Acute effects of moderate dehydration on the hepatic conversion of amino nitrogen into urea nitrogen in healthy men

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ABSTRACT

Intracellular hydration may play a role in the regulation of protein and nitrogen metabolism. The hepatic removal of nitrogen by urea synthesis has a key regulatory role in nitrogen balance. The purpose of the present study was to establish the acute effects of dehydration on the hepatic kinetics of urea synthesis, quantified by functional hepatic nitrogen clearance (FHNC), in healthy volunteers. Seven healthy men were studied twice in random order. On both study days, a primed continuous infusion of alanine was given. On the day of dehydration an intravenous bolus injection of a loop diuretic (furosemide, 1 mg/kg) was superimposed. FHNC was calculated as the ratio between measured synthesis rates of urea nitrogen and blood alanine concentrations. Furosemide induced a weight loss of 1 kg. During dehydration, FHNC decreased by approx. 25% (41 ± 11 to 54 ± 10 litres/h; \( P < 0.02 \)). On both occasions individual FHNC and glucagon values were positively correlated \( (r^2 = 0.6) \). In addition, dehydration more than halved the linear slope of the relationship \( (P < 0.05) \). The FHNC values were correlated with the urinary excretion of both potassium and sodium \( (r^2 = 0.68, P < 0.01 \) and \( r^2 = 0.62, P < 0.02 \) respectively). Changes in the reactivity of urea synthesis to glucagon (i.e. the ratio between FHNC and glucagon concentration) was negatively correlated with an indirectly estimated change in intracellular water \( (r^2 = 0.79, P < 0.05) \). We conclude that acute moderate dehydration down-regulates both total urea synthesis and its sensitivity to glucagon. The latter was related to estimated intracellular water loss. Dehydration may thus have nitrogen-saving consequences with regard to the hepatic contribution to whole-body nitrogen homoeostasis. The mechanism of this effect and the relationship with sodium and potassium fluxes is not known.

INTRODUCTION

All living cells possess metabolic mechanisms that actively counteract the effects of changes in hydration that otherwise threaten the integrity of the cell. Conversely, many metabolic regulatory pathways utilize secondary changes in hydration as a signal in metabolic homoeostasis; an example is the regulation of cellular protein synthesis by changes in hydration secondary to the influx of amino acids [1]. However, most of this information has originated from experiments on isolated cells or organs, and not much is known about the metabolic effects of changes in hydration in vivo under physiological or pathophysiological conditions.

The effects of changes in hydration on hepatic urea synthesis are of particular interest. Phylogenetically, urea is the archaic osmotic filler that protected cells against osmotic challenges [2]. Urea synthesis is orchestrated together with protein synthesis, in the sense that nitrogen can be utilized or excreted either way. Moreover,
increased urea synthesis seems to be a primary hepatic element of stress catabolism, during which changes in hydration are also frequent. A recent study in healthy men demonstrated that slow (> 15 h) gradual lowering of osmolality influenced whole-body protein, glucose and lipid metabolism [3]. Hypo-osmolality resulted in protein sparing [3].

The purpose of the present work, therefore, was to study the effects of acute dehydration on the regulation of urea synthesis in vivo. Substrate regulation of urea synthesis was quantified by means of the so-called functional hepatic nitrogen clearance (FHNC), which is the amino nitrogen substrate standardized rate of urea synthesis, thus representing the quantitative ability for urea synthesis.

MATERIALS AND METHODS

Subjects

Seven healthy men (median age 23 years; range 21–27 years), with normal blood pressure (median 115/80 mmHg; range 120–110/85–75 mmHg) and with no signs or symptoms of disease, volunteered to participate. Subjects did not receive any medical treatment for 2 weeks before each study day. Informed consent was obtained from all participants. The study was approved by the local Committee of Ethics, and was carried out in accordance with the Second Declaration of Helsinki.

Procedure

The study was performed as an open random sequence placebo-controlled cross-over study. All subjects were studied on two separate days, separated by an interval of 4 weeks: a control day and an intervention (dehydration) day. The procedures on the two study days were identical, except for the intravenous bolus injection of the loop diuretic furosemide (Diurall; DAK, Nycomed, Roskilde, Denmark; 1 mg/kg body weight), which was given at 11.15 hours on the day of intervention. Values for 24 h urinary sodium excretion did not differ between the study days (control, mean 171 (range 60–200) mmol/24 h; dehydrated, 186 (141–272) mmol/24 h).

On each study day, after a 12 h fast, an oral water load (200 ml of tap water) was given every 30 min from 07.00 hours until diuretics were given on the day of active treatment. The following period, the intervention period, lasted 45 min. Glucagon and insulin concentrations were measured as the time averages corresponding to the urine collections, unless stated otherwise.

Venous blood samples were taken from a cubital vein; infusions were given in the other arm. Blood samples were drawn at the beginning and end of the basal period, and after 45 min. Glucagon and insulin concentrations were measured by RIA using Wick chromatography. Glucagon was extracted by ethanol [6]. Plasma and urine sodium and potassium, haemoglobin, albumin and IgM were measured by routine methods in the Department of Clinical Chemistry, Aarhus University Hospital. Body weight was measured with a precision of ± 0.1 kg (Soehline, Germany).

Total body water (TBW) in the basal period was assessed using the nomogram [7]:

\[
TBW = 0.3265 \times bw + 0.2239 \times bh - 0.1387 \times age - 14.47
\]

where bw is body weight (kg), bh is body height (cm) and age is given in years. The decrease in body weight during the experiment was assumed to be equal to loss of TBW. Extracellular water volume (ECV) was estimated to be 33% of TBW in the basal period [8], and changes in plasma IgM concentration were taken to reflect changes in interstitial fluid IgM. In the intervention period, ECV was estimated as:

\[
ECV_{ip} = ECV_{bp} \times IgM_{bp}/IgM_{ip}
\]

where the subscripts ip and bp denote the intervention period and the basal period respectively (see above).

Intracellular water volume (ICV) was estimated as TBW minus ECV.

The loss of intracellular sodium (dICV Na⁺) in the intervention period was calculated as:

\[
dICV Na^+ = uNa^+_ip - (ECV_{ip} \times pNa^+_{ip} - ECV_{bp} \times pNa^+_{bp})
\]
where the prefixes u and p denote urinary and plasma respectively. The loss of intracellular potassium was calculated using the same formula, but substituting K⁺ for Na⁺.

FHNC was calculated as the ratio between the corresponding data sets of urea nitrogen synthesis rate (UNSR) and the average alanine concentration. FHNC thus standardizes UNSR with regard to substrate (i.e. alanine) drive. UNSR was calculated using the urinary rate of excretion of urea nitrogen (E) and the accumulation of urea nitrogen in TBW (A), corrected for the fractional loss by hydrolysis in the gut of newly synthesized urea (L), taken to be 0.17, i.e. [9]:

\[ \text{UNSR} = \frac{A + E}{1 - L}. \]

Glucagon sensitivity was quantified as the ratio between FHNC and glucagon concentration.

**Statistics**

Data are presented as means ± S.D. unless stated otherwise. Analyses were performed using paired Student’s t-tests. If data were not normally distributed, logarithmic (ln) transformation was performed. Two-tailed  P values of < 0.05 were considered statistically significant.

One subject was excluded from data analysis of changes in water volume, as he experienced nausea after the injection of diuretic, and retention of water in the gastrointestinal space was considered possible.

Comparison between regression analyses was done as regression between groups [10].

**RESULTS**

**Water and electrolytes**

Furosemide decreased body weight by 1% (Table 1). The blood concentrations of haemoglobin, albumin and sodium increased by 7%, 15% and 1% respectively, indicating extracellular dehydration. Plasma concentrations of potassium and IgM did not change significantly.

Dehydration increased urinary sodium loss by a factor of 13, and urinary potassium loss by a factor of 3.5 (Table 2). The calculated loss of potassium from the intracellular space was doubled (P < 0.01), while no change was found for sodium.

During dehydration, the calculated values of TBW and ECV decreased significantly, by 2.5% and 14% respectively. The approximate intracellular space decreased by 1%, which did not reach statistical significance.

**Table 1** Hydration and electrolyte response to alanine infusion with (dehydrated) and without (control) diuretic injection in seven healthy subjects in the intervention period. Values are means ± S.D. ns, not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Dehydrated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>76.5 ± 5.0</td>
<td>75.6 ± 5.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Haemoglobin (mmol/l)</td>
<td>9.0 ± 0.3</td>
<td>9.6 ± 0.5</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Plasma albumin (μmol/l)</td>
<td>655 ± 37</td>
<td>751 ± 28</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>IgM (μmol/l)</td>
<td>0.60 ± 0.29</td>
<td>0.65 ± 0.26</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma Na⁺ (mmol/l)</td>
<td>138 ± 1</td>
<td>140 ± 1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Plasma K⁺ (mmol/l)</td>
<td>4.3 ± 0.2</td>
<td>4.4 ± 0.3</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Table 2** Effects of dehydration on water and electrolytes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention period</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Na (mmol/l)</td>
<td>Control (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>11 ± 3</td>
</tr>
<tr>
<td>U-K (mmol/l)</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>dICV Na (mmol/l)</td>
<td>-2 ± 43</td>
</tr>
<tr>
<td>dICV K (mmol/l)</td>
<td>-3 ± 3</td>
</tr>
<tr>
<td>TBW (l)</td>
<td>48.2 ± 2.6</td>
</tr>
<tr>
<td>ECV (l)</td>
<td>15.6 ± 0.5</td>
</tr>
<tr>
<td>ICV (l)</td>
<td>32.6 ± 2.1</td>
</tr>
</tbody>
</table>

**Table 3** Metabolic responses to alanine infusion with (dehydrated) and without (control) diuretic injection in seven healthy subjects in the intervention period. Values are means ± S.D. A paired t-test was performed; ns, not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine (mmol/l)</td>
<td>Control (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>2.79 ± 0.43</td>
</tr>
<tr>
<td>UNSR (mmol/l)</td>
<td>151 ± 19</td>
</tr>
<tr>
<td>FHNC (l/h)</td>
<td>54 ± 10</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>153 ± 30</td>
</tr>
<tr>
<td>Insulin (units/l)</td>
<td>33 ± 14</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.3 ± 0.4</td>
</tr>
</tbody>
</table>

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All individuals showed a fall in FHNC during dehydration, except for one in which the value was unchanged.

Dehydration increased plasma glucagon by approx. 25%; insulin and glucose did not change (Table 3). In each experiment, FHNC was directly related to the glucagon concentration (Figure 2) \( (r^2_{\text{control}} = 0.68, r^2_{\text{dehydration}} = 0.60) \). Dehydration more than halved the linear slope of the relationship, i.e. the reactivity of urea synthesis to glucagon, from \( y = 19.5 + 0.22x \) to \( y = 21.2 + 0.09x \) \( (P < 0.05) \).

FHNC values were linearly related to urinary potassium secretion \( (r^2 = 0.68, P < 0.01) \) and sodium excretion \( (r^2 = 0.62, P < 0.02) \).

The changes in the reactivity of urea synthesis to glucagon were linearly related to the approximate changes in ICV \( (r^2 = 0.79; P < 0.05) \) (Figure 3). There was no relationship between FHNC and changes in body weight or losses of intracellular K⁺ or Na⁺.

**DISCUSSION**

The present study showed that moderate dehydration acutely decreased FHNC and the response of urea synthesis to glucagon in healthy volunteers. FHNC values were related to urinary losses of sodium and potassium. The changes in glucagon sensitivity and in estimated ICV paralleled each other. The results thus confirm that changes in hydration also have acute metabolic and regulatory consequences in vivo.

**Water and electrolytes**

It is difficult from a methodological point of view to describe acute changes in ICV in humans. Isotope techniques would require several hours to reach a steady state. In the present study, most of the water lost during dehydration was from the extracellular space, but a detectable amount of water was also lost from the intracellular space. This contrasts with an earlier study in which furosemide increased ICV slightly (5%) [11]. This difference may be due to the fact that our measurement was carried out at 45 min, whereas that in the quoted study took place at 90 min, by which time compensatory water movement may have occurred. Also, the bioimpedance technique used in the quoted study is subject to inaccuracy that, all in all, may be more marked than that of our weight-based indirect method [12,13]. An indirect indication of changes in ICV was the loss of intracellular potassium. Evidently, slight acute intra-
cellular dehydration can be obtained with furosemide, but the effect is probably transient.

The relationship between the urinary losses of sodium and FHNC might reflect the fact that loss of body water and sodium are coupled. However, to our knowledge there is no evidence for a role for sodium in the regulation of urea synthesis, while there is evidence for the importance of changes in ICV [14].

Urinary potassium loss was also related to FHNC. Potassium was lost primarily from the ICV space, and intracellular potassium depletion decreases the capacity for urea synthesis in rats [15]. However, in the present study the decrease in intracellular potassium would be at most 1 mmol/l if the loss was distributed uniformly. In rat liver tissue, a marked reduction of 10 mmol/l potassium induces a decrease in the ICV space of 14 % [16]. Extrapolated to our study, the change in ICV would be only 1.4 %, i.e. similar to our approximation of 1 % [14].

Metabolism
The decrease in FHNC caused by dehydration indicates that, at any given alanine concentration, less urea was synthesized, or that any given rate of urea synthesis required a larger alanine concentration drive during dehydration. In the present study the FHNC was quantified as the ratio between UNSR and the steady-state alanine concentration, which has the dimension of metabolic clearance and approximates the FHNC, as described in earlier papers as the slope of the regression of multiple corresponding data points of UNSR and total α-amino nitrogen [17,18]. Despite the simplicity of the present method, the conceptual basis of the two methods is the same. Estimating the FHNC as a ratio based on one set of observations allows the possibility of describing acute changes, so as to detect rapid metabolic effects. This approximate method has been used previously to describe the acute effects of glucagon [19] or pain on the hepatic capacity for conversion of amino nitrogen into urea nitrogen [20].

Furosemide has been shown not to have any effects on urea synthesis from ammonia in rat liver preparations and in human liver slices [21]. However, this is not necessarily the case for ureagenesis from alanine, which involves plasma membrane transport and liberation of ammonia as earlier steps. These steps seem to be sensitive to cell hydration [22], while the synthesis of urea from ammonia is not inhibited by hyperosmolarity [9]. Thus, although in the present study such an effect of furosemide is not likely, it cannot be ruled out entirely.

The changes in the reactivity of urea synthesis to glucagon, i.e. the ratio between FHNC and glucagon concentration, were negatively correlated with the changes in estimated ICV. Glucagon acutely up-regulates ureagenesis and FHNC, which mediates part of the nitrogen loss associated with stress. This mechanism was less effective during dehydration. The mechanisms by which dehydration and glucagon interfere with the regulation of urea synthesis are at present unknown. In vitro studies have shown that an increase in perfusate osmolality decreases the rate of transport of amino acids across the cell membrane, and thereby decreases the intracellular amino acid concentration and the rate of urea synthesis [23]. The present study does not allow us to differentiate between the possible effects of dehydration on intracellular urea synthesis and cellular amino acid transport.

Speculation and conclusions
Our results provide clues to the importance of the regulation of urea synthesis by dehydration. Dehydration decreases hepatocyte protein synthesis during an amino acid load, which leaves the hepatocyte with an increased requirement for the elimination of amino nitrogen. However, we found that urea synthesis was down-regulated by dehydration. This may support the notion that the regulation of urea synthesis by hydration represents an archaic defence mechanism against intrahepatocyte hyperosmolality, rather than being an aspect of metabolic regulation. To explore this further, it will be necessary to measure intracellular amino acid levels during changes in hydration.

In conclusion, we have shown that acute moderate dehydration caused by injection of a loop diuretic down-regulated both the capacity for urea synthesis and its response to glucagon in healthy men. The changes in the sensitivity to glucagon paralleled the estimated changes in ICV. Thus changes in hydration may have consequences for the hepatic contribution to whole-body nitrogen homoeostasis in healthy individuals, and even more so during disease states involving pathological hydration states together with stress and hyperglucagonaemia. The mechanism of this effect and its relationship to sodium and potassium fluxes are not known.

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REFERENCES

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