Enhanced renal Na\textsuperscript{+} reabsorption by carbohydrate in beverages during restitution from thermal and exercise-induced dehydration in men

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IT IS WELL KNOWN that relatively severe thermal dehydration by exercise and body weight loss within 30 min. During the 60 min before the start of drinking and the following 180 min, we measured plasma volume (PV), plasma glucose ([Glc]p), serum insulin ([Ins]s), plasma Na\textsuperscript{+} concentrations, and the renal clearances of insulin, lithium, and Na\textsuperscript{+} with plasma vasopressin ([AVP]p) and aldosterone concentrations ([Alld]p) every 30 min. After dehydration, PV decreased by ~5% and plasma osmolality increased by ~6 mosmol/kg H\textsubscript{2}O in all trials with no significant differences among them. We found in the high-carbohydrate trial that J PV increased faster than in the control trial and remained at the higher level than other trials for the last 60 min (P < 0.05); 2) accumulated urine volume was smallest after 90 min (P < 0.05); 3) the renal Na\textsuperscript{+} reabsorption rate was greatest for the first 120 min (P < 0.05); 4) during which period [AVP]p and [Alld]p were not significantly different from other trials (both, P > 0.9); and 5) [Glc]p and [Ins]s were highest from 45 to 105 min (P < 0.05) during rehydration. Thus carbohydrate in beverages enhances renal Na\textsuperscript{+} reabsorption, and insulin is possibly involved in this enhancement.

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Based on these findings as a background, in the present study we compared the renal Na\textsuperscript{+} reabsorption rate during recovery from thermal and exercise-induced dehydration while varying the carbohydrate concentration of beverages in three trials. Moreover, to assess the effects of carbohydrate-induced insulin secretion on the renal Na\textsuperscript{+} reabsorption rate, we gave subjects the same amount of liquid as total body weight loss by dehydration within the first 30 min of rehydration in all trials. Our hypothesis was that the renal Na\textsuperscript{+} reabsorption rate would be enhanced as carbohydrate concentration in beverage increased with increased plasma insulin concentration.

METHODS

Subjects

The procedures in this study conformed to the guidelines of the Declaration of Helsinki and were approved by the Review Board on Human Experiments, Shinshu University School of Medicine. After the experimental protocols had been fully explained, seven healthy and nonsmoking young men gave their written informed consent before participating in this study. All subjects were recreationally active in sports/exercise and had no history of cardiovascular or pulmonary diseases. The physical characteristics of the subjects were 25 ± 6 (SD) years old, 169 ± 6 cm height, 65.37 ± 10.83 kg weight, 3,081 ± 592 ml peak aerobic power (V\textsubscript{O}2peak), and 2,946 ± 535 ml plasma volume (PV).

Trials

Subjects performed high-carbohydrate (HC), low-carbohydrate (LC), and control (CNT) rehydration trials by drinking one of three beverages with 3.4 g glucose + 3.1 g fructose, 1.7 g glucose + 0.0 g fructose, or 0.0 g fructose per liter, respectively, in a common composition of electrolyte solution: 21 meq/l [Na\textsuperscript{+}], 5 meq/l [K\textsuperscript{+}], 16.5 meq/l [Cl\textsuperscript{−}], and 10 meq/l [citrate\textsuperscript{−}]. They drank the same amount of beverage as total body weight loss within 30 min. During the 60 min before the start of drinking and the following 180 min, we measured plasma volume (PV), plasma glucose ([Glc]p), serum insulin ([Ins]s), plasma Na\textsuperscript{+} concentrations, and the renal clearances of insulin, lithium, and Na\textsuperscript{+} with plasma vasopressin ([AVP]p) and aldosterone concentrations ([Alld]p) every 30 min. After dehydration, PV decreased by ~5% and plasma osmolality increased by ~6 mosmol/kg H\textsubscript{2}O in all trials with no significant differences among them. We found in the high-carbohydrate trial that J PV increased faster than in the control trial and remained at the higher level than other trials for the last 60 min (P < 0.05); 2) accumulated urine volume was smallest after 90 min (P < 0.05); 3) the renal Na\textsuperscript{+} reabsorption rate was greatest for the first 120 min (P < 0.05); 4) during which period [AVP]p and [Alld]p were not significantly different from other trials (both, P > 0.9); and 5) [Glc]p and [Ins]s were highest from 45 to 105 min (P < 0.05) during rehydration. Thus carbohydrate in beverages enhances renal Na\textsuperscript{+} reabsorption, and insulin is possibly involved in this enhancement.

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fructose, or 0.0 g glucose + 0.0 g fructose per deciliter, respectively, in a common composition of electrolyte solution: 21 meq/l [Na+], 5 meq/l [K+], 16.5 meq/l [Cl-], 10 meq/l [citrate−], and 1 meq/l [lactate−], after thermal and exercise-induced dehydration. The osmolarity of the beverages was 350, 277, and 77 mosmol/kgH2O in HC, LC, and CNT trials, respectively. Each trial was conducted at the same time of day separated by at least 7 days from the preceding trial to avoid any effects of circadian rhythm or thermal adaptation by exercise on the measurements. The order of the trials was randomized.

Protocol

Subjects performed the trials at least 1 wk after PV and \( V_{\text{O}2\text{peak}} \) were measured. Figure 1 shows an outline of the protocol. On the day before a trial, subjects were given the same meals prepared by us according to age-matched RDA for Japanese people (23): 2,569 kcal total energy and 10.8 g salt, and were asked to refrain from alcohol, caffeine, and strenuous physical activity during the day. In addition, they were asked to take 300 mg lithium carbonate (LITIOMAL; Fujinaga Pharmaceutical, Tokyo, Japan) and 500 ml water immediately before going to bed at ~22:00 to measure lithium clearance the next day, as described below.

On the morning of the trial day, subjects reported to the laboratory at 06:00, rested in a sitting position in a test room controlled to 28 ± 1°C atmospheric temperature (T\(_\text{a}\)) and 25 ± 17% relative humidity (RH) (mean ± range), and ate a light breakfast: 200 kcal, 0.4 g salt, and 500 ml water. After the first blood sample (14 ml) had been taken at 07:15 through an 18-gauge Teflon catheter placed in the left antecubital vein, they emptied their bladders, weighed themselves to the nearest 10 g (HW-100K; A&D, Tokyo), and entered an artificial climate chamber controlled to 36.0 ± 1°C (mean ± range), and sat in the contour chair of the cycle ergometer in an upright position while being attached to ECG electrodes. They then repeated a set of 30 min exercise at 50% \( V_{\text{O}2\text{peak}} \) and 10 min rest 1–3 times until they had lost ~2.3% weight, during which time sweat was collected with a vinyl bag over the forearm skin surface attached to the bag for collecting sweat over the forearm was removed, and the bag for collecting sweat over the forearm was removed, and the absorbance of a plasma sample at 10 min after the start of exercise (27). If subjects were not able to continue the intensity of exercise at the last set, we shortened the exercise period. The weight loss was 1.58 ± 0.11, 1.57 ± 0.10, and 1.53 ± 0.08 kg in HC, LC, and CNT trials, respectively, with no significant differences between them (P = 0.772).

After the targeted body weight loss had been attained at ~09:00, the bag for collecting sweat over the forearm was removed, and subjects rested in a sitting position until 11:00 without any fluid. They were infused with 1% inulin dissolved in 0.9% saline (INULEAD; Fuji Yakuhin, Saitama, Japan) from ~09:30 at 5 ml/min for 30 min through another 18-gauge Teflon catheter placed in the right antecubital vein. At ~10:00, the infusion was stopped for a few minutes for subjects to empty their bladders, and then inulin infusion was restarted at 1.67 ml/min until 14:00. Using this procedure, we confirmed that inulin concentration in plasma was maintained almost constant until the end of the experiment. At ~11:00, subjects drank the same amount of liquid at ~14°C as the body weight was lost within 30 min: 1,577 ± 115, 1,566 ± 97, and 1,531 ± 77 ml in HC, LC, and CNT trials, respectively. We defined the time at the onset of drinking as 0 min. They then rested in a sitting position for an additional 150 min with no fluids. Fourteen-milliliter blood samples were taken 45 min (~45) and 15 min (~15) before the start of drinking and at 15, 45, 75, 105, 135, and 165 min. Urine volume was measured using a cylinder at ~30, 0, 30, 60, 90, 120, 150, and 180 min.

All experiments were performed between October and June to avoid any effects of heat acclimatization in summer.

Measurements

Plasma volume. \( V_{\text{O}2\text{peak}} \) was determined with graded cycle-ergometer exercise in an upright position in an artificial climate chamber controlled to 25.0 ± 0.1°C T\(_\text{a}\) and 25 ± 1% RH (mean ± range) on the day after the PV measurement. After the ECG electrodes had been applied, the subjects started pedaling at 60 cycles/min at an initial intensity of 0 W. The intensity was increased by 60 W every 3 min until reaching 180 W; above this intensity, it was increased by 30 W every 2 min up to 240 W, and then 15 W every 2 min until subjects were not able to maintain the rhythm. \( V_{\text{O}2\text{peak}} \) was calculated every 15 s from the oxygen and carbon dioxide fractions in expired gas and the expired ventilatory volume (Aeromonitor AE300s; Minato, Tokyo). \( V_{\text{O}2\text{peak}} \) was determined after the three largest consecutive values at the end of exercise had been averaged.

Blood properties. A 1-ml aliquot of each 14-ml blood sample was used to determine hematocrit (Hct in%; microcentrifuge) and hemoglobin concentration ([Hb] in g/dl; sodium lauryl sulfate hemoglobin method). Three milliliters of aliquot were transferred to a heparin-treated tube and immediately centrifuged at room temperature for 3 min, and the plasma was placed in a chilled tube and stored at −85°C until assayed, which was used to determine plasma total protein concentration ([TP]p) by refractometry, plasma osmolality ([P]p) by the freezing point depression method (one-ten osmometer; FISKE, Norwood, MA), plasma Na+ and K+ concentrations ([Na+]p, [K+]p, respectively) by flame photometry (480 Flame Photometer; Corning, Medfield, MA), and plasma glucose concentration ([Glc]p) by an enzymatic method with glucose oxidase (YSI 2300 STAT PLUS; YSI, Yellow Springs, OH).

Fig. 1. Experimental protocol. After dehydration by exercise in a warm environment, subjects drank the same amount of one of 3 beverages (see details in text) as total sweat loss within 30 min. Before and after drinking, they were infused intravenously with inulin dissolved in saline. Blood and urine were sampled at the timings indicated with arrows. The samples were used to determine blood and urine compositions and to determine the glomerular filtration rate (GFR), and Li+, Na+, and water clearances. RH, relative humidity; T\(_\text{a}\), atmospheric temperature; \( V_{\text{O}2\text{peak}} \), peak aerobic power.
Seven milliliters of aliquot were transferred into a 10-ml glass serum separation tube, and at 30 min later these tubes were centrifuged at 4°C. The serum was placed in another chilled tube and also stored at −85°C until assayed. The serum was used to determine concentrations of insulin ([Ins]s), Li+, (Li+), and inulin by chemiluminescence enzyme immunoassay (LUMIPULSE L2400; Fujirebio, Tokyo), atomic absorption spectrometry (Z-6100; Hitachi High-Technologies), and using an F-kit (Roche Diagnostics, Mannheim, Germany), respectively.

In 6 of 7 subjects, the remaining 3 ml of aliquots were placed in a chilled tube (EDTA 2Na 1.5 mg/ml), which was centrifuged at 4°C and stored at −85°C until the determination of arginine vasopressin ([AVP]p) and aldosterone concentrations ([Ald]p) by radioimmunoassay (AVP-RIA Mitsubishi; Mitsubishi Chemical Medience, Tokyo and SPAC-S Aldosterone; TFB, Tokyo, respectively). The respective intra-assay coefficients of variation for the measurements of [AVP]p were 14.40 and 8.59% at the levels of 1.08 and 7.15 pg/ml, and those of [Ald]p were 15.05 and 6.35% at the levels of 32.1 and 386.7 pg/ml, respectively.

[Na+]p, [K+]p, (Li+), and [Glc]p were expressed in milliequivalents or millimoles per kilogram water after correcting for [TP]p (27). [Ins], (in μU/ml) was converted to nanomoles per milliliter(4).

**Sweat properties.** Sweat in the bag was mixed carefully, sampled into a 1.5-ml tube, and stored in a refrigerator at −85°C until assayed. Sweat Na+ concentration on the forearm ([Na+]sw) was determined by flame photometry.

**Urinary properties.** The urine was placed in a chilled tube and stored at −85°C until assayed. A 3-ml urine sample was used to determine urine osmolality (Uosmol) and Na+ ([Na+]u), K+ ([K+]u), and sweat Na+ concentration ([Na+]sw). Inulin concentrations were determined by the same assay as the blood analyses.

**Calculations.**

Averaged sweat Na+ concentration throughout the whole body ([Na+]sw) was estimated from [Na+]sw using the equation: [Na+]sw = 0.57[Na+]sw + 11.05 (3). We determined sweat loss after exercise from a decrease in body weight. Also, we calculated the body fluid volume from a decrease in body weight. Also, we calculated the body fluid volume from a decrease in body weight. Also, we calculated the body fluid volume from a decrease in body weight. Also, we calculated the body fluid volume from a decrease in body weight.

We tested any significant differences in the values before dehydration (Table 1) and total water and Na+ gains between trials using one-way (3 within groups) ANOVA for repeated measures. We tested any significant differences in the values in Tables 2 and Figs. 2–7 between trials using two-way [3 within groups and time] ANOVA for repeated measures. Because there were only seven subjects per trial, which limited the statistical power (1-β) and the effect size in the comparison of variables among the trials by two-way ANOVA (8), we confirmed that the statistical power and the effect size were 1.000 and 0.17 for [Glc]p, and 1.000 and 0.21 for [Ins]s, respectively, in Fig. 4, and 0.809 and 0.05 for RabNa, 0.644 and 0.04 for PRabNa, and 0.24 for DRabNa, respectively, in Fig. 7. Subsequent post hoc tests were performed using the Tukey-Kramer method. The standard least squares method was used to perform regression analyses between [Ins], versus PRabNa, and DRabNa in Fig. 8. All comparisons were regarded significant when P < 0.05.

**RESULTS.**

Table 1 shows PV, [TP]p, Posmol, [Na+]p, [K+]p, and [Glc]p before dehydration and [Na+]sw during dehydration. We confirmed that there were no significant differences between trials. Table 2 shows blood properties after and during dehydration. After dehydration, PV decreased by ~140 ml while [TP]p, [Na+]p, [K+]p, Pposmol, and [Na+]sw increased by 259 ± 204, 6.8 ± 0.1, 6.8 ± 0.1, and 5.73 ± 0.10, respectively, compared to baseline. [K+]p increased by 0.085 while [Na+]sw decreased by 0.24. Inulin clearance was measured from the measurement of inulin clearance every 30 min from baseline of PV by the Evans blue dye dilution method before the first trial and percent change in PV (%ΔPV) calculated from Hct and [Hb] (16). Assuming that red cell blood volume was constant throughout the experiments. Changes in PV during dehydration/rehydration in each trial were presented as differences from PV before dehydration. Also, %ΔPV in respect of the level before drinking (after dehydration) is presented.

The inulin (glomerular filtration rate, GFR), Na+, (CNa), and Na+, (Cosmol) clearances were calculated by standard methods. Considering Donnan’s effect, these clearances were corrected for [TP]p, presented as g H2O/min. CNa allows us to estimate fluid flowout from the proximal to distal tubules, because filtered Li+ is reabsorbed exclusively by the proximal tubules (20). We calculated the fractional tubular reabsorption of Na+ in the total (FRNa), proximal (FPRNa), and distal tubules (FDFNa), respectively. Also, we calculated the tubular reabsorption rate of Na+ in the total (RabNa), proximal (PRabNa), and distal tubules (DRabNa), respectively. These calculation were performed according to the equations shown in the appendix (24).

Although these renal functions were determined from urine samples taken 15 min after every blood sampling, we adopted them as the values corresponding to the blood samples since they were averaged values during 30 min urine collection, reflecting the composition of urine 15 min before sampling.

**Statistics.**

Table 1 shows Plasma properties before dehydration and Na+ concentration in sweat during dehydration in three trials

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>LC</th>
<th>CNT</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV, ml</td>
<td>2.978 ± 204</td>
<td>3.213 ± 259</td>
<td>3.185 ± 255</td>
<td>0.085</td>
</tr>
<tr>
<td>[TP]p, g/dl</td>
<td>6.8 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>0.580</td>
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<tr>
<td>Posmol, mosmol/kg H2O</td>
<td>288 ± 1</td>
<td>290 ± 1</td>
<td>289 ± 1</td>
<td>0.197</td>
</tr>
<tr>
<td>[Na+]p, meq/kg H2O</td>
<td>149.0 ± 0.5</td>
<td>149.0 ± 1.1</td>
<td>149.2 ± 0.9</td>
<td>0.976</td>
</tr>
<tr>
<td>[K+]p, meq/kg H2O</td>
<td>4.06 ± 0.07</td>
<td>4.04 ± 0.07</td>
<td>4.18 ± 0.03</td>
<td>0.243</td>
</tr>
<tr>
<td>[Glc]p, mmol/kg H2O</td>
<td>5.73 ± 0.10</td>
<td>5.78 ± 0.12</td>
<td>5.89 ± 0.21</td>
<td>0.704</td>
</tr>
<tr>
<td>[Na+]sw, meq/l</td>
<td>60.6 ± 6.4</td>
<td>58.6 ± 6.7</td>
<td>58.3 ± 8.1</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Values are the means ± SE of 7 subjects. HC and LC, high- and low-glucose trials, respectively; CNT, control trial; PV, plasma volume; [TP]p, [Na+]p, [K+]p, and [Glc]p; total protein, sodium, potassium, and glucose concentrations in plasma, respectively; Pposmol, plasma osmolality; [Na+]sw, Na+ concentration of sweat in the whole body during dehydration. [Na+]sw, estimated in the forearm measured in the present study using an equation reported previously (3). PV was measured by the Evans blue dye dilution method once before the first trial in each subject, and the values before dehydration in the following trials were estimated from hematocrit and hemoglobin concentrations assuming that blood cell volume remained unchanged throughout the experiments (16).

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Table 2. Plasma variables before and after drinking in three trials

<table>
<thead>
<tr>
<th>Trials</th>
<th>−15 min</th>
<th>15 min</th>
<th>45 min</th>
<th>75 min</th>
<th>105 min</th>
<th>135 min</th>
<th>165 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPV, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>−146 ± 28</td>
<td>−185 ± 35</td>
<td>4 ± 41</td>
<td>95 ± 43*</td>
<td>119 ± 24*</td>
<td>132 ± 27*</td>
<td>125 ± 51</td>
</tr>
<tr>
<td>LC</td>
<td>−116 ± 27</td>
<td>−178 ± 30</td>
<td>72 ± 82*</td>
<td>105 ± 60*</td>
<td>96 ± 31*</td>
<td>98 ± 27</td>
<td>72 ± 35</td>
</tr>
<tr>
<td>CNT</td>
<td>−163 ± 31</td>
<td>−242 ± 33</td>
<td>−69 ± 47</td>
<td>−59 ± 46</td>
<td>−33 ± 50</td>
<td>26 ± 32</td>
<td>51 ± 26</td>
</tr>
<tr>
<td>[TP]p, g/dl</td>
<td></td>
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<tr>
<td>HC</td>
<td>7.4 ± 0.1</td>
<td>7.5 ± 0.2</td>
<td>7.1 ± 0.2</td>
<td>6.9 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>LC</td>
<td>7.3 ± 0.1</td>
<td>7.4 ± 0.1</td>
<td>6.8 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>CNT</td>
<td>7.2 ± 0.1</td>
<td>7.3 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>[Na+]p, meq/kgH2O</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>294 ± 1</td>
<td>297 ± 1</td>
<td>297 ± 1*</td>
<td>294 ± 1†</td>
<td>291 ± 1*</td>
<td>289 ± 1</td>
<td>289 ± 1</td>
</tr>
<tr>
<td>LC</td>
<td>296 ± 1</td>
<td>297 ± 2</td>
<td>295 ± 2*</td>
<td>289 ± 1</td>
<td>290 ± 2</td>
<td>289 ± 1</td>
<td>289 ± 1</td>
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<tr>
<td>CNT</td>
<td>295 ± 1</td>
<td>296 ± 1</td>
<td>290 ± 2</td>
<td>289 ± 1</td>
<td>287 ± 1</td>
<td>289 ± 1</td>
<td>288 ± 1</td>
</tr>
<tr>
<td>[K+]p, meq/kgH2O</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HC</td>
<td>152.0 ± 0.7</td>
<td>152.9 ± 0.6</td>
<td>149.6 ± 0.7</td>
<td>149.6 ± 0.5</td>
<td>149.9 ± 0.6</td>
<td>149.8 ± 0.4</td>
<td>150.2 ± 0.8</td>
</tr>
<tr>
<td>LC</td>
<td>152.5 ± 0.9</td>
<td>152.7 ± 0.8</td>
<td>149.0 ± 0.5</td>
<td>148.7 ± 0.3</td>
<td>148.5 ± 0.6</td>
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<td>150.4 ± 0.6</td>
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<tr>
<td>CNT</td>
<td>153.0 ± 1.0</td>
<td>153.5 ± 1.1</td>
<td>149.7 ± 0.8</td>
<td>149.0 ± 0.9</td>
<td>148.9 ± 0.7</td>
<td>148.8 ± 1.2</td>
<td>148.7 ± 1.1</td>
</tr>
<tr>
<td>P_osmol, mosmol/kgH2O</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HC</td>
<td>4.48 ± 0.15</td>
<td>4.48 ± 0.12</td>
<td>4.19 ± 0.10*</td>
<td>3.98 ± 0.12*</td>
<td>4.01 ± 0.10*</td>
<td>4.17 ± 0.09</td>
<td>4.22 ± 0.12</td>
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<tr>
<td>LC</td>
<td>4.49 ± 0.09</td>
<td>4.50 ± 0.11</td>
<td>4.33 ± 0.13</td>
<td>4.24 ± 0.10</td>
<td>4.25 ± 0.08</td>
<td>4.30 ± 0.07</td>
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<tr>
<td>CNT</td>
<td>4.55 ± 0.12</td>
<td>4.53 ± 0.13</td>
<td>4.51 ± 0.11</td>
<td>4.45 ± 0.07</td>
<td>4.37 ± 0.06</td>
<td>4.26 ± 0.06</td>
<td>4.23 ± 0.06</td>
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</table>

Values are the means ± SE of 7 subjects. ΔPV, change in plasma volume from the baseline before dehydration; P_osmol, plasma osmolality. *P < 0.05 vs. CNT trial. †P < 0.05 HC vs. LC.

P_osmol, [Na+]p, and [K+]p increased by ~0.5 g/dl, 6 mosmol/kgH2O, 3 meq/kgH2O, and 0.4 meq/kgH2O, respectively, with no significant differences between trials. During rehydration, we found that ΔPV and P_osmol were generally higher in HC and LC trials than in CNT trial during 45–105 min, while [K+]p was lower in HC trial than CNT trial during this period.

As in Fig. 2, top left, total fluid gain by the end of rehydration by beverage intake and by saline infusion for measuring inulin clearance was 2,146 ± 111, 2,135 ± 94, and 2,106 ± 74 ml in HC, LC, and CNT trials, respectively, with no significant differences among trials (between trials, P = 0.860 in main factor for ANOVA). On the other hand, accumulated urine volume sampled every 30 min during dehydration and rehydration was significantly lower in the HC trial than the other trials after 90 min, and by the end of rehydration, 564 ± 73, 673 ± 100, and 691 ± 49 ml in HC, LC, and CNT, respectively, significantly less in the HC trial than other trials. As a
result, we found that the net volume gain was slightly but significantly higher in the HC trial than CNT trial (P < 0.05).

Similarly, as in Fig. 2, top right, total Na\(^+\) gain by the end of rehydration was 121 ± 2, 120 ± 2, and 121 ± 3 meq in HC, LC, and CNT trials, respectively, with no significant differences among trials. In addition, negative Na\(^+\) balance due to sweat loss and urine loss during dehydration was 91 ± 13, 84 ± 10, 94 ± 11 meq in HC, LC, and CNT trials, respectively, with no significant difference among trials (between trials, P = 0.857 in main factor for ANOVA). On the other hand, accumulated Na\(^+\) loss into urine every 30 min during dehydration and rehydration was significantly less in HC and LC trials than in CNT trial after 90 min, and by the end of rehydration, it was 22 ± 3, 26 ± 3, and 32 ± 3 meq in HC, LC, and CNT trials, respectively, which was less in HC and LC trials than in CNT trial. As a result, negative Na\(^+\) balance in HC and LC trials was recovered by 120 min of rehydration, respectively, earlier than 180 min in CNT trial, with significantly higher net Na\(^+\) gain in the HC trial than CNT trial after 120 min (P < 0.05).

As shown in Fig. 3, we found that %ΔPV was higher in HC and LC trials than CNT trial for the first 75 min of rehydration (both, P < 0.05); however, the increase in %ΔPV for LC trial was blunted after 105 min with no significant differences from CNT trial. On the other hand, %ΔPV in the HC trial remained significantly higher than LC and CNT trials at 135 min and LC trial at 165 min.

As shown in Fig. 4, [Glc]\(_p\) increased after drinking beverages and showed peak values of 10.6 ± 0.6 and 9.2 ± 0.4 mmol/kgH\(_2\)O at 45 min in HC and LC trials, respectively, and thereafter gradually decreased to the baseline, being significantly higher in the HC trial than LC trial from 45 to 135 min. Similarly, [Ins]\(_s\) showed peak values of 0.59 ± 0.08 and 0.27 ± 0.03 nmol/l at 45 min in HC and LC trials, respectively, and thereafter gradually decreased to the baseline, being significantly higher in the HC trial than LC trial from 15 to 135 min. On the other hand, both remained unchanged throughout measurements in the CNT trial.

Figure 5 shows GFR, free water clearance (C\(_{\text{H}_{2}\text{O}}\)), C\(_{\text{Li}}\), and C\(_{\text{Na}}\) from after dehydration to the end of rehydration. The figure also shows [AVP]\(_p\) and [Ald]\(_p\) from before dehydration to the end of rehydration. As in the figure, GFR was significantly higher in the HC trial than other trials from 15 to 45 min. C\(_{\text{H}_{2}\text{O}}\) was lower in the HC trial than other trials from 45 to 75 min. There were no significant differences in C\(_{\text{Li}}\) and C\(_{\text{Na}}\) between trials (between trials, P = 0.227 and 0.172 for the main factor of ANOVA, respectively). Also, there were no significant differences in the profiles of [AVP]\(_p\) and [Ald]\(_p\) until 105 min of rehydration among the trials (between trials, both, P > 0.9, for the main factor of ANOVA), but [Ald]\(_p\) in the HC trial increased thereafter with a significant difference from LC trial at 165 min (P < 0.05) and CNT trial at 135 and 165 min (both, P < 0.05).

Figure 6 shows FR\(_{\text{Na}_{\text{a}}}\), PFR\(_{\text{Na}_{\text{a}}}\), and DFR\(_{\text{Na}_{\text{a}}}\) after dehydration and during rehydration. FR\(_{\text{Na}_{\text{a}}}\) in HC and LC trials was higher than in CNT trial from 15 to 105 min and from 45 to 75 min, respectively. Also, FR\(_{\text{Na}_{\text{a}}}\) was higher in HC trial than LC trial at 15 min. Although PFR\(_{\text{Na}_{\text{a}}}\) during rehydration was generally similar between trials except for the slight differences between LC trial and CNT trial at 135 and 165 min, DFR\(_{\text{Na}_{\text{a}}}\) in HC and LC trials was significantly higher than CNT trial between 15 and 165 min except at 135 min and 45–75 min, respectively.
Since we found marked differences in FRNa between trials with 120-min rehydration and also since we found no significant differences in \([\text{AVP}]_p\) and \([\text{Ald}]_p\) between trials during the period, we determined RabNa, PRabNa, and DRabNa for the first 120 min and for the latter 60 min separately. As in Fig. 7, we found that RabNa for the first 120 min was 21.8 ± 2.5 meq/min in HC trial, significantly higher than 18.5 ± 2.4 and 18.0 ± 2.6 meq/min in LC and CNT trials, respectively, which was largely due to significantly higher PRabNa in HC group than in other groups (between trials, \(P = 0.0134\) in main factor for ANOVA). Also, we found that RabNa for the latter 60 min was 18.8 ± 2.2 meq/min in HC trial and significantly higher than 16.8 ± 2.1 and 16.4 ± 2.3 meq/min in LC and CNT trials, respectively (between trials, \(P = 0.0363\) in main factor for ANOVA).

Figure 8 shows the relationships between \([\text{Ins}]_s\) versus PRabNa and DRabNa when the values after dehydration and during the first 120-min rehydration were pooled, during which period, again, no significant differences in \([\text{AVP}]_p\) and \([\text{Ald}]_p\) among trials were observed. In the figure, mean values for seven subjects are presented with critical difference bars for \([\text{Ins}]_s\), PRabNa, and DRabNa at \(P = 0.05\). The critical differences were determined by the Tukey-Kramer method after confirming by two-way ANOVA for repeated measures that main effects of time on the variables were significant during the period (\(P < 0.0001, P = 0.028,\) and \(P = 0.0025,\) respectively). We found that \([\text{Ins}]_s\) was significantly correlated with PRabNa (\(r = 0.652; P = 0.022\)) and DRabNa (\(r = 0.649;\) respectively).
DISCUSSION

In the present study, we found that Na\(^+\) excretion into urine was lower, fluid and Na\(^+\) retentions were higher, and PV recovery was greater as carbohydrate concentration in beverages for rehydration was increased. In addition, we found that it was achieved by the enhanced renal Na\(^+\) reabsorption rate for the first 120 min of rehydration. Furthermore, we found that this enhanced renal Na\(^+\) reabsorption rate was significantly correlated with increased [Ins]\(_s\) by drinking beverages with carbohydrate, while there were no increases in [AVP]\(_p\) and [Ald]\(_p\) during the period.

**Body Fluid and Na\(^+\) Balances**

As in Fig. 2, although there were no significant differences in fluid volume and Na\(^+\) gains among trials, we found that urine volume and Na\(^+\) loss into urine were lower, and therefore net volume and Na\(^+\) gain were higher as carbohydrate concentration in beverage increased. In the HC trial, since the lower urine volume and Na\(^+\) loss into urine started from 60 min of rehydration and also since no significant increases in [AVP]\(_p\) and [Ald]\(_p\) were observed during the period (Fig. 5), these hormones were unlikely to be involved in the mechanisms.

**Fig. 7. Change in renal Na\(^+\) reabsorption (Rab\(_{Na}\))**, proximal (PRab\(_{Na}\)) and distal tubular Na\(^+\) reabsorption (DRab\(_{Na}\)) for the first 120 min and the latter 60 min during rehydration. Means and SE bars for 7 subjects are presented.

*Significant differences vs. CNT; †significant differences vs. LC; $significant differences from the first 120 min period at \(P < 0.05\).

**Fig. 8. Relationships between serum insulin concentration ([Ins]\(_s\)) vs. Na\(^+\) reabsorption in proximal (PRab\(_{Na}\)) and distal tubules (DRab\(_{Na}\)) when the values after dehydration and during the first 120-min rehydration were pooled are shown with critical difference bars (C.D.) for each variable at \(P < 0.05\). C.D. were determined by the Tukey-Kramer method after confirming using two-way ANOVA for repeated measures that main effects of time on the variables were significant during the period (\(P < 0.0001\), \(P < 0.028\), and \(P < 0.0025\), respectively). The symbol marked with parentheses in the upper panel is located outside the 95% range of the confidence limits, indicated with the gray lines, of the regression line.
Plasma Volume

As shown in Table 2 and Fig. 3, PV recovered more in HC and LC trials than in the CNT trial for the first 120 min of rehydration despite the similar net fluid and Na\(^+\) gains. This may be caused partially by accelerated intestinal water absorption due to enhanced Na\(^+\) and glucose cotransportation (15, 30). In addition, the lower reduction in \(P_{\text{osmol}}\) of which was explained by increased [Glcp] in HC and LC trials than in CNT trial would prevent fluid shift from extracellular to intracellular space (26), and decrease urine volume by increasing renal free water reabsorption, thereby accelerating PV recovery; however, the possibility might be minor. It has been reported that glucose and fructose move partially through the cell membrane and also they are metabolized quickly so that they do not work so strongly as osmotic active substances compared with other ionic molecules in the body (2). Indeed, we confirmed no significant increase in [AVP]\(_p\) during the period (Fig. 5), suggesting that the increases in glucose and/or fructose did not stimulate at least osmotic receptors in the brain. Thus the enhanced PV recovery for the first period of rehydration with increased carbohydrate concentration in beverages was caused mainly by enhanced absorption of fluid in the intestine.

As in Fig. 3, \(\%\Delta PV\) remained higher in the HC trial than other trials for the last 60 min of rehydration. It might be explained by prolonged absorption of fluid due to higher concentration of carbohydrate in the beverage. Experimentally, we found significantly higher [Glcp] in the HC trial than in LC trial at 135 min of rehydration (Fig. 4). Alternatively, less urine volume and Na\(^+\) loss into urine starting after the first 60 min of rehydration in the HC trial (Fig. 2) might contribute to higher \(\%\Delta PV\) by enhancing recovery of extracellular fluid volume.

\([\text{Ins}]_s\) and RabNa

We found that GFR was highest in the HC trial for the first 45 min of rehydration (Fig. 5) with the highest increases in \([\text{Ins}]_s\) and [Glcp] (Fig. 4). Also, FR\(_{Na}\) and RabNa in this period were highest in the trial (Figs. 6 and 7). Furthermore, we found that \([\text{Ins}]_s\) was significantly correlated with PRabNa and DRabNa (Fig. 8).

Cohen et al. (7) examined the effects of insulin on renal blood flow, GFR, and the Na\(^+\) reabsorption rate in the rat kidney perfused in vitro and suggested that insulin caused renal vasodilation and increased GFR. Recently, Tucker et al. (33) confirmed these results in awake rats. Moreover, there have been several studies suggesting that insulin enhanced Na\(^+\) reabsorption in the proximal as well as distal tubules in the rodent kidney using the microperfusion technique in vitro (6, 10–12, 19, 32). In the present study, we reconfirmed that RabNa and PRabNa were higher in the HC trial than in other trials for the first 120-min period of rehydration with significant correlations between \([\text{Ins}]_s\) versus PRabNa and DRabNa (Fig. 8). In Fig. 8, top panel, a point at 45 min of rehydration in the HC trial (marked with parentheses) is located outside the 95% range of the confidence limits of the regression line. This might have been because the PRabNa value, calculated by using a urine sample collected from 0 to 30 min, rapidly increased after 15 min at which blood was sampled to determine \([\text{Ins}]_s\). These results suggest that the highest RabNa in the HC trial was at least partially caused by the highest \([\text{Ins}]_s\).

When considering glomerular and tubular Na\(^+\) balance, FL\(_{Na}\) was 3.2 and 3.8 meq/min higher in the HC trial than LC and CNT trials, respectively, for the first 120 min on average while, as in Fig. 7, RabNa was 3.3 and 3.9 meq/min higher in the HC trial than in LC and CNT trials, respectively, in this period, ~0.1 meq/min more than the greater filtered load of Na\(^+\) in the HC trial than in other trials. Indeed, we confirmed that the accumulated Na\(^+\) loss into urine tended to be less in HC trial than other trials during rehydration with significant differences between HC and CNT trials after 120 min of rehydration. Thus the lower amount of Na\(^+\) loss into urine was likely caused by more enhanced RabNa than FL\(_{Na}\) in the HC trial compared with other trials for the first 120 min.

\([\text{Ald}]_p\) for Latter 60 min of Rehydration

As in Fig. 5, \([\text{Ald}]_p\) in the HC trial slightly but significantly increased after 135 min of rehydration with significant difference from other trials. The mechanism remained unknown; however, a possible reduction of Na\(^+\) concentration in the ascending loop of Henle due to increased PRabNa for the first period of rehydration might stimulate renin secretion via the juxtaglomerular body (21), although after some delay, which might partially contribute to the increase in DRabNa for the latter 60 min of rehydration (Fig. 7).

Plasma K\(^+\) Concentration

As in Table 2, \([\text{K}^+]_s\) was lower in the HC trial than in CNT trial from 45 to 105 min rehydration. This might have been caused by the action of insulin to stimulate Na\(^+\)-K\(^+\)-ATPase in the skeletal muscular membrane, enhancing K\(^+\) uptake and Na\(^+\) release from intracellular fluid space (18) or by increased K\(^+\) release from plasma to the intestinal lumen due to enhanced Na\(^+\) and glucose cotransportation through the intestinal epithelia (14).

Limitations

In the present study, we did not allow subjects to drink beverages ad libitum, the higher Na\(^+\) retention was caused primarily by the action of insulin to stimulate Na\(^+\)-K\(^+\)-ATPase in the skeletal muscular membrane, enhancing K\(^+\) uptake and Na\(^+\) release from intracellular fluid space (18) or by increased K\(^+\) release from plasma to the intestinal lumen due to enhanced Na\(^+\) and glucose cotransportation through the intestinal epithelia (14).

In the present study, we used male subjects to avoid any effects of menstrual cycle on the results as it has been suggested that sex hormones, such as estrogen and progesterone, have significant effects on body fluid regulation (31) and blood glucose homeostasis (34), therefore, our finding might be limited to only male subjects.

Finally, we estimated \([\text{Na}^+]_{\text{osm}}\) from \([\text{Na}^+]_s\) according to the equation previously reported by absorbent patch method for regional sweat collection (3), different from the method in the present study. However, since there was no significant differ-
Perspectives and Significance

The total renal reabsorbed Na⁺ for 180 min of rehydration was ~8 meq more in the HC trial than CNT trial, equivalent to only 9% of total Na⁺ loss into sweat and urine during dehydration. It did not seem to significantly contribute to Na⁺ retention during restitution from thermal dehydration; however, since this enhanced Na⁺ retention in the HC trial was caused by ~100 g (~400 kcal) carbohydrate in the beverage and also since caloric intake of a meal is generally 1,000 kcal, more Na⁺ retention and PV recovery are expected to occur from a meal due to more enhanced and prolonged insulin secretion. Indeed, Osterberg et al. (28) reported that fluid retention in the body significantly increased during 240-min rehydration by a drinking beverage with 12% carbohydrate, accompanied by a more increased and prolonged plasma insulin level, compared with beverages with less than 6% carbohydrate. Thus this assumption is consistent with the previous finding that voluntary dehydration was recovered with meals (1). In the present study, we have suggested one of the possible mechanisms. In conclusion, carbohydrate in diluted electrolyte solution enhanced renal Na⁺ reabsorption during restitution from thermal and exercise-induced dehydration in young male subjects, and insulin is possibly involved in this enhancement.

APPENDIX

We calculated the renal functions according the following equations.

\[
\begin{align*}
FL_{Na} &= [Na⁺]_p \times GFR / 1000, \\
UE_{Na} &= [Na⁺]_u \times UF / 1000, \\
Rab_{Na} &= FL_{Na} - UE_{Na}, \\
FR_{Na} &= Rab_{Na} / FL_{Na} \times 100\%, \\
PER_{Na} &= (1 - C_L/C_L) \times 100\%, \\
PRab_{Na} &= FL_{Na} / PER_{Na} \times 100\%, \\
DFR_{Na} &= (1 - C_O/C_L) \times 100\%, \\
DRAb_{Na} &= C_L \times [Na⁺]_p / DFR_{Na} \times 100\%, \\
C_{H₂O} &= UF - C_{osmol},
\end{align*}
\]

where FL_{Na} is filtered load of Na⁺ in the glomerulus (meq/min); [Na⁺]_p is Na⁺ concentration in plasma (meq/kgH₂O); GFR is glomerular filtration rate (ml/min); UE_{Na} is urinary loss of Na⁺ (meq/min); [Na⁺]_u is Na⁺ concentration in urine (meq/kgH₂O); UF is urine flow (ml/min); Rab_{Na} is the reabsorption rate in the total tubules (meq/min); FR_{Na} is the fractional reabsorption of Na⁺ in the total tubules (%); PER_{Na} is the fractional reabsorption of Na⁺ in the proximal tubules (%); PRab_{Na} is the reabsorption rate in the proximal tubules (meq/min); DFR_{Na} is the fractional reabsorption of Na⁺ in the distal tubules (%); DRAb_{Na} is the reabsorption rate in the distal tubules (meq/min); and C_{H₂O} is free water clearance (ml/min).

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