Substrate Utilization During Exercise Performed With and Without Glucose Ingestion in Female and Male Endurance-Trained Athletes

Michael C. Riddell, Sara L. Partington, Nicole Stupka, David Armstrong, C. Rennie, and Mark A. Tarnopolsky

Compared to males, females oxidize proportionately more fat and less carbohydrate during endurance exercise performed in the fasted state. This study was designed to test the hypothesis that there may also be gender differences in exogenous carbohydrate (CHOexo) oxidation during exercise. Healthy, young males \((n = 7)\) and females \((n = 7)\) each completed 2 exercise trials (90 min cycle ergometry at 60% VO\(_{2\text{peak}}\)), 1 week apart. Females were eumenorrheic and were tested in the midfollicular phase of their menstrual cycle. Subjects drank intermittently either 8% CHOexo \((1 \text{ g glucose} \cdot \text{kg} \cdot \text{h}^{-1})\) enriched with U-13C glucose or an artificially sweetened placebo during the trial. Whole-body substrate oxidation was determined from RER, urinary urea excretion, and the ratio of 13C:12C in expired gas during the final 60 min of exercise. During the placebo trial, fat oxidation was higher in females than in males \((0.42 \pm 0.07 \text{ vs. } 0.32 \pm 0.09 \text{ g} \cdot \text{min}^{-1} \cdot \text{kg LBM}^{-1} \times 10^{-2})\) at 30 min of exercise \((p < .05)\). When averaged over the final 60 min of exercise, the relative proportions of fat, total carbohydrate, and protein were similar between groups. During CHOexo ingestion, both the ratio of 13C:12C in expired gas \((p < .05)\) and the proportion of energy derived from CHOexo relative to LBM \((p < .05)\) were higher in females compared to males at 75- and 90-min exercise. When averaged over the final 60 min of exercise, the percentage of CHOexo to the total energy contribution tended to be higher in females \((14.3 \pm 1.2\%)\) than in males \((11.2 \pm 1.2\%; p = .09)\). The reduction in endogenous CHO oxidation with CHOexo intake was also greater in females \((12.9 \pm 3.1\%)\) than in males \((5.1 \pm 2.0\%; p = .05)\). Compared to males, females may oxidize a greater relative proportion of CHOexo during endurance exercise which, in turn, may spare more endogenous fuel. Based on these observations, ingested carbohydrate may be a particularly beneficial source of fuel during endurance exercise for females.

Key Words: sex differences, gender differences, carbohydrate drinks.
Introduction

An increasing number of studies indicate that there are gender differences in the metabolic responses to endurance exercise (34). It has been consistently reported that females have lower RER values than males during prolonged exercise in the fasted state (11, 15, 20, 26, 27, 33, 35). The lower RER observed during submaximal exercise in females indicates that they use more endogenous fat and less endogenous carbohydrate (CHOendo) as a metabolic substrate. However, we are unaware of any published studies exploring possible gender differences in metabolism during exercise performed with ingested carbohydrate.

Based on studies conducted primarily with adult male subjects, the availability of carbohydrate as a substrate for skeletal muscles and the central nervous system is thought to be a critical factor in the performance of endurance exercise (13). Since CHOendo stores are limited, exogenous carbohydrate (CHOexo) is often ingested either before and/or during exercise as an additional energy source to improve performance and delay fatigue (7). Indeed, CHOexo has been shown to delay the onset of fatigue in men by maintaining high rates of total CHO (CHOtotal) oxidation and blood glucose concentrations later in exercise (2, 6, 14, 16). These beneficial effects of CHOexo have also been observed in adolescent boys (28, 29). Surprisingly, the effects of CHOexo intake upon metabolism during exercise in females are not well documented. Bailey et al. (1) demonstrated that, compared with a placebo intake, 6% CHOexo increased exercise performance in the follicular and luteal phases of the menstrual cycle. Recently, Campbell et al. (3) found that exercise performed with placebo ingestion during the follicular phase had higher plasma glucose rates of appearance and disappearance than exercise performed during the luteal phase. When the females ingested CHOexo, however, there were no differences in substrate oxidation rates between menstrual phases, thereby indicating that variations in the ovarian hormones do alter substrate metabolism, but only in the absence of CHOexo (3). Interestingly, these authors reported that the percentage of plasma glucose oxidation, relative to total energy provision, was nearly 2-fold higher in females during exercise with CHOexo than values reported in the literature for males exercising at a similar relative intensity (9, 16, 19). As a result of higher rates of plasma glucose oxidation in females, as compared to males, females may also have higher relative rates of CHOexo oxidation.

The purpose of this study, therefore, was to compare substrate utilization between males and females during endurance exercise performed both with and without CHOexo ingestion. We hypothesized that without CHOexo intake, the percent energy contribution from fat would be greater, and the percent energy contribution from CHOtotal would be less in females compared with males, during exercise performed at the same relative intensity. We also hypothesized that during exercise performed with CHOexo intake, the percent energy contribution from CHOexo oxidation to the total energy provision would be higher in females than in males.

Methods

Subjects

Seven female and 7 male endurance trained athletes volunteered for the study. They were advised of the risks associated with the study and signed written consent forms
approved by the McMaster University Research Advisory Committee. Males were selected based upon a training history of consistent participation in endurance-type physical activity for at least 1 year (minimum of 5 days per week and 45 min per session) and peak oxygen consumption ($\text{VO}_2\text{peak}$) of at least 50 ml · kg$^{-1}$ · min$^{-1}$. The females were matched to the males based on similar training histories and by having a $\text{VO}_2\text{peak}$ of at least 45 ml · kg$^{-1}$ · min$^{-1}$. This matching was performed to result in similar $\text{VO}_2\text{peak}$ values when expressed as a percentage of lean body mass (LBM). The physical and functional characteristics of the subjects are shown in Table 1. All female subjects were eumenorrheic for a minimum of 6 months prior to the study and were tested in the mid-follicular phase of their menstrual cycle. Both men and women were predominantly runners, with cycling comprising less than 50% of their training volume.

**Preliminary Session**

$\text{VO}_2\text{peak}$ was determined within 2 weeks of the first experimental trial using a cycle ergometer and a computerized open-circuit gas collection system, as previously described (35). $\text{VO}_2\text{peak}$ was considered to be the highest value recorded during an incremental ergometer protocol, with termination of the test occurring when pedal revolutions could not be maintained at $>$ 60 revolutions/min in spite of vigorous encouragement and a respiratory exchange ratio (RER) $>$ 1.12. Total body fat mass and fat-free mass (FFM) was determined using dual energy X-ray absorptiometry (DEXA; QDR 1000W, Hologic, Waltham, MA, USA) in the late afternoon, a minimum of 4 h after the $\text{VO}_2\text{peak}$ test and after ad libitum rehydration. Four-day dietary records were collected from each subject (3 weekdays and 1 weekend day) for macronutrient analysis. Subjects were instructed to repeat their same nutrient intake on the 2 days preceding the experimental trials in order to minimize potential dietary differences in endogenous substrate availability. In order to keep a low background $^{13}$C enrichment in expired CO$_2$, subjects avoided the ingestion of CHO derived from C$_4$ photosynthetic plants, which are naturally enriched in $^{13}$C (32). Subjects were

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male $(n = 7)$</th>
<th>Female $(n = 7)$</th>
<th>Significance</th>
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</thead>
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<tr>
<td>Age (y)</td>
<td>25.7 ± 4.6</td>
<td>23.3 ± 1.5</td>
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<tr>
<td>Height (cm)</td>
<td>185 ± 8.0</td>
<td>166.0 ± 7.2</td>
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<tr>
<td>Mass (kg)</td>
<td>77.6 ± 6.8</td>
<td>61.5 ± 8.3</td>
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<tr>
<td>LBM (kg)</td>
<td>66.8 ± 5.7</td>
<td>49.4 ± 6.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Abs $\text{VO}_2\text{peak}$ (ml · min$^{-1}$)</td>
<td>4583 ± 510</td>
<td>3227 ± 362</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>$\text{VO}_2\text{peak}$ (ml · kg LBM$^{-1}$ · min$^{-1}$)</td>
<td>68.9 ± 8.2</td>
<td>65.7 ± 6.3</td>
<td>ns</td>
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</tbody>
</table>

*Note.* Values are means ± SD. LBM: lean body mass; Abs $\text{VO}_2\text{peak}$: absolute peak O$_2$ uptake; ns, not significant.
also instructed to keep the intensity and type of exercise consistent between trials for the 2 days prior to testing to ensure similar glycogen availability for each trial. Subjects were asked to exercise for no more than 60 min in the 2 days prior to the testing and to rest the day prior to testing.

**Experimental Design**

Each subject completed two experimental trials, spaced 1 week apart. All testing occurred between day 3 and day 14 of their menstrual cycle, with day 1 being the first day of menses. Trials were identical except for the CHOexo intake during exercise, and the order was counterbalanced among the subjects. During the trials, subjects intermittently drank either 8% exogenous glucose (CHOexo), equal to 1.0 g · kg body mass · hour exercise⁻¹, or a similar volume of an artificially sweetened placebo (see below). Drinks were grape flavored and contained 18 mmol·L⁻¹ NaCl. Subjects were blinded to the content of the drinks in each trial. Each experimental trial consisted of 90 min of cycling at 60% of their predetermined VO₂peak.

**Protocol**

Subjects were instructed to fast overnight and to abstain from caffeine the evening prior to the experimental trials. On the morning of the experiment, they consumed a defined formula snack (375 kcal for males, 235 kcal for females; 55% carbohydrate, 30% fat, 15% protein) provided by the investigators. The pre-test meal was provided to match the usual practice of consuming a snack prior to a race. Subjects reported to the laboratory after the snack was ingested, emptied their bladder, and their pre-trial weights were recorded. A 20-G Teflon plastic catheter (Insyte, Becton Dickinson) was inserted into an antecubital vein for blood sampling. The catheter was kept patent with a 2-ml saline flush following each sample. The start of exercise (time = 0 min) on the cycle ergometer occurred 90 min after the consumption of the defined formula. Subjects were instructed to ingest the provided beverages (CHOexo or placebo (Aspartame flavored drink) within 30 s at 20 min prior to the start of exercise and at 0, 15, 30, 45, 60, and 75 min of exercise (for a total of seven equal aliquots). The glucose in the CHOexo trial was derived from corn (BDH-Chemical, Toronto, ON) and artificially enriched with U⁻¹⁵C glucose (¹³C/¹²C > 99% excess, Isotec, Miamisburg, OH, USA) to achieve a final isotopic composition of +43.6 delta per 1000 difference (‰) versus the ¹³C-to-¹²C ratio from the international standard ¹³C Pee Dee Belemnita-1 (PDB-1; i.e., +43.6‰ [δ¹³C] PDB-1), as measured by dual-inlet isotope ratio mass spectrometry (VG-Sira 10, series II, Manchester, UK). This high level of enrichment, compared with that of normal expired gas (Figure 1), provided a strong measurement signal and reduced the error associated with a small shift in the isotopic composition of CO₂ arising from the oxidation of endogenous substrates during exercise (23).

**Measurements and Calculations**

Whole blood sampling and respiratory measurements were made at rest before the ingestion of the first exogenous beverage (−20 min), prior to the start of exercise (0 min) and every 15 min during the exercise period. Blood samples (12 ml) were drawn from the indwelling catheter and each sample was centrifuged at 5000 × g for
and the plasma supernatant was stored at –20 °C and subsequently analyzed for glucose and lactate concentrations using an enzymatic glucose-lactate analyzer (2300 Stat Plus, Fisher Scientific) and insulin concentration using a commercially available double-antibody RIA kit (Coat-a-Count kit # TKIN5; Diagnostics Products, Los Angeles, CA, USA).

Protein oxidation was determined based on the amount of urea excreted during the exercise period (total of 2 h, 10 min before start and 20 min after exercise) as compared to a resting sample taken for 2 h on the rest day prior to the testing under identical conditions (10). Urea excretion during exercise was estimated from urea concentration in urine (kit # 640-B; Sigma Diagnostics) and estimated sweat loss during the exercise period. CHOtotal and fatty acid oxidation were computed from indirect respiratory calorimetry corrected for protein oxidation (25). For this purpose, CO₂ production (VCO₂) and oxygen consumption (VO₂) were measured using open-circuit spirometry during 5-min collection periods every 15 min during exercise. During gas sampling, duplicate 10-ml expired gas samples were drawn directly from the mixing chamber and stored in 10-ml non-treated evacuated tubes for subsequent determination of ¹³C/¹²C in expired CO₂ and CHOexo oxidation. The isotopic composition of CO₂ in expired gas samples was determined using an isotope ratio mass spectrometer (BreathMat Plus, Finnigan MAT GmbH, Bremen, Germany) and expressed in ‰ (δ¹³C) PDB-1.

Figure 1 — Isotopic composition of expired CO₂ during the placebo and CHOexo intake trials in males and females. Exercise (60% VO₂peak) started at time zero. Values are means ± SE. δ¹³C PDB-1, difference in ¹³C Pee Dee Bilemmitella-1 (PDB-1) standard. *Gender difference in the CHOexo trial (p < .05).
CHOexo oxidation was calculated for the sampling periods using the Mosora et al. (21) formula:

$$\text{CHOexo} = \text{VCO}_2 \cdot \left[ \frac{(R_{\text{exp}} - R_{\text{ref}})}{(R_{\text{exo}} - R_{\text{ref}})} \right] (1/k)$$

where VCO$_2$ is in L · min$^{-1}$ STPD, R$_{\text{exp}}$ is the isotopic composition of expired CO$_2$ during exercise, R$_{\text{ref}}$ is the isotopic composition of expired CO$_2$ at rest prior to CHOexo ingestion, R$_{\text{exo}}$ is the isotopic composition of the CHOexo beverage, and k (0.7426 L · g$^{-1}$) is the volume of CO$_2$ provided by the complete oxidation of glucose (25). This method of determining CHOexo oxidation assumes that $^{13}$CO$_2$ recovery in expired gas during exercise is complete or almost complete (8), although there is a delay in recovery due to the large labile bicarbonate pool (22). To take into account this delay between $^{13}$CO$_2$ production in the tissues and at the mouth, a priming dose of CHOexo was given prior to exercise (~20 min), and the percent energy calculations were only made during the last 60 min of exercise. CHOendo oxidation was computed as the difference between CHOtotal and CHOexo oxidation. The contribution of the oxidation of the various substrates to the energy yield was computed from their respective energy potentials (3.9, 9.7, and 4.2 kcal · g$^{-1}$ for glucose, fat, and proteins, respectively; 10).

Statistical Analyses

Data are presented as means ± SEM. For measurements made repeatedly in both trials and in both genders, a three-way mixed ANOVA design (1 between factor = gender; 2 within factors = trial, time) was performed (Statistica, StatSoft). Tukey’s HSD post hoc test was used to identify the location of the significant differences when analysis of variance yielded a significant $F$ ratio. Inter-group differences in physical and functional characteristics were compared by using a non-paired $t$ test. A probability of $p < .05$ was taken to indicate significance.

Results

Physical and Functional Characteristics

Table 1 shows the physical and functional characteristics of the subjects. Compared with the females, males were taller, heavier, leaner, and had higher absolute VO$_{\text{2peak}}$ values. By design, there was no significant difference between the genders in relative VO$_{\text{2peak}}$, when this value was expressed per kilogram of LBM.

Expired Gases and Substrate Utilization

Resting RER values were similar between trials and between genders, averaging 0.844 ± 0.01 (pooled data). Resting absolute VO$_2$ was also similar between trials but was higher for the males (0.36 ± 0.013 L/min) than for the females (0.24 ± 0.018 L/min; $p < .01$). During exercise, absolute VO$_2$ was higher for males (2.76 ± 0.043 L/min) than for females (1.90 ± 0.024 L/min) ($p < .01$). By design, the percentage of VO$_{\text{2peak}}$ during exercise was similar between trials and between genders, averaging 59.6 ± 0.54% (pooled data). In both trials, and for both genders, the RER decreased throughout the 90 min of exercise (main effect of time, $p < .001$). The decrease in RER with time was less during CHO intake than during placebo intake ($p < .05$,
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trial × time interaction). RER values were higher for males than for females at 30 min exercise (gender × time interaction, \( p < .05 \); Figure 2). The \( ^{13}\text{C}/^{12}\text{C} \) in expired \( \text{CO}_2 \) at rest before beverage ingestion and during the exercise period is shown in Figure 1. \( ^{13}\text{C}/^{12}\text{C} \) in expired \( \text{CO}_2 \) at rest, prior to the first beverage ingestion, was similar between genders and between trials (averaging \(-22.7 \pm 0.3\% \) [\( ^{\delta-13}\text{C} \)] PDB-1, pooled data; \( n = 28 \)). Significant Gender × Time × Trial interactions in \( ^{13}\text{C} \) enrichment of expired \( \text{CO}_2 \) were seen (\( p < .05 \)). During the placebo trial, \( ^{13}\text{C}/^{12}\text{C} \) in expired \( \text{CO}_2 \) increased slightly but significantly in males from \(-22.8 \pm 0.4\% \) to \( 19.9 \pm 1.0\% \) [\( ^{\delta-13}\text{C} \)] PDB-1 (\( p < .001 \)) but remained unchanged in the females (Figure 1). During the glucose trial, both genders had a marked and progressive increase in \( ^{13}\text{C}/^{12}\text{C} \) in expired \( \text{CO}_2 \) throughout exercise, but the values were higher in the females than in the males at 75 min (\( p < .05 \)) and at 90 min (\( p < .05 \)).

Substrate oxidation rates during the last 60 min of exercise, expressed as percent of LBM, are shown in Table 2. Significant main effects of Trial and Time on CHOtotal oxidation, fat oxidation, and CHOendo oxidation were found (all \( p < .05 \)). For data collapsed across group and trial, fat oxidation increased and CHOtotal oxidation decreased with exercise time. Compared with the placebo trial, the CHOexo trial was associated with higher CHOtotal and lower fat oxidation rates. During the placebo trial, a significant Gender × Time interaction on fat oxidation was also found, with values at 30 min higher in females than in the males (\( p < .05 \)). During the CHOexo trial, a significant interaction between Gender × Time (\( p < .05 \)) on CHOexo oxidation was also found, with oxidation rates higher in females than in males at 75 and 90 min of exercise (both \( p < .05 \)).
Table 2 Substrate Oxidation Rates

<table>
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<th>Substrate</th>
<th>Placebo</th>
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<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>75</td>
<td>90 min</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>75</td>
<td>90 min</td>
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<tr>
<td>Female</td>
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<td></td>
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<tr>
<td>CHOexo</td>
<td>0.50 ± 0.03</td>
<td>0.66 ± 0.05</td>
<td>0.77 ± 0.06</td>
<td>*0.94 ± 0.08</td>
<td>*0.97 ± 0.08</td>
<td>0.49 ± 0.05</td>
<td>0.55 ± 0.05</td>
<td>0.67 ± 0.05</td>
<td>*0.70 ± 0.07</td>
<td>*0.77 ± 0.08</td>
</tr>
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<td>CHOendo</td>
<td>3.98 ± 0.24</td>
<td>3.92 ± 0.28</td>
<td>3.85 ± 0.22</td>
<td>3.87 ± 0.25</td>
<td>3.69 ± 0.29</td>
<td>4.14 ± 0.35</td>
<td>4.15 ± 0.28</td>
<td>4.05 ± 0.23</td>
<td>4.07 ± 0.23</td>
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<tr>
<td>CHOtotal</td>
<td>3.98 ± 0.24</td>
<td>3.92 ± 0.28</td>
<td>3.85 ± 0.22</td>
<td>3.87 ± 0.25</td>
<td>3.69 ± 0.29</td>
<td>4.14 ± 0.35</td>
<td>4.15 ± 0.28</td>
<td>4.05 ± 0.23</td>
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<tr>
<td>Fat</td>
<td>*0.42 ± 0.07</td>
<td>0.45 ± 0.10</td>
<td>0.52 ± 0.08</td>
<td>0.50 ± 0.07</td>
<td>0.60 ± 0.09</td>
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<td>0.43 ± 0.06</td>
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<tr>
<td>Males</td>
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<tr>
<td>CHOexo</td>
<td>4.59 ± 0.38</td>
<td>4.34 ± 0.35</td>
<td>4.13 ± 0.36</td>
<td>3.99 ± 0.27</td>
<td>3.94 ± 0.27</td>
<td>4.39 ± 0.31</td>
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<td>CHOendo</td>
<td>4.59 ± 0.38</td>
<td>4.34 ± 0.35</td>
<td>4.13 ± 0.36</td>
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<td>3.94 ± 0.27</td>
<td>4.89 ± 0.29</td>
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<tr>
<td>CHOtotal</td>
<td>4.59 ± 0.38</td>
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<tr>
<td>Fat</td>
<td>*0.32 ± 0.09</td>
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<td>0.63 ± 0.10</td>
<td>0.25 ± 0.09</td>
<td>0.40 ± 0.11</td>
<td>0.44 ± 0.10</td>
<td>0.40 ± 0.08</td>
<td>0.45 ± 0.10</td>
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Note. Values are expressed relative to lean body mass (g · min⁻¹ · kg LBM⁻¹ · 10⁻²). CHOexo: exogenous carbohydrate; CHOendo: endogenous carbohydrate; CHOtotal: total CHO. *Indicates significant difference between genders (p < .05). For statistical main effects and other interactions, see Results section.
The relative percentages of energy contribution from exogenous glucose, endogenous glucose, total fat, and total protein during the last 60 min of exercise are shown in Figure 3. During the placebo trial, percent energy contribution from CHOTotal (72.3 ± 4.2% vs. 70.3 ± 3.7%), fat (21.9 ± 3.7% vs. 22.8 ± 3.7%), and protein (5.8 ± 1.3% vs. 6.9 ± 1.1%) oxidation were similar in males and females. Compared with the placebo trial, CHOendo oxidation in the CHOexo trial was slightly but significantly \( p < .05 \) reduced in the males (72.3 ± 4.2% vs. 68.4 ± 3.7%) and females (70.3 ± 3.7% vs. 59.7 ± 3.3%). The percent reduction in CHOendo oxidation with CHOexo intake tended \( p = .05 \) to be higher in females (12.9 ± 3.1%) than in males (5.1 ± 2.0%). Compared with the placebo trial, protein oxidation in the CHOexo trial was also slightly but significantly \( p < .05 \) reduced in the males (5.8 ± 1.3% vs. 4.2 ± 1.1%) and females (6.9 ± 1.1% vs. 5.2 ± 1.4%). Compared with the placebo trial, fat oxidation in CHOexo trial was also significantly \( p < .05 \) reduced in the males (21.9 ± 3.7% vs. 16.2 ± 3.2%) and females (22.8 ± 3.7% vs. 19.8 ± 2.9%). There were no significant gender differences in the sparing of fat or protein with CHOexo intake. In the CHOexo trial, percent energy contribution from CHOexo tended \( p = .09 \) to be higher in females (14.3 ± 1.2%) than in males (11.2 ± 1.2%).

Figure 3 — Average percent energy contributions from fat, protein, CHOendo, and CHOexo oxidation during the final 60 min of exercise in the placebo and CHOexo intake trials in males and females. A = males-placebo; B = males-CHOexo; C = females-placebo; D = females- CHOexo. Main effect of trial \( p < .05 \) on percent energy contribution from CHOendo, protein \( p < .05 \), and fat \( p < .05 \) oxidations were seen. Percent energy contribution from CHOexo tended to be higher in females than in males \( p = .09 \).
Blood Variables

Blood glucose, lactate, and insulin concentrations are shown in Figure 4. During the placebo trial, blood glucose levels were similar between genders and remained relatively unchanged with exercise. Compared to the placebo trial, blood glucose levels were higher in the CHOexo trial at the start of exercise and after 45 min of exercise (trial × time interaction, p < .005). The increase in blood glucose in the CHOexo trial was slightly but significantly higher in females than in males (gender × trial interaction, p < .05). Lactate concentrations were similar between genders and between trials, decreasing slightly but significantly (time effect, p < .05) with time. During the placebo trial, plasma insulin levels decreased similarly in both genders. Compared with the placebo trial, plasma insulin levels in the CHOexo trial were higher at the start of exercise (p < .05). No gender differences in insulin concentration were found.

Figure 4 — Blood glucose, lactate, and insulin concentrations before and during exercise in the placebo and CHOexo intake trials in males and females. Trial × Time interactions on blood glucose (p < .005) and insulin (p < .05) were seen. A Gender × Trial interaction (p < .05) on blood glucose concentration was seen. A main effect of Time on lactate concentration (p < .05) was also seen.
Discussion

The main finding of this study is that, during the later stages of prolonged running, female athletes utilize more ingested CHOexo than male athletes exercising at the same relative intensity (60% of VO$_{2\text{peak}}$). In addition, it appears that CHOexo intake may spare more CHOendo in females than in males during prolonged running. These observations, made during the follicular phase of the menstrual cycle in females, extend the previous observations that gender differences in substrate utilization exist during endurance exercise in the absence of exogenous carbohydrate administration (11, 15, 20, 26, 27, 33, 35). Although a previous study investigated the influence of CHOexo intake and menstrual phase on glucose kinetics in females (3), this study is the first to directly compare substrate utilization during CHOexo intake in females and male participants. We found that during the final 60 min of 90 min of high intensity exercise, the percent energy contribution from CHOexo was ~25% higher for females than for males, and represented 14% of the overall energy contribution compared with the 11% observed for males in our study (Figure 3). The values reported during the final 60 min of exercise for the female subjects in our study is also ~25% higher than that reported for males in similarly conducted studies using $^{13}$C labeling (24). This difference in CHOexo oxidation between males and females persisted when absolute oxidation rates are expressed per kilogram of lean body mass (Table 2). It is important to note that the gender differences in CHOexo oxidation only existed at 75 and 90 min of exercise and, when the data were pooled over the final 60 min, the difference in oxidation rate did not reach statistical significance. Although we cannot rule out the possibility that gender differences in CHOexo oxidation exist only during the later stages of prolonged exercise, it is possible that intersubject variability in oxidation rate may have limited our ability to detect smaller differences during earlier time points.

An increasing number of studies indicate that there are gender differences in the metabolic responses to endurance exercise. Several studies have shown that females have lower RER values as compared to males during prolonged exercise, performed without CHOexo, indicating a greater reliance on fat as a fuel (11, 15, 20, 26, 33, 35, 36). Moreover, the higher relative rates of fat utilization in females are likely enhanced when they perform exercise during the luteal phase of their menstrual cycle (3). Our study also indicated that females tend to utilize more fat than males, although we only observed statistical significance at 30 min of exercise in the placebo trial (Table 2). Our failure to observe gender differences at other time points during the placebo trial may be related to the pre-exercise snack provided to the subjects. Indeed, the majority of previous studies comparing substrate utilization between males and females have been conducted with subjects exercising in the fasted state. Our study also suggests that CHOexo intake largely eliminates gender differences in whole-body substrate utilization (Figure 3). Together, these findings suggest that, compared with males, females tend to spare endogenous carbohydrate, particularly during periods of reduced CHO availability. When exogenous fuels are available, however, females may have a higher capacity to utilize these fuels as energy substrate.

Despite the overwhelming evidence that females utilize more fat and less CHOendo than males (34), we have shown that the percent energy contribution from CHOexo is higher in females than in males (Figure 3). This finding is in agreement with those of Campbell et al. (4) who showed that percent plasma glucose
oxidation was higher (~20% of the total energy contribution) during CHOexo intake for females than the 8–11% that they had previously reported for males (9). Indeed, higher rates of plasma glucose oxidation with CHOexo intake in females may explain the higher rates of CHOexo oxidation in our study. Unfortunately, we did not measure plasma glucose enrichment and thus are unable to relate the rate of plasma glucose oxidation with CHOexo oxidation in our subjects.

The differences between females and males in CHOexo oxidation and the sparing of CHOendo with CHOexo intake are somewhat modest and appear to occur only during the later stages of prolonged exercise (Table 2, Figure 3). In addition, it is unknown if these small differences in metabolism translate to meaningful differences in the effectiveness of CHOexo to improve exercise performance. The investigation by Campbell et al. (3) illustrates, however, that compared with placebo intake, CHOexo intake improved exercise performance by 19–26% in their females participants, values considerably higher than the 7% increase observed for males in their prior study (9). It may be, therefore, that the higher oxidation of CHOexo in females in the later stages of prolonged exercise (Figure 1, Table 2) may contribute the large increases in performance observed with CHOexo intake in prior investigations. Further investigation is required to directly compare the beneficial effects of CHOexo on exercise performance in males and females.

The mechanism(s) behind the higher rate of CHOexo oxidation for females compared with males in our study are unknown, but may be a result of several factors. First, higher CHOexo oxidation rates in females may be at least partially explained by higher relative rates of CHOexo intake. Although subjects ingested identical rates of exogenous glucose relative to their total body mass, females ingested more carbohydrate when the data are expressed per kilogram of LBM. Indeed, more CHOexo ingested relative to LBM may explain the higher rates of glucose concentrations and higher insulin levels observed in the females compared with the males. It is unlikely, however, that these small difference in CHOexo intake relative to LBM can fully explain the higher CHOexo oxidation rates in females compared with males, since CHOexo ingestions rates higher than 1.0 g/kg/h do not appear to increase CHOexo oxidation rates significantly, at least in male subjects (18). Second, higher rates of glucose oxidation in females may be a result of their sex hormone characteristics. Studies conducted in rats and in humans suggest that the female sex hormone, 17-β-estradiol, may mediate a number of metabolic effects during exercise. Indeed, the administration of 17-β-estradiol to male subjects results in significant muscle and liver glycogen sparing during exercise (17, 30). Furthermore, human studies have demonstrated that 17-β-estradiol treatment decreases glucose rates of appearance and disappearance in amenorrheic females (31) and males (5). It may be, therefore, that 17-β-estradiol limits endogenous CHO utilization in females, which causes a greater reliance on exogenous fuel sources like CHOexo. Further studies are necessary, however, to test this hypothesis. Third, higher rates of glucose oxidation may be explained by gender differences in GLUT4 levels or hexokinase activity. This final hypothesis seams unlikely, however, since no major gender differences in either GLUT4 transporter number in rats (12) or hexokinase activity in humans (37) have been observed.

Our study considered a number of important methodological considerations. Since females generally have a higher relative body fat content than males, and because subjects have a wide range of training habits, we choose to match the males
and females based upon assessment of both training history and VO\textsubscript{2peak} expressed relative to lean body mass (Table 1). This matching approach, which has been used in a number of our previous studies (26, 35, 36), takes into account both the genetic (VO\textsubscript{2peak} potential) and environmental (training state) factors contributing to VO\textsubscript{2peak} and expresses them relative to the mass of metabolically active tissues. This approach may be particularly important when comparing metabolic variables between genders during exercise. It is also important to mention that we selected eumenorrheic females for our study and that the experimental trials were conducted during the follicular phase of their menstrual cycle. During the follicular phase of the menstrual cycle (~first 14 days after the onset of menses) the 17-β-estradiol concentration starts at levels comparable to males and then increases until ovulation (which marks the onset of the luteal phase). During the luteal phase of the menstrual cycle, the concentrations of both estrogen and progesterone are markedly elevated until a rapid drop at the onset of menses to start another cycle (~28 days in total). The follicular phase of the menstrual cycle was chosen in this study, because 17-β-estradiol levels predominate with little contribution from progesterone, because gender differences in RER have repeatedly been shown to exist during this phase, and because the determination of the follicular phase is more accurate than the luteal phase. Based upon the work of Campbell et al. (3), it is likely that the gender differences that we observed would be present, or even more evident, if the comparison occurred in the luteal phase.

Our study has a number of practical considerations that may be particularly relevant to female athletes. Based on our results and others (3), it appears that exogenous CHO intake (1.0 k CHO/kg/h) provides a considerable energy source for oxidation, elevates blood glucose levels, and spares endogenous fuel sources in female athletes. Since CHO loading prior to exercise is considerably less effective in increasing muscle glycogen levels in females than in males (37), CHOexo intake during exercise may be even more important for females than for males. Indeed, dramatic increases in performance with CHOexo intake compared with placebo intake have been observed in females during both the follicular and luteal phases of the menstrual cycle (3). Further studies are necessary to confirm the hypothesis that increased CHO intake increases exercise performance in female athletes. On the basis of the results from this study—that females use more exogenous CHO than men during exercise performed at a similar intensity—female athletes preparing for endurance events should ensure adequate CHO ingestion during an exercise event. Failure to ingest CHO may result in accelerated glycogen depletion and premature fatigue.

References


**Acknowledgments**

These studies were conducted at the McMaster University Medical Centre, Hamilton, Ontario, Canada. This study was funded by NSERC Canada (Dr. Tarnopolsky). We wish to acknowledge M. Wolfe from the Department of Gastroenterology for the analysis of 13C/12C in expired CO2 gas samples. We also acknowledge the assistance of Dr. S. Phillips for his help with the metabolic cart measurements.