Energy substrate utilization with and without exogenous carbohydrate intake in boys and men exercising in the heat

Gabriela T. Leites¹, Giovani S. Cunha¹,², Lisa Chu¹, Flavia Meyer² and Brian W. Timmons¹

¹ Child Health & Exercise Medicine Program, Department of Pediatrics, McMaster University, Hamilton, ON, Canada.
² Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Author contributions: B.W.T. and G.T.L conceptualized and designed the research project; G.T.L acquired the data with assistance from G.S.C, L.C; G.T.L conducted the statistical analysis; G.T.L interpreted results of experiments with assistance from, G.S.C, L.C., F.M. and B.W.T; G.T.L wrote the final manuscript with manuscript revisions from all authors. All authors reviewed and agreed upon the final manuscript.

Running title: Substrate utilization during exercise in the heat

Keywords: children, substrate utilization, exercise, heat, hydration, stable isotope.

Contact Information:
Brian W. Timmons, PhD
Child Health & Exercise Medicine Program
Department of Pediatrics, McMaster University
1280 Main Street West, HSC 3N27G
Hamilton, ON, Canada, L8S 4K1
Tel: 905-521-2100, ext 77615
Fax: 905-521-1703
Email: timmonbw@mcmaster.ca
ABSTRACT

Little is known about energy yield during exercise in the heat in boys compared to men. To investigate substrate utilization with and without exogenous carbohydrate (CHOexo) intake, seven boys (11.2 ± 0.5 yr) and nine men (24.0 ± 3.3 yr) cycled (4×20 min bouts) at a fixed metabolic heat production (\(\dot{H}_p\)) per unit body mass (6 W·kg\(^{-1}\)) in a climate chamber (38° C and 50% relative humidity), on two occasions. Participants consumed a \(^{13}\)C-enriched 8% CHO beverage (CARB) or placebo beverage (CONT) in a double-blinded, counter balanced manner. Substrate utilization was calculated for the last 60 min of exercise. CHOexo oxidation rate (2.0 ± 0.3 vs. 2.5 ± 0.2 mg·kgFFM\(^{-1}\)·min\(^{-1}\), \(P = 0.02\)) and CHOexo oxidation efficiency (12.8 ± 0.6 vs. 16.0 ± 0.9 %, \(P = 0.01\)) were lower in boys compared to men exercising in the heat. Total carbohydrate (CHO\(_{\text{total}}\)), endogenous CHO (CHO\(_{\text{endo}}\)) and total fat (FAT\(_{\text{total}}\)) remained stable in boys and men (\(P > 0.05\)) during CARB, whereas CHO\(_{\text{total}}\) oxidation rate decreased (\(P < 0.001\)) and FAT\(_{\text{total}}\) oxidation rate increased over time similarly in boys and men during CONT (\(P < 0.001\)). The relative contribution of CHOexo to total energy yield increased over time in both groups (\(P < 0.001\)). In conclusion, endogenous substrate metabolism and the relative contribution of fuels to total energy yield were not different between groups. The ingestion of a CHO beverage during exercise in the heat may be as beneficial for boys and men to spare endogenous substrate.

Keywords: children, substrate utilization, exercise, heat, hydration, stable isotope.

NEW AND NOTEWORTHY

This study provides the new finding that during exercise in the heat boys experience a lower exogenous carbohydrate oxidation rate and oxidation efficiency compared to men. In contrast, endogenous substrate metabolism and the relative contribution of substrate for the total energy during exercise were not different in boys and men. These age-related differences observed in the heat are noteworthy because they expand our knowledge of exercise metabolism in children.
Introduction

A number of studies have described marked differences in energy metabolism during exercise between children and adults (12, 18, 30, 31). Understanding child-adult differences in energy metabolism might be particularly important for children because puberty is associated with a conservation of endogenous substrate, most likely due to growth-related energy requirements (31). Evidence suggests that CHO supplementation before or during exercise can shift the relative amount of substrate utilization (11, 31). More information on energy metabolism during exercise in the pediatric population would be beneficial because children have a greater reliance on CHOexo compared to adults (31). Environmental conditions have been described as an important factor that alter energy metabolism during exercise (14). Heat stress impairs the oxidation rate of CHOexo in adults (15); however, there is no information about CHOexo oxidation in children compared to adults under heat stress.

In adults, prolonged exercise combined with heat stress decreased CHOexo oxidation and increased reliance on endogenous fuels (15). Possible mechanisms that could explain the heat effect on CHOexo oxidation during exercise include: 1) reduction of intestinal blood flow and CHOexo absorption due to increased shift of blood flow to the skin for evaporative cooling 2) reduced gastric emptying, and 3) reduced glucose transport into the muscle. Heat stress contributed to impaired CHOexo oxidation during exercise in trained male cyclists (15), although the hypohydration levels could have also influenced this during exercise in the heat. Dumke, et al. (11) showed similar CHOtotal oxidation after consuming a placebo and a 6% glucose beverage in males during exercise in the heat. However, fat oxidation was 25% lower in the CARB trial compared to CONT, suggesting that CHOexo availability can alter substrate selection during exercise in the heat.
From a pediatric point-of-view, the potential for \( \text{CHO}_{\text{exo}} \) metabolism to be reduced as a result of heat stress requires special consideration, especially given the existing evidence that supports a greater reliance on \( \text{CHO}_{\text{exo}} \) during the exercise in youth compared with adults in thermoneutral conditions (26, 27, 30). Few studies have considered maturity status as a factor, which influences the rate of \( \text{CHO}_{\text{exo}} \) oxidation during exercise. Pre- and early-pubertal boys demonstrated a greater reliance on \( \text{CHO}_{\text{exo}} \) for energy compared to men, measured by \( ^{13}\text{C} \) isotope technique, during 60 minutes of cycling in thermoneutral conditions at 70% of \( \dot{V}\text{O}_{2\text{peak}} \) (31). It was suggested that a greater reliance on \( \text{CHO}_{\text{exo}} \) in boys may be related to pubertal status and important for preserving endogenous fuels (31). In addition, boys utilized ~ 70% more fat and 23% less \( \text{CHO}_{\text{total}} \) compared to men during exercise without \( \text{CHO}_{\text{exo}} \) (31). While the majority of studies have focused on substrate utilization in thermoneutral environments (29, 30), exercise in the heat may impair \( \text{CHO}_{\text{exo}} \) oxidation (7, 14, 15). An experimental design that directly compares the effects of \( \text{CHO}_{\text{exo}} \) on energy metabolism is lacking, and would be important to clarify possible age- or maturity-related differences. Therefore, more information is required to identify the effects of \( \text{CHO} \) intake before and during exercise in the heat for children, in light of its potential to alter metabolism.

The effect of \( \text{CHO} \) intake on endogenous substrate utilization has not yet been evaluated during exercise in the heat in the pediatric population. An important consideration for comparing children and adults exercising in the heat is to select an experimental design that guarantees similar thermoregulatory responses to avoid bias on child-adult comparisons. To date, most comparisons of children and adults exercising in the heat have employed similar relative exercise intensities (% \( \dot{V}\text{O}_{2\text{peak}} \)) (2, 13), and have not considered the impact of differential metabolic heat production. A recent study (17) suggested that exercise prescribed to elicit a fixed metabolic heat
production per unit body mass guaranteed similar thermoregulatory responses between boys and men exercising in the heat.

The purpose of this study was to compare the effects of CHO intake on energy metabolism between boys and men during exercise in the heat at the same metabolic heat production per unit of body mass. Specifically, we examined total CHO (CHO<sub>total</sub>), CHO<sub>exo</sub>, endogenous CHO (CHO<sub>endo</sub>), and total fat (Fat<sub>total</sub>) oxidation rates during exercise in the heat. We hypothesized that providing CHO<sub>exo</sub> during exercise in the heat would have a greater effect in boys compared to men, in terms of reducing fat oxidation and promoting CHO<sub>total</sub> oxidation. We also hypothesized that boys would use relatively more CHO<sub>exo</sub> as energy during exercise in the heat compared to men.

**Methods**

**Participants.** Seven healthy active boys (10- to 12-years-old) and nine healthy active men (20- to 30-years-old) participated in this study. Sample size was calculated with 95% statistical power and a 5% significance level based on CHO<sub>exo</sub> oxidation rate in the heat, according to Jentjens et al. (14). Participants’ physical and fitness characteristics are summarized in Table 1. Participants were not heat acclimatized as data collection took place during the winter months in Ontario, Canada. No medical conditions were reported and no medications were taken at the time of participation. Boys and their guardian and men were informed of the experimental protocol and potential risks and provided written informed assent, where appropriate, and/or consent to participate. The study was approved by the Hamilton Integrated Research Ethics Board.
**Experimental design and procedures.** A repeated-measures, crossover, counterbalanced, double-blinded, placebo-controlled study design was used. Participants were required to attend three visits, one preliminary and two experimental trials.

**Preliminary trial.** At the preliminary session, a physical activity questionnaire (3) was used to confirm similar activity levels between boys and men. Participants were also asked about their health status, including any previous diagnosis of chronic disease and use of medication. Biological maturation was determined using self-assessed Tanner staging (19). Standing height and body mass (BWB-800, Tanita Corporation, IL) were assessed in all participants; sitting height was also measured in boys to calculate estimated age of peak height velocity (20). Body surface area (BSA) was calculated from the measurements of body height and mass (10). Body composition was measured via Dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500A scanner, Hologic Inc., Waltham, MA).

To determine $\dot{V}O_{2\text{peak}}$, an incremental exercise test in a thermoneutral room was performed, using the McMaster All-Out Progressive Continuous Cycling Test (3). The test began at 25 W and had 25-50 W increments every 2 min, according to participant’s height, while maintaining a cadence between 60 and 80 rpm. All participants were verbally encouraged to give their best performance. A calibrated electronically-braked cycle ergometer (*Lode Excalibur*, Groningen, the Netherlands) was used for all testing. Measurements of expired $VO_2$ and $VCO_2$ were made continuously using a calibrated metabolic cart (*Vmax29*, SensorMedics, Mixing Chamber method, Yorba Linda, CA, U.S.A.). $\dot{V}O_{2\text{peak}}$ was considered the highest 30-s oxygen uptake value. To gauge the participant’s perception of their effort, Borg’s 6-20 categorical scale was used. The test ended when 2 of the 4 following criteria were reached: 1) inability to maintain
a cycling cadence above 60 rpm in spite of strong verbal encouragement; 2) HR (Polar S610; Polar Electro Oy, Kempele, Finland) >195 beats·min⁻¹; 3) rating of perceived exertion (RPE) >19; and (4) respiratory exchange rate > 1.1.

**Hydration, diet and activity before experimental trials.** Prior to the experimental trials, participants were asked to abstain from caffeine and alcohol (adults), to refrain from strenuous physical activity for 24 hours and avoid corn or corn-based products (28) to reduce background enrichment of expired CO₂ from naturally derived ^{13}C for 48 hours. On the day prior to the experimental trials, a hydration protocol was prescribed, whereby participants were instructed to consume 12 mL·kg⁻¹ of bottled water, in addition to their usual intake. This extra volume was divided between the morning and night. On the morning of the experimental trials, participants ingested 6 mL·kg⁻¹ of water. The same standardized meal was consumed prior to each experimental visit, consisting of 40% of total daily energy calculated individually, and contained 50% of energy from carbohydrate, 30% from protein, and 20% from fat. Participants arrived at the laboratory at least 3 h following their standardized meal.

**Experimental trials.** On two separate occasions, participants cycled for 80 min divided into 4 × 20 min bouts with 10-min of rest between bouts at a fixed metabolic heat production per unit body mass (~6 W·kg⁻¹). To avoid any influence of circadian variance, trials were completed as close as possible to the same time of day for each participant. The experimental trials were identical except for the beverage before and during the exercise in the heat. Participants were given either a ^{13}C-enriched 8% CHO beverage (CARB) or a placebo beverage (CONT). The CHO solution was artificially enriched with uniformly labeled ^{13}C-glucose to an estimated isotopic composition of +100.0 change per 1000 difference versus the ^{13}C/^{12}C ratio from the
international standard $^{13}$C Pee Dee Belemnita-1 (+100.0% \[\delta^{13}C\] PDB$^{-1}$). The placebo was artificially-sweetened with 3.2 g of Splenda per 1,000 mL of fluid and, therefore, was identical in flavour to the CHO beverage. The beverages were coded appropriately by one of the investigators who was not involved in the data collection or analysis. The total volume of beverage consumed by each participant was calibrated to his fat-free mass (FFM; 1.3 g CHO per kg FFM$^{-1}$). Participants were given their first drink (0.65 g CHO per kg FFM$^{-1}$) 30 min before the start of the exercise (time = -30 min) and consumed additional drinks (0.16 g CHO per kg FFM$^{-1}$ each) at four subsequent time points (0, 25, 55 and 85 min). Additional water was provided to maintain euhydration levels during CARB and CONT trials at the same time points.

**Experimental protocol.** Prior to the consumption of the experimental beverage, each participant provided a resting, baseline breath sample to measure the background enrichment of $^{13}$C in expired CO$_2$. CHO (total, endogenous and exogenous) and fat oxidation rates were calculated from 3-min gas collection periods (SensorMedics Vmax29, Yorba Linda, CA). A 60-mL syringe was used to draw a sample of the expired gas directly from the tube connecting the participant’s mouthpiece to the metabolic cart and later analyzed for the ratio of $^{13}$C/$^{12}$C. Participants ate a standardized meal that consisted of two portions of white bread (40 g) and one portion of strawberry jam - no sugar added (15 g) and drank the initial bolus of the experimental beverage (carbohydrate or placebo).

After the first beverage was consumed, participants waited 30 min in a thermoneutral room before entering the climate chamber. Within this time, the following procedures were performed: participants provided a urine sample, which was immediately analyzed for urine
specific gravity (USG) (Atago refractometer, 2722-E04; at Q17 a resolution of 1.000 to 1.050 density, Tokyo, Japan) and color using an 8-point scale that ranges from very pale yellow (number 1) to brownish green (number 8) to ensure similar initial hydration status (1). A USG cutoff value of 1.020 was applied (16), considering higher values in need of additional hydration prior to exercise. After emptying their bladder, nude body mass was recorded. A HR monitor (Polar Electro Oy, S610, Finland) was used, and $T_{re}$ was measured using a flexible thermometer (YSI 400 series thermistor, USA) inserted 10 cm for boys and 12 cm for men beyond the anal sphincter. Skin temperature ($T_{sk}$) was measured using skin thermistors (YSI 400 series temperature sensors, USA), placed on the upper back, arm and calf.

Participants then went into the climate chamber and mounted a cycle ergometer. Prior to exercise, participants rested for 5 min in the climate chamber, which was set to 38 °C and 50% relative humidity (verified by a 3M QUESTemp® QT34 Wet Bulb Globe Temperature Heat Stress Monitors, Illinois, USA) and another 3-min resting expired gas sample was collected. Participants then received an aliquot of the experimental beverage. Additional breath samples were collected for 3-min periods during the middle (7-10 min) and the end (17-20 min) of each cycling bout to verify metabolic heat production and calculate substrate utilization. Breath samples were collected from both CARB and CONT trials to determine $^{13}$C-enrichment.

$T_{re}$, $T_{sk}$ and HR were recorded every 5 min. Participants were weighed with shorts, shoes and electrodes after emptying their bladder at the beginning and end of each trial to determine body weight changes (post-exercise - pre-exercise body mass).
Calculations. The rate of metabolic energy expenditure (\( M \); in W\( \cdot \)m\(^{-2} \)) was estimated using the average of VO\(_2\) (L\( \cdot \)min\(^{-1} \)) and RER measured during the experimental trials, and calculated as

\[ M = \dot{V}O_2 \cdot \left[ \left( \frac{RER - 0.7}{0.3} \right) \cdot e_c \right] + \left[ \left( \frac{1.0 - RER}{0.3} \right) \cdot e_f \right] \cdot 60 \cdot BSA \cdot 1000 \]

where \( e_c \) is the caloric equivalent per liter of oxygen for the oxidation of carbohydrates (21.13 kJ), and \( e_f \) is the oxidation of fat (19.62 kJ). Metabolic heat production (\( \dot{H}_p \); in W\( \cdot \)m\(^{-2} \)) was calculated as the difference between \( M \) and the external work rate (\( W \)).

\[ \dot{H}_p = M - W \]

CHO\(_{\text{total}}\) and Fat\(_{\text{total}}\) oxidation rates were calculated according to the following equations

\[ \text{CHO}_{\text{total}}(g \cdot \text{min}^{-1}) = 4.59 \cdot \dot{V}CO_2(L \cdot \text{min}^{-1}) - 3.23 \cdot \dot{V}O_2(L \cdot \text{min}^{-1}) \]

\[ \text{Fat}_{\text{total}}(g \cdot \text{min}^{-1}) = -1.70 \cdot \dot{V}CO_2(L \cdot \text{min}^{-1}) + 1.69 \cdot \dot{V}O_2(L \cdot \text{min}^{-1}) \]

The energy provided from CHO\(_{\text{total}}\) and fat oxidation was calculated from their energy potentials (3.87 and 9.75 kcal\( \cdot \)g\(^{-1} \), respectively). Breath samples stored in Exetainer tubes (Labco Exetainer, Lampeter, Ceredigion, UK) were subsequently analyzed for \(^{13}\text{C}/^{12}\text{C}\) ratio in expired CO\(_2\) by isotope ratio mass spectrometry (IDMicro Breath Version 2.0, Compact Science Systems, Staffordshire, UK). CHO\(_{\text{exo}}\) oxidation was calculated using the equation modified from Mosora et al. (21):

\[ \text{CHO}_{\text{exo}}(g \cdot \text{min}^{-1}) = \dot{V}CO_2 \left\{ \left( \frac{R_{\text{exp}} - R_{\text{ref}}}{R_{\text{exo}} - R_{\text{ref}}} \right) / (l/\kappa) \right\} \]

Where \( \dot{V}CO_2 \) (L\( \cdot \)min\(^{-1} \)) is in STPD, \( R_{\text{exp}} \) is the isotopic composition of expired CO\(_2\) during CARB, \( R_{\text{ref}} \) is the isotopic composition of expired CO\(_2\) during CONT at the corresponding time point, \( R_{\text{exo}} \) is the isotopic composition of the CHO\(_{\text{exo}}\), and \( k \) (0.7426 L\( \cdot \)g\(^{-1} \)) is the volume of CO\(_2\)
produced by the complete oxidation of 1g of glucose. CHO_endo oxidation during CARB was calculated by subtracting CHO_exo from CHO_total. Because of the presence of a large bicarbonate pool in the body and because of the delay in measuring ^13^CO_2 production by the tissues at the mouth (23), computations of CHO_exo oxidation were made for the last 60 min of exercise. The oxidation efficiency of CHO_exo (%) was calculated as the area under the curve (AUC) of CHO_exo during the entire 80-min of exercise in the heat divided by the amount of glucose ingested (in g) and multiplied by 100.

**Statistical Analysis**

All data are expressed as mean ± SE. The significance level adopted was 5%, and the analysis was conducted using a statistical software package (STATISTICA for Windows 7.0, StarSoft). Independent t-tests were used for the inter-group comparisons (i.e. differences in physical and fitness characteristics, exercise intensity, urinary parameters, hypohydration levels). T_re, T_sk, HR, RER, substrate oxidation responses of boys and men over time were determined by using a group x trial x time mixed factorial ANOVAs (2 x 2 x 3 analysis). Where appropriate, a Tukey’s post hoc test was used to determine significance among means. Partial eta-squared (\(\eta^2\)) was calculated as a measure of effect size for ANOVAs, where values of 0.01, 0.06 and above 0.15 were considered as small, medium and large, respectively (8). Cohen’s d (d) was calculated as a measure of effect size for pairwise comparisons. Values of 0.2, 0.5, and above 0.8 were considered as small, medium and large, respectively (8).
Results

Hydration levels. All participants arrived with similar hydration levels for the experimental trials according to mean ± SE values of USG and urine colour, boys and men, respectively: 1.019 ± 0.003 and 2.2 ± 0.4, and 1.016 ± 0.002 and 2.0 ± 0.3 (CARB); 1.015 ± 0.003 and 1.7 ± 0.3, and 1.012 ± 0.002 and 2.1 ± 0.4 (CONT). The recorded body fluid balance at the end of exercise in the heat in boys and men respectively, were: -0.2 ± 0.2 and 0.2 ± 0.2 % in CARB, and -0.2 ± 0.3 and 0.2 ± 0.1 % in CONT. The volume of fluid (in L) required to maintain hydration during exercise for boys and men were, respectively: 0.8 ± 0.3 and 1.2 ± 0.3 L in CARB (P = 0.01), and 0.8 ± 0.2 and 1.4 ± 0.3 L in CONT (P = 0.01).

\( \dot{H}_p, T_{re}, T_{sk} \text{ and } HR \). Exercise intensity according to metabolic heat production per unit body mass was similar between boys and men, respectively: 5.7 ± 0.3 and 6.0 ± 0.2 W·kg\(^{-1}\) in CARB, and 6.0 ± 0.4 and 6.5 ± 0.2 W·kg\(^{-1}\) in CONT. Figure 1 depicts \( T_{re}, T_{sk} \) and HR over the 4 × 20-min bouts of exercise in the heat. No differences were found in resting \( T_{re} \) between boys and men in CARB (Figure 1A). \( T_{re} \) increased similarly during exercise in the heat in boys and men in CARB and CONT (\( P = 0.48, \eta^2 = 0.07 \)), resulting in a similar \( \Delta T_{re} \) between boys and men in CARB (0.8 ± 0.2 and 0.9 ± 0.2 °C, respectively) and CONT (0.8 ± 0.3 and 0.9 ± 0.1 °C). \( T_{sk} \) increased in the first 15 min of exercise in the heat, and then reached a steady-state value of ~37 °C, with no differences between CARB and CONT (\( P = 0.70, \eta^2 = 0.05 \)) (Figure 1B). A significant group × time interaction was found in HR (\( P < 0.001, \eta^2 = 0.17 \)) (Figure 1C), but no group x trial interaction was found (\( P = 0.63, \eta^2 = 0.02 \)). Average HR was similar between boys and men during exercise in the heat in CARB and CONT.
**RER.** The average resting RER was not different between boys and men in CARB (0.86 ± 0.01 and 0.89 ± 0.02, respectively) and CONT trials (0.84 ± 0.01 and 0.89 ± 0.02, respectively). The average RER during exercise was similar between boys (Figure 2A) and men (Figure 2B). In both groups, RER was greater in CARB compared to CONT over time ($P = 0.011$, $\eta^2 = 0.83$).

**Breath enrichment.** The isotopic composition of expired CO$_2$ during exercise in the heat in CARB and CONT are shown in Figure 3. No differences were observed at rest between groups and trials (pooled average was -19.7 ‰ [δ-13C] PDB$^{-1}$). During CARB, a marked increase in breath enrichment of $^{13}$CO$_2$ was observed over time in both boys and men, indicating a strong measurement signal compared to at rest (difference of 9.9 ‰ [δ-13C]PDB$^{-1}$), with no differences between groups ($P = 0.10$). During CONT, the ratio of $^{13}$C/$^{12}$C remained constant over time in both boys and men ($P = 0.08$), with a pooled average of -19.7 ± 0.07 and -20.9 ± 0.31 ‰ [δ-13C] PDB$^{-1}$, respectively.

**Substrate utilization.** CHO$_{exo}$, CHO$_{total}$, CHO$_{end}$, and FAT$_{total}$ oxidation rates were calculated over the last 60 min of exercise and presented in Table 2. To control for differences in body size, values are expressed relative to FFM (mg FFM$^{-1}$·min$^{-1}$). CHO$_{exo}$ oxidation rates were lower in boys compared to men (group effect: $P = 0.015$, $d = 0.9$). Both boys and men increased the oxidation rates of CHO$_{exo}$ over time with no differences between groups (group x time effect: $P = 0.73$, $\eta^2 = 0.022$). Oxidation efficiency of CHO$_{exo}$ was lower in boys compared to men (12.8 ± 0.6 vs. 16.0 ± 0.9 ‰, respectively; $P = 0.01$, $d = 1.5$). CHO$_{total}$ and CHO$_{endo}$ oxidation rates were not different between boys and men (group effect: $P = 0.10$ and $P = 0.13$, respectively), even when the experimental trial was considered (group x trial:
CHO total oxidation rates were stable in boys and decreased in men over time (group x time: P = 0.041, η^2 = 0.20). During CARB trial, CHO total and CHO endo did not change over time in both groups. However, during CONT trial, CHO total decreased over time (time x trial effect: P < 0.001; η^2 = 0.54). Fat total oxidation rates were not different between boys and men (group effect: P = 0.41). Fat total oxidation rates were greater in CARB compared to CONT (trial effect: P = 0.004). Fat total oxidation during CARB remained constant over time, but during CONT oxidation increased over time (trial x time: P < 0.001, η^2 = 0.36) similarly in both groups (group x trial: P = 0.80, η^2 = 0.04).

Energy yield. To account for inter-group differences in energy yield, the percent of total energy provided from CHOexo, CHO total, CHO endo, and Fat total was calculated for boys and men as shown in Figure 3.

The percent of energy contribution of CHOexo was similar between boys and men (group effect: P = 0.076, d = 0.7). The relative contribution of CHOexo oxidation increased over time in boys from 4.8 to 7.7 %, and in men from 6.0 to 8.9 %, showing no differences in the increment (group x time effect: P = 0.85, η^2 = 0.011). The percent of energy contribution of CHO endo for energy yield was not different between boys and men (group effect: P = 0.23). The relative contribution of CHO endo remained stable in CARB trial, but decreased over time in both groups in CONT trial (group x time: P = 0.073, η^2 = 0.3, group x trial: P = 0.81, η^2 = 0.04; and trial x time: P < 0.0001, η^2 = 0.69). The percent energy contributions of CHO total and Fat total to total yield were not different between boys and men (group effect: P = 0.15). The percent contribution of CHO total for energy yield was lower in CONT trial (66.8 ± 3.0 % in boys and 72.2 ± 3.7 % in men, Figure 3A and B) compared to CARB (72.2 ± 0.66 % in boys vs. 81.1 ± 1.06 % in men,
During CONT, the percentage of total energy provided from CHO_total decreased over time from 71.6 to 61.2% in boys, and from 78.5 to 65.8% in men. However, it remained stable in CARB trial (76.2 ± 0.7% in boys, and 81.1 ± 1.0% in men) (group x time effect: \( P = 0.054, \eta^2 = 0.19 \); and trial x time effect: \( P < 0.0001, \eta^2 = 0.69 \)). Consequently, Fat_total contribution increased over time in CONT trial, but remained stable over time in CARB trial.

**Discussion**

This study demonstrated that CHO_exo oxidation rate and CHO_exo oxidation efficiency were lower in boys compared to men during exercise in the heat, although the percent contribution of CHO_exo to total energy yield was similar. Furthermore, no differences were observed in CHO_total, CHO_endo and Fat_total utilization between boys and men. CHO_total, CHO_endo and Fat_total oxidation did not change over time during CARB trial; while CHO_total decreased and Fat_total increased over time similarly in boys and men during CONT trial. Finally, compared to CONT trial, a higher percent contribution of CHO_total and lower percent contribution of Fat_total to total energy yield were reported in the CARB trial.

Our findings with respect to age-related differences in CHO_exo oxidation rate and efficiency, and relative contribution of CHO_exo to total yield support some but not all previously reported studies (29, 30). Unlike our results, Timmons, et al. (31) showed a greater reliance on CHO_exo in boys compared to men (10.7 vs. 8.4 mg kg\(^{-1}\) min\(^{-1}\)) by the end of 60 min of cycling at 70% VO\(_{2}\)peak with an \(^{13}\)C-enriched CHO-solution (4% sucrose + 2% glucose) in a thermoneutral environment. The contribution of CHO_exo to total energy yield increased over time in both groups, but the average contribution was higher in boys.
Other studies that verified the impact of biological maturation on CHOexo oxidation and endogenous substrate using the same protocol showed younger boys had a greater reliance on CHOexo compared to older boys (30), but not girls (29). Although it is difficult to directly compare our results to those of the aforementioned studies, plausible explanations for our differences include the amount and type of CHOexo provided, exercise intensity and duration, as well as environmental conditions. More specifically, the amount of CHOexo provided by Timmons, et al. (31) was normalized by kg of body weight (6 aliquots x 4 mL/kg body weight), which may been advantageous for children compared to adults. In contrast, we provided CHOexo normalized by fat free mass (1.3 g CHO kg.FFM⁻¹) since this is related to the active muscle mass during exercise and allowed for a more realistic comparison between heterogeneous groups. The extent to which the rate of CHO ingestion influences metabolic responses during exercise in children has not been systematically investigated. Within the range of body masses typical of healthy adults, no correlation between exogenous CHO utilization and body mass was observed, suggesting that carbohydrate intake during exercise can be provided in absolute amounts rather than scaled to body mass (6). However, due to the much smaller body mass of children, the potential role of body size in CHOexo oxidation requires further examination. In adults (32), the highest rates of CHOexo oxidation were attained when CHO was ingested at rates of 1.0 g min⁻¹ (moderate dose). However, CHOexo oxidation did not further increase when larger amounts of CHO (1.5 g min⁻¹, high dose) were ingested during exercise. The source of CHO might have also contributed to the discrepant findings. Earlier studies (26, 27, 30, 31) that provided a combination of CHOs (fructose + glucose) in the experimental beverage reported a higher relative contribution of CHOexo oxidation to total energy yield in boys (15-30%) and in men (15-20%) compared to boys and men in the present study (~8.3%). However, when glucose alone
was provided during prolonged exercise in the heat, the reported relative contribution of CHO$_{exo}$ to total energy yield (~10%) for adults (15) was similar to our study.

In adults, heat stress has been suggested as an important variable that affects energy substrate utilization during exercise, resulting in a greater reliance on CHO metabolism, however CHO$_{exo}$ oxidation is usually lower in hot environments compared to cool environments (15). No previous study has examined the effect of CHO intake on endogenous substrate utilization during exercise in the heat in children. The possible mechanism for lower CHO$_{exo}$ oxidation during exercise in hot compared to cool environments might be related to an increased blood flow to the skin for evaporative cooling, resulting in a reduction of flow to other organs and limiting intestinal absorption of CHO$_{exo}$, which may be influenced by differences in $T_r$. For this reason, we chose a low-to-moderate exercise intensity to allow both groups to complete the prolonged exercise and to minimize potential effects of higher-intensity exercise on gastric emptying or intestinal fluid absorption. The same metabolic heat production, rather than % VO$_{2\text{peak}}$ or maximal power output, was used to match exercise intensity to induce the same thermoregulatory responses. The use of exercise intensity based on metabolic heat production normalized to body mass can provide an unbiased comparison of thermoregulatory responses (i.e. $\Delta T_r$) in heterogeneous groups, such as children and adults (17).

While most studies examining CHO$_{exo}$ oxidation during exercise in adults have been performed in cool or thermoneutral environments, a few studies have evaluated CHO$_{exo}$ oxidation and substrate utilization during exercise in the heat. Heat stress, compared to a cool environment, reduced CHO$_{exo}$ oxidation rate (0.76 vs. 0.84 g.min$^{-1}$) and increased CHO$_{total}$ oxidation rate (3.18 vs. 2.85 g.min$^{-1}$) in trained male cyclists pedaling for 90 minutes at 55% VO$_{2\text{peak}}$ (14). Unlike our study, an important limitation of this work is that hypohydration levels
and final $T_{re}$ were greater during exercise in the heat, which could have reduced muscle blood flow and in turn limited $\text{CHO}_{exo}$ oxidation. Dumke, et al. (11) showed that absolute $\text{CHO}_{total}$ oxidation ($\text{g} \cdot \text{min}^{-1}$) was similar between a placebo and a 6% $\text{CHO}$ beverage in males during exercise in the heat, but absolute fat oxidation was 25% lower in the experimental trial compared to placebo. Our study corroborates these findings by showing an increase in fat oxidation in CONT trial and maintenance of fat during CARB trial in boys and men. Altogether, these studies suggest that $\text{CHO}_{exo}$ availability during exercise in the heat can alter metabolic response and possibly increase reliance on fat stores when $\text{CHO}_{exo}$ availability is low.

In thermoneutral conditions, investigations have shown that children use proportionally more $\text{Fat}_{total}$ and less $\text{CHO}_{total}$ compared with adults exercising at the same relative intensity ($\%\dot{V}O_{2peak}$. This suggests that children may have a higher endogenous fat oxidation due to a higher intramuscular triglyceride availability compared to adults (4). A study showed (32) that $\text{CHO}_{total}$ oxidation was lower and $\text{Fat}_{total}$ oxidation was higher during exercise in boys compared to men at 70% $\dot{V}O_{2peak}$. In contrast, our findings showed that $\text{Fat}_{total}$ and $\text{CHO}_{total}$ oxidation rates were similar between boys and men during exercise at a lower intensity ($\sim$ 6 W.kg$^{-1}$). In our study, both boys and men demonstrated a similar decrease in $\text{CHO}_{total}$ oxidation rate and an increase in $\text{Fat}_{total}$ oxidation rate over time in the CONT trial. This was confirmed by changes in RER values, which decreased in both boys and men over time in CONT trial. During prolonged exercise, an increased reliance on fat stores is expected with glycogen depletion and/or when $\text{CHO}_{exo}$ availability decreases and fatty acids are released from triacylglycerol stores in adipose tissue and muscle (25).

As expected, $\text{CHO}_{exo}$ availability shifted substrate utilization from $\text{Fat}_{total}$ to a greater reliance on $\text{CHO}_{total}$ during exercise in both boys and men. In the present study, $\text{CHO}_{total}$ and
Fat_{total} were relatively constant overtime in CARB trial in boys and men, compared to the CONT
trial. Compared to the CONT trial, the oxidation rate of CHO_{total} was 18% greater in boys and
12% greater in men by the end of exercise in the CARB trial; and oxidation rate of FAT_{total} was
66% lower in boys and 104% lower in men by the end of exercise. Based on our results, CHO
intake spared endogenous substrate oxidation in both boys and men during exercise in the heat,
but future studies are needed to elucidate the location of this spared fuel (i.e. intra or extra
muscular stores).

It is generally accepted that carbohydrate supplementation during exercise can postpone
fatigue and improve exercise capacity and performance during prolonged exercise in both
children (9) and adults (5, 7). The present findings may be applicable for active boys and men
exercising in the heat and may vary for other populations (i.e female, sedentary individuals and
chronic conditions) and environmental conditions (i.e thermoneutral and cool). There is a lack of
studies comparing the effects of CHO intake on substrate utilization during exercise in the heat
between boys and men. Future studies should investigate the impact of different amounts of
CHO, different time points of CHO ingestion, different combinations of CHO (i.e glucose +
fructose), and compare the oxidation rate of CHO in other environmental conditions (i.e.,
thermoreutral and cool). The impact of different exercise intensities on CHO_{exo} oxidation
between children and adults should also be verified in future research.

In summary, boys had a lower CHO_{exo} oxidation rate and CHO_{exo} oxidation efficiency
compared to men. However, boys and men did not show differences in endogenous substrate
metabolism or the relative contribution of substrate to total energy yield over the last 60-min of
exercise at the same metabolic heat production in a hot environment. Recommendations for CHO
intake among active youth should consider that the ingestion of a CHO beverage during exercise
in the heat may be beneficial to spare endogenous substrate in children and adults; and it may translate into delayed fatigue onset and enhanced aerobic performance, as it results in energy and fluid availability.
Acknowledgements

We wish to thank the participants for their time and effort. GTL and GSC are supported by a fellowship from CNPq (Brazilian Research Council), Science without Borders. BWT was supported by a CIHR New Investigator Salary Award.

Conflict of interest

None of the authors had a conflict of interest regarding any aspect of this research.
References


CAPTION FOR FIGURES

**Figure 1.** Rectal temperature ($T_{re}$) (A), weighted skin temperature ($T_{sk}$) (B) and heart rate (HR) (C) in CARB trial (solid symbols) and CONT trial (open symbols) for boys (■ and □) — and for men (● and ○). Values are means ± SE. Hatched bars represent exercise in the heat. No significant differences were found between groups for $T_{re}$, $T_{sk}$ or HR ($P > 0.05$).

**Figure 2.** Respiratory exchange ratio (RER) during exercise in CARB trial (solid symbols) and CONT trial (open symbols) for boys (A; ■ and □) and for men (B; ● and ○). *Significant different between CARB and CONT in boys and in men ($P < 0.05$). Hatched bars represent exercise in the heat.

**Figure 3.** Breath enrichment of $^{13}$C in expired air [$\delta$% Pee Dee Bellemnitella (PDB)] at rest and during exercise in the heat with (CARB, solid symbols) and without (CONT, open symbols) CHO ingestion for boys (■ and □) and for men (● and ○). Values are expressed in mean ± SE. Hatched bar represent exercise. *Significantly different between CARB and CONT in boys and men ($P < 0.05$).

**Figure 4.** Percent of energy contribution from substrate during exercise in CONT trials (A and B) and CARB trial (C and D). The white portions represent total fat ($F_{a t \text{otal}}$). The hatched portions represent endogenous carbohydrate ($CHO_{endo}$). The black portions represent exogenous carbohydrate ($CHO_{exo}$). $CHO_{exo}$ percent contribution to total energy expenditure was similar between boy and men ($P = 0.076$). Additional statistical results are presented in the Results section.
Breath enrichment (delta $^{13}$C vs PDB)
Table 1. Physical and fitness characteristics of boys and men. *P < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 7)</th>
<th>Men (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.2 ± 0.2</td>
<td>24.0 ± 1.1*</td>
</tr>
<tr>
<td>Body mass (Kg)</td>
<td>38.7 ± 2.2</td>
<td>72.9 ± 1.9*</td>
</tr>
<tr>
<td>Body Height (cm)</td>
<td>148.3 ± 2.8</td>
<td>174.4 ± 1.8*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>19.1 ± 1.5</td>
<td>15.1 ± 1.5*</td>
</tr>
<tr>
<td>$VO_{2peak}$ (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>44.7 ± 1.7</td>
<td>44.9 ± 3.6</td>
</tr>
<tr>
<td>HR$_{max}$ (beats min$^{-1}$)</td>
<td>189 ± 2</td>
<td>182 ± 3</td>
</tr>
<tr>
<td>Tanner stage 1/2, n</td>
<td>3/4</td>
<td>—</td>
</tr>
<tr>
<td>Years from peak high velocity</td>
<td>-2.1 ± 0.2</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SE. *P < 0.05. $VO_{2peak}$: peak oxygen output; HR$_{max}$: maximal heart rate. Tanner stage 1 = pre-pubertal; tanner stage 2 = early-pubertal.
Table 2. Substrate utilization during exercise in the heat in CARB and CONT for boys and men.

<table>
<thead>
<tr>
<th></th>
<th>CHO total</th>
<th>CHOexo</th>
<th>CHOendo</th>
<th>Fat total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CARB</td>
<td>CONT</td>
<td>CARB</td>
<td>CONT</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 min</td>
<td>23.6 ± 1.5</td>
<td>24.0 ± 2.5</td>
<td>1.5 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>80 min</td>
<td>24.2 ± 1.5</td>
<td>22.7 ± 2.1</td>
<td>2.0 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>110 min</td>
<td>25.0 ± 1.8</td>
<td>20.4 ± 2.2</td>
<td>2.5 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 min</td>
<td>28.0 ± 1.0</td>
<td>28.0 ± 1.1</td>
<td>2.0 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>80 min</td>
<td>26.0 ± 1.0</td>
<td>25.5 ± 1.3</td>
<td>2.5 ± 0.1</td>
<td>—</td>
</tr>
<tr>
<td>110 min</td>
<td>26.6 ± 1.2</td>
<td>23.4 ± 1.6</td>
<td>2.8 ± 0.2</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are presented in means ± SE given in mg kgFFM⁻¹ min⁻¹. CHO_total, total carbohydrate oxidation; CHO_exo, exogenous carbohydrate oxidation; CHO_endo, endogenous carbohydrate oxidation; Fat_total, total fat oxidation. Statistical analysis is presented in the Results section.