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Endogenous versus exogenous carbohydrate oxidation measured by stable isotopes in pre-pubescent children plus ^{13}C abundances in foods consumed three days prior

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ABSTRACT

Purpose: The purposes of the present study were to (a) examine resting metabolism, substrate utilization, and endogenous versus exogenous carbohydrate (CHO) oxidation before and after 30-g rapidly-digesting carbohydrate (RDC) ingestion using indirect calorimetry and breath test analysis of stable isotope concentrations in pre-pubescent children and (b) report the ^{13}C abundances in foods consumed for three days prior.

Methods: Nineteen children (n = 10 boys, n = 9 girls) at Tanner stage I or II participated (mean age ± 95% CI = 9.84 ± 0.77 y) in this study. Food was administered to the children for three days preceding their scheduled breath tests. Breath tests and indirect calorimetry were performed after an 8-h fast before and 60 min following consumption of a 30-g simple RDC drink consisting of maltodextrin and sucrose. Open circuit spirometry and indirect calorimetry monitored resting metabolism and CHO oxidation. Separate breath samples were taken every 15 min. Samples of all foods and breath samples were analyzed for ^{13}C and ^{12}C abundances with a stable-isotope mass spectrometer.

Results: ^{13}C in expired breath samples were −23.81 ± 1.64‰ at baseline and increased every 15 min after consumption of the CHO drink ($p < 0.001-0.009$). Cumulative total, endogenous, and exogenous CHO utilization increased during the post-prandial period ($p < 0.001$). Endogenous CHO oxidation was consistently greater than exogenous CHO oxidation ($p < 0.001-0.002$).
Blood glucose was elevated from baseline at 30- and 60-min post-prandial ($p < 0.001$). Insulin did not change over time ($p = 0.184$).

Conclusions: The foods provided during the 3-day controlled diet effectively minimized ^{13}C variation prior to metabolic testing. The ^{13}C abundances of foods reported herein should serve as practical recommendations to reduce ^{13}C intake before breath tests. While endogenous CHO oxidation remained greater in proportion to exogenous CHO oxidation, these findings suggest that even a relatively small amount of RDC can increase exogenous CHO oxidation and blood glucose in normal-weight children. To further examine shifts in endogenous versus exogenous CHO utilization, we recommend that future studies take steps to minimize ^{13}C variation before breath tests and examine changes in substrate metabolism at rest and during exercise in normal weight and overweight pre-pubescent children.

Clinical trial registration number: NCT03185884.

1. Introduction

Sugar consumption in children has been under scrutiny in relation to the contributing role excess intake may have in unhealthy weight gain and associated health concerns [1]. Sugars are considered to negatively contribute to the overall energy density of

Abbreviations: CHO, carbohydrate; VO_2, rate of oxygen consumption; VCO_2, rate of carbon dioxide production; DXA, dual-energy x-ray absorptiometry; RDC, rapidly-digesting carbohydrate.

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the diet due to high palatability, which can promote overeating and result in a positive energy balance [2,3]. The influence of sugar intake on health continues to be a controversial topic [4–6], with recent suggestions that dietary sugars and other rapidly-digesting carbohydrates (RDC) may lead to metabolic dysregulation due to increased postprandial blood glucose and insulin, as well as suppressed utilization of stored energy substrates [3,7]. Therefore, even without overeating, it is postulated that important shifts in substrate utilization induced by consumption of RDC may promote insulin resistance, increased fat accumulation, and obesity.

Understanding shifts in substrate utilization from fasted to fed states can provide insight regarding the impact of RDC on the metabolic profiles and patterns of pre-pubescent children. To accurately assess substrate utilization, certain techniques and measurements of breath samples and indirect calorimetry should be performed. Breath tests involve the measurement of stable carbon isotopes in exhaled breath samples with mass spectrometry. The specific carbon isotopes measured during most breath tests are naturally-labeled ^{12}C and ^{13}C as a component of carbon dioxide (CO_2). After consumption of an exogenous carbohydrate (CHO) rich in ^{13}C, breath tests can be used to monitor the metabolic oxidation of endogenous CHO low in ^{13}C and the oxidation of the newly introduced exogenous CHO high in ^{13}C by tracking the ^{13}C:^{12}C isotopic ratios in exhaled CO_2 [8]. Although breath tests are more commonly used to examine CHO oxidation in adults [9–12], this technique has also been applied in children [13–16]. For example, Rueda-Maza et al. [14] utilized breath tests in obese and normal-weight prepubertal children to examine CHO oxidation. Despite similar ^{13}C abundances over time, exogenous CHO oxidation was greater in the obese children, implying less reliance on endogenous CHO from glycogenolysis than normal-weight children [14]. Timmons et al. [15,16] also used ^{13}C breath tests in children to examine the influence of pubertal status on CHO and fat metabolism during exercise. These studies demonstrated that in pre-pubertal boys, but not girls, there was a greater reliance on exogenous CHO oxidation during the last 15 min of a 60-min steady-state exercise test [15,16].

Due to the typically high intakes of CHO in most diets, minimizing the variation in ^{13}C abundance in expired CO_2 before the exogenous CHO is consumed becomes important when using breath tests. If accomplished, ^{13}C abundance should elevate from baseline to reflect exogenous CHO substrate utilization. While fasting has been recommended to reduce variation in ^{13}C abundance and increase test sensitivity [8], controlling dietary intake to minimize ^{13}C ingestion in the days preceding the breath tests can help ensure demonstrable ^{13}C abundance shifts [17]. Thus, understanding the ^{13}C abundances of common foods may help future researchers and clinicians better prescribe diets to minimize ^{13}C variability prior to breath tests.

Due to photosynthetic pathway differences resulting in isotopic discrimination, the ^{13}C:^{12}C ratio within plants are distinctly different [17,18]. Most plants, referred to as C3 plants, have a median ^{13}C abundance of about −27‰, while the median value for a smaller classification of plants, C4 plants, is much higher at −14‰ [19]. C4 plants include corn and sugar-cane based products, so foods containing these ingredients are generally higher in ^{13}C [18]. Consequently, prescribing controlled diets preceding breath tests with low corn and sugar-based contents may help reduce ^{13}C variances in baseline expired CO_2. However, little evidence is available regarding the ^{13}C abundances in common foods that children may eat on the days leading up to a breath test. Therefore, the purposes of the present study were to (a) examine resting metabolism, substrate utilization, and endogenous versus exogenous CHO oxidation before and after ingestion of a 30-g RDC drink using indirect calorimetry and breath test analysis of stable isotope concentrations in pre-pubescent children and (b) report the ^{13}C abundances in foods consumed for three days prior.

2. Materials and methods

2.1. Study design

This study was designed to examine responses to ^{13}C, ^{12}C, and ^{13}C:^{12}C ratio as well as substrate utilization from breath tests performed after a three-day dietary control period in pre-pubescent children. Subjects reported to the study site for a baseline visit and a test visit. The baseline visit consisted of anthropometric and body composition assessments and providing the pre-packaged foods to be consumed before the test visit. The test visit took place in the morning after an 8–12 h fast and having refrained from caffeine and heavy exercise for 12 h prior to the test visit. Breath tests were performed at baseline and were taken every 15 min after the consumption of a 30-g RDC drink consisting of 50% maltodextrin and 50% sucrose. Descriptive comparisons are reported for ^{13}C content and ^{13}C:^{12}C among pre-packaged foods consumed during the dietary control period and the CHO solution provided.

2.2. Participants

Nineteen subjects (boys, n = 10; girls, n = 9) volunteered for this study. Subjects were recruited, screened, and determined to be eligible for the study if they met the following inclusion criteria: a) male and 9–13 years of age, or female and 8–10 years of age; b) Tanner stage 1 [20,21] based on subject self-report; c) free of diabetes or any metabolic disease; d) born full term, defined as 37–40 weeks; e) Subject was born normal weight, defined as 2.5–4.3 kg; f) passed the Physical Activity Readiness Questionnaire (PAR-Q+ 2015) [22]; g) body mass index (BMI) > 5th percentile and <95th percentile; h) provided signed youth assent and signed parental consent as approved by the University of Nebraska Medical Center Institutional Review Board (IRB Protocol # 167-17-FB, title: *AL25 Proof of Concept Study: Carbohydrates and Children*).

2.3. Anthropometrics and body composition

Height, seated height, and body mass were measured using a digital scale and stadiometer (Seca 769, Hamburg, Germany). These measurements were also used to estimate maturity offset (years) from peak height velocity using the Mirwald equation [23]. Whole-body dual x-ray absorptiometry (DXA) scans (iDXA, GE Medical Systems Lunar, Madison, Wisconsin) measured lean mass, fat mass, and percent body fat. Participant demographics are reported in Table 1.

2.4. Pre-packaged foods

At the baseline visit, pre-packaged foods were provided to each subject to consume for three days immediately prior to the test visit. Thirty-six foods were chosen to reduce the intake of naturally occurring ^{13}C based on Jahren et al. [18] by attempting to limit corn- and sugar-based foods (Supplement 1). Each subject was given the pre-packaged food and was instructed to consume only the pre-packaged foods for the three days prior to the test visit. Meal plans including the pre-packaged foods were designed by a registered dietitian and individualized to each participants' energy needs and provided 50–60% energy from CHO sources.

2.5. Metabolic measurements and breath sample analysis

Subjects rested for 30 min before and 60 min after consuming a CHO drink while the rate of oxygen consumption ($\dot{V}O_2$) and carbon

dioxide production ($\dot{V}CO_2$) were measured continuously with calibrated metabolic carts (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah). The oxygen and carbon dioxide gas analyzers were calibrated with a gas mixture of 4% CO_2, 16% O_2, and 80% N. The spirometer was calibrated with a 3 L syringe (Series 5530, Hans Rudolf, Inc., Shawnee, KS). Face masks (7450 V2, Hans Rudolph, Inc., Shawnee, KS, size extra small), headgear (7450 V2, Hans Rudolph, Inc., Shawnee, KS), with attached two-way non-rebreathing valves (2700, Hans Rudolph, Inc., Shawnee, KS) were secured around the nose and mouth of each subject to form an air tight seal on the face. During the resting $\dot{V}O_2$ and $\dot{V}CO_2$ measurements, subjects remained seated in a reclined padded chair and refrained from talking. Breath − by − breath measurements were averaged over 15-min time epochs. CHO oxidation rate ($g \cdot min^{-1}$) was calculated using standard stoichiometric equations [24,25]:

$$\text{CHO Oxidation Rate } (g \cdot min^{-1}) = 4.55\ (\dot{V}CO_2) - 3.21\ (\dot{V}O_2) - 2.87\ (n)$$

where *n* represents urinary nitrogen excretion. In the above calculations, CHO oxidation is based on a $\dot{V}O_2$ (STPD) of 2.500 $L \cdot min^{-1}$ and a $\dot{V}CO_2$ (STPD) of 2.250 $L \cdot min^{-1}$ and negligible protein oxidation (n = 0) [25].

Breath samples were collected by asking subjects to breath into a plastic straw (VP116/C, Breath Sampling Straws, Labco Limited, Lampeter, UK) exiting into a 12 mL exetainer vial (439W, Exetainer® 12 mL coated vial-No Vacuum 439W, Labco Limited, Lampeter, UK). Sealed vials containing breath samples were labeled and stored prior to analysis. A baseline breath sample of expired gases for analysis of ^{13}C abundance was collected before consumption of the RDC drink, and subsequent measurements were obtained every 15 ± 5 min for 60 min post-RDC drink. All breath samples were analyzed by isotope ratio mass spectrometry (VG Optima dual inlet stable isotope ratio mass spectrometer, Isoprime Inc., Manchester, UK) for ^{13}C abundance in expired CO_2 [9]. Determination of the ^{13}C: ^{12}C ratio was based on the following equation [26]:

$$\Delta C_{13} = \frac{C_{13} : C_{12 sample} - C_{13} : C_{12 standard}}{C_{13} : C_{12 standard}} \times 1000\ ‰$$

where $\Delta^{13}C$, in parts per million (‰), reflects the abundance of ^{13}C in a sample relative to the abundance of that same isotope in a standard. The reporting standard is Vienna Pee Dee Belemnite Formation limestone (VPDB) with a ^{13}C: ^{12}C of 0.011237 [18].

Exogenous CHO oxidation rate ($g \cdot min^{-1}$) after CHO drink consumption was calculated using the following equation [27]:

$$\text{Exogenous CHO Oxidation} = \dot{V}CO_2 \left(\Delta^{13}C_{\text{timepoint}} - \Delta^{13}C_{\text{baseline}} \Big/ \Delta^{13}C_{\text{ingested}} - \Delta^{13}C_{\text{baseline}} \right) \times (1 / k)$$

where $\dot{V}CO_2$ ($L \cdot min^{-1}$) is in STPD, $\Delta^{13}C$ timepoint is the isotopic composition of expired CO_2 at the measured respective timepoint, $\Delta^{13}C$ baseline is the isotopic composition of expired CO_2 at baseline, $\Delta^{13}C$ ingested is the isotopic composition of the RDC drink, and k ($0.74326\ L \cdot g^{-1}$) is the volume of CO_2 produced when 1 g of glucose is completely oxidized. Endogenous CHO oxidation rate was then calculated as the difference of total CHO oxidation and exogenous CHO oxidation rate. Cumulative total, exogenous, and endogenous CHO oxidation (g) was calculated across the 60 min post-prandial period in 15-min increments.

2.6. Food sample ^{13}C analysis

Thirty-six commonly consumed foods were obtained from local grocery stores (Table 2 and Supplement 1). Aliquots of the different foods, in addition to the study CHO drink, were lyophilized, pulverized to a fine powder, and analyzed for their ^{13}C content using an automated combustion system (AP2003 with Isoprep continuous flow isotope ratio mass spectrometer, Isoprime Inc., Manchester, UK) in concurrence with a stable-isotope mass spectrometer (VG Optima duel inlet stable isotope ratio mass spectrometer, Isoprime, Inc., Manchester, UK). The abundance of the stable isotope ratios of all food samples was quantified as the deviation of the ^{13}C:^{12}C of the sample from the known standard as previously discussed in the analysis of ^{13}C abundance of the breath samples [18].

2.7. Blood samples

Capillary blood samples (200 μL) were collected in microvettes (Microvette® 200 μL, K3 EDTA, violet US code; 10.8 mm × 46.6 mm, Sarstedt, Inc., Newton, NC) after lancet finger punctures taken at baseline, and after 30 and 60 min of resting. Collected blood samples were centrifuged and stored at −80 °C until analysis. Serum

Table 1
Means ± 95% confidence intervals for age, BMI, height, body mass, fat mass, and fat-free mass.

	Boys, n = 10	Girls, n = 9	Combined, n = 19
Age (years)	11.00 ± 0.89*	8.56 ± 0.56	9.84 ± 0.77
BMI	18.88 ± 1.76*	16.16 ± 1.07	17.47 ± 1.14
Height (cm)	148.96 ± 6.94*	135.40 ± 5.47	142.54 ± 5.24
Body mass (kg)	41.10 ± 9.2*	29.82 ± 3.88	35.76 ± 4.49
Fat mass (kg)	9.06 ± 4.13	6.30 ± 1.85	7.75 ± 2.22
Fat mass (%)	22.80 ± 6.29	23.43 ± 3.76	23.00 ± 3.39
Fat-free mass (kg)	26.85 ± 2.71*	19.16 ± 1.92	23.21 ± 2.43
Fat-free mass (%)	74.00 ± 6.17	73.56 ± 3.69	74.00 ± 3.32
Peak Height Velocity (years)	14.61 ± 0.52*	11.88 ± 0.35	13.32 ± 0.74
Maturity Offset (years)	−3.05 ± 0.58	−2.75 ± 0.55	−2.91 ± 0.37

* Indicates a significant difference between boys and girls ($p \leq 0.05$).

Table 2
Relative ^{13}C isotope value ($\Delta^{13}_{VPDB}C$ (‰)) of common food items provided to pre-pubescent children (n = 19) to ensure a ^{13}C restriction diet. Food items are ranked by ^{13}C (‰) in ascending isotope value, as assessed by isotopic ratio mass spectrometry.

Food Item	$\Delta^{13}_{VPDB}C$ (‰)[a]	^{13}C:^{12}C of Sample[b]	Total Carbon (%)[c]
Crackers 1[d]	−28.77	0.0109138	51.38
Crackers 2	−28.60	0.0109156	56.37
Oatmeal	−28.60	0.0109187	41.09
Crackers 3	−28.22	0.0109199	47.62
Crackers 4	−27.89	0.0109236	48.85
Crackers 5	−27.79	0.0109247	48.24
Brown Rice	−27.75	0.0109251	46.64
Raisins	−27.50	0.0109280	39.97
Peanut Butter 1	−27.26	0.0109307	63.98
White Rice	−27.20	0.0109313	44.26
Breaded Chicken	−26.36	0.0109408	55.35
Peanut Butter 2	−26.33	0.0109412	60.94
Macaroni and Cheese	−25.49	0.0109506	42.81
Crackers 6	−24.90	0.0109572	49.63
White Whole Grain Bread	−24.52	0.0109615	45.16
Cinnamon Raisin Bread	−24.15	0.0109656	44.04
Chicken Alfredo	−24.02	0.0109670	45.04
Chips	−23.55	0.0109724	52.08
Canned Cheddar	−22.43	0.0109850	53.53
Portable Protein Pack	−22.40	0.0109852	60.21
Mandarin Oranges	−22.01	0.0109897	40.06
Chicken Noodle Soup	−21.85	0.0109915	40.19
Salami	−20.02	0.0110120	50.95
Turkey Ham and Cheese Wrap	−19.47	0.0110183	47.79
Gelatin	−19.40	0.0110190	35.17
Cheese Pizza	−19.15	0.0110218	64.46
Pepperoni Pizza	−19.09	0.0110225	53.51
Beef Jerky 1	−18.69	0.0110270	42.93
Pepperoni Pizza Wrap	−18.64	0.0110275	40.26
RDC Drink[e]	−18.13	0.0109322	44.79
Yogurt	−18.07	0.0110340	42.52
Beef Jerky 2	−17.69	0.0110382	60.29
String Cheese 1	−16.92	0.0110468	54.77
String Cheese 2	−16.91	0.0110469	56.21
Vitamin D Milk	−15.66	0.0110610	42.44
Sugar-Free Powdered Drink Mix	−15.52	0.0111075	37.87
Pineapple	−10.59	0.0111181	40.31

[a] The delta notation ($\Delta^{13}_{VPDB}C$ (‰)) for the ^{13}C abundances is used to describe isotopic differences between samples as these differences typically vary at or beyond the third significant digit as seen by the calculated ^{13}C:^{12}C ratio of the food sample.
[b] The calculated ^{13}C:^{12}C ratio of the food sample.
[c] Reflects the percent of total carbon within the food sample.
[d] See Supplement 1 for the complete list of the production company and ingredients, following the same descending order.
[e] The rapidly-digesting CHO (RDC) drink utilized in this study consisted of 50% sucrose and 50% maltodextrin. The mixture resulted in 120 kcals of CHO (30-g) dissolved in 237 mL sugar-free lemonade.

was collected after centrifugation and analyzed in duplicate by enzyme-linked immunosorbent assays (ELISA) to determine concentrations of insulin (Insulin ELISA kit, Crystal Chem High Performance Assays) per commercial kit instructions. Twenty-five μL of controls, standards, and samples were placed in each well. Absorbance was read at 450 and 630 nm. Glucose was measured with a handheld blood glucose monitoring system (Freestyle Freedom Lite®, Abbott Diabetes Care Inc. Alameda, CA) and recorded immediately.

2.8. Rapidly-digesting carbohydrate (RDC) drink

The RDC drink provided 30-g of CHO from a 50:50 ratio of maltodextrin:sucrose. Ingredients were provided by Abbott Nutrition (Columbus, Ohio) and individually weighed prior to administration at the study site and mixed in a sugar-free lemonade-flavor drink mix (Crystal Lite, Kraft Food, Glenview, IL) with 237 mL of distilled water. Drinks were mixed and administered by a staff member who was masked to the study participants and data. Table 2 reports the isotopic abundance within the RDC drink.

2.9. Statistical analysis

All variables were assessed for distributional normality with Shapiro Wilks tests and analyzed for outliers with stem and leaf plots. A one-way repeated measures ANOVA examined mean CHO oxidation rate across time (0, 15, 30, 45, 60 min). A 4 × 2 mixed factorial ANOVA [time (15, 30, 45, 60) x CHO type (Endogenous vs Exogenous)] compared the differences between type of CHO oxidation rate over time. A 5 × 2 mixed factorial ANOVA [time (0, 15, 30, 45, 60 min) x CHO type (Endogenous vs Exogenous)] compared differences in cumulative CHO oxidation over time. Changes in ^{13}C (‰), glucose concentrations, and insulin concentrations were analyzed with one-way repeated measures ANOVAs across time. When appropriate, follow up analyses included paired samples t-tests with Bonferroni corrections and independent samples t-tests. All statistical analyses were performed in IBM SPSS v. 25 (Chicago, IL, USA), while means and 95% confidence intervals were calculated in a spreadsheet software program (Microsoft Excel, version 16.10). An alpha of $p \leq 0.05$ was considered statistically significant for all comparisons.

3. Results

The ^{13}C (‰) content of the thirty-six food items plus the study RDC drink are shown in Table 2. Food items are arranged from lowest to highest ^{13}C content, ranging from the ^{13}C values of −28.77 to −10.59‰ (Table 2). Note that only 7 food items exhibited higher ^{13}C abundances than the study CHO drink, while 29 food items showed lower ^{13}C abundances. After consuming only items from the thirty-six food choices listed in Table 2 for three days, mean ± 95% confidence intervals breath samples of ^{13}C for the 19 pre-pubescent children were −23.81 ± 1.64‰ at baseline, and significantly increased every 15 min after consumption of the CHO drink ($p < 0.001$–0.009) (Table 3).

CHO oxidation rate was greater at 30, 45, and 60 min than baseline and 15 min ($p < 0.001$–0.001). CHO oxidation rate was also greater at 60 min than 45 min ($p = 0.05$), but there were no differences between baseline and 15 min, 30 and 45 min, or 30 and 60 min ($p = 0.112$–0.914). While there were no differences between the rate of endogenous CHO oxidation and exogenous CHO oxidation at 15 and 30 min, endogenous was greater than exogenous at 45 and 60 min ($p < 0.001$–0.003). Endogenous CHO oxidation rate increased over time ($p < 0.001$–0.028), except between 30 and 45 min ($p = 0.638$). There were no differences in exogenous CHO oxidation rate over time ($p = 0.434$–0.960) (Table 3). Cumulatively, total, exogenous, and endogenous CHO oxidation increased systematically over time ($p < 0.001$) (Table 4). Endogenous CHO oxidation was consistently greater than exogenous CHO oxidation ($p < 0.001$–0.002) (Fig. 1). Blood glucose concentrations increased from baseline at 30 and 60 min ($p < 0.001$) but were not different between 30 and 60 min ($p = 0.113$). There were no differences in insulin concentrations over time ($p = 0.184$) (Fig. 2).

4. Discussion

Despite being a major public health initiative, the prevalence of overweight/obesity and accompanying metabolic dysfunction in children and adolescents has continued to increase from 2000 to 2016 [28,29]. In fact, approximately 18.5% of children in the United States are considered obese [28]. Since obesity is directly associated with many health risks, monitoring metabolic patterns in children has become even more imperative. Excessive intake of sugar, and other RDC, is considered a major contributing factor to youth obesity. Sugar contributes to the diet's energy density [1] as well as the substrate partitioning [7] of energy sources, which may underlie childhood obesity and metabolic dysregulation. Therefore, assessing methodology to accurately examine resting metabolism, substrate utilization, and endogenous/exogenous CHO oxidation before and after consumption of RDC will provide insight regarding ^{13}C responses and CHO metabolism in pre-pubescent children.

The results of the present study indicated that the baseline ^{13}C abundances in pre-pubescent children after a 3-day controlled diet were −27.86 to −21.50‰ (Table 3). Schoeller et al. [17] reported higher fasted baseline values ranging from −21.8 to −20.8‰ in adults across the United States. Compared to Schoeller et al. [17], the lower baseline ^{13}C in the present study may have reflected the effectiveness of the 3-day diet and/or a lower, more stable baseline from which changes in ^{13}C could be monitored in expired CO_2. Similarly, Normand et al. [10], reported approximately −24.5‰ ^{13}C in young European adults who purposefully avoided foods rich in ^{13}C, which was well-matched to the average −23.81‰ ^{13}C baseline in the present study. However, we are aware of no previous reports of baseline ^{13}C abundances in children. Therefore, the results of the present study provide relatively novel ^{13}C abundances at baseline after a controlled diet in a protected population of pre-pubescent children.

Studies attempting to minimize ^{13}C variation in children prior to metabolic testing ask participants to refrain from eating corn-based products or foods derived from C_4 signature plants that are rich in ^{13}C the day before metabolic testing [14,16]. Examples of ^{13}C-rich foods include cane sugar, popcorn, maize, millet, polenta, pineapple, or any tropical fruit. Many of these foods have been tested for ^{13}C [17,18] and exhibit higher ^{13}C abundances than most of the foods tested and consumed by the children in the present study (Table 2). However, recommendations of foods to consume to minimize dietary ^{13}C enrichment in children have not been reported. Table 2 shows a list of conveniently accessible, commonly-consumed foods that were tested in the present study for isotopic ^{13}C abundance. Table 2 may serve as practical recommendations to limit ^{13}C intake before breath tests. Future studies are needed, however, to compare a simple overnight fast to a controlled diet for minimizing baseline ^{13}C abundance in expired air.

After consuming the RDC drink, ^{13}C abundance increased systematically at every 15-min period (Table 3). The RDC drink exhibited a much higher abundance (Table 2) than the mean baseline breath value (Table 3), thus, consuming the RDC drink increased ^{13}C abundance in expired CO_2. In fact, only 7 of the food items tested (Table 2) had ^{13}C abundances higher than the RDC drink. It is clear that the foods low in ^{13}C consumed by the children reduced variations in baseline ^{13}C abundance enough to allow subtle, but measurable shifts in substrate utilization.

As expected, capillary blood glucose significantly increased after consuming the 30 g RDC drink. Subsequently, total CHO oxidation rate also increased during the last 30–60 min of rest after consuming the 30-g RDC drink (Table 3). Interestingly, exogenous

Table 3
Means ± 95% confidence intervals of ^{13}C abundance (‰) and total, exogenous, and endogenous carbohydrate (CHO) oxidation rate analyzed from breath samples at baseline and every 15 min after consumption of a rapid-digesting CHO (RDC) drink, as well as concentrations of glucose and insulin analyzed from blood samples obtained at baseline and every 30 min after consumption of the RDC drink.

Time (minutes)	^{13}C Abundance (‰)	Total CHO Oxidation rate ($g \cdot min^{-1}$)	Exogenous CHO Oxidation Rate ($g \cdot min^{-1}$)	Endogenous CHO Oxidation Rate ($g \cdot min^{-1}$)	Glucose ($mg \cdot dL^{-1}$)	Insulin ($mU \cdot L^{-1}$)
0	−23.81 ± 1.64	0.109 ± 0.02	–	0.109 ± 0.02	80.94 ± 3.20	15.22 ± 2.99
15	−23.60 ± 1.54[a]	0.093 ± 0.02	0.054 ± 0.03	0.039 ± 0.02	–	–
30	−23.11 ± 1.48[a,b]	0.175 ± 0.05[b]	0.060 ± 0.03	0.115 ± 0.05[b]	129.22 ± 12.33[a]	20.38 ± 6.23
45	−22.82 ± 1.44[a,b,c]	0.177 ± 0.04[b]	0.054 ± 0.03	0.123 ± 0.04[b,e]	–	–
60	−22.65 ± 1.41[a,b,c,d]	0.212 ± 0.05[b,d]	0.053 ± 0.03	0.159 ± 0.05[b,c,d,e]	110.78 ± 8.70[a]	18.91 ± 6.4

[a] Indicates significantly greater than baseline (0 min).
[b] Indicates significantly greater than 15 min.
[c] Indicates significantly greater than 30 min.
[d] Indicates significantly greater than 45 min.
[e] Indicates a significant difference from exogenous CHO oxidation.

Table 4
Means ± 95% confidence intervals of cumulative total, exogenous and endogenous carbohydrate (CHO) utilization analyzed from breath samples obtained every 15 min of the 60-min post-prandial period before and after consumption of the rapidly-digested CHO (RDC) drink.

Time (min)	Cumulative CHO Utilization (g)	Cumulative Exogenous CHO Utilization (g)	Cumulative Endogenous CHO Utilization (g)
0	1.64 ± 0.25	0	1.64 ± 0.25[e]
15	2.15 ± 0.35[a]	0.27 ± 0.12[a]	1.89 ± 0.33[a,e]
30	3.57 ± 0.64[a,b]	0.85 ± 0.37[a,b]	2.72 ± 1.02[a,b,e]
45	5.98 ± 1.09[a,b,c]	1.78 ± 0.81[a,b,c]	4.20 ± 1.10[a,b,c,e]
60	9.39 ± 1.69[a,b,c,d]	2.96 ± 1.38[a,b,c,d]	6.43 ± 1.75[a,b,c,d,e]

[a] Indicates significantly greater than baseline (0 min).
[b] Indicates significantly greater than 15 min.
[c] Indicates significantly greater than 30 min.
[d] Indicates significantly greater than 45 min.
[e] Indicates a significant difference from exogenous CHO oxidation.

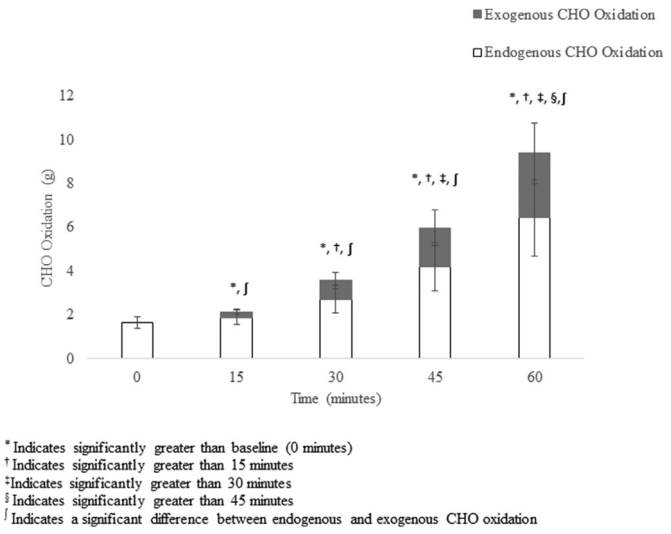

Fig. 1. Total carbohydrate (CHO) oxidation broken down as exogenous and endogenous CHO oxidation cumulatively across 60 min in pre-pubescent children before and after consuming a 30-g rapid-digesting carbohydrate (RDC) drink.

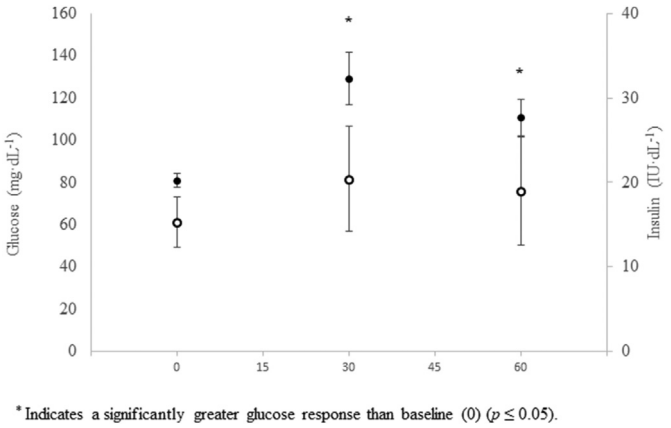

Fig. 2. Means ± 95% Confidence Intervals of glucose (closed circles) and insulin (open circles) responses of pre-pubescent children (n = 19) measured at baseline and 30 and 60 min after consuming a 30-g RDC drink.

CHO oxidation rate remained constant, whereas the endogenous CHO oxidation rate increased over time (Table 3). Cumulatively, CHO utilization reached 9.39 g over 60 min in the pre-pubescent children, with a greater proportion coming from endogenous sources (Table 4, Fig. 1). This is consistent with Rueda-Maza et al. [14] in non-obese children who showed that endogenous CHO continued to predominate as the main CHO substrate up to 2.5 h after eating a CHO-rich breakfast.

Younger children may rely less on exogenous energy sources overall as suggested by studies which have evaluated energy metabolism during exercise. Timmons et al. [15,16] reported that prepubertal boys and girls exhibited lower CHO oxidation when compared to same-sex older adolescents while exercising at the same intensity [15,16]. Young boys also exhibited lower total and exogenous CHO oxidation during exercise when compared to adult males [13]. In a resting state, the pre-pubescent children of the present study had a greater reliance on endogenous CHO oxidation after consuming an exogenous CHO RDC drink. Endogenous CHO utilization is an important metabolic regulator [30] and the ability to breakdown and oxidize stored glycogen is critical to maintain glucose homeostasis and energy, especially during periods of growth. Additionally, utilizing glycogen stores facilitates the re-uptake of glucose to replenish glycogen and is associated with improvements in insulin sensitivity [30]. In adults, this process of glycogen cycling is linked to reduced fat regain [31] and risk of type 2 diabetes [32]. Of interest, in the study by Rueda-Maza [14], exogenous CHO oxidation was found to increase more in obese children than in normal-weight children (12.7 vs 8.5 g per 2.5 h) after consuming a standard CHO meal, suggesting a relationship between dysregulated CHO metabolism and obesity in children. Future studies should evaluate both short- and long-term effects of different CHO energy sources on both metabolic substrate utilization, growth and future risk of obesity in children.

In conclusion, the foods provided to pre-pubescent children during a 3-day controlled diet seemed to effectively minimize ^{13}C variation in children prior to metabolic testing. The ^{13}C abundances of foods reported in Table 2 should serve as practical recommendations to reduce ^{13}C intake before breath tests. ^{13}C abundances steadily increased from baseline after consuming a 30-g RDC drink. CHO oxidation rate increased in the final 30 min of the 60-min period. Despite increases in ^{13}C abundances in expired CO_2, exogenous CHO oxidation rate did not increase. Yet, consuming the RDC did result in a three-fold increase in cumulative exogenous CHO utilization over the 60-min post-prandial period. While endogenous CHO oxidation remained greater in proportion to exogenous CHO oxidation, these findings suggest that relatively small amounts of RDC can increase exogenous CHO oxidation and blood glucose in normal-weight children. To further examine shifts in exogenous versus exogenous CHO utilization, we recommend that future studies take steps to minimize ^{13}C variation prior to breath tests and examine changes in substrate metabolism at rest and during exercise in normal and overweight pre-pubescent children.

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Declaration of competing interest

Current disclosures for JTC: From 2001-present, Dr. Cramer's research has been externally funded by 22 separate project grants from Abbott Nutrition, Nebraska Beef Council, Nebraska Extension, Stepan Lipid Nutrition, Rock Creek Pharmaceuticals, General Nutrition Corporation, Experimental & Applied Sciences, Nutricia, and the University of Nebraska Agriculture Research Division with funds provided by the Hatch Act (Agency: U.S. Department of Agriculture, National Institute for Food and Agriculture: Accession No: 1000080; Project No: NEB-36-078). From 2010 to 2013, Joel was an expert witness or expert consultant in 10 separate legal matters representing defendants Vital Pharmaceuticals, MusclePharm, and Celsius. From 2008 to 2016, Dr. Joel T. Cramer was a paid consultant for either Abbott Nutrition, General Nutrition Center, ErgoGenix/ErgoPharm, and/or Corr-Jensen Labs. From 2018-present, Joel has served as a paid consultant for Regeneron Pharmaceuticals. VAM is an employee and stockholder of Abbott Nutrition. MES, ZMG, BDM, and TJL did not report any potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2020.100041.

Author contributions

MES: Writing – original draft, review and editing, visualization, formal analysis.

ZMG: Conceptualization, Investigation, writing – review and editing, project administration.

BDM: Investigation, writing-review and editing.

TJL: Investigation, writing-review and editing.

VAM: Conceptualization, methodology, writing-review and editing.

JTC: Conceptualization, methodology, formal analysis, investigation, writing-original draft, review, and editing, supervision, project administration, funding acquisition.

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