Osmopressor Response

The Osmopressor Response Is Linked to Upregulation of Aquaporin-1 Tyrosine Phosphorylation on Red Blood Cell Membranes

You Hsiang Chu, Yu-Juei Hsu, Herng Sheng Lee, Shung-Tai Ho, Che-Se Tung, Ching-Jiunn Tseng, Min-Hui Li, Tso-Chou Lin, Chih-Cherng Lu

Abstract—Studies in patients with an impaired efferent baroreflex led us to discover that ingesting water induces a robust increase in blood pressure and vascular resistance. This response was also present in healthy subjects with intact baroreflexes, described as osmopressor response. This study was to discover the physiology of the osmopressor response by determining functional activation of the aquaporin-1 water channel receptor on red blood cell membranes in young healthy subjects. In a randomized, controlled, crossover fashion, 22 young healthy subjects (age, 19–27 years) ingested either 500 or 50 mL of water. Heart rate, blood pressure, cardiac index, and total peripheral vascular resistance were measured using a Finometer hemodynamic monitor. Blood sampling was performed at 5 minutes before and at 25 and 50 minutes after either the water ingestion or control session. Immunoblotting for aquaporin-1 tyrosine phosphorylation was performed before and after subjects ingested either 500 or 50 mL of water. At 25 minutes after the ingestion of 500 mL of water, total peripheral resistance increased significantly, and plasma osmolality decreased. Functional expression of aquaporin-1 tyrosine phosphorylation on red blood cell membranes increased significantly at 25 and 50 minutes after subjects ingested 500 mL of water compared with that before water ingestion. This study concludes that water ingestion produces upregulation of aquaporin-1 tyrosine phosphorylation on red blood cell, which presents as a novel biological marker that occurs simultaneously with the osmopressor response.

Key Words: aquaporin-1 ▪ drinking ▪ erythrocytes ▪ tyrosine phosphorylation

Previous studies in patients with an impaired efferent baroreflex led us to discover that water ingestion induces a robust increase in blood pressure (BP) and vascular resistance.1,2 Initially, Jordan et al1 demonstrated that ingesting 473 mL (16 oz) of water induces a profound increase in systolic BP averaging ≈40 mm Hg in patients with autonomic failure and also elevated BP by ≈11 mm Hg in elderly subjects. This effect appears within 10 minutes, is maximal at 25 to 40 minutes, and largely dissipates by 90 minutes after water ingestion.1 Although the presor effect of water is greatest in individuals with impaired baroreflex buffering, water induces a maximal rise in peripheral vascular resistance at 25 minutes after ingestion without an associated increase in BP in young healthy subjects.3-5 This prominent vascular response after water ingestion is termed the osmopressor response (OPR), reflecting that the stimulus setting of the response is hypo-osmolality rather than gastric distension.6,7

The hypo-osmotic stimulus of water has been thought to act through the osmosensitive structures in portal and splanchnic circulation.8 In animals, the osmopressor response seems to be mediated through transient receptor potential cation channel, subfamily V, member 4 (TRPV4) activation on osmosensitive hepatic spinal afferents, dorsal horn ganglia, and spinal cord.9 Aquaporin-1 (AQP1), the first discovered water channel protein, was found colocalized with portal osmosensor TRPV4 in the plasma membrane of axons and synaptic terminals in the superficial dorsal horn and the enteric plexus.10 We supposed that the AQP1, driven by osmotic gradients, contributes to the osmopressor response by acting as an osmotic sensing input carried by circulating red blood cells (RBCs).

Thus, we hypothesized water ingestion induces functional expression of the AQP1 in circulating RBC during the osmopressor response.11 The study presented here demonstrates that the hypo-osmotic stimulus of water increases AQP1 tyrosine phosphorylation simultaneously with the osmopressor response.

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Methods

This study was approved by the Institutional Review Board (098-032-312) of Tri-Service General Hospital, Taipei, Taiwan and was performed in the Pain Department Laboratory. Informed consent was obtained from each volunteer. We studied 22 healthy normal young adults who had no history of syncope and who were currently using no prescription or over-the-counter medications.

Study Protocol

The subjects were asked to empty their bladder before beginning the test to avoid any effect of the urinary bladder or stomach distension, which is known to affect peripheral sympathetic activity. Study sessions took place in a quiet, dimly lit room at a comfortable ambient temperature (21–24°C). We used a randomized, crossover study design. Each subject underwent the study protocol twice on separate days. Subjects received either 500 or 50 mL water in their initial study, with the alternative in their second test. Because water ingestion increases, total systemic vascular resistance in healthy young subjects, within 5 minutes after ingestion, reaches a maximum in 20 to 40 minutes, and is maintained for >1 hour; the 25-minute time point after water ingestion was taken as representative of the maximal osmopressor response, and the 50-minute time point as the terminal phase of OPR in comparison with the baseline level before water ingestion.

The Western blotting for AQP1 was performed in 18 subjects selected from 22 study subjects. The correlation between the change of tyrosine phosphorylation of AQP1 on RBC and TPR after water was examined in 18 subjects. For comparison, AQP1 tyrosine phosphorylation on RBC membrane was assessed by Western blot before and after intravenous infusion of 5% glucose in water (D5W) in 4 subjects.

Instrumentation

An antecubital venous catheter was inserted for blood sampling at least 15 minutes before the beginning of the test, with the patient in the sitting position. Hemodynamic parameters including heart rate, BP, stroke volume, cardiac index, and total peripheral vascular resistance (TPR) were measured using a Finometer (Finapres Measurement Systems, Arnhem, The Netherlands). Computation of stroke volume and cardiac output using the model flow method was based on the finger arterial pressure. The hemodynamic parameters were determined by the calculation of 1 minute average from the continuous recording of Finometer. Skin blood flow was recorded and measured using a Laser Doppler flowmeter (DRT4 Instrument, Moor Instrument, Axminster, UK) over the right palmar region.

Blood Sampling for Plasma Osmolality

Blood samples (2 mL) were collected at time points of 5 minutes (baseline), 25, and 50 minutes after water ingestion. Plasma osmolality was analyzed by the freezing point depression method using an Advanced osmometer model 3900 (Advanced Instruments, Norwood, MA).

Blood Sampling for AQP1 on RBC Membranes

Ten milliliters of blood was collected at 5 minutes before (baseline) and 25 and 50 minutes after water ingestion (Method S1 in the online-only Data Supplement).

Immunoprecipitation and Western Blot Analyses

Equal amounts of protein (500 μg) from the RBC membranes were used for immunoprecipitation and Western blot analysis (Method S2).

Statistical Analysis

A sample size of 22 was estimated to have 95% power to detect an effect size of 0.8 by the paired t test with a 2-sided significance level of 0.05. A repeated-measures ANOVA was used to assess changes of AQP1 tyrosine phosphorylation from baseline between the water-ingesting (500 mL) and control (50 mL) sessions. Appropriate parametric (Student t test) tests for paired data were used for the analysis. Values are reported as mean±standard deviation. P<0.05 were considered significant, and all tests were 2-tailed. Statistical analyses were performed using SPSS version 13.0 software (SPSS Inc, Chicago, IL).

Results

Demographic data, basal hemodynamic variables, and plasma osmolality for all 22 subjects are shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
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<tr>
<td>Sex, male:female</td>
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<td></td>
</tr>
<tr>
<td>Weight, kg</td>
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<tr>
<td>Height, cm</td>
<td>165.1±8.4</td>
<td>147–182</td>
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<td>Body mass index, kg/m²</td>
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<td>Blood pressure, mmHg</td>
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<td></td>
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<tr>
<td>Systolic</td>
<td>118.4±11.6</td>
<td>93–142</td>
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<tr>
<td>Diastolic</td>
<td>69.9±7.0</td>
<td>55–88</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>73.0±9.0</td>
<td>57–97</td>
</tr>
<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>3.3±0.5</td>
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</tr>
<tr>
<td>Stroke volume, mL</td>
<td>76.2±16.6</td>
<td>49–120</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
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<tr>
<td>Osmolality</td>
<td>285.1±3.1</td>
<td>277.0–291.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>39.2±5.5</td>
<td>30.0–57.0</td>
</tr>
<tr>
<td>Total peripheral resistance, dyne·sec·cm⁻²</td>
<td>1365±278</td>
<td>955–2205</td>
</tr>
<tr>
<td>Laser Doppler skin blood flow, perfusion units</td>
<td>188.8±87.9</td>
<td>113.1–395.0</td>
</tr>
</tbody>
</table>

The body mass index is the weight in kilograms divided by the square of the height in meters.

Table 1. Characteristics of the 22 Subjects Examined at 5 Minutes (Baseline) Before Water Ingestion
Table 2. Hemodynamic Variables at 5 Minutes Before (Baseline), 25 Minutes, and 50 Minutes After Ingestion of Either 50 or 500 mL of Water in the Same Subjects; n=22

<table>
<thead>
<tr>
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<th>Water 500 mL</th>
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<td>Baseline</td>
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<td>50 min</td>
<td>Baseline</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>72.6±8.3</td>
<td>69.5±9.7</td>
<td>70.4±7.9</td>
<td>73.4±9.9</td>
</tr>
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<td>SBP, mmHg</td>
<td>118.6±11.9</td>
<td>127.6±10.1</td>
<td>132.7±11.3</td>
<td>118.3±11.5</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>70.4±7.1</td>
<td>76.0±7.2</td>
<td>77.7±10.1</td>
<td>69.4±7.1</td>
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<tr>
<td>MABP, mmHg</td>
<td>90.7±7.8</td>
<td>98.2±9.6</td>
<td>100.9±10.3</td>
<td>91.0±7.3</td>
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<tr>
<td>SV, ml/min</td>
<td>76.8±14.7</td>
<td>75.4±14.0</td>
<td>78.7±16.0</td>
<td>75.6±18.7</td>
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<tr>
<td>CI, L·min⁻¹·m⁻²</td>
<td>3.29±0.56</td>
<td>3.09±0.49</td>
<td>3.11±0.53</td>
<td>3.21±0.52</td>
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<tr>
<td>TPR, dyne·sec·cm⁻⁵</td>
<td>1372±279.2</td>
<td>1549±294.9</td>
<td>1465±281.2</td>
<td>1358±283.6</td>
</tr>
<tr>
<td>SkBF, perfusion units</td>
<td>165.4±88.9</td>
<td>129.1±89.1</td>
<td>99.5±70.6</td>
<td>172.5±105.7</td>
</tr>
</tbody>
</table>

Values are the mean (SD). CI indicates cardiac index; DBP, diastolic blood pressure; MABP, mean arterial blood pressure; SBP, systolic blood pressure; SkBF, laser Doppler skin blood flow; SV, stroke volume; and TPR, total peripheral vascular resistance.

*P<0.05 indicates a significant difference between 50 and 500 mL of water ingestion at time points of 25 and 50 min after ingestion.

Discussion

This study first demonstrated that water ingestion upregulated the AQP1 tyrosine phosphorylation on red blood cell membrane in healthy volunteers simultaneously with elicitation of the osmopressor response, including increase of total peripheral vascular resistance and attenuation of skin blood flow. The upregulation of the AQP1 tyrosine phosphorylation presented as an important biological marker during the osmopressor response. Intravenous infusion of D5W did not alter the functional expression of the AQP1 tyrosine phosphorylation.

Drinking water increases leg vascular resistance and TPR in healthy young subjects proportional to the increase in muscle sympathetic nerve activity of nerves supplying the calf region. This change in vascular tone of the limbs after ingesting water is thought to be partially compensated for by opposing changes in other vascular beds. Our previous study demonstrated that ingesting water induces sympathetic vasoconstriction associated with a decrease in palmar skin blood flow, which could be a characteristic of the OPR in young healthy subjects. Direct evidence for sympathetic vasoconstrictor activation after water ingestion is an increase in muscle sympathetic nerve activity. Cardiovascular tone has also been suggested to contribute to buffering the vasoconstrictor response but leads to no apparent change in BP. Consistent with these previous studies, the present study showed that ingesting water produced a maximal rise in TPR and an apparent decrease in skin blood flow without a prominent change in BP.

To our knowledge, the actual site and type of stimulus that elicits the OPR remains controversial. Several potential stimuli have been considered, including duodenal stretch, vasoactive hormones, osmotic factors, and the change in plasma volume after water ingestion. Jordan et al found no measurable changes in plasma vasopressin at 30 and 60 minutes after subjects drank 500 mL of water and concluded that vasopressin did not participate in the OPR. Hydration status may affect BP, but acutely increasing plasma volume via an intravenous saline infusion fails to elicit the pressor response seen with duodenal water administration. The absence of a pressor response after a saline infusion via nasogastric tube makes luminal stretch an unlikely explanation for the increase...
in BP or osmopressor response to water, because both fluids would induce an equal duodenal stretch. Because saline ingestion does not elicit the OPR either, the potential reason to explain water elicitation of the OPR depends mainly on the hypo-osmotic nature of water itself to act as an afferent input via gastrointestinal stimulation. Thus, the maximal effect of the OPR that occurred through a decrease in plasma osmolality after water ingestion suggests that the hypo-osmolality of water acted as a major afferent stimulus.

According to May et al, liver afferents may contribute to water drinking–induced sympathetic activation in human subjects, and there is evidence for osmosensitive mechanisms in the portal system. In fact, it is not easy to investigate how water affects luminal osmolality of the gastrointestinal tract in humans. An animal study provides evidence that duodenal infusion of water produces relative hypo-osmolality in the portal circulation, which indicates the hypo-osmotic nature of water. However, it is still unclear how water interacts with osmoreceptors in the portal and splanchnic circulation to induce the OPR in human.

The nonwater transport functions of AQPs are increasingly being recognized as a property that may have physiological significance. To our knowledge, AQP1 participates in homeostatic control of cell swelling by water when cells encounter hypo-osmolality. It seems likely that AQP1 on circulating RBC membranes helps control cell homeostasis but also provides a potential target as mediation for multiple physiological functions. It has since been shown that the direct mechanism of AQP1 ion channel activation requires intracellular cGMP binding. Truncation of the C-terminal domain prevents activation of the AQP1 ion channel by cGMP, suggesting functional significance of this region. A central tetrameric pore is the ion channel in AQP1, and its function is regulated by intracellular signaling involving tyrosine phosphorylation. Accordingly, phosphorylation of tyrosine Y253 in the AQP1C-terminal domain acts as a master switch regulating responsiveness of AQP1 ion channels to cGMP. These findings expand our understanding of AQP1 as a multifunctional regulated channel.

This AQP1 signaling conduction is important to investigate the functional activation of AQP1 in human RBCs and opens new avenues for exploring the physiological roles of dual ion and water conductance properties of the AQP1 channel.

Previous studies have demonstrated that ingesting water may lower portal plasma osmolality to a greater extent than systemic plasma osmolality to produce the OPR because the intraduodenal saline infusion does not alter portal/systemic osmolality or raise BP in patient with autonomic failure. Our study demonstrated there was an excellent correlation between the increase of the AQP1 tyrosine phosphorylation on RBC membrane and the increase of TPR at 25th min after

Figure 1. Plasma osmolality decreased after ingestion of 500 mL water at 25 minutes. Plasma osmolality from the same subject with ingestion of either 50 or 500 mL water at 0, 25, and 50 minutes. ANOVA with repeated-measures for the difference of plasma osmolality over time between the water-ingesting (500 mL) and control (50 mL) sessions was significant (P=0.0002). The bars indicate mean±SD; n=22.

Figure 2. Water ingestion altered tyrosine phosphorylation of aquaporin-1 (AQP1) on red blood cell membrane. A, Red blood cells (RBC) membrane protein from the same subject with ingestion of either 50 or 500 mL water for 0, 25, and 50 minutes was extracted and subjected to immunoprecipitation (IP) and immunoblotting (IB). Baseline control was without ingestion of water before experiment. Total AQP1 protein showed no significant change throughout the course, whereas upregulated tyrosine phosphorylation of AQP1 was significantly induced by ingestion of 500 mL water for 25 and 50 minutes, not by ingestion of 50 mL water for 25 and 50 minutes. Shown in A is a representative gel. Actin was used as an input control. B, The semiquantitative analysis demonstrated significant increase of tyrosine phosphorylation of AQP1 on RBC membrane induced by 500 mL water ingestion for 25 and 50 minutes (**P<0.001 and *P=0.0135). The AQP1 tyrosine phosphorylation showed 1.5-fold and 3.0-fold increase at 25 and 50 minutes, respectively, after ingestion of 500 mL of water; n=18. C, The scatter-gram demonstrated the ratio of tyrosine phosphorylation of AQP1/total AQP1 on RBC membrane induced by either 50 or 500 mL water ingestion at baseline, 25th, and 50th min (**P=0.003; *P=0.0481).
The upregulation of AQP1 tyrosine phosphorylation on RBCs membrane and its physiological role in the osmopressor response still need to be clarified in a future study. Perhaps, manipulating osmosensitive AQP1 afferent signal transduction in circulating RBCs might have usability in the prevention of syncope or presyncope in young healthy subjects, as well as management of postural hypotension in patients with autonomic disorders.

**Perspectives**

Ingestion of water is proven to be therapeutic to relieve debilitating hypotension episodes. Identifying the exact mechanism of water-induced sympathetic activation may provide novel targets for treating orthostatic and vasovagal syncope. The water ingestion–induced AQP1 tyrosine phosphorylation is a new molecular candidate to implicate the physiology of autonomic cardiovascular regulation in humans.

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**Disclosures**

None.

**References**


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**Novelty and Significance**

**What Is New?**

- The upregulated tyrosine phosphorylation of aquaporin-1 on red cell membrane might contribute to the osmopressor response.
- To explain its molecular mechanism of autonomic cardiovascular physiology in human.

**What Is Relevant?**

- Identification of the exact molecular mechanism of the water-induced sympathetic activation.
- To provide novel targets for the treatment of orthostatic hypotension, orthostatic intolerance, and vasovagal syncope.

**Summary**

Hypo-osmotic nature of water elicits that aquaporin-1 tyrosine phosphorylation might link to the osmopressor response in young healthy subjects.
The Osmopressor Response Is Linked to Upregulation of Aquaporin-1 Tyrosine Phosphorylation on Red Blood Cell Membranes

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The osmopressor response is linked to upregulation of aquaporin-1 tyrosine phosphorylation on red blood cell membranes

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Method S1-S2
Table S1
Figures S1-2
Method S1

**Blood sampling for AQP1 on RBC membranes**

Ten milliliters of blood was collected in K$_2$ ethylenediaminetetraacetic acid (EDTA)-containing tubes (Becton Dickinson, Franklin Lakes, NJ, USA), and Western blot analyses for AQP1 and AQP1 tyrosine phosphorylation on RBC membrane protein were performed at time points of −5, 25, and 50 min after subjects ingested either 500 or 50 mL of water. The RBCs were isolated by Ficoll Plaque Plus (GE Healthcare Bio-Sciences AB, Björkgatan, Uppsala, Sweden). RBCs were lysed in three volumes of ice cold 1 mM Na$_2$HPO$_4$, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF, Gold Biotechnology, St. Louis, MO, USA), pH 7.4, for 1 h at 4°C. RBC membranes were washed free of hemoglobin by pelleting at 14,500 rpm for 20 min at 4°C over four wash cycles. 200 µL of lysis buffer (250 mM sucrose, 5 mM Tris-HCl, 1 mM MgCl, 1 mM Na$_3$VO$_4$, 1 mM PMSF, EDTA-free Complete Protease Inhibitor Cocktail Tablets) was added to the sample and vortexed for 30 s. After a 20-min incubation at 4°C with continuous stirring, the samples were centrifuged at 17,000 × g for 30 min to pellet the cytoskeletal and insoluble protein. Protein content was determined in triplicate with bovine serum albumin (BSA) as the standard (DC Protein Assay, BIO-RAD, Hercules, CA, USA).

Method S2

**Immunoprecipitation and Western Blot Analyses**

Equal amounts of protein (500 µg) from the RBC membranes were used for immunoprecipitation. RBC membrane lysates were incubated with monoclonal antiAQP1 antibody (MCA2099, AbD Serotec, clone 1/A5F6, IgG1 isotype, Oxford, OX5 1GE, UK) for 2 h at 4°C in a cold room with rotation and then immunoprecipitated with Protein G Agarose for 12 h using a Protein G Immunoprecipitation Kit (Sigma, St. Louis, MO, USA), according to the manufacturer’s protocol. The immunoprecipitated proteins or whole cell lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and transferred to a polyvinylidene fluoride membrane (Immobilon-P Transfer Membrane; Millipore, Billerica, MA, USA). The membranes were blocked overnight at 4°C with 3% BSA (Fraction V, Sigma) in Tris-buffered saline with Tween 20 (TBST) (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.2% Tween 20). After washing three times with TBST, the blots were incubated overnight at 4°C with antiphosphotyrosine-horseradish peroxidase (HRP)-conjugated antibody
(NIF-1353, Amersham Pharmacia Biotech, Little Chalfont, U.K.) diluted 1:1,000 in 3% BSA. In other experiments, blots were incubated with primary antiAQP1 antibody (AbD Serotec) and then with HRP-labeled secondary antibodies. The β-actin antibody (sc-47778, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted 1:20,000 in 3% non-fat milk and TBST. The antimouse secondary antibody was diluted 1:20,000 in 3% non-fat milk and TBST. The membranes were rewashed extensively, and binding was detected using the Immobilon Western blotting detection system (Millipore), according to the user’s guide.
Table S1. Hemodynamic variables at 5 min before (baseline), 25 min and 50 min after intravenous infusion of 5% glucose water. (n=4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Name</th>
<th>Baseline</th>
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<th>50 min</th>
</tr>
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<td></td>
<td></td>
<td>±7.0</td>
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<td>Heart rate,</td>
<td>beats/min</td>
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<td>92.0</td>
<td>98.0</td>
<td>99.3</td>
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<td>1798</td>
<td>1844</td>
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<td>SkBF, perfusion</td>
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<td>197.0</td>
<td>129.3</td>
<td>143.0</td>
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<td></td>
<td>281.0 ±5.0</td>
<td>283.3±6.0</td>
<td>279.8 ±1.7</td>
</tr>
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</table>

Values are the mean (SD); SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP, mean arterial blood pressure; SV, stroke volume; CI, cardiac index; TPR, total peripheral vascular resistance; SkBF, laser Doppler skin blood flow.
Figure S1. There was no significant correlation between the change of plasma osmolality and total peripheral vascular resistance at 25th min after ingestion of 500mL water (n=22; r=0.104; p=0.6446)
Figure S2. Ration of tyrosine phosphorylation of AQP1/AQP1 on red blood cell membrane protein before (baseline) and 25th, 50th min after intravenous infusion of 500mL 5% glucose water. Membrane protein was extracted and subjected to immunoprecipitation (IP) and immunoblotting (IB). Upper panel was a representative gel. Actin was used as an input control. The semi-quantitative analysis demonstrated no significant change of tyrosine phosphorylation of AQP1 on RBC membrane after 5% glucose water infusion in the lower panel (n=4).