Urinary aquaporin-2 in healthy humans and patients with liver cirrhosis and chronic heart failure during baseline conditions and after acute water load

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Urinary aquaporin-2 in healthy humans and patients with liver cirrhosis and chronic heart failure during baseline conditions and after acute water load.

Background. Patients with liver cirrhosis and chronic heart failure (CHF) have a reduced capacity to excrete water. Studies in healthy humans have shown that an acute water load reduces the excretion of aquaporin-2 in urine (u-AQP-2). We wanted to test the hypothesis that an acute water load reduces u-AQP-2 less in patients with liver cirrhosis or CHF than in healthy humans.

Methods. Fourteen healthy subjects, 14 patients with liver cirrhosis, and 14 patients with CHF were given an oral water load of 20 mL/kg. Urine was collected every 30 minutes for 4 hours for analysis of u-AQP-2. Blood samples were drawn at the beginning and at the end of the study for analysis of arginine vasopressin (AVP). u-AQP-2 was determined by radioimmunoassay.

Results. During the study period, urinary output was 22.8% higher than water intake in the healthy controls and increased 14-fold from baseline, but in patients with liver cirrhosis and CHF urinary output was 14% and 24% less than the intake, while urinary output increased 7- and 19-fold from baseline, respectively. u-AQP2 decreased significantly more in patients with CHF (39%) than in healthy controls (17%) but it was unchanged in those with liver cirrhosis. AVP decreased 46% in patients with CHF, but was unchanged in healthy controls and those with liver cirrhosis. A 24-hour urinary excretion of AQP-2 was significantly elevated in patients with CHF (median, 25.7 nmol/mol creatinine) compared to healthy controls (15.7 nmol/mol creatinine) and those with liver cirrhosis (17 nmol/mol creatinine).

Conclusion. The excretion of AQP-2 in urine is abnormal both in liver cirrhosis in which we find less suppression of u-AQP2 by an acute water load and in CHF in which we find a high baseline level and an exaggerated suppression of u-AQP2 by an acute water load.

Key words: urinary aquaporin-2, water load, healthy humans, water retention.

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and did not change after an acute water load. However, other studies have shown a decrease in AQP-2 expression in rats with compensated liver cirrhosis [15] and in decompensated liver cirrhosis [16].

The exact role of AQP-2 under conditions of sodium and water retention is not fully clarified in humans. The effect of an acute oral water load on u-AQP-2 and its relationship to AVP and urinary osmolality (u-osm) in healthy humans, in patients with liver cirrhosis, and in patients with CHF has not been studied by simultaneous measurement of other important regulatory hormones of the water and sodium homeostasis such as the renin-angiotensin-aldosterone system and the natriuretic peptides.

The purpose of the present study was to measure the effect of an acute water load on (1) urinary excretion of AQP-2, (2) plasma concentration of vasopressin, (3) urine osmolality, (4) the renin-angiotensin-aldosterone system, and (5) the natriuretic peptide system. We wished to test the hypotheses that urinary excretion of AQP-2 was reduced less in patients with liver cirrhosis and CHF than in healthy controls after an acute oral water load, and that baseline levels of u-AQP-2 were higher in liver cirrhosis and CHF than in controls.

METHODS

Subjects

For the healthy subjects, the following inclusion criteria were used: (1) men and women; and (2) age, 35 to 75 years old. Exclusion criteria were (1) a history or clinical signs of disease of the heart, lungs, liver, kidneys or endocrine organs; (2) neoplastic disease; (3) arterial hypertension; (4) alcohol or drug abuse; (5) medical treatment except for birth control pills; (6) unwillingness to participate; and (7) abnormal laboratory screening tests (i.e., abnormal blood hemoglobin, white cell count, platelets, p-sodium, p-potassium, p-creatinine, p-albumin, b-glucose, s-cholesterol, p-bilirubin and p-alanine aminotransferase, or albuminuria or glucosuria).

For the liver cirrhosis patients the following inclusion criteria were used: (1) men and women; (2) age, 35 to 75 years old; and (3) cirrhosis based on liver histology for 5 minutes at 1.6 rpm until assayed. For radioimmunoassay C_18 Sep-Pak filtration was placed in a forearm vein. After voiding, a water load of 20 mL/kg was given orally for 15 minutes. Urine was collected every 30 minutes for 4 hours. Voiding took place in the standing or sitting position. Blood samples were drawn at the beginning and at the end of the study. Plasma was analyzed for AVP, osmolality (osm), renin concentration (PRC), angiotensin II (Ang II), aldosterone, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), sodium, and hemotocrit. Urine was analyzed for AQP-2 concentration, urine osmolality (u-osm), urine creatinine concentration (u-creatinine), and urine sodium concentration.

Measurements

AQP-2 was measured by radioimmunoassay, as previously described [10]. Urine samples were centrifuged for 5 minutes at 1.6 × 100g (3000 rpm) and 125 to 3000 μL of the supernatant (depending on u-osm) was freeze-dried and kept frozen at −20°C until assayed. For radioimmunoassay rabbit anti-AQP-2 antibody was obtained from Søren Nielsen (Department of Cell Biology, Institute of Anatomy, Aarhus University, Aarhus, Denmark). The minimum detection level was 32 pg/tube. The coefficients of variation were 11.7% (interassay) and 5.9% (intra-assay).

AVP in plasma was measured by radioimmunoassay, which was a modification of the method described previously [17]. Before the assay procedure C_{18} Sep-Pak
(Water Associates, Milford, MA, USA) extraction was performed. The antibody was a gift from professor Jacques Dürr (Miami, Florida, USA). The minimum detection level was 0.5 pmol/L. The coefficients of variation were 13% (interassay) and 9% (intra-assay).

PRC was measured by a commercial immunoradiometric assay (Nichols Institute Diagnostics, Geneva, Switzerland). The coefficients of variation were 2.5% (intra-assay) and 9.9% (interassay). Minimal detection level was 1.4 µU/mL.

Ang II in plasma was determined by radioimmunoassay using a modification [18] of the method originally described by Kappelgaard, Damkjaer-Nielsen, and Giese [19]. Radioimmunoassay was performed after previous extraction of plasma by C₁₈ Sep-Pak cartridges (Water Associates). The antibody was obtained from the Department of Clinical Physiology, Glostrup Hospital (Glostrup, Denmark). Minimal detection level was 2 pmol/L plasma. The coefficients of variation were 12% (interassay) and 8% (intra-assay).

Aldosterone in plasma was measured by a modification [18] of the method originally described [20]. With a rabbit anti-Aldo antibody (Simoco, Denmark) radioimmunoassay was performed after extraction of plasma by C₁₈ Sep-Pak cartridges (Water Associates). The minimum detection level was 42 pmol/L plasma. The coefficients of variation were 13% (interassay) and 9% (intra-assay).

ANP in plasma was determined by radioimmunoassay, as previously described [18]. ANP was extracted from plasma by means of C₁₈ Sep-Pak cartridges (Water Associates), using ethanol, acetic acid, and water. For radioimmunoassay rabbit anti-ANP antibody was obtained from the Department of Clinical Chemistry, Bispebjerg Hospital (Copenhagen, Denmark). The minimum detection level was 0.5 pmol/l plasma. The coefficients of variation were 12% (interassay) and 10% (intra-assay).

BNP in plasma was measured by radioimmunoassay, previously described [21]. Immunoreactive BNP was extracted from plasma by use of C₁₈ Sep-Pak cartridges (Water Associates) eluted by 80% ethanol in a 4% acetic acid solution. Radioimmunoassay was performed using a rabbit anti-BNP antibody without cross-reactivity with α-ANP and urodilatin (URO). The minimum detection level was 0.5 pmol/L plasma. The coefficients of variation were 11% (interassay) and 6% (intra-assay).

Plasma and urinary concentrations of sodium and potassium were measured by routine methods at the Department of Clinical chemistry, Holstebro Centralgyhæus. Plasma and urinary osmolality was measured by freezing-point depression (Advanced model 3900 multisample osmometer).

Blood pressure was determined by UA-743 digital blood pressure meter (A&D Company, Ltd., Japan).

Statistics
Data from 14 healthy subjects, 14 patients with liver cirrhosis, and 14 patients with CHF were included in the statistical analyzes. Due to lack of normality or inhomogeneity of the variance we used Friedman’s ANOVA (analysis of variance) for paired comparisons within the groups followed by comparisons between baseline and the other periods as described by Siegel and Castellan [22]. Since baseline values were different between healthy subjects and patients, we compared relative changes from baseline by comparing healthy subjects on one hand and patients on the other. Between groups comparisons were performed with Kruskal-Wallis’s test for unpaired comparisons followed by Mann-Whitney’s rank sum test. P < 0.05 was considered the limit of significance.

RESULTS

Demographics
Fourteen healthy subjects of mean age of 60 years (range, 44 to 64 years old), six men and eight women, were studied.

Fourteen patients with liver cirrhosis of mean age 54 years (range, 42 to 62 years old), eight men and six women participated in the study. Nine patients had cirrhosis verified by a liver biopsy. The five patients in which a liver biopsy had not been performed had years of excessive alcohol abuse. All had ascites, and one had portal hypertension. Five patients had esophageal varices (three ascites patients) and two patients had portal hypertension (one ascites patient) verified by ultrasound. Nine patients (six patients with ascites) received diuretic treatment. The diuretic treatment consisted of either spironolactone (six patients) or a combination therapy of spironolactone and furosemide (three patients). The cirrhotic patients treated with diuretics were in a state of normohydration or with mild-to-moderate leg edema and ascites.

Fourteen patients with CHF of mean age 65 years (range, 49 to 74 years old), 11 men and three women were studied. The etiology of CHF was ischemic heart disease (93%) and cardiomyopathy (7%). Nine patients were in NYHA class II [left ventricular ejection fraction (LVEF), mean 28%] and five in NYHA class III (LVEF, mean 29%). Medication included digoxin, diuretics, angiotensin-converting enzyme (ACE) inhibitors, Ang II inhibitors, aspirin, warfarin, isosorbide mononitrate, statins, beta blockers, and amiodarone. Four patients had an ICD pacemaker. All patients were in a clinically stable condition. Clinical and laboratory data for all participants are given in Table 1.

Water balance during the study
The water intake was 1605 mL (25th percentile, 1362; 75th percentile, 1669) in healthy subjects, 1562 mL (1253...
Table 1. Clinical and laboratory data for 14 healthy subjects, 14 patients with liver cirrhosis, and 14 patients with chronic heart failure (CHF). Urine analyses are based on 24-hour urine collections the day before the studies

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Cirrhosis</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Men/women</td>
<td>6/8</td>
<td>8/6</td>
<td>11/3</td>
</tr>
<tr>
<td>Years</td>
<td>60 (44–64)</td>
<td>54 (42–62)</td>
<td>24.7 (22.4–29.3)*</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.6 (24.3–28.4)</td>
<td>29.2 (23.6–32)*</td>
<td>24.7 (22.4–29.3)*</td>
</tr>
<tr>
<td>Systolic blood pressure mm Hg</td>
<td>120 (114–136)</td>
<td>110 (98–116)*</td>
<td>115 (103–129)*</td>
</tr>
<tr>
<td>Diastolic blood pressure mm Hg</td>
<td>75 (72–80)</td>
<td>67 (60–72)*</td>
<td>64 (60–74)*</td>
</tr>
<tr>
<td>Pulse rate min</td>
<td>65 (62–68)</td>
<td>67 (62–81)*</td>
<td>60 (55–66)*</td>
</tr>
<tr>
<td>p-sodium mmol/L</td>
<td>141 (139–142)</td>
<td>136 (135–139)*</td>
<td>138 (136–139)*</td>
</tr>
<tr>
<td>P-potassium mmol/L</td>
<td>3.8 (3.7–3.9)</td>
<td>3.9 (3.6–4.3)*</td>
<td>3.9 (3.9–4.3)*</td>
</tr>
<tr>
<td>p-creatinine l mol/L</td>
<td>290 (288–292)</td>
<td>287 (280–291)*</td>
<td>287 (284–292)*</td>
</tr>
<tr>
<td>s-osmolality mosmol/kg</td>
<td>195 (160–227)</td>
<td>156 (84–226)*</td>
<td>199 (130–262)*</td>
</tr>
</tbody>
</table>

Median with 25% and 75% percentiles.
*Not significant compared to healthy subjects
P < 0.05 compared to healthy subjects

Plasma AVP

The absolute values of AVP in healthy subjects, in patients with liver cirrhosis, and patients with CHF are shown in Figure 3. AVP was unchanged during the study in healthy subjects and in patients with liver cirrhosis. AVP was significantly higher in patients with liver cirrhosis compared to healthy subjects at baseline (48%, P < 0.05), but no significant difference in relative values was observed between healthy subjects and patients with liver cirrhosis during the experiment. AVP decreased by 46% during the study in patients with CHF (P < 0.005). AVP was significantly elevated at baseline in CHF patients (155%, P < 0.006) compared to healthy subjects, and the changes in relative values were significantly greater in CHF patients (43%, P < 0.004) compared to healthy subjects (−13%).

Osm in urine and urinary output

Figure 4 shows u-osm in healthy subjects, in patients with liver cirrhosis, and patients with CHF. In healthy subjects, u-osm decreased significantly with a maximum decrease of 88% after 120 minutes. At the end of the study, u-osm did not deviate significantly from baseline in healthy subjects. In patients with cirrhosis u-osm decreased significantly after 90 minutes. The maximal decrease was seen after 120 minutes and was 72%. Subsequently, u-osm slowly increased but was still significantly lower than baseline at the end of the study. There was no significant difference between healthy subjects and patients with liver cirrhosis at baseline. The relative decrease in u-osm was significantly less in patients with liver cirrhosis compared to healthy subjects between 30 and 180 minutes. In CHF patients, u-osm decreased significantly after 90 minutes. The maximal decrease was seen after 150 minutes and was 80%. Subsequently there was a slow increase, but u-osm was still significantly lower than baseline at the end of the study. There was no significant difference between
increases were significantly greater in CHF patients compared to healthy subjects (data not shown).

**PRC, Ang II, aldosterone, ANP, and BNP**

Table 2 shows PRC, Ang II, aldosterone, ANP, and BNP in healthy subjects, patients with liver cirrhosis, and patients with CHF. PRC and aldosterone were significantly decreased during the study in healthy subjects and in CHF patients with only a tendency toward a decrease in patients with liver cirrhosis. The decrease in PRC was significantly greater in CHF patients compared to healthy subjects. Ang II and ANP were unchanged in all the groups. BNP increased significantly in patients with CHF with no change in patients with liver cirrhosis and healthy subjects. All the hormones were significantly increased at baseline in CHF patients and in patients with liver cirrhosis compared to healthy subjects.

**Blood pressure and heart rate**

Mean arterial blood pressure (MAP) and heart rate were measured every 30 minutes. There was no significant change in MAP in healthy subjects during the study. MAP was significantly increased in patients with cirrhosis at 30 minutes and at 120 minutes [baseline, 80 mm Hg (73 to 87); 30 minutes, 90 mm Hg (86 to 98, \( P < 0.05 \); 120 minutes, 114 mm Hg (104 to 133, \( P < 0.001 \)]. The same pattern was seen in CHF patients [baseline, 82 mm Hg (73 to 91); 30 minutes, 94 mm Hg (87 to 103, \( P < 0.05 \); 120 minutes, 120 mm Hg (110 to 142, \( P < 0.001 \)]. Subsequently MAP decreased to baseline level during the rest of the study in all patients. The heart rate showed no change during the study in either of the groups (data not shown).

**DISCUSSION**

In the present study of healthy subjects and patients with liver cirrhosis and CHF, we investigated the effect of an acute oral water load on u-AQP-2, water excretion, and hormones in plasma of importance for renal function and sodium and water homeostasis in 30-minute periods from 8:00 a.m. to 12:00 a.m. In healthy subjects, the total urinary output was greater than the total water intake after an acute water load. The acute water load resulted in a small but significant decrease in u-AQP-2 but no change in AVP. In addition, there was a substantial increase in urinary output, and the relative decrease in u-osm was less than in healthy subjects. Patients with liver cirrhosis had a reduced ability to excrete an acute water load. In addition, they showed only a tendency toward a decrease in u-AQP-2 and AVP. The relative increases in urinary output, and the relative decrease in u-osm was less than in healthy subjects. Patients with CHF also had a diminished capacity to excrete an acute water load with a 24% less urinary output than water intake. In patients with
Fig. 2. Relative changes in urinary aquaporin-2 (u-AQP2) after an acute water load in 14 healthy subjects (□), 14 patients with liver cirrhosis (■), and 14 patients with chronic heart failure (CHF) (□). Baselines are from the end of the 24-hour urine collection to the beginning of the study. Results are means ± SD. †P < 0.05 vs. healthy subjects.

CHF, u-AQP2 and AVP decreased throughout the study. The decrease in u-AQP2 was larger in patients with CHF than in healthy subjects. The relative increases in urinary output were larger in patients with CHF than in healthy subjects, whereas the decrease in u-osm was smaller. For ethical reason, we did not find it justified to discontinue the medical treatment of the patients for more than 24 hours. It is, therefore, possible that the drugs still have a small effect on water balance.

The role of AQP-2 in cirrhosis has been studied extensively in rats. The results have been conflicting with regard to the renal expression of AQP-2. The initial studies showed an increased level of AQP-2 protein and AQP-2 mRNA in carbon tetrachloride (CCL4)–induced cirrhotic rats with ascites [14, 23]. In addition, an acute water load did not decrease AQP-2 mRNA within 1 hour [14]. Liver cirrhosis induced by common bile duct ligation (CBL) showed a decreased expression of AQP-2 both in rats with and without ascites [15, 16]. Recently, Jonassen et al [24] showed no difference in the expression of AQP-2 protein in rats with CCL4-induced liver cirrhosis and control rats. The rats had hyponatremia, ascites, and increased plasma level of AVP and showed an impaired ability to excrete an intravenous water load. We are the first to study the role of AQP-2 in humans with liver cirrhosis, and our results seem to be in agreement with...
Table 2. Plasma levels of renin (PRC), angiotensin II (Ang II), aldosterone, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) at baseline and 240 minutes after an acute water load in 14 healthy subjects, 14 patients with liver cirrhosis, and 14 patients with chronic heart failure (CHF)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>240 min</th>
</tr>
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<tbody>
<tr>
<td><strong>PRC μU/mL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>14 (12–22)</td>
<td>12 (9–15)*</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>29 (13–54)b</td>
<td>23 (11–40)</td>
</tr>
<tr>
<td>CHF</td>
<td>73 (46–142)</td>
<td>41 (22–86)*</td>
</tr>
<tr>
<td><strong>Ang II pmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>8.6 (4.5–11.7)</td>
<td>8.1 (5.5–13.6)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>9.1 (3.8–14.8)</td>
<td>7.2 (4.8–14.8)</td>
</tr>
<tr>
<td>CHF</td>
<td>13.4 (7.4–18.7)b</td>
<td>10.2 (9.6–18.2)</td>
</tr>
<tr>
<td><strong>Aldo pmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>61.2 (45.9–105.0)</td>
<td>41.7 (35.4–50.0)b</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>122.3 (74.4–182.1)b</td>
<td>70.9 (40.3–207.1)</td>
</tr>
<tr>
<td>CHF</td>
<td>94.5 (61.1–149.4)b</td>
<td>50.0 (41.7–100.8)b</td>
</tr>
<tr>
<td><strong>ANP pmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>6.1 (5.5–6.9)</td>
<td>5.4 (4.4–7.0)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>8.0 (3.9–10.5)</td>
<td>7.3 (4.4–9.8)</td>
</tr>
<tr>
<td>CHF</td>
<td>20.2 (13.3–41.1)b</td>
<td>21.0 (11.5–30.8)</td>
</tr>
<tr>
<td><strong>BNP pmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.9 (1.1–3.3)</td>
<td>2.0 (1.2–3.9)</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>4.2 (2.9–9.7)b</td>
<td>4.9 (3.0–11.9)</td>
</tr>
<tr>
<td>CHF</td>
<td>16.9 (9.1–44.8)b</td>
<td>18.7 (10.2–59.8)b</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. baseline; bP < 0.05 vs. healthy subjects at baseline; 'P < 0.05 vs. healthy subjects

It has been demonstrated that AQP-2 protein and mRNA expression is higher in rats with severe CHF [12, 13], but, in the study by Nielsen et al [13], rats with compensated CHF showed no increase in AQP-2 expression. In our study, the patients had hyponatremia, increased basal AVP concentration, and a 24-hour urine sample showed an increased excretion of AQP-2. Despite a larger increase in urinary output and a decrease in u-AQP-2, the ability to dilute urine was impaired in patients with CHF. They did not excrete all the ingested water and the decrease in u-osm was less than in healthy subjects. Thus, we have demonstrated that u-AQP-2 is increased in patients with CHF, and that an acute water load can suppress AVP and decrease u-AQP-2, but water excretion was not normalized.

The results of the present study show that AQP-2 was not up-regulated in patients with liver cirrhosis, despite an increase in AVP and an inability to excrete an acute water load normally. However, the increase in AVP was only moderate and only six patients had ascites. This might at least partly explain the absence of an increase in u-AQP-2 excretion. Possibly u-AQP-2 is increased in more advanced liver cirrhosis. The inability to excrete an acute water load by the cirrhotic patients in our study must, therefore, be due to a different mechanism, which is unknown at present. One question to be answered is why the increase in AVP does not increase u-AQP-2 excretion. Ecelbarger et al [25] raised the question of the existence of AVP-independent mechanisms for the
regulation of AQP-2 expression and showed that, despite a continuous subcutaneous infusion of 1-desamino-8-D-AVP (synthetic AVP V_2-receptor agonist), AQP-2 was down-regulated after water loading. In addition, thirsting of rats in the presence of chronic V_2-receptor blockade (OPC-31260) increases AQP-2 expression levels [26]. Thus, vasopressin-independent mechanisms might play a significant role in the abnormal water balance in liver cirrhosis.

Earlier studies have shown that AVP decreases in response to an acute water load in CHF patients [27, 28]. The decrease was maximal at 2 hours and AVP was still significantly lower at 5 hours. AVP was significantly higher in patients with CHF compared to healthy subjects throughout the study except after 5 hours. In our study, we measured AVP after 4 hours, and there was no difference between healthy subjects and patients with CHF. However, the relative change in AVP was larger in patients with CHF than in healthy subjects, probably because AVP had returned to baseline values in healthy subjects and not in patients with CHF. It seems likely, therefore, that AVP and AQP-2 may play a role in the inability to excrete an acute water load in patients with CHF. Recently Martin et al [29] demonstrated the role of AQP-2 in the enhanced water reabsorption in CHF, because u-AQP-2 was significantly decreased by the administration of a selective V_2-receptor AVP antagonist (VPA-985) in CHF patients. Although both AVP and u-AQP-2 were reduced by an oral water load in CHF to an even greater extent than in healthy control subjects, our study clearly demonstrated an abnormal water excretion in CHF. Thus, the reduced capacity to excrete water cannot be attributed to the short-term AVP and AQP-2 regulation only.

Both the renin-angiotensin-aldosterone system and the natriuretic peptides are involved in the pathogenesis of CHF and liver cirrhosis. Both patients with liver cirrhosis and CHF patients showed an elevated level in PRC and aldosterone at baseline compared to healthy subjects. Ang II was only increased in patients with CHF. In patients with liver cirrhosis, there was no change in the renin-angiotensin-aldosterone system after the acute water load consistent with a previous study [30]. In patients with CHF the renin-angiotensin-aldosterone system was suppressed in response to the water load, but this did not normalize water excretion.

It has been shown previously that ANP is normal or increased and BNP is increased in patients with liver cirrhosis presumably as a compensatory phenomenon to promote sodium excretion. This is consistent with our results. In patients with CHF, it is also well known that both ANP and BNP is increased. ANP is secreted in response to increased atrial stretching and BNP is secreted in response to ventricular dilatation. In healthy subjects and patients with liver cirrhosis, water loading did not change ANP and BNP, while BNP increased in patients with CHF. This difference may be attributed to differences in cardiac output, which is reduced in CHF but often normal in liver cirrhosis.

CONCLUSION
We have demonstrated that 24-hour urinary AQP-2 excretion was increased in patients with CHF but not in cirrhotic patients compared to healthy subjects. An acute water load did not change u-AQP-2 in patients with cirrhosis but decreased u-AQP-2 in patients with CHF and even more than in healthy subjects. Our results indicate that excretion of AQP-2 in urine is abnormal both in liver cirrhosis and in CHF.

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