Prolonged exercise after diuretic-induced hypohydration: effects on substrate turnover and oxidation

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In an attempt to protect cardiovascular integrity and fluid and electrolyte balance during exercise, increased activation of the sympathetic nervous system (SNS) occurs, which results in elevated blood concentrations of norepinephrine (NE), as a result of spillover from the nerve endings, and epinephrine (Epi), secreted from the adrenal medulla (38). Changes in SNS activation are also accompanied by increased levels of plasma renin activity, aldosterone, arginine vasopressin, and a decreased level of α-atrial natriuretic peptide (6, 38). Collectively, the major effects of these hormonal changes during exercise are to increase electrolyte and water reabsorption from the kidney, reduce sweat rates, and promote cardiac contractility and vasoconstriction in the cutaneous and splanchnic vasculature (6, 38). If the hypohydration is so severe as to result in a lower cardiac output compared with euhydration, increases in systemic vascular resistance occur (14, 18), probably in conjunction with reductions in blood flow to the working muscles (29).

The compensatory responses to hypohydration and exercise, both cardiovascular and hormonal, suggest that a shift in substrate turnover and oxidation may occur. The exaggerated increase in catecholamine concentration, particularly Epi, as an example, would be expected to promote an increase in the mobilization and utilization of carbohydrates (CHO) (8). In addition, if blood flow is compromised, O₂ availability to the mitochondria may be threatened. Under such conditions, CHO oxidation becomes more emphasized, ostensibly because it provides the highest ATP yield per mole of O₂ (1). An increase in blood glucose utilization has been reported to occur during submaximal exercise in hypoxia, even though oxidative phosphorylation is not compromised (2).

In this study, we have hypothesized that hypohydration resulting in a reduction in PV would alter glucose kinetics, promoting an increase in both glucose release from the liver and glucose oxidation by the working muscle. These changes would be accompanied by a decline in both the mobilization and utilization of blood free fatty acids (FFA).

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METHODS

Participants. Ten healthy young males were recruited and screened to ensure that they were healthy and not active on a regular basis. Their mean (±SE) age, weight, peak aerobic power (VO₂ peak), and maximal heart rate were 20.3 ± 0.4 yr, 78.1 ± 3.0 kg, 3.96 ± 0.14 l/min, and 199.0 ± 2.5 beats/min, respectively. All of the experimental procedures, risks, and benefits were explained to each subject before written consent was obtained and after approval of the study by the Office of Human Research (University of Waterloo, Waterloo, ON).

Design and procedures. The basic experimental design consisted of having the participants perform a standardized, prolonged submaximal cycling test on two separate occasions: under control conditions (CON) and after 4 days of diuretic administration (DIU). On each of four days before each submaximal test, subjects consumed either a diuretic (Novotriamide; 100 mg triamterene and 50 mg hydrochlorothiazide) or a placebo. The exercise tests were separated by a minimum of 1 wk and were administered in a randomized, single-blind order. All exercise measurements were conducted at approximately the same time of day for each subject. The exercise test, which was performed at ~61% VO₂ peak, was planned for a 90-min duration. However, because some subjects were unable to complete the 90 min and because blood sampling was a problem in some subjects, we report only on the first 60 min of exercise. Exercise was performed in ambient temperatures (22–24°C) and humidities (35–45%). Each participant consumed a standardized snack 4 h before each exercise test (Ensure liquid, 1,045 kJ: 14.8% protein, 31.5% fat, and 53.7% carbohydrate; Ross Laboratories, Montreal, QC, Canada). Only water (ad libitum) was allowed between consumption of the snack and arrival at the laboratory. All participants were instructed not to engage in any vigorous physical activity for the duration of the experiment and to follow a normal balanced diet.

Participants reported to the laboratory ~150 min before the start of the exercise test. During the preexercise period, body weight was determined, a 20-gauge catheter was inserted into an antecubital vein, and a blood sample was collected. A primed constant infusion of stable isotope tracers was then initiated into the antecubital vein catheter. Subjects then rested quietly for ~90 min. A second 20-gauge catheter was then inserted into a heated dorsal hand vein of the opposite arm to allow for arterialized blood sampling. Fluid consumption by the subjects was not permitted after the preparatory period or during the prolonged exercise tests.

After placement of the second catheter, the participants were positioned upright on an electronically braked cycle ergometer, and the seat height was adjusted to provide slight flexion at the knee while the pedal was at its lowest point. After a 15-min period of quiet sitting, during which time a blood sample was taken and respiratory gas exchange was measured, subjects began cycling.

DIU resulted in a 14.6 ± 3.3% decrease in resting PV as calculated from changes in resting hematocrit (37). Hematocrits were determined after subjects were seated at rest for at least 15 min.

O₂ uptake measurements. The submaximal exercise bouts were performed on an electrically braked cycle ergometer (Quinton 870, Excalibur Sport, Groningen, Netherlands). Each of the participants had performed progressive exercise until fatigue for measurement of VO₂ peak ~1 wk before the first submaximal test by means of a protocol that has been previously described (16). Gas exchange and ventilatory measures were determined using an open-circuit gas collection system (23). The gas analyzers (Beckman OM-11 and LB-2) and the pneumotachograph (Hewlett-Packard 4750A) were calibrated on each testing day. Common reference gases, with the gas percentages precisely determined, were used for the calibration. The pneumotachograph was calibrated by using a 3-liter syringe emptied to produce a flow rate similar to that found in exercise. For the submaximal cycling tests, the same absolute power output (170 ± 6.7 W) was used for the two conditions. Measurements of respiratory gas exchanges during steady state were made over a 3- to 4-min collection period before and intermittently during each test (0, 15, 30, and 60 min). Steady state was confirmed by comparing consecutive 20-s periods of gas exchange.

Indirect calorimetry. Stoichiometric equations and appropriate caloric equivalents (12, 40) were used to calculate carbohydrate (CHO) and fat oxidation rates during the exercise. We assumed that the nitrogen excretion rate was 135 μg·kg⁻¹·min⁻¹ (34). Although indirect calorimetry technically provides for an estimation of total glucose oxidation, we have followed the general practice of labeling it as CHO oxidation.

Blood sampling. Arterialized blood samples were collected before and at regular intervals during exercise (0, 15, 30, 45, and 60 min). The samples were used for the determination of blood lactate, glucose, glyceral, serum FFA, insulin, and glucagon concentrations and for plasma glucose and glyceral isotopic enrichment. For determination of blood metabolites (lactate, glucose, and glyceral), whole blood was deproteinized using ice-cold perchloric acid. After centrifugation for removal of the precipitated proteins, ice-cold KHCO₃ was added to neutralize the samples. For analysis of serum FFA, ~1.5 ml of blood were allowed to clot; the sample was then centrifuged, and the resulting serum was stored until analysis. Blood samples for the determination of glucose and glyceral enrichment were added to heparinized tubes, the tubes were then centrifuged, and the resulting plasma was stored for later analysis. All samples were stored at ~80°C before analysis. Blood concentrations of lactate, glucose, glyceral, and serum FFA were determined by fluorometric methods as described previously (17). Standard radioimmunoassay methods were used to determine the serum concentration of insulin and glucagon (Coat-A-Count, Diagnostic Products, Intermedico, Toronto, ON, Canada).

For a given individual, all of the samples for a given measurement were analyzed in duplicate and during the same analytical session.

Isotopes. The stable isotope tracers used for determination of substrate turnover, [6,6-²H₂]glucose and [²H₂]glycerol (98% enriched; MassTrace, Woburn, MA), were diluted in sterile 0.9% saline under aseptic conditions and then filtered through a 0.2-μm filter (Pall Gelman Sciences, Ann Arbor, MI). Immediately before infusion, the infusate was passed through another 0.2-μm filter (Pall Gelman Sciences). A priming dose of glucose (14 μmol/kg) and glyceral (1.3 μmol/kg) was administered before the initiation of a constant infusion (0.22 ± 0.03 μmol·kg⁻¹·min⁻¹ for [6,6-²H₂]glucose and 0.1 ± 0.03 μmol·kg⁻¹·min⁻¹ for [²H₂]glycerol). The specific infusion rates for each tracer were calculated by multiplying the infusion concentration (determined fluorometrically) by the infusion rate. The infusion rate was doubled (as compared with rest) for both tracers at the onset of exercise. To avoid biasing the data, the specific infusion rates and infusates were kept constant for each subject over each condition.

Tracer enrichment. Glucose and glyceral enrichments were determined using the pentacetate derivative of glucose and the trimethylsilyl derivative of glyceral. For glucose, 250 μl of...
plasma were deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The resulting supernatant was then deionized by passing it over a mixed-bed anion-cation exchange chromatographic column (AG-1-X8 and AG 50W-X8; Sigma Chemical, St. Louis, MO). The eluted fluid from this column was then lyophilized to dryness. To the lyophilized extract 100 μl of a 2:1 solution of acetic anhydride and pyridine were added to create the final derivative. Samples were then incubated at 80°C for 15 min. For glycerol, 1,000 μl of plasma were deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The resulting supernatant was then deionized by passing it over a mixed-bed anion-cation exchange chromatographic column (AG-1-X8 and AG 50W-X8; Sigma Chemical, St. Louis, MO). The eluted fluid from this column was then lyophilized to dryness. To the lyophilized extract 100 μl of a 2:1 \( N,O\)-bis(trimethylsilyl)trifluoroacetamide-pyridine were added. Extracts were then incubated at 80°C for 30 min.

Gas chromatography-mass spectrometry. Enrichment of each of the derivatives was measured by injection of 1 μl of extract into a Hewlett-Packard 6890 gas chromatography (GC) oven (Palo Alto, CA). An HP-5 fused silica capillary column (15 m × 0.32 μm, 0.25-μm film thickness) was used in the GC oven (Hewlett-Packard). Mass analysis was performed using a Hewlett-Packard 5973 mass spectrometer operating in EI+ mode. Data were processed using HP-Chemstation software (Hewlett-Packard).

Selected ion masses were monitored, depending on which derivative was injected. Mass-to-charge ratios (m/z) were determined for 200, 202, 205, and 208 atomic mass units for determination of glucose and glycerol enrichment, respectively. These enrichments gave the expected \( M + 2M + 0 \) or \( M + 3M + 0 \) ratios, indicating that there were no other interfering products or masses. All masses were also corrected according to a linear peak area standard curve for glucose and glycerol.

Calculations. The rates of appearance (Ra) and disappearance (Rd) of glucose and glycerol were calculated using the steady-state tracer dilution equation at rest (31). During exercise, isotope kinetics were calculated with the Steele steady-state tracer dilution equation at rest (31). During exercise, Rd of glucose and glycerol were calculated using the following equation:

\[
\text{Rd} = \frac{\text{Ra} - \text{Rd}}{\text{Kd}}
\]

where Kd is the metabolic clearance rate. The effective volumes of distribution were assumed to be 230 ml/kg for glycerol and 100 ml/kg for glucose. We had previously observed such volumes of distribution to be adequate under a variety of conditions (30–32). Increasing (+50%) or decreasing (~50%) the volume of distribution had little effect on the results obtained; therefore it appears that these volumes of distribution were adequate (30).

The total amount of glycerol released during exercise is the total of release from peripheral and intramuscular lipolysis. We have assumed that glycerol Ra is representative of whole body lipolysis, because the activity of glycerol kinase within skeletal muscle is negligible (28, 34) and the majority of glycerol phosphorylation occurs in the liver during exercise (34). With these assumptions, therefore, three times the rate of lipolysis represents the maximum FFA availability, since there are very few monoglyceride or diglyceride pools within skeletal muscle or adipocytes (34).

We also estimated the minimal rate of muscle glycogen oxidation, as previously described (30). Briefly, whole body CHO oxidation was calculated from indirect calorimetry. We also assumed that 100% of glucose Rg was oxidized during exercise (31, 34). It was assumed that the difference between whole body CHO oxidation and glucose oxidation represented muscle glycogen oxidation. The current assumptions would lead to an underestimation of the minimal rate of glycogen oxidation in situations where plasma glucose is converted directly to lactate instead of being completely oxidized or when plasma glucose is converted into glycogen in muscle (34).

Statistics. Data were analyzed using a two-way repeated-measures ANOVA for experimental condition (CON, DIU) and time (0–60 min). When a significant interaction was found \( P < 0.05 \), the Newman-Keuls post hoc technique was used to determine pairwise differences. A paired t-test was used to analyze area under the curve data for glycerol Rg.

RESULTS

Respiratory gases. Hypohydration had no effect on changes in \( \dot{V}_{O_2} \) and carbon dioxide production (\( \dot{V}_{CO_2} \)) observed during prolonged exercise (Table 1). Exercise led to an increase in both \( \dot{V}_{O_2} \) and \( \dot{V}_{CO_2} \) compared with rest. During exercise, \( \dot{V}_{O_2} \) was higher at 90 min than at 15 min. The respiratory exchange ratio also significantly increased as a result of the exercise; however, DIU had no effect. The loss of PV resulted in an increase in hemoglobin (g/100 ml) from 15.6 ± 0.6 to 17.5 ± 0.6.

Blood metabolite concentration and turnover. Arterialized venous blood lactate concentrations were increased at 15 min of exercise (Fig. 1). No further changes were observed as the exercise progressed; in addition, no differences were observed for DIU compared with CON. Serum FFA decreased during the early phase of exercise but returned to resting levels by 60 min (Fig. 2). As with plasma lactate, DIU had no effect on serum FFA response to exercise compared with CON.

Plasma glucose concentrations remained constant throughout the 60 min of exercise for both conditions (Fig. 3A). Blood glucose was generally higher during both rest and exercise with DIU. Exercise resulted in a progressive rise in glucose Rg (Fig. 3B). Glucose Rg was lower at rest and at 15 and 30 min of exercise and was greater at 60 min of exercise for DIU compared with CON. The metabolic clearance rate of glucose was not different between the experimental conditions. The onset of exercise led to an increase in glucose Rd. No further increases were observed until 60 min of exercise (Table 2).

Table 1. Effects of DIU on \( \dot{V}_{O_2} \), \( \dot{V}_{CO_2} \), and RER during exercise

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}_{O_2} ), l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.47±0.03</td>
<td>2.38±0.07</td>
<td>2.39±0.09</td>
<td>2.56±0.08</td>
</tr>
<tr>
<td>DIU</td>
<td>0.51±0.05</td>
<td>2.43±0.01</td>
<td>2.43±0.08</td>
<td>2.53±0.12</td>
</tr>
<tr>
<td>( \dot{V}_{CO_2} ), l/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.42±0.03</td>
<td>2.30±0.08</td>
<td>2.26±0.08</td>
<td>2.35±0.08</td>
</tr>
<tr>
<td>DIU</td>
<td>0.45±0.05</td>
<td>2.37±0.06</td>
<td>2.35±0.07</td>
<td>2.40±0.08</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.89±0.02</td>
<td>0.97±0.02</td>
<td>0.94±0.02</td>
<td>0.92±0.01</td>
</tr>
<tr>
<td>DIU</td>
<td>0.91±0.05</td>
<td>0.98±0.01</td>
<td>0.97±0.01</td>
<td>0.96±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE \((n = 10\) subjects). \( \dot{V}_{O_2} \), oxygen consumption; \( \dot{V}_{CO_2} \), carbon dioxide production; DIU, diuretic administration; RER, respiratory exchange ratio. Main effect for time \((P < 0.01)\) was found for \( \dot{V}_{O_2} \) and \( \dot{V}_{CO_2} \). For \( \dot{V}_{O_2} \) and \( \dot{V}_{CO_2} \), \( P < 0.01\) at all exercise times.
In general, glycerol concentrations were greater with DIU than in CON (Fig. 4A). Exercise resulted in an increase in plasma glycerol by 30 min of exercise, with further increases observed at 45 and 60 min of exercise. Glycerol Ra was unaffected by DIU (Fig. 4B). Exercise resulted in a general increase in glycerol Ra regardless of condition. The increase, which was first observed at 15 min, was progressive with time. No differences were observed between the two conditions for whole body lipolysis, as estimated from the area under the glycerol Ra curve (Fig. 5).

**Substrate oxidation.** Hypohydration had no effect on whole body CHO or fat oxidation during exercise (Table 3). With the onset of exercise, whole body CHO oxidation increased. Whole body fat oxidation also increased during exercise but only at 60 min. Blood glucose oxidation was different between the two experimental conditions (Table 3). DIU resulted in lower blood glucose oxidation at 15 and 30 min of exercise, but by 60 min of exercise, blood glucose oxidation was not different between DIU and CON. Exercise also led to a progressive increase in blood glucose oxidation in both conditions. Calculated muscle glycogen oxidation was not different between the two conditions (Table 3). In general, exercise led to a decline in muscle glycogen oxidation at 60 min (Table 3).

**Hormones.** In both conditions, exercise resulted in an increase and a decrease in plasma glucagon and insulin, respectively (Table 4). For plasma glucagon, the concentration was greater by the end of exercise (60 min) compared with rest. In contrast, exercise led to a rapid decline in plasma insulin that was evident by 30 min of exercise. No further reduction was observed after 30 min. DIU had no effect on plasma insulin concentration; for plasma glucagon, however, the concentration with DIU was greater than in CON.

**DISCUSSION**

As hypothesized, the diuretic-induced reductions in PV altered the glucoregulatory response to prolonged moderate-intensity exercise. Both plasma glucose Ra and Rd and the rate of glucose oxidation were depressed early in exercise with DIU compared with CON, which was unexpected. However, by 60 min of exercise, these changes were either reversed (Ra) or not statistically different (glucose oxidation). Interest-
ingly, the alterations in blood glucose regulation and metabolism were not accompanied by changes in fat oxidation, whole body lipolysis, and total CHO oxidation. In addition, although no differences were observed for plasma insulin concentration between the two conditions, plasma glucagon was elevated after the reduction in PV. The sympathetic drive also appeared to be increased after DIU, as indicated by the progressive difference in NE concentration that was observed during exercise (35). No differences were found between the two conditions in the EPI response (35). It should be emphasized that, even though the exercise was conducted at the same absolute intensity before and after hypohydration, the relative work load might increase if the PV loss induced a decrease in V̇O₂ peak. At least for diuretic-induced reductions in PV, however, this does not appear to occur (36).

In the current study, we used a model of diuretic administration to induce a reduction in plasma volume. The 4 days of Novotriamazide administration (100 mg triamterene and 50 mg hydrochlorothiazide) led to an ~14.6% decline in resting PV. Hydrochlorothiazide is a moderately potent diuretic that acts on the early distal convoluted tubule, whereas triamterene is a mildly potent diuretic that acts on the late distal convoluted tubule (33). Triamterene is considered a potassium-sparing diuretic that, when used in combination with diuretics like hydrochlorothiazide, prevents the kaliuresis that is normally associated with its (hydrochlorothiazide) use (33). In addition, there is evidence to suggest that the combination of these drugs, taken over both the long term and the short term, have little influence on serum Na⁺, K⁺, Cl⁻, Mg²⁺, Ca²⁺, urate, creatinine, and blood glucose concentrations (25). We have previously used similar doses of hydrochlorothiazide and triamterene that resulted in similar PV losses (41). In the current and the previous studies (41), the diuretic induced an isosmotic hypovolemia. Diuretics act primarily to decrease extracellular fluid volume, both plasma volume and interstitial volume. Other models used to induce hypohydration, such as heat dehydration, exercise, and heat and fluid restriction, all result in an elevation in osmolality (36). With these forms of hypohydration, the hyperosmolality mobilizes fluid from the intracellular to the extracellular space in an attempt to defend against the PV loss (36). As a consequence, the manner in which the reduction in PV is induced may result in different physiological responses during exercise. It is acknowledged that the possibility exists that the effects that we have observed on glucose Ra and Rd may not be due to the hypohydration but could be a direct effect of the diuretic itself.

Table 2. Effect of exercise and DIU on glucose metabolic clearance rate

<table>
<thead>
<tr>
<th>Time, min</th>
<th>CON</th>
<th>DIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.89 ± 0.37</td>
<td>2.03 ± 0.28</td>
</tr>
<tr>
<td>15</td>
<td>6.10 ± 0.72</td>
<td>4.91 ± 0.40</td>
</tr>
<tr>
<td>30</td>
<td>6.41 ± 0.70</td>
<td>5.44 ± 0.53</td>
</tr>
<tr>
<td>45</td>
<td>6.97 ± 0.71</td>
<td>6.28 ± 0.62</td>
</tr>
<tr>
<td>60</td>
<td>7.86 ± 0.69</td>
<td>7.92 ± 1.03</td>
</tr>
</tbody>
</table>

Values are means ± SE in μl·kg⁻¹·min⁻¹; n = 10. Main effect of time (P < 0.01) was found: 0 < 15, 30, 45 < 60 min.

Fig. 4. Blood glycerol concentration (A) and glycerol Ra (B) with exercise and DIU. Values are means ± SE (n = 10). Main effects were found for both time and condition for blood glycerol (P < 0.05): For condition, DIU > CON. For time, 0, 15 < 30 < 45 < 60 min. A main effect for time was found for glycerol Ra (P < 0.05): 0 < 15, 30, 45, and 60 min, 0, 15, 30 < 60 min.

Fig. 5. Whole body lipolytic response during exercise with DIU, expressed as area under the glycerol Ra curve (μmol/kg). Values are means ± SE (n = 10).
Table 3. Effect of exercise and DIU on calculated substrate oxidation

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Carbohydrate oxidation</th>
<th>Fat oxidation</th>
<th>Glucose oxidation</th>
<th>Glycogen oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DIU</td>
<td>CON</td>
<td>DIU</td>
</tr>
<tr>
<td>0</td>
<td>27.8 ± 4.2</td>
<td>32.2 ± 8.6</td>
<td>1.3 ± 0.3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>15</td>
<td>199 ± 12</td>
<td>200 ± 11</td>
<td>3.1 ± 0.7</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>30</td>
<td>186 ± 12</td>
<td>199 ± 12</td>
<td>3.6 ± 0.9</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>60</td>
<td>178 ± 11</td>
<td>182 ± 12</td>
<td>5.1 ± 1.1</td>
<td>6.2 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE in µmol·kg⁻¹·min⁻¹ (n = 10). Glucose oxidation, plasma glucose oxidation; glycogen oxidation, muscle glycogen oxidation. *Significantly different from CON (P < 0.05); †significantly different from 0 min (P < 0.05); ‡significantly different from 15 and 30 min (P < 0.05). Significant main effect for time was found for carbohydrate oxidation, muscle glycogen oxidation, and fat oxidation (P < 0.05). Carbohydrate oxidation, 0 < 15, 30, 60 min; glycogen oxidation, 0 < 15, 30 > 60 min; fat oxidation, 0, 15, 30 < 60 min.

Hypohydration altered the hormonal response to exercise in a manner opposite to what is normally observed with endurance training. With training, there is a significant reduction in the sympathoadrenal drive, as indicated by the reduction in NE concentration, which is most dramatic late in exercise (3). Unlike in the DIU model, there is also a large reduction in the blood Epi response (3). In addition, plasma glucagon is decreased and not increased, as was observed during exercise after DIU (3). Despite these differences, glucose R₉ was significantly lower at rest and at 15 and 30 min of exercise. This suggests that the elevated levels of glucagon did not influence glucose Rₐ, despite suggestions that glucagon is one of the major regulators of hepatic glucose production (24, 39). It appears that the elevated plasma glucose concentration occurred as a result of a decline in the uptake of plasma glucose by the various tissues in the body. Support for this possibility is found with the glucose R₉ data, which demonstrate that glucose R₉ with DIU was attenuated both at rest and early in the exercise (15 and 30 min) compared with CON. It is also possible that the elevated blood glucose level was also involved in inhibiting hepatic glucose output, as has been previously observed when blood glucose levels are elevated (20). Gonzalez-Alonso et al. (13) also observed that exercise-induced dehydration leads to similar alterations in blood glucose concentrations late during prolonged exercise (13). They reported greater increases in the concentration of plasma glucagon with dehydration. Therefore, it appears that diuretic-induced alterations in PV and dehydration both have an impact on blood glucose regulation. However, it should be noted that, in the current study, all measures of blood metabolites and hormones were taken from a peripheral vein and may not reflect concentrations in the portal vein. Finally, other factors have also been implicated in the control of endogenous glucose production (39), and further work is required to determine the mechanisms that are involved in the observed changes.

Generally, the onset of exercise leads to a very rapid and large increase in glucose Rₐ, which then continues to increase at a much more moderate and linear rate of increase as the exercise progresses (3, 24). In the present study, CON demonstrated a linear glucose Rₐ response to continued dynamic exercise; however, DIU led to a curvilinear increase in glucose Rₐ as the exercise progressed. The uptake of glucose as estimated by glucose R₄ followed a similar pattern to what was observed with endogenous glucose production. It appears that, early in exercise, DIU led to a decreased reliance on endogenous glucose, but as exercise continued, there was an almost exponential increase in endogenous glucose production and in glucose uptake. These changes do not appear to be related to alterations in plasma glucose, insulin, and glucagon concentrations. Despite the main effects for both plasma glucagon and glucose, the relative changes with the exercise were similar for both experimental conditions. Therefore, if the alterations in glucose regulation had been due to changes in either plasma glucose or glucagon, a differential response between these variables would have been expected during exercise between CON and DIU.

The alterations in glucose R₉ and R₄ in the current study may have been related to the differences in thermoregulation that were also observed (35). The DIU condition resulted in a greater and more rapid rise in core temperature. It has been previously observed that heat stress significantly alters endogenous glucose production during exercise (21). Specifically, exercise in a higher ambient temperature increased glucose R₉ and respiratory exchange ratio (RER), indicating an increased rate of carbohydrate oxidation (21). That increase in glucose R₉ and RER was associated with an increase in plasma glucagon that was also observed late in exercise with the added heat stress (21). As part of the same study, an increased rate of muscle glycogenolysis was also observed, which supports the notion of a shift toward increased CHO utilization (9).

Table 4. Effect of DIU and exercise on plasma insulin and glucagon

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Insulin, nIU/ml</th>
<th>Glucagon, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>DIU</td>
</tr>
<tr>
<td>0</td>
<td>14.1 ± 1.1</td>
<td>15.8 ± 1.9</td>
</tr>
<tr>
<td>30</td>
<td>7.8 ± 1.1</td>
<td>8.3 ± 0.9</td>
</tr>
<tr>
<td>60</td>
<td>6.4 ± 0.9</td>
<td>7.5 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 10). Main effect for condition was observed for glucagon (P < 0.05), DIU > CON; main effect for time was observed for insulin and glucagon (P < 0.01). For insulin, 0 > 30 and 60 min; for glucagon, 0 and 30 < 60 min.
In the current study, total CHO oxidation did not differ during exercise in DIU compared with CON. Similarly, there was no difference in muscle glycogen oxidation between conditions. In contrast, plasma glucose oxidation, particularly early in exercise, was decreased. Because glucose oxidation represents <15% of the total CHO oxidation, and because the reduction in glucose oxidation was small with DIU, the impact on total CHO oxidation was minimal. With our protocol, fat oxidation represents only a small percentage of the total substrate oxidized. As expected, time-dependent increases in fat oxidation occurred during the exercise. Although not significant ($P = 0.06$), there was a strong trend for fat oxidation to increase and become more pronounced later in the exercise with DIU. A similar effect was also indicated for glucose oxidation. Unfortunately, due to fatigue the exercise could not be sustained for a longer period of time. Although it is tempting to suggest that differences in substrate utilization between conditions might have become more emphasized if the exercise could have been prolonged beyond 60 min, we do not know what would happen.

Abnormal elevations in muscle temperature, which would also be expected to occur with DIU given the increase in core temperature that was observed (35), have been demonstrated to alter muscle metabolism and substrate oxidation (7, 9, 10, 21). Work in the heat, which resulted in elevations in muscle temperature, led to increased rates of muscle glycogenolysis and a decreased reliance on extramuscular substrates (9, 21, 22). Our results appear to be consistent with the decreased reliance on blood substrates, at least for glucose. Interestingly, the fact that we did not find any differences in blood Epinephrine concentrations during exercise between the two conditions suggests that other factors are involved in mediating the increase in glycogenolysis previously observed (9). Increased Epi levels were shown previously to enhance muscle glycogenolysis during exercise (8).

The reduction in PV induced by DIU did not have an effect on glycero Rg, or whole body lipolysis during exercise but did lead to elevated concentrations of plasma glycerol both at rest and during exercise. The trend toward an increase in lipolysis with DIU was not significant. It is noteworthy that rest values for both blood glycerol and lipolysis appear to account for any change observed during exercise between conditions. Because plasma NE was not elevated during this period after PV loss, the elevated glucagon levels appear to be involved. Plasma NE levels were greatly exaggerated late in the exercise (35). However, no changes in either lipolysis or utilization were observed at this time. These observations appear to challenge the role of NE in fat turnover (27). On the other hand, it is possible that measurements of plasma NE concentration have limited significance, given that NE control of lipolysis is based on local release from adipose tissue (39). Collectively, it appears that the reduction in plasma volume may influence lipolysis, possibly through an alteration in the endocrine response to exercise. The increases in blood glycerol concentration with DIU were unexpected, since no changes were observed in glycero Rg. With the reductions in cardiac output discussed earlier, it is possible that splanchnic blood flow could have been reduced with DIU. A reduction in flow could result in a decline in glyceral clearance, as the liver is the primary site of glyceral uptake (4, 26).

In summary, we observed that reductions in PV mediated by diuretic administration altered glucose kinetics during prolonged exercise of moderate intensity. The specific effect depended on the time of exercise. Early in exercise, plasma glucose release from the liver and utilization by muscle decreased, whereas late in exercise these effects were reversed. These changes occurred in the absence of changes in whole body lipolysis and fat oxidation. Hypohydration would appear to be an important factor in understanding the changes in control of substrate turnover and oxidation that occur with exercise and training.

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