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Water immersion is associated with an increase in aquaporin-2 excretion in healthy volunteers

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Abstract

Here, we report the alterations in renal water handling in healthy volunteers during a 6 h thermoneutral water immersion at 34 to 36 °C. We found that water immersion is associated with a reversible increase in total urinary AQP2 excretion.

Water immersion in humans induces upward fluid shift. As a consequence, central and total blood volume are expanded, which is accompanied by an increase in cardiac dimension with heart stretching causing release of atrial natriuretic peptide. The increase in urinary cGMP during immersion confirms the role of circulating atrial natriuretic peptide as a vasodilator and promoter of vascular permeability [1]. This is associated with diuresis and natriuresis likely due to stimulation of cardiopulmonary volume receptors [1].

In the present study we tested the hypothesis that the vasopressin sensitive Aquaporin 2 (AQP2) water channel might contribute towards the diuresis observed during water immersion. Twelve healthy male subjects (age 24.1 ± 1.7 years, weight 74.6 ± 7.2 kg, height 180.5 ± 5.7 cm) participated in the study after having given their written informed consent.

The subjects were not allowed strenuous physical exercise in the week before the study, they were not allowed to drink any alcohol or coffee, to smoke or take any medication that influences diuresis for the whole duration of the study.

The study consisted of two parts: 6 h of thermoneutral Head Out Water Immersion (HOWI, water temperature 34–36 °C) and – with a time interval of at least 3 weeks in between – 6 h under appropriate control conditions of sitting on a chair in the same room (room temperature about 28 °C, air humidity about 62%). The study design is illustrated in Fig. 1.

Each time, the study lasted for 3 days. The subjects arrived on the morning of day 1 before the intervention, underwent a physical examination and started collecting their first twelve-h urine sample (US1, 8 am–8 pm). They spent the night in the guestrooms of the institute and collected the overnight urine sample (US2, 8 pm–6 am). At 6 am of the following day they were woken up and physiological parameters were measured. Urine sample US3 (6 am–8 am) was collected. After breakfast, the subjects arrived at the immersion facility (day 2). There, both parts of the study took place. Each part started at 8 am and lasted until 2 pm. Subjects sat still in the immersion tank respectively on a chair in the same room. During this 6 h study period, three two-h urine samples (US4: 8 am–10 am, US5: 10 am–noon, US6: noon–14 pm) were collected.

At the end of the 6 h study period, physiological parameters were measured. Urine samples US7 (2 pm–8 pm) and US8 (8 pm–8 am) were collected. On the morning of day 3 the subjects

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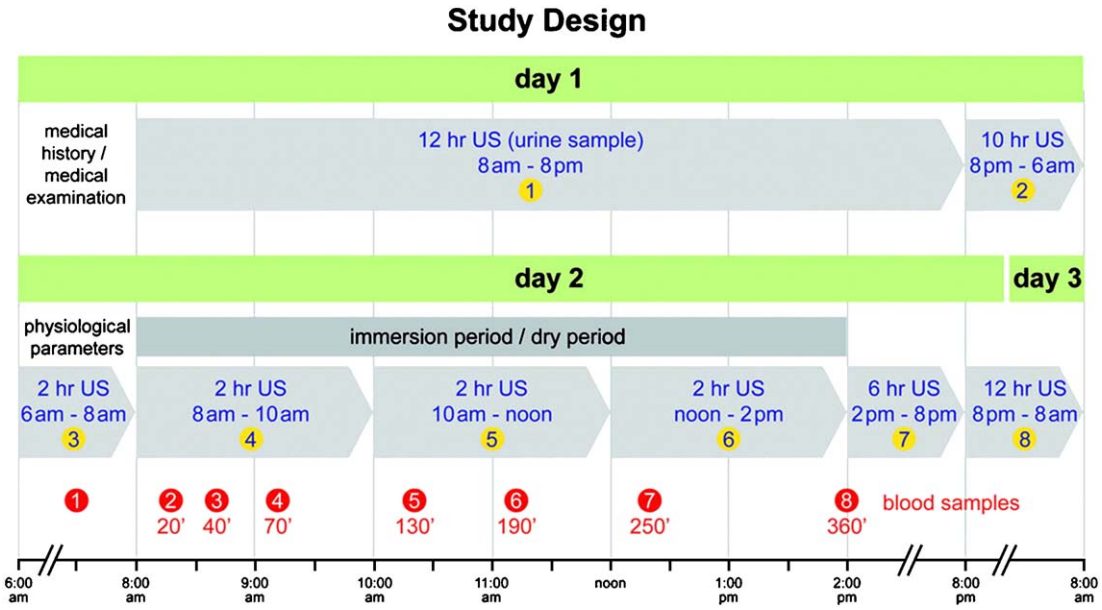

Fig. 1. Schematic drawing of the study design. Twelve male healthy volunteers participated in this study. The experiment was conducted in 3 days. Subjects were exposed to a 6 h water immersion in a thermoneutral bath and to a 6 h control condition of sitting on a chair in the same room under thermoneutral conditions. Urine samples as well as blood samples were collected at the indicated times.

left the Institute. Urine samples were stored at −20 °C until examined. There were no restrictions on fluid and food intake except for the 6 h study period when fluid intake was regulated at 200 ml·h^{-1}. The subjects kept protocol of their food and fluid intake all throughout the study.

The blood samples were taken from an antecubital vein at the following time points: 30 min before the start of the intervention (Basal condition) and 20 min, 40 min, 70 min, 130 min, 190 min, 250 min and at 360 min after the beginning of the intervention. Whole blood was taken into EDTA-plasma vacutainer tubes without stasis, immediately cooled and centrifuged and plasma was drawn off.

Plasmatic proANP concentrations were determined using the proANP enzyme immunoassay (Biomedica Medizinprodukte GmbH&Co KG, Vienna, Austria).

To investigate the renal water handling during immersion, fluid balance was calculated as the difference between the fluid loss (renal and extrarenal) and fluid intake. The importance of maintaining a thermoneutral temperature throughout the study for the calculation of fluid balance has to be underlined. In fact, since an increase in temperature results in a peripheral vasodilatation, it is absolutely necessary to maintain a thermoneutral temperature to avoid confounding influences on renal haemodynamics and excretory function.

Extrarenal fluid loss was calculated as 540 ml·24 h^{-1} (perspiratio insensibilis), which corresponds to 22.5 ml·h^{-1}. As the immersion tank was surrounded by saturated air and with high humidity in the whole room, perspiratio insensibilis was not taken into account for the calculation of fluid balances during the 6 h study period but for all the other collection periods. To achieve most accurate volume measurements, fluid intake, urine output and fluid balances were calculated in ml·kg^{-1}·h^{-1}, using the subjects' body weight.

Fig. 2 reports the measured values of water loss and water intake that allow the calculation of fluid balance. No differences in fluid intake and urine output could be seen before the immersion period. Throughout the 6 h study period, urine volume (ml·kg^{-1}·h^{-1}) was significantly higher in immersion than in control (8 am–10 am: 5.1±0.8>1.5±0.3, $P<0.01$; 10 am–noon: 6.1±0.52>2.8±0.37, $P<0.01$; noon–2 pm: 4.8±0.46>3.0±0.29, $n=12$, $P<0.05$) (Fig. 2A).

In the 6-h post-immersion period (2 pm–8 pm), immersed subjects showed higher fluid intake (2.00±0.17>1.4±0.14, $n=12$, $P<0.05$) (Fig. 2B) and lower urine output (0.8±0.08<1.50±0.17, $n=12$, $P<0.05$) than control. Therefore the immersed subjects found themselves in negative fluid balance for the duration of the immersion period, while dry control did not (Fig. 2C).

No differences between the two groups could be seen in the last collection period (US8, 8 pm–8 am).

To test whether the increase in urine volume during immersion was due to a possible reduced insertion of the vasopressin sensitive water channel AQP2 into the plasma membrane, we next evaluated the urinary AQP2 excretion in all subjects.

It is well established that AQP2 represents the chief target for the regulation of collecting duct water permeability and is translocated from intracellular vesicles to the apical membrane of collecting duct cells after vasopressin stimulation [2–4]. Interestingly, it has been reported that AQP2 is in part excreted in urine and that the excretion increases in response to vasopressin and is altered in several pathological states [5–8]. Therefore, urinary excretion of Aquaporin 2 (AQP2) has been proposed as a biomarker of renal concentrating ability. In this study, AQP2 excretion was measured to evaluate whether AQP2 might account for the higher urine output.

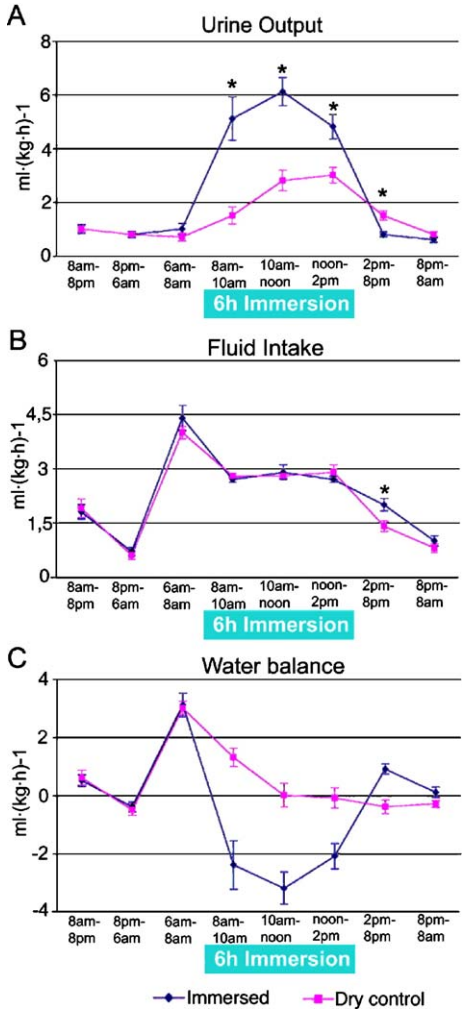

Fig. 2. Water handling. (A) Urine Output. Throughout the 6 h study period, urine output was significantly higher in immersed volunteers than in dry control. In contrast, in the 6 h post-immersion period (2 pm–8 pm), immersed subjects showed lower urine output (0.8 ± 0.08 ml·kg^{-1}·h^{-1} $<1.50\pm0.17$ ml·kg^{-1}·h^{-1}, $P<0.05$) than control. (B) Fluid Intake. During the 6 h study period, fluid intake was regulated at 200 ml·h^{-1} whereas in all the other periods, subjects had no limitation on their fluid intake. In the 6 h post-immersion period, subjects showed higher fluid intake than control. (C) Water Balance. This parameter was calculated as difference between water loss and water intake. With respect to the control condition, immersed subjects found themselves in negative fluid balance for the duration of the immersion period and in positive fluid balance in the 6 h post-immersion period. All values are presented as mean±S.E. *$P<0.05$, Student's t test for paired data.

In our study, we used an enzyme-linked immunosorbent assay (ELISA) as previously described [9] with some modifications.

Briefly, 5 or 10 µl of urine sample were diluted to 50 µl in PBS containing 0.01% SDS placed in MaxiSorp 96-well microplate (Nunc, Rochester, NY, USA) and incubated for 16 h at 4 °C. In parallel wells, increasing concentrations (50, 100, 200, 300, 400, 500, 1000 pg/50 µl) of a synthetic peptide reproducing the C-terminal region of the human AQP2 were incubated as internal standard. After washing and blocking, 50 µl of affinity purified anti-AQP2 antibodies were added to each well, incubated for 3 h followed by incubation with anti-rabbit IgG conjugated with horseradish peroxidase (Sigma). Wells were washed and 50 µl of the substrate solution [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid);

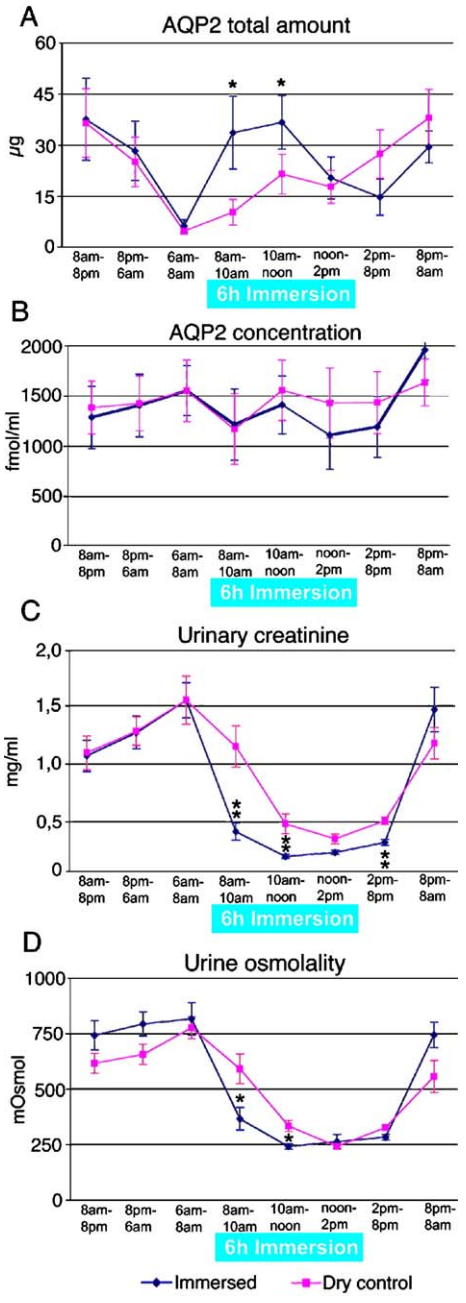

Fig. 3. AQP2 excretion, Creatinine, Osmolality. (A) Total urinary AQP2 excretion. To measure the total urinary AQP2 excreted, the amount of excreted AQP2 in each urine sample was calculated considering the urine output in a given time interval. In immersed subjects, total urinary AQP2 excretion was significantly increased in the first and the second period of water immersion followed by a rapid return to basal values after few hours of recovery. (B) AQP2 concentration. AQP2 concentration (fmol/ml) did not increase throughout the entire study period. (C) Urinary creatinine excretion. Urinary creatinine was significantly lower than in dry control during the first 4 h of water immersion and in the 6-h post-immersion period. (D) Urine osmolality. Throughout the first 4 h of the immersion period, urine osmolality was significantly lower in immersed volunteers than in dry control and returned to baseline. Values are presented as mean±S.E.; *$P<0.05$; **$P<0.01$, Student's t test for paired data.

Sigma] were added in each well and incubated for 30 min in the dark. Absorbance was measured with a microplate reader at 405 nm (BioRad model 550).

In all subjects, thermoneutral HOWI caused a marked increase in total urinary AQP2 excretion during the first 4 h of water immersion compared to dry control (8 am–10 am: 36.56±10.64 μg>10.19±3.73 μg, $P<0.05$; 10 am–noon: 36.56±7.87 μg>31.41±0.37 μg, $n=12$, $P<0.05$) (Fig. 3A). However, this increase in the total amount of AQP2 excretion was mainly due to the higher urinary output. In fact, when AQP2 concentration was calculated, no significant differences between immersion and control conditions were observed throughout the entire tested period (Fig. 3B). Consistent with these data, a significant decrease in both urinary creatinine and urine osmolality was observed during the first 4 h of water immersion compared to control conditions (Fig. 3C–D). Following this time, the 6 h post-immersion period was associated with a prompt return to pre-immersion levels in both AQP2 and creatinine excretion with a progressive increase of urine osmolality.

In agreement with these data, the creatinine clearance measured during the first 2 h of water immersion was significantly higher in immersed subjects indicating that immersion causes an increase in the glomerular filtration rate (GFR) (Table 1).

Several studies reported that vasopressin levels decrease during water immersion [1]. The mechanisms of vasopressin suppression during water immersion are incompletely defined, but it appears that not only cardiopulmonary mechanoreceptors but also arterial baroreceptors mediate the response [1]. Urinary vasopressin concentration (U AVP) was measured by RIA (Arginine vasopressin RIA kit, DSL-Webster, TX, USA) and obtained data confirmed a significant decrease in the concentration of this hormone during the first 4 h of water immersion compared to dry control (8 am–10 am: 4.01±1.1 pmol/L<31.49±11.37 pmol/L, $n=6$, $P<0.05$; 10 am–noon: 7.18±1.47 pmol/L<19.47±6.42 pmol/L, $n=6$, $P<0.05$) (Fig. 4). No significant differences in U AVP levels were found after 4 h water immersion (Fig. 4). This finding is in apparent discrepancy with the observed increase in the total urinary AQP2 excretion. A possible explanation for this observation might come from recent data by Wang et al. [10] who found an increase in the apical labeling of AQP2 after 90 min in renal collecting ducts in rats subjected to continuous Atrial Natriuretic Peptide (ANP) infusion. On the other hand, ANP infusion induced a sustained natriuresis and transient diuresis.

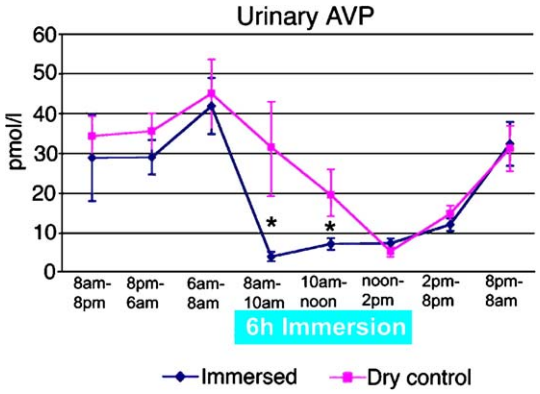

Fig. 4. Urinary AVP. Urinary concentration of vasopressin (AVP). Throughout the first 4 h of the immersion period, U AVP was significantly lower in immersed volunteers than in dry control. Values are presented as mean±S.E.; *$P<0.05$; **$P<0.01$, Student's t test for paired data.

The authors suggested that these effects might represent direct or compensatory effects to increase sodium and water reabsorption to prevent the development of volume depletion in response to ANP infusion.

ANP values were measured in the subjects enrolled in this study. While proANP-concentrations before immersion and dry control were within the reference range (reference range <1945 fmol ml^{-1}), 20 min after the beginning of the intervention, an increase in concentration was seen in immersed subjects (20 min after intervention: 1994±140 fmol·ml^{-1}>1678±112 fmol·ml^{-1}, $P<0.05$). This increase lasted throughout the 6 h study period, with immersed subjects displaying significantly higher proANP concentrations than dry controls (Table 2).

Plasma osmolality was found significantly higher in immersed subjects which is consistent with an increase of water output (Table 2).

The effect of ANP to induce AQP2 accumulation in the inner medullary collecting duct would be consistent with the described observation of cGMP-mediated increase in AQP2 accumulation in the apical plasma membrane of principal cells mainly in the medullary collecting ducts [11,12]. Therefore, since HOWI induces a significant increase in proANP and AQP2 urinary excretion in the presence of a decrease in vasopressin levels, we might speculate that ANP may stimulate AQP2 targeting to the luminal membrane. These data are in line with previous observations by Buemi et al. [13] who found an increase of AQP2 excretion during 2 h water immersion. In this study the authors followed the

Table 1
Creatinine Clearance (ClCr) measured in the immersion day

		Immersion period/dry period				
	Collecting time	6 am–8 am	8 am–10 am	10 am–noon	noon–2 pm	2 pm–8 pm
ClCr (ml/min)	Immersed subjects	159+34.0	190.0±20.5*	144.2±13.3	138.8±12.2	32.0±5.8
	Dry control	169±62.4	155.6±16.2	136.5±14.9	118.5±8.3	76.5±10.2

All values are expressed as Mean+S.E.
* $P<0.05$ Student t test for paired data.

Table 2
ProANP concentrations and plasma osmolality in immersed subjects respect with dry control during 6 h intervention period

			Immersion period/dry period						
	Collecting time	Basal	20′	40′	70′	130′	190′	250′	360′
Plasmatic proANP (fmol ml^{-1})	Immersed subjects	1840±152	1994±140*	2152±137*	2327±167*	2394±176*	2302±144*	2192±127*	2092±102*
	Dry control	1760±143	1678±111	1612±104	1590±107	1523±91	1502±91	1544±126	1553±124
Plasma osmolality (mosmol kg H_2O^{-1})	Immersed subjects	288±0.86	287±0.86	287±1.15*	287±0.86*	285±1.73*	284±1.15*	286±1.44*	284±0.86*
	Dry control	287±0.57	286±0.86	285±0.86	284±0.86	282±0.86	282±0.86	282±0.86	281±0.86

All values are expressed as Mean±S.E.
* $P<0.05$ Student t test for paired data.

effect of water immersion in healthy humans for only 2 h whereas in the present contribution the volunteers were subjected to water immersion for 6 h and were monitored also for 6 h of recovery. Regarding AQP2 excretion it has to be underlined that Buemi and coll. calculated AQP2 excretion normalized to creatinine which may not be a reliable parameter for AQP2 normalization because of the altered renal hemodynamic during immersion. Moreover, the evidence that AQP2 concentration does not change during the immersion period in our experimental conditions would underscore the role of AQP2 in the diuresis associated to the immersion as well as to the post-immersion period characterized by a decrease in urine output. Our data show that, despite identical water intake during control and immersion periods, flow rate throughout immersion exceeded that observed during control, putting the immersed subjects into a negative fluid balance. Moreover, in the post-immersion period they showed higher fluid intake and lower urine output thus reaching a fluid balance. Again, this ability seems not to be related to a modulation of AQP2. Another different result we obtained in this study is a significant increase in the creatinine clearance during the immersion period consistent with an increase in the GFR as well as a significant increase in plasma osmolality which should be expected because of a reduction of vasopressin levels. The increase in the GFR may contribute to the higher urine output observed during the immersion. To conclude, our results suggest that the increase in urinary flow rate is rather attributable to both a decreased reabsorption of filtrate proximal to the diluting site and to an increase in the distal delivery of filtrate observed during immersion [14–16] and AQP2 modulation does not seem to play a role in these processes. Instead, the increase in total AQP2 excretion might reflect a secondary kidney response to counteract fluid loss after immersion. Together, all these effects are a complex response to central blood volume expansion caused by immersion and involve several hormones regulating extracellular fluid volume and osmolality.

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