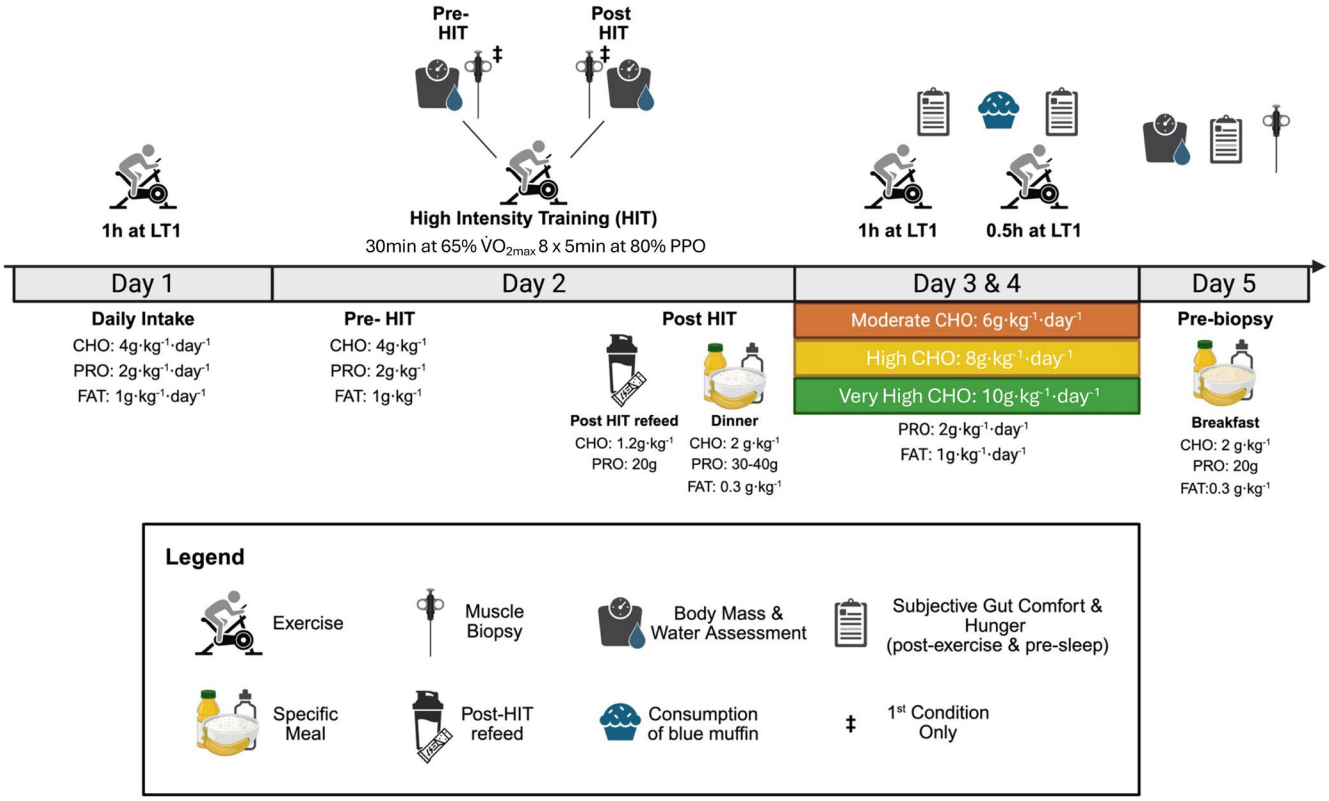

FIGURE 1 | Schematic overview of study design, where dietary intake and exercise was controlled for 5 days to replicate pre-competition practices of endurance athletes, with CHO intake manipulated between conditions, providing either 6, 8 or 10 g kg^{-1} day^{-1} of CHO for 48 h. Blue muffin; gut transit time measurement; CHO, carbohydrate; GI, gastrointestinal; h, hours; LT, lactate threshold; PPO, peak power output; PRO, protein; SS, steady state.

(block randomization), participants completed 3 × 5-day periods of dietary control and prescribed exercise designed to mimic pre-competition practices of competitive endurance cyclists (anecdotal evidence from the field). Participants were unaware of upcoming treatments until the first day of each condition. Days 1 and 2 standardized physiological condition and muscle glycogen concentration through ~36 h of a low-moderate CHO intake (4 g kg^{-1} day^{-1}), followed by a high intensity interval cycling exercise session (see Experimental Trials). Days 3 and 4 participants were provided a custom-made pre-packaged diet with moderate, high or very high amounts of CHO (6, 8 or 10 g kg^{-1} day^{-1}, respectively), before returning to the laboratory the morning of day 5 for a high CHO breakfast (2 g kg^{-1}), 2 h rest period and muscle biopsy. Experimental conditions were separated by ≥ 7 days, with participants instructed to continue habitual training during each washout period.

2.3 | Preliminary Testing

Participants arrived at the laboratory following 24 h rest, at least 2 h post-prandial. Height and body mass were measured semi-nude (SECA, Hamburg, Germany), before participants were seated for resting measures of heart rate (HR; Polar H10, Kempele, Finland), blood glucose and lactate using capillary fingertip blood samples, which were immediately analyzed (Biosen C-Line, EKF Diagnostics, Cardiff, UK). Participants then completed incremental lactate threshold and maximal oxygen consumption ($\dot{V}O_{2max}$) 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 analyzed for blood glucose and lactate, with HR collected in the final 10 s of each stage. The test was terminated when participants reached a blood lactate concentration of ≥ 4 mmol L^{-1}.

Following 10 min rest, participants completed a $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ 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. Peak power output (PPO) was determined using the equation outlined by Kuipers et al. [25]. 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 [26].

2.4 | Experimental Trials

2.4.1 | Prescribed Exercise

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 (mean power output at

LT1 [defined as baseline lactate concentration +1 mmol L^{-1}] for all training sessions: 164 ± 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 [27]. Participants completed no additional exercise outside prescribed trials.

2.4.2 | High Intensity Cycling Session

Day 2, participants arrived at the laboratory at 1500 h to complete a high intensity training session (HIT; [28]), previously shown to lower muscle glycogen concentration ~50% [29]. The session consisted of a 5 min warm-up at 100 W, 30 min cycle at an intensity ~65% $\dot{V}O_{2max}$, followed by 2 min active recovery at 100 W. Following this, participants completed 8 × 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 match absolute work done and attempt to standardize level of glycogen depletion.

2.4.3 | Dietary Manipulation

Days 1 and 2 (pre-HIT) participants consumed a low-moderate CHO intake (4 g kg^{-1} day^{-1}) as well as 2, 1 and 0.5–0.6 g kg^{-1} day^{-1} of protein, fat and dietary fiber respectively. Immediately post-HIT session, participants were provided with 1.2 g 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 g kg^{-1} CHO, 30–40 g protein and 0.3 g kg^{-1} of fat. Days 3 and 4, participants consumed either 6, 8 or 10 g kg^{-1} day^{-1} CHO as well as 2, 1 and 0.5–0.6 g kg^{-1} day^{-1} of protein, fat and dietary fiber respectively (Table 2). Plans provided a standardized healthy mixed diet in the form of usual meals (breakfast, lunch and dinner) with various snacks throughout each day. Participants were instructed to consume pre- and post-exercise meals or snacks at least 1–2 h pre/post-exercise to standardize CHO timing. On the morning of day 5 (0715–0730 h) participants consumed a high CHO breakfast providing 2 g kg^{-1} CHO, 20 g protein and 0.3 g kg^{-1} of fat. Information regarding estimated energy intake, energy expenditure and energy availability is presented in Table S3.

All foodstuff for each 5-day experimental period was provided custom-made and pre-packaged (weighed to nearest 0.05 g), along with instructions and a checklist. Meal plans were created using an online nutrition software (Nutritics, Dublin, Ireland). Participants were asked to send photos of each meal at the time of consumption via online messenger to confirm adherence (WhatsApp, Meta, USA). High and very high CHO conditions were supplemented with high CHO, zero fiber products such as jelly sweets, orange juice and sports drinks, making the total amount of fiber between diets comparable (Table 2). Food containers were weighed pre and post provision to account for leftovers (97.1% ± 1.0% compliance), with participants instructed to only consume foodstuffs 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 [30, 31].

2.4.4 | 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 movement and stool type using the Bristol stool chart [32]. Post-exercise and pre-sleep on days 3 and 4, and pre-biopsy day 5, participants completed subjective gut comfort and hunger scales. GI discomfort was recorded on a 0–10 scale for symptoms of nausea, reflux, stomach fullness, abdominal cramps, flatulence, and urge to defecate [33]. Hunger scales were completed on a series of 0–100 mm lines which corresponded to 'not at all' and 'extremely', respectively, where participants drew a vertical line corresponding to how they felt [34]. Post-lunch day 4, participants consumed two muffins colored with a blue dye (~60 g each, containing 0.75 g of royal blue food coloring), and were asked to note down the exact time of consumption as

TABLE 2 | Participant actual nutritional intake during habitual free living and experimental conditions (based on % daily compliance).

		Habitual	4 g kg^{-1} day^{-1}	6 g kg^{-1} day^{-1}	8 g kg^{-1} day^{-1}	10 g kg^{-1} day^{-1}
CHO	(g)	273 ± 70	277 ± 49	419 ± 74	560 ± 100	691 ± 120
	(g kg^{-1})	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^{-1})	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^{-1})	1.4 ± 0.5	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Fiber	(g)	—	35 ± 6	35 ± 8	36 ± 6	36 ± 6
	(g kg^{-1})	—	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Energy	(kcal)	2539 ± 679	2283 ± 402	2863 ± 507	3427 ± 610	3940 ± 682

well as the day and time of the first excretion event with visible blue color within stool [35].

2.4.5 | Body Water

Total body and extracellular water (TBW and ECW) were measured in standardized conditions on 3 occasions per condition (pre- and post-exercise 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 [36]. Intracellular water was calculated as the difference between TBW and ECW.

2.4.6 | Muscle Biopsies

Five biopsies were obtained in total, pre- and post-exercise day 2 in the first experimental condition (to determine baseline glycogen concentration following ~36 h of 4 g kg^{-1} day^{-1} CHO and confirm level of glycogen depletion post HIT session, respectively) and 2 h post-breakfast day 5 in each condition, to report muscle glycogen concentration post CHO loading interventions. Biopsies were obtained from the vastus lateralis under local anesthesia (Marcaine 0.5%), using the Weil-Blakesley conchotome technique. To avoid the impact of multiple biopsies on muscle glycogen [37], day 2 pre- and post-exercise 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.

2.4.7 | Biochemical Analysis of Muscle Glycogen

Muscle glycogen concentration was determined with acid hydrolysis as previously detailed [10]. Prior to hydrolysis, samples were freeze dried and dissected of visible blood, fat, or connective tissue under a microscope (Zeiss, Germany). Glucose concentration was quantified using a commercially available kit (GLUC-HK, Randox Laboratories, Antrim, UK). Accuracy of glycogen measurements was ratified against a standard with known concentration of glycogen (Rabbit liver glycogen, Sigma-Aldrich, Merck group, Germany). Coefficient of variation between duplicates of the same muscle sample was 10.5% ± 9.3%.

2.5 | Statistical Analysis

All data is presented as means ± SD unless otherwise stated. Sample size estimation was determined based on muscle glycogen data of Maunder et al. [38] where consumption of 2.4, 5.0 and 6.5 g kg CHO during a loading period provided a partial eta^2 (η^2_p) effect size of 0.62 and corresponded to an a priori sample size of 4 to achieve an alpha of 0.05 and power of 0.95 (G*Power, version 3.1.9.7). Linear mixed models were used to analyze skeletal muscle glycogen and body water variables due to 4 missing at random data points (3 for glycogen due to missed biopsy samples [pre-exercise day 2 for two participants, and post load for 8 g kg^{-1} day^{-1}] and 1 across body water variables due to technical error [6 g kg^{-1} day^{-1} post-load]), 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. Simple Pearson's correlation was used to supplement the primary mixed model analysis by assessing the strength of the linear relationship between CHO intake (relative and absolute) and skeletal muscle glycogen. Ordinal data (stool type and gut discomfort symptoms) were analyzed 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 analyzed using a one- or two-way repeated measures analysis of variance (ANOVA), with Bonferroni post hoc tests used to explore significant pairwise comparisons (unless otherwise stated). ANOVA effect sizes are η^2_p with values of 0.01, 0.06 and 0.14 corresponding to a small, medium and large effect respectively [39]. All data was analyzed using SPSS (version 29, IBM, USA) with significance set at $p < 0.05$. Figures were created using GraphPad Prism (version 10, Massachusetts, USA).

3 | Results

3.1 | Nutrition, Fluid Intake, Hunger and Satiety

Nutritional intake across each experimental period was successfully controlled, with prescribed and actual nutritional intake closely matched (Table 2). As a result, CHO consumption on days 1 and 2 was similar across all conditions ($p = 1.00$), whilst being significantly different across loading days for 6, 8 and 10 g kg^{-1} 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 ± 0.5, 2.5 ± 1.0 and 2.6 ± 0.8 L across day 3 and 4 for 6, 8 and 10 g kg^{-1} day^{-1} conditions, respectively. Participants scored significantly higher feelings of hunger (condition effect, $p = 0.03$; Figure 2a) when consuming 6 compared to 10 g kg^{-1} day^{-1} CHO ($p = 0.05$), and significantly greater fullness (condition effect, $p = 0.01$) consuming 10 versus 6 g kg^{-1} day^{-1} CHO ($p = 0.02$; Figure 2b). Desire for savory showed a significant main effect for time ($p = 0.02$; Figure 2c), post hoc tests revealed a 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 g kg^{-1} day^{-1} CHO (Figure 2d). There was no main effect for condition on subjective scores of tiredness ($p = 0.38$, Figure 2e), sleepiness ($p = 0.72$, Figure 2f), energy ($p = 0.42$, Figure 2g) or lethargy/sluggishness ($p = 0.36$, Figure 2h).

3.2 | Muscle Glycogen

Day 2 pre-exercise muscle glycogen concentration following 4 g kg^{-1} day^{-1} of CHO decreased by −266.9 ± 79.4 mmol kg^{-1} DM (−71%, $p < 0.001$) following the HIT session. There was a significant main effect of CHO intake on muscle glycogen

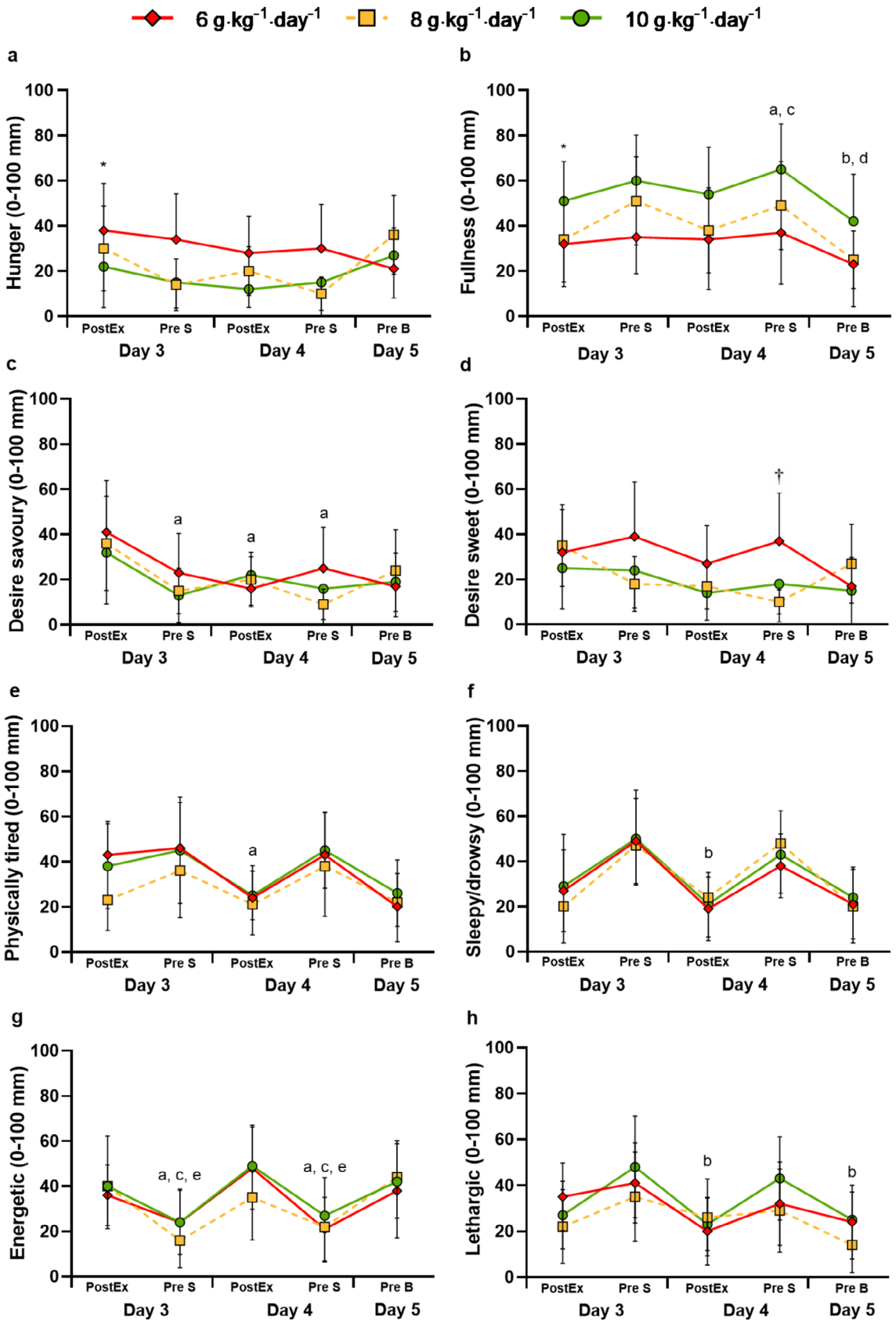

FIGURE 2 | Subjective visual analogue scale scores for hunger (a), stomach fullness (b), desire for savory (c) or sweet (d), physical tiredness (e), sleepiness (f), energy/liveliness (g) and lethargy/sluggishness (h) post-exercise (PostEx), pre-sleep (Pre S) on days 3 and 4, as well as pre-breakfast (Pre B) in the morning of day 5, under conditions of high CHO availability (6, 8 or 10 g kg^{-1} day^{-1} CHO). *Significant main effect of condition (6 vs. 10 g kg^{-1} day^{-1}). †Significant interaction effect. [a]Significant time effect vs. day 3 post exercise. [b]Significant time effect vs. day 3 pre-sleep. [c]Significant time effect vs. day 4 post exercise. [d]Significant time effect vs. day 4 pre-sleep. [e]Significant time effect vs. day 5.

concentration ($p < 0.001$), where consuming a high or very high relative CHO intake (8 or 10 g kg^{-1} day^{-1}) for 48 h resulted in significantly greater glycogen concentrations compared to baseline (Figure 3). There was no significant difference following consumption of 4 versus 6 ($p = 0.20$) or 6 versus 8 g kg^{-1} day^{-1} of CHO ($p = 1.00$, respectively), whilst 10 g kg^{-1} day^{-1} resulted in significantly higher muscle glycogen compared to all other conditions ($p < 0.05$; Figure 3a). Delta between conditions were similar, as 2 g kg^{-1} day^{-1} increases in CHO intake increased muscle glycogen by 107.1 ± 123.2, 35.3 ± 118.8 and 117.8 ± 137.3 mmol kg^{-1} DM from 4 to 6, 6 to 8 and 8 to 10 g kg^{-1} day^{-1} respectively ($p = 0.47$, $\eta^2_p = 0.09$; Figure 3b). There was a strong significant positive correlation between relative ($r = 0.71$, $p < 0.001$; Figure 4a) and absolute CHO intake ($r = 0.64$, $p < 0.001$; Figure 4b) versus muscle glycogen concentration.

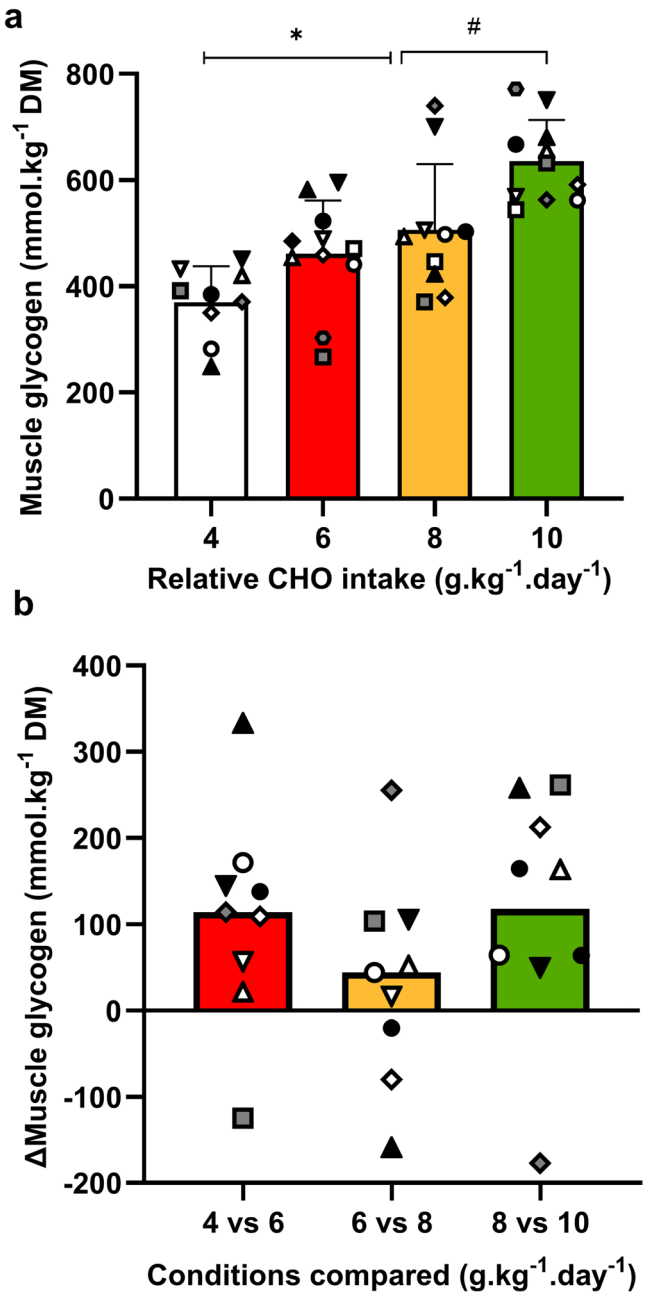

FIGURE 3 | Skeletal muscle glycogen concentration following consumption of a diet containing either 4, 6, 8 or 10 g kg^{-1} day^{-1} of CHO for 36–48 h (a) and the delta between CHO conditions (b). *Significantly different from 10 g kg^{-1} day^{-1}. #Significantly different from 4 g kg^{-1} day^{-1}. Each symbol represents individual participant data.

3.3 | Gastrointestinal Measures

Median (IQR) stool type did not differ (χ^2 [2] = 0.29, $p = 0.87$) whilst consuming 6, 8 or 10 g kg^{-1} 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, with 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 ± 5.0, 19.0 ± 4.0 and 18.7 ± 5.8 h following 6, 8 and 10 g kg^{-1} day^{-1} CHO respectively. Gut comfort symptoms were similar between conditions ($p > 0.05$), apart from stomach fullness, where participants

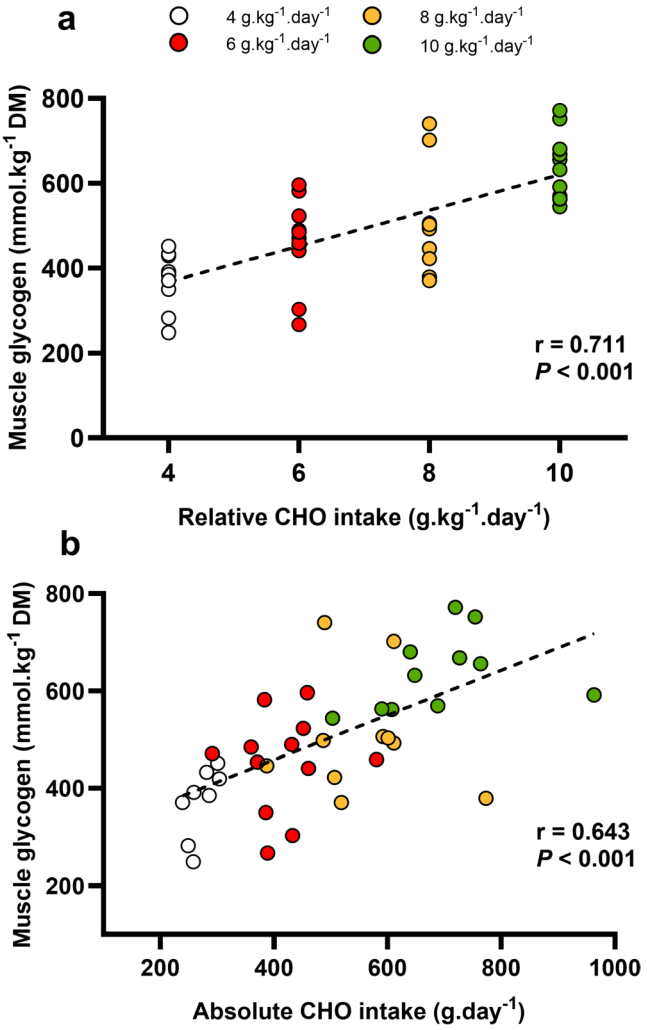

FIGURE 4 | Correlation of skeletal muscle glycogen concentration and relative (a) or absolute CHO intake (b) following consumption of a diet containing either 4, 6, 8, or 10 g kg^{-1} day^{-1} of CHO for 36–48 h, with significance at $p < 0.05$ and r value reported. Each data point represents individual participant data, with symbols indicating CHO intake during experimental conditions.

TABLE 3 | Median (IQR) gut comfort symptoms (0–10) across 48 h loading period when participants consumed 6, 8 or 10 g kg^{-1} day^{-1} CHO.

GI symptom	6 g kg^{-1} day^{-1}	8 g kg^{-1} day^{-1}	10 g kg^{-1} day^{-1}	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)	**$p = 0.006$***	6 vs. 8 $P = 0.53$ **6 vs 10 $p = 0.017$*** **8 vs 10 $p = 0.014$***
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)	$p = 0.156$	—

Note: Wilcoxon signed rank test significance (*) set at $p \leq 0.017$ (Bonferroni correction). Bold values indicate significant differences between conditions.

reported significantly greater feelings of fullness when consuming 10 compared to 6 or 8 g kg^{-1} day^{-1} CHO (Table 3). However, there were a higher number of moderate–severe symptoms (scores ≥ 5) reported during the 10 g kg^{-1} day^{-1} condition for nausea, reflux, stomach fullness and gas/flatulence (Table 3). One female participant accounted for 55% of scores ≥ 5 across symptoms of nausea, reflux, stomach fullness and flatulence.

3.4 | Body Mass and Body Water

Body mass decreased from pre- to post-exercise on day 2 (−0.59 ± 0.36, −0.54 ± 0.37, −0.56 ± 0.34 kg), despite consumption of 0.96 ± 0.33, 0.93 ± 0.28 and 0.87 ± 0.16 L of water during the session for 6, 8 and 10 g kg^{-1} day^{-1}, respectively. However, this decrease did not result in changes in total body water from pre- to post-exercise, which saw no main effect for condition, time or interaction ($p = 0.20$, $p = 0.53$, $p = 0.36$). Body mass measured on the morning of day 5 did not differ between conditions ($p = 0.70$, $\eta^2 = 0.03$; Figure 5a). This was also the case for total body, extra- and intra-cellular water content ($p = 1.00$, $p = 0.25$ and $p = 0.58$) as there was no difference between conditions (Figure 5b). Individual participant data included within Table S5.

4 | Discussion

This study reports for the first time the dose–response of dietary CHO intake and skeletal muscle glycogen in endurance trained cyclists with CHO intakes from 6 to 10 g kg^{-1} day^{-1}, whilst replicating a real-world pre-competition exercise taper. Results show a linear increase in muscle glycogen concentration with increasing CHO intake; however, 10 g kg^{-1} day^{-1} of CHO resulted in greater muscle glycogen stores compared to all other conditions. Hence, in contrast to our hypothesis, there was no apparent saturation point of CHO intake on muscle glycogen. All conditions were well tolerated in terms of gut comfort, with minimal impact on markers of gut function and we report no changes in body mass, total body, extra- or intracellular water between conditions.

In line with the history of CHO loading [3, 6, 7, 18] a high and very high relative CHO intake (8 and 10 g kg^{-1} day^{-1}) for 48 h resulted in significantly increased muscle glycogen concentrations compared to baseline (day 2 pre-exercise), where participants consumed a low-moderate CHO intake (4 g kg^{-1} day^{-1}) for ~36 h (Figure 3). The 172% increase in glycogen concentration with 10 g CHO kg^{-1} day^{-1} was smaller than the ~200% increase suggested by Burke et al. [8] and previously reported [6, 7, 18, 40]. A possible reason for this discrepancy is that current study conditions mimicked real-world settings where athletes, albeit less, keep training pre-competition. This is different from other studies where participants were fully rested throughout the high to very high CHO intake loading period (8.3–10.5 g kg^{-1} day^{-1} for 24–72 h) [7, 18, 40], likely resulting in a higher net CHO availability compared to the current study. This is a relevant consideration for real-world training conditions, where higher CHO intakes may be necessary during CHO loading conducted during a pre-competition taper, as shown by Sherman and colleagues [17], where 10 g kg^{-1} day^{-1} of CHO maintained baseline muscle glycogen concentrations following 7 days training in cyclists and runners, whilst 5 g kg^{-1} day^{-1} did not. Notably, exercise intensities were higher compared to the current protocol (1 h at 70% $\dot{V}O_{2max}$ followed by five 1-min sprints vs. steady state sessions at LT1). We also observed a dose–response between CHO intake and muscle glycogen concentration between 4 and 10 g kg^{-1} day^{-1} (Figure 4) and no tapering in the increase of muscle glycogen synthesized with 2 g kg^{-1} day^{-1} increases in CHO intake (Figure 3b).

Accordingly, the 10 g kg^{-1} day^{-1} condition resulted in significantly higher muscle glycogen stores compared to 8 g kg^{-1} day^{-1}, suggesting this level of CHO intake in the simulated days leading up to competition was best to ensure a 'supercompensation' of muscle glycogen stores. In agreement, increased CHO intake overall also inadvertently optimized post-exercise CHO ingestion following the exercise sessions conducted during the loading period (days 3 and 4). Retrospective analysis revealed participants ingested ~4 g kg^{-1} within the 0–4 h post-exercise period on both days during the 10 g kg^{-1} day^{-1} condition, in line with acute post-exercise refueling guidelines [9] (compared to 2 and 3 g kg^{-1} for 6 and 8 g kg^{-1} day^{-1}; Table S4). Considering glycogen utilization during exercise is a strong driver for post-exercise glycogen resynthesis [5], increased CHO intake immediately post-exercise, and the related insulin-mediated upregulation of glycogen synthase activity, likely resulted in greater muscle glycogen synthesis rates during this period [41, 42]. It remains to be determined whether higher CHO intakes (> 10 g kg^{-1} day^{-1}) would result in even higher post-load glycogen concentrations.

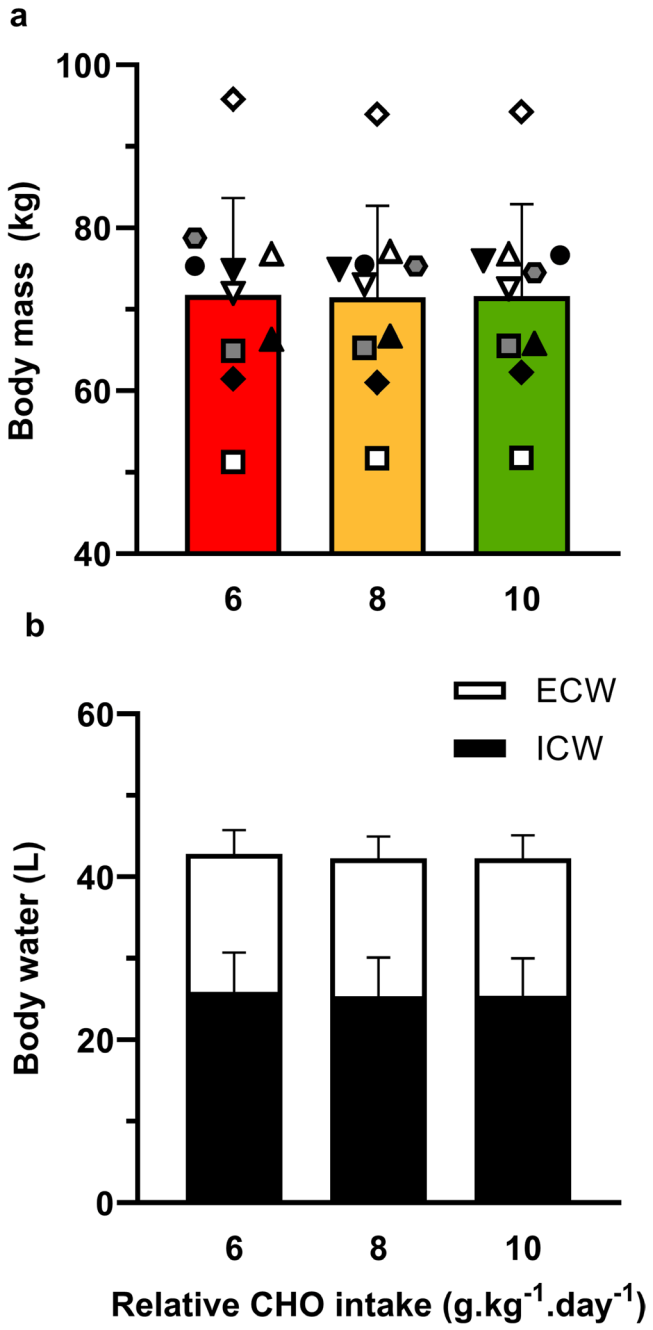

FIGURE 5 | Body mass (a) and total body water (b) expressed as intra and extra-cellular water (ICW and ECW) of endurance trained males and females following consumption of a diet containing 6, 8 or 10 g kg^{-1} day^{-1} of CHO for 48 h. Each symbol represents individual participant data.

Correlation data and the linear dose–response relationship (Figure 4) suggest higher amounts of CHO may have resulted in even higher glycogen concentrations. These findings contrast with previous reports which suggested a saturation point or 'ceiling' effect for muscle glycogen synthesis at 7–10 g kg^{-1} day^{-1} [2, 8, 15]. Burke et al. [15] originally proposed this threshold after 24 h of 7 vs. 11.8 g kg^{-1} day^{-1} of CHO resulted in no difference in glycogen concentrations (522.8 ± 41.8 and 519.4 ± 25.2 mmol kg^{-1} DM respectively). Values are comparable with the 8, but not the 10 g kg^{-1} day^{-1} condition in the current study, where muscle glycogen was 22.4% higher, despite a lower relative CHO intake (–1.8 g kg^{-1} day^{-1}). A possible reason behind this discrepancy was total time of enhanced 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 [15]. Bussau et al. [7] suggested an 'improved' protocol only required 24–36 h to 'supercompensate' glycogen stores, with additional time providing no further benefit to glycogen concentration. However, numerous others have shown a continued enhancement of muscle glycogen stores over multiple days of a high-very high CHO intake [14, 43–45], suggesting the ~2.5-fold longer loading period of increased CHO availability in the current study was responsible for the higher muscle glycogen stores, 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 high CHO availability would not further augment glycogen synthesis (and concentration), however it cannot be concluded where this threshold occurs from current study data. Findings also suggest up to 10 g kg^{-1} day^{-1} of CHO results in no measurable negative effects on gut function or body mass.

High and very high dietary CHO intakes were well-tolerated in terms of gut comfort in a cohort of endurance trained individuals, with a negligible effect on markers of gut transit time and function. To the authors' knowledge, this is one of the only times the effects of CHO loading on gut comfort and function have been measured [4], with previous studies focussed on GI discomfort symptoms associated with high CHO intakes during exercise (90–120 g h^{-1}) [46, 47]. The high tolerance in the current study likely resulted from the control of other known influencers of gut comfort and function during exercise, such as nutritional intake (specifically dietary fiber, fat and protein intake), intensity of exercise conducted during the loading period (light to moderate intensity; LT1) and participant training status, where most endurance trained individuals commonly use CHO loading strategies pre-competition [19, 48]. Stomach fullness was greater consuming 10 vs. 6 and 8 g kg^{-1} day^{-1} CHO (Table 3). However, median stomach fullness in the 10 g kg^{-1} day^{-1} condition was below a moderate severity score of 5 and total number of severe scores were low across all symptoms. Interestingly, one female accounted for 55% of all symptoms scored ≥ 5, which may be related to specific characteristics of this individual. The high tolerability of the diet in terms of gastrointestinal function was also accompanied by no detectable changes in body composition.

Contrary to our hypothesis and at odds with previous reports, body mass, TBW, ECW and ICW were similar between conditions (Figure 5). This contrasts with current literature where body mass increased following CHO loading [21, 22, 49], which supported the idea that water binds to glycogen in a 4:1 ratio [20, 49, 50]. In our study, assuming a 4:1 water: glycogen ratio, a post load total muscle mass of 28.6 kg (BIA data), and that the increase in muscle glycogen would have been homogenous across all muscles, the increase in total muscle glycogen would have been 30, 175 and 144 g in 6 vs. 8, 6 vs. 10 and 8 vs. 10 g kg^{-1} day^{-1} CHO, respectively. This in turn, would have been reflected in increases of body mass (water) of 0.12, 0.70 and 0.58 kg, respectively, which was not detected. A recent narrative review by Shiose et al. [51] suggested the glycogen: water relationship was less clear than commonly believed, as early animal studies [52, 53] and recent studies in humans showed no change in body

water following manipulation of glycogen stores [22, 49, 54]. Interestingly, human studies that reported this (current study included) used multifrequency BIA or BIS [22, 49, 54], which despite being validated against gold standard isotope dilution techniques [36], can be affected by changes in fluid and electrolyte balance [55], which were not controlled in the current study (in fact, retrospective analysis revealed differences in sodium intake between conditions; 1751 ± 382, 2223 ± 588 and $3009 \pm 518\,mg\,day^{-1}$ for 6, 8 and $10\,g\,kg^{-1}\,day^{-1}$, respectively). In agreement, Shiose et al. [49] found a 0.9 L (2.4%) increase in TBW following 72 h of a very high CHO intake ($12\,g\,kg^{-1}\,day^{-1}$) measured with deuterium dilution, whereas BIS showed no significant difference, despite a 0.4 L increase (~1%). Therefore, it is likely the use of BIS/BIA, the relatively small study sample sizes ($n \leq 11$) and long nature of the current study protocol (potential noise introduced by fluctuations in body mass over time), limited sensitivity to detect small, localized changes in TBW expected with $2–4\,g\,kg^{-1}\,day^{-1}$ increments in CHO intake (1%–2% or 0.5–0.7 L). Moreover, previous reports of the positive relationship between glycogen storage and water [20, 21, 50] have often compared extreme conditions of CHO availability, where participants underwent upper and lower body depletions and consumed vastly different CHO intakes ($\geq 6\,g\,kg^{-1}\,day^{-1}$ difference between conditions), resulting in exaggerated differences in muscle glycogen and body water compared to the real world. Additionally, diets with divergent amounts of CHO intake, may also be divergent in dietary residue such as fiber, which may be a contributor to higher body mass in high CHO diets [56]. Future studies to determine the relationship between high-CHO diets and body mass, and its mechanisms are warranted.

This study reports for the first time the dose–response of a moderate, high, and very high dietary CHO intake (6, 8, and $10\,g\,kg^{-1}\,day^{-1}$, respectively) on skeletal muscle glycogen concentration in endurance trained individuals. Results show that, mimicking the pre-competition practices of endurance cyclists, a very high dietary CHO intake ($10\,g\,kg^{-1}\,day^{-1}$) resulted in the highest muscle glycogen concentrations. The lack of plateau in the increase in muscle glycogen with increasing dietary CHO suggests higher CHO amounts may have led to higher muscle glycogen. These findings both support and challenge the current nutritional guidelines by providing evidence that a 'ceiling' for muscle glycogen resynthesis is yet to be determined. Our findings also show that high CHO strategies were well tolerated in relation to GI function and discomfort symptoms, apart from modest increases in stomach fullness, whereas changes in body mass and water were not detected (possibly due to current study procedures). Further research should evaluate not only the mechanisms behind these observations but also the effectiveness of stepwise increases in CHO intake on prolonged endurance performance with rigorous methodologies.

5 | Perspectives

Future research should incorporate CHO loading strategies with intakes $> 10\,g\,kg^{-1}\,day^{-1}$ to identify where the 'ceiling' of CHO loading for increased muscle glycogen concentrations ('supercompensation') truly occurs, as well as confirming the upper limit of tolerability for athletes in terms of gut comfort, in practical training and nutritional conditions. It remains to be determined whether current findings are directly applicable to elite endurance athletes, given metabolic differences between trained amateurs and those competing at the highest level. Elite endurance athletes may achieve supercompensation with lower CHO intakes (i.e., $8\,g\,kg^{-1}\,day^{-1}$), however greater absolute exercise energy expenditure in training sessions conducted during the loading period and favored CHO utilization with increased CHO availability, also suggest higher rates of glycogenolysis, and perhaps a similar requirement to consume at least $10\,g\,kg^{-1}\,day^{-1}$ to optimize pre-competition glycogen stores. Methodological limitations of the current study include the descriptive Pearson's correlation, which does not account for repeated observations, and body mass/water measurements, which were possibly influenced by study procedures. Nonetheless, in agreement with Shiose et al. [46], the relationship between muscle glycogen and body water appears inconclusive, despite the commonly accepted belief of a 1:4 glycogen: water relationship, further research work is required to confirm this.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Self-reported hormonal contraceptive use by female participants during screening and preliminary measures laboratory visit. **Table S2:** Alignment of estimated menstrual cycle phase and experimental conditions for the only naturally menstruating female participant, in line with Elliot-Sale et al. (2021). **Table S3:** Estimated energy intake, total and exercise energy expenditure and energy availability for each study participants during 48 h CHO loading periods consuming either 6, 8 or 10 $g\,kg^{-1}\,day^{-1}$ of CHO. **Table S4:** Distribution of CHO intake ($g\,kg^{-1}$) in relation to exercise sessions conducted for each study participant during a 48 h CHO loading period consuming a total of either 6, 8 or 10 $g\,kg^{-1}\,day^{-1}$ of CHO. **Table S5:** Individual study participant data for total body, extra and intracellular water (liters) following a 48 h CHO loading period consuming either 6, 8 or 10 $g\,kg^{-1}\,day^{-1}$ of CHO.