Preventing hemodilution abolishes natriuresis of water immersion in humans

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Danish Aerospace Medical Centre of Research, Rigshospitalet 7805, DK-2200 Copenhagen; Department of Medical Physiology, University of Copenhagen, DK-2200 Copenhagen; and Department of Internal Medicine and Endocrinology, Herlev Hospital, DK-2730 Herlev, Denmark.

Johansen, Lars Bo, Bettina Pump, Jørgen Warberg, Niels Juel Christensen, and Peter Norsk. Preventing hemodilution abolishes natriuresis of water immersion in humans. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R879–R888, 1998.—The hypothesis was tested that hemodilution is one of the determinants of the water immersion (WI)-induced natriuresis. Eight males were subjected to 3 h of 1) WI to the midchest (Chest), 2) WI to the neck combined with thigh cuff-induced (80 mmHg) venous stasis (Neck + stasis), and 3) a seated time control (n = 6). Central venous pressure and left atrial diameter increased to the same extent during Chest and Neck + stasis (P < 0.05), whereas renal sodium excretion only increased during Chest from 77 ± 7 to 225 ± 13 µmol/min (P < 0.05). During Chest, plasma colloid osmotic pressure (COP) decreased from 27.7 ± 0.7 to 25.1 ± 0.7 mmHg (P < 0.05), and plasma volume (PV) increased from 3,263 ± 129 to 3,581 ± 159 ml (P < 0.05), whereas these variables remained unchanged during Neck + stasis. Plasma norepinephrine concentration decreased similarly during Chest and Neck + stasis by 45 ± 7 and 34 ± 4%, respectively (P < 0.05), whereas plasma renin activity decreased only during Chest (P < 0.05). In conclusion, during WI in humans 1) hemodilution (decrease in COP and increase in PV) is a pivotal stimulus for the natriuresis and 2) central blood volume expansion without hemodilution does not augment renal sodium output.

body fluids; blood proteins; kidney; pressoreceptors; hormones

IT IS GENERALLY ACCEPTED that cardiopulmonary low-pressure receptors are stimulated during thermoneutral (34.5°C) water immersion (WI) to the neck in humans and that this stimulation through hormonal and neuronal mediators constitutes the primary mechanism of the WI-induced natriuresis and diuresis (5).

During WI in humans, however, plasma volume (PV) is also increased (12, 13) and plasma colloid osmotic pressure (COP) decreased (12) as a consequence of a fluid shift from the interstitial to the intravascular space (14). Because a decrease in COP may induce a natriuresis during a volume stimulus in dogs (3), we have previously investigated the contribution of this variable to the augmented renal sodium excretion during graded WI in humans (12). During hip immersion, when no change in central venous pressure (CVP) occurs, renal sodium excretion is doubled and amounts to 25% of the natriuresis of immersion to the neck. Simultaneously, COP is decreased and PV increased to almost the same extent as during neck immersion. We have therefore concluded that a decrease in COP is a contributing stimulus for the natriuresis of WI in humans.

Because central blood volume expansion and hemodilution (defined as increase in PV and decrease in COP) usually occur simultaneously during WI, the relative contribution of each to the natriuresis of WI is difficult to estimate. In our previous study (12), we investigated the effects of hemodilution on renal function when CVP did not increase (hip immersion). It is conceivable, however, that because central blood volume expansion and hemodilution normally occur in concert, the effect of each is augmented and dependent on the effect of the other. In other words, without hemodilution during WI to the neck, the natriuresis might be attenuated to a larger extent than expected from our previous observations (12).

A WI study was conducted, therefore, during which cuffs around the thighs of the subjects were inflated. The use of venous thigh cuff inflation has been used previously as a means of investigating the effects of a reduced circulating blood volume on renal function in supine subjects (8). Our intention, however, was not to reduce total blood volume but instead to counteract the WI-induced hemodilution (decrease in COP and increase in PV) by the use of venous stasis and simultaneously maintain an unchanged central blood volume expansion by increasing the depth of immersion.

Thus the purpose was to test the hypothesis that hemodilution is one of the determinants of the WI-induced natriuresis in humans.

MATERIALS AND METHODS

Eight healthy males [age 23.2 ± 0.9 (SE) yr, weight 82 ± 4 kg, and height 1.85 ± 0.03 m] participated in the WI experiments. Six of the subjects [age 24.5 ± 1.2 yr, weight 76.7 ± 3.4 kg, and height 1.82 ± 0.01 m] participated in a time control experiment. All had a negative history of cardiovascular or kidney diseases and exhibited normal results of routine clinical examinations, including measurements of blood hemoglobin concentration (Hb, 8.0–11.0 mmol/l), arterial pressure (systolic pressure range: 110–132 mmHg; diastolic pressure range 76–94 mmHg), and electrocardiogram recordings. All subjects had their urine tested for protein, Hb, erythrocytes, and glucose with test-strip-based urinalysis. All subjects denied taking any medication at the time of the study. The experimental protocol was approved by the Ethics Committee of Copenhagen (KF 01–107/96), and, after careful oral and written explanation, written consent was obtained according to the Declaration of Helsinki.

Each subject underwent the two WI sessions in the upright seated posture with the sequence in a randomized, balanced order between the subjects separated by at least 3 wk. In
addition, 1 yr later six of the subjects performed a time
time control experiment. The sessions consisted of 1) WI to the
midchested for 3 h without thigh cuff inflation (Chest), 2) WI to
the neck for 3 h with simultaneous thigh cuff inflation of 80
mmHg (Neck + stasis), and 3) a seated time control study for
3 h in the empty water tank without thigh cuff inflation. The
different water levels were used to ensure similar increases in
central blood volume whether the cuffs were inflated or not,
because results of previous studies from our laboratory have
indicated that increasing the depth of WI can further aug-
ment central blood volume without inducing hemodilution
during venous thigh cuff inflation (12, 14). Each session was
preceded and followed by the subjects being seated outside
the water for 1.5 and 1 h, respectively. Before each experi-
ment, the subject was provided with food containing a fixed
content of sodium (135 mmol/24 h) for 4 days. Water intake
was ad libitum. No food or fluid intake was allowed during the
12-h before the experiment.

From 10 PM the evening before the experiment, the subject
was confined to the laboratory. H He was awakened at 7:30 AM.
A polyethylene central venous catheter (Cavafix, Braun) was
inserted through a cubital vein into the intrathoracic region
for measurements of CVP and collection of blood. Intratho-
racic placement of the central venous catheter was confirmed
by typical CVP waveforms and responses to respiratory
maneuvers. In the opposite arm, a peripheral venous catheter
(Venflon) was placed in a forearm vein for injection of Evans
blue. After emptying his bladder, the subject drank 400 ml of
tap water immediately afterwards. Measurements and proce-
sures were always performed in the following sequence: blood
sampling, 2 ml of blood were drawn to empty dead space.
After each sampling of blood, the catheter was flushed with
an amount of saline equal to that of the collected blood.
Finally, at an hourly interval the subject stood briefly on
a foot support to void outside the water and drank 200 ml of tap
water immediately afterwards. Measurements and proce-
sures were always performed in the following sequence: blood
sampling, Evans blue measurements, arterial blood pres-
sures, CVP, HR, left atrial diameter, and urine collection.

WI was performed by using an electrical hoist to lower a
chair suspended from the ceiling with the subject into an
insulated plastic tank filled with tap water. During the pre-
and postimmersion periods outside the water, the subject sat
in the chair above the water surface. Average water tempera-
ture varied over time between 34.60 ± 0.02 and 34.66 ±
0.05°C, room temperature between 25.4 ± 0.1 and 26.3 ±
0.2°C, and relative air humidity between 46 ± 3 and 58 ± 1%.

At the beginning of the experiment, a thigh cuff (20 × 88
cm) was placed around each thigh of the subject as close to the
genitofemoral region as possible. Immediately before start of
Neck + stasis, the cuffs were manually inflated to 80 mmHg
within 60 s. After inflation of the cuffs, the subject was
lowered into the water within 30 s. A loose strap around the
thighs prevented the subject from floating upwards. During
Chest and control, the cuffs remained around the thighs of the
subjects without being inflated. Subjects reported no feeling
of discomfort while wearing the inflated thigh cuffs.

Urine volume was measured at hourly intervals in a
graded cylinder. Urine and plasma osmolality (Uo) and
P values were measured on fresh samples by freezing-point
depression (Advanced Osmometer 3MO Plus). Concentra-
tions of Na+ and K+ in urine and plasma were measured on
fresh samples with an ion-selective electrode system (KNA-2,
Radiometer), and concentrations of creatinine in urine and
plasma were determined by a conventional Jaffe’ method.
Excretion rates of Na+, K+, and osmole (Uo, U, UoV, and
UoV); creatinine clearance (Cer); fractional excretions of
Na+ (FE Na+) and K+; and free water clearance (Ch2O) were
determined by conventional formulas.

PV was determined 15 min before and at 0.5 and 3 h after
the subject was lowered into the water tank using an Evans
blue dye dilution technique modified for repeated determina-
tions (9, 13).

Plasma concentrations of norepinephrine (NE) and epineph-
rine (Epi) were measured as described previously (11) with
a radioenzymatic assay (17), and plasma renin activity (PRA)
(11, 22) and atrial natriuretic peptide (ANP) (11, 25) were
measured with radioimmunoassays. Plasma aldosterone was
measured by radioimmunoassay with a commercially avail-
able kit (Coat-A-Count; Diagnostic Products, Los Angeles,
CA).

Hemocrit (Hct) was measured in quadruplicate on micro-
hematocrit tubes after centrifugation for 5 min at 15,000
rpm values were not corrected for trapped plasma and whole
body Hct. Hb in blood was measured in duplicate by a
spectrophotometric method as described previously (13).

Plasma protein concentration (Pprot) was measured in
duplicate in a refractometer (pocket refractometer, Belling-
ham & Stanley). Plasma density (PD) was determined in a
density meter (model DMA 46, Paar). COP was measured in
colloid osmometer (model 4400 Wescor).

CVPs were measured with the use of a disposable pressure
transducer, and HR was calculated from electrocardiogram
recordings as described previously (12).

Left atrial diameter was measured by M-mode echocardio-
ography (Aloka SSD 500). Standard images were obtained from
the parasternal long axis view during the end-expiratory
phase of respiration and recorded on video. Left atrial diam-
eter was then determined by an independent observer accord-
ing to Feigenbaum (7) from an average of three printouts
from the video recorder.

Systolic and diastolic arterial pressures (SAP and DAP)
were measured in a brachial artery by sphygmomanometry.
DAP was defined as the cuff pressure at the appearance of
the fourth sound of Korotkoff. The arm rested 20 cm above
heart level on all occasions before, during, and after Neck
+ stasis, Chest, and control to prevent the arms from being
immersed. Therefore, SAP, DAP, and mean arterial pressure
(MAP) are −15 mmHg lower than usually observed. Arterial
pulse pressure (PP) was calculated from SAP minus DAP, and
MAP was calculated from DAP + 1/3 PP.

Measurements of body weight were performed on the
naked subject before and after the experiment on an elec-
tronic scale.

Data are presented as means ± SE. ANOVA (Statgraphics
plus for Windows, version 3.0) for repeated measures with
the variable as the main variate and time and subjects as
factors was used to evaluate the effects on the variable at the
same experimental periods, an ANOVA for
repeated measures was used with the variable as the main variate and intervention (Neck + stasis, Chest, and control, respectively) and subjects as factors. Differences between mean values were evaluated by a post hoc multiple range test (Newman-Keuls). Paired t-tests were applied when appropriate. A significance level of 0.05 was chosen.

Additional methodological study. To investigate whether Chest and Neck + stasis caused an asymmetric change in the shape of the left atrium so that the similar increase in left atrial diameter during Chest and Neck + stasis, respectively, might not have reflected a similar increase in left atrial volume, an additional methodological study in another group of four subjects (age 34.2 ± 4.2 yr, weight 76.5 ± 3.0 kg, height 1.84 ± 0.03 m) was performed. Left atrial diameter was measured in two different planes after 15 min of Chest and Neck + stasis, respectively, and after 15-min periods of subjects being seated before and after immersion. Left atrial diameter was measured once during each intervention in two subjects being seated before and after immersion. Left atrial volume, an additional methodological study in another group might not have reflected a similar increase in left atrial diameter where

RESULTS

Cardiovascular variables. Left atrial diameter (Fig. 1) increased during Neck + stasis and Chest from 28.4 ± 0.4 and 28.2 ± 0.4 mm, respectively, to maxima of 33.0 ± 1.0 mm during Neck + stasis and 33.0 ± 0.5 during Chest (P < 0.05). During control, values varied insignificantly between 29.1 ± 0.9 and 30.0 ± 1.5 mm.

CVP (Fig. 1) increased from −3.4 ± 0.9 to a maximum of 4.7 ± 1.2 mmHg during Neck + stasis (P < 0.05) and from −2.6 ± 0.5 mmHg to 3.9 ± 0.5 mmHg during Chest (P < 0.05). There was no significant difference between values of the two WI sessions. During control, CVP values varied between −4.5 ± 0.7 and −2.8 ± 0.8 mmHg (NS).

PP (Fig. 1) increased during Chest from 36 ± 2 mmHg to a peak of 45 ± 1 mmHg during the second hour of immersion (P < 0.05). No significant changes occurred during Neck + stasis and control. The means of PP over the whole period of immersion of 41.9 ± 0.6 and 41.1 ± 2.3 mmHg during Chest and Neck + stasis, respectively, did not differ, but were higher than the corresponding mean value of 34.5 ± 2.3 mmHg during control (P < 0.05).

Changes in SAP, DAP, MAP, and HR are presented in Table 1. HR decreased during both WI sessions (P < 0.05) but not during control. The mean HR value over the whole period of immersion in Chest of 58 ± 2 beats/min was significantly lower than the computed value of 61 ± 2 beats/min during Neck + stasis (paired t-test, P < 0.05).

Renal responses. Despite similar increases in left atrial diameter and CVP during the two WI sessions, Neck + stasis abolished the natriuretic response (Fig. 2), because U_{Na}V varied insignificantly between 86 ± 18 and 110 ± 17 µmol/min. During Chest, however, U_{Na}V increased from 77 ± 7 µmol/min to a peak of 225 ± 13 during the third hour of immersion (P < 0.05).

Values during control varied insignificantly between 65 ± 9 and 78 ± 14 µmol/min. Cumulated U_{Na}V during Chest amounted to 31.5 ± 1.4 mmol/3 h, which was higher than the values of 18.1 ± 2.7 and 12.1 ± 1.6 mmol/3 h during Neck + stasis and control, respectively (P < 0.05). Cumulated U_{Na}V of Neck + stasis and control did not differ. The temporal profiles of FENa and U_{Na}V (Table 2) followed that of U_{Na}V. U_{Na}V (Table 2) decreased during Chest and Neck + stasis (P < 0.05) and decreased in the recovery period during control (P < 0.05).

During Chest, V (Fig. 2) increased from 1.1 ± 0.2 ml/min to a peak of 4.8 ± 0.3 ml/min during the second hour. During Neck + stasis and control, respectively, V varied insignificantly between 1.1 ± 0.2 and 2.8 ± 0.8 ml/min and 1.3 ± 0.2 and 3.0 ± 0.3 ml/min. The cumulated urine output of 748 ± 60 ml/3 h during Chest was higher (P < 0.05) compared with the values of 505 ± 97 and 400 ± 65 ml/3 h during Neck + stasis.

Fig. 1. Left atrial diameter (A), central venous pressure (CVP; B), and arterial pulse pressure (PP; C) before, during, and after 3 h of water immersion to the neck with thigh cuff inflation of 80 mmHg (Neck + stasis; ○), water immersion to the midchest without thigh cuff inflation (Chest; ●), and a seated time control (□). Values are means ± SE of n = 8 (Chest, Neck + stasis) and n = 6 (control) except for CVP where n = 7 during Neck + stasis and Chest, left atrial diameter where n = 5 during control. *Significant difference compared with average pre-water immersion value.

Values during control varied insignificantly between 65 ± 9 and 78 ± 14 µmol/min. Cumulated U_{Na}V during Chest amounted to 31.5 ± 1.4 mmol/3 h, which was higher than the values of 18.1 ± 2.7 and 12.1 ± 1.6 mmol/3 h during Neck + stasis and control, respectively (P < 0.05). Cumulated U_{Na}V of Neck + stasis and control did not differ. The temporal profiles of FENa and U_{Na}V (Table 2) followed that of U_{Na}V. U_{Na}V (Table 2) decreased during Chest and Neck + stasis (P < 0.05) and decreased in the recovery period during control (P < 0.05).

During Chest, V (Fig. 2) increased from 1.1 ± 0.2 ml/min to a peak of 4.8 ± 0.3 ml/min during the second hour. During Neck + stasis and control, respectively, V varied insignificantly between 1.1 ± 0.2 and 2.8 ± 0.8 ml/min and 1.3 ± 0.2 and 3.0 ± 0.3 ml/min. The cumulated urine output of 748 ± 60 ml/3 h during Chest was higher (P < 0.05) compared with the values of 505 ± 97 and 400 ± 65 ml/3 h during Neck + stasis.
Table 1. Effect on cardiovascular variables of water immersion to the neck with venous stasis of the legs with thigh cuffs (80 mmHg) and immersion to the midcalf without thigh cuff inflation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Experiment, h</th>
<th>During Experiment, h</th>
<th>After Experiment, h</th>
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<tr>
<td>SAP, mmHg</td>
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<tr>
<td>Control</td>
<td>103 ± 3</td>
<td>108 ± 1</td>
<td>105 ± 2</td>
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<tr>
<td>Neck + stasis</td>
<td>102 ± 3</td>
<td>104 ± 2</td>
<td>105 ± 2</td>
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<tr>
<td>Chest</td>
<td>107 ± 4</td>
<td>108 ± 4</td>
<td>109 ± 4</td>
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<tr>
<td>DAP, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>67 ± 4</td>
<td>74 ± 4</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>61 ± 1</td>
<td>68 ± 1</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Chest</td>
<td>71 ± 3</td>
<td>73 ± 4</td>
<td>73 ± 3</td>
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<tr>
<td>MAP, mmHg</td>
<td></td>
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<tr>
<td>Control</td>
<td>79 ± 3</td>
<td>85 ± 3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>75 ± 1</td>
<td>80 ± 2</td>
<td>82 ± 3</td>
</tr>
<tr>
<td>Chest</td>
<td>83 ± 3</td>
<td>85 ± 4</td>
<td>85 ± 3</td>
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<tr>
<td>HR, beats/min</td>
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<tr>
<td>Control</td>
<td>62 ± 3</td>
<td>60 ± 2</td>
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<td>Neck + stasis</td>
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<td>66 ± 2</td>
</tr>
<tr>
<td>Chest</td>
<td>64 ± 2</td>
<td>65 ± 2</td>
<td>67 ± 3</td>
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</table>

Values are means ± SE of 8 subjects except for control where n = 6. Control, seated time control without thigh cuff inflation; Neck + stasis, water immersion to the neck with thigh cuff inflation of 80 mmHg; Chest, water immersion to the midcalf without thigh cuff inflation; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate. *Significant difference compared with mean of pre-experiment values (P < 0.05).

and control, respectively. Values during Neck + stasis and control did not differ.

$C_{\text{H}_2\text{O}}$ (Fig. 2) increased from $-0.9 \pm 0.2$ ml/min to between $0.4 \pm 0.6$ and $1.4 \pm 0.4$ ml/min during Chest (P < 0.05) and from $-1.2 \pm 0.3$ ml/min to between $0.1 \pm 0.6$ and $0.5 \pm 0.7$ ml/min during Neck + stasis (P < 0.05). During control, values varied insignificantly between $-0.6 \pm 1.0$ and $0.3 \pm 1.4$ ml/min. There was no significant differences between $C_{\text{H}_2\text{O}}$ values of the three experimental conditions.

$U_{\text{Na}}$ (Table 2) increased during Chest (P < 0.05). Furthermore, a transient increase was observed during the first hour of Neck + stasis (P < 0.05). No significant changes were observed in $C_{\text{O}2}$ (Table 2).

$U_{\text{Na}}$ during the 24 h before the experiments varied insignificantly between 126 ± 15 and 136 ± 8 mmol/24 h. During Chest and Neck + stasis body weight decreased by $0.51 \pm 0.08$ kg (P < 0.05) and 0.25 ± 0.10 kg (P < 0.05), respectively. During control, body weight was unchanged.

Hemodilution. During Chest, PV (Fig. 3) increased from 3,263 ± 129 to 3,581 ± 159 ml within the initial 30 min (P < 0.05) and remained at this level. During Neck + stasis, PV varied insignificantly between 3,218 ± 151 and 3,293 ± 177 ml and during control varied between 3,205 ± 85 and 3,264 ± 72 ml.

COP (Fig. 3) decreased from 27.7 ± 0.7 mmHg to between 24.7 ± 0.6 and 25.7 ± 0.6 mmHg during Chest. Except for a single nadir point of 26.8 ± 0.7 mmHg 15 min into the Neck + stasis period (P < 0.05), COP did not change compared with the preimmersion value of 27.6 ± 0.7 mmHg. One hour after Neck + stasis, COP reached a peak of 29.0 ± 0.7 mmHg (P < 0.05). During control, COP varied insignificantly between 28.6 ± 0.7 and 29.1 ± 0.6 mmHg. The temporal profiles of $P_{\text{prot}}$ and PD (Table 3) almost followed the same trend as that of COP. $P_{\text{prot}}$, however, exhibited a statistically significant decrease during Neck + stasis compared with the preimmersion values.

$P_{\text{osmol}}$ (Table 1) decreased during control (P < 0.05) but not during Chest and Neck + stasis.

During Chest, Hct and Hb (Table 3) clearly decreased (P < 0.05), whereas only a slight temporary decrease occurred during the initial 15 min of Neck + stasis (P < 0.05).

Endocrine responses. PRA (Fig. 4) exhibited a significant decrease during Chest from 2.6 ± 0.3 to between 1.4 ± 0.3 and 1.0 ± 0.2 ng·ml$^{-1}$·h$^{-1}$. During Neck + stasis, no changes occurred. During control, PRA increased from 2.0 ± 0.2 to between 2.5 ± 0.2 and 2.9 ± 0.3 ng·ml$^{-1}$·h$^{-1}$ (P < 0.05). Plasma concentration of aldosterone (Fig. 4) decreased to almost the same extent during Chest and Neck + stasis. The mean value of aldosterone during Chest over the whole period of immersion of 64 ± 7 pg/ml was significantly lower than the value of 98 ± 14 pg/ml during Neck + stasis (paired t-test, P < 0.05).

Plasma concentration of NE (Fig. 4) decreased similarly during Chest and Neck + stasis by between 36 ± 7 and 45 ± 7% and between 31 ± 4 and 34 ± 4%, respectively (P < 0.05). During control, no changes occurred. Due to the fact that there was an inexplicable difference of ~100% between the absolute values of NE during control and those before Neck + stasis and Chest, we have chosen to present the relative changes in Fig. 4 and the absolute NE values in Table 4. There was no statistically significant difference comparing the mean NE value over the whole period of immersion of 51 ± 11 pg/ml during Chest with the mean value of 72 ± 14 pg/ml during Neck + stasis. Epi values are presented in Table 3. Except for an initial decrease during Neck + stasis (P < 0.05), no changes occurred.

Plasma ANP is presented in Table 4. In two subjects, for inexplicable reasons, during Chest and Neck +
stasis, preimmersion values of ANP were higher by several factors compared with the other preimmersion values. Therefore, values from these two subjects were excluded from further analysis and are not presented here. Plasma ANP did not change in a statistically significant manner during any of the WI sessions. However, during Chest and Neck + stasis, five of seven subjects exhibited increases.

Additional methodological study. Compared with the mean of the pre- and postimmersion values, left atrial diameter measured in M-mode from the parasternal long-axis view increased from 30.3 ± 3.0 to 36.3 ± 2.8 mm during Neck + stasis and from 30.0 ± 3.2 to 36.3 ± 2.5 mm during Chest (n = 4, P < 0.05). Thus comparing the echocardiographic measurements of left atrial diameter during Chest and Neck + stasis, respectively, reveals very similar increases in left atrial diameter in the two planes and thus also in left atrial volume.

**DISCUSSION**

The results indicate that when hemodilution (decrease in COP and increase in PV) is prevented by thigh cuff-inflated venous stasis, the natriuretic response to WI in humans is abolished. Thus, during WI in humans, hemodilution is a pivotal stimulus for the natriuresis, and central blood volume expansion without hemodilution does not augment renal sodium output. It is noteworthy that suppression of release of renin and aldosterone was attenuated when hemodilution was prevented. We suggest that the natriuresis of WI in humans is initiated as a result of the combined effects of hemodilution and central blood volume expansion with associated neuronal and endocrine changes.

Hemodilution and renal responses. It has previously been indicated by us that hemodilution, including a decrease in COP and an increase in PV, accounts for up to 25% of the natriuresis of WI in humans (12). In light of our previous results, the present observations are surprising, because we had expected that preventing hemodilution without affecting central blood volume expansion would attenuate and not abolish the WI-induced natriuresis.

The effects of hemodilution per se on the renal responses might theoretically be caused by several mechanisms: 1) a direct effect of a decrease in COP on peritubular capillary reabsorption of fluid and solutes, 2) lowering of the hematocrit, 3) increased renal blood flow, and 4) increased tubular delivery of solutes with effects on the macula densa.

Regarding 1, the decrease in COP in the efferent glomerular arteriole could result in a diminished net peritubular capillary reabsorption of fluid (16, 20). This in turn would increase peritubular interstitial pressure and thereby attenuate the absolute proximal reabsorption rate of solutes from the tubules. As a result, delivery of fluid and solutes to the distal segments of the nephron would be increased.

Regarding 2, studies in dogs have demonstrated that an isoncotic lowering of hematocrit, without change in PV, depresses proximal tubular reabsorption of sodium (15). By simultaneously expanding PV, a natriuresis was induced. Thus intravascular volume expansion seems necessary for a natriuresis to occur when Hct is lowered. These observations agree with the results of our study, where the natriuresis was abolished when Hct and PV remained unchanged.

Regarding 3, the increased PV might per se induce an increase in renal plasma flow (2). This could induce an increase in medullary interstitial hydrostatic pressure and/or a decrease in medullary interstitial tonicity through a "wash-out" of solutes induced by the increased flow. These effects would reduce passive fluid and sodium reabsorption from the loop of Henle (16, 20, 24).
Table 2. Effect on kidney variables of water immersion to the neck with venous stasis of the legs with thigh cuffs (80 mmHg) and immersion to the midchest without thigh cuff inflation

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<tbody>
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<td>FE_{Na}, %</td>
<td>0.46±0.08</td>
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<td>0.55±0.09</td>
<td>0.47±0.06</td>
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<tr>
<td>FE_{K}, %</td>
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<td>17.3</td>
<td>17.2</td>
<td>15.3</td>
<td>16.2</td>
<td>11.1</td>
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<tr>
<td>C_{cre}, ml/min</td>
<td>108±9</td>
<td>91±7</td>
<td>98±5</td>
<td>106±7</td>
<td>100±10</td>
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<td></td>
</tr>
<tr>
<td>U_{eK}, µmol/min</td>
<td>56.9</td>
<td>65.9</td>
<td>68.9</td>
<td>65.9</td>
<td>64±14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U_{oK}, µmol/min</td>
<td>44.8</td>
<td>65.11±*</td>
<td>62.12</td>
<td>60.10</td>
<td>50±8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U_{enaK}, mosmol/kg</td>
<td>40.6</td>
<td>62.12±*</td>
<td>83±12</td>
<td>7511*</td>
<td>62±14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 subjects except for control where n = 6. FE_{Na}, fractional sodium excretion; FE_{K}, fractional potassium excretion; C_{cre}, creatinine clearance; U_{eK}, potassium excretion; U_{oK}, osmolar excretion; U_{enaK}, urine osmolality. *Significant difference compared with pre-experiment values (P < 0.05).

Regarding 4, the increased distal tubular delivery of solutes resulting from an increase in glomerular filtration pressure caused by the volume load and the previously mentioned mechanisms might induce a decrease in renin release and thus generation of angiotensin II (18) through the macula densa mechanism. A decreased level of angiotensin II might increase sodium excretion directly through modulation of tubular transport mechanisms and/or through a direct effect on renal medullary blood flow (18, 20, 26). In addition, the decreased rate of generation of angiotensin II could account for the attenuated aldosterone secretion (18). The resulting natriuresis might thus be a result of the effect of changes in physical factors per se and the effect of these on release of humoral mediators of sodium excretion.

Cardiovascular variables. MAP was unchanged during all of the experimental interventions. Therefore, it is unlikely that a decrease in renal perfusion pressure could have contributed to the lack of natriuresis during Neck + stasis.

Because the natriuretic response to WI was abolished during Neck + stasis despite similar increases in CVP and left atrial diameter, stimulation of cardiopulmonary low-pressure receptors and the resulting decrease in renal sympathetic nervous activity do not seem to play a major role for induction of a natriuresis during WI in humans. This observation is in agreement with results of a study by Myers et al. (21), who observed a natriuretic response in human cardiac transplant recipients undergoing WI similar to that of a group of normal controls. In a WI study by Rabelink et al. (23), the natriuresis of a group of recent kidney transplant recipients was similar to that of a control group. These observations indicate that interruption of either the afferent (cardiac denervation) or efferent (kidney denervation) limb of the cardio-renal nervous connection has no or only little effect on the renal responses to immersion. Finally, in a study in intact conscious monkeys, Cornish and Gilmore (1) observed that increases in left atrial pressure induced by the application of left atrial snares produced no significant renal responses.

Because stimulation of cardiopulmonary low-pressure receptors per se does not seem to constitute a major determinant of the natriuresis induced by volume stimuli in humans, central blood volume expansion might instead through neuroendocrine reflexes function as a modulator of the natriuretic response to an acute volume stimulus. This postulate is substantiated by previous results from our laboratory: by immersing subjects to the hips (12), hemodilution almost similar to that of neck immersion occurs without central blood volume expansion. This results in a natriuresis amounting to some 25% of the one of neck immersion. In a saline infusion study (11), also from our laboratory, central blood volume expansion was prevented by the use of lower body negative pressure without affecting the degree of hemodilution. This induced an attenuated natriuresis amounting to some 50% of the one of saline infusion with central blood volume expansion. Thus stimulation of cardiopulmonary low-pressure volume receptors might potentiate the natriuretic response to PV expansion and a de-
A possible mechanism for this is a decrease in renal sympathetic nervous activity (4), which in combination with natriuresis-induced increases in renal blood flow, could account for the increase in COP. A possible mechanism for this a decrease in renal sympathetic nervous activity (4).

Table 3. Effect on blood variables of water immersion to the neck with venous stasis of the legs with thigh cuffs (80 mmHg) and immersion to the midchest without thigh cuff inflation

<table>
<thead>
<tr>
<th>Before Experiment, h</th>
<th>During Experiment, h</th>
<th>After Experiment, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.5</td>
<td>-0.25</td>
</tr>
<tr>
<td>Hct, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>46.4 ± 0.6</td>
<td>46.6 ± 0.5</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>45.9 ± 0.4</td>
<td>46.1 ± 0.5</td>
</tr>
<tr>
<td>Chest</td>
<td>45.7 ± 0.6</td>
<td>45.7 ± 0.5</td>
</tr>
<tr>
<td>Hb, mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.9 ± 0.2</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>9.9 ± 0.1</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>Chest</td>
<td>9.9 ± 0.2</td>
<td>9.9 ± 0.1</td>
</tr>
<tr>
<td>P_prot, g/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>73.0 ± 1.9</td>
<td>72.8 ± 1.8</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>72.0 ± 1.4</td>
<td>72.0 ± 1.4</td>
</tr>
<tr>
<td>Chest</td>
<td>71.9 ± 1.2</td>
<td>71.3 ± 1.2</td>
</tr>
<tr>
<td>PD, g/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,025.9 ± 0.4</td>
<td>1,028.5 ± 0.4</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>1,025.6 ± 0.3</td>
<td>1,026.6 ± 0.3</td>
</tr>
<tr>
<td>Chest</td>
<td>1,025.4 ± 0.2</td>
<td>1,025.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 subjects except for control where n = 6. Hct, hematocrit; Hb, hemoglobin concentration; P_prot, plasma protein concentration; PD, plasma density.

* Significant difference compared with mean of pre-experiment values (P < 0.05).
Hemodilution and Renal Responses

Fig. 4. Plasma renin activity (PRA; A), plasma aldosterone concentration (Aldo; B), and relative changes in plasma norepinephrine concentration (ΔNE; C) before, during, and after 3 h of water immersion to the neck with thigh cuff inflation of 80 mmHg (Neck + stasis; ○), water immersion to the midchest without thigh cuff inflation (Chest; ●), and a seated time control (■). Values are means ± SE of n = 8 (Chest, Neck + stasis) and n = 6 (control).

Table 4. Effect on plasma osmolality and hormones of water immersion to the neck with venous stasis of the legs with thigh cuffs (80 mmHg) and immersion to the midchest without thigh cuff inflation

<table>
<thead>
<tr>
<th></th>
<th>Before Experiment, h</th>
<th>During Experiment, h</th>
<th>After Experiment, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.5</td>
<td>-0.25</td>
<td>0</td>
</tr>
<tr>
<td>P-osmol, mosmol/kgH2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>284 ± 1</td>
<td>284 ± 2</td>
<td>283 ± 1</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>285 ± 1</td>
<td>281 ± 1</td>
<td>281 ± 1</td>
</tr>
<tr>
<td>Chest</td>
<td>283 ± 1</td>
<td>283 ± 1</td>
<td>282 ± 1</td>
</tr>
<tr>
<td>P-norepinephrine, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>220 ± 19</td>
<td>223 ± 17</td>
<td>228 ± 19</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>97 ± 18</td>
<td>108 ± 20</td>
<td>69 ± 13*</td>
</tr>
<tr>
<td>Chest</td>
<td>100 ± 24</td>
<td>94 ± 22</td>
<td>54 ± 12*</td>
</tr>
<tr>
<td>P-epinephrine, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40 ± 7</td>
<td>48 ± 7</td>
<td>42 ± 7</td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>33 ± 12</td>
<td>35 ± 8</td>
<td>21 ± 4*</td>
</tr>
<tr>
<td>Chest</td>
<td>25 ± 5</td>
<td>25 ± 5</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>P-atrial natriuretic peptide, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck + stasis</td>
<td>34 ± 2</td>
<td>28 ± 2</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Chest</td>
<td>32 ± 4</td>
<td>32 ± 5</td>
<td>36 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 subjects except for control where n = 6. P-osmol, plasma osmolality; P-norepinephrine, plasma norepinephrine concentration; P-epinephrine, plasma epinephrine concentration; P-atrial natriuretic peptide (n = 7 for Chest and Neck + stasis). *Significant difference compared with mean of pre-experiment values (P < 0.05).
occurred this could totally account for the lack of natriuresis during Neck + stasis.

Whether changes in left atrial diameter reflect changes in left atrial volume during WI could theoretically be questioned, because it could be postulated that the increase in water level during Neck + stasis from midchest to neck could have induced a change in atrial configuration so that changes in left atrial diameter in one plane did not accurately reflect changes in atrial volume. However, our demonstration that left atrial diameter increases in a similar fashion in two planes during Chest and Neck + stasis renders this postulate unlikely.

Could increased physical and mental stress due to thigh cuff inflation have abolished the natriuresis during Neck + stasis? That the level of stress was increased does not seem likely for the following reasons: 1) plasma Epi did not increase during either of the experimental conditions, 2) HR decreased during both WI sessions in contrast to unchanged values during control, and 3) subjects did not report any discomfort when wearing the inflated thigh cuffs during Neck + stasis. Considering these indirect indicators, increased physical and mental stress during Neck + stasis most likely does not constitute a mechanism of the attenuated renal responses.

It might be argued that the lack of natriuresis during Neck + stasis is a result of a reduced blood flow to the kidneys because of translocation of blood to the thorax and legs, respectively. In this manner, the intra-abdominal blood volume could have been decreased compared with that of Chest. To address this question, measurements of renal blood flow should be performed in future studies combining WI with thigh cuff inflation.

Conclusion. It is concluded that during WI in humans 1) hemodilution (decrease in COP and increase in PV) is a pivotal stimulus for the natriuresis and 2) central blood volume expansion without hemodilution does not augment renal sodium output. It is noteworthy that suppression of release of renin and aldosterone was attenuated when hemodilution was prevented. We suggest that the natriuresis of WI in humans is initiated as a result of the combined effects of hemodilution and central blood volume expansion with associated neuro- and endocrine changes.

Perspectives

This study on the contribution of hemodilution to the WI-induced natriuresis in humans has produced surprising results, because we had only expected the natriuretic response to be attenuated and not abolished. Thus new questions can be raised regarding the importance of cardiopulmonary low-pressure receptors for the diuretic and natriuretic responses to volume stimuli in humans. On the basis of the present observations, these low-pressure reflexes seem to act as modulators rather than determinants of the natriuretic responses. To further elucidate the relative contribution of central blood volume expansion and hemodilution to the diuresis and natriuresis of volume stimuli, respectively, we suggest that more detailed studies be performed on renal hemodynamics combined with clamping of either central blood volume or hemodilution.

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