The American Journal of Clinical Nutrition xxx (xxxx) xxx

The American Journal of CLINICAL NUTRITION

journal homepage: www.journals.elsevier.com/the-american-journal-of-clinical-nutrition

Original Research Article

Liver glycogen stores via ^{13}C magnetic resonance spectroscopy in healthy children: randomized, controlled study☆

Astrid MH. Horstman [1], Stephen J. Bawden [2,3], Abi Spicer [3], Noura Darwish [1], Amélie Goyer [1], Léonie Egli [1], Natacha Rupp [1], Kaori Minehira [1], Penny Gowland [2,3], Denis Breuillé [1], Ian A. Macdonald [1,4], Elizabeth J. Simpson [2,4,*]

[1] *Nestlé Institute of Health Sciences, Nestlé Research, EPFL Innovation Park, Lausanne, Switzerland;* [2] *National Institute for Health Research Biomedical Research Centre, Queen's Medical Centre, Nottingham, United Kingdom;* [3] *Sir Peter Mansfield Imaging Centre, School of Physics and Astronomy, University of Nottingham, Nottingham, United Kingdom;* [4] *David Greenfield Human Physiology Unit, MRC/ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham School of Life Sciences, Queen's Medical Centre, Nottingham, United Kingdom*

A B S T R A C T

Background: Owing to its role in glucose homeostasis, liver glycogen concentration ([LGly]) can be a marker of altered metabolism seen in disorders that impact the health of children. However, there is a paucity of normative data for this measure in children to allow comparison with patients, and time-course assessment of [LGly] in response to feeding has not been reported. In addition, carbon-13 magnetic resonance spectroscopy (^{13}C-MRS) is used extensively in research to assess liver metabolites in adult health and disease noninvasively, but similar measurements in children are lacking.

Objectives: The main objectives were to quantify the depletion of [LGly] after overnight fasting and the subsequent response to feeding.

Methods: In a randomly assigned, open-label, incomplete block design study, healthy, normal-weight children (8–12 y) attended 2 evening visits, each separated by ≥5 d and directly followed by a morning visit. An individually tailored, standardized meal was consumed 3-h prior to evening assessments. Participants then remained fasted until the morning visit. [LGly] was assessed once in the fed (20:00) and fasted state (08:00) using ^{13}C-MRS. After the 8:00 assessment, 200 ml of a mixed-macronutrient drink containing 15.5 g (402 kJ) or 31 g carbohydrates (804 kJ), or water only, was consumed, with ^{13}C-MRS measurements then performed hourly for 4 h. Each child was randomly assigned to 2 of 3 drink options across the 2 mornings. Data are expressed as mean (SD).

Results: Twenty-four children including females and males (13F:11M) completed the study [9.9 (1.1) y, BMI percentile 45.7 (25.9)]. [LGly] decreased from 377.9 (141.3) to 277.3 (107.4) mmol/L overnight; depletion rate 0.14 (0.15) mmol/L min. Incremental responses of [LGly] to test drinks differed ($P < 0.001$), with incremental net area under the curve of [LGly] over 4 h being higher for 15.5 g [−67.1 (205.8) mmol/L·240 min; $P < 0.01$] and 31 g carbohydrates [101.6 (180.9) mmol/L·240 min; $P < 0.005$] compared with water [−253.1 (231.2) mmol/L·240 min].

Conclusions: After overnight fasting, [LGly] decreased by 22.9 (25.1)%, and [LGly] incremental net area under the curve over 4 h was higher after subsequent consumption of 15.5 g and 31 g carbohydrates, compared to water. *Am J Clin Nutr* 20XX;xx:xx–xx.

Keywords: muscle glycogen concentration, fasting, feeding, carbohydrate metabolism, gastric emptying

Introduction

With the increased prevalence of obesity and metabolic disorders in the general population globally [1–3], there is a need for a comprehensive understanding of the impact of diet and lifestyle on energy metabolism in humans across the lifespan. Although multiple studies have explored this in healthy and diseased adult cohorts, less work has focused on children.

In adults, glycogen provides the primary acute-phase carbohydrate energy store and contributes to blood glucose concentration regulation between meals. Although muscle contains the largest reservoir of glycogen, liver glycogen metabolism contributes ~45% of total endogenous glucose production during the initial periods of fasting and

Abbreviations: $AUC_{240\ min}$, AUC over 4 h; BIBD, balanced incomplete block design; ^{13}C-MRS, carbon-13 magnetic resonance spectroscopy; $i.netAUC_{240\ min}$, incremental net AUC over 4 h; [LGly], liver glycogen concentration; MR, magnetic resonance; TR, repetition time.

☆ This trial was registered at clinicaltrials.gov (NCT04278209).

* Corresponding author. National Institute for Health Research Biomedical Research Centre, Queen's Medical Centre, Nottingham, United Kingdom.
E-mail address: liz.simpson@nottingham.ac.uk (E.J. Simpson).

https://doi.org/10.1016/j.ajcnut.2023.01.014
Received 28 July 2022; Received in revised form 4 January 2023; Accepted 13 January 2023; Available online xxxx

thus plays a fundamental role in blood glucose homeostasis [4, 5]. Liver glycogen content rapidly depletes with a period of fasting [6, 7] and is readily replenished in adults following consumption of carbohydrates. By contrast, fasting results in a limited reduction of the glycogen depots in adult skeletal muscle [7], with muscle switching its energy substrate metabolism predominantly to lipid oxidation during fasting [8], and insulin-stimulated muscle glucose uptake is reduced [9]. However, similar information in children is lacking.

To our knowledge, only 2 studies have measured liver glycogen concentration ([LGly]) in children using carbon-13 magnetic resonance spectroscopy (^{13}C-MRS) previously [10, 11]. One study [10] measured [LGly] following overnight fasting and 4-h after 2 standardized meals (at breakfast and lunch) and reported [LGly] after the final meal was 36% above fasting values. In the other [11], daytime liver glycogen accumulation in children with type 1 diabetes mellitus was compared with that of controls without diabetes. Liver glycogen content was ascertained in the fasted (morning) and fed (afternoon) state, and the ability of young children with type 1 diabetes mellitus to replace glycogen stores in the liver (depleted after an overnight fast) appeared to be comparable to control children. However, to what extent liver and muscle glycogen concentrations are depleted in healthy children after overnight fasting and the temporal dynamics occurring after eating is unknown.

^{13}C-MRS is a well-validated, noninvasive tool for organ-specific glycogen measurement [12–14] and has been used in multiple adult studies over many years [15–17]. The technique allows for safe and amenable sequential measurements of glycogen stores and is particularly suited to more vulnerable cohorts, such as children, but has not, to date, been widely exploited in this latter population.

The current study aimed to expand current knowledge of normative glycogen stores in children by using ^{13}C-MRS to assess the depletion of both liver and muscle glycogen after overnight fasting and to investigate to what extent these stores are replenished with the intake of a small breakfast (equivalent to 1 or 2 servings of a chocolate malt beverage).

Methods

Study design

A 2-phase, randomly assigned, controlled, open-label, single-center study was conducted to investigate the effect of consuming carbohydrates on liver and muscle glycogen stores in healthy children after overnight fasting. Specifically, the primary outcome measure was the incremental net area under the concentration/time curve over 4 h (i.net$AUC_{240\ min}$) for liver glycogen. The secondary outcome measures were the change in liver and muscle glycogen concentrations after an overnight (12-h) fast and the i.net$AUC_{240\ min}$ of the concentration/time curve for muscle glycogen. Although there was a theoretical potential for bias because of the lack of blinding of participants to drink allocation, all analyses of magnetic resonance (MR) scans were carried out by individuals blinded to the intervention drink given. Furthermore, no subjective measures were collected in participants that could have been impacted by them knowing the drink that they had consumed.

An interim analysis was carried out after the first phase had been completed to compute the number of additional subjects required to reach conditional statistical power. In phase 1 (9 participants; Aug 2020–Dec 2020), a balanced incomplete crossover block design was employed to assess the effects of consuming 15.5 g or 31 g of available carbohydrates on [LGly] (compared to water; 6 participants per drink), with a second phase (15 participants; Apr 2021–Nov 2021) using a balanced crossover design to measure the effects of consuming 15.5 g of carbohydrate compared to water (15 participants per drink). The random allocation sequence for each phase was generated using an online system (iMedidata Rave) hosted by study sponsor (Société des Produits Nestlé SA), and individuals were randomly assigned (using unstratified randomization) at the point of entering the study, with their participant number allocated sequentially. Enrollment was carried out independently by the research team at Nottingham. After a successful screening, participants attended the Sir Peter Mansfield Imaging Centre (University of Nottingham, UK) on 4 occasions; 2 evening visits, each separated by $\geq$5 d and each directly followed by a morning visit.

Ethics

This study was conducted according to the Declaration of Helsinki 1973 guidelines (revised 2013), and all procedures involving human participants were approved by the University of Nottingham Medical School Ethics Committee (reference 426-1911). Written consent was obtained from all volunteers and their legal guardians, and the protocol was registered at www.clinicaltrials.gov (reference NCT04278209).

Participants

Healthy, normal-weight children at Tanner stage 1 or 2 [18, 19] and aged 8–12 y were recruited from the local population through advertisements on social and traditional media platforms. Twenty-nine children who were interested in participating in the study and who fulfilled age and general health criteria attended the initial screening at the David Greenfield Human Physiology Unit (Medical School, Nottingham, UK) with their legal guardians. BMI percentile was calculated from height and weight percentile using ethnicity-appropriate growth charts, with healthy BMI defined as being between the 5th and 85th percentile. Those with self-reported food allergies related to ingredients in the trial drinks, including lactose intolerance, were not recruited. See Supplemental Figure 1 for Consort Diagram illustrating participant flow through the recruitment and experimental pathway.

Standard meal

Three hours prior to evening assessments (17:00), an individually tailored meal (Table 1) (60% of energy as carbohydrate, 20% protein, and 20% fat) providing 35% of the daily energy requirement (determined as described below) was consumed by participants at home, with no further food intake from this meal until the following morning visit. Habitual diet and food preferences were previously determined from a 4-d dietary record (3 weekdays and 1 weekend day), with daily PAL assessed over 5 d using a triaxial accelerometer GT3X (ActiGraph LLC) worn at the waist. Participants collected both measurements after recruitment and returned to researchers before the first experimental visit. Dietary records were analyzed, and the standard evening meal was designed using a food composition database (Nutritics) [20], with the activity data interrogated using the manufacturer's software Actilife V6 (Actigraph LLC). The individual's PAL index was subsequently used as a multiplier for resting energy expenditure estimated from standard equations [21] to calculate daily energy requirement. Guardians were asked to record when their child ate the standard meal (start and end time) and if any of the meal was not consumed. Where the meal was not fully consumed, guardians were requested to photograph

TABLE 1
Energy and macronutrients content of the prescribed standardized evening meal presented to, and consumed by the participants, according to drink allocation (water, 15.5 g beverage, and 31 g beverage) the following morning

Drink allocation	Meal prescribed			
	Energy (kJ)	Proteins (g)	Carbohydrates (g)	Fat (g)
Water ($n = 21$)	2732 (360)	33 (5)	97 (13)	15 (2)
15.5 g beverage ($n = 21$)	2699 (347)	32 (4)	95 (14)	15 (2)
31 g beverage ($n = 6$)	2812 (346)	32 (6)	99 (15)	17 (2)
	Meal consumed			
	Energy (kJ)	Proteins (g)	Carbohydrates (g)	Fat (g)
Water ($n = 21$)	2657 (448)	32 (6)	94 (17)	15 (2)
15.5 g beverage ($n = 21$)	2594 (473)	31 (6)	91 (18)	15 (3)
31 g beverage ($n = 6$)	2774 (351)	32 (6)	97 (14)	17 (2)

Data are the mean with standard deviation in parentheses.

the food remaining, and the actual intake (macronutrient and energy content) was estimated from these.

Study visits

On each evening visit (20:00), participants underwent an ~1 h MR scanning session (details below) at the Sir Peter Mansfield Imaging Centre to assess volume of stomach contents and liver and muscle glycogen concentration. They then returned the following morning (08:00), having been instructed to have nothing to eat or drink other than water in the intervening period. Baseline MR measurements for stomach contents' volume and [LGly] were made before the consumption of a test drink according to the randomly assigned drink allocation, with allocation for both sessions revealed after the first evening scan had been completed. Because of the extended fasting time required of the young participants when randomly assigned to consuming water, the ~30-min scan protocol to determine initial muscle glycogen concentration on all morning visits was scheduled immediately after drink ingestion to shorten the study day. Assessments of the volume of stomach contents, liver, and muscle glycogen concentration were then made every hour for 4 h following drink consumption.

Interventional products

The test drinks were 200 ml of water or an equivalent volume of a mixed-macronutrient chocolate malt beverage Milo (Société des Produits Nestlé S.A.); the latter consisting of malt extract, skimmed milk powder, sugar, vegetable oil, and cocoa powder, which provided either 15.5 g ("15.5 g beverage") or 31 g of carbohydrate ("31 g beverage"); powder equivalent to 1 and 2 servings. The macronutrient and energy content of drinks are shown in Supplemental Table 1. A mixed-macronutrient test drink was used in this study as it was a palatable means to provide the carbohydrate to these young participants and was expected to have a more rapid gastric emptying than an equivalent solid meal, reducing the postdrink data collection time and, by extension, the fasting time for children on the water visit. The chocolate malt beverages were supplied as powdered mixes, which were reconstituted with 200 ml of warm water immediately before consumption and were ingested within 10 min.

MR protocol

All MR measurements were acquired using a Philips 3 Tesla Achieva MRI system (Philips), with an integrated proton body coil used for image acquisition. ^{13}C-MR spectra were obtained using a single-loop ^{13}C surface coil with integral butterfly proton decoupling channels (PulseTech). A ^{13}C-labeled urea sample was positioned in the center of the coil and used as a calibration reference signal.

At each timepoint, participants were placed in the scanner in the supine position, and initial images were acquired to determine gastric contents volumes (~2 min). The ^{13}C surface coil was then placed over the liver [22] for the acquisition of ^{13}C-MRS to determine [LGly] (~14 min). Finally, the ^{13}C surface coil was repositioned over the right thigh to acquire ^{13}C-MRS to determine muscle glycogen concentration (~26 min at the evening and initial morning scan, with subsequent assessments taking ~6 min).

Volumes of gastric contents were measured using a fast gradient echo sequence [repetition time (TR) = 2 ms, the field of view = 300 × 200 × 300 mm, total scan time = 16 s, slice resolution = 120 × 192, 25 slices]. Images were imported into Analyze9 (Mayo Foundation), and the regions of interest were manually drawn around stomach contents to calculate volume [22, 23].

Liver glycogen concentrations were measured using a nonlocalized short-TR block pulse-acquire sequence (bandwidth = 7 kHz, TR = 280 ms, sample points = 1024, number of averages = 3072, total scan time ~15 min) with pencil beam shimming. Scout images were initially acquired to confirm correct coil positioning, followed by a long-TR ^{13}C-reference scan for signal calibration (TR = 1500 ms, number of averages = 20). The area under the glycogen doublets at ~101 ppm was determined by fitting Gaussian curves using an in-house Matlab (MathWorks Inc) script and scaling to the signal from the ^{13}C-Urea reference peak (~175 ppm). Absolute concentrations were then calculated by comparison with a 200 mmol/L glycogen phantom after correcting for differences in transmit-receive field (B_1) sensitivity [22, 24, 25]. A decrease in [LGly] from the evening (fed) assessment to the next morning (fasted) assessment was summarized as a percentage decrease, and the between-visit coefficient of variation for fasting [LGly] was computed. The overnight depletion rate of [LGly] over the fasting period was calculated across all visits, with the depletion rate from the initial morning to 240 min assessment calculated at the water visit. The incremental net area under the curve over the 4-h was computed using the trapezoid method, accounting for areas both below and above the fasting glycogen concentration.

Muscle glycogen concentrations were measured using a nonlocalized adiabatic half passage hyperbolic-secant pulse-acquire sequence (bandwidth = 7 kHz, TR = 1358 ms, sample points = 512) with narrow-band proton decoupling [15] (adiabatic half passage and decoupling used because of the variability and smaller size of muscle compared to the liver in children). Eleven hundred and eighty-four

spectra were acquired and averaged at the evening and morning fasted time points (~26 min), whereas only 296 were averaged at all postdrink time points (~6 min) because of time constraints in the postprandial phase. The area under the single decoupled glycogen peak at ~101 ppm was determined by fitting a Gaussian curve using in-house Matlab script and scaling to the signal for the ^{13}C-Urea reference peak at (~175 ppm). Because the muscle size variations affect volumes within the field of view, MRI was used to estimate participant-specific B_1 sensitivity effects and correct the final signal. Absolute concentrations were then calculated by comparison with a 200 mmol/L glycogen phantom [17].

Sample size

The initial sample size for the study was calculated for the primary objective, which was to determine the effects of consuming a mixed-macronutrient drink containing 15.5 g or 31 g of carbohydrates on [LGly] after an overnight fast. The effects to be interrogated were the differences in [LGly] area under the curve over 4 h ($AUC_{240\ min}$) between each carbohydrate amount and water, with the purpose being to confirm the observed effect while controlling the experiment-wise error rate at 0.05. In order to show a difference of 1.5 units within participant SD in [LGly] $AUC_{240\ min}$ after consuming 31 g of carbohydrate, with a power of 80% and an α-level (2-sided) of 0.05, it was estimated that 8 participants for each drink would be needed. This corresponded to a balanced incomplete block design (BIBD) with the allocation of the sequences 0–1, 1–0, 1–2, 2–1, 0–2, and 2–0 for 24 participants. An interim analysis was carried out after 9 participants had been completed to assess the conditional power of the primary variable, with a maximum of 24 participants retained.

Interim analysis

The stopping rule for success was, according to Pocock, $P < 0.028$ for [LGly] $AUC_{240\ min}$ at interim and at final analysis [26]. The randomization scheme of the BIBD was applied for 9 subjects, and the purpose of the interim analysis was to allow any design changes based on conditional power. Interim analysis sample size assessment resulted in dropping the 31 g beverage visit because significant differences with the water were already obtained at this stage despite the small number of participants in this group. The recruitment of a further 15 participants to undergo the water and 15.5 g beverage visit was recommended in order to reach 67% conditional power.

Statistical analysis

All data were coded and analyzed using SAS Life Science Analytical Framework version 5.2.3 (SAS Institute Inc.). Data were initially checked for normality of distribution (using qq-plot and residuals compared with fitted values plot). Parametric data are described in the text and tables as the mean with SD in parentheses. Normally distributed data in figures are the mean with error bars showing the SEM.

Glycogen overnight depletion was calculated as the average from all participants' overnight depletion measurements pooled across the 2 visits. Glycogen concentration $AUC_{240\ min}$ assessments, made across the 3 drink options, were carried out using a linear mixed-effect model adjusting for drink, baseline [LGly] concentration values, BMI percentile, and the visit as fixed effects and participant as a random effect. Gastric contents evaluations, made across the 3 drink options, were carried out using a linear mixed-effect model adjusting for drink, baseline values, timepoint, and the drink × time interaction as fixed effects and participant as a random effect. The macronutrient and energy contents of the standard meals provided before evening visits were compared across visits using the linear mixed-effect model with a drink as a fixed effect and a participant as a random effect. Analysis was unpaired (because of incomplete crossover block design), used a 2-tailed assessment, and statistical significance was assumed where $P < 0.028$ for [LGly] $AUC_{240\ min}$ analysis and where $P < 0.05$ for all other analyses. Post hoc analysis was used to probe differences in repeated measures data.

Finally, associations between i.net$AUC_{240\ min}$ and carbohydrate content of the 3 drinks were investigated using correlation analysis and are expressed as Pearson's R.

Results

Participants

Twenty-four children, including females and males (13F:11M), completed the study [9.9 (1.1) y, BMI percentile 45.7 (25.9)]. In phase 1 recruitment, 9 children (4F:5M) were randomly assigned to 2 of the 3 drinks (31 g beverage, 15.5 g beverage or water) in the BIBD ($n = 6$/drink allocation), with 15 (9F:6M) being randomly assigned in phase 2 (after interim analysis) to the 15.5 g beverage and water. Participant characteristics are shown in Table 2. No participant withdrew from the study after randomization.

Standard meal

The macronutrient and energy content of the standard meal provided before evening visits and the actual amounts consumed are shown in Table 1. There were no differences in total energy or carbohydrate intake at the evening meal between drink allocation.

[LGly]

Mean [LGly] decreased from 377.9 (141.3) to 277.3 (107.4) mmol/L overnight [−22.9 (25.1)%; equivalent to a depletion rate of 0.14 (0.15) mmol/L·min], with a between-visit coefficient of variation for fasting [LGly] being 21.5 (15.1)%. The impact of the test drink consumption on [LGly] is shown in Figure 1. There was a difference in response of [LGly] between drinks across the 4-h postingestion period ($P < 0.05$). Both the consumption of the 15.5 g as well as the 31 g beverage showed a different response in [LGly] compared to water ($P < 0.05$ and $P < 0.001$, respectively).

Similarly, there was a difference in 4-h i.netAUC seen across the visits ($P < 0.05$), with this variable being significantly different after consumption of the 15.5 g and 31 g beverages compared to water ($P < 0.005$ and $P < 0.01$, respectively). Moreover, exploratory analysis of the relationship between the amount of carbohydrate consumed and i.net$AUC_{240\ min}$ showed a linear carbohydrate "dose" response in i.net$AUC_{240\ min}$ ($R = 0.51$; $P < 0.001$; Figure 2). At 2 h after test drink consumption, mean liver glycogen repletion (from fasting) was 5.8 (29.6)% following consumption of the 15 g beverage and 34.6 (57.0)% after the 31 g beverage, whereas following water consumption, [LGly] decreased by 21.2 (29.4)% during the first 2 h after intake ($P < 0.005$). However, because of the small sample size in the 31 g group, inferences made for this group should be interpreted with caution.

TABLE 2
Participant baseline characteristics according to each drink option

Variable	Water ($n = 21$)	15.5 g beverage ($n = 21$)	31 g beverage ($n = 6$)
Age (y)	9.8 (1.1)	10.0 (1.2)	10.0 (1.4)
BMI percentile	43.2 (26.6)	42.8 (25.4)	64.0 (17.0)
Female (n)	13	12	1

Values are the mean with standard deviation in parentheses.

Muscle glycogen

Muscle glycogen concentrations in the fed (evening) and fasted (morning) states were not different; 114.9 (38.2) and 116.3 (40.4) mmol/L, respectively. Moreover, there was no difference in the response of muscle glycogen concentrations, or 4-h i.netAUC, between the 15.5 g beverage and water visit. Technical difficulties resulted in a complete data set for only 1 participant at the 31 g beverage visit for muscle glycogen concentration; thus, the differences between this test drink and water could not be compared.

Gastric emptying

Because gastric emptying will influence the uptake of carbohydrates into the circulation, and thus [LGly] replenishment, we also measured the volumes of gastric contents over the 4-h postprandial timepoints (Figure 3). At the 1 h assessment, water appeared to have completely emptied from the stomach, with mean gastric content volumes reaching fasted volumes by 2 h and 3 h after consumption of the test drinks containing 15.5 g and 31 g carbohydrates, respectively. Gastric emptying half-life was estimated as 32.4 (0.5) min for water, 62.5 (6.1) min for 15.5 g, and 88.3 (8.4) min for 31 g beverages ($P < 0.001$).

There was a difference in response of mean volume of gastric contents between drinks across the 4-h postingestion period, with the time × drink interaction being statistically significant ($P < 0.001$). Both the consumption of the 15.5 g as well as the 31 g beverage showed a different response in the volume of gastric contents compared to water at the timepoint of 60 min after drink consumption ($P < 0.001$ for both comparisons). At the remaining timepoints, comparisons between drinks did not reach significance.

Discussion

The current study expands knowledge of normative glycogen stores in children by using ^{13}C-MRS to assess the impact of overnight fasting on both liver and muscle glycogen. Moreover, the time-course response and extent to which these stores were replenished with intake of 15.5 g and 31 g available carbohydrates (equivalent to 1 or 2 servings of a chocolate malt beverage) are presented. To our best knowledge, it is the first time that these have been quantified in children.

Liver glycogen concentration, depletion, and repletion could be important markers of altered metabolism seen in disorders that impact

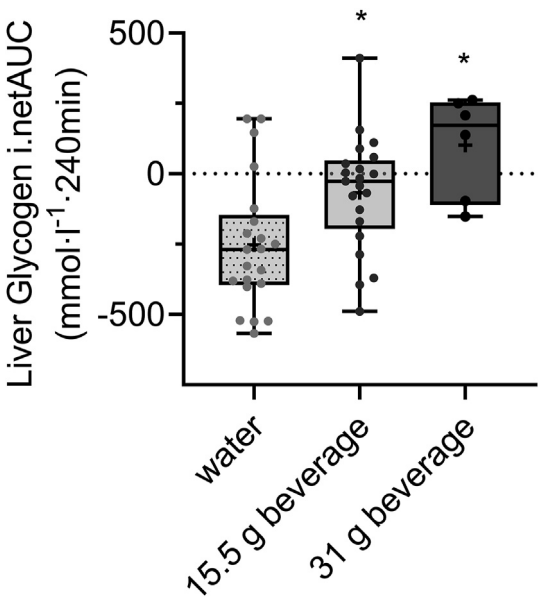

FIGURE 2. Incremental net area under the curve (i.netAUC) for liver glycogen concentrations over the 4-h postprandial periods for each of the 3 drinks ($n = 21$, 21, and 6 for water and 15.5 g and 31 g beverage, respectively). The figure shows the individual values, with boxes indicating the 25th and 75th percentile, and error bars showing the data range. The bold lines within the boxes indicate the median, with crosses showing the group's mean. 15.5 g beverage: drink containing 15.5 g of carbohydrates. 31 g beverage: drink containing 31 g of carbohydrates. * $P < 0.05$ compared to water.

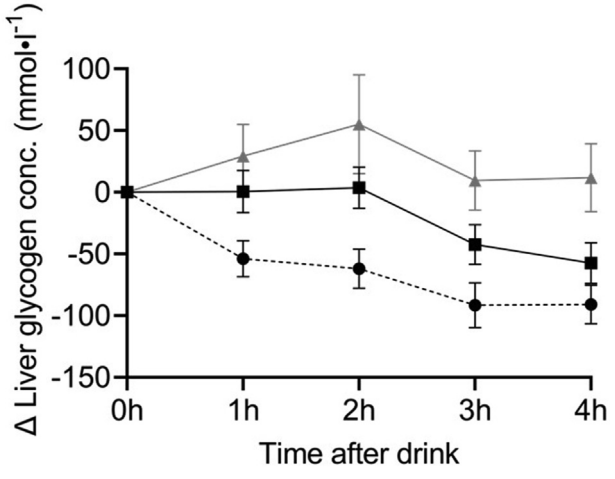

FIGURE 1. Incremental change in liver glycogen concentrations from fasted morning state, measured hourly for 4 h after the drink. Data are the mean with error bars indicating SEM. ●- Black circles: water ($n = 6$). ■- Black squares: drink containing 15.5 g of carbohydrates ($n = 21$). ▲- Gray triangles: drink containing 31 g of carbohydrates ($n = 21$). Drink effect: $P < 0.05$. $P < 0.05$ for 15.5 g beverage vs. water and $P < 0.001$ for 31 g beverage vs. water.

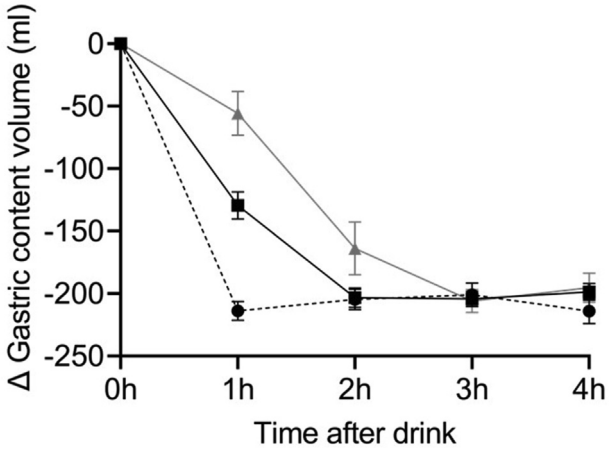

FIGURE 3. Incremental change in gastric content volume compared to fasted morning state, measured hourly for 4 h after the drink. Data are the mean with error bars indicating SEM. ●- Black circles: water ($n = 6$). ■- Black squares: drink containing 15.5 g of carbohydrate ($n = 21$). ▲- Gray triangles: drink containing 31 g of carbohydrate ($n = 21$). Time × Drink: $P < 0.001$. At t = 60 min, $P < 0.001$ for 15.5 g beverage vs. water and $P < 0.001$ for 31 g beverage vs. water.

the health of children because of the liver's role in maintaining glucose homeostasis. Biopsies would traditionally be taken to assess liver glycogen content, but concerns around the safety of this invasive technique limit its use in healthy children and make it unsuitable for sequential measurements to assess depletion or repletion rates in any child. ^{13}C-MRS has been used extensively in research to assess liver metabolites in adult health and disease noninvasively. However, this technique has not been widely exploited in pediatric cohorts. Consequently, there is a paucity of normative data for [LGly] in children to allow comparison with patient cohorts. Indeed, we are aware of only 2 such reports in the literature [10, 11]. In the present study, the MR scanning protocols were well tolerated by the young participants, making it an acceptable technique to use in this age group.

Our data indicated that [LGly] decreased by ~23% overnight. In adults, [LGly] has been reported to decrease by ~0.2 mmol/L·min in the first 22 h of fasting, a rate that was near constant over this time frame [5]. In the current study, this depletion rate in children over the 12-h overnight fast was lower, at 0.14 mmol/L·min, although it has been hypothesized that depletion rates could be greater than seen in adults [27]. The prefasting [LGly] in the current protocol was assessed 3-h after the standard evening meal, and at this timepoint, the stomach still showed evidence of the meal being present. As participants had not fully digested and absorbed the meal at this "fed" assessment, it is possible that [LGly] may not have reached the postprandial "peak" 3 h after the meal and, therefore, could be underestimated. Consequently, the liver glycogen depletion rate in children over the 12-h fast may be higher than calculated by the current data. Indeed, on the "water" study day, the reduction of [LGly] with continued fasting over the morning (which, according to adult data, would continue with the same depletion rate as seen overnight) showed a higher "morning" depletion rate [0.38 (0.30) mmol/L·min] than observed overnight. Logistical considerations around meal and bedtimes of this age group (and the duration that the young participants remained fasted during "water" visits) meant that scheduling a later evening visit or an earlier evening meal was not possible. Future studies would benefit from using a standard prefast meal which has a faster gastric emptying and absorption time.

The fasting [LGly] of children in the current cohort was 277 mmol/L. A previous study using ^{13}C-MRS showed that fasting [LGly] in healthy children aged 6–12 y was lower [median (range) 178 (120–203) mM glycosyl units] [11]. However, before the overnight fast in this study, no standardized meal was provided (and information about the composition and size of prior meals was not described), whereas the current protocol provided a high carbohydrate meal (35% of total daily energy requirement; 60% of energy as carbohydrate) which may have contributed to the higher [LGly] seen the following morning. Because of the limited information available in the literature, it is difficult to determine whether fasting values obtained in the current study were higher than would be expected.

Breakfast is considered an important meal of the day, and it is recommended that breakfast should contribute ~20% of a child's daily energy requirement, with 60% of energy being provided by carbohydrates [28]. Research has shown that breakfast consumption improves diet quality, and functional benefits may be associated with breakfast eating in children [29–31]. Consumption of the 15.5 g test drink after the overnight fast (equivalent to ~0.5 g of available carbohydrate/kg body weight) maintained fasting [LGly] for 2 h and delayed the decrease seen with continued fasting, whereas consumption of the 31 g test drink led to a 35% increase (from fasting levels) in [LGly] at the 2-h timepoint, which decreased to overnight fasting concentrations over the subsequent 2 h. A standard serving-size equivalent of breakfast cereal and milk, or a slice of bread and jam, provides ~25–32 g of carbohydrates, which approximately equates to the 31 g beverage in the current study. However, in the United Kingdom, it is estimated that the average intake of carbohydrates at breakfast in 5–12-y-olds is approximately double this amount [31]. Inferences from the linear "dose" response obtained in the current study would suggest that this higher carbohydrate intake would result in a further increased net.iAUC$_{240\ min}$ for [LGly]. The implications of delaying glycogen depletion or increasing glycogen concentrations in the liver on function (for example, cognitive and physical performance) in children were not measured in this study, and existing evidence around the benefits of breakfast eating on cognitive function is ambiguous [29]. However, the relationship between functional measures and carbohydrate stores, plus the impact of breakfast macronutrient composition, glycemic load, or GI on those stores, is warranted. These future studies would be feasible in a young cohort using MR methods.

The glycogen concentration of the children's muscle was lower in the fasted state than previously observed in adults in our laboratory [32], yet higher than reported by others [14, 33]. Although data are scarce, muscle glycogen content, determined in biopsies, suggests that this variable in 11–13-y old males may be lower than reported in adults [34–36]. There were a number of technical difficulties in acquiring muscle glycogen measurements in the current study because of the B_1 field inhomogeneities and the variability in the size and shape of the quadriceps muscles. In order to overcome these challenges, a Biot–Savart static field approximation was used to estimate total change compared to a standard phantom based on acquired images. In addition, the signal from muscle was much smaller than the liver because of the distribution of glycogen through all musculoskeletal tissue, resulting in a low signal-to-noise ratio. This was addressed by acquiring the signal using adiabatic pulses [37] and decoupling [38] and by using a longer muscle scan time at the evening "fed" and morning "fasted" scans; the latter increased signal-to-noise ratio at these timepoints. Scheduling restrictions did not allow the extended scan time at postprandial timepoints; consequently, the variability in these data was greater. Future studies investigating muscle glycogen in children will need to consider the impact of these factors on imaging time and analysis. However, the absence of a change in muscle glycogen concentrations detected in the current study following overnight fasting (using the extended acquisition time) or with the intake of carbohydrates is similar to what has previously been observed in adults [39].

In conclusion, [LGly] decreased by 23% in healthy children (8–12 y) after an overnight fast. Subsequent consumption of 15.5 g of available carbohydrate maintained [LGly] at overnight fasting levels for 2 h and delayed the further decrease seen after water intake. At this 2-h timepoint, [LGly] was 35% higher than fasting values after ingestion of 31 g of carbohydrate, with this measure staying above fasting concentrations for 4 h after consumption. The 4-h i.netAUC for [LGly] was higher after consuming 15.5 g and 31 g carbohydrates compared with water. Muscle glycogen concentration at rest did not change significantly with fasting or refeeding. Results from this study expand the current limited knowledge of normative glycogen stores in children.

Acknowledgements

We thank the participants and their guardians for their support and enthusiasm for the project.

Author contribution

The authors' responsibilities were as follows: AMHH, SJB, ND, AG, LE, KM, PG, DB, IAM, and EJS contributed to the study design. AG, NR, and EJS managed the project. SJB, AS, NR, and EJS acquired data. AMHH, SJB, ND, PG, DB, IAM, and EJS interpreted the results. AMHH, SJB, ND, and EJS wrote the initial draft of the manuscript. All authors read, revised, and approved the final manuscript.

AMHH, AG, LE, NR, KM, DB, and IAM were employees of Société des Produits Nestlé. SJB, AS, PG, and EJS report no conflicts of interest.

Funding

This study was funded, and the drink products were supplied by Société des Produits Nestlé SA. EJS was financially supported by the National Institute for Health Research Nottingham Biomedical Research Centre. All other authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application to the corresponding author and approval from the study team.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.01.014.

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