american physiological society

JOURNAL OF APPLIED PHYSIOLOGY

J Appl Physiol 130: 517–527, 2021.
First published December 10, 2020; doi:10.1152/japplphysiol.00311.2020

RESEARCH ARTICLE

Systemic but not local rehydration restores dehydration-induced changes in pulmonary function in healthy adults

Hannah Marshall,[1] Oliver R. Gibson,[1,2] Lee M. Romer,[1,2] Camilla Illidi,[1] James H. Hull,[1,3] and Pascale Kippelen[1,2]

[1]*Centre for Human Performance, Exercise, and Rehabilitation, Brunel University London, Uxbridge, United Kingdom;* [2]*Division of Sport, Health, and Exercise Sciences, College of Health, Medicine, and Life Sciences, Brunel University London, Uxbridge, United Kingdom; and* [3]*Department of Respiratory Medicine, Royal Brompton Hospital, London, United Kingdom*

Abstract

Water transport and local (airway) hydration are critical for the normal functioning of lungs and airways. Currently, there is uncertainty regarding the effects of systemic dehydration on pulmonary function. Our aims were *1*) to clarify the impact of exercise- or fluid restriction-induced dehydration on pulmonary function in healthy adults; and *2*) to establish whether systemic or local rehydration can reverse dehydration-induced alterations in pulmonary function. Ten healthy participants performed four experimental trials in a randomized order (2 h exercise in the heat twice and 28 h fluid restriction twice). Pulmonary function was assessed using spirometry and whole body plethysmography in the euhydrated, dehydrated, and rehydrated states. Oral fluid consumption was used for systemic rehydration and nebulized isotonic saline inhalation for local rehydration. Both exercise and fluid restriction induced mild dehydration (2.7 ± 0.7% and 2.5 ± 0.4% body mass loss, respectively; $P < 0.001$) and elevated plasma osmolality ($P < 0.001$). Dehydration across all four trials was accompanied by a reduction in forced vital capacity (152 ± 143 mL, $P < 0.01$) and concomitant increases in residual volume (216 ± 177 mL, $P < 0.01$) and functional residual capacity (130 ± 144 mL, $P < 0.01$), with no statistical differences between modes of dehydration. These changes were normalized by fluid consumption but not nebulization. Our results suggest that, in healthy adults: *1*) mild systemic dehydration induced by exercise or fluid restriction leads to pulmonary function impairment, primarily localized to small airways; and *2*) systemic, but not local, rehydration reverses these potentially deleterious alterations.

NEW & NOTEWORTHY This study demonstrates that, in healthy adults, mild systemic dehydration induced by exercise in the heat or a prolonged period of fluid restriction leads to negative alterations in pulmonary function, primarily localized to small airways. Oral rehydration, but not nebulized isotonic saline, is able to restore pulmonary function in dehydrated individuals. Our findings highlight the importance of maintaining an adequate systemic fluid balance to preserve pulmonary function.

airway; dehydration; exercise; fluid; nebulization

INTRODUCTION

Systemic dehydration, defined as a deficit of total body water (1), commonly occurs in individuals who perform sustained physical activity in hot environments (2), as well as in patients and older adults (3). Even at mild levels [i.e., ~2–3% body mass loss (1)], systemic dehydration can have unfavorable effects on multiple organ systems, including the cardiovascular, renal, and nervous systems (4) and can compromise physical and cognitive performance (1). Limited and contradictory data currently exist regarding the effects of systemic dehydration on the respiratory system.

Two previous studies showed deleterious alterations in expiratory flow or lung volumes in healthy adults (5) and in athletes with mild asthma (6) following mild systemic dehydration (up to 2.5% body mass loss). A third study (7) however showed improvements in selected measures of pulmonary function [including increases in forced expiratory volume in 1 s (FEV_1) and in the FEV_1 to forced vital capacity ratio (FEV_1/FVC)] in healthy adults following moderate dehydration (4.5% body mass loss). The different population groups and severities of dehydration, as well as the various modes of dehydration [i.e., fluid restriction (5) vs. exercise (6) vs. diuretic drug (7)], have generated uncertainty regarding the impact of systemic dehydration on pulmonary function.

Fluid supply to the airways stems primarily from the bronchial circulation, which itself arises from the systemic circulation (8). Optimal lung fluid balance is a critical component of normal pulmonary functioning (9), with airway surface liquid dehydration implicated in several respiratory diseases, such as cystic fibrosis (10) and exercise-induced bronchoconstriction (11). Water flows across the airway epithelia in response to an osmotic gradient. Thus, when individuals become

Correspondence: P. Kippelen (pascale.kippelen@brunel.ac.uk).
Submitted 27 April 2020 / Revised 16 November 2020 / Accepted 7 December 2020

dehydrated and bronchial blood flow and/or composition changes, water movement to the airways is modified and airway hydration may become compromised. Alterations in airway surface liquid thickness ("depth"), composition, and/or rheology can promote peripheral or small airway instability and provoke premature airway closure (12), potentially increasing respiratory symptoms (in particular, breathlessness) and worsening respiratory reserve in susceptible individuals.

The aim of this study was to clarify the impact of mild systemic dehydration on pulmonary function in healthy adults. Since exercise in hot environment and/or insufficient fluid intake are two common causes of systemic dehydration, we compared the effects of 2 h of exercise in the heat with 28 h of fluid restriction on pulmonary volumes, capacities, and flows. In line with our findings in athletes with asthma (6), we hypothesized that pulmonary function would be compromised in dehydrated healthy adults, as evidenced by changes in spirometry and whole body plethysmography parameters. We did not anticipate any difference between dehydration modalities, as both exercise and fluid restriction cause the same type of dehydration, i.e., hyperosmotic hypovolemia (13). Additionally, we aimed to establish: *1*) whether dehydration-induced changes in pulmonary function are reversible with immediate rehydration; and *2*) whether local rehydration delivered directly at the site of the airways (via nebulized isotonic saline) is superior to systemic rehydration (via oral fluid intake) in restoring pulmonary function.

METHODS

Participants

Ten healthy, nonsmoking individuals (2 females; age: 29 ± 8 yr; body mass: 62.8 ± 8.5 kg; and stature: 173 ± 10 cm), with no history of respiratory illness (including asthma and exercise-induced bronchoconstriction) completed this study. Pulmonary function was checked for normality via spirometry, with FEV_1 >80% predicted and FEV_1/FVC >70% used as inclusion criteria and Global Lung Function Initiative 2012 equations used as reference (14). Participants provided written informed consent and the study was approved by the Brunel University London Research Ethics Committee (Reference No. 6639-TISS-Jul/2017–7774-2).

Experimental Overview

A repeated-measures, randomized crossover design was utilized, with all participants completing four experimental trials on separate days. The trials comprised: *1*) fluid restriction plus systemic rehydration; *2*) fluid restriction plus local rehydration; *3*) exercise plus systemic rehydration; and *4*) exercise plus local rehydration.

Alcohol, caffeine, and strenuous exercise were prohibited in the 24 h before testing. Before the first trial, participants completed a 24 h food diary, which they subsequently replicated before each trial.

Participants arrived at the laboratory at 0900 h (±1 h) in a euhydrated state. Basic anthropometrics, hydration status, and pulmonary function were assessed. Resting minute ventilation ($\dot{V}_E$) was then recorded to allow for estimation of airway surface liquid loss over the 28 h fluid restriction trials. Following baseline measurements, participants underwent one of two dehydration trials: 28 h fluid restriction or exercise-induced dehydration. Upon completion of the dehydration trials, hydration status and pulmonary function were reassessed; this was followed by a period of rehydration with oral fluid or isotonic saline nebulization. Spirometry was performed 15 and 35 min within the rehydration periods. Hydration status and pulmonary function were recorded 60 min after commencing rehydration.

Hydration Status

Capillary blood samples were collected in triplicate from the participant's fingertip. The samples were analyzed for hemoglobin (Hb) concentration (HemoCue Ltd., Dronfield, Derbyshire, UK) and hematocrit (Hct). Hematocrit tubes were centrifuged at 12–14,000 rpm for 3 min (Hematospin 1300 Centrifuge, Hawksley & Sons Ltd., West Sussex, UK) and assessed via microscopy. Hemoglobin and Hct were then used for the calculation of plasma volume (15). Plasma osmolality (P_{osm}) was analyzed via freezing point depression osmometry (Advanced 3320 Micro-Osmometer, Vitech Scientific Ltd., West Sussex, UK). Urine osmolality (U_{osm}) was measured using a portable refractometer (Pocket PalOsmo, Atago Vitech Scientific, Scotland, UK), and the threshold for euhydration was set at <700 mosmol/kgH_2O (2). Nude body mass was recorded to the nearest 0.1 kg.

Pulmonary Function

Pulmonary function was measured via spirometry then whole body plethysmography. All tests were performed in accordance with American Thoracic Society/The European Respiratory Society guidelines (16, 17). At 15 and 35 min of rehydration, forced maneuvers were performed in duplicate only (as long as FEV_1 and FVC were reproducible). Specific airway resistance (sRaw) was measured using the interrupter (i.e., airway occlusion) technique (18).

Dehydration Protocols

Fluid restriction.

As done previously (19), participants were prohibited from consuming any fluid and were restricted to consuming foods with low water content (<30%) from a list of acceptable/prohibited foods (4) for 28 h. Participants kept a diary of all food consumed, which they replicated during the second fluid restriction trial. Participants were fitted with an activity monitor (ActivPal, PAL Technologies Ltd., Glasgow, UK) and asked to limit physical activity for the entire duration of the fluid restriction. Local environmental temperature and humidity were recorded throughout 28 h using a portable logger (Hygrochron, iButton, Maxim Integrated, CA) and were later used to estimate absolute water content of inspired air and airway surface liquid loss (11, 20). Expired water content was assumed to equal 33 mgH_2O·L^{-1} of air (11). The difference between inspired and expired absolute water content was calculated and multiplied by resting $\dot{V}_E$. Total water loss over the duration of the fluid restriction trial was estimated assuming negligible variations in $\dot{V}_E$ over the 28 h period.

Exercise-induced dehydration.

Participants completed 2 h of low-intensity exercise in hot conditions [37°C, 50% relative humidity (RH)] with total fluid

restriction. The exercise protocol was identical to that used in our previous study (6), alternating 20 min of cycling on a stationary bike (Excalibur Sport, Lode, Groningen, The Netherlands) and 10 min of stepping. At the end of each bout of cycling and stepping, the following measurements were taken: heart rate (FT1, Polar Electro Oy, Kempele, Finland), tympanic temperature (Thermoscan Exactemp 6022, Braun, Germany), overall rating of perceived exertion [RPE; on a scale of 6–20 (21)] and rating of breathing discomfort [on a scale of 1–10 (22)]. During the final 5 min of each bout of cycling/stepping, $\dot{V}_E$ and oxygen uptake ($\dot{V}O_2$) were recorded breath-by-breath (Vyntus CPX, Carefusion, Germany), with mean $\dot{V}_E$ over the final minute used for analysis. Mean $\dot{V}O_2$ over 2 h of exercise was used for estimation of airway surface liquid loss, based on calculations provided by Mitchell et al. (23):

$$\dot{m}_e = 0.019\,\dot{V}O_2\,(44 - Pa)$$

where $\dot{m}_e$ is the rate of evaporative water loss in the expired air ($g \cdot min^{-1}$) and $\dot{V}O_2$ is the oxygen uptake ($L \cdot min^{-1}$ STPD).

Rehydration Protocols

Systemic rehydration.

Participants gradually rehydrated by ingesting water at room temperature, mixed with 3 g NaCl·L H_2O^{-1} to improve fluid retention (24). The volume of fluid ingested (liters) matched the loss of body mass (kg). Participants ingested water in three equal boluses (550 ± 176 mL) over 15 min, with a 5-min break between boluses to perform spirometry.

Local rehydration.

An ultrasonic nebulizer (UltraNeb, DeVilbiss Healthcare Ltd., UK) and isotonic saline (0.9%) were used to locally rehydrate the airways at a measured output of 1.4 ± 0.2 $mL \cdot min^{-1}$. Participants were required to breathe tidally through a two-way nonrebreathing valve (Series 1410, Hans Rudolph, Inc., KS) with a nose clip in place. Participants were exposed to three 15-min bouts of nebulization, with 5 min breaks in between.

Sample Size

Sample size was based on our previous work that investigated the impact of exercise-induced dehydration upon pulmonary function in athletes with mild asthma (6). We hypothesized that *1*) while still present, the reduction in FVC (i.e., primary outcome measure) following dehydration will be less severe in the healthy participants recruited in the present study compared with individuals with asthma (6); and *2*) the change in FVC will be independent to the mode of dehydration. To detect a 200 mL difference in FVC between pre- and postdehydration, with a standard deviation of the difference in the response of matched pairs of 50 mL, we calculated that a sample size of eight participants would give ~80% power for an alpha level of 5%.

Statistics

Statistical analyses were performed using dedicated software (SPSS version 26, SPSS, IBM Corp., Armonk, NY). All data were normally distributed, as confirmed by the Shapiro-Wilk test. To assess changes in spirometry, plethysmography, and hydration within and between trials, three-way repeated measures ANOVA were used (with mode of dehydration, mode of rehydration, and time as main factors). Post hoc Bonferroni-adjusted pairwise comparisons were used where significant main or interaction effects were detected. A within-subjects repeated measures correlation (25) was used to determine the relationship between changes in hydration and pulmonary function. Statistical significance was set at $P < 0.05$. Descriptive statistics are shown as means ± SD, unless otherwise stated. Differences between modes of dehydration/rehydration are expressed as means and 95% confidence intervals (CI).

RESULTS

Dehydration Protocols

Fluid restriction.

Over the 2 × 28 h periods of fluid restriction, physical activity levels did not differ significantly, with participants spending 22 ± 2 and 22 ± 3 h sedentary, 5 ± 2 and 5 ± 2 h standing, and 2 ± 1 and 2 ± 1 h stepping/walking. Resting $\dot{V}_E$ was well matched (i.e., not statistically different) between trials (9 ± 3 and 10 ± 2 $L \cdot min^{-1}$), as were environmental conditions (23.3 ± 2.7°C, 54 ± 8% RH and 24.2 ± 2.5°C, 51 ± 11% RH). As a result, estimated water loss from the airways was not different between the two fluid restriction trials (total water loss: 325 ± 125 and 357 ± 91 mL; rate of water loss: 12 ± 4 and 13 ± 3 $mL \cdot h^{-1}$).

Exercise.

Participants cycled at 70 ± 9 W. Physiological (heart rate, tympanic temperature, and $\dot{V}_E$) and perceptual responses (overall RPE and rating of breathing discomfort), as well as estimated total water loss from the airways were not different between trials (Table 1). Compared with fluid restriction, the estimated total water loss from the airways during exercise was smaller ($P < 0.001$), whereas the estimated rate of water loss was larger ($P < 0.001$). The mean differences between exercise and fluid restriction were 262 mL (95% CI: 201–323 mL) for total water loss and 28 $mL \cdot h^{-1}$ (95% CI: 22–33 $mL \cdot h^{-1}$) for rate of water loss.

Hydration Status

Data for hydration status are shown in Table 2.

Table 1. *Physiological and perceptual responses to 2 h of exercise in the heat with no fluid replacement*

	EX-Systemic	EX-Local
Heart rate, $beats \cdot min^{-1}$	131 ± 23	130 ± 27
Tympanic temperature. °C	37.1 ± 0.4	37.0 ± 0.4
Minute ventilation, $L \cdot min^{-1}$	37 ± 7	39 ± 7
Overall rating of perceived exertion	12 ± 2	11 ± 3
Rating of breathing discomfort	3 ± 2	2 ± 2
Airway water loss, mL	79 ± 16	80 ± 19
Airway water loss, $mL \cdot h^{-1}$	39 ± 8	40 ± 10

Data are means ± SD; $n = 10$. EX-Systemic, exercise with systemic rehydration trial; EX-Local, exercise with local rehydration (nebulized isotonic saline) trial. The overall rating of perceived exertion was rated on a scale of 6–20, while breathing discomfort was rated on a scale of 0–10. All $P > 0.05$ for between-trial differences.

Table 2. *Hydration status at baseline, immediately after fluid restriction- and exercise-induced dehydration, and following systemic and local (airway) rehydration*

	FR-Systemic	EX-Systemic	FR-Local	EX-Local
Body mass, kg				
Baseline	62.9 ± 8.7	62.5 ± 8.6	63.1 ± 8.8	63.0 ± 8.5
Dehydration	61.2 ± 8.4[a]	61.0 ± 8.5[a]	61.4 ± 8.5[a]	61.4 ± 8.3[a]
Rehydration	62.5 ± 8.7[b,c]	62.2 ± 8.6[b,c]	61.3 ± 8.5[a]	61.3 ± 8.3[a]
Plasma osmolality, mosmol/kgH_2O				
Baseline	294 ± 5	294 ± 6	291 ± 5	293 ± 7
Dehydration	300 ± 4[a]	300 ± 4[a]	300 ± 5[a]	300 ± 4[a]
Rehydration	293 ± 4[c,d]	292 ± 5[c,d]	301 ± 5[a]	300 ± 7[a]
Hemoglobin, g·L^{-1}				
Baseline	147 ± 15	150 ± 16	145 ± 14	148 ± 18
Dehydration	151 ± 14	148 ± 11	150 ± 18	150 ± 15
Rehydration	143 ± 13	142 ± 20	147 ± 13	148 ± 18
Hematocrit, %				
Baseline	45 ± 4	45 ± 6	45 ± 5	45 ± 5
Dehydration	45 ± 5	45 ± 3	45 ± 5	45 ± 5
Rehydration	44 ± 4[c]	44 ± 5[c]	46 ± 5	46 ± 5
Urine osmolality, mosmol/kgH_2O				
Baseline	201 ± 137	166 ± 137	255 ± 199	132 ± 134
Dehydration	897 ± 103[a,e]	477 ± 188[a]	966 ± 135[a,e]	442 ± 232[a]
Rehydration	544 ± 261[a,c]	694 ± 224[a,c]	1,024 ± 99[b]	851 ± 171[b]
Plasma volume, %				
Baseline	55.4 ± 4.2	55.3 ± 5.8	54.8 ± 4.6	55.1 ± 5.4
Dehydration	53.5 ± 6.1	55.7 ± 2.7	53.6 ± 6.6	54.0 ± 5.1
Rehydration	60.1 ± 6.3[b,c]	59.5 ± 5.4[b,c]	55.4 ± 4.4	54.7 ± 6.3

Data are means ± SD; $n = 10$. FR-Systemic, fluid restriction with systemic rehydration (oral fluid intake); EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration (nebulized isotonic saline); EX-Local, exercise with local rehydration. [a]$P < 0.05$ vs. baseline; [b]$P < 0.05$ vs. baseline and dehydration; [c]$P < 0.05$ vs. FR-Local and EX-Local at the corresponding time point; [d]$P < 0.05$ vs. dehydrated; [e]$P < 0.05$ vs. EX-Systemic and EX-Local at the corresponding time point.

Effect of dehydration.

Both modes of dehydration induced a mild level of dehydration, with a reduction in body mass of 2.5 ± 0.4% after exercise and 2.7 ± 0.7% after fluid restriction (both $P < 0.001$ vs. baseline). No significant difference was noted between trials [mean difference in body mass loss between exercise and fluid restriction: 0.1 kg (95% CI: −0.1–0.3 kg)]. The reduction in body mass was associated with an increase in P_{osm} in all trials ($P < 0.001$), but no difference between modes of dehydration [mean difference in dehydration-induced P_{osm} change between exercise and fluid restriction: 0.8 mosmol/kgH_2O (95% CI: −1.8–3.3 mosmol/kgH_2O)]. U_{osm} increased following exercise and fluid restriction ($P < 0.001$), but the increase was greater following fluid restriction ($P = 0.001$). Mean difference in dehydration-induced U_{osm} change between exercise and fluid restriction was 408 mosmol/kgH_2O (95% CI: 267–549 mosmol/kgH_2O). Plasma volume, hemoglobin and hematocrit were not different after exercise or fluid restriction compared with baseline.

Effect of rehydration.

Following systemic, but not local rehydration, body mass increased ($P < 0.001$ vs. dehydration). Mean difference in body mass gain between systemic and local rehydration was 1.4 kg (95% CI: 1.2–1.5 kg). Postsystemic rehydration, body mass remained slightly lower compared with baseline ($P = 0.002$). P_{osm} was restored following systemic rehydration but remained elevated following local rehydration ($P < 0.001$ vs. baseline). Mean difference in P_{osm} change following systemic versus local rehydration was 7.9 mosmol/kgH_2O (95% CI: 5.7–10.1 mosmol/kgH_2O). Neither systemic nor local rehydration normalized U_{osm} ($P < 0.001$ vs. baseline). In fact, following local rehydration, U_{osm} increased ($P = 0.001$ vs. dehydration). Consequently, U_{osm} was higher after local compared with systemic rehydration ($P = 0.001$). Mean difference in U_{osm} change between modes of rehydration was 286 mosmol/kgH_2O (95% CI: 162–412 mosmol/kgH_2O). Systemic rehydration increased plasma volume ($P = 0.013$ vs. dehydration, $P = 0.012$ vs. baseline) and reduced hemoglobin concentration ($P = 0.017$), while no changes were noted following local rehydration. Mean differences between systemic and local rehydration were 4.0% (95% CI: 1.7–6.4%) and 4.6 g·L^{-1} (95% CI: −0.2–9.3 g·L^{-1}) for changes in plasma volume and hemoglobin, respectively. Hematocrit was lower following systemic versus local rehydration ($P = 0.004$). Mean difference for the change in hematocrit between both modes of rehydration was 1.9% (95% CI: −0.2–3.6%).

Spirometry

Due to a technical issue, data from one participant had to be excluded from the analysis ($n = 9$). At study entry, baseline spirometry data were within normal range for all participants, with group mean data: 3.9 ± 0.76 liters (102 ± 12% pred.) for FEV_1; 5.06 ± 1.09 liters (108 ± 12% pred.) for FVC; 80 ± 7% for FEV_1/FVC; and 9.42 ± 1.92 L·s^{-1} (104 ± 12% pred.) for peak expiratory flow (PEF).

Effect of dehydration.

As illustrated in Fig. 1, mild dehydration induced by exercise and fluid restriction led to a reduction in FVC ($P < 0.001$) but not FEV_1. The change in FVC was not different between modes of dehydration (exercise: −173 ± 169 mL; fluid restriction: −131 ± 70 mL), with a mean difference of 43 mL (95% CI:

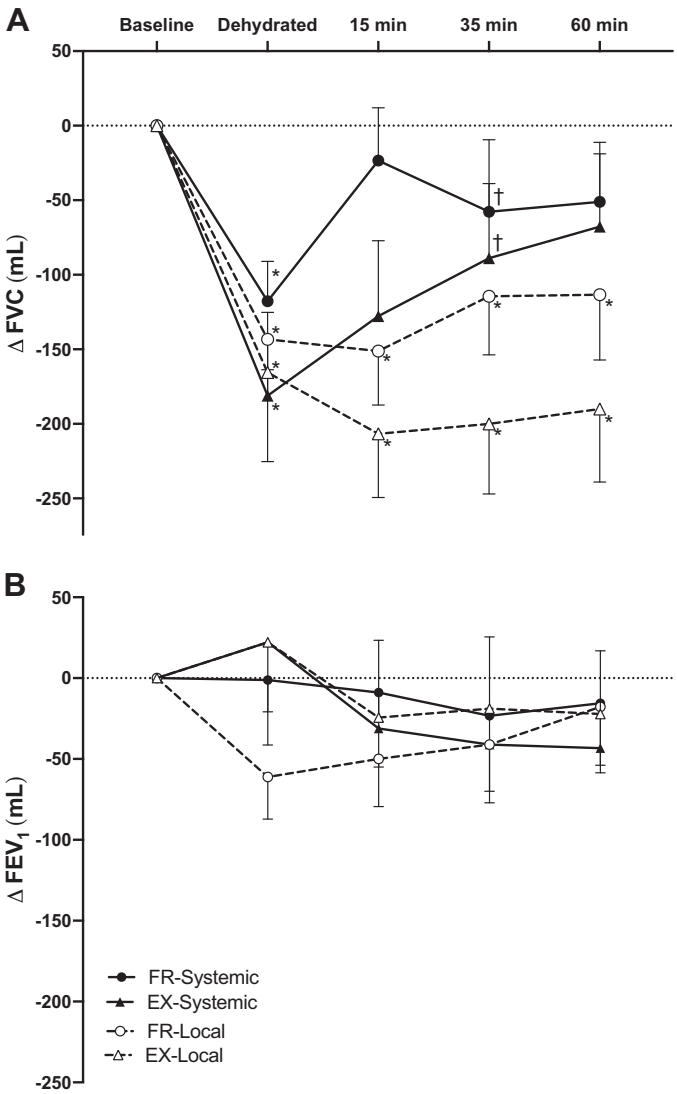

Figure 1. Changes in forced vital capacity (*A*; FVC) and forced expiratory volume in 1 s (*B*; FEV_1) following fluid restriction- and exercise-induced dehydration and at 15, 35, and 60 min of systemic (oral fluid intake) and local (nebulized isotonic saline) rehydration. Values are means ± SE for 9 healthy adults (2 females). Closed circles, fluid restriction with systemic rehydration; closed triangles, exercise with systemic rehydration; open circles, fluid restriction with local rehydration; open triangles, exercise with local rehydration. *$P < 0.05$ vs. baseline, †$P < 0.05$ vs. dehydration.

−50–136 mL). As FEV_1 did not change, the ratio FEV_1/FVC increased postdehydration ($P = 0.002$), with the increase slightly larger after exercise (3.5 ± 2.2 vs. 1.5 ± 1.8% for fluid restriction, $P < 0.001$). Mean difference in FEV_1/FVC change between exercise and fluid restriction was 2.0% (95% CI: 0.9–3.1%). No differences were noted between or within trials for PEF (data not shown).

Effect of rehydration.

After only 15 min of systemic rehydration, FVC was restored ($P > 0.05$ vs. baseline) (Fig. 1*A*). At that point, the mean difference in FVC improvement between systemic and local rehydration was 98 mL (95% CI: 18–178 mL). With local rehydration, FVC remained below baseline over the entire 60-min period of rehydration (Fig. 1*A*). FEV_1/FVC was restored by systemic rehydration ($P > 0.05$ vs. baseline at all recovery time points), while only transient improvements in FEV_1/FVC were noted at 15 and 35 min during local rehydration ($P > 0.05$ vs. baseline). Following 60 min of rehydration, FEV_1/FVC was 0.4 ± 1.9 and 2.1 ± 1.7% above baseline in the systemic and local rehydration trials, respectively ($P = 0.029$). Mean difference in FEV_1/FVC change between both modes of rehydration was 1.8% (0.7–2.9%). FEV_1 (Fig. 1*B*) and PEF (data not shown) remained unaltered following rehydration.

Whole Body Plethysmography

Whole body plethysmography data are presented in Table 3.

Effect of dehydration.

Static lung volumes and capacities were not different at baseline between trials. Following dehydration with both fluid restriction and exercise, increases in functional residual capacity (FRC; $P < 0.001$) and residual volume (RV; $P < 0.001$) were noted. Postdehydration changes in FRC (fluid restriction: 134 ± 152 mL; exercise: 131 ± 151 mL) and RV (fluid restriction: 151 ± 131 mL; exercise: 282 ± 194 mL) were not different between modes of dehydration (Fig. 2). The mean differences in FRC and RV increases between exercise and fluid restriction were 3 mL (95% CI: −113–119 mL) and 116 mL (95% CI: −9–240 mL), respectively. As total lung capacity (TLC) remained unchanged following dehydration, the RV/TLC ratio increased ($P < 0.001$), with exercise inducing a larger change ($P = 0.005$ vs. fluid restriction). Mean difference in RV/TLC increase between exercise and fluid restriction was 1.6% (95% CI: 0.1–3.1%). Expiratory reserve volume (ERV) and sRaw were unaffected by dehydration.

Effect of rehydration.

FRC and RV were both restored following systemic rehydration (both $P < 0.05$ vs. baseline) but did not change following local rehydration (FRC: $P = 0.010$ vs. baseline; RV: $P = 0.007$ vs. baseline; Fig. 2*B*). The mean differences in FRC and RV changes between systemic and local rehydration were 126 mL (95% CI: −20–271 mL) and 153 mL (95% CI: 25–281 mL), respectively. TLC was not impacted by rehydration. Consequently, RV/TLC was returned to baseline after systemic ($P > 0.05$ vs. baseline), but not after local rehydration ($P = 0.007$ vs. baseline). Mean difference in RV/TLC change following systemic versus oral rehydration was 2.0% (95% CI: 0.5–3.6%). ERV was slightly reduced after 60 min of systemic and local rehydration ($P = 0.011$ vs. baseline). Mean difference in ERV reduction between systemic and oral rehydration was 7 mL (95% CI: −9–106 mL). sRaw was unaltered by rehydration.

Correlation Analysis

The percent change in body mass in response to dehydration and rehydration (60 min) showed a moderate positive correlation with the change in FVC ($r = 0.643$, $P < 0.001$; Fig. 3*A*) and moderate negative correlations with the change in FRC ($r = −0.644$, $P < 0.001$; Fig. 3*C*) and RV ($r = −0.693$, $P < 0.001$; Fig. 3*E*). The change in P_{osm} following the dehydration and rehydration interventions showed a moderate negative correlation with the change in FVC ($r = −0.653$, $P < 0.001$;

Table 3. *Whole body plethysmography data at baseline, after fluid-restriction- and exercise-induced dehydration, and after 60 min of systemic and local (airway) rehydration*

	FR-Systemic	EX-Systemic	FR-Local	EX-Local
Total lung capacity, L				
Baseline	7.05 ± 1.48	7.06 ± 1.54	7.06 ± 1.49	7.10 ± 1.45
Dehydration	7.09 ± 1.46	7.10 ± 1.55	7.12 ± 1.40	7.14 ± 1.56
Rehydration	7.07 ± 1.46	7.19 ± 1.48	7.15 ± 1.50	7.17 ± 1.60
Functional residual capacity, L				
Baseline	4.03 ± 1.00	4.01 ± 1.07	3.91 ± 0.99	4.07 ± 1.03
Dehydration	4.08 ± 1.08[a]	4.15 ± 1.01[a]	4.12 ± 0.93[a]	4.19 ± 1.02[a]
Rehydration	3.90 ± 0.97[b,c]	4.02 ± 1.00[b,c]	4.09 ± 1.02[a]	4.15 ± 1.11[a]
Residual volume, L				
Baseline	2.14 ± 0.52	2.19 ± 0.55	2.19 ± 0.53	2.23 ± 0.53
Dehydration	2.27 ± 0.54[a]	2.49 ± 0.62[a]	2.36 ± 0.51[a]	2.49 ± 0.69[a]
Rehydration	2.13 ± 0.47[b,c]	2.29 ± 0.55[b,c]	2.35 ± 0.60[a]	2.47 ± 0.72[a]
Residual volume/total lung capacity, %				
Baseline	30.3 ± 4.9	31.0 ± 5.0	31.0 ± 4.4	31.4 ± 4.3
Dehydration	32.1 ± 5.2[a,d]	35.3 ± 6.3[a]	33.5 ± 5.4[a,d]	34.9 ± 6.5[a]
Rehydration	30.4 ± 3.3[b,c]	31.9 ± 4.1[b,c]	32.9 ± 5.2[e]	34.4 ± 6.7[e]
Expiratory reserve volume, L				
Baseline	1.89 ± 0.55	1.83 ± 0.63	1.72 ± 0.48	1.83 ± 0.53
Dehydration	1.81 ± 0.59	1.69 ± 0.54	1.76 ± 0.46	1.70 ± 0.49
Rehydration	1.75 ± 0.55[a]	1.73 ± 0.50[a]	1.74 ± 0.48[a]	1.68 ± 0.52[a]
Specific airway resistance, kPa·s^{-1}				
Baseline	0.99 ± 0.19	1.01 ± 0.22	1.05 ± 0.21	1.00 ± 0.25
Dehydration	1.03 ± 0.22	1.06 ± 0.22	1.08 ± 0.27	1.03 ± 0.24
Rehydration	0.97 ± 0.23	1.04 ± 0.29	1.06 ± 0.26	1.05 ± 0.26

Data are means ± SD; n =10. FR-Systemic, fluid restriction with systemic rehydration (oral fluid intake); EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration (nebulized isotonic saline); EX-Local, exercise with local rehydration. [a]$P < 0.05$ vs. baseline; [b]$P < 0.05$ vs. dehydration; [c]$P < 0.05$ vs. FR-Local and EX-Local at the corresponding time point, [d]$P < 0.05$ vs. EX-Systemic and EX-Local at the corresponding time point; [e]$P < 0.05$ vs. baseline and dehydration.

Fig. 3*B*) and moderate positive correlations with FRC (r = 0.524, $P < 0.001$; Fig. 3*D*) and RV (r = 0.587, $P < 0.001$; Fig. 3*F*). No other significant relationships were noted.

DISCUSSION

The findings from this study show negative alterations in pulmonary function in mildly dehydrated healthy adults following both 2 h of exercise in the heat and 28 h of fluid restriction. The observed reduction in FVC combined with an elevated RV and FRC suggest that the dehydration-induced pulmonary impairment is primarily localized to the small airways. Dehydration-induced alterations in pulmonary function were reversed following acute systemic rehydration (via oral fluid intake) but not following local rehydration of the airways (via nebulized isotonic saline). Systemic hydration, via plasma osmolality, may therefore play a regulatory role in the maintenance of small airway patency in healthy humans.

Effects of Dehydration

This study shows that mild systemic dehydration, induced by both exercise and fluid restriction, leads to a reduction in FVC (~150 mL) and elevations in RV (~220 mL) and FRC (~130 mL) in healthy adults. These findings are in line with our previous work that demonstrated negative alterations in FVC, RV and FRC in athletes with mild asthma following 2 h of exercise in the heat (6). That we were able to replicate our previous findings in a healthy population suggests that dehydration-induced pulmonary impairment is a general phenomenon that is present irrespective of the presence of pulmonary or airway disease. The reduced severity of the pulmonary function alterations (mean reduction in FVC following exercise ~170 mL vs. ~300 mL in asthmatic individuals (6), with only 30% of our healthy participants reaching the "clinical threshold" of 200 mL (26) suggests that, while still affected, the airways of healthy individuals have a higher tolerance to systemic dehydration in comparison with individuals with preexisting lung conditions.

Our spirometry results are in contrast to those previously obtained in healthy individuals (see introduction). Govindaraj (5) reported that mild dehydration (2.0 ± 0.9% loss of body mass) induced by 16 h of fluid restriction caused negligible changes in FVC but was associated with a significant reduction in FEV_1 (~180 mL). While we cannot explain the differences observed in FEV_1, the absence of a detectable change in FVC may be explained by the fact that in the study by Govindaraj (5) only 5 out of the 20 participants lost >2% body mass, whereas all our participants reached this threshold. According to Cheuvront and Kenefick (1), a day-to-day change in body mass >2% provides 95% probabilistic certainty that dehydration has occurred. A further study conducted by Javaheri et al. (7) showed improvements in pulmonary function (incl. FEV_1, FEV_1/FVC, and flow rates at all lung volumes) following moderate dehydration (i.e., 4.0 to 4.5% loss of body mass) induced by diuretics in a small sample (n = 6) of healthy men. The use of chlorthalidone could however explain the divergent findings, as diuretics cause iso-osmotic hypovolemia, whereas exercise and fluid restriction lead to hyperosmotic hypovolemia (13).

Effects of Rehydration

A novel finding of the current study is that systemic rehydration was effective at restoring selected lung volumes and

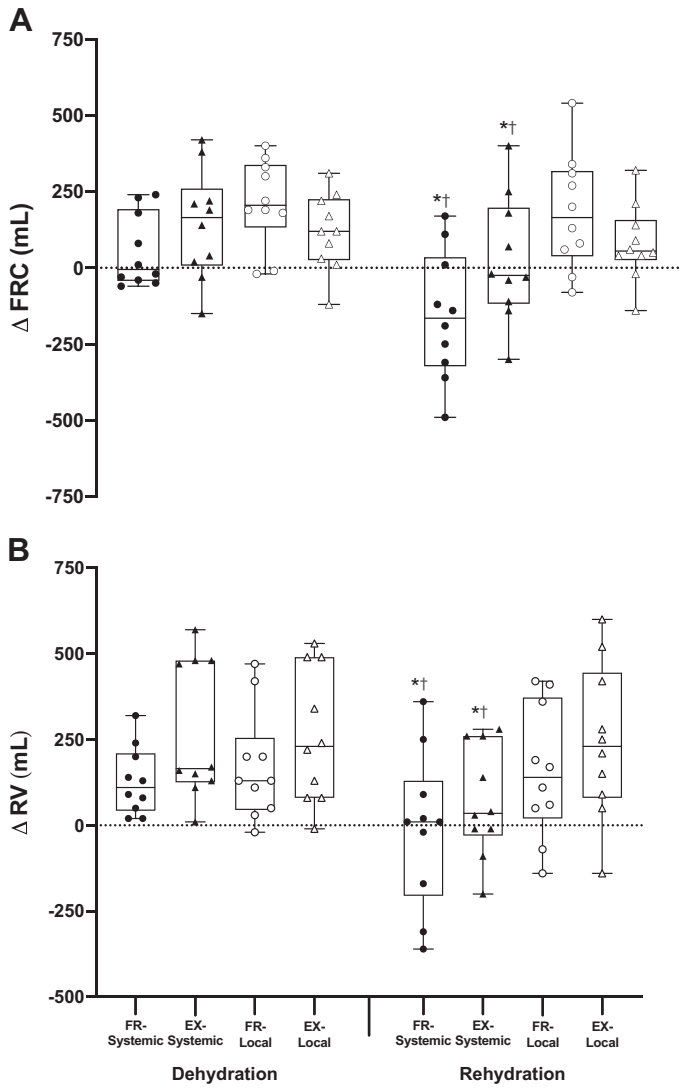

Figure 2. Changes in functional residual capacity (*A*; FRC) and residual volume (*B*; RV) after fluid restriction- and exercise-induced dehydration and following systemic (oral fluid intake) and local (nebulized isotonic saline) rehydration in 10 healthy adults (2 females). Boxplots represent the median and interquartile range, with whiskers representing the minimum and maximum values. FR-Systemic, fluid restriction with systemic rehydration; EX-Systemic, exercise with systemic rehydration; FR-Local, fluid restriction with local rehydration; EX-Local, exercise with local rehydration. *$P < 0.05$ vs. dehydration, †$P < 0.05$ vs. FR-Local and EX-Local at corresponding time point.

capacities (i.e., FVC, RV, and FRC). The positive effect of oral fluid intake on FVC was noted after only 15 min, which suggests a rapid reversal of the pulmonary alterations. Previously, dehydration-induced alterations in FVC, RV, and FRC were not restored following 40 min ad libitum water intake in individuals with asthma (6). The use of a matched-volume rehydration strategy [with 100% of fluid replaced vs. 61 ± 19% in previous work (6)], together with administration of a hypertonic solution known to improve fluid retention (24), enabled us to return body mass close to baseline within an hour. In contrast, following nebulized isotonic saline rehydration, body mass was maintained below (−1.7 ± 0.5 kg) and P_{osm} above (9 ± 4 mosmol/kgH_2O) baseline, and FVC, RV, and FRC were not restored. Our findings therefore suggest that oral hypertonic fluid intake, but not nebulized isotonic saline solution, is an effective strategy to reverse dehydration-induced pulmonary alterations.

Interpretation of Findings

A decrease in FVC alongside concomitant increases in RV, RV/TLC, and FRC is usually indicative of airway closure and air trapping (12). Our findings therefore suggest that systemic dehydration may selectively impair small airway function. Alterations in spirometry and plethysmography were noted conjointly with increases in P_{osm} following both dehydration modalities. Together with the reversal of FVC, RV, FRC, and RV/TLC under systemic rehydration only (i.e., when P_{osm} was normalized) and the significant association between P_{osm} and lung volumes, our finding points toward P_{osm} as a key determinant of the small airway impairment.

Pogson et al. (27) reported an inverse correlation between increased serum osmolality and decreased FVC and FEV_1 in a large population (>10,000) of patients suffering from chronic obstructive pulmonary disease. The authors suggested a causal relationship, mediated by airway epithelial cells, between increased serum plasma osmolality and reduced pulmonary function. Airway epithelial cells are "osmotic transducers" (28) that respond to changes in osmolality of both their extracellular and intracellular environments. Through controlled secretion and/or absorption of salt and water, airway epithelial cells preserve hydration of the airways and maintain water and osmolyte homeostasis in human lungs (29). In our dehydration trials, we postulate that airway epithelia "detected" the increase in P_{osm} in bronchial vasculature, which, in turn, would have influenced water supply to the airways and altered the composition and/or content of the airway surface liquid. The common functional implication of pertubations to the airway surface liquid is peripheral airway instability and premature airway closure (12), which aligns with the lung volume changes observed in our participants. During the systemic rehydration trials (i.e., oral fluid consumption), the rapid normalization of P_{osm} is likely to have facilitated the return of airway surface liquid to its hydrated state; this would have decreased surface tension and reopened the collapsed airways, thereby explaining the rapid restoration of lung volumes to baseline. That previous studies (30, 31) have evidenced restoration of plasma volume and extracellular osmolality (i.e., P_{osm}) within the timeframe used for our rehydration trials (30 to 60 min) supports the idea that extracellular hypervolemia following fluid consumption facilitates the recovery of small airway patency, even if full recovery of intracellular and interstitial compartments may take up to 4 h following rehydration (32). During the local rehydration trials (i.e., nebulized isotonic saline), the mucus layer may have acted as a "liquid reservoir" (33), with no or little influence on airway surface liquid ionic composition and, thereby, no ensuing reversal of small airway collapse and no restoration of pulmonary function.

In airway epithelia, numerous mechanisms ensure efficient control of airway surface fluid "depth" and composition. These mechanisms involve both passive surface forces (dependent on hydration status of the mucus layer) and active ion transport mechanisms (located within the airway

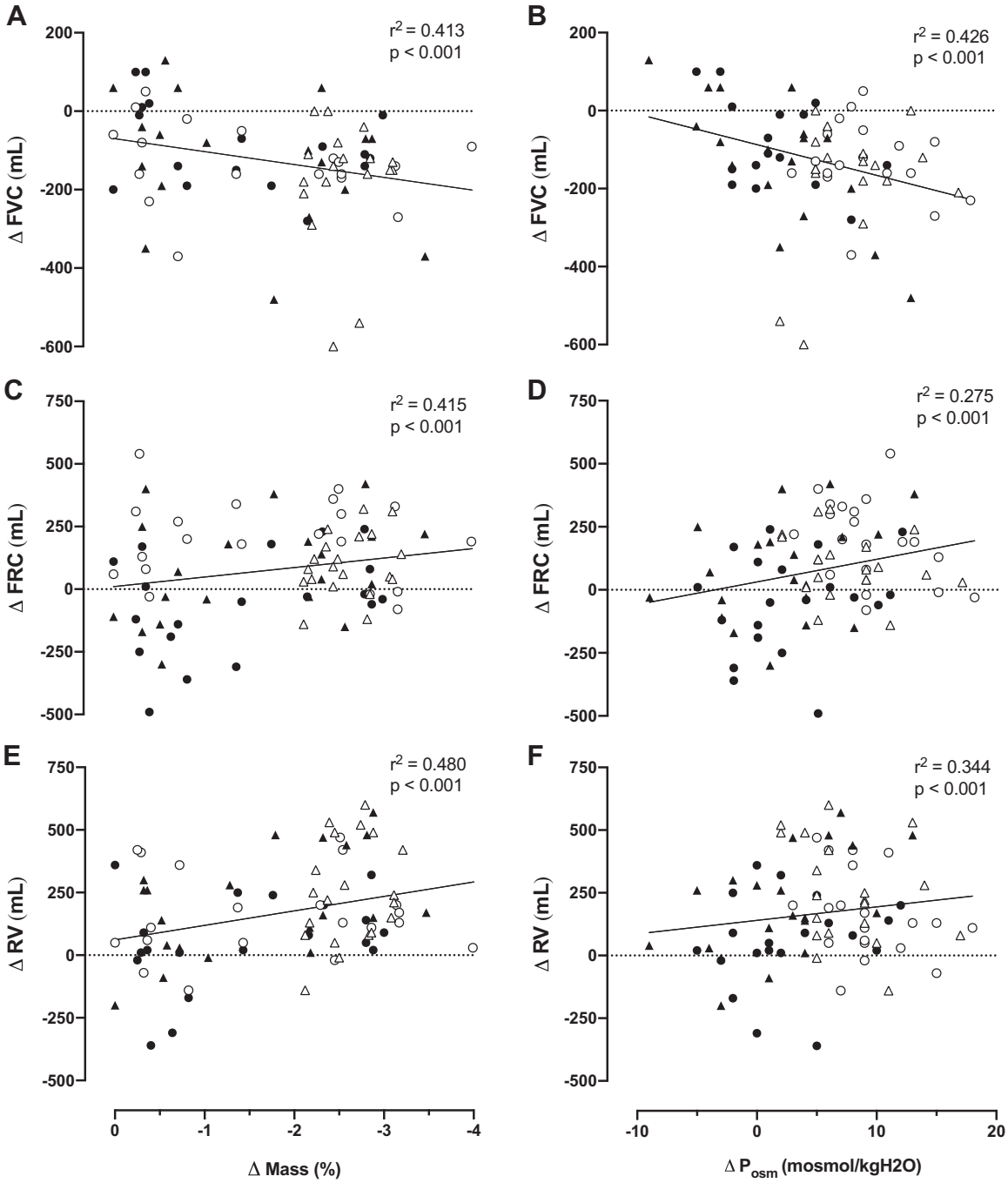

Figure 3. Relationships between change in body mass and changes in forced vital capacity (*A*; FVC), function residual capacity (*C*; FRC), and residual volume (*E*; RV) and relationships between change in plasma osmolality (P_{osm}) and changes in FVC (*B*), FRC (*D*), and RV (*F*) over the course of 4 trials (at dehydration and rehydration) in healthy adults ($n = 9$ for FVC and $n = 10$ for FRC and RV; 2 females). Closed circles, fluid restriction with systemic rehydration; closed triangles, exercise with systemic rehydration; open circles, fluid restriction with local rehydration; open triangles, exercise with local rehydration.

lining cells) (9). In our experiment, while the fluid restriction trials would have provided a prolonged window (28 h) for active ion transport to take place, thereby favoring maintenance of airway hydration, the involvement of such mechanism during the shorter (2 h) exercise trials is questionable. However, in cultures of differentiated human airway epithelia, rapid (within minutes) and transient increases in paracellular sodium conductance have been observed (34). Ion flow through the epithelial cells may therefore have counteracted the effects of systemic dehydration on local (airway) fluid availability in both sets of trials. The extent of this effect and implication for the severity of airway impairment require further work.

Methodological Considerations

The average ~2.6% loss of body mass in the present study was well matched with the ~2.3% loss in our previous study

of athletes with mild asthma (6) and across the four experimental trials. While the 28-h duration was required to induce the target degree of dehydration (19), it did not allow us to record pulmonary function at the same time of day within trials. To exclude diurnal variation as a confounder to the observed changes, we invited a subset of the participants (n = 5; 50% of our initial sample) to perform an additional "control" visit. Spirometry and whole body plethysmography were performed in a euhydrated resting state at matched time points to the experimental trials. The difference between morning and afternoon values was 85 ± 92 mL for FVC, −76 ± 200 mL for RV, and −54 ± 198 mL for FRC. As the directions of change were opposite to those found following dehydration, we can exclude diurnal variation as a confounding influence on the observed alterations in pulmonary function.

To account for the possible effect of evaporative water loss, through pulmonary ventilation, on airway surface liquid osmolarity (35), we estimated airway water losses during our dehydration trials. Airway water loss was greater during fluid restriction (~340 mL) compared with exercise (~80 mL) and accounted for 23% of total body water loss during the 28-h fluid restriction period versus only 5% during the 2 h of exercise. The nonsignificant differences in pulmonary impairments between the two modes of dehydration suggest that evaporative water loss was not a significant contributor to the observed changes. However, as typically reported in the literature (36, 37), relatively large variability was noted for some of our outcome measures (including RV and FRC). Therefore, to ascertain a lack of differential effect of exercise versus fluid restriction on dehydration-induced pulmonary impairment, our findings require replication using a larger sample and across a range of dehydration severities.

In the absence of a "gold standard" method for assessment of small airway function (38), we used a combination of highly standardized functional tests (i.e., spirometry and whole-body plethysmography). Alongside these functional tests, imaging techniques such as high-resolution computed tomography or magnetic resonance imaging could have helped to quantify small airway dysfunctions (38). An ultrasonic nebulizer was used to ensure a high flow rate and even distribution of water vapor was delivered to the airways; however, the rate of delivery ranged from 1.0 to 1.8 mL·min^{-1}. These values are lower than expected (39) and may have limited our ability to restore lung volumes and capacities. Prior work has shown that isotonic saline delivered as small droplets (as generated by the nebulizer used in the current study) penetrates to the lung periphery (40). We are confident therefore that our solution reached the small airways. However, our nebulized isotonic solution may have become hypotonic upon delivery, as the solution was only isotonic when delivered in a euhydrated state. In individuals with asthma, both hyper- and hypo-osmotic nebulized saline can compromise pulmonary function (41). Thus we cannot exclude the possibility that our local rehydration strategy modified airway surface liquid ion concentration, ultimately preventing restoration of pulmonary function.

Finally, it is possible that oral fluid consumption might have led to psychological benefits and, thereby, contributed to improved effort during volitional respiratory maneuvers. Cognitive task performance and mood have indeed been shown to improve following rehydration with oral fluid in healthy men (42). However, PEF, an effort-dependent variable, did not significantly change at any time in our study. We are therefore confident that the effort produced by our participants remained consistent throughout the trials and that any psychological effects were likely minimal.

Clinical and Functional Significance

Our findings have potential significance to both healthy and clinical populations. In particular, endurance athletes are at increased risk for exercise-induced dehydration and commonly report respiratory symptoms (including breathlessness and cough) while exercising (43). Older adults, especially those with pulmonary disease, often experience exertional breathlessness (44) and are particularly prone and vulnerable to dehydration (3). Further work is now needed to determine the impact of dehydration-induced pulmonary alterations on susceptibility to respiratory symptoms and to understand the risk of pulmonary function deterioration in dehydrated states. Whether dehydration, by increasing gas trapping, triggers or exacerbates dynamic lung hyperinflation, thereby promoting breathlessness during physically demanding tasks, remains to be determined. It is also conceivable that the changes induced by dehydration impair airway surface liquid and thus mucociliary clearance mechanisms. Further work is needed to explore the role of systemic dehydration on mucociliary dysfunction and pulmonary exacerbations.

Conclusions

Mild systemic dehydration was associated with a reduction in pulmonary function, primarily localized to the small airways. These changes occurred in healthy adults after both acute exercise in the heat and prolonged periods of fluid deprivation. Oral fluid consumption, but not nebulized isotonic saline, quickly reversed these alterations in pulmonary function. Future work is needed to explore the implications of dehydration-induced changes in pulmonary function in older adults, especially in those with pulmonary disease. In the meantime, oral rehydration appears to be the most effective strategy for reversing dehydration-induced pulmonary impairments.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

H.M., O.R.G., L.M.R., and P.K. conceived and designed research; H.M. and C.I. performed experiments; H.M. analyzed data; H.M., O.R.G., and P.K. interpreted results of experiments; H.M. prepared figures; H.M. drafted manuscript; H.M., O.R.G., L.M.R., C.I., J.H.H., and P.K. edited and revised manuscript; H.M., O.R.G., L.M.R., C.I., J.H.H., and P.K. approved final version of manuscript.

ENDNOTE

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at https://doi.org/10.6084/m9.figshare.12191496.v1. These materials are not a part of the manuscript and have not

REFERENCES

1. **Cheuvront SN**, **Kenefick RW.** Dehydration: physiology, assessment, and performance effects. *Compr Physiol* 4: 257–285, 2014. doi:10.1002/cphy.c130017.
2. **Sawka MN**, **Burke LM**, **Eichner ER**, **Maughan RJ**, **Montain SJ**, **Stachenfeld NS.** Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 377–390, 2007. doi:10.1249/mss.0b013e31802ca597.
3. **Allison SP**, **Lobo DN.** Fluid and electrolytes in the elderly. *Curr Opin Clin Nutr Metab Care* 7: 27–33, 2004. doi:10.1097/00075197-200401000-00006.
4. **Popkin BM**, **D'Anci KE**, **Rosenberg IH.** Water, hydration, and health. *Nutr Rev* 68: 439–458, 2010. doi:10.1111/j.1753-4887.2010.00304.x.
5. **Govindaraj M.** The effect of dehydration on the ventilatory capacity in normal subjects. *Am Rev Respir Dis* 105: 842–844, 1972. doi:10.1164/arrd.1972.105.5.842.
6. **Simpson AJ**, **Romer LM**, **Kippelen P.** Exercise-induced dehydration alters pulmonary function but does not modify airway responsiveness to dry air in athletes with mild asthma. *J Appl Physiol* 122: 1329–1335, 2017. doi:10.1152/japplphysiol.01114.2016.
7. **Javaheri S**, **Bosken CH**, **Lim SP**, **Dohn MN**, **Greene NB**, **Baughman RP.** Effects of hypohydration on lung functions in humans. *Am Rev Respir Dis* 135: 597–599, 1987.
8. **Baile EM.** The anatomy and physiology of the bronchial circulation. *J Aerosol Med* 9: 1–6, 1996. doi:10.1089/jam.1996.9.1.
9. **Boucher RC.** Regulation of airway surface liquid volume by human airway epithelia. *Pflugers Arch* 445: 495–498, 2003. doi:10.1007/s00424-002-0955-1.
10. **Haq IJ**, **Gray MA**, **Garnett JP**, **Ward C**, **Brodlie , M.** Airway surface liquid homeostasis in cystic fibrosis: pathophysiology and therapeutic targets. *Thorax* 71: 284–287, 2016. doi:10.1136/thoraxjnl-2015-207588.
11. **Anderson SD**, **Daviskas E.** Pathophysiology of exercise-induced asthma: role of respiratory water loss. In: *Allergic and Respiratory Disease in Sports Medicine*, edited by Weiler J. New York: Marcel Dekker Inc., 1997, p. 87–114.
12. **Macklem PT**, **Proctor DF**, **Hogg JC.** The stability of peripheral airways. *Respir Physiol* 8: 191–203, 1970. doi:10.1016/0034-5687(70)90015-0.
13. **Cheuvront SN**, **Kenefick RW**, **Charkoudian N**, **Sawka MN.** Physiologic basis for understanding quantitative dehydration assessment. *Am J Clin Nutr* 97: 455–462, 2013. doi:10.3945/ajcn.112.044172.
14. **Quanjer PH**, **Stanojevic S**, **Cole TJ**, **Baur X**, **Hall GL**, **Culver BH**, **Enright PL**, **Hankinson JL**, **Ip MS**, **Zheng J**, **Stocks J**, **Schindler C**, **ERS Global Lung Function Initiative.** Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 40: 1324–1343, 2012. doi:10.1183/09031936.00080312.
15. **Dill D**, **Costill D.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247–248, 1974. doi:10.1152/jappl.1974.37.2.247.
16. **Miller MR**, **Hankinson J**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Coates A**, **Crapo R**, **Enright P**, **van der Grinten CP**, **Gustafsson P**, **Jensen R**, **Johnson DC**, **MacIntrye N**, **McKay R**, **Navajas D**, **Pedersen OF**, **Pellegrino R**, **Viegi G**, **Wagner J**, **ATS/ERS Task Force.** Standardisation of spirometry. *Eur Respir J* 26: 319–338, 2005. doi:10.1183/09031936.05.00034805.
17. **Wanger J**, **Clausen JL**, **Coates A**, **Pedersen OF**, **Brusasco V**, **Burgos F**, **Casaburi R**, **Crapo R**, **Enright P**, **van der Grinten CP**, **Gustafsson P**, **Hankinson J**, **Jensen R**, **Johnson D**, **MacIntyre N**, **McKay R**, **Miller MR**, **Navajas D**, **Pellegrino R**, **Viegi G.** Standardisation of the measurement of lung volumes. *Eur Respir J* 26: 511–522, 2005. doi:10.1183/09031936.05.00035005.
18. **Goldman MD**, **Smith HJ**, **Ulmer WT.** Whole-body plethysmography. In: *European Respiratory Monograph*, edited by Gosselink R, Stam H. Sheffield, UK: European Respiratory Society Journals Ltd, 2005, p. 15–43.
19. **Szinnai G**, **Schachinger H**, **Arnaud MJ**, **Linder L**, **Keller U.** Effect of water deprivation on cognitive-motor performance in healthy men and women. *Am J Physiol Regul Integr Comp Physiol* 289: R275–R280, 2005. doi:10.1152/ajpregu.00501.2004.
20. **Daviskas E**, **Gonda I**, **Anderson SD.** Local airway heat and water vapour losses. *Respir Physiol* 84: 115–132, 1991. doi:10.1016/0034-5687(91)90023-C.
21. **Borg GA.** Psychophysical bases of perceived exertion. *Med Sci Sport Exerc* 14: 377–381, 1982.
22. **Mahler DA**, **Horowitz MB.** Perception of breathlessness during exercise in patients with respiratory disease. *Med Sci Sports Exerc* 26: 1078–1081, 1994.
23. **Mitchell JW**, **Nadel ER**, **Stolwijk JA.** Respiratory weight losses during exercise. *J Appl Physiol* 32: 474–476, 1972. doi:10.1152/jappl.1972.32.4.474.
24. **Evans GH**, **James LJ**, **Shirreffs SM**, **Maughan RJ.** Optimizing the restoration and maintenance of fluid balance after exercise-induced dehydration. *J Appl Physiol* 122: 945–951, 2017. doi:10.1152/japplphysiol.00745.2016.
25. **Bland JM**, **Altman , DG.** Statistics notes: calculating correlation coefficients with repeated observations: Part 1–correlation within subjects. *BMJ* 310: 446–446, 1995. doi:10.1136/bmj.310.6977.446.
26. **Pellegrino R**, **Viegi G**, **Brusasco V**, **Crapo RO**, **Burgos F**, **Casaburi R**, **Coates A**, **van der Grinten CPM**, **Gustafsson P**, **Hankinson J**, **Jensen R**, **Johnson DC**, **MacIntyre N**, **McKay R**, **Miller MR**, **Navajas D**, **Pedersen OF**, **Wanger J.** Interpretative strategies for lung function tests. *Eur Respir J* 26: 948–968, 2005. doi:10.1183/09031936.05.00035205.
27. **Pogson ZE**, **McKeever TM**, **Fogarty A.** The association between serum osmolality and lung function among adults. *Eur Respir J* 32: 98–104, 2008. doi:10.1183/09031936.00144207.
28. **Willumsen NJ**, **Davis CW**, **Boucher RC.** Selective response of human airway epithelia to luminal but not serosal solution hypertonicity. Possible role for proximal airway epithelia as an osmolality transducer. *J Clin Invest* 94: 779–787, 1994. doi:10.1172/JCI117397.
29. **Bartoszewski R**, **Matalon S**, **Collawn JF.** Ion channels of the lung and their role in disease pathogenesis. *Am J Physiol Lung Cell Mol Physiol* 313: L859–L872, 2017. doi:10.1152/ajplung.00285.2017.
30. **Maughan RJ**, **Leiper JB.** Sodium intake and post-exercise rehydration in man. *Eur J Appl Physiol Occup Physiol* 71: 311–319, 1995. doi:10.1007/BF00240410.
31. **Nose H**, **Mack GW**, **Shi X**, **Nadel ER.** Role of osmolality and plasma volume during rehydration in humans. *J Appl Physiol* 65: 325–331, 1988. doi:10.1152/jappl.1988.65.1.325.
32. **Armstrong LE.** Hydration assessment techniques. *Nutr Rev* 63: S40–S54, 2005. doi:10.1111/j.1753-4887.2005.tb00153.x.
33. **Tarran R**, **Grubb BR**, **Gatzy JT**, **Davis CW**, **Boucher RC.** The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J Gen Physiol* 118: 223–236, 2001. doi:10.1085/jgp.118.2.223.
34. **Flynn AN**, **Itani OA**, **Moninger TO**, **Welsh MJ.** Acute regulation of tight junction ion selectivity in human airway epithelia. *Proc Natl Acad Sci USA* 106: 3591–3596, 2009. doi:10.1073/pnas.0813393106.
35. **Boucher RC.** Molecular insights into the physiology of the 'thin film' of airway surface liquid. *J Physiol* 516: 631–638, 1999. doi:10.1111/j.1469-7793.1999.0631u.x.
36. **Hankinson JL**, **Stocks J**, **Peslin R.** Reproducibility of lung volume measurements. *Eur Respir J* 11: 787–790, 1998.
37. **Houghton CM**, **Woodcock AA**, **Singh D.** A comparison of plethysmography, spirometry and oscillometry for assessing the pulmonary effects of inhaled ipratropium bromide in healthy subjects and patients with asthma. *Br J Clin Pharmacol* 59: 152–159, 2005. doi:10.1111/j.1365-2125.2004.2262.x.
38. **Konstantinos Katsoulis K**, **Kostikas K**, **Kontakiotis T.** Techniques for assessing small airways function: Possible applications in asthma and COPD. *Respir Med* 119: e2–e9, 2016. doi:10.1016/j.rmed.2013.05.003.
39. **Katial RK**, **Reisner C**, **Buchmeier A**, **Bartelson BB**, **Nelson HS.** Comparison of three commercial ultrasonic nebulizers. *Ann Allergy Asthma Immunol* 84: 255–261, 2000. doi:10.1016/S1081-1206(10)62763-9.
40. **Eberl S**, **Chan HK**, **Daviskas E**, **Constable C**, **Young I.** Aerosol deposition and clearance measurement: A novel technique using dynamic SPET. *Eur J Nucl Med* 28: 1365–1372, 2001 doi:10.1007/s002590100586.

41. **Schoeffel RE**, **Anderson SD**, **Altounyan RE.** Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline. *Br Med J (Clin Res Ed)* 283: 1285–1287, 1981. doi:10.1136/bmj.283.6302.1285.
42. **Zhang N**, **Du SM**, **Zhang JF**, **Ma GS.** Effects of dehydration and rehydration on cognitive performance and mood among male college students in Cangzhou, China: a self-controlled trial. *Int J Environ Res Public Health* 16: 1891, 2019. doi:10.3390/ijerph16111891.
43. **Couto M**, **Moreira A.** The athlete "out of breath. *Eur Ann Allergy Clin Immunol* 48: 36–45, 2016.
44. **O'Donnell DE**, **Laveneziana P**, **Webb K**, **Neder JA.** Chronic obstructive pulmonary disease: clinical integrative physiology. *Clin Chest Med* 35: 51–69, 2014.