Increase in Skeletal-Muscle Glycogenolysis and Perceived Exertion With Progressive Dehydration During Cycling in Hydrated Men

Heather M. Logan-Sprenger, George J.F. Heigenhauser, Graham L. Jones, and Lawrence L. Spriet

This study investigated the effects of progressive mild dehydration during cycling on whole-body substrate oxidation and skeletal-muscle metabolism in recreationally active men. Subjects (N = 9) cycled for 120 min at ~65% peak oxygen uptake (VO_2peak 22.7 °C, 32% relative humidity) with water to replace sweat losses (HYD) or without fluid (DEH). Blood samples were taken at rest and every 20 min, and muscle biopsies were taken at rest and at 40, 80, and 120 min of exercise. Subjects lost 0.8%, 1.8%, and 2.7% body mass (BM) after 40, 80, and 120 min of cycling in the DEH trial while sweat loss was not significantly different between trials. Heart rate was greater in the DEH trial from 60 to 120 min, and core temperature was greater from 75 to 120 min. Rating of perceived exertion was higher in the DEH trial from 30 to 120 min. There were no differences in VO_2 respiratory-exchange ratio, total carbohydrate (CHO) oxidation (HYD 312 ± 9 vs. DEH 307 ± 10 g), or sweat rate between trials. Blood lactate was significantly greater in the DEH trial from 20 to 120 min with no difference in plasma free fatty acids or epinephrine. Glycogenolysis was significantly greater (24%) over the entire DEH vs. HYD trial (433 ± 44 vs. 349 ± 27 mmol · kg^{-1} · dm^{-1}). In conclusion, dehydration of <2% BM elevated physiological parameters and perceived exertion, as well as muscle glycogenolysis, during exercise without affecting whole-body CHO oxidation.

Keywords: hydration, exercise, fluid intake, body-mass loss, substrate oxidation, sweat rate
Methods

Subject Characteristics

Nine men, $M \pm SE$ age 21.6 ± 0.5 years, height 178.1 ± 0.9 cm, body mass 77.3 ± 2.2 kg, and VO$_{2\text{peak}}$ 4.4 ± 0.2 L/min, volunteered to participate in the study. All subjects engaged in light- to moderate-intensity physical activity 3 or 4 days/week. Subjects were informed both verbally and in writing of the experimental protocol and potential risks before giving their written consent to participate. The research ethics boards of the University of Guelph and McMaster University approved the study.

Preexperimental Protocol

In preparation for the experiment, subjects visited the laboratory on three separate occasions. On the first visit, they performed an incremental cycling test to exhaustion on an electronically braked cycle ergometer (LODE Excalibur, Quinton Instrument, Groningen, The Netherlands) for the determination of VO$_{2\text{peak}}$. Respiratory gases were collected and analyzed using a metabolic cart (MOXUS metabolic system, AEI Technologies, Pittsburgh, PA). After a 30-min break, subjects cycled for ~20 min at ~65% VO$_{2\text{peak}}$ to establish the power output for the subsequent 120-min trials.

On two subsequent occasions, subjects reported to the laboratory for practice trials and cycled at ~65% VO$_{2\text{peak}}$ for 120 min without fluid (DEH) or with fluid (HYD) to replace sweat losses. DEH trials occurred first to ascertain sweat losses over the 120-min trial and to determine how much fluid subjects needed to drink during the HYD trial to maintain fluid balance. All subjects abstained from strenuous exercise and caffeine and recorded their diet in the 24 hr before the trials. Two hours before the practice rides, subjects ingested a meal provided for them (787 kcal; 144 g carbohydrate, 15 g fat, 19 g protein) and 250 ml of fluid. Subjects also drank 300 ml of water 90 and 45 min before each trial to ensure that they were hydrated before cycling. On arrival at the laboratory, subjects provided a small midstream urine sample to determine urine specific gravity (USG) and completely voided their bladder. A pretrial body-mass measurement was made wearing dry shorts only. After 40, 80, and 120 min of exercise, subjects stopped cycling and a muscle biopsy was taken with the Bergström technique (Bergström, 1962). Three incisions were made in the skin and deep fascia under local anesthesia (2% xylocaine without epinephrine) for three separate biopsies. Immediately before exercise, a venous blood sample (~5 ml) and a muscle biopsy were obtained while the subject rested on a bed. All muscle samples were immediately frozen in the needle in liquid nitrogen and stored in liquid nitrogen for subsequent analyses. Subjects then cycled for 120 min at ~65% VO$_{2\text{peak}}$ at a constant cadence (80–95 rpm). Venous blood samples were obtained at 20, 40, 60, 80, 100, and 120 min of exercise. HR, Tc, and RPE were recorded every 15 min during exercise. RPE was determined using the Borg scale (rating 6–20; Borg, 1970). During the HYD trial subjects were given fluid every 15 min to match sweat loss and drank the fluid after HR, Tc, and RPE measurements were recorded. At 40, 80, and 120 min subjects stopped cycling and a muscle biopsy was taken with the subjects sitting on the cycle ergometer. After the muscle biopsy was taken, subjects removed their shoes and shirt, towed dry, and were weighed for the determination of body-mass loss over the previous 40 min of exercise. The same procedure was replicated for the second trial, with muscle biopsies taken from the opposite leg, and the trials were randomized and separated by 7 days.

Analyses

**Trial Conditions.** Laboratory temperature (°C) and relative humidity (%) were measured using a digital thermometer (Fisher Scientific, Ottawa, ON). USG was collected every 20 min during exercise to determine the volume of oxygen consumed (VO$_2$) and the volume of carbon dioxide produced (VCO$_2$) and to calculate the RER and whole-body CHO and fat oxidation with use of the nonprotein RER table and the following equations:

\[
\text{CHO oxidation (g)} = 4.585 \times (\text{VCO}_2) - 3.226 \times (\text{VO}_2)
\]

(Peronnet & Massicotte, 1991)

\[
\text{Fat oxidation (g)} = 1.695 \times (\text{VO}_2) - 1.701 \times (\text{VCO}_2)
\]

(Peronnet & Massicotte, 1991)
measured via handheld pocket refractometer (Model PAL10S, Atago USA Inc., Bellevue, WA) to assess hydration status from the preexercise urine sample. The refractometer was calibrated with distilled water before each measurement. Stover, Zachwieja, Stefan, Murray, and Horswill (2006) reported that USG measured with refractometry correlated strongly with urine osmolality (r = .995), and a USG of 1.020 correlated with an urine osmolality of ~800 mOsm/kg. In light of this and the published position stand from the American College of Sports Medicine, a USG below 1.020 was considered to indicate a hydrated state (Sawka et al., 2007).

**Blood Measurements.** Venous blood was collected in sodium heparin tubes. A portion of whole blood (200 μl) was added to 1 ml of 0.6-M perchloric acid and centrifuged. The supernatant was stored at −20 °C and later analyzed for blood glucose and lactate with fluorometric techniques (Bergmeyer, 1974). A second portion (1.5 ml) was centrifuged and the supernatant was analyzed for plasma free fatty acids with an enzymatic colorimetric technique (NEFA C test kit, Wako Chemicals, Richmond, VA). A third portion (1.5 ml) was added to 30 ml of EGTA and reduced glutathione and centrifuged (10,000 g) for 3 min, and the supernatant was analyzed for epinephrine with an enzymatic immunoassay kit (Epinephrine RIA kit, Rocky Mountain Diagnostics Inc., Colorado Springs, CO). The percent plasma volume change was calculated using the measured ATP, creatine, and phosphocreatine values, an estimated H+ concentration, and the creatine kinase constant of 1.66 × 109 (Sahlin, Harris, Nylind, & Hultman, 1976; Saltin, 1990). AMpf was calculated from the estimated ADPf and measured ATP content using the adenylyl kinase equilibrium constant of 1.05.

**Statistical Analysis**

All data were tested for normality of distribution and presented as M ± SE. Time-versus-trial data were assessed using a two-way ANOVA, and specific differences were located using the Student-Newman-Keuls post hoc test. A paired t test was used to compare single-parameter data between trials. Statistical significance was accepted as p < .05.

**Results**

**Trial Conditions**

No significant pretrial differences existed between the HYD and DEH trials for laboratory temperature (HYD 22.6 ± 0.1 °C vs. DEH 22.8 ± 0.2 °C, p = .29), relative humidity (32% ± 2.8% vs. 33% ± 2.7%, p = .49), pretrial body mass (77.1 ± 2.2 kg vs. 77.5 ± 2.2 kg, p = .10), or hydration state (USG 1.013 ± 0.003 vs. 1.015 ± 0.003, p = .60).

**Body-Mass Loss, Sweat Loss, and Fluid Intake**

Body mass was maintained in the HYD trial by consuming a mean of 2.3 ± 0.2 L of fluid over the 120 min of cycling. In the DEH trial, body mass was significantly lower at 40 (0.8 ± 0.1%, p = .04), 80 (1.8 ± 0.2%, p = .003), and 120 min (2.7 ± 0.2%, p < .001, Table 1). There was no significant difference in sweat loss between the HYD and DEH trials (p = .15). Only 1 subject micturated after both the HYD trial (350 ml) and the DEH trial (400 ml).

**VO2 and Whole-Body Substrate Use**

Subjects started the exercise trial at 63% ± 0.7% VO2peak, which then increased to 65% ± 0.7% VO2peak. There was no significant difference between trials (Figure 1[a], p = .09). The RER progressively decreased in both trials over time and was significantly lower than 20 min at 80, 100, and 120 min in both trials (p < .05), with no significant differences between trials (p = .47, Figure 1[b]).

There was no significant difference in total CHO oxidation (53 ± 8 vs. 55 ± 17 g, p = .33) between trials. CHO oxidation significantly decreased over time from zero to 40 (HYD 312 ± 9 g vs. DEH 307 ± 10 g, p = .38) or fat oxidation (53 ± 8 vs. 55 ± 17 g, p = .33) between trials. CHO oxidation significantly decreased from zero to 40 (HYD 115 ± 6 g, p = .03), 40 to 80 min (105 ± 5 vs. 107 ± 6 g, p = .05), and 80 to 120 min (91 ± 4 vs. 85 ± 5 g, p = .03), with no significant differences between trials (p = .19). Fat oxidation increased
### Table 1 Whole-Blood and Plasma Parameters During 120 min of Cycling at ~65% Peak Oxygen Uptake in the Hydrated (HYD) and Dehydrated (DEH) Trials, $M \pm SE (N = 9)$

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Trial</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pvol loss (%)</td>
<td>HYD</td>
<td>——</td>
<td>6.0 ± 1.3†</td>
<td>4.9 ± 1.2†</td>
<td>4.5 ± 1.3†</td>
<td>5.1 ± 1.4†</td>
<td>4.9 ± 1.5†</td>
<td>4.1 ± 1.2†</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>——</td>
<td>7.1 ± 1.2†</td>
<td>8.3 ± 1.2†*</td>
<td>8.9 ± 1.5†*</td>
<td>8.7 ± 1.5†*</td>
<td>8.5 ± 1.4†*</td>
<td>9.4 ± 1.5†*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>HYD</td>
<td>4.0 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>HYD</td>
<td>0.5 ± 0.1</td>
<td>2.0 ± 0.3†</td>
<td>1.5 ± 0.3†</td>
<td>1.4 ± 0.3†</td>
<td>1.4 ± 0.2†</td>
<td>1.2 ± 0.2†</td>
<td>1.5 ± 0.3†</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.7 ± 0.3</td>
<td>2.6 ± 0.6†*</td>
<td>2.3 ± 0.5†*</td>
<td>1.9 ± 0.3†*</td>
<td>1.9 ± 0.3†*</td>
<td>2.0 ± 0.5†*</td>
<td>2.1 ± 0.5†*</td>
</tr>
<tr>
<td>Plasma FFA (mM)</td>
<td>HYD</td>
<td>0.15 ± 0.02</td>
<td>——</td>
<td>——</td>
<td>0.21 ± 0.03†</td>
<td>0.38 ± 0.1†</td>
<td>——</td>
<td>0.89 ± 0.1†</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.14 ± 0.02</td>
<td>——</td>
<td>——</td>
<td>0.19 ± 0.04†</td>
<td>0.44 ± 0.1†</td>
<td>——</td>
<td>0.84 ± 0.1†</td>
</tr>
<tr>
<td>Plasma EPI (nM)</td>
<td>HYD</td>
<td>0.41 ± 0.03</td>
<td>——</td>
<td>——</td>
<td>0.93 ± 0.1†</td>
<td>1.13 ± 0.1†</td>
<td>——</td>
<td>1.65 ± 0.2†</td>
</tr>
<tr>
<td></td>
<td>DEH</td>
<td>0.41 ± 0.04</td>
<td>——</td>
<td>——</td>
<td>1.06 ± 0.1†</td>
<td>1.11 ± 0.1†</td>
<td>——</td>
<td>1.66 ± 0.2†</td>
</tr>
</tbody>
</table>

*Note.* Pvol = plasma volume; FFA = free fatty acids; EPI = epinephrine.

†Significantly different from 0 min ($p < .05$). *Significantly greater than HYD ($p < .05$).

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**Figure 1** — (a) Oxygen uptake (VO₂) and (b) respiratory-exchange ratio (RER) during 120 min of cycling at ~65% VO₂peak in the hydrated (HYD) and dehydrated (DEH) trials. Data are $M \pm SE (N = 9)$. Arrows indicate approximately 1%, 2%, and 3% body-mass loss.
over time from zero to 40 (HYD 12 ± 2 vs. DEH 13 ± 2 g, p = .05), 40 to 80 (18 ± 2 vs. 18 ± 2 g, p = .02), and 80 to 120 min (23 ± 2 vs. 24 ± 3 g, p = .005), with no significant trial differences (p = .11).

**HR, Tc, and RPE**

HR increased significantly over time in both trials and was significantly higher in the DEH than in the HYD trial from 60 to 120 min of cycling (p = .002, Figure 2[a]). Tc increased significantly over time in both trials (p < .002) and was significantly higher in the DEH than in the HYD trial from 75 to 120 min (p = .003, Figure 2[b]). RPE significantly increased over time in both trials (p = .01) and was significantly higher in the DEH trial from 30 to 120 min (p = .001, Figure 2[c]). The mean RPE over the entire trial was also significantly greater in the DEH than in the HYD trial (14.4 ± 0.6 vs. 12.9 ± 0.3, p = .01).

**Blood Measurements**

Hemoglobin and hematocrit were significantly higher than rest from 20 to 120 min of exercise in both trials. In the DEH trial, hemoglobin was significantly greater from 40 to 120 min (p < .001) and hematocrit was significantly greater from 60 to 120 min (p = .013). Plasma volume loss was significantly greater in the DEH trial from 40 to 120 min of exercise (p < .001, Table 1). Blood glucose was unaffected by time (p = .07) or trial (p = .41), and blood lactate was significantly increased above rest at all time points in both trials (p < .001 and was greater in the DEH trial at all exercise time points (p < .001, Table 1). Plasma free fatty acids and epinephrine significantly increased from rest in both trials (p < .001), with no significant differences between trials (free fatty acids p = .84, epinephrine p = .44; Table 1).

**Muscle Fuels and Metabolites**

Skeletal-muscle phosphocreatine content significantly decreased in the first 40 min of exercise (p = .03) and remained lower than rest at 80 and 120 min of exercise in both trials, with no significant differences between trials (p = .99, Table 2). Skeletal-muscle creatine changes were reciprocal to the phosphocreatine changes, and muscle ATP content was unaffected by exercise (p = .21) or hydration state (p = .10). ADPf and AMPf were significantly higher than rest at all time points during exercise in both trials (p = .01), with no differences between trials (p = .41, Table 2). Muscle lactate content increased with exercise (p = .02), peaked at 40 min in both trials, and was significantly greater in the DEH trial at 40 (p = .02), 80 (p = .01), and 120 min (p = .009) of exercise (Table 2).

Muscle glycogen content was similar in the two trials before exercise (p = .11) and significantly lower at 40, 80, and 120 min in both trials compared with rest (p < .01). Total glycogen use (0–120 min) was significantly greater (24%) in the DEH trial (433 ± 44 vs. 349 ± 27 mmol · kg⁻¹ · dm⁻¹, p = .02, Figure 3). However there was no significant difference in glycogen use from 0 to 40 (19%, HYD, 209 ± 30 vs. DEH, 249 ± 43 mmol · kg⁻¹ · dm⁻¹, p = .13), 40 to 80 (19%, 77 ± 14 vs. 92 ± 19 mmol · kg⁻¹ · dm⁻¹, p = .47), and from 80 to 120 min (46%, 63 ± 12 vs. 92 ± 24 mmol · kg⁻¹ · dm⁻¹, p = .09), although there were strong trends in each time period.

**Discussion**

This study investigated the effects of mild progressive dehydration during exercise at ~65% VO₂peak on whole-body substrate oxidation and skeletal-muscle metabolism, as well as cardiovascular, thermal, and perceived exertion responses in active, hydrated men. In the control trial (HYD) of this study, we prevented dehydration by having subjects drink enough fluid to precisely replace their sweat losses over the 120-min cycling trial. HR increased from 150 ± 4 beats/min at 15 min to 160 ± 5 and 165 ± 4 beats/min at 60 and 120 min, while Tc increased from 37.2 ± 0.1 °C at rest to 37.8 ± 0.1 °C at 15 min and reached a plateau of 38.1 ± 0.1 and 38.2 ± 0.1 °C at 60 and 120 min, respectively. In the DEH trial, the subjects lost approximately 1%, 2%, and 3% body mass at 40, 80, and 120 min through sweating, adding to the physiological demands of exercising for 120 min at ~65% VO₂peak. All physiological responses to exercise were exacerbated in the DEH trial, as HR and Tc were higher at 60 and 120 min by 6–7 beats/min and by 0.2 °C and 0.5 °C, respectively, in the DEH-versus-HYD trial. Even in the first 40 min of exercise in DEH (~1% body-mass loss), RPE, plasma volume loss, and blood [La] were all higher and there was a significantly greater muscle lactate content and a trend for increased muscle glycogen use (p = .17). From 40 to 80 and 80 to 120 min, body-mass loss progressed from 1% to 2% and from 2% to 3%, and all physiological parameters remained higher in the DEH trial. The 3% body-mass loss over 120 min of exercise increased overall muscle glycogen use by 24% but had no effect on whole-body carbohydrate oxidation in the DEH trial.

**The Effects of Progressive Dehydration on Muscle Metabolism**

Previously, Hargreaves et al. (1996) reported that net muscle glycogen use was 16% greater over a 120-min trial at 67% VO₂peak when trained men were dehydrated by 2.9% body mass. The RER was significantly higher in the fluid-restricted trial after 60 and 120 min of exercise, with the difference between trials being greater in the second hour of cycling. The current study examined time-course changes to muscle metabolism at 40, 80, and 120 min of cycling at ~65% VO₂peak with progressive dehydration to ascertain how much dehydration was necessary to see a shift in substrate oxidation and muscle glycogen use. There were no differences in whole-body substrate oxidation when subjects were dehydrated by 1%, 2%, or 3% body mass. In addition, there were trends for accelerated muscle glycogen use in each 40-min exercise segment, resulting in a significant 24% increase over the entire
Figure 2 — (a) Heart rate during 120 min of cycling at ~65% peak oxygen uptake (VO$_{2\text{peak}}$) in the hydrated (HYD) and dehydrated (DEH) trials. Heart rate was significantly greater than 15 min at all time points in both trials ($p < .05$). bpm = beats per minute. (b) Core temperature during 120 min of cycling at ~65% VO$_{2\text{peak}}$ in the HYD and DEH trials. Core temperature was significantly greater than 15 min for all time points in both trials ($p < .05$). (c) Rating of perceived exertion (RPE) during 120 min of cycling at ~65% VO$_{2\text{peak}}$ in the HYD and DEH trials. RPE was significantly greater than 15 min from 45–120 min in both trials ($p < .05$). Data are $M \pm SE$ ($N = 9$). *Significantly higher than HYD trial ($p < .05$). Arrows indicate approximately 1%, 2%, and 3% body-mass loss.
An interesting finding in this study was the greater total muscle glycogen use in the DEH trial with no difference in whole-body carbohydrate oxidation. This suggests that the extra glycogen metabolized to pyruvate was not oxidized but converted to lactate. This speculation is supported by the augmented blood and muscle lactate contents throughout the DEH trial.

Three potential mechanisms may account for the increased muscle glycogenolysis and glycogen phosphorylase (PHOS) activity in the DEH trial without also affecting the activity of pyruvate dehydrogenase. They include an increased sympathoadrenal response leading to elevated circulating epinephrine and activation of PHOS, a decreased energy status in the cell manifested by elevated ATP:ADP ratio and AMPf levels (as they act as allosteric activators of PHOS), and increased muscle temperature (Tm; Febbraio, 2000).

Hargreaves et al. (1996) reported no difference in plasma epinephrine at 60 or 120 min of cycling with 2.9% body-mass loss at 120 min, and in the current study we also found no significant difference in epinephrine response with fluid restriction up to 3% body-mass loss in men. Estimates of free ADP and AMP in the DEH and HYD trials of the current study did not reveal any apparent differences, downplaying the first suggestion that these allosteric regulators can explain the increased PHOS activity and glycogenolysis in the DEH trial.

Second, studies examining the importance of local hyperthermia to muscle glycogenolysis increased the Tm of one leg during exercise and reported increased muscle glycogenolysis and [La] in the hot leg only, independent of changes to Tc or circulating epinephrine. This demonstrated that increasing local Tm increased glycogenolysis at a given Tc, when both legs were exposed to the same

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Skeletal-Muscle Fuel and Metabolite Contents (mmol · kg⁻¹ · dm⁻¹) During 120 min of Cycling at ~65% Peak Oxygen Uptake in the Hydrated (HYD) and Dehydrated (DEH) Trials, M ± SE (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HYD</td>
</tr>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>PCr</td>
<td>80.3 ± 4.9</td>
</tr>
<tr>
<td>Creatine</td>
<td>74.7 ± 3.2</td>
</tr>
<tr>
<td>ATP</td>
<td>25.6 ± 1.2</td>
</tr>
<tr>
<td>ADPf</td>
<td>144 ± 19</td>
</tr>
<tr>
<td>AMPf</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Glycogen</td>
<td>522 ± 58</td>
</tr>
</tbody>
</table>

Note. PCr = phosphocreatine; ATP = adenosine triphosphate; ADPf = free adenosine diphosphate; AMPf = free adenosine monophosphate.
†Significantly different from 0 min (p < .05). *Significantly greater than HYD (p < .05).
epinephrine (Febbraio, Carey, Snow, Stathis, & Hargreaves, 1996; Starkie, Hargreaves, Lambert, Proietto, & Febbraio, 1999). In a review article, Febbraio (2000) stated that increases in Tc of >0.5 °C increased intramuscular CHO utilization consistently during moderate-intensity exercise in the heat. In the current study, Tc was 0.3–0.5 °C higher in the final 30–45 min of exercise in the DEH trial (~20 °C). While Tm was not measured in this study, the work of Hargreaves et al. (1996) and Febbraio (2000) predicts that Tm would have been higher during exercise in the DEH trial of the current study (Starkie et al., 1999). Similar results without Tm measures have been reported in a DEH versus HYD trial when exercising in the heat (35 °C), as Gonzalez-Alonso, Calbet, and Nielsen (1999) had male subjects cycle until volitional exhaustion (135 ± 4 min) while progressively dehydrating to ~3.9% body-mass loss. They reported a 45% increase in muscle glycogen use across the contracting leg compared with the euhydrated trial.

Therefore, the last mechanism proposed—an increased Tm and the Q10 effect—appears to be the most plausible explanation for the increased muscle glycogenolysis reported during progressive dehydration in men in the current study. It is currently unknown why dehydration preferentially increases CHO metabolism and not fat metabolism, but it may be related to the ability to quickly mobilize muscle CHO versus the relatively slower mobilization of fat fuels not coming from intramuscular triglycerides. The current data also suggest that activity of PHOS may be more sensitive to increased Tm (increased pyruvate production) as compared with the activity of pyruvate dehydrogenase, resulting in no increase in pyruvate oxidation and more lactate formation. As this was the case, the increased glycogen use with dehydration appeared to be wasted as the excess pyruvate produced was converted to lactate and not oxidized. Others have reported that dehydration in the heat increased muscle glycogen use (45% greater), muscle lactate accumulation, and net lactate release across the contracting leg compared with the euhydrated trial (Gonzalez-Alonso et al., 1999), but there was also an increase in CHO oxidation, unlike the current study. At the present time, there does not appear to be an explanation for this finding except that the subjects in Hargreaves et al.’s (1996) and Gonzalez-Alonso et al.’s (1999) studies were trained cyclists, and the subjects in the current study were only recreationally trained.

**Effects of Dehydration on Cardiovascular and Thermal Responses**

It is well established that fluid ingestion attenuates the increases in HR and Tc and the decreases in stroke volume and cardiac output that occur during prolonged exercise without fluid ingestion (Armstrong et al., 1997; Cheuvront, Kenefick, Montain, & Sawka, 2010; Febbraio et al., 1996; Hamilton, Gonzalez-Alonso, Montain, & Coyle, 1991; Morimoto, 1990; Nadal et al., 1980; Sawka et al., 1985). An early study demonstrated that when heat-acclimatized male subjects were dehydrated to 3%, 5%, and 7% body-mass loss by an exercise-heat regimen and then walked in a hot environment (49 °C) at a low intensity for 140 min, HR and Tc increased linearly with the severity of dehydration (Sawka et al., 1985). In a similar way, our results demonstrated that as dehydration increased from zero to 1%, 1% to 2%, and 2% to 3% body mass during exercise in the DEH trial, HR and Tc became progressively higher than the elevations in the HYD trial.

Hypovolemia and the displacement of blood to the skin for evaporative cooling make it difficult to maintain central venous pressure during exercise when fluid is restricted (Sawka, Montain, & Latzka, 2001). Central venous pressure is regulated by the continuous adjustment of blood volume to the changing size of the vascular bed to maintain cardiac output, and heat stress or exercise-induced dehydration provides a threat to this control as inadequate fluid intake during periods of sweat loss reduces plasma volume (Morimoto, 1990). In light of the significantly greater loss in plasma volume found in the DEH versus the HYD trial after ~40 min of cycling, a reduction in central venous pressure and stroke volume may account for the significantly elevated HR to maintain cardiac output when stroke volume was compromised. An accompanying baroreflex that would decrease cutaneous blood flow leading to heat storage may account for the augmented Tc found in the DEH trial. In support of this, Nadal et al. (1980) reported that diuretic-induced dehydration of 2.7% body mass led to restrictions in core-to-skin heat transfer, which forced esophageal temperature to nearly 39 °C during 30 min of cycling at 55% VO2peak in the heat. Further support was provided by Montain and Coyle (1992a), who investigated whether fluid ingestion attenuated the hyperthermia and cardiovascular drift that occurred during exercise dehydration due to increases in blood volume. Subjects exercised at ~65% VO2peak for 2 hr in three conditions: no fluid replacement, infusion with a blood-volume expander, or given fluid to replace ~80% of sweat loss. They reported that fluid replacement and the blood-volume expander maintained blood volume compared with the no-fluid trial, but only fluid replacement resulted in lower Tc. The authors argued that the decreased hyperthermia during exercise in the fluid-replacement trial was due to the measured increase in skin blood flow. In the current study, subjects had higher Tc values in the last 45 min of exercise in the DEH (38.7 °C) versus HYD (38.1 °C) trials, while the sweat rates were the same, suggesting that the lack of heat transfer to the periphery accounted for the elevated Tc in the DEH trial with as little as ~1–2% body-mass loss.

**Effects of Dehydration on RPE**

In this study, RPE became significantly higher in the DEH trial after only 30 min of cycling when subjects had lost <1% body mass. Similar results have been reported in other studies investigating the effects of progressive dehydration on RPE (Ishijima et al., 2009; McGregor,
Nicholas, Lakomy, & Williams, 1999). It is speculated that hypovolemia associated with exercise dehydration leading to a reduction in brain blood flow may exacerbate the sense of effort associated with exercising without fluid leading to greater perceived exertion (Maughan, Shirreffs, & Watson, 2007). More simply, it may be that the temperature and cardiovascular centers that sense elevations in Tc, HR, and reduced plasma volume feed back to the brain and increase the RPE during exercise at the same relative intensity in a mildly dehydrated state. Shirreffs, Merson, Fraser, and Archer (2004) reported that as subjects became progressively more dehydrated to 2.7% body-mass loss they reported feelings of headache, reductions in their ability to concentrate, and reduced alertness, which may all contribute to an elevated RPE during exercise. Finally, it is not possible to blind subjects, so the mere knowledge that they are not drinking during exercise. Dill, D.B., & Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. 

**Summary and Conclusions**

This study investigated the time course of changes in whole-body substrate oxidation and skeletal-muscle metabolism, as well as cardiovascular, thermal, and perceived-exertion responses in recreationally active, hydrated men with progressive mild dehydration during exercise at ~65% VO2peak. All changes in physiological parameters accompanying exercise in a hydrated state were exacerbated with mild dehydration of ~1–2% body-mass loss. Muscle glycogenolysis was significantly increased in the DEH versus HYD condition over the entire trial (0–120 min) with no difference in whole-body CHO oxidation between trials. We speculate that the increased glycogenolysis was due to increases in Tm and the Q10 effect, as there appear to be no differences in plasma epinephrine or the energy status of the cell (free ADP or AMP) between the HYD and DEH trials. There does not appear to be an obvious explanation for the lack of increased whole-body CHO oxidation in the face of the dehydration-induced increase in muscle glycogenolysis.

**References**


Ishijima, T., Hashimoto, H., Satou, K., Muraoka, I., Suzuki, K., & Hiquchi, M. (2009). The different effects of fluid...


