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CLINICAL STUDY

Aquaporin-2 (AQP2) Urinary Excretion and Assumption of Water with Different Mineral Content in Healthy Subjects

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The aquaporin-2 (AQP2) plays a key role in AVP-induced absorption of water, and its urinary excretion is related to its function. We aimed to test if the assumption of water with different mineral content can modify the expression of AQP2, leading to a change in AQP2 urinary concentration, in 20 healthy young subjects. Each subject received an oral water load (LM or HM) of 250 mL/hour for four hours, and several variables were measured. Plasmatic osmolality after water assumption was significantly reduced with no differences after the low (LM) or the high mineral (HM) water load. Urinary osmolality and plasmatic vasopressin concentration were significantly reduced after an assumption of both kinds of water. However, serum vasopressin was lower after HM water assumption than after LM. AQP2 urinary excretion was significantly reduced after water assumption with respect to the basal level and it was lower after LM than after HM water assumption. The different mineral content of water was investigated as a factor contributing to the development of hypertension. Considering that AQP2 can play a role in pathogenesis of hypertension, our demonstration that AVP-mediated AQP2 urinary excretion is strictly influenced by the consumption of water with different mineral content suggests a new, interesting field of investigation related to the link between blood pressure alterations and nutritional habits.

Keywords aquaporin-2, vasopressin, mineral water

INTRODUCTION

The mechanisms underlying water balance maintenance depend on the integration of different hormones, including vasopressin (AVP) and the atrial natriuretic factor (ANF).

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AVP increases water permeability in the collecting duct by binding AVP to its V2 receptor in the basolateral membrane of the collecting duct cells, as well as by the exocytic insertion of intracellular vesicles containing aquaporin-2 (AQP2) water channels in the apical plasma membrane.[1–5] Aquaporins (AQP) allow water to pass rapidly through the permeable epithelia of tissues in which they are distributed. They were found in different forms in the kidney (AQP1–AQP4, AQP6, and AQP7).[6–8] AQP2 plays a key role in AVP-induced reabsorption of water.[9–13] It has been suggested that some diseases associated with an altered urinary osmolality are linked to a pathologic regulation and expression of AQP in the kidney. Moreover, it seems that AQP family, AQP-2 in particular, could be involved in the pathogenesis of hypertension, as recently demonstrated in a mouse model.[14] AQP-2 urinary excretion has a close correlation with kidney AQP2 expression. It can be used as a marker for the action of AVP on the collecting ducts.[15,16] In the present study, we aimed to test whether the assumption of water with different mineral content could modify the renal expression of AQP2, leading to a change in AQP2 urinary concentration.

MATERIAL AND METHODS

Study Subjects

Our study group consisted of 20 healthy subjects (10 males and 10 females, mean age 23.8 ± 2.5 yrs.) without a history of arterial hypertension; neoplastic disease; cardiovascular disease; diabetes; or renal, lung, or endocrine disease. Body weight ranged between 55 and 70 Kg. Alcohol, tea, and coffee were prohibited for a week before and during the study. Subjects were not under medical treatment, including oral contraceptives. The local Ethics Committee approved the study, and informed consent was obtained from all subjects.

Study Design

The study was conducted in rooms with a stable temperature (18°C) to minimize perspiration variations. The subjects were asked to avoid physical activity during the experiment. The designed study consisted of a low mineral content water assumption experiment (LM) and a high mineral content water assumption experiment (HM). Every experiment was conducted in two different consecutive midweek days. Each subject drank commercial tap water with either low mineral content or high mineral content. Mineral contents of both kinds of water are shown in Table 1.

All subjects were given a standardized diet containing 10 mEq of sodium and 10 mEq of potassium. We performed a protocol similar to our previous work.[9] After a ten-hour period of fluid restriction and after voiding and completely emptying his/her bladder at 08.00, the subject resumed the sitting position for 4 h (08:00–12:00 h). Urine was collected for basal measurement. Immediately after the subject had voided, a 20-gauge 3.8-cm Teflon catheter with a flash chamber and an accompanying Teflon stylet was inserted into left forearm vein to allow sampling of blood without using an anticoagulant or intravenous infusion. The sterile stylet was re-inserted into the catheter after each sample had been drawn. After the basal blood sample at 08:00, every subject received an oral water load (LM or HM) of 250 mL/hour from 8:00 to 12:00.

The following variables were measured: arterial pressure (AP), heart rate (HR), body weight (BW), creatinine clearance (Cl cr), plasma osmolality (posm), urinary osmolality (Uosm), urinary volume (Uvol), plasma arginine-vasopressin (P-AVP), and urinary aquaporin 2 (U-AQP2). The blood samples were placed immediately into chilled Vacutainer tubes containing potassium ethylenediaminetetraacetate, and the plasma was promptly separated in a refrigerated centrifuge. The samples were then stored at –80°C until assayed.

The Plasma Arginine-Vasopressin RIA Kit was from Buhlmann Laboratories, Basel, Switzerland. The Plasma Aldosterone RIA Kit was from DPC-Diagnostic Products Corp., Los Angeles, California, USA. The Plasma hANF was from Peninsula Laboratories, Inc., Belmont, California, USA. The Plasma Renin Activity RIA Kit was from Pasteur Institute, Paris, France.

Urinary AQP2 (U-AQP2) Measurement

Control peptide AQP2 and affinity pure anti-AQP2 antibodies generated in rabbits were supplied by Alpha Diagnostics International, San Antonio, Texas, USA, in PBS pH 4 at 1 mL/mL. Biotinylation of affinity pure antibodies was mainly performed using the Biotin tag-MicroBiotinylation Kit (Sigma Chemicals, St. Louis, Missouri, USA).

Enzyme Cycling System

The signal generated by alkaline phosphatase was amplified by the Dako ampliQ System, with which the signal from the primary enzyme is multiplied numerous times and an amplification factor of 100-fold can easily be achieved.

Sample Diluent

Tris Buffer containing pH 7.4 with 20 g/L BSA, 0.15 mol/L NaCl, 0.05% Triton.

Conjugate Buffer

Tris Buffer containing 15 g/L BSA, 15 g/L sodium caseinate, 0.15 mol/L NaCl, 0.02% Triton was passed through a 0.45 mm filter.

Aquaporin Calibrators

Control peptide AQP2 was diluted in a sample diluent to the working concentration range. The minimal detectable quantity of AQP2 was 2.5 pg/mL.

Table 1
Main chemical/physical properties of mineral waters utilized

Type of water	pH	TDS	mS	Ca++	Mg ++	Na +	K +	HCO3–	SO4–	Cl–	NO3–
Low mineral	9.2	6.00	66.5	2.0	5.8	1.4	9.8	0.8	26.5	4.5	12.0
High mineral	6.1	1283	1800	362	18	47	45	1385	5	22	6

Abbreviations: pH=acidity, alkalinity; TDS=total dissolved solids at 180°C; mS=electric conductivity (μS/cm).

Dissolved substances in one litre of water (mg): Ca=calcium, Mg=magnesium, Na=sodium, K=potassium, HCO3=carbonates, SO4=sulfates, Cl=chlorides, NO3=nitrates.

Assay Procedure

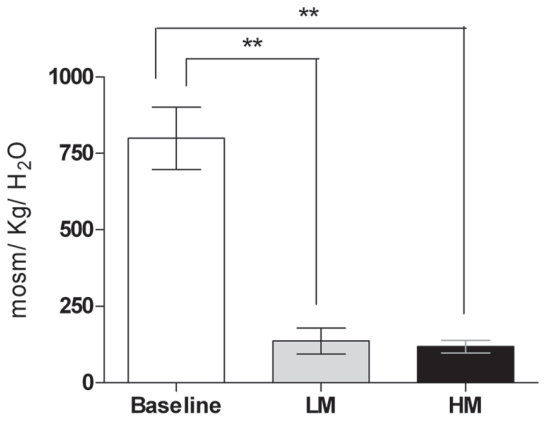
Flat-bottomed Micro-Elisa plates (Nunc Covalink, Denmark) were coated overnight at 4°C in a 200-mL/well coating buffer. The plate was washed three times with a wash buffer (PBS pH 7.4 with 0.15 mol/L NaCl), blocked with a 250 mL/well blocking buffer (BSA 30 g/L in PBS with NaCl 0.15 mol/L, pH 7.4), incubated for 1 h at 37°C, and then washed five times and stored dry at 4°C. Urine samples were concentrated five times (Micropure 0.22 mm, Microcon 3, Amicon) and diluted 1:4 in a sample diluent. Aquaporin control, the calibrator, was diluted in sample diluent to the working concentration range. Calibrators and samples (200 ml) were added in duplicate to blocked wells and incubated for 3 h at room temperature, and then washed four times. 200 mcl of biotynilate rabbit antiserum, diluted 1:1000 in conjugated buffer, was applied to each well. After incubation for 2 h at 37°C, the plate was washed four times and filled with 200 mcl/well of avidin conjugated with alkaline phosphatase (Sigma), diluted 1:1000 in a conjugated buffer, and incubated for 1 h at 37°C.After five washings, the enzyme activity was amplified by an alcohol dehydrogenase-diaphorase cycling system, and after adding the stop solution, the absorbance values were read at 492 nm.

Statistical Analysis

Data are expressed as mean value ±SEM. A comparison was made between groups using a paired Student *t* test and analysis of variance. The SPSS 11.0 statistical package and Microsoft Excel were used for tabulation and analysis. Graphs were drawn by using Prism Statistical software (version 4.00; Graphpad, San Diego, California, USA).

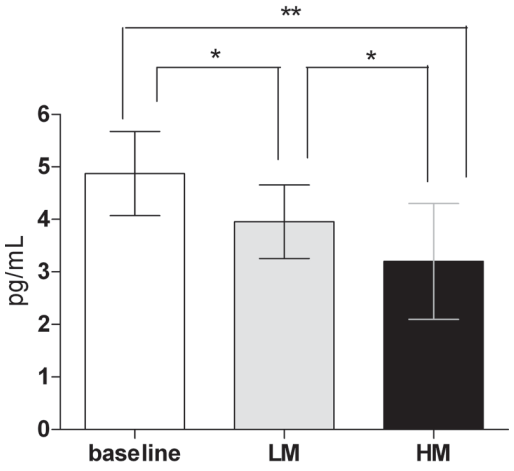
RESULTS

Plasmatic osmolality after water assumption appeared reduced in all the subjects. No statistical differences in this parameter were seen between low mineral and high mineral load. Urinary osmolality (see Figure 1) and plasmatic vasopressin (see Figure 2) concentrations were significantly reduced, compared with the basal level, after the assumption of both the kinds of water. However, serum vasopressin was lower after high mineral content water assumption than after low mineral content water assumption. On the other side, AQP2 urinary excretion was significantly reduced after water assumption with respect to the basal level, and it was lower after LM than after HM water assumption (see Figure 3). No statistical differences



HM = High mineral content water. LM = Low mineral content water. ** $p < 0.01$

Figure 1. Effects of two different mineral water types assumption in healthy humans compared to baseline values on urinary osmolarity.



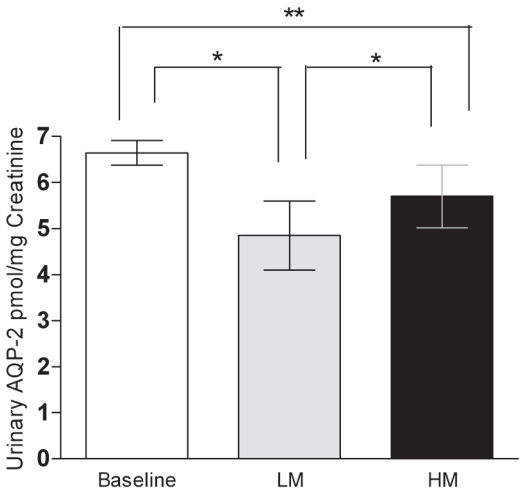
HM = High mineral content water. LM = Low mineral content water. **$p < 0.01$, *$p < 0.05$

Figure 2. Effects of mineral water types assumption compared to baseline values on plasmatic AVP.

were seen in other parameters (arterial pressure, heart rate, body weight, creatinine clearance) after water load compared to basal levels. Data collected are summarized in Table 2.

DISCUSSION

Water represents about 60% of body weight in adults and 80% in children. Maintenance of hydro-electrolytic equilibrium is due to the oral introduction of food and in particular mineral water. Drinkable waters are classified



HM = High mineral content water. LM = Low mineral content water, $**p < 0.01$, $*p < 0.05$.

Figure 3. Urinary AQP respect to baseline levels after LM and HM water assumption.

basing on mineral content, indicated as Total Dissolved Solids (TDS): the quantity of mineral salts deposited after evaporation at 180°C of one liter of water. Consequently, we have low mineral content water when TDS is <500 mg/L.[17] A further classification takes into account mineral quality. On this basis, the assumption of some water types might be preferable in different pathological states.

In post-menopause women, water with a high content of bicarbonate could reduce lipemia with a protective effect on cardiovascular disease and metabolic syndrome,[18,19] while the assumption of water with a high calcium content may represent a weapon against osteoporosis.[20–22] High mineral content water drinking might increase lithiasis risk, but this finding is a controversial issue in the literature.[23–25]

In our study, we verified a hypothesis that water with different mineral content influences AQP-2 urinary excretion. AQP-2 channel mediates water transport across the apical plasma membrane of the renal collecting ducts and allow the reuptake of water. AQP-2 is regulated by short- and long-term mechanisms. Short-time regulation implies AVP-stimulated trafficking of AQP-2-bearing intracellular vesicles to the apical membrane of the collecting ducts, thus increasing water permeability. When AVP levels decline, the vesicles are retrieved by endocytosis. Long-term alteration regulation implies a rapid increase in the intracellular protein levels.[26] Moreover, it has been shown how AQP-2 urinary excretion is a reliable indicator of its function.[27] It was already demonstrated that AQP-2 urinary excretion can be independent from serum AVP increasing and other factors can regulate it.[9,13,28] In this sense, low mineral content water and high mineral content water induced a statistically significant decrease in AQP-2 urinary excretion respect to basal conditions. When comparing AQP-2 levels after the assumption of LMW, we had a statistically significant decrease with respect to HMW assumption. This finding is probably related to a mechanism of physiologic compensation of central osmotic receptors to reduce plasmatic osmolality after a water load enhanced after the introduction of water with a low mineral content. Simultaneously, we had the same trend in AVP plasmatic levels, confirming that this hormone is the link between water mineral content and urinary AQP-2 variations. Many studies have previously demonstrated that AQP-2 can be implicated in the

Table 2
Effects of different mineral water types on several parameters

		Water type	
	Baseline	LM water	HM water
Urine volume after six hours from basal, mL	—	1307.5	1212.5
Serum osmolality, mosm/Kg/H_2O	289 ± 10.91	285 ± 12.75	286 ± 14.70
Urinary osmolality, mosm/Kg/H_2O	799 ± 102.19	136 ± 42.63*	118 ± 20.94*
Plasma arginine-vasopressin, pg/mL	4.87 ± 0.78	3.95 ± 0.64†	3.30 ± 0.22‡
Urinary AQP-2, pmol/mg creatinine	6.64 ± 0.26	4.85 ± 0.75†	5.70 ± 0.68‡
Systolic arterial pressure, mmHg	117	118	120
Diastolic arterial pressure, mmHg	73	77	76
Cr clearance, mL/min	122.9	124.6	123.5
Body weight, Kg	69.0	69.7	69.9
Heart rate, beat/min	77	75	75

*$p < 0.01$ vs. baseline.
†$p < 0.05$ vs. baseline.
‡$p < 0.01$ vs. baseline and < 0.05 vs. LM water.

pathogenesis of various diseases, such as hypertension. In a previous work, we have verified that AVP and AQP-2 modifications could play a crucial role in this sense.[29] Another recent study seems to confirm this hypothesis, where the expression of AQP-2 channels was found to be increased in the kidney in association with enhanced activity of the AVP/cAMP pathway in spontaneous hypertensive rats.[14] Such evidence is in accord with an experimental work that demonstrated that the induction of a DOCA-salt hypertension is probably related mainly to an enhancement of cAMP generation with a consequent improvement of expression/shuttling of AQP2 water channels in the kidney.[30] Moreover, it was been shown how AQP2 expression, which is regulated by dietary salt, is greatly involved in the mechanism of salt adaptation, and its altered regulation could contribute to the pathogenesis of hypertension.[31] Mineral water was already investigated as a factor contributing to the development of hypertension. In a randomized double-blind crossover trial, sodium chloride- and sodium bicarbonate-rich mineral water increased blood pressure in elderly normotensive individuals.[32] At the same time, regular mineral water ingestion in subjects with a low calcium and magnesium urinary excretion results after three weeks in a decrease in blood pressure.[33] Although they are interesting, these studies do not clarify the exact role played by different mineral water content in blood pressure alterations and, above all, do not explain at which level this influence could be realized.

Considering the role of aquaporin regulation in the pathogenesis of hypertension, as we have previously seen, our finding that AVP-mediated AQP-2 urinary excretion is strictly influenced by the consumption of water with different mineral content suggests a new, interesting field of investigation related to the link between blood pressure and nutritional habits.

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