*Kidney International, Vol. 67 (2005), pp. 613–621*

ION CHANNELS – MEMBRANE TRANSPORT – INTEGRATIVE PHYSIOLOGY

# Defining conditions that lead to the retention of water: The importance of the arterial sodium concentration

**MOHAMMAD A. SHAFIEE, ANDRE F. CHAREST, SURINDER CHEEMA-DHADLI, DANIEL N. GLICK, OLGA NAPOLOVA, JAMSHID ROOZBEH, ELENA SEMENOVA, ASHEER SHARMAN, and MITCHELL L. HALPERIN**

*Renal Division, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada*

**Defining conditions that lead to the retention of water: The importance of the arterial sodium concentration.**

*Background.* A water diuresis occurs when a large volume of water is ingested rapidly. Nevertheless, water conservation is required to provide a source of water for evaporative heat dissipation throughout the day. Therefore, the objective was to define conditions that permit the retention of ingested water.

*Methods.* Volunteers collected urine q2h plus an overnight specimen; water loading was conducted after overnight food and water restriction; paired arterialized and venous blood samples were analyzed.

*Results.* When 20 mL water/kg was consumed in <15 minutes, the peak urine flow rate was 11 ± 0.6 mL/min. The volume of water retained after water intake stopped, and when the urine was hyperosmolar, correlated directly with the daily excretion of sodium plus potassium ($r^2 = 0.63$). The plasma sodium concentration ($P_{Na}$) was 4.0 ± 0.5 mmol/L lower in arterialized than paired venous blood 30 to 40 minutes after water ingestion began ($P < 0.01$). In preliminary studies, the smallest water load consumed in 15 minutes that would reproducibly cause a water diuresis was defined in each subject. This same acute water load was retained, however, if it contained 150-mmol/L fructose, but not glucose, or if it was consumed slowly (sipping). The arterialized $P_{Na}$ was not significantly lower than in paired venous samples when water was sipped.

*Conclusion.* A large fall in arterialized and not venous $P_{Na}$ best reflected the signal to induce a water diuresis. Although a *very* large water load can induce a water diuresis, smaller water loads can be retained for future heat dissipation.

Water is essential for survival because almost every enzymatic reaction occurs in an aqueous solution. Moreover, because water is a critical component of the extracellular fluid volume, it also serves to maintain perfusion pressure. In the long term, there is water balance in the body because its intake is equal to its output. When a large volume of water is ingested rapidly, it should be excreted promptly or a dangerous rise in intracranial pressure may occur. Nevertheless, water intake is not precisely matched to its loss in the short term. This raises questions about how the water control system is regulated after water is ingested. From a broader perspective, a source of water is needed for loss by evaporation to dissipate some of the heat produced by metabolism, especially during exercise [1, 2]. In quantitative terms, the evaporation of 1 L of water dissipates 500 to 600 kcal, one fourth of the heat produced under usual resting conditions [3].

The water control system has a sensor that detects a positive balance for water. The signal is a lower plasma sodium (Na) concentration ($P_{Na}$) that causes cells in the hypothalamic osmostat to swell [4, 5]. The net result is inhibition of both thirst [6] and the release of vasopressin [7]; the latter can induce a large water diuresis. On the other hand, there might be a mechanism to retain a safe volume of ingested water for future evaporative loss to achieve thermoregulation.

Experiments were designed to answer three questions. First, under what conditions might a water load be retained? Second, does a fall in the $P_{Na}$ in venous blood provide a reliable indicator of the stimulus to suppress the release of vasopressin? Third, once a water diuresis begins, what will determine how much of the ingested water will be excreted? Results to be reported indicate that the main signal that leads to a water diuresis is a large fall in the arterial $P_{Na}$. Based on these data, new insights into the integrative physiology of water homeostasis will be proposed.

## METHODS

### Studies in human subjects

*Subjects.* The Research Ethics Board of St. Michael's Hospital approved the protocols described herein. Volunteers were in good health and did not take medications in the week prior to study.

*Procedures.* Urine was obtained by voluntary voiding; the time and volume of each sample were recorded. The rate of excretion of creatinine was used to assess completeness of collection [8]. In the diurnal collections,

**Key words:** antidiuretic hormone, fructose, heat loss, integrative physiology, intestinal tract, water diuresis.

Received for publication March 30, 2004
and in revised form August 3, 2004
Accepted for publication August 17, 2004

subjects voided q2 to 3h during the day plus an overnight sample to permit undisturbed sleep. Each subject performed 2 or more 24-hour urine collections with unrestricted oral intake and activity.

All water load procedures and blood sampling were carried out in the morning after overnight food and water restriction (average 12-hour). To ensure consistency, each water load was repeated on at least two occasions, 2 to 7 days apart; the average value for each subject is reported. In the experiments where arterialized and venous $P_{Na}$ values were measured, blood samples were collected simultaneously. In studies designed to evaluate the ability of a water load to induce a water diuresis, blood was not drawn to minimize the risk of inducing a nonosmotic stimulus for the release of vasopressin.

*Studies to induce a water diuresis.* Subjects ingested 20 mL water/kg in 15 minutes ($N = 16$). To maintain this positive water balance, subjects drank a volume of water that was equal to the volume of each urine collection. After reaching a plateau for the urine flow rate (3 or more consecutive values that did not differ by more that 10%), no more water was ingested. When the urine osmolality ($U_{osm}$) exceeded the plasma osmolality ($P_{osm}$), balances for electrolyte-free water were calculated.

### Studies to define conditions that would lead to the retention of ingested water

*Preliminary experiment.* On separate days, each subject ($N = 14$) drank progressively smaller water loads over 15 minutes to define the minimum intake that would consistently induce a water diuresis. They also drank a volume of water equal to each urine specimen. This minimum water load was used in the next series of experiments.

**Determine if adding fructose to the acute water load would prevent the induction of a water diuresis.** In 11 subjects, fructose (150 mmol/L) was added to the water to slow its rate of intestinal absorption [9]. Each subject maintained a positive water balance by ingesting a volume of water equal to the prior urine output. This fructose load did not cause gastrointestinal symptoms. Control subjects ($N = 8$) consumed the same volume of a solution that contained equimolar glucose instead of fructose.

**Determine if slowing the rate of water ingestion would prevent the induction of a water diuresis.** In this protocol, 14 subjects drank a similar or larger volume of water than defined in the preliminary experiment, but its ingestion occurred over >150 minutes. They consumed 1.5 to 3 mL water/kg at the outset and 0.5 to 1 mL/kg every 30 minutes thereafter. The study was terminated when the urine flow rate reached 3 mL/min (equivalent to ~4.5 L/day) and/or when the $U_{osm}$ was less than the $P_{osm}$. The volume of electrolyte-free water retained was calculated.

### Assessment of the arteriovenous $P_{Na}$ difference

To determine the magnitude of the difference between the $P_{Na}$ in arterial and venous blood after ingesting a water load, blood samples were drawn simultaneously using a 22-gauge angiocatheter inserted in the antecubital vein of one arm and a vein on the dorsum of the contralateral hand. An infrared lamp from the top and a heating pad from below were used to heat the hand to 45 to 50°C before collecting arterialized blood [10, 11]. Catheters were kept patent by irrigation with a saline solution that contained 20 IU heparin/mL (Hepalean 1000 IU/mL; Organon Teknika, Toronto, Canada). The first portion of each sample was discarded because of possible contamination with the saline/heparin solution. Three blood samples were drawn q10 minutes from each site at baseline. Each subject then ingested 20 mL of water/kg in 15 minutes; blood samples were drawn at 10- to 15-minute intervals for 60 minutes. There were no urine specimens in these experiments because of its short duration. Moreover, it was deemed to be too stressful to collect a urine sample while having both arms immobilized. A sipping protocol of 10 mL/kg was also performed over 150 minutes in 4 subjects, with similar blood sampling technique.

### Studies in rats

These studies were carried out to obtain $P_{Na}$ values in portal venous and jugular venous blood during a water load.

*Animals.* Adult male Wistar rats (weight 300–400 g) were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. The Animal Care Committee of St. Michael's Hospital approved the study protocol.

*Procedures.* To avoid an electrolyte-rich stomach content, rats ($N = 12$) were fed an electrolyte-poor diet for 4 days (ICN Pharmaceuticals, Montreal, Canada; Na 1 mmol/kg, $K^+$ 0 mmol/kg, $Cl^-$ 0 mmol/kg). On day 4, they were given 20 mL of water per kg body weight by gavage tube. After 5 minutes, rats were anesthetized and a catheter was inserted into the femoral and portal veins. Blood was drawn simultaneously from these veins and then the aorta at the 8-minute time. In a second series of experiments, the degree of fall in the $P_{Na}$ in the jugular and femoral veins was compared in 6 rats that were fed their regular diet. Following anesthesia, catheters were inserted into the femoral and the internal jugular vein. The infusate contained 50 mmol NaCl/L, and it was administered into the femoral vein at 0.9 mL/min over 30 minutes. This infusion was stopped for 2 minutes and samples were drawn simultaneously from the jugular and femoral veins and then the aorta. The $P_{Na}$ values were compared as paired differences in each animal.

*Calculation of electrolyte-free water balance.* The volume of electrolyte-free water in each urine sample was calculated as previously described [12]. This volume was

**Table 1.** Description of subjects

| ID | Age *years* | Weight *kg* | Daily urine volume *mL* | Daily Na excretion *mEq* | Daily K excretion *mEq* | Daily osmoles excretion *mosmoles* |
|---|---|---|---|---|---|---|
| F-1 | 32 | 65 | 1577 | 100 | 66 | 753 |
| F-2 | 28 | 55 | 848 | 126 | 66 | 645 |
| F-3 | 59 | 60 | 1760 | 69 | 57 | 493 |
| F-4 | 29 | 57 | 1204 | 166 | 51 | 818 |
| M-1 | 35 | 65 | 855 | 196 | 33 | 856 |
| M-2 | 37 | 70 | 900 | 145 | 49 | 745 |
| M-3 | 36 | 70 | 901 | 208 | 62 | 879 |
| M-4 | 27 | 75 | 1016 | 194 | 69 | 969 |
| M-5 | 62 | 60 | 1133 | 119 | 59 | 828 |
| M-6 | 18 | 60 | 617 | 97 | 34 | 701 |
| M-7 | 18 | 60 | 565 | 121 | 89 | 608 |
| M-8 | 37 | 60 | 1119 | 211 | 84 | 1062 |
| M-9 | 29 | 84 | 1166 | 205 | 73 | 1149 |
| M-10 | 35 | 80 | 1080 | 231 | 68 | 1028 |
| M-11 | 29 | 60 | 1024 | 111 | 57 | 692 |
| M-12 | 37 | 80 | 1357 | 276 | 72 | 1180 |
| M-13 | 34 | 85 | 1910 | 207 | 80 | 945 |
| Mean | 34 | 67 | 1120 | 164 | 63 | 844 |
| SEM | 3 | 2 | 89 | 14 | 4 | 47 |

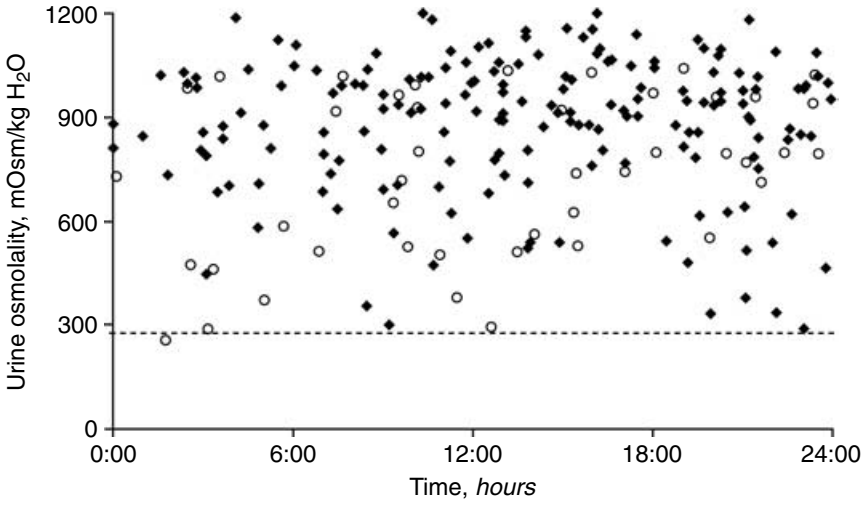


**Fig. 1. Urine osmolality in urines collected throughout the day.** Urines were provided q2 to 3h during daytime plus overnight if the subject rose to void in all the subjects in this study. Data are shown for male (◆) and female (O) subjects. The dashed horizontal line represents the $P_{osm}$ at 290 mOsm/kg $H_2O$. The time of day is shown on the x-axis and the $U_{osm}$ on the y-axis.

compared to the total volume of ingested electrolyte-free water to calculate this balance.

*Analytic techniques.* Urine pH and blood gas analysis were performed at 37°C with a digital pH/blood gas analyzer (Corning 178 blood pH analyzer, Corning, NY, USA); Na and potassium (K) were measured by flame photometry (Radiometer, FLM-3; London, ON, Canada); chloride (Cl) was measured by electromimetic titration (Chloride meter, CMT 10; London Scientific Ltd., London, Ontario); osmolality was measured by freezing point depression (Advanced Instruments, Inc., Needham Heights, MA, USA); urea and creatinine in plasma and urine [13] and vasopressin in plasma [14] were measured as previously described.

### Statistical analyses

Results are reported as the mean ± SEM. Comparisons within individuals were done by paired *t* test. Comparisons between groups were done with analysis of variance (ANOVA) when there were more than 2 groups. A $P$ value that was $< 0.05$ was considered to be statistically significant.

## RESULTS

Studies were performed in 4 female and 13 male volunteers (Table 1). Although there were large differences in the daily excretions of water, Na, K, and total osmoles between subjects (Table 1), the values did not vary by more than 20% in multiple daily collections in each individual (results not shown). The average rates of excretion of Na, K, osmoles, and water were 164 ± 14 mmol/day, 63 ± 4 mmol/day, 844 ± 47 mosmol/day, and 1120 ± 89 mL/day, respectively. The $U_{osm}$ was greater than the $P_{osm}$ in all but a single urine specimen, and this difference was very small (Fig. 1).

**Table 2.** Studies during a 20-mL/kg water load

| ID | Baseline $U_{FR}$ *mL/min* | Time to 1/2 peak $U_{FR}$ *minutes* | Peak $U_{FR}$ *mL/min* | % Water retained | VP levels *pg/mL* |
|---|---|---|---|---|---|
| F-1 | 0.7 | 70 | 12.3 | 57% | <0.5 |
| F-2 | 0.9 | 43 | 9.5 | 35% | <0.5 |
| F-3 | 1.7 | 50 | 7.8 | 64% | <0.5 |
| F-4 | 0.44 | 80 | 11.3 | 26% | <0.5 |
| M-2 | 0.56 | 108 | 7.7 | 51% | <0.5 |
| M-3 | 0.60 | 70 | 8.9 | 8% | <0.5 |
| M-4 | 0.45 | 45 | 17.6 | 48% | <0.5 |
| M-5 | 0.81 | 75 | 11.8 | 13% | <0.5 |
| M-6 | 0.46 | 50 | 13.8 | 32% | <0.5 |
| M-7 | 0.39 | 80 | 11 | 16% | <0.5 |
| M-8 | 0.64 | 67 | 11.5 | 62% | <0.5 |
| M-9 | 0.80 | 66 | 10.5 | 22% | <0.5 |
| M-10 | 0.60 | 83 | 10.7 | 57% | <0.5 |
| M-11 | 0.54 | 60 | 10.7 | 7% | <0.5 |
| M-12 | 0.63 | 150 | 10.7 | 74% | <0.5 |
| Mean | 0.68 | 73 | 11.1 | 38 | <0.5 |
| SEM | 0.08 | 7.0 | 0.6 | 5.8 | – |

Abbreviations are: $U_{FR}$, urine flow rate; VP, vasopressin.

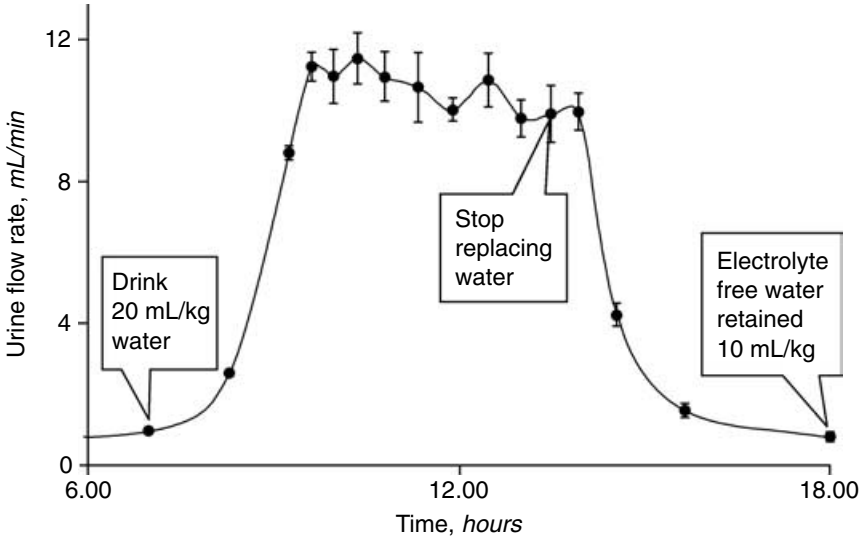


**Fig. 2. Representative study of a 20-mL/kg water load.** The time of day in hours is shown on the x-axis, and the urine flow rate is shown on the y-axis. After obtaining a second-voided urine, the subject consumed 20 mL water/kg in 15 minutes. After reaching a plateau for the urine flow rate at peak diuresis, water intake was stopped and the subject excreted only 10 mL/kg before his $U_{osm}$ was greater than his $P_{osm}$.

### Renal response to an acute 20-mL/kg water load

A brisk water diuresis began 73 ± 7 minutes after the acute intake of this water load. The peak urine flow rate was 11 ± 0.6 mL/min. Vasopressin levels were maximally suppressed when peak flow rates were achieved in every subject (Table 2). A representative study is shown in Figure 2. After the intake of water was stopped, and when the $U_{osm}$ exceeded the $P_{osm}$, 38 ± 5.8% of this electrolyte-free water load was retained (Table 2); 59 ± 3.3% of this water load was retained in 7/15 subjects.

### Studies to define conditions where ingested water would be retained

The objective in this series of experiments was to determine if slowing the rate of ingestion of water or its absorption from the intestinal tract could influence the ability to retain this ingested water. In preliminary experiments, we defined the minimum volume of water that, when ingested in 15 minutes, would reproducibly cause a water diuresis in each subject—this volume was 8.3 ± 0.8 mL/kg (Table 3). Under these conditions, the peak urine flow rate was 7.0 ± 0.6 mL/min; this flow rate was maintained by drinking a volume of water equal to the prior urine output.

When the same volume of water defined in the preliminary experiment contained 150 mmol fructose per liter and was consumed in 15 minutes, there was no water diuresis in 10/11 subjects (Table 3). In contrast, when the solution contained equimolar glucose instead of fructose, a water diuresis occurred (6.0 ± 1.0 mL/min).

When the same volume of water defined in the preliminary experiment was ingested slowly (sipping), again there was no water diuresis (Table 3); the volume of water retained in each subject was reproducible when the procedure was repeated on as many as 4 separate days.

**Table 3.** Influence of rate of water absorption on the urine flow rate

| ID | Amount of water intake *mL/kg* | Peak $U_{FR}$ during gulping *mL/min* | Peak $U_{FR}$ during gulping + glucose *mL/min* | Peak $U_{FR}$ during gulping + fructose *mL/min* | Peak $U_{FR}$ during sipping *mL/min* | $U_{osm}$ during sipping *mOsm/L* |
|---|---|---|---|---|---|---|
| F1 | 6 | 5.3 | – | 6.1 | 0.6 | 720 |
| F2 | 4 | 3.4 | 2.5 | 0.2 | 0.9 | 486 |
| F3 | 7 | 2.7 | 3.3 | 1.8 | 1.0 | 238 |
| F4 | 9 | 8.7 | N.D. | N.D. | 1.8 | 328 |
| M2 | 8 | 6.3 | 5.9 | 0.8 | 0.7 | 761 |
| M3 | 8 | 6.2 | N.D. | N.D. | 1.3 | 563 |
| M4 | 14 | 10.1 | N.D. | 1.5 | 0.8 | 664 |
| M5 | 5 | 6.8 | 7.9 | 1.8 | 1.9 | 488 |
| M6 | 6 | 7.7 | 8.8 | 0.9 | 1.0 | 518 |
| M7 | 10 | 6.1 | N.D. | 1.6 | 0.6 | 589 |
| M8 | 11 | 9.2 | 4.8 | 1.2 | 1.9 | 410 |
| M9 | 7 | 8.2 | N.D. | 1.7 | 2.4 | 470 |
| M10 | 10 | 6.6 | 9.0 | 1.4 | 0.8 | 893 |
| M12 | 15 | 10.5 | N.D. | N.D. | 1.0 | 504 |
| Mean | 8.3 | 7.0 | 6.0 | 1.7 | 1.2 | 545 |
| SEM | 0.8 | 0.6 | 1.0 | 0.5 | 0.2 | 46 |

Abbreviations are: $U_{FR}$, urine flow rate; N.D., not determined. The peak urine flow rate during gulping was significantly greater than during fructose ingestion or during sipping ($P < 0.05$).

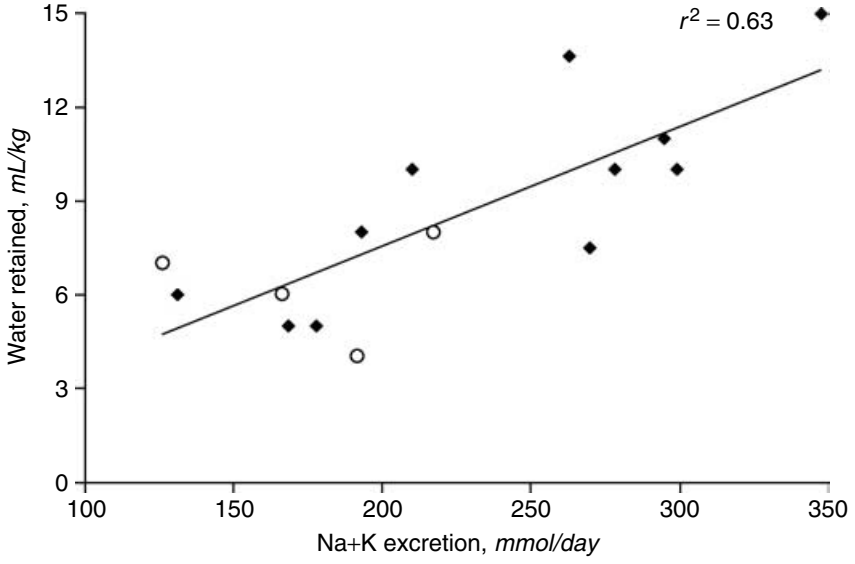


**Fig. 3. Influence of the Na + K content of the diet on the volume of water retained during the slow intake of water.** The daily excretion of Na + K in mmol/day is shown on the x-axis, and the volume of water retained after slow water ingestion in mL/kg is shown on the y-axis. Data are shown for male (◆) and female (O) subjects. Subjects consuming more salt might raise their level of vasopressin more quickly when their arterialized $P_{Na}$ increased due to the renal excretion of a large volume of electrolyte-free water when the input from the GI tract declined. There is a direct and statistically significant relationship between these two parameters ($r^2 = 0.63$, $P < 0.01$).

Moreover, if there was no further positive water balance (volume ingested = urine output), there was no rise in the urine flow rate. When the volume of water retained was examined in conjunction with their 24-hour Na + K excretion rates in the diurnal collections, a positive correlation was observed ($r^2 = 0.63$, Fig. 3).

### Assessment of the arteriovenous $P_{Na}$ difference

The final series of experiments in human subjects was performed to evaluate the signal to suppress the central release of vasopressin. The arterialized blood reflected arterial blood because its $PO_2$ and $O_2$ saturation were only minimally different both at baseline and during the water load period and much higher than in paired venous samples ($PO_2$ 76 ± 3 mm Hg, 95 ± 1% saturation vs. $PO_2$ 36 ± 3 mm Hg, 68 ± 5% saturation). Also, the $PCO_2$ was lower than in arterialized than in venous blood (39 ± 3 vs. 46 ± 4 mm Hg).

After obtaining control values, 7 subjects drank 20 mL of water/kg in 15 minutes. The arterialized $P_{Na}$ fell promptly, whereas the decline in the venous $P_{Na}$ was much smaller in this period (Fig. 4). The maximum fall in the arterialized $P_{Na}$ from baseline was 5.6 ± 0.6 (range 4 to 8) mmol/L. The largest difference between the arterialized and the venous $P_{Na}$ occurred at 33 ± 5 minutes and was 4.0 ± 0.5 mmol/L ($P < 0.001$). In contrast, after 60 minutes, the arterialized and venous $P_{Na}$ values were no longer significantly different.

Four subjects sipped 10 mL of water per kg over 150 minutes. The arterialized $P_{Na}$ was not significantly lower than in paired venous blood samples (Fig. 5).

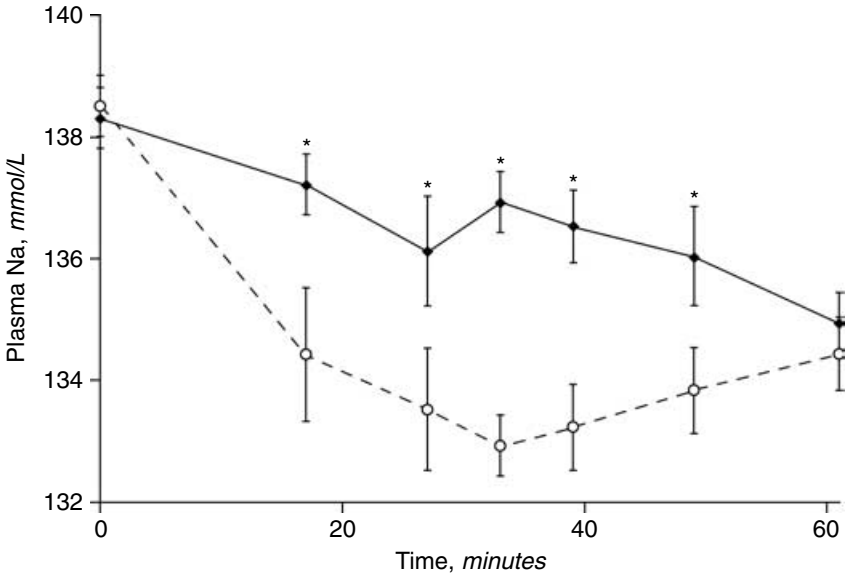


**Fig. 4. Fall in the $P_{Na}$ in arterialized and venous plasma during a 20-mL/kg water load.** The time in minutes after consuming 20 mL of water in <15 minutes is shown on the x-axis, and the $P_{Na}$ in mmol/L is shown on the y-axis. The dashed line depicts the arterialized $P_{Na}$ and the solid line depicts the $P_{Na}$ in the antecubital vein. The mean value for all control bloods is shown as the 0-time value. The results are the mean ± SEM for 7 subjects. All $P_{Na}$ values except the 0-time and the 60-minute times were significantly lower in the arterialized blood as compared to the venous $P_{Na}$ values by paired value analysis ($^*P < 0.01$).

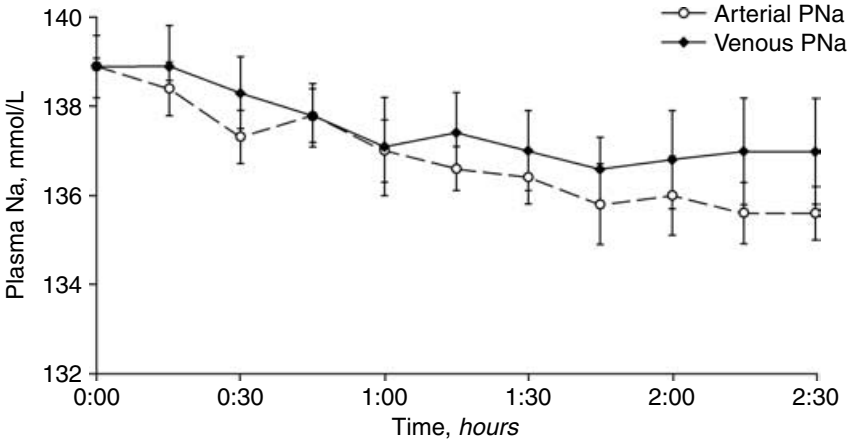


**Fig. 5. Fall in the $P_{Na}$ in arterialized and venous plasma while sipping a 10 mL/kg water load.** The time in hours after beginning the consumption of the water load is shown on the x-axis, and the $P_{Na}$ in mmol/L is shown on the y-axis. The dashed line depicts the arterialized $P_{Na}$ and the solid line depicts the $P_{Na}$ from the antecubital vein. The mean value for all control bloods is shown as the 0-time value. The results are the mean ± SEM for 4 subjects. The $P_{Na}$ values were not significantly lower in the arterialized as compared to the corresponding venous values.

### Experiments in rats

In the first series of experiments, the $P_{Na}$ was lowest in the portal vein (139 ± 0.5 mmol/L), intermediate in arterial plasma (141 ± 0.3 mmol/L), and highest in femoral vein plasma (143 ± 0.3 mmol/L) after an oral water load (Table 4, set 1). In the second series of experiments, the $P_{Na}$ was lowest in arterial blood (134 ± 0.5 mmol/L), intermediate in the jugular vein (136 ± 0.5 mmol/L), and highest in the femoral vein (138 ± 0.5 mmol/L) after an intravenous electrolyte-free water load (Table 4, set 2).

## DISCUSSION

The purpose of this study was to examine conditions needed to retain ingested water so that it could be available later for evaporative loss when heat production is high. We also investigated the source of the signal that might initiate a water diuresis.

Our principal results reveal that while a water diuresis was always produced in overnight-fasted subjects that drank 20 mL of water per kg body weight in 15 minutes, 38 ± 5.8% of this water was retained after water intake stopped, and the $U_{osm}$ was > the $P_{osm}$ (Table 2). In the experiments designed to study factors that might lead to water retention, we first determined the smallest acute oral water load that would reproducibly cause a water diuresis in each subject. When the absorption of water from the intestinal tract was retarded because it contained a poorly absorbed sugar (fructose) or when it was consumed slowly (sipping), a water diuresis did not occur, despite producing the same positive water balance. The final series of experiments was designed to help reveal the signal perceived by the hypothalamic water control system. There was a significantly larger fall in the arterialized $P_{Na}$ than in a paired peripheral venous blood in the first hour after drinking this water load in 15 minutes (Fig. 4), but not when this water load was consumed slowly (Fig. 5).

Four factors can influence the ability of an oral water load to lower circulating vasopressin levels sufficiently to

**Table 4.** Plasma Na concentration in different vascular compartments in rats after water loading

| Set 1 | Portal vein | Δ | Aorta | Δ | Femoral vein |
|---|---|---|---|---|---|
| Plasma Na *mmol/L* | 139 ± 0.5 | | 141 ± 0.3 | | 143 ± 0.3 |
| Difference *mmol/L* | | 1.7 ± 0.5 | | 2.5 ± 0.3 | |
| Set 2 | Jugular vein | Δ | Aorta | Δ | Femoral vein |
| Plasma Na *mmol/L* | 136 ± 0.5 | | 134 ± 0.5 | | 138 ± 0.5 |
| Difference *mmol/L* | | 1.9 ± 0.6 | | 3.8 ± 0.5 | |

Studies in set 1 were carried out with 12 rats in set 1 and in 6 rats in set 2. All the comparisons were made by paired differences and were statistically significant ($P < 0.05$).

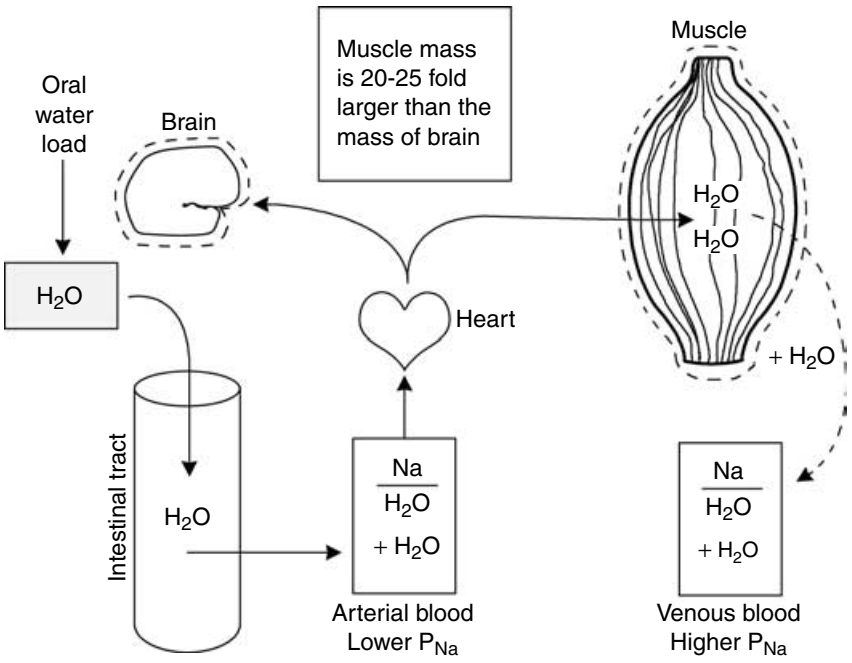


**Fig. 6. Water distribution after the rapid consumption of water.** When water is ingested (gray filled box on the left), it is absorbed from the intestinal tract and enters the portal vein; this leads to a large fall in the arterial $P_{Na}$. When arterial blood is delivered to the brain and skeletal muscle, the muscle cells will continue to swell for a longer period than for brain cells because the mass (water content) of muscle is much larger than that of the brain, yet their blood flow rates are similar. As a result, the $P_{Na}$ in venous blood draining muscle will be much higher in the earlier time points before equilibrium has been reached.

induce a water diuresis. First, there are events that occur before water enters the systemic circulation. Second, enough water must enter cells of the central osmostat to suppress the release of vasopressin. Third, nonosmotic stimuli for the release of vasopressin and $V_2$ receptor up-regulation must be taken into account. Fourth, an increased ability to shift water rapidly into muscle cells can decrease the degree of fall in the arterial $P_{Na}$ and thereby the ability of a given water load to induce a water diuresis.

## Events acting prior to the absorption of water

Oropharyngeal factors were evaluated in dogs with a gastric fistula [15]. Interestingly, the acute ingestion of water or isotonic saline caused a similar, rapid small fall in circulating vasopressin without changing the venous $P_{Na}$ or $P_{osm}$. This fall was independent of the absorption of water because the inhibition of the release of vasopressin occurred when virtually all ingested water was removed via the gastric fistula. In humans, oropharyngeal inhibitory signals were influenced by the temperature of the ingested fluids—ice chips caused an early, small suppression of the release of vasopressin, independent of a fall in $P_{Na}$ [16]. Nevertheless, oropharyngeal factors are not sufficient to initiate a water diuresis.

Published data do not permit an unambiguous evaluation of gastrointestinal or hepatic signals to detect the rate of input of ingested water prior to its entry into the systemic circulation because the arterial $P_{Na}$ was not measured in these studies [17]. If there were such a system, it would have to distinguish between electrolyte-free water containing glucose versus fructose because ingesting identical volumes of the glucose solution induced a water diuresis, whereas ingesting the fructose solution did not do so (Table 3). Moreover, the bulk of ingested glucose and fructose was removed rapidly because their concentrations in the systemic circulation do not rise more than a few mmol/L.

## Degree of cell swelling in the central osmostat

A large water diuresis was consistently seen following the ingestion of 20 mL of water per kg in <15 minutes presumably because this caused a sufficient degree of brain cell swelling. The arterial $P_{Na}$ should be a better early indicator of the signal to control the release vasopressin than the peripheral venous $P_{Na}$ (Fig. 6). There are a number

of factors that can influence the arterial $P_{Na}$ after water is ingested. For example, the content of electrolytes, poorly absorbed solutes, and water in the lumen of the stomach or small intestine prior to the ingestion of water can influence the amount of osmole-free water. In addition, the rate of water absorption from the gastrointestinal tract depends on the rates of water ingestion, stomach emptying, and factors such as poorly absorbed solutes in this fluid, which could influence the rate of absorption of water in the small intestine.

To evaluate these issues more directly, studies were carried out in rats. As expected, the $P_{Na}$ in portal venous blood was lower than the $P_{Na}$ of paired arterial samples following an oral water load in rats (Table 4). Second, an even better indicator of the degree of swelling of brain cells should be to measure the $P_{Na}$ in the internal jugular venous plasma because this $P_{Na}$ is closest to the value in the interstitial compartment of the brain. Because the $P_{Na}$ in the jugular vein was lower than in peripheral venous blood (Table 4), brain cells are more swollen than muscle cells at early times.

There were two observations during the 20-mL/kg water load that suggested that there might be a different $P_{Na}$ in arterialized and peripheral venous blood. First, a strong aversion to drink the entire volume of water in the 20-mL/kg water load developed well before completing the ingestion of water as previously described [18]. Second, a new symptom was commonly seen after achieving the peak water diuresis. In the first 30 minutes after water intake had stopped, 7/15 subjects complained of thirst despite the retention of $\sim$ 50% of the positive water balance. A possible mechanism for thirst could be a rise in the renal venous $P_{Na}$ and thereby in arterial blood due to an ongoing water diuresis when water absorption from the intestinal tract had declined. Accordingly, it might be helpful to follow arterialized $P_{Na}$ values to better understand the signal recognized by the central osmostat, especially if one wants to correlate $P_{Na}$ values and plasma vasopressin levels.

### Nonosmotic stimuli for the release of vasopressin and $V_2$ receptor levels

With a large nonosmotic stimulus for the release of vasopressin [7], a water diuresis might not occur despite achieving a low arterial $P_{Na}$. Because pain and anxiety are nonosmotic stimuli for the release of vasopressin, we did not draw arterialized or venous blood samples when the objective of the study was to determine the magnitude and duration of a water diuresis and the subsequent retention of this ingested water.

Subjects with a higher intake of Na might begin a study with a slightly higher $P_{Na}$. Therefore, they might need a larger positive water balance to suppress the release of vasopressin sufficiently to cause a water diuresis. In this context, subjects with the higher intake of NaCl had higher circulating levels of vasopressin in the first morning blood samples (median 0.86 vs. <0.5 pg/mL) and retained more water after stopping the 20-mL/kg-water load (Fig. 2 and Table 2). The extremely low plasma vasopressin levels (<0.5 pg/mL) in these latter 4 volunteers were accompanied by lower first morning Na excretion rates ($42 \pm 9$, $N = 4$, vs. $135 \pm 25$, $N = 4$, μmol/min). Despite having undetectable levels of vasopressin in the first morning plasma, the corresponding urine flow rate was <1 mL/min and the $U_{osm}$ was >600 mOsm/kg $H_2O$. We speculate that, although they had very low circulating levels of vasopressin, this amount was sufficient to induce enough water permeability in the distal nephron because of upregulation of their vasopressin $V_2$ receptor level caused by the chronic low circulating levels of vasopressin. Consistent with this impression, the acute water load needed to induce a water diuresis in these subjects was much smaller (i.e., <5 mL/kg) than the subjects on a higher NaCl intake ($\sim$10 mL/kg).

### Ability to shift water rapidly into muscle cells

If more ingested water could be 'stored' quickly in muscle cells, there would be a smaller early fall in the arterial $P_{Na}$ for a given positive water balance. Said another way, this water becomes 'occult' to the central osmostat, and it would be less able to induce a water diuresis. In more detail, when arterial plasma with its lower $P_{Na}$ traverses the capillary of skeletal muscle, water is drawn rapidly by osmosis into muscle cells (Fig. 6). Because the mass of skeletal muscle ($\sim$24 kg in a 70 kg adult) is very large relative to its blood supply at rest ($\sim$1 L/min), the capacity to draw the extra water in arterial plasma into muscle cells will not be saturated at early times. Hence, it will take longer to reach osmotic equilibrium between the ICF compartment and venous blood draining muscle as compared to brain cells and their venous effluent because of the much higher blood flow rate per kg in brain versus muscle. In support of this interpretation, the $P_{Na}$ in the jugular vein of rats receiving an intravenous water load was lower than in their femoral vein (Table 4, set 2).

Two factors can accelerate water entry into skeletal muscle cells. First, if the blood flow rate to skeletal muscle was larger than usual (e.g., mild exercise), more of the ingested water will be delivered to and retained in this compartment at earlier times (Fig. 6). Second, if the number of osmoles in the ICF compartment were to rise (e.g., during a sprint [19]), more water will enter the ICF of that organ. Hence, in Paleolithic times, a hunter could retain even more of the ingested water for use later in heat dissipation.

An interesting example of a mechanism of water retention in animals is illustrated in the water-deprived donkey or camel in the desert [20]. These animals can retain

rapidly ingested water (i.e., a 100-kg dehydrated donkey will drink 12 L of water in <5 minutes without inducing a water diuresis). If much of this ingested water were stored temporarily in their stomach, it would not cause an acute fall in the arterial $P_{Na}$. This permits a shorter stay in an oasis, and minimizes the risk of contact with dangerous predators.

### Integrative physiology

It is not immediately obvious why a very abundant nutrient in prehistoric times (fructose) would be poorly absorbed in the intestinal tract (for review, see reference [21]). This occurs because the intestinal tract lacks a specific transporter for fructose [22]. Because fructose is a poor substrate for glucose transporters, much of this sugar is delivered to bacteria in the colon, where it is metabolized to produce absorbable organic acids [23]. Although this bacterial metabolism results in decreased regeneration of ATP from the ingested fructose in the host, there could still be advantages for the host. First, these organic acids have very useful functions, such as being excellent fuels for the colon [24] and possibly for the heart [25]. Second, as shown in this study, by slowing the rate of absorption of ingested water, a larger volume of water could be retained for future heat dissipation.

## CONCLUSION

It appears that the water control system is poised for water retention, and rarely for water excretion because all urines collected during a 24-hour period had a $U_{osm}$ equal to or higher than their $P_{osm}$ (Fig. 1). Ingested water after an overnight fast could be retained if it contained poorly absorbed organic solutes such as fructose, or if it was consumed slowly. Although the rapid ingestion of a 20-mL/kg water load always caused a water diuresis, 7/15 subjects retained much of this water after its intake was stopped (Table 2). The signal perceived to initiate a water diuresis is closely related to an initial fall in the arterial $P_{Na}$. Based on these data, the emphasis on how water can be retained for 'future sweat' and heat dissipation should be included in the integrative physiology of the expected renal response to an ingested water load.

## ACKNOWLEDGMENT

This study was supported by a grant from the Canadian Institutes for Health Research (FRN 15485).

*Reprint requests to Mitchell L. Halperin, MD, FRCP(C), FRS, Emeritus Professor of Medicine, University of Toronto, St. Michael's Hospital Annex, Lab #1, Research Wing, 38 Shuter Street, Toronto, Ontario, M5B 1A6, Canada.*
*E-mail: mitchell.halperin@utoronto.ca*

## REFERENCES

1. Quinton P: Physiology of sweat secretion. *Kidney Int* 32:S102–S108, 1986
2. Sato K, Kang WH, Saga K, Sato KT: Biology of sweat glands and their disorders. *J Am Acad Dermatol* 20:537–563, 1989
3. Schmidt-Nielsen K: *Animal Physiology: Adaptation and Environment*, 5th ed., Cambridge, UK, Cambridge University Press, 1997
4. Wilson Y, Nag N, Davern P, *et al*: Visualization of functionally activated circuitry in the brain. *PNAS* 99:3252–3257, 2002
5. Sterns RH, Silver SM, Spital A: Hyponatremia, in *The Kidney: Physiology & Pathophysiology* (vol 4), edited by Seldin DW, Giebisch G, Philadelphia PA, Lippincott Williams & Wilkins, 2000, pp 1217–1238
6. Fitzsimons JT: Physiology and pathophysiology of thirst and sodium appetite, in *The Kidney: Physiology & Pathophysiology* (vol 4), edited by Seldin DW, Giebisch G, Philadelphia PA, Lippincott Williams & Wilkins, 2000, pp 1153–1174
7. Robertson GL: Vasopressin, in *The Kidney: Physiology & Pathophysiology* (vol 4), edited by Seldin DW, Giebisch G, Philadelphia PA, Lippincott Williams & Wilkins, 2000, pp 1133–1152
8. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
9. Jungas RL: *Lectures on Gastrointestinal Physiology and the Hormonal Regulation of Energy Metabolism*, Farmington, CT, University of Connecticut Health Center, 2002
10. Collis JM, Neaverson MA: Arterialized venous blood: A comparison of pH, $PCO_2$, $PO_2$ and oxygen saturation with that of arterial blood. *Br J Anaesth* 39:883–886, 1967
11. Forster HV, Dempsey JA, Thomson J, *et al*: Estimation of arterial $PO_2$, $PCO_2$, pH, and lactate from arterialized blood. *J Appl Physiol* 32:134–137, 1972
12. Mallie J-P, Bichet DG, Halperin ML: Effective water clearance and tonicity balance: The excretion of water revisited. *Clin Invest Med* 20:16–24, 1997
13. Cheema-Dhadli S, Halperin ML: Relative rates of appearance of nitrogen and sulphur: Implications for postprandial synthesis of proteins. *Can J Physiol Pharm* 71:120–127, 1993
14. Bichet DG, Arthus MF, Lonergan M: Platelet vasopressin receptors in patients with congenital nephrogenic diabetes insipidus. *Kidney Int* 39:693–699, 1991
15. Thrasher TN, Nistal-Herrera JF, Keil LC, Ramsay DJ: Satiety and inhibition of vasopressin secretion after drinking in dehydrated dogs. *Am J Physiol* 240:E394–E401, 1981
16. Salata RA, Verbalis JG, Robinson AG: Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. *J Clin Endocrinol Metab* 65:561–567, 1987
17. Choi-Kwon S, Baertschi AJ: Splanchnic osmosensation and vasopressin: mechanisms and neural pathways. *Am J Physiol* 261:E18–E25, 1991
18. Cherney DZI, Davids MR, Edoute Y, Halperin ML: The importance of thirst for the development of hyponatraemia when 3,4-methylnedioxymethamphetamine ("Ecstasy") is ingested. *Quart J Med* 95:475–483, 2002
19. Welt LG, Orloss J, Kydd DM, Oltman JE: An example of cellular hyperosmolarity. *J Clin Invest* 29:935–939, 1950
20. Schmidt-Nielsen K, Schmidt-Nielsen B: Water metabolism of desert mammals. *Physiol Rev* 32:135–161, 1952
21. Davids MR, Segal AS, Brunengraber H, Halperin ML: An unusual cause for ketoacidosis. *Q J Med* 97:365–376, 2004
22. Helliwell PA, Richardson M, Affleck J, Kellett GL: Stimulation of fructose transport across the intestinal brush-border membrane by PMA is mediated by GLUT2 and dynamically regulated by protein kinase C. *Biochem J* 350:149–154, 2000
23. Ladas SD, Raptis SA: Honey, fructose absorption, and the laxative effect. *Nutrition* 15:7–8, 1999
24. Cherbuy C, Andrieux C, Honvo-Houeto E, *et al*: Expression of mitochondrial HMGCoA synthase and glutaminase in the colonic mucosa is modulated by bacterial species. *Eur J Biochem* 271:87–95, 2004
25. Leclerc J, des Rosiers C, Montgomery JA, *et al*: Metabolism of R-β-hydroxypentanoate and of β-ketopentanoate in conscious dogs. *Am J Physiol* 268:E446–E452, 1995