Gender differences in carbohydrate loading are related to energy intake

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Tarnopolsky, Mark A., Carol Zawada, Lindsay B. Richmond, Sherry Carter, Jane Shearer, Terry Graham, and Stuart M. Phillips. Gender differences in carbohydrate loading are related to energy intake. J Appl Physiol 91: 225–230, 2001.—We demonstrated that female endurance athletes did not increase their muscle glycogen concentration after an increase in the dietary carbohydrate intake (58%–74%), whereas men did (Tarnopolsky MA, SA Atkinson, SM Phillips, and JD McDougall, J Appl Physiol 78: 1360–1368, 1995). This may have been related to a lower energy intake compared with men.

The aforementioned gender differences could have implications for nutritional recommendations for female endurance athletes. For example, our laboratory previously observed that women increase muscle glycogen in response to a high dietary carbohydrate intake “but the magnitude was smaller than that previously observed in men” (25). Both groups proposed several mechanisms to explain the apparent gender difference in carbohydrate loading.

First, dietary energy intake is lower in women compared with men, even when expressed relative to lean mass (17, 23). This translates into a lower carbohydrate intake for women compared with men when expressed relative to fat-free mass (FFM; the largest pool of glucose disposal). For example, we found that the carbohydrate intake for men was 7.7–9.6 g·kg FFM−1·day−1 and for women was 5.9–7.9 g·kg FFM−1·day−1, when the subjects increased their carbohydrate intake from 58 to 74% of energy intake (23).

The majority of studies examining carbohydrate loading have been conducted using predominantly or exclusively male subjects, and the carbohydrate intake on the high carbohydrate diet was usually >8.5 g·kg FFM−1·day−1 (3, 11, 20). Thus the provision of extra energy during a carbohydrate-loading regimen may allow women to increase their carbohydrate intake to >8.5 g·kg FFM−1·day−1 and permit muscle glycogen supracompensation. We have recently shown that, when men and women consume carbohydrate expressed relative to body weight (glucose 1 g·kg−1·day−1, immediately and 1 h post) after endurance exercise, the rate of glycogen resynthesis over the next 4 h was similar between the genders (24). Finally, in the study by Spriet and colleagues (25), the carbohydrate intake on the high carbohydrate diet was 10.1 g·kg FFM−1·day−1, and the women did increase muscle glycogen by 13% compared with their habitual intake, although this increase was less than that reported previously for men (3, 11, 20).

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A second hypothesis that could explain the apparent inability of women to carbohydrate load is that there are gender differences in the muscle enzymatic and/or transport capacity for glycogen storage. One candidate enzyme is hexokinase, because the mRNA for this enzyme has been shown to increase significantly after insulin infusion in humans (14) and is reported to be higher in untrained to moderately trained men compared with women (8, 21). Work in animals did not show an effect of estradiol on GLUT-4 content (9). Furthermore, if GLUT-4 migration to the sarcolemma were differentially affected between the genders, we would not have found similar rates of glycogen resynthesis during the first 4 h after exercise in an earlier study (24). Thus we do not feel that glucose transport can explain a potential gender difference in glycogen synthesis.

More recently, there has been a renewed interest in the characterization of the two forms of muscle glycogen, termed pro- and macroglycogen (1, 2). Proglycogen has a smaller mass compared with macroglycogen, and the latter is the main portion that increases in response to the consumption of a high carbohydrate intake (2). To date, studies have not examined whether gender differences exist in the proportion of these two forms of glycogen, nor whether there is a gender-specific response in each of the pools in response to dietary carbohydrate loading.

The purpose of the current study was to examine whether the provision of extra energy, in conjunction with additional absolute carbohydrate, would allow women to increase muscle glycogen concentration by a similar amount compared with men in response to a modified dietary carbohydrate-loading protocol. Secondary objectives were to determine whether hexokinase activity and/or differences in the proportion of pro- and macroglycogen could explain the previously described gender differences in dietary carbohydrate loading.

METHODS

Subjects

Seven female and six 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. Men were selected on the basis of a training history of consistent participation in endurance-type physical activity for at least 1 yr (minimum of 5 days/wk and 45 min/session) and peak oxygen consumption (VO2 peak) of at least 55 ml·kg−1·min−1. The women met the same training criteria and had a VO2 peak of at least 45 ml·kg−1·min−1.

All female subjects were eumenorrheic with a normal cycle length. Four of the women were taking triphasic-type oral contraceptives. The women were tested during the midfollicular phase of their menstrual cycle (Hab, day 6.2 ± 3; CHO, day 8.2 ± 5.3; CHO + E, day 6.8 ± 4; see below for definition of groups); during the midfollicular phase of the menstrual cycle, we have not found significant differences in the plasma 17-β-estradiol concentration between women taking oral contraceptives and those not (Carter and Tarnopolsky, unpublished observations). VO2 peak was determined within 2 wk of the first trial by using a cycle ergometer and a computerized open-circuit gas collection system as previously described (23). VO2 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 despite vigorous encouragement and the RER was >1.12. Total body fat mass and FFM were determined by using dual-energy X-ray absorptiometry (QDR 1000W, Hologic, Waltham, MA) in the late afternoon, a minimum of 4 h after the VO2 peak test and after ad libitum rehydration. Each subject completed prospective diet records for three weekdays and one weekend day during this 2-wk period. Subjects were given detailed instructions about diet recording and used measuring cups during this period. The composition and energy content of each subject’s diet was then analyzed using a computer-based nutrient analysis program (Nutritionist V, N-Squared Computing, San Bruno, CA) (Table 1).

Design

Each of the subjects completed three 5-day trials in a randomized, double-blinded fashion. On the basis of each individual’s dietary analyses, diets were packaged and provided to each subject at the beginning of each 5-day trial: Hab, habitual intake; CHO, isonenergetic, but 75% of energy was from carbohydrates; and CHO + E, carbohydrate proportion at 75% plus ~34% additional energy (the difference between men’s and women’s habitual energy intake). Subjects were instructed to adhere strictly to each diet and were permitted to consume water ad libitum. Each participant was to return any uneaten food and recorded daily consumption. Compliance was >96%; however, all of the women complained that they were very full on the CHO + E diet and correctly identified this diet. The dietary characteristics are given in Table 2.

On the day before the first day of an experimental trial, subjects cycled for 60 min at 65% of their VO2 peak. After the 60-min ride, they performed 2-min intervals at 85% of their VO2 peak five times to further deplete muscle glycogen concentration. They then cycled for 60, 45, and 30 min at 65% VO2 peak on days 1, 2, and 3 of the 5-day trial, respectively. Day 4 was a rest day. On day 5, the subjects consumed breakfast and lunch from the prepackaged diet and then arrived for muscle biopsy 4 h after the last meal (late afternoon). The muscle biopsy was taken from the vastus lateralis by using suction modification and 2% lidocaine without epinephrine. The sample was immediately dissected free of any fat or connective tissue. The biopsy was immediately frozen in liquid nitrogen, weighed, and stored at −80°C until analysis.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Men</th>
<th>Women</th>
<th>Significance (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>74.1 ± 10.6</td>
<td>59.4 ± 4.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>66.3 ± 9.0</td>
<td>46.1 ± 2.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9.2 ± 1.8</td>
<td>20.9 ± 2.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.3 ± 7.4</td>
<td>163.5 ± 4.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age, yr</td>
<td>22.8 ± 4.7</td>
<td>21.7 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>VO2peak, ml·kg−1·min−1</td>
<td>62.8 ± 5.4</td>
<td>53.3 ± 6.4</td>
<td>NS</td>
</tr>
<tr>
<td>VO2peak, ml·kg FFM−1·min−1</td>
<td>70.0 ± 4.6</td>
<td>68.6 ± 8.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. FFM, fat-free mass; VO2peak, peak O2 uptake; NS, not significant.
buffer, 100 μL of HCl and incubation at 95°C for 3 min to stop the reaction. This reaction was allowed to proceed for 1 h at room temperature until addition of HCl and incubation at 95°C for 3 min to stop the reaction. After the reaction was stopped, 1 ml of 50 mM imidazole buffer, 100 μM NADP⁺, 30 mM ammonium acetate, 5 mM MgCl₂, 1 mM EDTA, and phosphogluconate dehydrogenase (0.1 U/ml) were added and allowed to stand at room temperature for 15 min. HK activity was determined by the amount of NADPH fluorescence measured against standards of glucose-6-phosphate (3–10 mM). Protein concentration of the homogenate was also determined by using a Bradford method (Biorad, Hercules, CA). Values were expressed as moles per kilogram protein per hour.

**Pro- and macroglycogen.** The other piece of muscle was lyophilized overnight and dissected free of visible blood and connective tissue. Approximately 2–3 mg were extracted into perchloric acid, and pro- and macroglycogen were determined by using an enzymatic method as previously described (1). Total glycogen was taken as the sum of proglycogen + macroglycogen and was reported as millimoles glucosyl units per kilogram dry mass.

**Statistical Analysis**

An independent t-test was used to compare the physical characteristics of the subjects. The glycogen, diet analysis, and hexokinase activity were analyzed by using a two-way ANOVA (between variable = men and women; within variable = 3 diets). Given that our a priori hypothesis was that men and women would have differing glycogen responses (23), we also used a one-way ANOVA to analyze the glycogen data. A Newman-Keuls post hoc test was used to locate pairwise differences. A probability of \( P < 0.05 \) was taken to indicate significance. Correlations between hexokinase activity and glycogen concentration were performed by using Pearson product-moment correlation analysis. All data were analyzed using a computerized statistical program (Statistica, V. 5.1, Statsoft, Tulsa, OK). All data in tables and figure are means ± SD.

**RESULTS**

**Diet Analysis**

Women consumed less energy than men when expressed as total energy intake \(( P < 0.01)\) and per kilogram of body weight \(( P < 0.05)\), yet values were similar when expressed relative to FFM. By design, both men and women consumed ~34% more energy on the CHO + E diet compared with either Hab or CHO \(( P < 0.001)\). Also by design, the percent of energy derived from protein and fat was lower and from carbohydrates was higher on the CHO and CHO + E diets compared with Hab \(( P < 0.01)\). Carbohydrate intake expressed per kilogram was lower during each trial for women compared with men \(( P < 0.05)\), yet there were no gender differences when expressed relative to lean mass. As expected, carbohydrate intake was higher for both men and women on the CHO vs. Hab diet and the CHO + E vs. both the CHO and Hab diets \(( P < 0.01 \) and 0.001, respectively). A summary of the diet analysis is found in Table 2.

**Hexokinase Activity**

There were no significant differences between genders or within diet conditions for hexokinase activity (Table 3). Furthermore, there was no correlation between hexokinase activity and muscle glycogen concentration (not significant).

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**Table 2. Diet analysis**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Energy kcal/day</th>
<th>%EN</th>
<th>Carbohydrate g·kg⁻¹·day⁻¹</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g·kg⁻¹·day⁻¹</td>
<td>g·kg FFM⁻¹·day⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hab</td>
<td>3,039 ± 954</td>
<td>15 ± 3</td>
<td>58 ± 5</td>
</tr>
<tr>
<td>Men</td>
<td>2,000 ± 487a</td>
<td>14 ± 2</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>Women</td>
<td>3,042 ± 973</td>
<td>15 ± 1d</td>
<td>75 ± 1e</td>
</tr>
<tr>
<td>CHO</td>
<td>1,993 ± 512b</td>
<td>15 ± 2e</td>
<td>75 ± 1e</td>
</tr>
<tr>
<td>Men</td>
<td>4,038 ± 1,206</td>
<td>14 ± 1d</td>
<td>75 ± 1e</td>
</tr>
<tr>
<td>Women</td>
<td>2,711 ± 691c</td>
<td>15 ± 2e</td>
<td>75 ± 1e</td>
</tr>
</tbody>
</table>

Values are means ± SD. Hab, habitual diet; CHO, high-carbohydrate diet; CHO + E, extra energy + CHO diet; %EN, percent energy intake. *Women lower than men \(( P < 0.01)\); **women lower than men \(( P < 0.05)\); †higher for CHO + E compared to CHO and Hab \(( P < 0.01)\); ‡CHO and CHO + E lower than Hab \(( P < 0.01)\); *CHO and CHO + E higher than Hab \(( P < 0.01)\); ‡CHO higher than Hab \(( P < 0.01)\); *CHO + E higher than CHO and Hab \(( P < 0.1 \) and 0.001, respectively).

**Table 3. Hexokinase activity**

<table>
<thead>
<tr>
<th></th>
<th>Hexokinase activity, mol·kg⁻¹·protein⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hab</td>
</tr>
<tr>
<td>Men</td>
<td>0.46 ± 0.09</td>
</tr>
<tr>
<td>Women</td>
<td>0.39 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SD.
Glycogen

For total glycogen, there was a significant main effect for diet ($P < 0.05$) with the CHO and CHO + E diets showing higher concentrations compared with Hab. Post hoc analysis showed that the delta values for men were above the critical value ($P < 0.05$) for an increase from Hab to CHO ($537.2 \pm 78.6 \rightarrow 660.2 \pm 120.1$) and Hab to CHO + E ($537.2 \pm 78.6 \rightarrow 741.1 \pm 187.7$), whereas the women did not significantly increase muscle glycogen from Hab to CHO ($629.1 \pm 231.1 \rightarrow 650.6 \pm 187.9$; not significant), yet they did increase from Hab to CHO + E ($629.1 \pm 231.1 \rightarrow 737.7 \pm 198.1$) (all values are mmol glucosyl units $\cdot kg$ dry mass $^{-1}$). When a one-way repeated-measures ANOVA was used for each of the men and women, the results were identical. Macroglycogen was higher for the CHO + E compared with the Hab trial for men only ($P < 0.05$). There were no gender effects for proglycogen and macroglycogen measurements (Fig. 1).

DISCUSSION

These results confirm an earlier report that concluded that women did not increase muscle glycogen stores in response to an increase in the dietary proportion of carbohydrates from $58$ to $75\%$ of energy intake, whereas men did show an increase (23). The novel finding was that the women were able to increase their muscle glycogen stores by a magnitude that was similar to that seen for men in response to a higher energy and carbohydrate intake.

We have previously reported that women did not increase their muscle glycogen concentration in response to an increase in dietary carbohydrate intake from $58$ to $74\%$ of energy intake, yet men showed a significant increase (23). One of the proposed mechanisms was that the carbohydrate intake was too low for the women to supracompensate glycogen stores because of their low energy intake (23). In that study, we demonstrated that the carbohydrate intake for the women on the high-carbohydrate diet ($75\%$ of energy intake) was $7.9 \, g \, carbohydrate \cdot kg \, FFM^{-1} \cdot day^{-1}$, which was similar to the male intake of $7.7 \, g \, carbohydrate \cdot kg \, FFM^{-1} \cdot day^{-1}$ on the habitual carbohydrate diet ($58\%$ carbohydrate) (23). Our results contrast slightly with those of Spriet and colleagues (25) in that they found that women did increase muscle glycogen storage in response to an increase in dietary carbohydrate intake. However, there are important differences between the studies that could explain these inconsistencies. First, the low-carbohydrate diet in their study provided $78\%$ of the energy from carbohydrates whereas the high-carbohydrate diet provided $78\%$ of energy from carbohydrates (25), compared with our values of $58$ and $75\%$, respectively. Second, in their study the women were tested in the luteal phase of the menstrual cycle (25), when the rate of glycogen resynthesis is greater compared with the follicular phase (16). We specifically tested each of our women in the midfollicular phase of the menstrual cycle to control for the potential variance introduced by the differential rates of glycogen storage seen between the phases (16).

In the current study, women achieved a carbohydrate intake above $8.0 \, g \cdot kg \, FFM^{-1} \cdot day^{-1}$ on the CHO diet due to an unexpectedly high percent body fat for the women (hence, a lower FFM). In spite of this, the women did not show an increase in their muscle glycogen stores in response to an increase in the percent of

Fig. 1. A: total muscle glycogen in response to the habitual diet (Hab), high carbohydrate diet (CHO), and the CHO + extra-energy diet (CHO + E) (see text for further definitions). *Significant increase compared with Hab diet ($P < 0.05$). B: macroglycogen concentration in response to dietary modification. *Significant increase compared with Hab diet ($P < 0.05$). C: proglycogen concentration in response to dietary modification. Units are millimole glucosyl units per kilogram dry mass ($dm$) for A, B, and C.
dietary carbohydrate, yet the men did show an increase. This may also relate to the fact that the women only increased their carbohydrate intake relative to total mass to 6.4 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\) on the 75% energy diet, whereas the men were at 7.9 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\). Many of the previously reported studies of carbohydrate supracompensation have used male subjects and a carbohydrate intake of >8.0 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\) (3, 11, 20). Therefore, our results are in agreement with review articles stating that a carbohydrate intake of 8.0–10.0 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\) is required to carbohydrate load (4). For a female athlete consuming 2,000 kcal/day, a carbohydrate intake of 8.0–10.0 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\) would equate to ~93–120% of habitual energy intake, assuming a body mass of 60 kg. Clearly, it would be impractical (and impossible for the higher intake) for an athlete to consume a diet with this proportion of carbohydrates. Therefore, the only practical method for a female athlete to glycogen supracompensate is to consume more energy. Given that an elite athlete would only have to perform this dietary intervention a few times per year, and that the total extra energy would amount to only 2,800 kcal over 4 days, it is a dietary strategy that is likely to be acceptable.

Another interesting finding in the current study was that the men increased their muscle glycogen stores with the extra energy intake. It is possible that they were just below a threshold for maximal supracompensation at 7.9 g carbohydrate·kg\(^{-1}\)·day\(^{-1}\) and the increase in energy allowed their carbohydrate intake to be optimized. A limitation of the current study is the absence of performance data; however, now that we have established that women can increase muscle glycogen, future studies should use carefully controlled and well-powered studies to demonstrate whether the expected increase in performance is realized from this increase. In an earlier gender-comparative study, our laboratory found that exercise performance was correlated with the increased muscle glycogen content for both men and women (women did not load or increase endurance time, whereas men increased glycogen concentration by 41% and performance by 45%) (23). Another study did find an increase in endurance exercise performance of 8.5% and an increase in muscle glycogen of 13% in women after a carbohydrate-loading protocol (25). Together, these studies provide strong evidence that, if women can increase their muscle glycogen using the recommendations contained in this paper, their endurance exercise capacity should be enhanced (23, 25).

In addition to the current results with carbohydrate loading, we have previously demonstrated that men and women show a similar increase in muscle glycogen after endurance exercise (first 4 h) when given a diet supplying 1.0 g carbohydrate·kg\(^{-1}\) immediately and at 1 h postexercise (24). These results provided evidence that, at least in the early postexercise period, there were no gender differences in the rate of glycogen resynthesis when the carbohydrate provision was expressed relative to body weight (24). From a practical standpoint, the current study and our previous one (24) provide strong evidence that nutritional recommendations for athletes need to consider habitual energy intake and to express carbohydrate intake as grams carbohydrate per kilogram per day or grams carbohydrate per kilogram FFM per day (4).

Two groups have reported that muscle hexokinase activity was higher in men compared with women (8, 21). There is some evidence that hexokinase activity may be a factor in determining muscle glycogen concentration (14). Insulin, which increases in response to an increase in dietary carbohydrate, increases the mRNA for hexokinase but not glycogen synthase (14). For these reasons, we measured hexokinase activity to see whether gender differences could explain the apparent inability of women to carbohydrate load. The results of the current study suggested that gender differences in absolute and relative carbohydrate intake can explain the apparent inability of women to carbohydrate load and that hexokinase activity per se is not a major determinant of this phenomenon. Our results also show that hexokinase activity is not different between well-trained men and women (7). Although it is possible that there are gender differences in other enzymes (i.e., glycogen synthase) or transporters (i.e., GLUT-4) important in glycogen synthesis, the fact that merely increasing energy intake (and hence absolute and relative carbohydrate intake) allowed for similar rates of glycogen supracompensation would mitigate against this hypothesis. With respect to GLUT-4, there is one report that found that complete ovarian hormone deficiency did not alter GLUT-4 protein content (9). Although indirect, the fact that the rate of glycogen resynthesis was the same for men and women in the first 4 h after endurance exercise (when changes in GLUT-4 would be most likely to influence glycogen resynthesis rates) after various nutritional supplements (24) provided evidence that insulin and contraction stimulated GLUT-4 sarcolemmal migration was not likely to be different between the genders.

In addition to the practical and mechanistic observations mentioned above, we have also shown that there are no inherent gender differences in basal protein and macroglycogen concentration in human skeletal muscle. However, we did demonstrate that macroglycogen increased on the carbohydrate + energy diet for the men only. This increase in response to a higher carbohydrate intake is similar to the conclusions based on observations in another study using only men (2) but does indicate that there may be subtle gender differences that should be considered in future research. A recent study also measured glycogenin concentration in human skeletal muscle and found a strong correlation between glycogenin activity and glycogen resynthesis rates (19). Although we found that energy intake/absolute carbohydrate intake is the major determinant of the ability to carbohydrate load, potential gender differences in glycogenin activity could have subtle gender-specific effects on muscle glycogen concentration and should be examined in future studies.
Overall, these findings provide support for the hypothesis that the previously reported gender difference in carbohydrate loading was due to gender differences in absolute and relative carbohydrate intakes. Therefore it is unlikely that gender differences in either enzymatic capacity and/or glucose transport could explain the earlier observations. Future studies should test the potential performance benefits from the dietary carbohydrate and energy manipulation proposed in this paper.

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REFERENCES


