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Saliva Parameters as Potential Indices of Hydration Status during Acute Dehydration

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ABSTRACT

WALSH, N. P., S. J. LAING, S. J. OLIVER, J. C. MONTAGUE, R. WALTERS, and J. L. J. BILZON. Saliva Parameters as Potential Indices of Hydration Status during Acute Dehydration. *Med. Sci. Sports Exerc.*, Vol. 36, No. 9, pp. 1535–1542, 2004. **Purpose:** Firstly, to identify whether saliva flow rate, osmolality, and total protein are potential markers of hydration, we compared changes in these parameters with changes in plasma osmolality during progressive dehydration. Secondly, we compared the sensitivity of saliva parameters to track hydration changes with the sensitivity of urine osmolality. Thirdly, to test the hypothesis that dehydration, rather than neuroendocrine regulation, is responsible for the decrease in saliva flow rate during prolonged exercise, we compared flow rate and catecholamine responses to prolonged exercise with and without fluids. **Methods**: Fifteen males (plasma osmolality 289 ± 4 mOsmol·kg^{-1}; mean ± SD) exercised (30°C, 70% RH) with no fluid intake (NFI) until body mass loss (BML) of 1.1, 2.1, and 3.0% and on another occasion with fluid intake (FI) to offset losses. **Results**: Plasma and urine osmolality increased during NFI (plasma osmolality 3.0% BML: 298 ± 4 mOsmol·kg^{-1}; $P < 0.01$). Saliva flow rate decreased ($P < 0.01$), saliva total protein increased ($P < 0.01$), and saliva osmolality increased from preexercise (50 ± 11 mOsmol·kg^{-1}) to 3.0% BML (105 ± 41 mOsmol·kg^{-1}) during NFI ($P < 0.01$). Saliva osmolality, urine osmolality, and saliva total protein correlated strongly with plasma osmolality during dehydration (r 0.87, 0.83, and 0.91, respectively; $P < 0.01$). During the FI trial, saliva flow rate and osmolality remained unchanged. Plasma catecholamine concentration increased during exercise ($P < 0.01$) with no difference between trials. **Conclusions**: Saliva osmolality and total protein appear to be as sensitive as urine osmolality to track hydration changes during hypertonic-hypovolemia. These results also suggest that dehydration has a greater involvement in the decrease in saliva flow rate during prolonged exercise than neuroendocrine regulation. **Key Words:** EXERCISE, HYPERTONIC-HYPOVOLEMIA, SALIVARY, MARKERS

Fluid losses of as little as 2–3% body mass have been associated with reduced heat dissipation, cardiovascular function, and exercise performance (1,5). Therefore, for a marker of hydration status to be of use it must be able to identify body water losses equivalent to 2–3% body mass (27). The best method for assessing hydration status involves expensive stable isotope methodology, which has obvious practical limitations (22). Whereas body weight changes may be used to assess acute changes in hydration status during a single exercise bout (27), over longer periods of time body weight changes may be influenced by food intake, fluid intake, fecal losses, urine production, body weight changes associated with substrate metabolism, and changes in body composition (19). More widely accepted and frequently used markers of hydration status include hematological (20,27) and urinary (2,14,20) parameters. Hematological markers require an invasive blood sample, and urinary markers track changes in hydration status with varied success (2,14,20).

The main purpose of the present study was to assess the utility of noninvasive saliva parameters to track hydration status during progressive acute dehydration. Saliva flow rate, osmolality, and total protein concentration have recently been shown to track changes in body mass during progressive acute dehydration (30). A progressive decrease in saliva flow rate and increase in saliva osmolality and total protein concentration were observed during acute dehydration evoked by a combination of exercise and heat stress (30). However, for saliva parameters to be considered as markers of hydration status, they must be shown to correlate strongly with plasma osmolality, a widely accepted hydration index, which is known to quantitatively reflect changes in hydration during dehydration evoked by exercise and heat stress (hypertonic-hypovolemia) (20,21). A neuroendocrine role (increase in sympathetic nervous system activity) is most often proposed (7,10), and a role for hypohydration (30) has recently been proposed to explain the secretion of smaller amounts of more concentrated saliva (e.g., with increased osmolality and total protein concentration) after exercise. An increase in sympathetic nervous system activity may explain the decrease in saliva flow rate during

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prolonged exercise by causing vasoconstriction of the blood vessels to the salivary glands (7,10). However, β-blockade by propanolol had no effect on the saliva flow rate response to submaximal exercise designed to elicit cardiovascular activity consistent with β-adrenergic activation (32). Furthermore, studies using combined exercise and fluid restriction (4) and exercise in a hot environment (13) show large reductions in saliva flow rate that were prevented when subjects received sufficient water to offset fluid losses during exercise. These studies suggest a more likely role for dehydration *per se*, than a neuroendocrine role, in the decrease in saliva flow rate with prolonged exercise. To test this hypothesis, we examined saliva flow rate and plasma catecholamine responses to prolonged exercise performed with and without fluid restriction.

As the predominant fluid constituent of saliva is water (97–99.5%), which enters saliva from plasma across acinar cells, it follows that hypohydration may cause salivary gland hypofunction (25). In order for water to move from plasma through acinar cells to form primary saliva, a trans-acinar cell sodium gradient must be generated. During hypertonic-hypovolemia, the extracellular fluid sodium concentration increases and this is reflected in a graded increase in plasma osmolality with progressive dehydration (20,21). As a result, a greater sodium concentration must be generated across the salivary acinar cell to drive fluid into the acinar lumen to form primary saliva. An increase in extracellular sodium concentration during hypertonic-hypovolemia might therefore result in the production of smaller amounts of more concentrated saliva. This mechanism may account for the decrease in saliva flow rate during water deprivation (6,25,26), intense thirst (6), after hot water immersion (31), and after prolonged exercise with a fluid deficit (4,29,30). In addition, this mechanism may also explain why saliva flow rate is unchanged after prolonged exercise with sufficient water to offset fluid losses (4). If changes in the extracellular sodium concentration influence saliva output as described, then a progressive increase in plasma osmolality during hypertonic-hypovolemia should be associated with the production of smaller amounts of more concentrated saliva. Our specific objectives were therefore: 1) to identify whether saliva flow rate, osmolality, and total protein concentration are potential markers of hydration status by comparing changes in these parameters with changes in plasma osmolality during progressive acute dehydration; 2) to compare the sensitivity of saliva parameters to track changes in hydration status with the sensitivity of urine osmolality, a regularly used hydration marker; and 3) to test the hypothesis that dehydration, rather than neuroendocrine regulation, is responsible for the decrease in saliva flow rate during prolonged exercise by reporting saliva flow rate and plasma catecholamine responses to prolonged exercise performed with and without fluid restriction.

METHODS

Subjects. Fifteen healthy males (mean ± SD: age, 23 ± 3 yr; height, 180 ± 4 cm; body mass, 73.9 ± 3.9 kg; lean body mass 62.7 ± 3.7 kg; body fat 14.9 ± 4.2%; $\dot{V}O_{2max}$ 57.3 ± 9.5 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. Volunteers were all nonsmokers and had no significant oral, dental, or systemic disease and were not taking any prescription or nonprescription medication at the time of the study. All subjects gave written informed consent before the study, which received local ethics committee approval.

Preliminary measurements. Maximal oxygen uptake was estimated by means of a continuous incremental exercise test to volitional exhaustion on a stationary cycle ergometer (Monark 814e, Varberg, Sweden) (29). After a 5-min warm-up at 70 W, subjects began cycling at 175 W, with increments of 35 W every 3 min. Expired air was analyzed continuously using an on-line breath-by-breath system (Cortex Metalyser 3B, Biophysik, Leipzig, Germany) to determine $\dot{V}E$, $\dot{V}O_2$, and $\dot{V}CO_2$. From the $\dot{V}O_2$–work rate relationship, the work rate equivalent to 60% $\dot{V}O_{2max}$ was interpolated. Before beginning the experimental trials, subjects cycled at 60% $\dot{V}O_{2max}$ in the heat (30°C and 70% relative humidity) without fluids until 3% body mass loss (BML) to estimate whole body sweating rate and exercise duration for the experimental protocol.

Experimental procedures. Subjects reported to the laboratory on two occasions, separated by 7 d, at 08:00 h after an overnight fast. On the morning of the trial, subjects were not permitted to perform any oral hygiene or consume any water before arrival at the laboratory. To ensure subjects arrived at the laboratory in a euhydrated state, they were given 30 $mL \cdot kg^{-1}$ body mass of bottled water to drink between 09:00 and 21:00 h the day before the trial (3). Subjects emptied their bladder and bowels, and baseline nude body mass was recorded on a digital platform scale to the nearest 50 g (Model 705, Seca, Hamburg, Germany). Dual energy x-ray absorptiometry (Hologic, QDR1500, software version V5.72, Bedford, MA) was used to determine lean and fat mass.

Dressed in shorts and shoes, subjects cycled on a stationary cycle ergometer (Monark 814e) in an environmental chamber (Delta Environmental Systems, Chester, UK) maintained at a dry-bulb temperature of 30°C and 70% relative humidity. The exercise in the environmental chamber consisted of 10-min of cycling at a power output corresponding to 60% $\dot{V}O_{2max}$. Subjects then left the chamber for a 5-min rest period during which they removed their clothing, dried with a towel, and were weighed nude to monitor BML. Subjects repeated the 10-min of exercise in the chamber followed by 5-min rest periods in the laboratory on one occasion with no fluid intake (NFI) until progressive BML of 1, 2, and 3% and on another occasion with fluid intake as water to offset fluid losses (FI). The order of trials was randomized ($N = 8$ subjects completed the NFI trial first). The exercise duration, which was the same in both trials, and the volume of water required to offset fluid losses on the FI trial were determined during preliminary measurements. During the FI trial, subjects received equal volumes of water immediately after exercise was begun and at 15-min intervals thereafter. Subjects received no food during

the NFI or FI trial. Heart rate (Polar Electro, Kempele, Finland) and rectal temperature (T_{rec}: Edale, Cambridge, UK) were monitored continuously during exercise to ensure that subjects did not exceed 180 bpm or 39.5°C, respectively. Expired air was analyzed for 3 min (7–10 min) during each period of cycling in the environmental chamber using an on-line breath-by-breath system (Cortex Metalyser 3B). Saliva, blood, and urine samples were collected at preexercise, 1, 2, and 3% BML on the NFI trial and pre- and postexercise on the FI trial. Additional saliva, blood, and urine samples were collected at 75, 135, and 195 min postexercise on the NFI trial. Saliva and blood samples were collected at least 15 min after fluid consumption after subjects had remained seated for 5–10 min. After exercise on the NFI trial, subjects were given a volume of carbohydrate (CHO)-electrolyte solution (6% CHO and 25 $mmol \cdot L^{-1}$ Na+) equivalent to 25% BML to consume between 0–15, 15–30, and 30–45 min in both the first and second hour of recovery (total drink volume: 150% BML).

Saliva collection and analysis. Unstimulated whole saliva samples were collected over a 2-min period using preweighed salivette tubes (Sarstedt, Leics, UK) according to the manufacturer's instructions (29). All saliva samples were collected while the subjects sat quietly in the laboratory in temperate conditions. Each subject was asked to swallow in order to empty the mouth before saliva was collected. The saliva sample was collected by the subject placing the polyester salivette swab (diameter 1 cm, length 2.5 cm) under the tongue for exactly 2 min with minimal orofacial movements during the collection. The polyester swab was replaced in the inner snap seal container and back into the salivette outer centrifuge tube. Saliva volume was estimated by weighing the salivette tube immediately after collection to the nearest milligram, and saliva density was assumed to be 1.00 $g \cdot mL^{-1}$ (9). From this, the saliva flow rate was determined by dividing the volume of saliva by the collection time. The salivette centrifuge tube was then stored at −40°C. After thawing, the salivette centrifuge tube comprising the inner snap seal container and the polyester swab was spun at 3000 × *g* for 5 min at room temperature allowing the collection of saliva in the bottom of the tube for analysis. Osmolality measurements on saliva were made using a freezing point depression Osmometer (Model 3 MO, Advanced Instruments, Massachusetts). Saliva total protein concentration was measured on a spectrophotometer using a commercially available kit (Kit No. 610, Sigma, Poole, UK) using protein standards supplied with the kit. Saliva total protein secretion rate and solute (electrolyte) secretion rate were calculated by multiplying saliva flow rate by saliva total protein concentration and osmolality, respectively. The intra-assay coefficient of variation was 2.0% for osmolality and 4.0% for total protein concentration.

Blood and urine collection and analysis. Blood samples were collected without venostasis from an antecubital vein into separate Vacutainer tubes containing lithium heparin and K_3EDTA (Becton Dickinson, Oxford, UK). Blood in the tube containing K_3EDTA was used to determine hemoglobin concentration in triplicate using a hematology analyzer (Beckman Coulter Gen S, Fullerton, U.S.). Hematocrit was determined in triplicate using the capillary method, and plasma volume changes were estimated according to Dill and Costill (11). Blood samples were spun at 1500 × *g* for 10 min in a refrigerated centrifuge to obtain plasma which was immediately stored at −40°C. Urine samples were collected into universal containers and frozen at −40°C. Osmolality measurements on heparinized plasma and urine were made using a freezing point depression Osmometer (Model 3 MO, Advanced Instruments). Plasma epinephrine and norepinephrine concentrations were determined on K_3EDTA plasma ($N = 8$) using ELISA kits (Labor Diagnostica Nord, Nordhorn, Germany). The intra-assay coefficient of variation was 2.0, 6.9, and 9.8% for osmolality, epinephrine, and norepinephrine concentration, respectively.

Statistical analysis. Data in the text and figures are presented as mean ± SD. The sample size was estimated to be $N = 12$ (8) using previous data examining the effects of dehydration on saliva parameters (30). We estimated the effect size for the decrease in saliva flow rate during dehydration to be 0.9–1.2. Alpha and power levels were set at 0.05 and 0.8, respectively, both of which are standard estimates. To allow for dropout, we recruited $N = 15$ subjects. The NFI trial data were examined using one-way repeated measures ANOVA (seven sample collections) with significant differences assessed by applying the *post hoc* Tukey test. Assumptions of homogeneity and sphericity in the data were checked, and, where appropriate, adjustments in the degrees of freedom for the ANOVA were made. A two-way repeated measures ANOVA was used to compare NFI and FI trial data at pre- and postexercise (two trials × two sample collections) with significant differences assessed by applying the *post hoc* Tukey test. For each subject a Pearson's correlation coefficient was calculated between plasma osmolality (criterion) and a predictor variable (e.g., saliva flow rate). Each Pearson's correlation coefficient was then converted using Fisher's Zr transformation to result in a more normal distribution. The mean Fisher's Zr was converted into the Pearson's correlation coefficient (mean r) using appropriate statistical tables (28). Based on the sample size and number of Pearson's correlations conducted, an adjustment was made to the value at which the mean correlation coefficient was deemed significant (24). Differences between mean correlation coefficients were determined using an adjusted z score equation (17). Statistical significance was accepted at $P < 0.05$.

RESULTS

At 1, 2, and 3%, target BML subjects reached an average BML of 1.1 ± 0.1 (range = 0.9–1.3%), 2.1 ± 0.1 (range = 1.9–2.3%), and 3.0 ± 0.1% (range = 2.7–3.2%) on the NFI trial. At postexercise on the FI trial, a BML of 0.2 ± 0.4% was recorded. It took on average 38 ± 9 min to reach 1.1% BML, an additional 33 ± 14 min to reach 2.1% BML, and an additional 29 ± 9 min to reach 3.0% BML. Subjects lost on average 2.2 ± 0.1 kg body mass to achieve 3.0% BML in 100 ± 24 min on the NFI trial. There was no significant

difference in T_{rec} at preexercise on the NFI trial compared with the FI trial (37.1 ± 0.2 and 37.1 ± 0.2°C, respectively). T_{rec} increased during exercise (main effect of time: $F_{(1.0, 11.0)} = 172$, $P < 0.01$). T_{rec} was significantly elevated immediately postexercise compared with preexercise on the NFI trial (interaction: $F_{(1.0, 11.0)} = 14.8$, $P < 0.01$) but was not significantly different from immediately postexercise on the FI trial (NFI: 38.5 ± 0.4; FI: 37.9 ± 0.3°C). Fluid provision during the FI trial blunted the increase in heart rate observed on the NFI trial during exercise (NFI: 173 ± 10; FI: 155 ± 11 beats·min^{-1}; interaction: $F_{(1.0, 12.0)} = 7.7$, $P < 0.05$; final exercising heart rate).

Plasma volume change and plasma osmolality. Plasma volume decreased by 5.0 ± 2.0% at 3.0% BML on the NFI trial (main effect of percent BML: $F_{(3.0, 36.0)} = 26.1$, $P < 0.01$). Plasma volume did not change significantly on the FI trial (+0.2 ± 3.2%). At preexercise on the NFI trial, plasma osmolality was 289 ± 4 (range 284–297) mOsmol·kg^{-1}. Plasma osmolality increased during progressive dehydration on the NFI trial (main effect of % BML: $F_{(3.5, 49.2)} = 26.4$, $P < 0.01$; Fig. 1A). *Post hoc* analysis revealed that plasma osmolality was significantly greater than preexercise by 2.1% BML ($P < 0.01$). At 3.0% BML on the NFI trial, plasma osmolality reached 298 ± 1 (range 293–306) mOsmol·kg^{-1}. The rehydration protocol reestablished preexercise plasma osmolality levels by 75 min postexercise on the NFI trial. Plasma osmolality was not significantly different at preexercise on the FI trial compared with the NFI trial. On the FI trial, postexercise plasma osmolality decreased compared with preexercise ($P < 0.01$) and was significantly lower than on the NFI trial at postexercise (interaction: $F_{(1.0, 14.0)} = 66.6$, $P < 0.01$).

Urine osmolality. At preexercise on the NFI trial urine osmolality was 353 ± 237 (range 89–704) mOsmol·kg^{-1}. Urine osmolality increased during progressive dehydration on the NFI trial (main effect of % BML: $F_{(6.0, 60.0)} = 47.1$, $P < 0.01$; Fig. 1B). *Post hoc* analysis revealed that urine osmolality was significantly greater than preexercise by 2.1% BML ($P < 0.01$). At 3.0% BML on the NFI trial, urine osmolality reached 728 ± 233 (range 451–955) mOsmol·kg^{-1}. It is noteworthy that two subjects could not provide a urine sample at 2.1% BML and three subjects could not provide a urine sample at 3.0% BML on the NFI trial. All 12 urine samples collected at 3.0% BML gave greater osmolality readings compared with preexercise. Urine osmolality remained significantly elevated at 75 min postexercise ($P < 0.01$) and was significantly lower than preexercise at 135 and 195 min postexercise on the NFI trial ($P < 0.01$). Urine osmolality was significantly greater at preexercise on the FI trial compared with the NFI trial ($P < 0.05$). On the FI trial, postexercise urine osmolality decreased compared with preexercise ($P < 0.01$) and was significantly lower than on the NFI trial at postexercise (interaction: $F_{(1.0, 8.0)} = 46.6$, $P < 0.01$).

Saliva flow rate. At preexercise on the NFI trial, saliva flow rate was 435 ± 320 (range 71–993) μL·min^{-1}. Saliva flow rate decreased during progressive dehydration on the NFI trial (main effect of % BML: $F_{(3.2, 44.9)} = 14.4$, $P <$

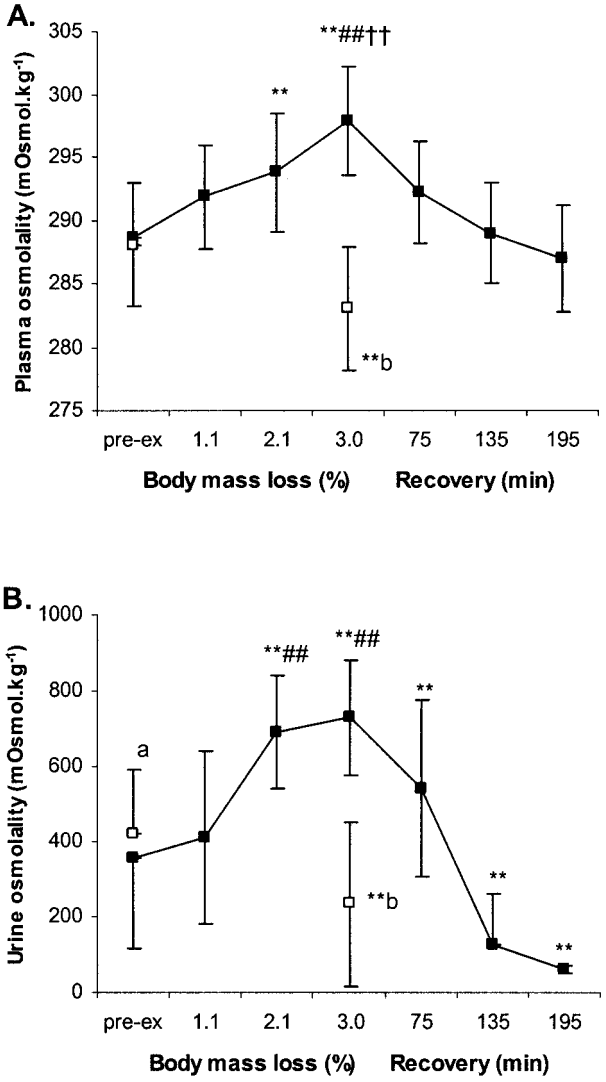

FIGURE 1—Plasma (A) and urine (B) osmolality during progressive acute dehydration (NFI ■) and with sufficient fluids to offset fluid losses (FI □). Values are means ± SD ($N = 15$). Significantly different from preexercise, ** $P < 0.01$. Significantly different from 1.1% BML, ## $P < 0.01$. Significantly different from 2.1% BML, †† $P < 0.01$. Significantly different from preexercise on NFI trial, a $P < 0.05$. Significantly different from postexercise on NFI trial, b $P < 0.01$. BML, body mass loss; NFI, no fluid intake; FI, fluid intake.

0.01; Fig. 2A). *Post hoc* analysis revealed that saliva flow rate was significantly lower than preexercise at 3.0% BML ($P < 0.01$). At 3.0% BML on the NFI trial, saliva flow rate decreased to 165 ± 43 (range 13–587) μL·min^{-1}. At 3.0% BML, saliva flow rate was lower than preexercise in 12 of 15 samples. The rehydration protocol reestablished preexercise saliva flow rate levels by 75 min postexercise on the NFI trial. Saliva flow rate was not significantly different at preexercise on the FI trial compared with the NFI trial. Fluid provision during the FI trial prevented the decrease in saliva flow rate observed on the NFI trial. At postexercise, saliva flow rate was significantly greater on the FI trial than on the NFI trial (interaction: $F_{(1.0, 14.0)} = 17.3$, $P < 0.01$).

Saliva osmolality. At preexercise on the NFI trial, saliva osmolality was 50 ± 11 (range 38–71) mOsmol·kg^{-1}. Saliva osmolality increased during progressive dehydration

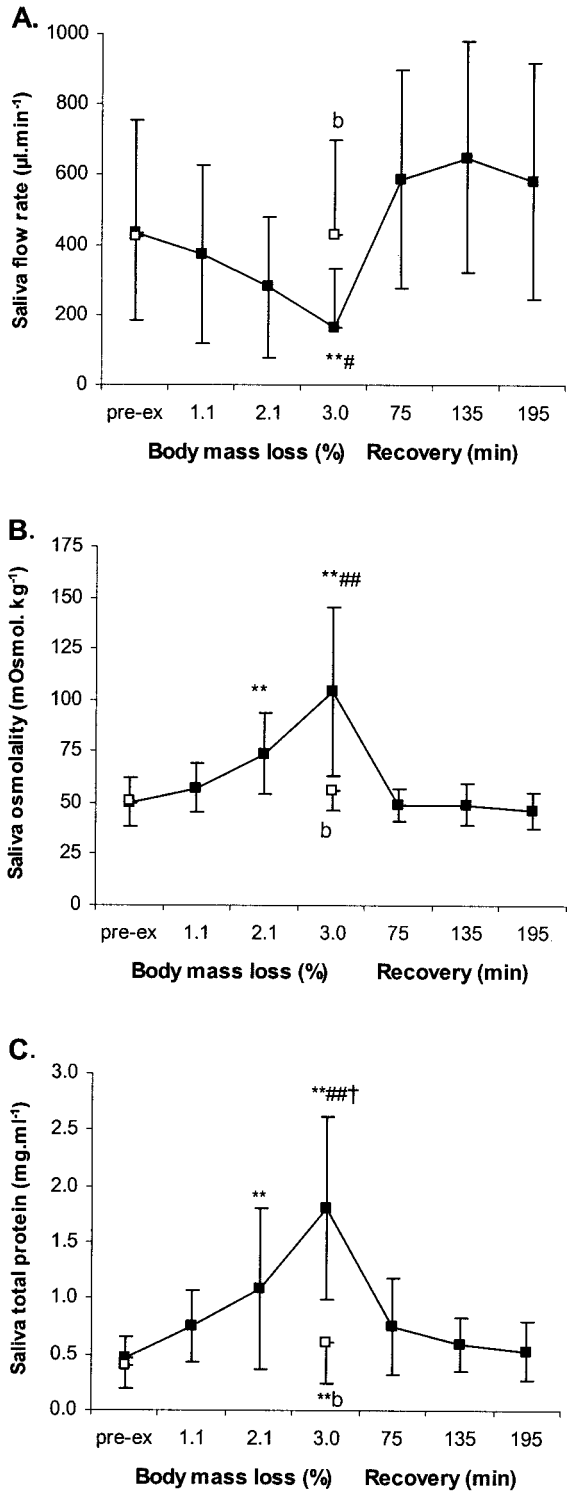

FIGURE 2—Saliva flow rate (A) osmolality (B) and total protein concentration (C) during progressive acute dehydration (NFI ■) and with sufficient fluids to offset fluid losses (FI □). Values are means ± SD (N = 15). Significantly different from preexercise, ** $P < 0.01$. Significantly different from 1.1% BML, # $P < 0.05$, ## $P < 0.01$. Significantly different from 2.1% BML, † $P < 0.05$. Significantly different from postexercise on NFI trial, b $P < 0.01$. BML, body mass loss; NFI, no fluid intake; FI, fluid intake.

on the NFI trial (main effect of percent BML: $F_{(3.0,\ 41.3)} = 30.5$, $P < 0.01$; Fig. 2B). *Post hoc* analysis revealed that saliva osmolality was significantly greater than preexercise by 2.1% BML ($P < 0.01$). At 3.0% BML on the NFI trial, saliva osmolality reached 105 ± 41 (range 55–200) mOsmol·kg^{-1}. At 3.0% BML, saliva osmolality was higher than preexercise in all samples. The rehydration protocol reestablished preexercise saliva osmolality levels by 75 min postexercise on the NFI trial. Saliva osmolality was not significantly different at preexercise on the FI trial compared with the NFI trial. Fluid provision during the FI trial prevented the increase in saliva osmolality observed on the NFI trial. At postexercise, saliva osmolality was significantly lower on the FI trial than on the NFI trial (interaction: $F_{(1.0,\ 14.0)} = 37.2$, $P < 0.01$). Saliva solute secretion rate did not change significantly during exercise on either the NFI or the FI trial (preexercise NFI: 23 ± 20; 3.0% BML: 16 ± 16 μOsmol·min^{-1}).

Saliva total protein concentration. At preexercise on the NFI trial saliva total protein concentration was 0.47 ± 0.19 (range 0.24–0.82) mg·mL^{-1}. Saliva total protein concentration increased during progressive dehydration on the NFI trial (main effect of % BML: $F_{(6.0,\ 54.0)} = 9.9$, $P < 0.01$; Fig. 2C). *Post hoc* analysis revealed that saliva total protein concentration was significantly greater than preexercise by 2.1% BML ($P < 0.01$). At 3.0% BML on the NFI trial, saliva total protein concentration reached 1.80 ± 0.82 (range 0.85–3.22) mg·mL^{-1}. At 3.0% BML, saliva total protein concentration was higher than preexercise in all samples. The rehydration protocol reestablished preexercise saliva total protein concentration levels by 75 min postexercise on the NFI trial. Saliva total protein concentration was not significantly different at preexercise on the FI trial compared with the NFI trial. Fluid provision during the FI trial blunted but could not prevent an increase in saliva total protein concentration ($P < 0.01$). At postexercise, saliva total protein concentration was significantly lower on the FI trial than on the NFI trial (interaction: $F_{(1.0,\ 10.0)} = 15.8$, $P < 0.01$). An increase in saliva protein secretion rate was observed at 75 min postexercise compared with preexercise (preexercise NFI: 0.22 ± 0.20; 75 min postexercise: 0.42 ± 0.29 μg·min^{-1}; $F_{(6.0,\ 54.0)} = 3.3$, $P < 0.01$).

Correlational comparisons. Urine osmolality, saliva osmolality, and saliva total protein concentration each correlated strongly with plasma osmolality during dehydration on the NFI trial (r = 0.83; r = 0.87; r = 0.91, respectively; $P < 0.01$) with no significant difference between the correlation coefficients. The correlation coefficients for urine osmolality, saliva osmolality, and saliva total protein concentration with plasma osmolality were greater ($P < 0.01$) than the corresponding correlation coefficient between saliva flow rate and plasma osmolality (r = −0.78; $P < 0.01$).

Plasma catecholamines. At preexercise on the NFI trial plasma epinephrine concentration was 0.064 ± 0.046 (range 0.017–0.154) ng·mL^{-1}. Plasma epinephrine concentration, corrected for plasma volume change, increased during progressive dehydration on the NFI trial (main effect of % BML: $F_{(3.0,\ 21.0)} = 6.4$, $P < 0.01$; Fig. 3A). *Post hoc* analysis revealed that plasma epinephrine concentration was significantly greater than preexercise at 3.0% BML only ($P < 0.01$). At 3.0% BML on the NFI trial plasma epineph-

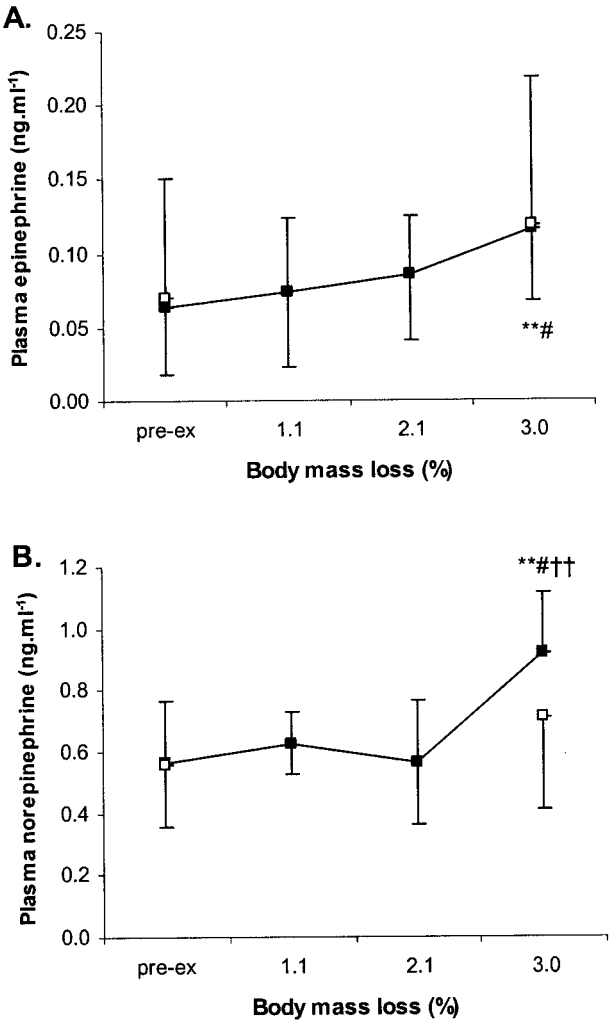

FIGURE 3—Plasma epinephrine (A) and norepinephrine concentration (B) during progressive acute dehydration (NFI ■) and with sufficient fluids to offset fluid losses (FI □). Values are means ± SD (N = 8). Significantly different from preexercise, ** $P < 0.01$. Significantly different from 1.1% BML, # $P < 0.05$. Significantly different from 2.1% BML, †† $P < 0.01$. BML, body mass loss; NFI, no fluid intake; FI, fluid intake.

rine concentration reached 0.116 ± 0.048 (range 0.063–0.213) ng·mL^{-1}. The two-way repeated measures ANOVA comparing plasma epinephrine concentration at pre- and postexercise in NFI and FI trials found no significant interaction, although there was a main effect of sample collection: plasma epinephrine concentration was significantly greater at postexercise compared with preexercise ($F_{(1.0, 6.0)}$ = 22.0, $P < 0.01$; Fig. 3A).

At preexercise on the NFI trial plasma norepinephrine concentration was 0.564 ± 0.179 (range 0.368–0.892) ng·mL^{-1}. A main effect of % BML was observed for plasma norepinephrine concentration during dehydration on the NFI trial ($F_{(3.0, 21.0)}$ = 7.6, $P < 0.01$; Fig. 3B). *Post hoc* analysis revealed that plasma norepinephrine concentration, corrected for plasma volume change, was significantly greater than preexercise at 3.0% BML only ($P < 0.01$). At 3.0% BML on the NFI trial, plasma norepinephrine concentration was 0.916 ± 0.229 (range 0.643–1.338) ng·mL^{-1}. The two-way repeated measures ANOVA comparing plasma norepinephrine concentration at pre- and postexercise in NFI and FI trials found no significant interaction, although there was a main effect of sample collection: plasma norepinephrine concentration was significantly greater at postexercise compared with preexercise ($F_{(1.0, 6.0)}$ = 9.6, $P <$ 0.05; Fig. 3B).

DISCUSSION

The primary objective of the present study was to identify whether saliva flow rate, osmolality, and total protein concentration are sensitive noninvasive markers of hydration status. To do this, we compared changes in these parameters with changes in plasma osmolality during progressive acute dehydration evoked by a combination of exercise and heat stress (hypertonic-hypovolemia). The results show a reduction in saliva flow rate and an increase in saliva osmolality and total protein concentration during dehydration (Fig. 2). The decrease in saliva flow rate during prolonged exercise without fluids is in agreement with others (4,13,30). The strong relationships between plasma osmolality and saliva osmolality (mean r = 0.87) and plasma osmolality and saliva total protein concentration (mean r = 0.91) show that changes in these saliva variables reflect changes in hydration status during hypertonic-hypovolemia. The changes in saliva osmolality and total protein concentration during dehydration were able to identify a BML of 2.1%, thus satisfying an established criteria for a marker of acute dehydration (27).

The second objective was to compare the sensitivity of saliva parameters to track changes in hydration status with the sensitivity of urine osmolality, a regularly used hydration marker. The sensitivity of saliva osmolality and total protein concentration compared favorably with urine osmolality, which also increased significantly by 2.1% BML (Fig. 1B) and was strongly associated with plasma osmolality during dehydration (mean r = 0.83). Saliva osmolality, saliva total protein concentration, and urine osmolality increased during dehydration in all subjects. From a practical perspective, however, it is noteworthy that three subjects could not provide a urine sample at 3.0% BML on the NFI trial. Changes in saliva flow rate during dehydration were less sensitive to whole body water losses, with only 12 of 15 subjects exhibiting a decrease in flow rate at 3.0% BML. The correlations for saliva osmolality, saliva total protein concentration, and urine osmolality with plasma osmolality were significantly greater than the corresponding correlation between saliva flow rate and plasma osmolality during dehydration (mean r = −0.78). Therefore, during hypertonic-hypovolemia, changes in saliva osmolality, saliva total protein concentration and urine osmolality appear to be more sensitive to changes in hydration status than changes in saliva flow rate.

We have observed large interindividual variation in saliva parameters, particularly saliva flow rate, as indicated in the large standard deviations (Fig. 2A), at preexercise (euhydration) and during both dehydration and rehydration. Large

interindividual variation in parotid saliva flow rate has been documented where mean ± SEM euhydrated parotid flow rate in young adults was 69 ± 30 $\mu L \cdot min^{-1}$ (25). In the present study, unstimulated whole saliva flow rate ranged from 71 to 993 $\mu L \cdot min^{-1}$ at euhydration and 13 to 587 $\mu L \cdot min^{-1}$ at 3.0% BML on the NFI trial. Clearly, defining a range for saliva flow rate to represent a state of euhydration in all individuals would be very difficult with such large interindividual variation. If we were to assume that the mean ± SD represents a reasonable range, euhydration might be determined by, for example, a saliva osmolality <61 $mOsmol \cdot kg^{-1}$ (euhydrated mean ± SD: 50 ± 11 $mOsmol \cdot kg^{-1}$) and a urine osmolality <590 $mOsmol \cdot kg^{-1}$ (euhydrated mean ± SD: 353 ± 237 $mOsmol \cdot kg^{-1}$). At preexercise, 3 of the 15 subjects would be incorrectly classified as dehydrated for both saliva osmolality and urine osmolality. At 3.0% BML, 3 of the 15 subjects would be incorrectly classified as euhydrated for saliva osmolality and 2 (of only 12 samples) for urine osmolality. For saliva parameters, and urine osmolality, to have practical use as markers of whole body hydration status, we recommend that euhydrated readings be determined for each individual. Other factors that may limit the usefulness of saliva parameters as potential markers of whole body hydration status include a possible short-term effect of neural control on saliva flow rate and composition (10), and a possible short-term effect of food intake and oral hygiene on saliva flow rate (16). It is encouraging that unstimulated whole saliva flow rate and osmolality do not show significant diurnal variation (29) and remain unchanged immediately after and 15 min after fluid consumption. Pilot data in $N = 8$ subjects in our laboratory show no effect of drinking 7 $mL \cdot kg^{-1}$ body mass of water or a CHO-electrolyte solution (6% CHO) on unstimulated whole saliva flow rate or osmolality either immediately after or 15 min after consuming the drink (Oliver et al., unpublished observations). These findings agree with those showing no change in unstimulated parotid saliva flow rate immediately after drinking up to 1 L of water (23).

The third objective was to test the hypothesis that dehydration, rather than neuroendocrine regulation (increase in sympathetic activity), is the most likely explanation for the decrease in saliva flow rate during prolonged exercise. Until now, a role for neuroendocrine regulation has been widely acknowledged to explain the decrease in saliva flow rate during prolonged exercise (7,10). To test this hypothesis, we have reported saliva flow rate and plasma catecholamine responses to prolonged exercise performed with and without fluid restriction. The results show that performing prolonged exercise with sufficient fluids to offset fluid losses prevents the decrease in saliva flow rate (Fig. 2A). Prolonged exercise with fluid intake has previously been shown to blunt the decrease in saliva flow rate observed with restricted fluid intake (4). In the present study, plasma epinephrine and norepinephrine concentration increased in similar magnitude during exercise performed on the NFI and FI trial (Fig. 3). In agreement with the present study, the plasma epinephrine response to 2 h of exercise in the heat (35°C) was similar when exercise was performed with no fluids (4.9% BML) or with fluids equivalent to 95% of losses (15). However, the same authors noted significantly greater plasma norepinephrine concentration after 2 h of exercise with no fluids compared with when fluids were provided. In contrast, we observed no difference in plasma norepinephrine concentration between the NFI and FI trial at postexercise. This discrepancy may be explained by the fact that the exercise in the previous study (15) was continuous in nature and performed in a hotter environment (35°C vs 30°C here) resulting in a significant core temperature difference of +1.2°C on the no fluid trial (final core of 39.4 ± 0.2°C) compared with the fluid trial. In the present study, we observed a nonsignificant core temperature difference of +0.6°C on the NFI trial (final core of 38.5 ± 0.4°C) compared with the FI trial (final core of 37.9 ± 0.3°C). Indeed, the magnitude of the plasma norepinephrine response to exercise is known to be associated with the magnitude of the rise in core temperature during exercise (12). To summarize, we have shown that performing prolonged exercise with sufficient fluids to offset fluid losses prevents the decrease in saliva flow rate but does not prevent the increase in plasma catecholamines. These results support the hypothesis that dehydration has a greater involvement in the decrease in saliva flow rate during prolonged exercise than neuroendocrine regulation.

The decrease in saliva flow rate during exercise without fluids appears to be largely responsible for the observed increase in saliva osmolality and saliva total protein concentration via a concentrating effect on saliva. The finding that saliva secretion rates for both solute and total protein did not change significantly during dehydration on the NFI trial suggests that the decrease in saliva flow rate during dehydration almost entirely accounted for the increase in both osmolality and total protein concentration. Results showing no change in saliva flow rate and osmolality after exercise on the FI trial also support a concentrating effect. Although we did observe a significant increase in saliva total protein concentration (+53%) after exercise on the FI trial, a much greater response (+383%) was observed after exercise on the NFI trial. An increase in β-sympathetic activity during exercise may account for this 53% increase in saliva total protein concentration on the FI trial. Increased β-sympathetic activity in the saliva glands has been reported to increase saliva total protein concentration (10). As the saliva total protein response to exercise was about sevenfold greater on the NFI versus the FI trial, it is reasonable to conclude that the decrease in saliva flow rate (concentrating effect) is largely responsible for the increase in saliva total protein concentration observed during prolonged exercise without fluids.

The mechanism(s) by which dehydration evoked such large reductions in saliva flow rate (−62% at 3.0% BML during the NFI trial) in the present study remains unclear. Reduction of body fluids during dehydration may cause salivary gland hypofunction because the predominant fluid constituent of saliva is water, which enters saliva from plasma through acinar cells (25). In order for water to move from plasma through acinar cells to form primary saliva, a trans-acinar cell sodium gradient must be generated. It is well established that during hypertonic-hypovolemia the

extracellular fluid sodium concentration increases, and this is reflected in a graded increase in plasma osmolality (Fig. 1A) (20,21). As a result, a greater sodium concentration must be generated across the salivary acinar cell to drive fluid into the acinar lumen to form primary saliva. An increase in extracellular sodium concentration during hypertonic-hypovolemia may account for the production of smaller amounts of more concentrated saliva (e.g., with increased osmolality) during dehydration in the present study. This mechanism may also account for why saliva flow rate and osmolality did not change during exercise on the FI trial when fluid intake prevented the increase in plasma osmolality. An increase in extracellular sodium concentration may explain the decrease in saliva flow rate observed during hot water immersion (31) and prolonged exercise with a fluid deficit (4,13,30) where a hypertonic type hypovolemia most likely occurred. Alternatively, as vasopressin responses to exercise are closely coupled to plasma osmolality (18), a direct effect of vasopressin on saliva production (possibly by increasing water reabsorption at the striated ducts) might also explain the saliva flow rate responses to exercise with and without fluids in the present study (16). Future research should compare saliva flow rate and osmolality responses to a hypertonic type hypovolemia (through sweat losses) with an isotonic type hypovolemia (diuretic induced) to elucidate the mechanism responsible for the saliva responses observed during dehydration in the present study.

In conclusion, our data show that saliva osmolality and total protein concentration appear to be as sensitive as urine osmolality to track changes in hydration status during hypertonic-hypovolemia. However, for saliva markers to be of use to track changes in hydration status in an applied setting, they must be shown to be sensitive to both hypertonic and isotonic hypovolemia, during acute and chronic dehydration. Finally, these results suggest that dehydration has a greater involvement in the decrease in saliva flow rate during prolonged exercise than neuroendocrine regulation.

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