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Effects of Water Loading on Observed and Predicted Plasma Sodium, and Fluid and Urine Cation Excretion in Healthy Individuals

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ABSTRACT

Rationale & Objective: Recently, it was discovered that significant amounts of sodium (Na^+) can be stored without concurrent water retention, indicating the presence of an additional compartment for Na^+ distribution. The osmoregulatory role of this compartment under hypotonic conditions is not known.

Study Design: Experimental interventional study.

Setting & Participants: Single-center study including twelve healthy male subjects

Intervention: To investigate whether Na^+ can be released from its nonosmotic stores after a hypotonic fluid load, a water loading test (WL; 20 ml water/kg in 20 min) was performed.

Outcomes: During a 240-min follow-up, we compared the observed plasma [Na^+], fluid and urine cation excretion with values predicted by the Barsoum-Levine and Nguyen-Kurtz formula. These formulas are used for guidance of fluid therapy during dysnatremia, but do not account for nonosmotic Na^+ stores.

Results: Thirty min after WL, plasma [Na^+] decreased 3.2±1.6 mmol/L (mean (SD)), after which plasma [Na^+] increased gradually. Hundred twenty min after WL, plasma [Na^+] was significantly underestimated by the Barsoum-Levine (-1.3±1.4 mmol/L, p=0.047) and Nguyen-Kurtz formula (-1.5±1.5 mmol/L, p=0.026). In addition, the Barsoum-Levine and Nguyen-Kurtz formula overestimated urine volume, while cation excretion was significantly underestimated with a cation gap of 57±62 mmol (p=0.009) and 63±63 mmol (p=0.005), respectively. After 240 min, this gap was 28±59 mmol (p=0.2) and 34±60 mmol (p=0.08), respectively.

Limitations: The compartment from which the mobilized sodium originated was not identified.

Conclusion: These data suggest that healthy individuals are able to mobilize osmotically inactivated Na^+ after an acute hypotonic fluid load. Further research is needed to expand

knowledge on the Na^+ buffer regarding the physiology of osmoregulation and dysnatremia therapy.

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Keywords: sodium, electrolyte, sodium homeostasis, dysnatremia, water loading.

PLAIN-LANGUAGE SUMMERY

For years, it was thought that the kidney is the key organ responsible for the body water and sodium (Na^+) content. However, recently it was discovered that significant amounts of Na^+ can be stored without concurrent water retention. These observations indicate the presence of an additional compartment for Na^+ distribution. The role of this compartment under hypotonic conditions is not known. This study shows that healthy individuals may be able to mobilize osmotically inactivated Na^+ after an acute water load. This observation suggests that the Na^+ buffer might play an important role in hypotonic stress. Moreover, osmotic inactivation and reactivation of Na^+ might be an essential variable for accurate prediction of the plasma [Na^+] change in treatment of patients with hypernatremia.

INTRODUCTION

Sodium (Na^+) is the principal osmotic force in the extracellular compartment. High Na^+ intake increases extracellular fluid osmolality resulting in water retention to maintain isotonicity of body fluids. For years, it was thought that the kidney is solely responsible for regulation of total body Na^+ content, and thereby regulation of the extracellular fluid compartment and blood pressure. However, several studies have shown that significant variations in total body Na^+ did not induce the expected variation in body weight or blood pressure in healthy male subjects.[1, 2] These findings indicate that an additional compartment exists where Na^+ can be stored without retention of water. Based on studies in animals and humans, interstitial negatively charged glycosaminoglycans (GAGs) seem to represent the principal Na^+ binding site.[3, 4] Skin ashing experiments in rats that were put on either a high or a low Na^+ diet intake showed increased skin Na^+ content after high Na^+ intake.[5, 6] In humans, Na^+ imaging studies using ^{23}Na-MRI indicate that the skin and muscle comprise the most important tissues where significant amounts of Na^+ can be stored.[7-9] While the exact biological function of nonosmotic Na^+ storage is yet unknown, the evidence so far indicates that it may serve as a buffer for excessive Na^+ [1, 2, 10] but may also have osmoregulatory effects.[11]

In a recent study conducted by our group, we aimed to quantify the capacity of this Na^+ buffer after hypertonic saline infusion in healthy male volunteers by comparing observed plasma [Na^+] and urine cation excretion with values estimated by formulas based on the Edelman equation. The Edelman equation describes a strong correlation between plasma [Na^+] and exchangeable cations [$Na_e + K_e$] per liter body water, which is in line with the two-compartment model for body fluids and solutes.[12] However, the equation is based on steady state conditions and does not take into account an additional compartment that can store and

release Na^+. After infusion of hypertonic saline, a large discrepancy was found between observed and estimated plasma [Na^+] changes and urine cation excretion.[13] In fact, only half of the cations that were cleared from the body water could be retraced in the urine. These results underscore that healthy individuals display the capacity to osmotically inactivate Na^+, indicating that the widely adopted two-compartment model oversimplifies Na^+ homeostasis.

So far, the role of this compartment in the context of hypotonic stress has not been studied in humans. Animal data suggest that osmotically inactive Na^+ in the skin may be mobilized after long-term salt deprivation in order to prevent hypotonicity.[14] This potential osmoregulatory role of Na^+ buffers may be particularly relevant for our still incomplete understanding of the pathophysiology of dysnatremia development.[15] In this interventional study in healthy male individuals, we investigated whether a hypotonic fluid load induced osmotic activation of nonosmotically stored Na^+ by comparing actual changes in plasma [Na^+] and cation excretion with changes predicted by formulas based on the Edelman equation.[16, 17]

METHODS

Participants

This interventional study was conducted in the Amsterdam University Medical Center (AUMC, Amsterdam, The Netherlands). Healthy, non-smoking male subjects between 18 and 40 years of age were included. Subjects were excluded if they had an office blood pressure >135/85 mmHg, body mass index (BMI) >30kg/m^2, a renal, cardiovascular or auto-immune disease, or a history of any type of malignancy within the past 5 years. The study was approved by the local Medical Ethics Committee (METC)(METC number 2014_241). All subjects signed informed consent.

Study design

The day before the study visit subjects were asked to collect 24-hour urine. After at least eight hours of fasting (food and drinks) subjects visited our research department for assessment of baseline blood pressure, heart rate and body weight (Table S1). In addition, we collected venous blood samples. After baseline measurements, a standardized water loading (WL) test was performed. Subjects had to drink 20 mL/kg body weight lukewarm tap water within 20 minutes (min).

Laboratory analysis

Blood was drawn at baseline and 30, 60, 120 and 240 min after start of the WL test. As blood was drawn at several time points subjects received an intravenous catheter in the antecubital vein of the right arm. Hemoglobin, hematocrit and mean corpuscular volume (MCV), and plasma [Na^+], [K^+], [Cl^-], [HCO_3^-], glucose, urea and osmolality were measured at all time points. At baseline, two and four hours after WL plasma uric acid and creatinine were measured as well. We collected urine 120 and 240 min hours after WL in which we measured

[Na^+], [K^+], [Cl^-], urea, osmolality uric acid and creatinine. All samples were analysed by the Laboratory of Clinical Chemistry, AUMC, using routine laboratory techniques (Roche cobas 8000 modular analyzer).

Hemodynamic measurements

Brachial blood pressure and heart rate were measured 5 times in seated position after a 5 min rest using a validated oscillometric device (Omron M7, Omron Healthcare, Hoofddorp, The Netherlands). The mean values of the last two measurements were used for statistical analysis.

Calculations

We compared observed changes in plasma [Na^+] with values predicted by the Barsoum-Levine (BL) and Nguyen-Kurtz (NK) formula (Item S1). To compare observed fluid and cation excretion with predicted cation excretion we rearranged the BL and NK formula. Total body water (TBW) was assessed as 60% of the total body weight. The Adrogué-Madias formula, which is frequently used in the clinical setting, was not used to estimate changes in plasma [Na^+], fluid and cation excretion, because it does not account for fluids added and lost in the same period of time as can be observed after water ingestion. Also, the BL formula combines the original AM formula and AM fluid loss formula, thereby correcting for fluid losses.[18]

Statistical analyses

Continuous data are expressed as mean and standard deviation (SD). Data that followed skewed distribution are shown as median and interquartile range (IQR). To assess whether changes in laboratory and hemodynamic parameters after water loading were significantly

different we used a general linear model for repeated measure with Least Significant Difference (LSD) post-hoc tests. Paired t-tests were used to compare observed and calculated excretion. To assess correlation between variables, we used Pearson's correlation coefficient. Significant differences were assumed to be at $p<0.05$. (SPSS, Version 24.0, Inc., Chicago, IL).

RESULTS

Study population

Sixteen subjects were screened of which twelve completed the study. Two subjects did not meet inclusion criteria because of hypertension and elevated plasma aspartate aminotransferase level. One subject was unable to comply with the study procedures and one subject was lost to follow-up after screening. All subjects that completed the study had a normal BMI, blood pressure and kidney function (Table 1).

Average plasma [Na^+] after WL was higher than estimated

To assess whether nonosmotically stored Na^+ is mobilized after WL, we compared the observed decrease in plasma [Na^+] with the estimated decrease. The mean amount of WL was 1.5±0.3 L. The maximal decrease in hematocrit was observed 60 min after WL (Figure 1A), indicating that most of the ingested water was absorbed at that time point. MCV did not significantly change. The decrease in average plasma [Na^+] was largest 30 min after the start of the WL test (Figure 1B). Plasma [Na^+] gradually increased after 60 min and was not significantly different from baseline at 120 and 240 min after WL. The change in plasma [Na^+] was highly heterogeneous; 30 min after WL, plasma [Na^+] was 0 to 5 mmol/L lower than at baseline. In one subject, plasma [Na^+] reached its lowest values 240 min after WL.

Plasma [K^+] was measured to assess whether transcellular shifts of K^+ occurred after changes of plasma [Na^+]. Plasma [K^+] did not significantly change after WL (Figure 1B). Lowest plasma osmolality was observed 30 min after WL (-6.3±2.6 mOsm/kg, $p<0.001$ vs. baseline). In line with changes of plasma [Na^+], plasma [Cl^-] significantly decreased in the first hour after WL. The correlation between plasma [Na^+] and plasma [Cl^-] changed during the four-hour follow-up (Figure S1). A significant correlation was present at baseline, 120 and

240 min, while 30 and 60 min after WL a non-statistically significant correlation was observed. WL did not cause significant changes in plasma [HCO_3^-].

The observed changes in plasma [Na^+] after WL were not in line with changes predicted by the NK and BL formula. Two hours after WL both formulas significantly underestimated average plasma [Na^+] (Figure 2). No difference between observed and estimated plasma [Na^+] was found 240 min after water ingestion. Both 120 and 240 min after WL a significant correlation was found between the observed plasma [Na^+] change and values predicted by the BL and NK formula (Figure S2).

Both formulas overestimated fluid excretion and underestimated cation excretion

Furthermore, we compared the observed urine volume and cation excretion with values predicted by the rearranged BL and NK formula. The total urine volume during 240 min follow-up was 1.7±0.7 L of which 1.2±0.6 L was excreted between 0-120 min and 0.5±0.3 L between 120-240 min. The expected urine volume during the 240-min follow-up according to BL and NK was 1.9±1.0 L (p=0.3) and 1.8±0.8 L (p=0.3), respectively. During the first 120 min after water ingestion, there was, however, a discrepancy between the observed and calculated urine excretion. The expected urine volume was significantly higher than observed. No statistically significant differences were found between the observed and calculated fluid excretion 120 min to 240 min after WL (Figure 3). Two hours after WL, a significant correlation was found between observed values and values predicted by the BL formula and NK formula (Figure S3A). However, from 120 min to 240 min after WL no significant association was observed (Figure S3C).

Total urine $Na^+ + K^+$ excretion was 75.3±28.5 mmol of which 48.3±20.3 mmol was excreted in the first two hours and 27.0±11.1 mmol was excreted in the last two hours. Fractional excretion of urine urea increased significantly two hours after WL (p=0.03), while fractional Na^+ and K^+ excretion did not (Table 2). During the entire four-hour follow-up after WL, urine cation excretion was similar to the expected values (Figure 4). However, according to the BL (-8.2±72.3 mmol, p=0.009 vs. observed) and NK formula (-15.1±75.5 mmol, p=0.005 vs. observed) the calculated urine cation excretion in the first two hours after WL was negative, meaning that no cation should have been excreted, which is not in line with the observation that on average 48 mmol of cations were excreted. Between 120-240 min after WL no significant differences were found between observed and estimated urinary cation excretion. Two hours after WL a strong correlation was found between the observed cation excretion and cation excretion predicted by the BL and NK formulas (Figure S3B). However, non-statistically significant correlations were present between observed and estimated cation excretion between two to four hours after WL (Figure S3D).

Since fluid excretion after WL may be affected by the ability to shift water into muscle cells we examined the correlation between 24-hour urine creatinine excretion, as a reflection of muscle mass, and the mismatch between observed and estimated fluid excretion after WL.[19] We found a non-statistically significant correlation. Furthermore, 24-hour urine Na^+ excretion, which may relate to the amount of sodium stored nonosmotically prior to the experiment, was not associated with changes in plasma [Na^+], urine cation excretion after WL or the mismatch between the observed and predicted plasma [Na^+], urine cation and fluid excretion.

Effect of WL on hemodynamics

To assess the effect of WL on hemodynamic parameters, blood pressure and heart rate were measured at fixed time points during the 240-min follow-up. Diastolic blood pressure was significantly increased 30 min after WL (7±1 mmHg, $p<0.001$ vs. baseline) but normalized to baseline values thereafter. No significant changes in systolic blood pressure and heart rate were observed during four-hour follow-up (Figure S4).

DISCUSSION

The current study suggests that healthy subjects are able to mobilize significant amounts of nonosmotically stored Na^+ from an additional compartment in response to a hypotonic stimulus. As a result, formulas based on the two-compartment model are not able to fully predict changes in sodium homeostasis induced by WL.

WL is a commonly used test to investigate physiologic responses to hypotonic stimuli. A previous study demonstrated that the ingested water is absorbed within approximately 75-120 minutes.[20] In our study, the nadir of hematocrit was seen 60 minutes after WL indicating that the ingested water had been completely absorbed. However, lowest values of plasma [Na^+], representing water balance, were already observed 30 minutes after WL. As subjects were not allowed to ingest Na^+, the discrepancy between hematocrit and plasma [Na^+] is not in line with current concepts of water and Na^+ homeostasis. According to current textbooks, the ingested water is distributed over the intra- and extracellular compartment to maintain isotonicity, leading to dilution and a subsequent decrease in plasma [Na^+]. Supposing that dilution is maximal after complete water absorption, lowest plasma [Na^+] and hematocrit should be reached at the same time. The fact that plasma [Na^+] reached nadir before lowest hematocrit values were observed indicates that extra Na^+ was added to the extracellular volume. As no external Na^+ intake was allowed during the experiment, this Na^+ is likely to come from an internal Na^+ compartment in which Na^+ can be temporarily stored and released upon hypotonic stimuli such as WL. The observed association between the cation excretion that was measured and the predicted cation excretion by the BL and NK formula *(BL: measured cation excretion = 0,2* predicted cation excretion + 49,8; NK: measured cation excretion = 0,2* predicted cation excretion + 51,1)* suggests that the average amount of mobilized Na^+ is about 50 mmol. The finding that Na^+ may be mobilized from an internal

buffer is in line with data from a previous study in endurance athletes that demonstrated that 70% of the athletes had similar or increased plasma [Na^+] despite gaining significant amounts of weight due to excessive fluid consumption.[11] Further analyses of 18 athletes that developed exercise induced hyponatremia showed that athletes were able to mobilize up to 214 mmol Na^+ around the race while others osmotically inactivated Na^+. The opposite, i.e., osmotic inactivation of Na^+, has also been shown in healthy volunteers at rest. In a controlled environment like the current study, infusion of hypertonic saline (2.4%) led to inactivation of 57 mmol of Na^+ during a four-hour follow-up.[13] This is very similar to the amount Na^+ that was mobilized in the current study.

Plasma sodium concentration is the key determinant of plasma osmolarity. Variations in plasma osmolarity are paralleled by changes in sodium and water excretion by the kidneys. Anti-diuretic hormone increases renal water reabsorption and plays a crucial role in lowering plasma osmolarity, while suppression of anti-diuretic hormone leads to an increase in water excretion and rise in plasma osmolarity. In addition to water diuresis that is induced by the kidney and is efficacious within hours, these data demonstrate that osmotic (re)activation of nonosmotically stored Na^+ may represent a more acute mechanisms that helps to neutralize a hypotonic load. We demonstrate that two hours after WL the average urine volume was 1.2 L, which was on average 300 mL less than the amount of water that was ingested during WL. The delay in onset of water diuresis is not because of water absorption, but because of the elimination half-life of circulating anti-diuretic hormone.[21] Our data show that the free water clearance significantly increased two hours after WL, indicating that the concentration of anti-diuretic hormone decreased. A study in healthy men showed that after ingestion of 1 L water, after 18 hours dehydration, urine flow starts to rise after 60 min and peak flow is reached after 90 min.[21] Consequently, in order to prevent hypotonicity during the first hours after hypotonic stress, Na^+ needs apparently to be mobilized from its nonosmotic stores.

As high Na^+ intake has been shown to increase nonosmotic Na^+ storage, subjects with high 24-hour Na^+ excretion may have less capacity for additional nonosmotic Na^+ storage and may be able to release more Na^+ after hypotonic stimuli. Although no correlation was present between the size of the cation gap and 24-hour Na^+ excretion in this study, the amount of Na^+ intake might play a role under more extreme hypotonic conditions, like exercise-induced hyponatremia. In addition, a previous study demonstrated that water is absorbed by muscle cells.[19] To investigate this effect we assessed whether baseline 24-hour urine creatinine excretion, as a reflection of muscle mass, was correlated to the observed inconsistency in sodium homeostasis. However, no correlation was found suggesting that the discrepancy between observed and measured data can probably not be explained by water storage in muscles.

In this study we used the BL and NK formula to predict changes in plasma [Na^+] following WL. Both formulas, like the Adrogue-Madias formula, are based on the Edelman equation. As stated earlier, the Edelman equation describes the correlation between plasma [Na^+] and exchangeable cations [$Na^+_e + K^+_e$] per liter body water. Unlike the BL formula, the NK formula includes the y-intercept and slope of the Edelman equation, which are considered clinically relevant as they are thought to reflect nonosmotic Na^+ storage and the Gibbs-Donnan and osmotic equilibrium, respectively.[17] However, since the Edelman equation is based on steady-state observations in different subjects, one may question whether this interindividual association takes into account intra-individual nonosmotic Na^+ storage as the additional compartment for nonosmotic sodium storage is likely to be dynamic and may be of particular relevance during hypo or hypertonic stimuli. The Edelman equation may therefore not be able to predict individual changes in sodium homeostasis after water or sodium loading or loss.

Interestingly, despite the large inaccurateness of the BL and NK formula two hours after WL we were able to predict plasma [Na^+] and urine cation excretion more accurately in the last two hours of the experiment. This may indicate that the initially osmotically activated sodium was inactivated again via renewed nonosmotic storage in the additional Na^+ compartment. This hypothesis is supported by the fact that two and four hours after water loading a significant correlation was present between plasma [Na^+] and plasma [Cl^-], but no correlation was found thirty and sixty minutes after WL. These data indicate that the Na^+ buffer is particularly relevant for immediate osmoregulation under extreme hypotonic conditions, such as the consumption of 1.5 L of water within short time in our experiment.

The clinical utility of formulas based on the Edelman equation has been questioned in previous studies.[22, 23] In intensive care unit patients with hyper and normonatremia, for example, the mean differences between predicted and observed plasma [Na^+] ranged from 3.4-4.5 mmol/L.[22] Also, in patients with hyponatremia due to polydipsia and patients with hyponatremia as a result of volume resuscitation, these formulas significantly underestimated plasma [Na^+].[23] Likewise, in the current study in healthy subjects we demonstrated that the discrepancy between observed and predicted plasma [Na^+] ranged from -8 to +0.5 mmol/L in the first hour after WL. As overcorrection of plasma [Na^+] or undertreatment can be harmful for patients, future research should identify the additional variables that impact plasma [Na^+] after hypo or hypertonic stimuli.

A potential limitation of this study is that we did not account for water lost with sweat, exhaled air, and water excreted in the feces in our calculations. This may account for a fluid loss of approximately 1100 mL/day. [24] However, within two hours after WL a 400-mL discrepancy was observed between estimated and observed urine volume, while insensible

fluid loss would only account for 92 mL (1100/12). The same applies for post-voiding urine retention, which may be around 36 mL.[13] Yet, these amounts are small and do not explain the observed discrepancy when including this residual volume into our calculations. Secondly, we have not measured gastro-intestinal water absorption and did not standardize diets prior to the experiment. Both factors may have contributed to the heterogenic responses to WL that were observed among subjects in our study. Another matter of consideration is estimation of the TBW. TBW was assessed as 60% of the total body weight, but TBW can be variable and depends on age, volume status, nutritional status and lean body mass. Still, our study population was very homogenous, all subjects were young and had a normal body composition and nutritional status. Lastly, actual Na^+ storage was not measured while ^{23}Na-MRI techniques can be used to measure Na^+ content of the skin and muscles.[8]

Our data show that healthy individuals are able to mobilize osmotically inactivated Na^+ after a hypotonic fluid load. The Na^+ buffer seems therefore to have an immediate osmoregulatory function that acts more rapidly than water diuresis in order to prevent hypotonicity. Furthermore, because a large discrepancy was found between observed and estimated plasma [Na^+], the Na^+ buffer seems to represent an important variable for accurate prediction of the plasma [Na^+] changes when treating dysnatremias. This may be particularly relevant in the context of hypernatremia. Yet, as our study was limited to healthy subjects and no alternative formulas that include ongoing Na^+ shifts from its nonosmotic stores are at hand, both formulas may still be useful as an initial guide for therapy in daily clinical practice, but frequent measurements of plasma [Na^+] in the follow-up are crucial.

SUPPLEMENTARY MATERIAL

Figure S1

Title: Plasma [Na^+] and plasma [Cl^-] after water loading (WL).

Figure S2

Title: Correlation between observed and estimated plasma [Na^+] after water loading (WL).

Figure S3

Title: Correlation between observed and estimated urine volume and cation excretion after water loading (WL).

Figure S4

Title: Blood pressure and heart rate after water loading (WL).

Table S1

Title: Description of the study visit.*

Item S1

Title: Barsoum-Levine and Nguyen-Kurtz formula.

AUTHOR CONTRIBUTIONS

Authors' Contributions: Designed study: LV; carried out experiments: SEID, JR; analyzed data: RDW; provided supervision: LV, RHGOE. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved

SUPPORT

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CONFLICT OF INTEREST

None

DISCLOSURES

None

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TABLES

Table 1 Baseline characteristics

Subjects (n)	**12**
Age (years)	23.0±3.8
BMI (kg/m²)	22.5±2.5
Systolic BP (mmHg)	119±10
Diastolic BP (mmHg)	62±10
Heart rate (bpm)	57±8
Plasma	
Sodium (mmol/L)	140.6±1.1
Potassium (mmol/L)	4.0±0.2
Chloride (mmol/L)	102.1±1.8
Bicarbonate (mmol/L)	25.9±1.7
Osmolality (mOsm/kg)	292±2
Creatinine (µmol/L)	82±12
24-hour urine	
Volume (mL)	1837±802
Sodium (mmol)	186±77
Potassium (mmol)	96±43
Chloride (mmol)	181±72
Creatinine (mmol)	24.4±8.8
Creatinine clearance (ml/min)	135±26
Urine morning sample on study visit	
Osmolality (mOsm/kg)	639±198
Solute-free water clearance (mL/min)	-1.6±0.7
Elektrolyte-free water clearance (mL/min)	-0.3±0.6

Caption: Values are means ± SD.

Table 2 Urine data before and after WL

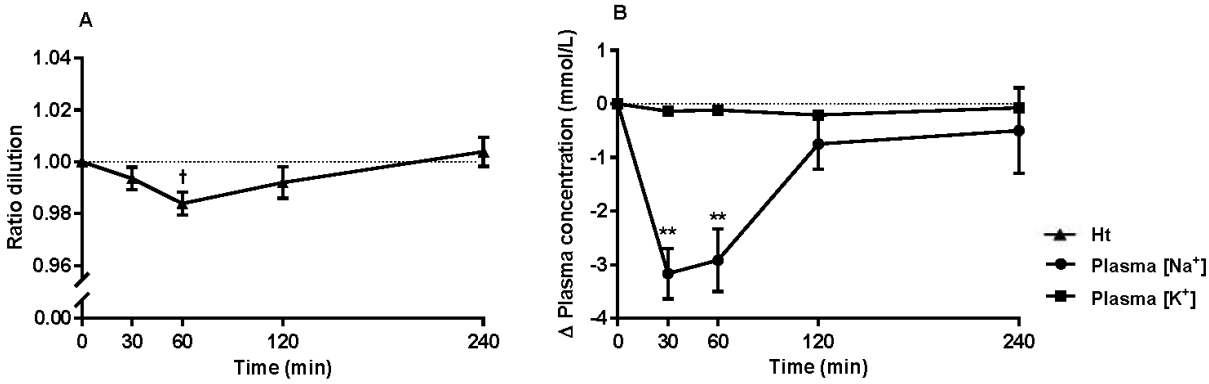
Urine		T0	T120	T240
Sodium	mmol/L	117.8±37.1	37.3±37.0**	58.8±36.3**
	mmol	39.9±18.7	32.1±16.5	19.6±8.3†
Potassium	mmol/L	65.3±32.6	23.3±33.5*	25.8±32.6††
	mmol	21.5±11.8	16.3±6.2	7.4±4.9
Chloride	mmol/L	121.7±38.0	34.9±44.4**	47.8±40.1**
	mmol	41.0±17.6	27.6±11.2**	14.5±13.7†, #
Urea	mmol/L	279.7±102.0	71.1±51.1**	125.1±69.2**
	mmol	92.6±38.9	67.0±25.0†	47.3±15.8**
Osmolality	mOsm/kg	639.3±198.4	186.4±176.1**	293.3±175.6**
Solute-free water clearance	mL/min	-1.6±0.7	4.4±3.1**	1.0±2.1*,#
Electrolyte-free water clearance	mL/min	-0.3±0.6	5.7±3.5**	2.1±2.2*,#
Fractional Na^+ excretion		0.69±0.27	0.84±0.33	0.71±0.27
Fractional K^+ excretion		0.12±0.05	0.16±0.05	0.10±0.06##
Fractional Urea excretion		0.45±0.07	0.52±0.09†	0.48±0.07##

Caption: Values are means ± SD. Univariate GLM, post-hoc test: LSD.

†p<0.05, ††p<0.01, *p<0.005, **p<0.001 vs. T0, #p<0.05, ##p<0.005 vs T120.

FIGURES

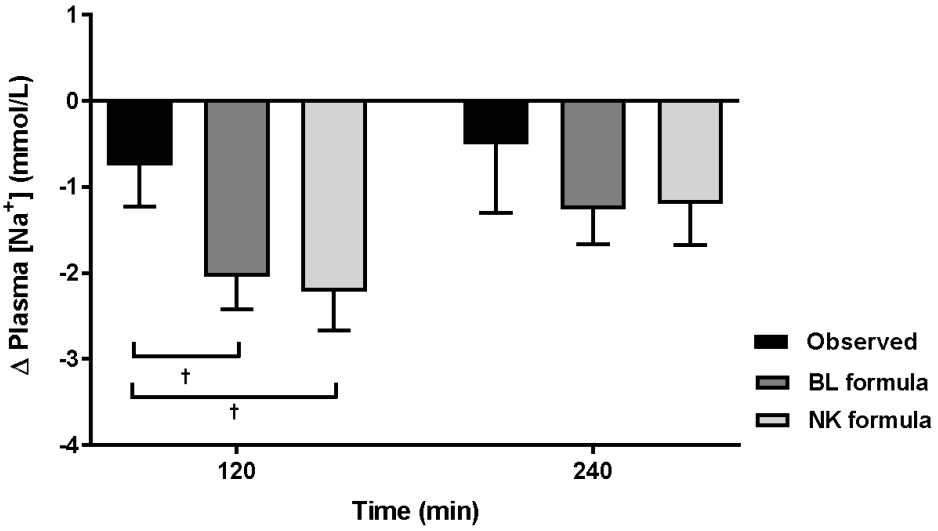
Figure 1



Title: Changes of laboratory parameters after water loading (WL).

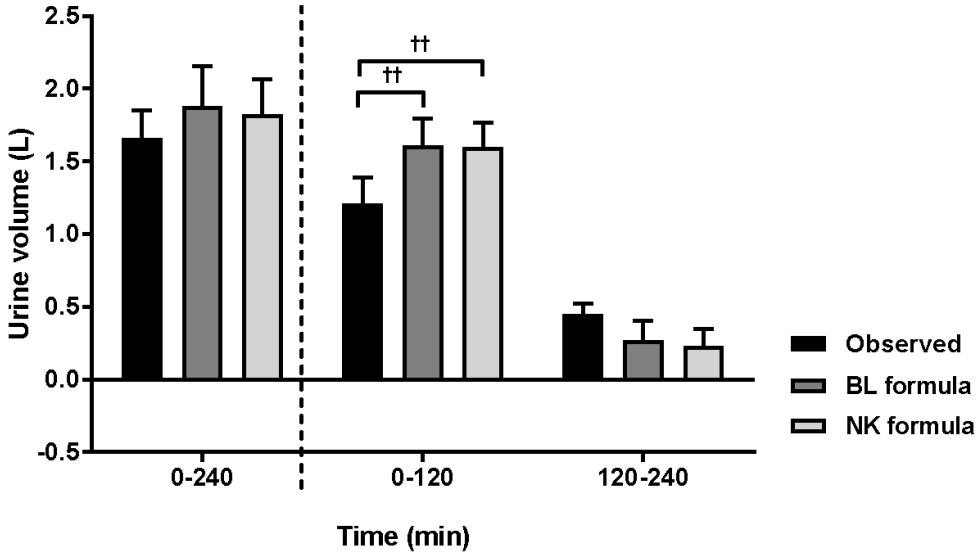
Caption: (A) The maximal decrease in hematocrit was observed 60 min after WL (ratio of dilution -2±0.7%, p=0.03 vs. baseline). (B) The decrease in average plasma [Na^+] was largest 30 min after the start of the WL test (-3.2±0.5 mmol/L, p<0.001 vs. baseline). Values are means ± SEM, **p<0.001, †p<0.05 vs baseline.

Figure 2



Title: Mismatch observed and estimated changes in plasma [Na^+] after water loading (WL).
Caption: Two hours after WL, significant inconsistencies were present for both formulas (Barsoum-Levine (BL): -1.3±0.4 mmol/L, p=0.05 vs. observed; Nguyen-Kurtz (NK): -1.5±0.4 mmol/L, p=0.03 vs. observed), whereas no differences were found four hours after water ingestion (BL: -0.8±0.6 mmol/L, p=0.4 vs. observed; NK: -0.7±0.5 mmol/L, p=0.4 vs. observed). Values are means ± SEM. †p<0.05 *p<0.005 and **p=<0.001 vs. observed values.

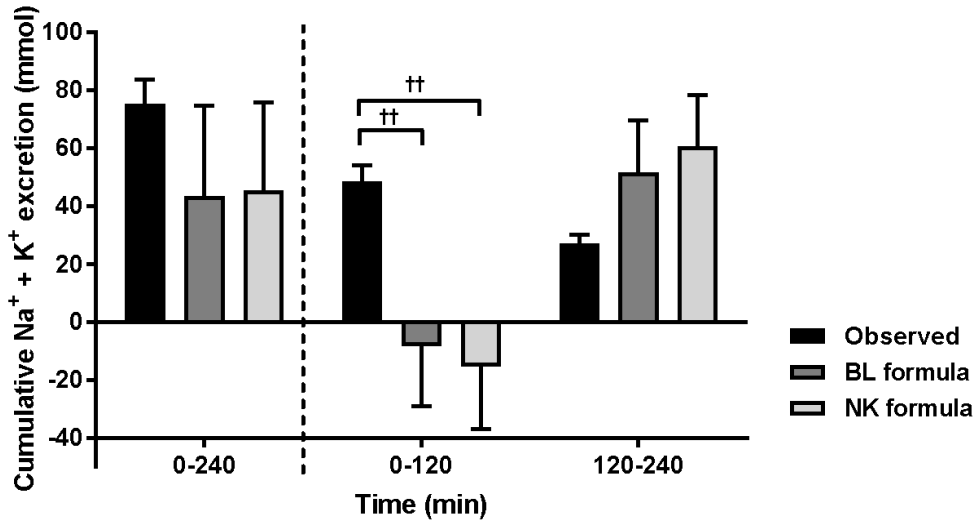
Figure 3



Title: Mismatch between observed and estimated urine volume.

Caption: 120 min after water loading (WL) the expected urine volume was significantly higher than observed (Barsoum-Levine (BL): 0.4±0.1 L, p=0.009 and Nguyen-Kurtz (NK): 0.4±0.1 L, p=0.006 vs. observed). No significant differences were found between the observed and calculated fluid excretion 120 min to 240 min after WL (BL: 0.2±0.1 L, p=0.2 and NK: 0.2±0.1 L, p=0.07 vs. observed). During the entire four-hour follow-up after WL urine volume was similar to expected values (BL: 1.9±0.3 L, p=0.3 and NK:1.8±0.2 L, p=0.3 vs. observed) Values are mean ± SEM. ††p<0.01 vs. observed volume.

Figure 4



Title: Mismatch between observed and estimated urine cation excretion after water loading (WL).

Caption: 120 min after WL the estimated cation excretion was significantly lower than observed (Barsoum-Levine (BL): 56.5±17.8 mmol, p=0.009 and Nguyen-Kurtz (NK): 63.5±18.3 mmol, p=0.005 vs. observed). No significant differences were found between the observed and calculated cation excretion 120 min to 240 min after WL (BL: 27.0±17.1 mmol, p=0.2 and NK: 33.5±17.4 mmol, p=0.4 vs. observed). During the entire four-hour follow-up after WL urine cation excretion was similar to expected values (BL: 43.5±31.2 mmol, p=0.3; NK: 45.4±30.4 mmol, p=0.3 vs. observed). Values are mean ± SEM. ††p<0.01 vs observed cation excretion.