BRECHUE, W. F., J. M. STAGER, AND H. C. LUKASKI. Body water and electrolyte responses to acetazolamide in humans. J. Appl. Physiol. 69(4): 1397-1401, 1990.—Acetazolamide (ACZ), a potent carbonic anhydrase inhibitor, is a known diuretic and causal agent in metabolic acidosis. Its diuretic qualities are well established with respect to urine flow and electrolyte excretion. However, the impact of ACZ on body hydration status has not been adequately quantified. Thus, to establish the influence of ACZ treatment on body water, nine healthy males were evaluated for hydration status after clinically prescribed doses of ACZ. The drug was administered in three 250-mg oral doses at 14, 8, and 2 h before determination of body water compartments. ACZ led to a significant 1.7-liter reduction in total body water (3.4%). A significant reduction in extracellular water of 3.3 liters is partitioned as the loss of total body water and a significant increase in intracellular water (1.6 liters). Venous blood pH and plasma HCO₃⁻ were significantly reduced 0.09 units and 5.9 mM, respectively, with ACZ. Plasma protein concentration was increased, but plasma osmolality did not change. Plasma Na⁺, K⁺, and Cl⁻ concentrations were not different with ACZ, but total electrolyte content was significantly decreased 45.2, 1.17, and 44.1 meq, respectively, for all three. Urine K⁺, HCO₃⁻, flow, and pH were elevated after ACZ treatment, whereas Na⁺ and Cl⁻ were the same as placebo levels. In conclusion, acute clinical doses of ACZ reduce body fluid compartments, leading to a moderate isosmotic hypovolemia with an intracellular volume expansion as well as metabolic acidosis.

INHIBITION of carbonic anhydrase with acetazolamide (ACZ) leads to increased urinary excretion of HCO₃⁻ and fixed cations (Na⁺ and K⁺) and decreased urinary excretion of titratable acid and ammonium (21). The loss of base precedes the development of metabolic acidosis, an effect that is well established (21). Urine volume is increased during acute carbonic anhydrase inhibition as a result of an osmotic effect of electrolyte loss (21). Beyond measurement of urine flow and changes in electrolyte patterns following ACZ administration, little is known about the impact of this drug on hydration status.

Presently, one clinical use for ACZ is as a prophylaxis for acute mountain sickness (AMS) (18). The aided acclimatization to altitude due to reduction or prevention of debilitating AMS symptoms allows susceptible individuals to perform work or recreational activity immediately on arrival at altitude. However, based on changes in body weight, hematocrit, and hemoglobin (23; unpublished data), it is suggested that acute doses of ACZ, such as those generally prescribed for AMS, may lead to a moderate dehydration before altitude exposure. Reductions in hydration status are known to impair cardiovascular and thermoregulatory function during exercise and ultimately limit submaximal exercise (12, 13, 22). Acute ACZ treatment reduces the ability to sustain normoxic and hypoxic submaximal exercise by ~30% (29). The mechanisms for this reduction are presently unknown but may be related to dehydration. Thus the purpose of the present investigation was to characterize hydration status after acute doses of ACZ and to evaluate the possible implications of such treatment on exercise tolerance.

METHODS

Nine healthy active males, aged 30.3 ± 5.1 (SD) yr, height 183.1 ± 6.3 cm, and weight 81.53 ± 11.0 kg, participated in the study. Each subject received a written and oral explanation of the procedures and was informed of potential risks and benefits in accordance with the Indiana University Committee for the Protection of Human Subjects. All subjects gave informed consent of the previously approved protocol before commencement of testing.

Experimental Protocol

The experimental protocol is summarized in Fig. 1. Body water compartments and blood and urine electrolytes were determined at time 0 (t = 0) after oral ingestion of either a placebo or ACZ. ACZ was administered in three 250-mg oral doses given at 6-h intervals. The last dose was administered 2 h before the determination of body water compartments. Treatments (placebo or ACZ) were administered in a repeated-measures crossover design. Treatment order was randomized and carried out in double-blind fashion. Flour placebos and ACZ were packaged in identical gelatin capsules to conceal their identities. All treatments (placebo or ACZ) were separated by ≥1 wk and were carried out at the same time of day. Compliance with treatment protocols was verified by the establishment of a metabolic acidosis and elevated urine pH.

All experiments were conducted in a normoxic thermoneutral environment (ambient temperature 25 ±
HCO₃⁻ was calculated from and was calculated as the difference between total body and extracellular volumes. Interstitial water was calculated as the corrected bromide space volume.

Measurements

Body water compartments were determined from estimates of total body water, extracellular water, and blood volume.

Total body water. Total body water was estimated by electrical impedance (20). The BIA-102 electrical impedance analyzer (RJL Systems, Detroit, MI) and the tetrapolar electrode arrangement were used (19). All measurements were made in the supine position after 15 min of rest in that position. The BIA-102 system was calibrated before each measurement with a simple resistance-capacitance circuit. The equation of Lukaski et al. (20) was used to calculate total body water from height, weight, age, and total body resistance.

Extracellular water. Extracellular water was estimated by sodium bromide dilution (28). Sodium bromide (Sigma Chemical, St. Louis, MO) was given orally at 0.15 ml/kg of a 3% (wt/vol) solution in 200 ml of distilled deionized water at t = -4 h. Venous blood samples were collected at t = -4 h and t = 0 h. All urine voided during the equilibration period was collected, pooled, and analyzed for bromide concentration to correct for excreted tracer. Assays for plasma and urine bromide levels were performed by fluorescent excitation analysis (28). Extracellular water was calculated as the corrected bromide space according to Lukaski and Bolonchuk (19). Technical and analytic error of the method is <1%.

Intracellular and interstitial water. Intracellular water was calculated as the difference between total body and extracellular volumes. Interstitial water was calculated as the difference between extracellular and plasma volumes.

Blood volume. Blood volume was determined by CO rebreathing (26). Subjects reported to the laboratory and rested in a seated position for 30 min before blood sampling and rebreathing. Subjects rebreathed 50 ml CO diluted in 5 liters 100% O₂ for 15 min. O₂ was added to replace the volume consumed. Before and after the 15-min rebreathing period a 1-ml venous blood sample was collected from an antecubital vein for spectrophotometric (Radiometer OSM 3) determination of total hemoglobin and carboxyhemoglobin. Hematocrit was determined in quadruplicate by microcapillary centrifugation, and values were corrected for trapped plasma and venous-to-whole body hematocrit ratios (7), 1-2%. Total body hemoglobin and blood volume were calculated according to Dahms and Horvath (8). Plasma volume was calculated from blood volume and hematocrit. Changes in blood and plasma volume were estimated from changes in hematocrit and hemoglobin (9).

Blood variables. A 10-ml blood sample and a 15-ml blood sample were collected from an antecubital vein without stasis at t = -4.5 h and t = 0, respectively. The samples were added to plastic heparinized tubes to prevent coagulation. One milliliter of whole blood from only the t = 0 blood sample was analyzed for blood gases, pH, hematocrit, and hemoglobin. The t = -4 h sample and the remainder of the t = 0 blood sample were centrifuged for 15 min. A 5-ml aliquot of the plasma was drawn off and stored at -70°C until it was analyzed for sodium bromide levels. The remainder of the plasma was placed on ice, and within 3 h of sampling it was used for determination of electrolytes and osmolality. Plasma Na⁺ and K⁺ concentrations were determined by flame photometry, plasma Cl⁻ concentrations by a chloridometer (Buchler-Cotlove, Buchler Instruments, Fort Lee, NJ), plasma osmolality by vapor pressure depression, and total proteins by refractometry.

Urine variables. Subjects emptied their bladders at t = -2 h, and the urine was added to the isotope equilibration pool, as described above for extracellular water determination. An aliquot of the urine collected between t = -2 h and t = 0 was sampled for electrolyte determination. At t = 0, a 5-ml aliquot was sampled from the isotope equilibration pool and stored at -70°C until analysis for sodium bromide. All urine was collected under oil and stored on ice during the experiment.

Urine volume was recorded, and pH was determined by glass pH electrode. Urine HCO₃⁻ was calculated from total CO₂ measured manometrically. Urine Na⁺, K⁺, and Cl⁻ were measured as described for plasma. Urinary electrolyte output between t = -2 h and t = 0 was calculated by multiplying urine electrolyte concentration by the urine volume.

Statistics

Dependent variables were tested for statistical difference by analysis of variance for repeated measures with the SPSS-X statistical package. Family-wise type I error rate was set at 0.05 and controlled during multiple F tests by correcting the per comparison error rate with Bonferroni's method.
the modified Bonferroni technique (15). Bonferroni adjustments resulted in an alpha level of 0.04 for omnibus F tests. Post hoc analyses were performed by simple main effects. All data are reported as means ± SE.

RESULTS

Body Water Compartments

Body weight decreased 2.1 kg (2.5%) after ACZ treatment. ACZ treatment led to a 1.7-liter (3.4%) decrease in total body water and a 3.3-liter (21%) decrease in extracellular water. The loss of extracellular water is partitioned into a 2.98-liter (27%) loss of interstitial water and a 0.32-liter (8.8%) loss of plasma water. Part of the extracellular water loss is accounted for by a 1.6-liter shift into the intracellular space. All changes in body water compartments with ACZ were statistically significant. Body water compartments are summarized in Fig. 2.

Blood Variables

ACZ resulted in a metabolic acidosis marked by significant decreases in venous blood pH of 0.09 units and plasma HCO₃⁻ of 5.9 mM. Hematocrit and hemoglobin increased significantly, 7.9 and 4.5%, respectively. Total protein concentration in plasma increased 7.5% after ACZ treatment. The classical response to carbonic anhydrase inhibition leads to a diuresis (21). It is unknown which fluid compartments are significantly affected by ACZ treatment. The blood data are summarized in Table 1.

Urine Variables

ACZ led to significant increases in urine volume (47%) and pH (1.47 units). Urinary excretion of HCO₃⁻ and K⁺ was significantly elevated 90 and 68%, respectively, with each successive dose causing less of a renal response (21). By the third or fourth dose (dose/8 h) urinary Na⁺ and HCO₃⁻ outputs are back to baseline levels. The magnitude of base depletion is similar after a single dose, several doses in 1 day, or repetitive daily doses in that subsequent doses of ACZ prevent recovery of initial base loss without further or additional base decrement (21). This is due to a carbonic anhydrase-independent route of HCO₃⁻ reabsorption (27). Cl⁻ output is not affected by carbonic anhydrase inhibition (21). Urinary K⁺ excretion remains elevated until the fourth or fifth dose, 32-40 h (21).

In the present study, venous plasma HCO₃⁻ and pH were reduced by 6 mM and 0.09 pH units, respectively, after ACZ treatment, in good agreement with previous

![Figure 2: Body water changes following acetazolamide. Open bars, placebo trial; hatched bars, ACZ trial. TBW, total body water; ECW, extracellular water; ISF, interstitial fluid; PV, plasma volume; ICF, intracellular fluid. Values are means ± SE. *Statistical difference, P ≤ 0.04.](image)
studies in dogs (21) and humans (23) and indicative of a metabolic acidosis. Urinary \( \text{HCO}_3^- \) excretion was elevated after ACZ compared with placebo levels. The repetitive dose schedule used here is within the time and dose magnitude in which renal \( \text{HCO}_3^- \) response is still possible. Because the magnitude of blood pH and plasma \( \text{HCO}_3^- \) changes is in the range of previous reports, it is assumed that the blood pH response had peaked.

Urinary electrolyte output was similar to previously reported responses in dogs (21) and humans (23) with repetitive doses of ACZ. Na\(^+\) and Cl\(^-\) outputs after ACZ were not different from placebo levels. Urinary K\(^+\) output was significantly greater than control after ACZ treatment. This is in agreement with previous work where K\(^+\) excretion remained elevated for up to 40 h with repeated ACZ doses (21).

ACZ administration significantly reduced body weight 2.5%. This is in agreement with previous reports of changes in body weight after acute ACZ treatment (23; unpublished work). Using body weight as an indirect assessment of total body water (TBW), one might suspect that ACZ leads to significant dehydration. In the present study, ACZ led to a 1.7-liter decrease in TBW, which differs from that predicted by body weight (2.1 kg). These differences are probably due to the analytic errors in the equations used to estimate body water from whole-body impedance and variations between the population used in the present study and that used to determine the prediction equations. Water compartment calculations and conclusions will be based on the TBW value, which may underestimate actual changes in hydration status based on body weight.

ACZ resulted in a 3.4% reduction of TBW and a 21% decrease in extracellular water (ECW). Other regimes of dehydration have resulted in similar compartmental changes. The decrease in TBW is similar in magnitude to reductions following thermal dehydration in a sauna (17), long-term intense exercise (11), or exercise in the heat (6). With 1.5- to 3-liter decreases in body water, thermal dehydration and exercise have been shown to reduce ECW between 0.8 and 1.5 liters (6, 17, 25). These decrements in ECW are less than one-half of the reduction found with ACZ at the same total water loss. Thus, differences must exist in the intracellular water (ICW) response. ICW was increased 1.6 liters with ACZ, contrasting the 0.6- to 2.2-liter decrease in ICW with thermal and exercise dehydration (17). Given the same TBW loss as dehydration and ACZ, thiazide and loop diuretics significantly decrease ECW but with a small increase in muscle ICW (3). Changes in plasma volume are quite similar (9-14%) with ACZ, dehydration (6, 12, 17), and diuretic therapy (1, 3) when TBW loss is ~2.4%.

The striking difference between ACZ and other dehydrating regimens is the intracellular fluid (ICF) response. During thermal or exercise dehydration there is increased sweating, which promotes the loss of water and NaCl from blood (25). To maintain blood volume, fluid is shifted from intracellular to intravascular spaces. This shift is mediated by an osmotic gradient between extracellular fluid (ECF) and ICF and is determined by the amount of Na\(^+\) and free water loss in sweat and the plasma protein concentration (25). Additionally, K\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\) loss from the intracellular space would potentiate this osmotic gradient (25, 30).

ICW would not be expected to decrease with ACZ administration because plasma osmolality and total circulating proteins were not significantly different from controls. Likewise, the weak saluretic effects of ACZ result in a minimal urinary loss of Na\(^+\) and Cl\(^-\). ACZ is also known to be a weak diuretic; yet it possess one of the greatest kaliuretic responses of any diuretic. This kaliuretic effect results in an intracellular depletion of K\(^+\) because urinary loss is greater than plasma loss (11). During periods of K\(^+\) depletion, K\(^+\) loss from the cell is matched by increased cellular Na\(^+\) and H\(^+\) uptake, 2 Na\(^+\) and 1 H\(^+\) to 3 K\(^+\) (5). This suggests that there may be a Na\(^+\) shift into the cells during ACZ treatment. This would account for the loss of plasma Na\(^+\) without a sustained renal excretion. Muscle, heart, kidney, and liver substantially increase their Na\(^+\) concentration during ACZ treatment (11). The loss of plasma Cl\(^-\) would also be explained by an extracellular to intracellular shift, perhaps following Na\(^+\). Thus the shift of ECF to ICF would follow a significant NaCl shift into the cell. Other more potent diuretics, the thiazides and furosemide, result in smaller increases in ICW during maximal diuresis (3). These diuretics result in smaller NaCl uptake by the cell than with ACZ. This is probably related to the lesser kaliuretic responses and less K\(^+\) loss by the cell. Additionally, the greater renal loss of NaCl and water than seen with ACZ would set a condition much like that of thermal dehydration.

Acute ACZ treatment results in several alterations that have implications for exercise tolerance. ACZ has been shown to decrease normoxic and hypoxic submaximal endurance (29) by unknown mechanisms. The body water losses with ACZ are of the magnitude, shown previously, to impair cardiovascular and thermoregulatory function during exercise (12, 17, 22). Likewise, increases in ICW also lead to greater rates of fatigue during exercise (2). After 1-4% decreases in TBW, submaximal exercise time to exhaustion was reduced 19% (24) while 10-km race times were increased 2.62 min (1). Acid-base status affects exercise (14), and the ACZ-induced metabolic acidosis could limit exercise tolerance. Furthermore, the depletion of K\(^+\) can lead to reduced glycogen synthesis (4) and can affect local blood flow (16).

In conclusion, acute ACZ treatment leads to significant decrements in TBW and ECW and plasma volume and a significant increase in ICW. These decreases are isosmotic and are without changes in plasma electrolyte concentrations. It appears that the volume of water loss is sufficiently large and could affect the ability to sustain submaximal exercise thermoregulation and, ultimately, exercise tolerance. Furthermore, alterations in acid-base status and K\(^+\) stores may also have a role in a reduced exercise tolerance by affecting local blood flow, muscle energy stores, or muscle contractility.

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REFERENCES


