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Elevated Cerebrospinal Fluid Sodium in Hypertensive Human Subjects with a Family History of Alzheimer's Disease

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Abstract

High salt (sodium) intake leads to the development of hypertension despite the fact that plasma sodium concentration ([Na^+]) is usually normal in hypertensive human patients. Increased CSF sodium contributes to elevated sympathetic activity and high blood pressure (BP) in rodent models of hypertension. However, whether there is an increased accumulation of sodium in the cerebrospinal fluid (CSF) of humans with chronic hypertension is not well defined. Here, we investigated CSF [Na^+] in samples from hypertensive and normotensive human subjects with family history of Alzheimer's disease collected in a clinical trial, as spinal tap is not a routine clinical procedure for hypertensive patients. The [Na^+] and osmolality in plasma and CSF were measured using flame photometry. Daytime ambulatory BP was monitored while individuals were awake. Participants were deidentified and data were analyzed in conjunction with a retrospective analysis of patient history and diagnosis. We found that CSF [Na^+] was significantly higher in participants with high BP compared with normotensive participants; there was no difference in plasma [Na^+], or plasma and CSF osmolality between groups. Subsequent multiple linear regression analyses controlling for age, sex, race, and body mass index revealed a significant positive correlation between CSF [Na^+] and BP, but showed no correlation between plasma [Na^+] and BP. In sum, CSF [Na^+] was higher in chronic hypertensive individuals and may play a key role in the pathogenesis of human hypertension. Collectively, our findings provide evidence for the clinical significance of CSF [Na^+] in chronic hypertension in humans.

Key words: Hypertension, Sodium, Cerebrospinal fluid, Alzheimer's disease, Osmolality.

Introduction

Hypertension, an important risk factor for cardiovascular disease (CVD), is the leading cause of death and one of the most significant public health burdens worldwide. The American Heart Association (AHA) estimates that hypertension affects 34% of US adults, or approximately 100 million individuals (7). Hypertension is independently associated with increased age, body mass index (BMI), race, and a high-salt diet (7, 41). In particular, excess dietary sodium has been linked to elevations in blood pressure (BP) (18). Previous studies have shown that high levels of tissue sodium are associated with a variety of conditions ranging from elevated BP to brain tumors, stroke, and bipolar disorder (8, 9, 28, 36, 42, 47, 51, 55). Recent research has highlighted the importance of cerebral spinal fluid (CSF) sodium in regulating sympathetic activity in experimental rat (26, 37, 39) and mouse models (40, 53). However, data on CSF sodium concentration ($[Na^+]$) in hypertensive humans is limited; thus, its clinical significance is unclear.

We know that, over time, hypertension has harmful effects on the brain. For example, hypertension has been linked to an increased risk of Alzheimer's disease (AD) later in life, and it has been shown that controlling BP protects against AD (6, 17, 52). Studies have shown that the renin angiotensin system (RAS), which regulates BP, plays a role in promoting AD, possibly through accumulation of the AD biomarkers, amyloid beta and tau protein, in CSF (3, 10). The use of BP medications that target the RAS can slow the conversion of mild cognitive impairment (MCI) to AD (5, 19, 57). Thus, studying the effects of sodium on hypertension in the middle-aged, at-risk population (i.e., those with a parental history of AD) is of particular clinical importance to our understanding, not only of AD, but also of hypertension itself and its association with sodium and eventual effects

on the brain. Here, we examined the relationship between sodium and BP by examining CSF and plasma [Na^+] in middle-aged, normotensive (NTN) and hypertensive (HTN) humans at risk for AD.

Materials and Methods

Human subjects

All clinical and biospecimen data in the present study were obtained at Emory University in Atlanta. Spinal fluid was collected from 43 middle-aged (45–65 years old) human subjects at risk for AD. Participants were recruited from the ASCEND (Association between Cardiovascular Risk and Preclinical Alzheimer's Disease Pathology, ClinicalTrials.gov NCT02471833) study, an ongoing observational study among individuals with a parental history of AD. Participants have parents with autopsy-confirmed or probable AD, as defined by NINDS-ADRDA criteria (38). AD diagnosis was verified using the validated Dementia Questionnaire and medical records, where available (31). Participants were free of cognitive impairment or major CVD, as determined by inclusion and exclusion criteria below. Relevant details of subject recruitment and collection of blood and CSF are also detailed below. Clinical data, including patient histories and diagnoses, were used in this retrospective analysis.

Informed consent for participation in the study was obtained from all subjects in accordance with the human subjects Institutional Review Boards of all participating centers. The study was performed in accordance with the World Medical Association Declaration of Helsinki, International Conference on Harmonization Note for Guidance on Good Clinical Practice (ICH Topic E6, 1996). Study protocols were also approved by The

Research Integrity Offices at the University of Nevada, Reno, and Emory University, and the Institutional Review Board.

Inclusion criteria (i): A biological parent with or who had AD; (ii) ≥45 years of age; (iii) willingness to fast for 8 hours (iv); and willingness to undergo all procedures, including blood draw, lumbar puncture (LP), and magnetic resonance imaging (MRI).

Exclusion criteria (i): Contraindication for LP or MRI; (ii) significant neurological disease, (iii) heart failure, (iv) type I or II diabetes, (v) history of significant head trauma, (vi) major depression within the past 2 years, (vii) HIV/AIDS, (viii) history of alcohol or substance abuse, (ix) significant systemic illness or unstable medical condition that could affect cognition or cause difficulty complying with or completing the protocol, (x) diagnosis of AD, (xi), subjective cognitive impairment (MCI) or residence in a skilled nursing facility, (xii) use of another investigational medication or AD medication, or (xiii) unwillingness to fast.

Ambulatory BP monitoring

Ambulatory BP monitoring (ABPM) has many advantages in relation to clinic BP measurement since it is conducted while individuals perform their normal daily activities and because it provides estimates of BP over the entire monitoring period, including separately during nighttime and daytime (56). BP was measured every 20 minutes while participants were awake. Ambulatory BP endpoints included mean daytime ambulatory BP (systolic and diastolic). Ambulatory measures provide superior predictive value for cardiovascular events compared with BP measurements in the clinic and have been used in dementia research(50).

Participants were separated into normotension or hypertension groups according to their diagnosis and awake ABPM, as summarized in Table 1. They were further sub-categorized as Normal BP (systolic BP [sBP]<120 mmHg and diastolic BP [dBP]<80 mmHg), Elevated BP (120≤sBP≤129 mmHg and dBP<80 mmHg), HTN-Stage 1 (130≤sBP≤134 mmHg or 80≤dBP≤84 mmHg), or HTN-Stage 2 (sBP≥135 mmHg or dBP≥85 mmHg) according to the 2017 High Blood Pressure Clinical Practice Guideline (HBPCPG) of the American College of Cardiology and American Heart Association (56). Participant data were de-identified, and measurements of plasma and CSF [Na^+] were performed in a blinded fashion.

CSF collection

CSF samples were acquired by LP after an 8-hour overnight fast according to the guidelines put forth in the “Biospecimens Best Practice Guidelines for the ADCs”, published by the National Alzheimer's Coordinating Center (NACC). Participants were placed in a sitting position and asked to maximally flex their knees, hips, back, and neck. The skin over L4-L5 was prepped and draped in a sterile manner; 1% lidocaine was used as a local anesthetic, followed by insertion of a spinal needle with introducer into the L4-L5 interspace using sterile technique. Approximately 22 ml of CSF was collected into sterile polypropylene collection tubes using a 24-gauge Sprotte needle and a gentle extraction technique. Samples were centrifuged at 2500 rpm for 10 minutes, then aliquoted into 500 µL polypropylene cryovials and stored at -80°C for assay after all participants had been sampled.

Blood collection

Blood was drawn from participants after an 8-hour overnight fast, and plasma was collected for [Na^+] measurements. Blood and CSF samples were obtained during the same study visit.

Flame photometer measurement of [Na^+] in plasma and CSF

The [Na^+] in plasma and CSF samples obtained from study participants was determined using a flame photometer (Model ISE IL 943; GMI Inc., Ramsey, MN, USA). The method was calibrated and performed according to the manufacturer's specifications using quality controls.

Plasma and CSF osmolality measurements

Plasma and CSF osmolality was measured with an Advanced Micro Osmometer (Model 3300; Advanced Instruments, Norwood, MA, USA), which was maintained in a dry room with a steady room temperature, as recommended by the manufacturer. Calibration was carried out as per instructions using 50 and 850 mOsm/kg H_2O standards. Twenty microliters of human plasma and CSF samples were used for these measurements. A Clinitrol 290 mOsm/kg H_2O reference solution (Ref. 3MA029; Advanced Instruments) was used to evaluate and ensure the performance of the instrument. Measurements were only accepted if the control provided a result of 290±2 mOsm/kg H_2O, as per the calibration instructions provided.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). Data were analyzed using Student's *t*-test or one-way analysis of variance (ANOVA) with Bonferroni post hoc tests to compare replicate means, as appropriate, using Prism8 (GraphPad Software,

San Diego, CA, USA). Multiple linear regressions were performed using SAS 9.4. All statistical tests were two-tailed, and differences were considered statistically significant at $P<0.05$.

Results

Subject characteristics

Participants were divided into two groups based on daytime ABPM, as outlined in Table 1. Participants in the normotension group (n=25) were defined as those with sBP < 130 mmHg and dBP < 80 mmHg with no previous history of hypertension diagnosis, whereas those in the hypertension group (n=18) had sBP ≥ 130 mmHg or a dBP ≥ 80 mmHg, and a history of hypertension diagnosis. Racial and gender profiles of both groups were similar; however, BMI was significantly higher in the high BP group ($P<0.05$). There were more females than males in both groups (~75% in the Normal BP group and 62% in the High BP group), but there was no significant difference in systolic (120±3.2 vs. 123.5±2.6 mmHg, $P=0.4959$) or diastolic (77.6±1.5 vs. 75.1±1.7 mmHg, $P=0.4361$) BP between male and female participants. Some subjects were currently using antihypertensive drugs (Table 2), and medications used by subjects were not withheld. Because salt sensitivity and hypertension rates increase in women after menopause, it is important to highlight that only 5 of a total of 33 women reported being on hormone replacement therapy. Since this percentage is small and the BP, CSF or plasma [Na^+] values of these individuals were not outliers, we did not further segregate female subjects into different groups.

Increased CSF [Na^+] in hypertensive humans

To determine if sodium levels in the CSF are higher in hypertensive humans, we analyzed CSF [Na^+] in normotensive and hypertensive participants (Table 1). As expected, we found an increase in the average sBP and dBP in the hypertension group compared with the normotension group (Figure 1A and 1B). Importantly, we found a significant elevation in CSF [Na^+] in hypertension participants compared with those in the normotension group (148.8±3.4 mM vs. 138.1±1.9 mM, P=0.0053), as shown in Figure 1C. In contrast, plasma [Na^+] was similar between the two groups (Figure 1D). There was no difference in CSF potassium concentration ([K^+]) between groups (Figure 1E).

To further determine whether CSF [Na^+] was related to hypertensive stage, we divided study participants into four sub-groups according to 2017 HBPCPG guidelines (56). As expected, sBP (Figure 2A) was significantly higher in Elevated BP (P<0.01), HTN-Stage 1 (P<0.0001), and HTN-Stage 2 (P<0.0001) subjects compared with individuals with normal BP; whereas dBP (Figure 2B) was significantly higher in HTN Stage 1 (P<0.05) and HTN Stage 2 (P<0.0001) subjects compared with normal BP individuals. Interestingly, we found that CSF [Na^+] was significantly higher specifically in HTN-Stage 2 subjects (155.7±6.8 mM; P=0.0051) compared with all other groups (Figure 2C). There was a tendency toward higher CSF [Na^+] in HTN-stage 1 subjects (144.8±3.1 mM) compared to individuals with normal BP (139.3±2.5 mM), but this difference did not reach statistical significance. As shown in Figure 2D, we found no difference in plasma [Na^+] among groups.

Human CSF [Na^+] is positively correlated with BP

Distinct gender differences in the incidence and severity of human hypertension are well established; race, age, and BMI are also factors known to affect BP (16, 33, 34). As shown

in Table 1, our study population contained more females than males. In addition, BMI was significantly higher in hypertensive subjects and was correlated with BP (Figure 3). To accurately determine correlations between CSF or plasma [Na^+] and sBP or dBP, we performed a multiple regression analysis between [Na^+] and BP, controlling for age, sex, race, and BMI. As shown in Table 3 and the regression curves in Figure 4A and 4B, [Na^+] in CSF was positively correlated with sBP (R^2=0.4189, *P*=0.0007) and dBP (R^2=0.205, *P*=0.032). Interestingly, we identified a significant contribution of BMI to sBP (*P*=0.005), but not dBP (*P*=0.102) (Table 3 and Figure 3). We found no correlation between plasma [Na^+] and sBP (R^2=0.0009, *P*=0.9759) or dBP (R^2=0.0222, *P*=0.2995), as shown in Table 3 and the linear regression curves presented in Figure 4C and 4D. To determine whether the elevation in CSF [Na^+] was caused by higher plasma [Na^+] in each individual, we performed linear regression analyses. These analyses showed no correlation between plasma and CSF [Na^+] (R^2=0.0002174, *P*=0.9252; Figure 4E), suggesting that the elevation in CSF [Na^+] is not attributable to passive diffusion from the circulation, and instead indicates that active transport mechanisms may be involved in elevating CSF [Na^+]. Collectively, our results indicate that CSF [Na^+] is positively correlated with BP, whereas plasma [Na^+] is not.

Osmolality is not affected by increased [Na^+] in CSF

We further analyzed CSF and plasma osmolality in normotension and hypertension groups and were surprised to find no difference in either CSF or plasma osmolality between the two groups (Figure 5A and 5B). Furthermore, there was no difference in CSF or plasma osmolality between groups at different hypertensive stages (Figure 5C and 5D), indicating that CSF [Na^+] may be more clinically relevant than CSF osmolality in the

pathogenesis of chronic hypertension. Notably, plasma and CSF osmolality were not correlated with sBP or dBP (Figure 5E–5H).

Discussion

High levels of dietary sodium are associated with elevated BP and adverse effects on cardiovascular health (11). Animal experiments, epidemiological studies, and clinical trials have provided compelling evidence for a detrimental effect of sodium intake on BP in both HTN and NTN individuals (1, 44). In addition to its effects on BP, excess dietary sodium consumption has been directly linked to coronary heart disease (12, 24), stroke (2) and non-cardiovascular diseases (4). Nevertheless, data on CSF and plasma [Na^+] in HTN humans is limited and its significance in cardiovascular health remains undetermined. Only one study with a small sample size has investigated the association between CSF or plasma [Na^+] and BP in young hypertensive humans consuming their normal, habitual diet (29). In this study, we found a significant increase in CSF [Na^+], but not plasma [Na^+], in hypertensive participants compared with normal BP individuals, suggesting the importance of CSF [Na^+] in human hypertension. We further showed that CSF [Na^+] is positively correlated with both systolic and diastolic BP in human subjects on their normal diet.

It has been suggested that small increases in plasma [Na^+] may be a mechanism by which dietary salt raises BP (15, 21, 48); however, our data showed no correlation between plasma [Na^+] and BP in normal or high BP individuals under a habitual diet. The conclusion that plasma [Na^+] is linked to BP in humans is mostly based on acute effects

of dietary sodium intake (15, 48). For example, in both NTN and HTN humans, a large and sudden increase in dietary sodium causes a 2- to 4-mM rise in plasma [Na^+] (25, 30, 43, 49). Studies of gradual increases or decreases in salt intake have similarly shown increases or decreases in plasma [Na^+], respectively, of approximately 3 mM after 5 days of treatment and reported a significant relationship between the increase in plasma [Na^+] and an increase in pulse pressure (22, 23, 45). In humans, the effect of an abrupt increase in sodium intake for 5 to 28 days on BP is also related to age; in younger subjects (<26 years of age), BP and plasma [Na^+] do not rise (25, 32). In contrast, in individuals over 60 years old, an acute increase in sodium intake is associated with a rise in plasma [Na^+] of 1.6 to 3 mM and an increase in mean arterial pressure (27, 30). These reports support the effects of acute high-salt intake on plasma [Na^+] and BP regulation. Nevertheless, an elegant commentary by Wardener et al. (15) that summarized seven independent studies on baseline plasma [Na^+] in a total of 108 NTN and 57 HTN humans concluded that there were no obvious differences in plasma [Na^+] between NTN and HTN subjects. The changes in plasma [Na^+] reported in our study are in the same range, and our overall findings agree with these reports indicating no correlation between plasma [Na^+] and BP in humans.

An important finding from this study is that CSF [Na^+], but not plasma [Na^+], is significantly correlated with BP in humans on their natural, habitual diet. To date, there have been only few independent reports on CSF [Na^+] in HTN humans (20, 29, 30). In one study, Kawano *et al*. examined CSF [Na^+] levels in 15 patients with essential hypertension on a high-salt diet (16–18 g/d) compared with a low salt diet (1–3 g/d) for 7 days. They reported that [Na^+] was significantly elevated in both CSF and serum during the high-salt-diet

period compared with the low-salt-diet period (30). In another study, 24 hypertensive individuals were provided 7 g or 25 g of daily dietary salt during low- and high-salt intake periods, respectively, for 7 days each. The authors of this study concluded that changes in CSF [Na^+] were related to the patient's salt sensitivity. With a daily salt intake of 25 g/d, CSF [Na^+] significantly increased in 13 salt-sensitive patients compared with salt-resistant individuals. However, the 7 g/d dietary salt regimen did not affect CSF [Na^+] in any of the patients (20). Although these studies did not perform correlation analyses of CSF [Na^+] and BP, taken together with our data, they indicate that elevated CSF [Na^+] might be a key mechanism by which sodium raises BP in humans. Our results provide the direct demonstration of a positive correlation between CSF [Na^+] and BP in humans. The mechanism by which a high-salt diet elevates CSF [Na^+] remains an important topic for future studies.

In this study, the daily intake of sodium was not monitored. However, the main focus of this study was on examining the levels of sodium in CSF versus plasma in populations on their regular habitual diets regardless of the cause (e.g., relative sodium intake, renal dysfunction). In addition, the increase in CSF [Na^+], but not plasma [Na^+], suggests important unknown mechanisms for active sodium re-distribution (accumulation) in the CSF in hypertension, which is a key finding of this study. Our finding that plasma and CSF [Na^+] were not correlated with each other also supports our hypothesis of enhanced active transport or retention of sodium in the CSF of HTN humans. CSF—the fluid in the brain ventricles and spinal cord—is predominantly, but not exclusively, produced by the choroid plexus. CSF plays an important role in protecting the brain and regulating brain interstitial electrolyte and fluid homeostasis (46). It has been shown that several sodium

transporters and antiport systems regulate CSF sodium (35). Among them, the Na-K-ATPase is probably the main transporter of sodium from the epithelium to CSF (35), whereas movement of sodium from CSF to the blood or interstitial space is mediated primarily by epithelial sodium channels (54). Whether there are changes in sodium transporters and/or the biophysical properties of these channels during the development of human hypertension remains to be determined. We propose that the increase in sodium levels in CSF might be related to dysfunction of the processes that redistribute or actively transport sodium into the brain, possibly in combination with an inadequacy of kidney sodium excretion or pressure diuresis mechanisms (13, 14).

One limitation of this study is that the subjects all have a family history of AD. Because, in current clinical practice, spinal tap is not a routine procedure for hypertensive patients without other complications, it is clinically difficult to justify and challenging to carry out such a study in this patient population. To achieve our goal, we chose this population, which is otherwise healthy but with a family history of AD, for both hypertensive and normotensive groups. Since all subjects in this study have a family history of AD, we believe that the data obtained reflect the relationship between CSF sodium and BP. However, we acknowledge that the observed elevation in CSF Na^+ and the positive correlation of CSF Na^+ with BP may be more significant in hypertensive patients with family history of AD, and may or may not be universal among regular hypertensive subjects.

Interestingly, when the subjects were subdivided into different groups according to their BP levels (Normal BP, Elevated BP, HTN Stages 1 and 2), we found that CSF [Na^+] was significantly higher only in HTN Stage 2 subjects. Although there was a trend toward an

increase in CSF [Na^+] in HTN Stage 1 subjects, this difference was not statistically significant, possibly due to the small sample size. On the other hand, since CSF [Na^+] was not increased in earlier stage HTN, another possibility is that CSF [Na^+] may reflect high BP-induced compromised BBB integrity or altered sodium transport resulting from HTN. Whether elevated CSF sodium is a cause or result of human hypertension needs further investigation.

In conclusion, our data show a significant increase in CSF [Na^+], but not plasma [Na^+], in HTN patients with a family history of AD that is positively correlated with BP, suggesting the potential importance of CSF Na^+, but not plasma Na^+, in the development or maintenance of chronic human hypertension. Our findings thus support the biological plausibility of a functional relationship between high sodium in the CSF and arterial pressure in humans, although the underlying mechanisms remain to be determined.

Clinical Perspectives

High dietary sodium intake is associated with elevated BP and adverse effects on cardiovascular health. The importance of CSF sodium in the regulation of sympathetic activity has been highlighted by recent experimental animal studies. However, the clinical significance of CSF sodium in hypertensive humans is not clear. In this study, we show that CSF sodium concentration is increased in hypertensive compared to normotensive individuals with family history of AD and that CSF sodium concentration is positively correlated with blood pressure. It is important to understand the mechanisms associated with the increase in CSF sodium concentration in hypertensive individuals, and to determine whether the elevated CSF sodium is the cause of high blood pressure, or

simply a phenomenon that accompanies the hypertension. Addressing these questions will be important for advancing our understanding of hypertension development and may contribute to the discovery of new therapeutics for hypertension.

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Author contribution

Y.FE. and W.W. designed the study.
V.K. and W.W. collected samples and edited the manuscript
W.Y. performed statistical analyses and edited the manuscript.
P.G.K. and W.W. provided valuable comments and edited the manuscript
F.T, L.A.C.S. and V.K. analyzed the data, and wrote and edited the manuscript.
Y.FE. conceived and supervised the study, analyzed the data, and wrote the manuscript.

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Conflict of Interests

The authors declare that there are no competing interests associated with the manuscript.

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Table 1. Characteristics of normal and high BP study subjects

	Normotension (n = 25)	**Hypertension (n = 18)**
Demographics		
Sex (M/F)	5/20	5/13
Race (White/African American/other)	14/10/1	13/5/0
Age (years)	60.6±1.09	62.0±1.4
BMI (kg/m^2)	25.8±1.0	30.1±1.4*

Values are presented as means±SEM. *P<0.05, ****P<0.0001 vs. Normal BP group; unpaired t-test. Abbreviations: **BP**, blood pressure; **M**, male; **F**, female; **BMI**, body mass index.

Table 2. Antihypertensive drugs used in Stage 1 and Stage 2 hypertensive subjects

Antihypertensive drugs	**HTN Stage 1 (n=11)**	**HTN Stage 2 (n=7)**
ACE inhibitors (n)	**0**	**1**
CC blockers (n)	**0**	**1**
Beta blockers (n)	**1**	**1**
Diuretics (n)	**0**	**2**
AT_1 receptor antagonists (n)	**0**	**1**

Abbreviations: ACE, angiotensin converting enzyme; CC, calcium channels; Beta, β-adrenergic receptor; AT1, angiotensin II type 1 receptor; HTN, hypertension.

Table 3. Multiple regressions between plasma or CSF [Na^+] and sBP or dBP, controlling for age, sex, race, and BMI

Multiple regressions between CSF [Na^+] and sBP (n=43)					
Variable	**df**	**Parameter estimate**	**SEM**	**t-value**	**Pr>|t|**
Intercept	1	21.75612	27.96648	0.78	0.4416
CSF [Na^+]	1	0.50138	0.13580	3.69	*0.0007
Age	1	0.06915	0.32899	0.21	0.8347
Sex	1	-1.80857	4.29869	-0.42	0.6764
Race	1	-2.81272	3.80568	-0.74	0.4645
BMI	1	0.97866	0.32867	2.98	*0.0051
Multiple regressions between CSF [Na^+] and dBP (n=43)					
Variable	**df**	**Parameter estimate**	**SEM**	**t-value**	**Pr>|t|**
Intercept	1	30.75332	20.85935	1.47	0.1489
CSF [Na^+]	1	0.22576	0.10129	2.23	*0.032
Age	1	0.01925	0.24539	0.08	0.9379
Sex	1	3.35840	3.20626	1.05	0.3017
Race	1	-1.22560	2.83854	-0.43	0.6684
BMI	1	0.41123	0.24515	1.68	0.1019
Multiple regressions between plasma [Na^+] and sBP (n=43)					
Variable	**df**	**Parameter estimate**	**SEM**	**t-value**	**Pr>|t|**
Intercept	1	92.14064	77.68614	1.19	0.2432
Plasma [Na^+]	1	-0.01595	0.52365	-0.03	0.9759
Age	1	0.09842	0.39147	0.25	0.8029
Sex	1	-5.00660	4.93816	-1.01	0.3172
Race	1	-0.95823	4.43967	-0.22	0.8303
BMI	1	1.03084	0.38856	2.65	*0.0117
Multiple regressions between plasma [Na^+] and dBP (n=43)					
Variable	**df**	**Parameter estimate**	**SEM**	**t-value**	**Pr>|t|**
Intercept	1	113.32486	51.98281	2.18	0.0357
Plasma [Na^+]	1	-0.36870	0.35039	-1.05	0.2995
Age	1	-0.01754	0.26195	-0.07	0.9470
Sex	1	1.67961	3.30431	0.51	0.6143
Race	1	-0.05791	2.97075	-0.02	0.9846
BMI	1	0.47508	0.26000	1.83	0.0757

Abbreviations: **df**, degrees of freedom; **SEM**, standard error of the mean; **CSF**, cerebrospinal fluid; **[Na^+]**, sodium concentration; **sBP**, systolic blood pressure**; dBP**, diastolic blood pressure; **BMI**, body mass index.

Figure Legends

Figure 1. **Increased [Na^+] in the CSF of high BP patients. (A, B)** sBP (A) and dBP (B) of study subjects. **(C, D)** CSF [Na^+] (C) and plasma [Na^+] (D) in study subjects. (E) CSF [K^+] in study subjects. Normotension, n=25; Hypertension, n=18; ***P*<0.01, *****P*<0.0001 vs. Normotension; unpaired *t*-test.

Figure 2. **Increased CSF [Na^+] in HTN stage 2 patients**. **(A, B)** sBP (A) and dBP (B) of study subjects. **(C, D)** CSF [Na^+] (C) and plasma [Na^+] in study subjects (D). Normal BP, n=20; Elevated BP, n=5; HTN-Stage 1, n=11; HTN-Stage 2, n=7; **P*<0.05, ***P*<0.001, *****P*<0.0001 vs. Normal BP group; $^{\#\#}P$<0.01, $^{\#\#\#\#}P$< 0.0001 vs. HTN-Stage 1 group; one-way ANOVA with Bonferroni post hoc tests.

Figure 3. Elevated BP in hypertensive participants. (A, B) Correlation of sBP (A) and dBP (B) with BMI (n=43). ** *P*<0.01; linear regression. **(C)** BMI of study subjects. Normal BP, n=20; Elevated BP, n=5; HTN-Stage 1, n=11; HTN-Stage 2, n=7; **P*<0.05, vs. Normal BP group; one-way ANOVA with Bonferroni post hoc tests.

Figure 4. Human CSF [Na^+] is positively correlated with BP. (A, B) Correlation of sBP (A) and dBP (B) with CSF [Na^+]. **(C, D)** Correlation of sBP (C) and dBP (D) with plasma [Na^+]. **(E)** Correlation of CSF [Na^+] with plasma [Na^+]. N=43, * *P*<0.05, *** *P*<0.01 ; linear regression.

Figure 5. Human CSF and plasma osmolality are not correlated with BP. (A, B) CSF and plasma osmolality in normal and high BP. N=25 (Normotension) and 18 (Hypertension), unpaired t test. **(C, D)** CSF and plasma osmolality in study subjects subdivided according to BP levels. Normal BP, n=20; Elevated BP, n=5; HTN-Stage 1, n=11; HTN-Stage 2, n=7, One-way ANOVA with Bonferroni post hoc tests. **(E, F)** Correlation of sBP (C) and dBP (D) with CSF osmolality (n=37). **(G, H)** Correlation of sBP (E) and dBP (F) with plasma osmolality (n=38), linear regression.