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<th><strong>Journal:</strong></th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
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<td><strong>Manuscript ID:</strong></td>
<td>apnm-2017-0777.R4</td>
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<tr>
<td><strong>Manuscript Type:</strong></td>
<td>Article</td>
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<tr>
<td><strong>Date Submitted by the Author:</strong></td>
<td>26-Jun-2018</td>
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<tr>
<td><strong>Complete List of Authors:</strong></td>
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<td><strong>Keyword:</strong></td>
<td>carbohydrate electrolyte beverage, endurance, performance, sports drink, women</td>
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<td><strong>Is the invited manuscript for consideration in a Special Issue?</strong></td>
<td>Not applicable (regular submission)</td>
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https://mc06.manuscriptcentral.com/apnm-pubs
The Effect of Carbohydrate Beverage Ingestion on Central versus Peripheral Fatigue: A Placebo-Controlled, Randomized Trial in Cyclists

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ABSTRACT

Purpose: We investigated whether carbohydrate ingestion delays fatigue in endurance-trained cyclists via peripheral or central mechanisms.

Methods: Ten men [35±9 yrs] and ten women [42±7 yrs] were assigned, in a double-blind, cross-over design to a sports drink [CHO] and placebo [PL]. Strength measures were made 3 times [pre-exercise, post-time trial and post-ride to exhaustion]: 1) Maximal voluntary contraction [MVC]; 2) MVC with superimposed femoral nerve magnetic stimulation to measure central activation ratio [CAR]; 3) femoral nerve stimulation in a 3-second pulse train on relaxed muscle. Subjects cycled 2 hours at ~65% of peak oxygen consumption [VO_{2peak}], with 5 1-minute sprints interspersed, followed by a 3-km time trial. After strength testing, cyclists remounted their bikes, performed a brief warmup, and pedaled at ~85% VO_{2peak} until unable to maintain workload. Changes in metabolic and strength measurements were analyzed with repeated measures ANOVA.

Results: From pre-exercise to post-time trial: MVC declined in men (17%) and women (18%) [p=0.004] with no effect of beverage [p>0.193]; CAR decreased in both sexes with PL [P=0.009], the decline was attenuated by CHO in men only [time×treatment, p=0.022]; there was no evidence of peripheral fatigue in either sex with either beverage [p>0.122]. Men rode faster in the time trial with CHO [p=0.005] but did not improve performance in the ride to exhaustion [p=0.080]. In women, CHO did not improve performance in the time trial [p=0.173], or in the ride to exhaustion [p=0.930].

Conclusion: Carbohydrate ingestion preserved central activation and performance in men, but not women, during long-duration cycling.

Key Words: carbohydrate-electrolyte beverage, endurance, performance, sports drink, women,
**Introduction**

Fatigue can be described as an inability to maintain force production despite increased effort (St Clair Gibson and Noakes 2004). The traditional theory of exercise-induced fatigue held that fatigue is based in the peripheral muscle (Hill and Lupton 1923). However, exercise performance is also regulated by the central nervous system [CNS] (Noakes et al. 2005) and a loss of drive from the CNS is known as central fatigue. Since fatigue is task-dependent (Millet and Lepers 2004, Hunter 2009), findings from studies which examine types of fatigue [central vs. peripheral] by employing short-duration contractions of specific muscle groups (Hunter et al. 2006, Yoon et al. 2007b) may not be applicable to dynamic contractions performed over a long period of time such as those experienced during endurance running or cycling. For example, Thomas et al. (2016b) demonstrated that peripheral fatigue was greater after exercise protocols which were performed at higher intensities.

Fatigue induced by short-duration exercise has been shown by some to result in greater peripheral fatigue in men compared to women (Russ et al. 2005), while others have found central fatigue to be greater in men (Martin and Rattey 2007). By contrast, fatigue induced by continuous prolonged cycling in our lab has been shown to be largely central in both sexes (Kremenic et al. 2009, Glace et al. 2013). Others (e.g., Jubeau et al. 2014) have additionally found a significant peripheral contribution to fatigue in long-duration cycling. Interestingly, women athletes may experience less peripheral fatigue than men after prolonged exercise (Glace et al. 2013, Temesi et al. 2015). These differences between sexes may be due to metabolic or mechanical factors (Hunter and Enoka 2001, Kent-Braun et al. 2002, Larivière et al. 2006, Yoon et al. 2007b).
It is well known that there is a beneficial effect of ingested carbohydrates during prolonged exercise (Thomas et al. 2016a). Carbohydrates may permit the exerciser to maintain a given intensity for a longer period of time (Hulston and Jeukendrup 2009). It had generally been accepted that carbohydrates serve as substrate for the peripheral muscle (Coyle et al. 1986, Tsintzas et al. 1995), but they may also alter central mechanisms by serving as substrate for the CNS, as shown in rats (Matsui et al. 2012). The effect of glucose on CNS function may be mediated by either blood glucose, as hypoglycemia results in disrupted electroencephalogram activity (Jensen et al. 2014), or from decreased glycogen levels. In a rodent model, Matsui et al. (2011) demonstrated significant reductions of brain glycogen levels after 2 hours of running. Levels of stored carbohydrate in the muscle may also provide feedback to the CNS to prevent catastrophic muscle glycogen depletion (Noakes et al. 2004). An additional mechanism for decrements in central activation after exercise may be a substrate-mediated increase in serotonin. As blood glucose concentration falls and fats are increasingly oxidized, serotonin levels increase (Davis et al. 1992), resulting in lethargy and fatigue (Meeusen and De Meirleir 1995).

While it is known that carbohydrate feeding during prolonged exercise can prevent hypoglycemia and improve performance and endurance capacity compared to a placebo (Jeukendrup 2014), research is lacking which specifically identifies which component, central or peripheral, is altered by carbohydrate ingestion or whether both are, in each of the sexes.

Thus, the primary purpose of this study was to compare the effect of carbohydrate ingestion on central and peripheral fatigue after prolonged cycling. Temesi et al. (2015) have stressed the importance of examining sex differences in studies of fatigue. Since similar numbers of women and men participate in endurance sports, ("Demographics: TeamUSA Triathlon" n.d., “Statistics | Running USA” n.d.) examining differences in how carbohydrates
affect fatigue during prolonged exercise in both sexes is especially warranted. Therefore, our secondary purpose was to examine whether the effects of carbohydrate ingestion on central versus peripheral fatigue differed between male and female cyclists. We hypothesized that a] strength loss after prolonged cycling (in this case, strength measured following 2 hours of cycling at the athlete’s ventilatory threshold plus a 3-km time trial, and then after a subsequent ride to exhaustion; see Methods below for details) would be mitigated by carbohydrate, primarily via central mechanisms, and b] prolonged cycling would result in a greater change in maximal voluntary contraction [MVC] and in peripheral fatigue in men than in women.

Materials and Methods

Ten healthy men and 10 healthy women, 35±9 and 42±7 years [p=0.080], with body masses of 78.6±10.1 and 60.0±6.6 kg, for men and women, respectively, were recruited from local triathlon and cycling clubs. It was explained to the subjects that they would be comparing 2 commercially available electrolyte-containing sports drinks; they were unaware that one was a placebo. Inclusion criteria required that they: cycled ≥80 km/week for at least the previous 3 months, were currently competing in cycling events, were 18-50 years of age, and that women were premenopausal, by self-report. Informed consent to participate in the study, which had been approved by the Institutional Review Board, was obtained from cyclists who met inclusion criteria.

Protocol Overview

Subjects reported to the lab on three separate days. On the first day they were familiarized with testing protocols and performed a test to determine peak oxygen consumption [VO2peak]. On their second and third days of testing, subjects were assigned to either a placebo beverage [PL] or to a carbohydrate-containing beverage [CHO], in a pseudo-randomized,
double-blind, cross-over design. Subjects cycled as follows: 2 hours at their ventilatory threshold [VT] with interspersed higher-intensity intervals, immediately followed by a 3-km time trial [TT], thus simulating races on varying terrain and with a push during the final kilometers. Cyclists then completed a ride to exhaustion at their respiratory compensation threshold [RCT]. Distance completed during this final effort differed between individuals, and resulted in fatigue of substantial severity, which limited their ability to continue at the chosen intensity. Measures of strength were obtained pre-ride, post time-trial and post-ride to exhaustion. Exact protocols for each day are described in detail, below.

**Maximal Testing and Familiarization**

A ramp test was performed to determine $VO_{2peak}$ and ventilatory thresholds on a cycle ergometer [Ergoselect, Ergoline, Bitz, Germany]. Workload was increased each minute by 15-30 watts, depending upon the cyclist’s ability. Wattages were selected in order that cyclists reach their maximal workload within 8-10 minutes (Yoon et al. 2007a). Cyclists were verbally encouraged to continue pedaling for as long as possible. Oxygen consumption and heart rate were measured throughout the test [TrueOne Metabolic System, Parvo Medics, Sandy, UT, USA]. Gas exchange measurements were made using the breath-by-breath mode, and data were averaged every 20 seconds. VT and RCT were determined by visual inspection of the graphs of ventilatory equivalents for oxygen [$Ve/VO_2$] and carbon dioxide [$Ve/VCO_2$] according to the methods described by Santos and Giannella-Neto (2004). The highest $VO_2$ achieved during the ramp protocol was considered the $VO_{2peak}$. Volunteers were then familiarized with the cycling protocol and with the strength testing and magnetic stimulation protocol.

Cyclists were sent home with a breakfast consisting of a liquid meal replacement [Ensure™] calculated to provide 6 kcal per kg body mass, to be consumed on testing days, and
were given food records on which to record their food intake on the days preceding the test sessions. Subjects were asked to make sure their diets were easily replicable and to try to keep food as similar as possible the day prior to each experimental session.

*Exercise with Placebo or Sports Drink*

Testing was scheduled for 2 days, 7-30 days apart. See the time line in Figure 1. A staff member, who was not involved with testing of subjects, prepared the CHO or PL beverages each day. The primary investigators were blinded to the treatment assignments. One hour before their 9 a.m. arrival at the laboratory, subjects consumed the Ensure™ previously provided, and 1% of body weight in water. Immediately upon arrival at the laboratory, subjects were asked to void and body mass was recorded. Blood glucose was determined via finger stick [FreeStyle Lite, Abbott Diabetes Care Inc., Alameda, CA, USA] pre-exercise, after 1 hour of cycling, and within 1 minute of cessation of exercise after the TT, and the ride to exhaustion, while still seated on their bikes.

*Quadriceps Strength Testing*

Isometric quadriceps strength was measured at 60° knee flexion, with subjects seated in a semi-reclined position [trunk extended 40° beyond upright position to facilitate femoral nerve stimulation]. A crossover shoulder strap and a belt around the abdomen were used to secure the subject to the chair. The right ankle was attached to a chain that was attached a force transducer [Kistler, Inc., Amherst, NY, USA]. Subjects then performed two maximal 5-second voluntary contractions [MVC] in order to determine voluntary quadriceps strength. Following this, the femoral nerve was stimulated for two 3-second contractions. Finally, the subjects performed two voluntary 5-second isometric contractions with a 3-second femoral nerve stimulation superimposed 2 seconds after the initiation of the voluntary contractions. All contractions were
performed one minute apart. Femoral nerve stimulations were performed using a magnetic stimulating device [MagStim Rapid Stimulator, MagStim Corp, Wales, UK]. Peripheral magnetic stimulation [PMS] was performed at a frequency of 40 Hz with intensity of the stimulus set at 100% of the output of the unit, duplicating a previously used protocol on which we have reported (Kremenic et al. 2009, Glace et al. 2013). A double-circular 90-mm [inside diameter] coil was used to deliver the stimulation to the femoral nerve slightly distal to the anterior-superior iliac crest. Before administration of the pulse trains, optimal location of the stimulating coil over the femoral nerve was determined by identifying the position giving the greatest twitch response to stimulation with single pulses. Central activation ratio [CAR] was measured using the contractions with PMS superimposed to identify central sources of fatigue (Kent-Braun and Le Blanc 1996). CAR was defined as the ratio of maximal volitional force generation to maximal volitional effort with PMS superimposed with values <1.0 indicating incomplete activation. During all contractions force was recorded continuously at 1000 Hz into a computer using BTS EMGAnalzyer [BTS Bioengineering, Brooklyn, NY, USA].

Cycling Protocol

After strength testing, subjects’ own bicycles were set on a cycle trainer [CompuTrainer Lab; RacerMate, Seattle, WA, USA], and they cycled for two hours at the workload which elicited their VT [216±38 vs. 118±32 watts, for men and women, respectively]. Throughout cycling either an artificially sweetened, non-caloric, electrolyte beverage [“PL”, 4C Totally Light2Go, 4C Foods Corp., Brooklyn, NY, USA] or a commercially available sports drink [“CHO”, Gatorade, PepsiCo, Purchase, NY, USA] was provided at a rate of 1% of body weight each hour. Both products were “Fruit Punch” flavored. No subjects were aware that one product was artificially sweetened. CHO supplied 59 grams of sugar/L, 109 mg/L of potassium, and 423
mg/L of sodium. The PL contained no sugar, and 20 mg/L and 30 mg/L of sodium and potassium, respectively.

Respiratory gas measurements were made every 20 minutes, and workload was adjusted to maintain oxygen consumption at VT in the first session. The same workloads were used in the subsequent session. Immediately following each gas measurement workload was increased for one minute to that which elicited RCT. Five 1-minute RCT-intervals were performed during the 2-hour ride. The 6-20 Borg scale was used to assess ratings of perceived exertion [RPE] at each gas measurement and during each RCT-interval (Borg 1982). Heart rate was recorded continuously [Polar CIC, Port Washington, NY, USA]. After cycling for two hours subjects performed a 3-km TT with instruction to pace themselves to ride the distance as quickly as possible. Feedback about distance covered was given every 0.5 km.

Following the completion of the TT, strength testing with magnetic stimulation was repeated. Subjects were taken directly from the bike to the dynamometer, and strength testing began within five minutes following cessation of exercise. After the post-TT strength tests the subjects immediately re-mounted their bicycles and, after a 0.5 km low-intensity warm-up, cycled at the RCT workload until it could no longer be maintained. Cyclists were verbally encouraged to ride as long as possible. Strength testing was repeated immediately upon cessation of the ride, with measurements again beginning within five minutes.

Statistical Analysis

Effect of carbohydrate beverage on strength, CAR, and other dependent variables was assessed by Time by Treatment repeated measures analysis of variance [ANOVA]. Sex was used as a between subjects factor in the ANOVA for all measurements except those related to strength since men were twice as strong as women. Normality of the data was verified using
Shapiro-Wilk test. All ANOVA were checked for violation of the sphericity condition and Greenhouse-Geisser corrections applied if appropriate. Pairwise difference between carbohydrate and placebo treatments were assessed using planned Bonferroni corrections. Differences in performance between beverages in cycling trials were analyzed using paired t-tests. An alpha level 0.050 was used as the threshold for statistical significance and all statistical calculations were performed using IBM SPSS Statistics Version 21 [International Business Machines, Armonk, NY, USA] Results in the text are presented as mean±standard deviation; graphs are presented with standard errors. Previous testing in our lab has shown that we can detect a voluntary force decrement of 24% and a change in CAR of 0.09 with 80% power [GraphPad StatMate Version 2.0, GraphPad Software Inc., La Jolla, CA, USA] using 10 subjects with magnetic stimulation of the femoral nerve (Glace et al. 2013).

**Results**

The mean VO$_{2\text{peak}}$ for men was 60.3±7.9 ml/kg/min vs. 46.5±7.9 ml/kg/min for women [p=0.001]. Determined during their maximal tests, VT occurred at 64.2±4.3% and RCT at 83.8±5.5%, of peak oxygen consumption.

Analysis of the diets from the days prior to testing showed no significant differences in kcals/kg body mass [effect of: treatment, p=0.720; treatment×sex, p=0.239; sex, p=0.399] nor carbohydrate/kg [effect of: treatment, p=0.489; treatment×sex, p=0.858; sex, p=0.639] for CHO or PL in men or in women. On the mornings of the beverage trials subjects drank Ensure™ for breakfast. Average energy provided to men was 472±174 kcal and to women was 360±126 kcal. During the ride, men drank a mean of 790±101 ml/hour of the randomized beverage, and women drank 603±66 ml/hour. This resulted in a decrease in body mass of approximately 1 kg during
both treatments, with ANOVA revealing an effect of time \( p<0.001 \) but no differences for time×treatment \( p=0.912 \), time×sex \( p=0.923 \) nor time×treatment×sex \( p=0.660 \). The change in body mass expressed as a percent of initial mass \(<2\%\) was not different between men and women \( p=0.539 \).

**Strength Measurements**

Sample force curves used to calculate central activation ratio for an actual subject, demonstrating changes in force from pre-exercise- to post-2 hour ride/TT and post-TT to exhaustion, are shown in Figure 2, for both PL and CHO treatments. Data for strength measures and CAR are shown in Table 1. From pre-exercise to post-2-hour ride/TT, men and women lost voluntary strength \( \text{time: men, } p=0.001; \text{ women, } p=0.004 \), regardless of the beverage ingested \( \text{time×treatment: men, } p=0.193; \text{ women, } p=0.214 \): men lost 17% [from 582±73 N to 489±95 N] and women lost 18% [from 270±117 N to 222±92 N] of voluntary strength. Peripheral fatigue, defined by loss of PMS-induced strength, was seen in neither men nor women \( \text{men, } 628±168 \text{ N pre; 650±120 post; women, } 290±148 \text{ N pre; 320±145 N post} \) at the end of the 2-hour ride/TT with either beverage [effect of time: \( \text{men, } p=0.917; \text{ women, } p=0.451 \); treatment: \( \text{men, } p=0.740; \text{ women, } p=0.772 \); time×treatment: \( \text{men, } p=0.972; \text{ women, } p=0.888 \)]. Both men and women showed a decrease in CAR at the end of the 2-hour ride/TT regardless of treatment [time: \( \text{men, } p=0.005; \text{ women, } p=0.001 \)]. Men, but not women, demonstrated an attenuation in this decrease in CAR with CHO ingestion [time×treatment: \( \text{men, } p=0.022; \text{ women, } p=0.802 \)].

After completing the ride to exhaustion, there were no further decreases in voluntary strength [men at exhaustion: 455±98 N, \( p=0.561 \); women at exhaustion: 223±104 N, \( p=0.757 \)], PMS-induced strength, our measure of peripheral fatigue, [men at exhaustion: 667±140 N, \( p=0.001 \); women at exhaustion: 464±75 N, \( p=0.004 \)].
p=0.562; women at exhaustion: 300±123 N, p=0.122] or CAR [men, p=0.566; women, p=0.717].

CAR was depressed similarly at end exercise in both treatments and in both sexes.

**Metabolic Measurements**

Oxygen consumption measures verified that exercise intensity during the two-hour ride was not different between treatments: there was no effect of time [p=0.664] or treatment [p=0.831], nor any interaction [p=0.374]. There was, however, an effect of treatment on substrate used. As expected, during the 2-hour ride RER declined more with PL than with CHO [treatment×time, p<0.001] Figure 3. RER was not different overall between PL and CHO in either sex. Heart rate was higher with CHO throughout [treatment, p=0.015], but the increase in heart rate over time was not different between treatments or sexes [time×treatment×sex, p=0.249]. RPE was consistently lower with CHO treatment, and increased similarly in men and women, from 12±1 to 13±1 from beginning to end of the 2 hours of cycling with CHO, and from 12±1 to 14±1 with PL [time p≤ 0.001; treatment p=0.029; time×treatment×sex p=0.424]. Blood glucose was higher in the CHO trial for both men and women [treatment, p=0.005, time×treatment×sex, p=0.980]. The better maintenance of blood glucose concentration was especially apparent in women, with higher glucose concentrations at every measurement point [treatment×sex, p=0.017], Figure 4.

Work performed during the 1-minute sprints was standardized for each cyclist and RPE during the sprints did not differ between treatments [p=0.592]. However, heart rate was higher with CHO [p=0.007] during the sprints in both men and women [time×treatment×sex, p=0.880]. There was neither a difference between sexes [p=0.150] nor an interaction over time [p=0.720].

**Performance Measurements**
Three-km TT and ride to exhaustion performance are shown in Figure 5. Performance in the 3-km TT was improved with CHO for men: 5.55±1.39 min with CHO vs. 5.98±1.55 min with PL [p=0.005]. Women did not exhibit the same performance-enhancement with CHO: their time for the TT with CHO was not faster than with PL [6.93±1.39 min vs 7.51±1.55 min, p=0.173]. Men did not ride longer with CHO in the ride to exhaustion [12.2±15.8 min CHO vs 7.3±7.9 min PL, p=0.080] and their RPEs were similar with CHO and PL [18±6 vs 19±4, p=0.126]. Women did not ride for a longer time in the ride to exhaustion with CHO [18.6±10.0 min CHO vs. 18.8±11.7 min PL, p=0.930] nor did CHO reduce their RPEs [18±3, CHO vs. 18±3, PL, p=0.269]. Women rode longer than men in the ride to exhaustion, but due to high variability this difference was not statistically significant [women: 18.7±11.4 min; men: 9.8±10.7 min; p=0.090].

Discussion

Our hypothesis that CHO preserves performance by primarily affecting central mechanisms was supported by our findings in men. Interestingly, we found no evidence of peripheral fatigue after our cycling intervention and therefore cannot comment upon whether CHO attenuates peripheral fatigue. Therefore, our second hypothesis, that peripheral fatigue would be more affected in men than women, was not supported. In women, neither cycling performance nor central fatigue was affected by consumption of CHO.

Women are under-represented in research (Johnson et al. 2014) despite evidence that findings in males often cannot be applied to females (Martin and Rattey 2007). Most research which examine the role of any particular strategy to enhance endurance performance have been done solely in men (Devries 2016) and very few studies examining the effect of carbohydrate
beverages on exercise performance have included women (e.g., Jeukendrup et al. 1997, Davis et al. 1997, Welsh et al. 2002, Winnick et al. 2005). Similarly, most studies which have evaluated central fatigue have used men, despite evidence that women may be more fatigue-resistant (Glace et al. 1998, Hunter et al. 2006, Yoon et al. 2007b, Ansdell et al. 2017) and that they may be less responsive to nutritional interventions such as carbohydrate loading (Tarnopolsky et al. 2001).

**Fatigue**

Both sexes experienced fatigue, as defined by a loss in voluntary force during MVCs. Men and women fatigued similarly and the loss of volitional force was not moderated by carbohydrate in either sex. This similar loss of voluntary strength is consistent with findings in cyclists (Glace et al. 2013) but is in contrast to a study of runners, which indicated that women were more fatigue-resistant than men (Glace et al. 1998). Strength loss during MVC was apparent after the 2-hour ride and TT, with no significant further loss of strength after the ride to exhaustion. Consistent with the similar fatigue of knee extensors in men and women which we observed, Hunter (2009, 2014) has suggested that there may be no sex differences in the fatigability of knee extensor muscles after dynamic exercise.

Our proxy measure for peripheral fatigue was stimulated strength. We were able to activate the muscle well: magnetic stimulation-induced force was >80% of voluntary strength. We found no indication of peripheral fatigue with or without carbohydrate intake: neither sex lost peripheral contractile capability when magnetic stimulation-driven. It is possible that there was a lack of potentiation in this measurement prior to cycling. However, peripheral fatigue has been shown to decrease as exercise duration increases, and intensity decreases, during time-trial cycling with completion times ranging from 6 to 66 minutes (Thomas et al. 2015). That study
differed from ours methodologically in that they used a single twitch for the induced contraction rather than a pulse train. We feel that the pulse train should give a better indication of the force-generating capacity of the muscle, leading to a more sensitive measurement; this has previously been demonstrated for CAR (Kent-Braun and Le Blanc 1996). Single pulses were used in our study only to determine optimal location for stimulation. Using pulse trains results in very substantial force generation: in previous work (Glace et al. 2013) we demonstrated high force generation [near subject’s voluntary max] in cyclists. In this study we were able to, on average, produce greater force in our subjects with PMS than they could produce voluntarily. We feel that this demonstrates the superiority of the technique, in addition to cyclists’ lack of experience with exercise [maximal isometric] performed in an unfamiliar posture [seated in hip extension rather than flexion].

In the current study, peripheral fatigue was absent after exhausting exercise that continued for more than 130 minutes. The lack of peripheral fatigue in men was in contrast to the findings in a previous study which we conducted in which men experienced both peripheral and central fatigue after 2 hours of cycling plus a 3-km time trial (Glace et al. 2013). While the protocols were similar between the current study and that of Glace et al. (2013), the earlier study allowed cyclists to do repeated sprints at an intensity of their own choosing, with RPEs ranging from 16-17. This study prescribed a sprint intensity corresponding to cyclist’s RCT, with RPEs which ranged from 14-16. It is possible that the lower sprint efforts/workloads contributed to the lack of peripheral fatigue.

The fatigue which we observed could be explained in both sexes by losses in central drive. CAR decreased by 15-20% in men and women with placebo, similar to the percent loss in MVC force. Central mechanisms have been well-documented to be the primary cause of fatigue...
of the knee extensors during long-duration running in men (Millet et al. 2002) and have been found to be the primary explanation for fatigue in women cyclists (Glace et al. 2013). With CHO, men, but not women, maintained central activation through 2 hours of cycling and the 3-km TT, as seen by the smaller decrement in CAR with CHO versus PL. When carbohydrate is present in the mouth, brain regions believed to be involved in reward and motor control are stimulated, and this activation may explain carbohydrate’s ergogenic effects (Chambers et al. 2009). It is unknown if women differ from men in their responsiveness to sugars within the mouth. At the end of the ride to exhaustion, during which no carbohydrate was ingested, both sexes showed similar decrements in central drive, regardless of treatment. The similar CAR depression at end exercise, regardless of treatment or sex, suggests that all subjects cycled with maximal effort. Further, it appears that when central drive decreased to a critical level, exercise at the intensity we set was no longer possible, regardless of beverage intake or sex.

**Metabolic and Cycling Performance Measures**

It is generally accepted that women utilize fatty acids during exercise to a greater degree than do men (Tarnopolsky 2008). Had we found that women in this study were less dependent upon carbohydrate than the men, it may have explained why they were less responsive to potential ergogenic effects of CHO, but we found no difference in substrate usage between men and women. Tremblay et al. (2010) also showed that women did not oxidize more lipids than men in a non-fasted state. Our data of RER in exercising women provides further evidence that non-fasting women who then consume a sports drink while exercising may not use more lipid as substrate than do men. While pedaling for 2 hours at their VT, both women and men perceived exercise to be easier with CHO, but during the self-paced TTs CHO did not affect RPE as cyclists were encouraged to give their best effort. It is possible that perception of effort might
differ according to menstrual phase, which was not controlled for in this study. However, Stephenson et al. (1982) found that RPE did not vary at set workloads across the menstrual cycle. The effect of CHO on men’s central fatigue was reflected in their cycling performances. With CHO, men rode faster in their TTs, at which time CAR was better maintained compared to PL. This is supported by Jeukendrup and Chambers (2010), who suggest that oral carbohydrate produces a positive afferent signal capable of increasing motor output. In contrast, we observed no ergogenic effect of CHO in women; they had no improvements in time to exhaustion or in TT performance, consistent with the lack of attenuation of central fatigue.

We chose the volume of fluid replacement according to American College of Sports Medicine’s general hydration recommendations of 0.4-0.8 L/hour, which take into account body mass and environmental conditions (American College of Sports Medicine et al. 2007). Gastric emptying is negatively affected as exercise intensity increases (Leiper et al. 2001). Robinson et al. (1995) found that exercising men who drank 1.5 L/hour while exercising at 85% of VO$_{2\text{peak}}$ only absorbed 600 ml/hour. In addition, women may be particularly susceptible to fluid overload: Twerenbold et al. (2003) found that nearly half the women consuming 1 liter of high sodium fluid per hour developed hyponatremia. The women in our study ingested approximately 600 ml of sports drink per hour, and the men took in nearly 800 ml/hour. This rate of intake was within recommendations, has previously limited body mass loss to less than 2% during similar exercise protocols conducted in our laboratory, and in this study led to body mass losses of approximately 1 kg [<2%].

**Limitations**

While we found that men and women responded differently to CHO, there were confounding factors that could have contributed. Although men and women met our inclusion...
criteria for training and racing, women may have been relatively less well trained than men, with a 20% difference in VO$_{2\text{peak}}$. However, Daniels and Daniels (1992) reported women to have a 15% lower VO$_{2\text{peak}}$ than similarly trained men.

Strength measurements were made as quickly as possible following cessation of cycling for both post-TT and post exhaustive ride. While every effort was made to minimize the delay prior to strength testing, as recovery can occur very quickly (Froyd et al. 2013), we were unable to control precisely for this. We began strength testing within 5 minutes of cessation of cycling, with some inconsistency between subjects in the exact amount of time.

We did not control for menstrual phase since there is scant evidence for its effect on performance in temperate conditions (Janse de Jonge et al. 2012) even when provided with carbohydrate (Bailey et al. 2000). Carbohydrate oxidation is greater in the follicular phase and it is possible that women may respond differently to a carbohydrate supplement during a particular phase of their cycles (Zderic et al. 2001). Smith et al. (1999) have shown that cortical excitability changes throughout the menstrual cycle; as this could affect the entire motor pathway, it is possible that this might have affected our measurements. Despite these possible confounding effects, women race during all phases of their menstrual cycle making our findings broadly applicable: carbohydrate-containing drinks should not be presumed to have an ergogenic effect in women.

**Perspective**

This study is one of few that include women in the evaluation of the effects of CHO upon performance during prolonged exercise, despite the ubiquitous participation of women in endurance sports. While our cycling protocol induced strength loss in both men and women, the use of CHO attenuated central fatigue in men only. Also, neither group experienced peripheral
fatigue. In both men and women engaging in endurance sports, research should focus on strategies for mitigating central fatigue. The finding that women were less responsive to the ergogenic effects of CHO is novel and deserves further investigation. In the United States alone, more than 6 billion dollars is spent annually on sports drinks (Statista 2016). To maximize the benefits women obtain from their purchases of these beverages, future research should explore the effect of CHO on fatigue and performance in women during each phase of the menstrual cycle.

Acknowledgement

We especially thank our volunteers for tolerating what was, at times, a very unpleasant protocol. Our appreciation also to Daniel Hogan for randomizing and preparing the beverages to maintain the blinded protocol. This study received no funding.

Conflict of Interest

The authors have no conflict of interest to report.
References


Table 1: Strength data during voluntary MVC, stimulated torque and CAR. Data are presented as mean±standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrate Treatment</th>
<th>Placebo Treatment</th>
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<tbody>
<tr>
<td></td>
<td>Pre-Exercise</td>
<td>Post 2h+TT</td>
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<tr>
<td>Voluntary MVC (% pre)</td>
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<td>100%</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>100%</td>
</tr>
<tr>
<td>Stimulated Torque (% pre)</td>
<td>Men</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>100%</td>
</tr>
<tr>
<td>CAR</td>
<td>Men</td>
<td>0.77±0.13</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>0.77±0.16</td>
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</tbody>
</table>

* Different from Pre-Exercise, p < 0.050. † Different from Pre-Exercise, p < 0.010.
Figure Captions

**Fig. 1** Protocol timeline. Aside from pre-exercise measurements, all fingersticks for blood glucose were done while subjects were still on the bike, prior to dismounting; strength measurements were made within five minutes of cessation of exercise.

**Fig. 2** Sample strength data from voluntary contractions with magnetic stimulation superimposed for one subject under carbohydrate (left) and placebo (right) conditions. Solid line: prior to fatigue. Dot-dash line: following two hours of cycling and time trial. Dotted line: following ride to exhaustion. Arrows indicate start of superimposed stimulation.

**Fig. 3** Respiratory Exchange Ratio: Effect of treatment x time, p <0.001. Numbers indicate minutes of exercise at which measurement was taken. M denotes men; W denotes women. *: Higher with CHO than PL, p<0.010.

**Fig. 4** Data for men are shown on the left, women’s data are shown on the right. Pre: Prior to exercise. 60: 60 minutes through 2-hour ride. TT: After 2-hour ride and Time Trial. Final: After ride to exhaustion. M: Men. W: Women. Blood glucose was better maintained with CHO: effect of time, p=0.005; sex x treatment, p=0.017.

**Fig. 5 a:** Time to perform time trial, *performance in the time trial was improved for men with CHO, p=0.005. **b:** Distance completed during ride to exhaustion.
Time Trial Performance

Ride to Exhaustion

(a) Time Trial Performance

(b) Ride to Exhaustion