Edelman’s equation is valid in acute hyponatremia in a porcine model: plasma sodium concentration is determined by external balances of water and cations

Christian Overgaard-Steensen,1,2 Anders Larsson,3 Henrik Bluhme,4 Else Tønnesen,1,2 Jørgen Frøkiær,2,5 and Troels Ring6

1Department of Anesthesiology, Aarhus University Hospital, Aarhus, Denmark; 2Institute of Clinical Medicine, Aarhus University Hospital, Skejby, Denmark; 3Department of Anesthesiology and Intensive Care Medicine, Uppsala University Hospital, Uppsala, Sweden; 4Department of Clinical Physiology and Nuclear Medicine, Aarhus University Hospital, Aarhus, Denmark; 5The Water and Salt Research Center, Aarhus University, Aarhus, Denmark; and 6Department of Nephrology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

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Overgaard-Steensen C, Larsson A, Bluhme H, Tønnesen E, Frøkiær J, Ring T. Edelman’s equation is valid in acute hyponatremia in a porcine model: plasma sodium concentration is determined by external balances of water and cations. Am J Physiol Regul Integr Comp Physiol 298: R120–R129, 2010. First published October 28, 2009; doi:10.1152/ajpregu.00412.2009.—Acute hyponatremia is a serious condition, which poses major challenges. Of particular importance is what determines plasma sodium concentration ([Na⁺]). Edelman introduced an explicit model to describe plasma [Na⁺] in a population as [Na⁺] = α·(exchangeable Na⁺ + exchangeable K⁺)/[(total body water) − β]. Evidence for the clinical utility of the model in the individual and in acute hyponatremia is sparse. We, therefore, investigated how the measured plasma [Na⁺] could be predicted in a porcine model of hyponatremia. Plasma [Na⁺] was estimated from in vivo-determined balances of water, Na⁺, and K⁺, according to Edelman’s equation. Acute hyponatremia was induced with desmopressin acetate and infusion of a 2.5% glucose solution in anesthetized pigs. During 480 min, plasma [Na⁺] and osmolality were reduced from 136 (SD 2) to 120 mmol/l (SD 3) and from 284 (SD 4) to 252 mosmol/kgH2O (SD 5), respectively. The following interpretations were made. First, Edelman’s model, which, besides dilution, takes into account Na⁺ and K⁺, fits plasma [Na⁺] significantly better than dilution alone. Second, a common value of α = 1.33 (SD 0.08) and β = −13.04 mmol/l (SD 7.68) for all pigs explains well the plasma [Na⁺] in the individual animal. Third, measured exchangeable Na⁺ and calculated exchangeable Na⁺ + K⁺ per weight in the pigs are close to Edelman’s findings in humans, whereby the methods are cross-validated. In conclusion, plasma [Na⁺] can be explained in the individual animal by external balances, according to Edelman’s construct in acute hyponatremia.

animal model; desmopressin acetate; exchangeable sodium; total body water; regulatory volume decrease

The question of what determines plasma sodium concentration ([Na⁺]) is of particular importance to understand the pathophysiology of hyponatremia. Knowing the determinants of plasma [Na⁺] is the first crucial step in understanding the mechanisms behind the development of hyponatremia and in enabling us to rationally correct and prevent the disorder in the patient. The most explicit model describing plasma [Na⁺] was proposed by Edelman in 1958 (11). According to Edelman’s study of 98 heterogeneous patients in surmounted steady state, the measured plasma [Na⁺] in the group can be described as a function of exchangeable Na⁺ (eNa⁺), exchangeable K⁺ (eK⁺), and total body water (TBW):

[Na⁺] = α·(eNa⁺ + eK⁺)/TBW + β

where α and β are constants estimated from the linear regression.

However, this formula is of little use, unless α and β are relatively precisely known for all patients. Edelman analyzed different factors with a possible influence on α and β, including acid-base disturbances and osmotic inequalities between cells and extracellular fluid (11). When treating patients with hyponatremia, what is needed is not a cross-sectional steady-state population relationship with fixed, common parameters, but a relationship that describes how intervention will predictably influence plasma [Na⁺] in each individual in a dynamic setting rather than in steady state. This is important, for understanding both why and how dysnatremia develops and, in particular, for understanding how to treat it.

Previous studies have implicitly assumed that the dynamic relationship within each patient is identical to the steady-state population relationship (1, 4, 25, 37), but formal assessment of this conjecture has not been carried out. This may partly be due to the practical problems in measuring eNa⁺ and eK⁺. Moreover, eNa⁺, eK⁺, and the measurement of TBW are difficult to handle in the nonsteady state (dynamic setting), which is of interest when modeling changes in plasma [Na⁺] over short periods.

A versatile application of Edelman’s model was advanced by Rose (37). He assumed that changes in the balance of Na⁺ and K⁺ would be directly reflected in changes in the sum of eNa⁺ and eK⁺, and that changes in the water balance would similarly lead to corresponding changes in TBW. Furthermore, Rose suggested a useful simplification of Edelman’s equation by equaling α to 1 and β to 0, and he showed how to employ this in a series of clinical encounters (37). Consistent with this interpretation, Boling finds that, in Edelman’s equation, β is

HYPONATREMIA IS AMONG THE most common electrolyte disorders in clinical practice, with a prevalence of 30% in hospitalized patients (47), and it may be associated with severe cerebral dysfunction and high mortality (2). Conversely, correction of hyponatremia may also cause severe morbidity and mortality (43, 51). Despite the common and severe nature of hyponatremia, its variable pathophysiology remains incompletely understood (51), and it may, therefore, be difficult to treat the condition in a rational way.

Address for reprint requests and other correspondence: C. Overgaard-Steensen, Institute of Clinical Medicine, Aarhus Univ. Hospital, Skejby, Brendstrupgaardvej 100, 8200 Aarhus N, Denmark (e-mail: christian.overgaard.steensen@ki.au.dk).
poorly defined, since no measurements are close to \((e\text{Na}^+ + e\text{K}^-)/\text{TBW} = 0\). Boling also finds that \(\alpha\) is not significantly different from 1 (6).

However, clinical and physiological studies of dysnatremia indicate possible violations of the assumptions underlying Edelman’s equation. First, the phenomenon of regulatory volume decrease (RVD) with cellular efflux of \(K^+\), and organic osmoties to restore volume under hypertone stress (19, 44) would decrease plasma \([\text{Na}^+]\) without changing TBW, and possibly without changing \(e\text{Na}^+\), \(e\text{K}^-\), or the external balances. This would hamper the logic of Edelman’s equation. Second, in various situations, large osmotic activation and inactivation of \(\text{Na}^+\) have been suggested to occur (18, 33, 46). This violates the notion that the external balances of \(\text{Na}^+\) and \(K^+\) determine the changes in exchangeable cations.

Hence, the purpose of the present study was to assess the applicability of Edelman’s equation in acute hyponatremia. This was achieved by a reformulation of Edelman’s equation to obtain a formula in which all ensuing values of plasma \([\text{Na}^+]\) are explicit functions of initial plasma \([\text{Na}^+]\), and the balances of cations and water given an estimate of initial TBW (TBW\(_0\)) in the individual pig. Furthermore, this formulation makes it possible to separate the contribution to the predicted \([\text{Na}^+]\) caused by dilution and electrolyte balance.

In an experimental setting, we then tested the following hypotheses. 1) \([\text{Na}^+]\) can be estimated according to Edelman’s equation in a model of acute hyponatremia. This implies testing the hypotheses. 2) Cation balances contribute to the estimate of \([\text{Na}^+]\) according to Edelman; and 3) \([\text{Na}^+]\) can be estimated with common and specific values of \(\alpha\) and \(\beta\) in the individual pig. Finally, we tested clinical used formulas to estimate plasma \([\text{Na}^+]\). To evaluate this, we developed a clinically relevant model in swine with acute progressive hyponatremia.

**Materials and Methods**

**Animal Preparation**

Sixteen female Landrace/Yorkshire crossbred pigs (28–37 kg) were studied. The study complies with national and international regulations for animal research and welfare. The experiment has been approved by the National Animal Ethics Committee (Dyreforsøgstilsynet, Copenhagen, Denmark; licence 2007-561-1399).

The pigs were fasted overnight with free access to water. Before the animals were transported to the research facility, they were sedated with 0.5 mg/kg midazolam (Dormicum, Hameln Pharmaceuticals, Hameln, Germany) and 1 mg/kg im azaperone (Stresnil, Janssen-Cilag, Neuss, Germany). Anesthesia was induced with 0.5 mg/kg iv etomidate (Hypnomidate, Janssen Pharmaceutica, Beerse, Belgium) and maintained with a continuous infusion of S-ketamine (5–14 mg·kg\(^{-1}\)·h\(^{-1}\); Pfizer, Ballerup, Denmark) and midazolam (1–3 mg·kg\(^{-1}\)·h\(^{-1}\)). The animals were endotracheally intubated (6.5 mm Portex Tracheal Tube, Smiths Medical International) and mechanically ventilated (S/5 Avance, Datex Ohmeda, Madison, WI) by using volume-controlled positive pressure ventilation (positive end-expiratory pressure: 5 cmH\(_2\)O; tidal volume: 8–10 ml/kg; respiratory frequency: 15–20 min\(^{-1}\); inspiratory-to-expiratory ratio: 1:1). Ventilation was adjusted to keep end-tidal CO\(_2\) between 5.5 and 6.0 kPa (17). Monitoring with ECG and pulse oxymetry was established. The temperature was held within 38–40°C (normal physiological range: 37.0–39.6°C (17)) by using an electric heating blanket.

**Surgical Procedures**

Before the surgical procedures, the animal received 750 mg iv of cefuroxime Stragen (Stragen Nordic, Stenlose, Denmark), and the skin was prepared with a 0.5% chlorhexidine/85% ethanol solution. A Radifocus Introducer II Fr.8 (Terumo, Tokyo, Japan) was inserted by an open approach into the external jugular vein (for fluids, drug administration, and a Swan-Ganz catheter). For blood pressure, heart rate, and for blood sampling, a Radifocus Introducer II Fr.6 (Terumo) was inserted into the common carotid artery. A pulmonary artery catheter (CCombo, Edwards Lifesciences, Irvine, CA) was advanced via the external jugular vein into a branch of the pulmonary artery for measurements of cardiac output and temperature (Baxter Vigilance, Edwards Lifesciences). A suprapubic bladder catheter was inserted by an open procedure for precise urine collection (20 Ch Foley Catheter: Unomedical, Kedah, Malaysia). Skin wounds were closed meticulously with 2-0 Ethilon (Johnson and Johnson, Hamburg, Germany).

**Experimental Protocol**

Animals were randomized to a hyponatremia group (n = 10) or a control group (n = 6). After instrumentation, the hyponatremia group received a single intravenous dose of 4 μg of desmopressin acetate (DDAVP) (Minirin, Ferring, Limhamn, Sweden) and infusion of 2.5% glucose (glucose 50 mg/ml; Fresenius Kabi, Uppsala, Sweden) mixed 1:1 with sterile water (Baxter, Lessines, Belgium) at a rate of 10 ml·kg\(^{-1}\)·h\(^{-1}\), together with 5 ml·kg\(^{-1}\)·h\(^{-1}\) Ringer lactate (Na\(^+\): 130 mmol/l, K\(^+\): 4 mmol/l, Ca\(^{2+}\): 1.5 mmol/l, Cl\(^-\): 109 mmol/l, lactate: 28 mmol/l; osmolality: 260 mosmol/kgH\(_2\)O; Fresenius Kabi, Uppsala, Sweden) for 8 h. The DDAVP dose chosen was based on pilot experiments with measurement of DDAVP concentration and the effect on \([\text{Na}^+]\). The control group was infused with Ringer lactate at a rate of 5 ml·kg\(^{-1}\)·h\(^{-1}\) for 8 h. Blood pressure, heart rate, cardiac output, and temperature were monitored in all animals throughout the experiment. We measured the weight before and after the experiment (Soehnle Professional and Backnang).

**Laboratory Measurements**

Blood samples were drawn before intervention and successively every hour from the common carotid artery into lithium-heparin tubes and centrifuged for 10 min at 3,500 RPM. Plasma was analyzed for \([\text{Na}^+]\), \([\text{K}^+]\), [creatinine], [urea], [albumin], and [Mg\(^{2+}\)] (where brackets denote concentration) by using a Vitros 5.1 FS analyzer (Ortho-Clinical Diagnostics, Copenhagen, Denmark).

Arterial blood was analyzed (ABL system 615/700, Radiometer, Copenhagen, Denmark) to determine pH, Po\(_2\), PCO\(_2\), [base excess], [standard bicarbonate], [hemoglobin], hematocrit level, [glucose], [lactate], [Cl\(^-\)], and [Ca\(^{2+}\)].

Urine was collected, and diuresis was recorded every hour. In the urine samples, \([\text{Na}^+]\) and \([\text{K}^+]\) were determined by means of an Eppendorf Flame Photometer (Eppendorf, Hamburg, Germany), and [creatinine], [urea], [albumin], [glucose], and [Mg\(^{2+}\)] were determined by using a Vitros 5.1 FS analyzer (Ortho-Clinical Diagnostics, Copenhagen, Denmark). Urine [Cl\(^-\)] was determined by using an ion-selective electrode, ADVIA 1650 Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY).

\(^1\) In pilot experiments (n = 10), 5% glucose infusion was used. This resulted in significant hyperglycemia (up to 30 mmol/l). Using 2.5% glucose infusions resulted in a plasma glucose value of 7.3 mmol/l (SD 1) compared with 6.3 mmol/l (SD 1) in the nonhypotone group. Urine glucose was measured in experiments 18-26. One pig had glucosuria with 38 mmol/l in the total urine. A slight hemolytic reaction was observed in the first blood samples after insertion of the arterial introducer. There was no conjunction with infusion of a hypotone solution, as the hemolytic reaction was manifest before the infusion began and also present in the control animals. Pilot studies showed no increase in free bilirubin (<22 μmol/l) or lactate dehydrogenase at time points of 0, 300, and 450 min after infusion of the hypotone solution.

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Plasma and urine for determination of osmolality were immediately frozen at minus 80°C. Osmolality was measured after thawing by using an Advanced model 3900 Multi-Sample Osmometer (Advanced Instruments).

Deuterium oxide space (V-D2O) was used to determine TBW. To determine V-D2O, the principle of dilution was applied (see APPENDIX for details):

\[
V - D2O = (V_i - V_o)(Dplasma_{480} - Dplasma_0)
\]

where \(V_i\) is injected volume D2O; \(V_o\) is D2O volume in urine; and \(Dplasma_{480}\) and \(Dplasma_0\) are fractional abundances of deuterium in the plasma at time points 0 and 480 min, respectively.

The animals were given a bolus of 10 g of D2O 100.0 atom%D (Sigma-Aldrich Chemie, Steinheim, Germany). The weight of the syringe was measured before and after injection (Mettler AJ150, Struers Chem, Roedovre, Denmark), and the weight difference (\(\Delta W\)) was calculated. Deuterium analysis was carried out as follows. Samples were pipetted into septum sealed containers (in duplicate). Platinum (5%) on alumina catalyst (in insert vials) was then added to the containers. The containers were sealed, and the head space was flushed with pure hydrogen. Reference waters (including a quality control standard) were prepared in the same manner. When all containers had been flushed, they were left for a period of 3 days to ensure complete equilibration of the water with the hydrogen. The samples and references were then analyzed by continuous flow-isotope ratio mass spectrometry, where a Europa Scientific ANCA-GSL and a Geo 20–20 IRMS were used. Samples for each subject were bracketed together for analysis. The analyses were performed by Iso-Analytical (Cheshire, UK).

TBW to time 0 was calculated as TBW to time 480 (V-D2O time 480) minus the accumulated water balance after 480 min (\(\Delta TBW\) time 480). \(\Delta TBW\) is the sum of fluid input minus the urine output. We did not take evaporation into account, because the animals were ventilated with a closed circuit system, and the 5-cm wounds on the neck and the abdomen were firmly closed.

eNa+ was determined by using Na-22 (PerkinElmer Danmark A/S, Hvidovre, Denmark). Na-22 (0.6 MBq) was intravenously injected, and blood samples were drawn 29 times from the time of injection to 480 min. The activity of Na-22 in 2.00-ml standards and plasma samples were counted in a Cobra II Auto-Gamma well scintillation counter (Packard Instruments) preset for 10,000 counts. Counting was performed in the first 4 h and over the last 4 h. The Na-22 activity and the Cr-51 activity were determined in the same samples. There was no spill from Cr-51 up to the Na-22 window, but there was ~10% spill from Na-22 down to the Cr-51 window when counting the activities. We have accounted for this in the calculations.

Calculations and Statistical Analysis

To assess the applicability of Edelman’s equation, Eq. A1 was reformulated to Eq. A5 (see APPENDIX for derivation):

\[
[Na^+]_s = [Na^+]_i + \frac{TBW_{i,0}}{TBW_{i,0} + \Delta TBW_{i,j}} \Delta (Na^+ + K^+)_{i,j} + \frac{TBW_{i,0} + \Delta TBW_{i,j}}{TBW_{i,0} + \Delta TBW_{i,j}} \Delta (Na^+ + K^+)_{i,j} \Delta [Na^+ + K^+]_{i,j}
\]

where all ensuing values of [Na+]_s are explicit functions of initial [Na+]_i,0 and the balances of cations (\(\Delta (Na^+ + K^+)_{i,j}\)) and water (\(\Delta TBW_{i,j}\)), given an estimate of initial TBW (TBW_0). Subscript “i” denotes pig, and subscript “j” denotes time point. A formula like this is used by Kurtz and Nguyen (24), but, in contrast to their equation, Eq. A5 makes it straightforward to separate the effect of dilution alone from the entire Edelman construct.

R version 2.7.1 was used for calculations and statistics. Data are presented as means with SD. The models were fitted by using multiple linear regressions. The statistical significance between different models was assessed by using ANOVA. Differences between groups were assessed by unpaired t-test when normally distributed or, if not, by the Mann-Whitney rank-sum test. Hierarchical modeling was accomplished by using Imer (5).

Altogether, we made 126 complete observations on 16 pigs over 9 h, which means that we had 18 missing values. Missing values were caused by unforeseeable technical mishaps unrelated to the status of the pigs and, therefore, assumed to be completely at random and ignored in the analysis.

RESULTS

DDAVP Induces Acute Hyponatraemia in Pigs

In the present study, a reproducible, progressive hyponatremic pig model was established. Intravenous DDAVP administration caused progressive hyponatremia (Fig. 1A). The plasma [Na+] was reduced from 136 mmol/l (SD 2) to 120 mmol/l (SD 3) after 480 min. In parallel, there was a progressive reduction in the plasma [Cl–] [from 101 mmol/l (SD 3) to 86 mmol/l (SD 2)] (Fig. 1B) and in the plasma osmolality [from 284 mosmol/kgH2O (SD 4) to 252 mosmol/kgH2O (SD 5)] (Fig. 1D). The plasma [K+] was unchanged. Thus the plasma [Na+] determines the plasma osmolality in this model (excluding substantial translocational hyponatremia). The animals were hemodynamically stable, with a mean arterial pressure of 94 mmHg (SD 17) and a heart rate of 77 beats/min (SD 18). The renal parameters are shown in Table 1.

Edelman’s Model Fits Plasma [Na+] Significantly Better Than Dilution Alone

To examine how either dilution alone or the complete Edelman model was able to explain the observed values of

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and so the external balances of $K^+$ and $Na^+$ are also taken into account.

Since the hyponatremia model is primarily dilutional in nature, it is not surprising that the dilutional model describes the data well, with $R = 0.86$ and $P < 0.01$. Nevertheless, Edelman’s model (Eq. A5), which is based on external balances of both water and electrolytes, fits the observed values better ($R = 0.98$, $P < 0.001$). When comparing the two models by using ANOVA, Edelman’s model is significantly better than the dilutional model, with an $F$ of 142 on 2 and 122 degrees of freedom, yielding a $P$ value $< 0.001$. Finally, examining the residuals from the dilution-only model revealed these to be significantly correlated ($P < 0.001$) with the electrolyte balance, indicating a clear formal defect in the dilution model, which was exactly corrected for in the complete Edelman model.

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**Fig. 1.** Plasma concentration of $Na^+$ ($[Na^+]$), (A); $Cl^-$ ([Cl$^-$]), (B); and $K^+$ ([K$^+$]), (C). D: plasma osmolality. ●, Hyponatremic animals; ○, control animals.
model. Thus Edelman’s model is significantly better than the dilution model when describing plasma [Na\(^+\)] in this pig model (Fig. 2).

This model, fitting a common \(\alpha\) and \(\beta\) for all measurements in all pigs, is the format of Edelman’s construct, which would make it most useful, since knowing the \(\alpha\) and \(\beta\) would make accurate estimates available in the individual pig. Regression of Eq. A5 by pooling all observations from all pigs yields a population estimate with \(\alpha = 1.33\) (SD 0.08) and \(\beta = -13.04\) mmol/l (SD 7.68) \((R = 0.98\) and \(P < 0.001)\). The model was well behaved as assessed by the residuals.

However, the measurements are not independent, as we have 9 measurements clustered in 16 pigs. To examine whether the clustering between measurements, which were obtained in each individual pig, influences the results, analysis was carried out using a hierarchical model.

In this hierarchical model, where the coefficients are assigned a distribution with random values for each animal, the clustering is taken appropriately into account, but at the price of a higher complexity. Hence, by fitting more parameters, this yields an even closer match between the measured and the fitted values (Fig. 3).

The hierarchical model yields a fixed effect for \(\alpha\) of 0.9784 (SD 0.12), and a random effect for \(\alpha\) with mean = 0 and SD 0.28, and, hence combined, a 95% confidence interval for \(\alpha\) between 0.66 and 1.49, and likewise for \(\beta\), a 95% confidence interval of \(-47.312\) to 56.640. No significant autocorrelation in residual error was found. However, the increased complexity of the hierarchical model does not seem to lead to a worthwhile improvement of the ability to explain the changes in [Na\(^+\)]. Hence, the complete pooling model yields a compact and useful summary, also of the hierarchical model. This implies that determination of the [Na\(^+\)] changes in the individual animal is possible by using the population-based coefficients in the pig model, thereby making Edelman’s construct useable in practice.

Measured eNa\(^+\) vs. Calculated eNa\(^+\) + eK\(^+\) in Pigs Are Close to Edelman’s Findings in Humans

To validate the model (Eq. A5), which is derived from Edelman’s Eq. 1, where successive balance data from pigs are used to estimate \(\alpha\), \(\beta\), and plasma [Na\(^+\)], we plot the data with Edelman’s original data from humans. The most straightforward method would be to plot plasma [Na\(^+\)] with (eNa\(^+\) + eK\(^+\))/TBW, but, unfortunately, we were not able to measure eK\(^+\). To use both estimated values (eNa\(^+\) + eK\(^+\)) and measured values (eNa\(^+\)), which are accessible in the pig study and in Edelman’s original work (11), we plot the computed (eNa\(^+\) + eK\(^+\)) kg (Eq. A3) vs. the measured eNa\(^+\)/kg (Fig. 4). This indicates that, in our model, Edelman’s construct, which is based on external balances, yields parameter estimates, which together determine essential bodily measurements, which are almost congruous when comparing pigs to humans.

**DISCUSSION**

The study demonstrate that Edelman’s model, which, besides dilution, also takes into account Na\(^+\) and K\(^+\), fits plasma [Na\(^+\)] significantly better than dilution alone and also leads to
explains the plasma [Na\(^+\)] and osmotic activation of Na\(^+\) urine output and have a time frame of 12–24 h, where RVD (26), are lacking the complete balance: they do not account for damage. The clinical studies, which have been carried out (8, 36, 45). Third, a large animal was chosen to provide accurate hourly measurements of urine production, uniform blood sampling with the possibility of consecutive sampling, and accurate monitoring of hemodynamics and ventilation.

**Edelman’s Model Works in an Experimental Model Based Primarily on Dilution**

In clinical practice, it has been assumed that changes in plasma [Na\(^+\)] can largely be understood on the basis of changes in hydration alone, since this is often by far the most important single effect (38). If this is true, we do not need Edelman’s more complicated construct to predict plasma [Na\(^+\)] in acute hyponatremia. By comparing the simple dilution model of the initial plasma [Na\(^+\)] to fit plasma [Na\(^+\)] with the entire Edelman construct, we demonstrate that Edelman’s construct estimates plasma [Na\(^+\)] significantly better with a \(P\)-value < 0.001. Furthermore, when examining the residuals from the two models against the cation balances, the residuals from the dilution model have a significant correlation (\(P < 0.001\)), which was accounted for in the full Edelman model. This implies that, even in a model, where the hyponatremia is based primarily on hypotonic dilution, the balances of Na\(^+\) and K\(^+\) are important. An experimental model with larger shifts in electrolyte balances would logically magnify the difference between the simple dilutional and complete Edelman model.

**Cross-Validation Using eNa\(^+\)**

To validate the model (Eq. A5), a plot using measured eNa\(^+\)/kg against estimated eNa\(^+\) + eK\(^+\) per weight in the pigs is close to Edelman’s findings in humans, whereby the methods are cross-validated.

**Animal Model**

The pig model of hyponatremia described here was developed in an attempt to study whether plasma [Na\(^+\)] can be explained when [Na\(^+\)] is declining in an experimental animal. The use of experimental animals in this area is crucial, because a lowering of plasma [Na\(^+\)] implies a substantial risk of brain damage. The clinical studies, which have been carried out (8, 26), are lacking the complete balance: they do not account for urine output and have a time frame of 12–24 h, where RVD and osmotic activation of Na\(^+\) would have occurred. Exceptions are the work of Deming and Gerbode (10), Steele et al. (42), Guglielminotti et al. (16), and Mallié et al. (28), but here the overall changes in plasma [Na\(^+\)] are <4.5 mmol/l. These small changes may mask model defects.

Several studies using vasopressin and infusion in dogs have been described (21, 23, 40, 41). But the objectives have not been to estimate [Na\(^+\)] as the hyponatremia developed. A model of peroral water load is described by Chan (9), but it is not suitable for acute studies of [Na\(^+\)]. Furthermore, dogs are generally small compared with humans, and they have a different renal physiology (36).

In contrast to previous models with reproducible hyponatremia in other species (13, 15, 48, 49), we needed a model of progressive hyponatremia over a few hours with accurate balances. First, we chose an infusion model because we needed a stable model with accurate balances, and because we find this model clinically relevant. Second, pigs were chosen because of their physiological similarity with humans; especially the water and electrolyte regulation in the multirenulate and multipapillate kidneys are very similar to the regulation in humans (32, 36, 45). Third, a large animal was chosen to provide accurate hourly measurements of urine production, uniform blood sampling with the possibility of consecutive sampling, and accurate monitoring of hemodynamics and ventilation.

**Edelman’s Model Works in an Experimental Model Based Primarily on Dilution**

In clinical practice, it has been assumed that changes in plasma [Na\(^+\)] can largely be understood on the basis of changes in hydration alone, since this is often by far the most important single effect (38). If this is true, we do not need Edelman’s more complicated construct to predict plasma [Na\(^+\)] in acute hyponatremia. By comparing the simple dilution model of the initial plasma [Na\(^+\)] to fit plasma [Na\(^+\)] with the entire Edelman construct, we demonstrate that Edelman’s construct estimates plasma [Na\(^+\)] significantly better with a \(P\)-value < 0.001. Furthermore, when examining the residuals from the two models against the cation balances, the residuals from the dilution model have a significant correlation (\(P < 0.001\)), which was accounted for in the full Edelman model. This implies that, even in a model, where the hyponatremia is based primarily on hypotonic dilution, the balances of Na\(^+\) and K\(^+\) are important. An experimental model with larger shifts in electrolyte balances would logically magnify the difference between the simple dilutional and complete Edelman model.

**Cross-Validation Using eNa\(^+\)**

To validate the model (Eq. A5), a plot using measured eNa\(^+\)/kg against estimated eNa\(^+\) + eK\(^+\) was made in Fig. 4, together with Edelman’s original data. To determine eNa\(^+\), other investigators use the principle of dilution 24 h after injection of the isotope (11, 40), and others argue for even longer periods in the edematous patient (34). To investigate the pigs in acute hyponatremia over 8 h, we estimated the steady-state

![Fig. 3. Fit of plasma [Na\(^+\)] vs. measured [Na\(^+\)] by using the entire Edelman construct in a hierarchical model. ●, Hyponatremic animals; ○, control animals.](http://ajpregu.physiology.org/)

![Fig. 4. (Exchangeable Na\(^+\) + exchangeable K\(^+\))/kg vs. measured exchangeable Na\(^+\)/kg. ●, Edelman’s original data; ○, pig data; vertical lines, standard deviation pig data; solid line, regression line from Edelman’s data; dashed line, regression line from pig data.](http://ajpregu.physiology.org/)

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activity by fitting the Na-22 disappearance curve from plasma to three monoeXponential functions and a constant (Fig. 5). The fit obtained was excellent ($P < 0.001$). At 8 h, the mean relative difference from the steady-state activity was 9% (SD 6). Extrapolation of the curve to 12 h gives a mean relative difference of 4% (SD 3). We do not know if an additional exponential function is hiding after 24 h. If this is the case, it would tend to overestimate eNa$^+$. We observed eNa$^+$/kg = 54.7 mmol/kg (SD 6.8). This is consistent with the literature: Johnson et al. (22) measured eNa$^+$/kg = 49.7 in swine (70 kg). That we observe higher values of eNa$^+$/kg in young pigs is comparable with the results in children, where eNa$^+$/kg declined from 82.5 mmol/kg in the newborn to 43 mmol/kg at the age of 14 yr (12). Furthermore, no difference was seen between eNa$^+$/kg in the hypotone group and in the control group ($P = 0.42$, Mann-Whitney rank-sum test).

**Physiological Consequences**

No acute global RVD. Using different methods, we demonstrate that changes in plasma [Na$^+$] can be estimated from external balances of water and electrolytes in acute hyponatremia. This indicates that global RVD is unlikely to have strongly influenced the results. Since the bulk of water and electrolytes in the body is in the muscles, this organ is of primary importance on a global level. If the muscles [60% of the pig mass, containing 80% water (30)] were making 100% RVD after they have increased 10% in volume (consistent with a 10% decrease in plasma [Na$^+$] and osmolality) with efflux of K$^+$ and Cl$^-$(minutes) and organic osmolytes (hours), as described in the literature (19, 20, 27, 35, 44), it would lower the plasma [Na$^+$]$_{180}$ from 120 to 112 mmol/l. This does not exclude the well-described acute adaptive response in smaller organs, such as the brain (3, 29, 52), since the impact on the entire system would be small and not detectable from plasma measurements of [Na$^+$]. On the global level, the excretion of 0.11 mmol·kg$^{-1}$·h$^{-1}$ K$^+$ in the urine is equal in the control animals and in the hypotone animals, excluding a substantial loss of K$^+$ in the hypotone state as a consequence of RVD. Therefore, acute RVD is unlikely to occur at a global level in this model. This indicates difficulties in extrapolation from the studies of acute RVD in very specialized cells [tubulus cell in the kidney (27), mucosa cell in the gut, Ehrlich ascites tumor cells (19)] to the entire organism. Furthermore, the cells are studied in conditions where the tonicity is momentarily decreased by 50%, and, although this might be of interest in connection with the particular cell (kidney tubulus cell or the gut mucosa cell), it is hardly relevant for the entire organism. On the other hand, it is well described that a chronic down-regulation of whole body volume occurs in maintained hyponatremia (50). How that occurs and is mediated, for instance in the skeletal muscle, is not well described.

No substantial osmotic activation/inactivation. That the changes in plasma [Na$^+$] are determined by external balances of Na$^+$, K$^+$, and water also implies that no substantial osmotic activation of stored osmotically inactive Na$^+$ is taking place in our model. Previously, it was demonstrated that there may be substantial osmotic activation of Na$^+$ (18, 33, 46). This would then lead to a decline in plasma [Na$^+$], which is less than expected from the dilution. In a recent balance study in Na$^+$-loaded dogs, it was shown that the sum of changes in total body Na$^+$ and K$^+$ was accompanied by osmotically adequate TBW changes (39). Consistent with this, the results from the balance study in water-loaded pigs demonstrate that the external cation balances and water balances perfectly match the plasma [Na$^+$] decline, leaving no space for substantial osmotic activation of osmotically inactive Na$^+$.

In a study of exercise-induced hyponatremia, Noakes et al. (33) found that the majority of athletes, who gained weight, had no corresponding decrease in plasma [Na$^+$] after exercise. This requires the addition of significant amounts of Na$^+$ into an expanded volume of TBW. This Na$^+$ is likely to originate from osmotically inactive eNa$^+$ stores (33). In the present study, pigs gained weight, but the plasma [Na$^+$] decline was predictable from the balances. Therefore, no significantly internal addition of osmotically active Na$^+$ violates Edelman’s equation in this setting. However, pigs were anesthetized and not exhausted by physical activity as the athletes in the work by Noakes et al. (33). Furthermore, it could be speculated that the effect of RVD would lower [Na$^+$] further and osmotic activation of Na$^+$ (tends to increase [Na$^+$]) might cancel each other, so we only observe the dilution effect of water loading plus the change in cation balances in these animals. Hence, we cannot exclude that whole body RVD and osmotic activation could occur together, but the finding of equal K$^+$ excretion in the two animal groups makes it unlikely. Likewise, the reported strong relationship between the decline in measured osmolality and the decline in plasma [Na$^+$] indicates no room for the organic osmolytes, which are thought to be involved in RVD or translational hyponatremia.

Simplification of Edelman’s equation may be clinically valuable. A simplified approach, where $\alpha = 1$ and $\beta = 0$ mmol/l (37), has previously been suggested to determine changes in plasma [Na$^+$]. Fitting the changes in plasma [Na$^+$] from the pig balances by using these values for $\alpha$ and $\beta$ in Eq. A5 shows that the simplification tends to overestimate [Na$^+$] by 2 mmol/l (Fig. 6). Nevertheless, 2 mmol/l is within the acceptable uncertainty range when plasma [Na$^+$] is determined in most clinical laboratories. On the other hand, using $\alpha$ equal to 1 and $\beta$ equal to 0 mmol/l results in a mean difference of 9 mmol/l in the cross-sectional assessment using Edelman’s data set (11). However, if our results that $\alpha$ and $\beta$ are stable within

![Fig. 5. Example that illustrates the fitting procedure to estimate the steady-state activity of Na-22 in pig 25. Solid line, entire fit; dashed lines, fit components. cpm, Counts/min.](http://ajpregu.physiology.org/ by 10.220.32.247 on October 14, 2017)
changes, but further clinical studies in humans are needed to find and validate simplifications of the Edelman construct. Hereby also, account must be taken of models in which dysnatremia is developed primarily by electrolyte imbalances compared with our model focused on dilutional changes.

Adrogue’s formula is overestimating [Na⁺] in our model of hyponatremia. The capability to estimate plasma [Na⁺] can be compared with the widely used formula made by Adrogue and Madias (1): changes in serum Na⁺ = [(infusate Na⁺ + infusate K⁺) − serum Na⁺]/(TBW + 1).

When using data from the pigs, where quantity and quality of the input are known and TBW = 60%, Adrogue’s formula gives an estimate of [Na⁺] after 8 h of 135 mmol/l (SD 3), which is significantly higher (P < 0.001) than the measured [Na⁺] = 126 mmol/l (SD 8). In this formula, only the input side of the water and cation balances is considered. The consequence is an overestimate of anticipated [Na⁺] compared with measured [Na⁺]. In clinical studies (26, 31) using Adrogue’s construct, measured [Na⁺] was significantly underestimated as hyponatremia was corrected. That the causes of hyponatremia are diverse and [Na⁺] is not only a matter of input are exemplified also by the phenomenon of desalination found in postoperative patients by Steele et al. (42) and in experimental models (13, 14).

In conclusion, we have demonstrated a feasible model for studying the effects of progressive hyponatremia in a large animal. Edelman’s concept works in this setting: first, cation balances contribute significantly to the estimate of [Na⁺] besides water balance, and, second, a common value of α and β for all pigs explains well the plasma [Na⁺] in the individual animal.

**Perspectives and Significance**

In clinical practice, it is of paramount importance that we understand plasma [Na⁺] in acute hyponatremia, so patients are treated rationally and cerebral disasters are avoided. To the extent that the study can be generalized, Edelman’s model is a valuable tool in guiding the management and prevention of acute hyponatremia. This implies that plasma [Na⁺] can be estimated simply from the initial plasma [Na⁺] and the balances of water and electrolytes in the patient, given a reasonable assessment of TBW. Future studies are needed to investigate different situations where plasma [Na⁺] is changing: more severe hyponatremia, faster decline in plasma [Na⁺], exercise-induced hyponatremia, hypernatremia, and hyponatremia with larger shifts in electrolyte balances and in a more chronic setting. Furthermore, since plasma [Na⁺] is determined by external balances in this acute pig model, it is not strongly influenced by global RVD. Hence, interest is focused on the transition between acute and chronic hyponatremia, in which global volume regulation related to RVD is well documented. Future studies at organ and cellular levels in the intact organism are warranted to address these aspects.

**APPENDIX: FORMULA AND CALCULATIONS**

**Derivation of α and β**

Assuming Edelman’s equation fits all points of measurement, we have the following general equation:

$$[\text{Na}^+]_\text{2} = \frac{[\text{Na}^+]_\text{1} \times \text{TBW}_1 + \Delta(\text{Na}^+ + \text{K}^+)}{\text{TBW}_1 + \Delta\text{TBW}}$$

The formula is also described by Mallié et al. (28), but without investigation of the underlying assumptions and with verification only in patients with minor changes in plasma [Na⁺]. The experimental data from the study of hyponatremic pigs indicate that the underlying assumptions are not violated, and that the formula may be useful in a wide range of plasma [Na⁺]

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**Fig. 6.** Fitted plasma [Na⁺] vs. measured [Na⁺] by using α = 1 and β = 0 (A), and α = 1 and β = 0, with all of the estimated values subtracted with 2 mmol/l (B). ●, Hyponatremic animals; ○, control animals.
\[ [Na^+]_{ij} = \frac{(eNa^+ + eK^+)}{TBW_{ij}} + \beta_{ij} \]  

(A1)

where subscript “j” denotes index time point, and subscript “i” denotes pig number.

The following assumptions are made for further analysis. 1) Changes in external balances for Na\(^+\) and K\(^+\) \((\Delta(Na^+ + K^+))\) are equal to the change in exchangeable cations \((eNa^+ + eK^+)\). 2) \(\alpha\) and \(\beta\) are the same in all measurements. 3) Changes in TBW in can be measured by external balances of water (\(\Delta TBW\)) (see below).

For an individual pig \(i\) at measurement \(j\) (time \(0\) is baseline), we have:

\[ [Na^+]_{ij} = \frac{(eNa^+ + eK^+)}{TBW_{ij}} + \beta \]

(A2)

Since

\[ [Na^+]_{i,0} = \frac{(eNa^+ + eK^+)}{TBW_{i,0}} + \beta \Leftrightarrow (eNa^+ + eK^+),0 \]

\[ = \frac{TBW_{i,0}([Na^+]_{i,0} - \beta)}{\alpha} \]

(A3)

Substituting \((eNa^+ + eK^+)\) in Eq. A2 with Eq. A3 gives

\[ [Na^+]_{ij} = \frac{TBW_{i,0}([Na^+]_{i,0} - \beta)}{\alpha} + \Delta(Na^+ + K^+)_{ij} \]

(A4)

\[ [Na^+]_{j} = \frac{[Na^+]_{i,0}TBW_{i,0} + \Delta TBW_{ij}}{TBW_{i,0} + \Delta TBW_{ij}} \]

(A5)

\[ [Na^+]_{j} = \frac{[Na^+]_{i,0} + \Delta(Na^+ + K^+)_{ij}}{TBW_{i,0} + \Delta TBW_{ij}} \]

By using this equation (Eq. A5), the measured balances of water and cations can be fitted to the initial plasma \([Na^+] = [Na^+]_{i,0}\) and the initial TBW = \(TBW_{i,0}\). Using the hourly data from the pigs as independent variables, the only unknown parameters are \(\alpha\) and \(\beta\), which can be determined with multiple regressions.

**TBW Determination**

\[ V - D_{2O} \] was used to determine TBW. To determine \(V - D_{2O}\), the principle of dilution was applied:

\[ V - D_{2O} = (D_{2O_{\text{input}}} - D_{2O_{\text{output}}})/ (\text{Steady-state}[D_{2O}] - \text{initial}[D_{2O}]) \]

\(D_{2O}\) input = injected volume \(D_{2O} = V_i = (\Delta W \cdot W\%) / \delta_{D_{2O}}\)

\(\Delta W = \text{weight of injected } D_{2O}, W\% = \text{weight } \% \text{ of } D_{2O} = 0.996, \) and

\(\delta_{D_{2O}} = \text{density of } 100\% \text{ of } D_{2O} = 20^\circ\text{C} = 1.105 \text{ g/ml}\)

\(D_{2O_{\text{input}}} = \text{Total output in urine} = V_u = \text{volume of urine} \cdot D_{\text{urine,48h}}\)

\(\frac{D_{\text{urine,48h}}}{D_{\text{plasma,0}}} = \text{fractional abundance of deuterium in the total amount of urine}

\(D_{\text{urine,0}} = D_{\text{plasma,0}} \) (since the deuterium fraction in all of the body fluids is the same at time \(0\)).

In steady state

\[ [D_{2O}]_{\text{plasma}} - \text{initial} = [D_{2O}]_{\text{plasma,480}} = D_{\text{plasma,480}} - D_{\text{plasma,0}} \]

\(D_{\text{plasma,480}}\) and \(D_{\text{plasma,0}}\) are fractional abundances of deuterium in plasma at \(t = 480 \text{ min} \) and \(t = 0\).

Therefore, we have

\[ V - D_{2O} = (V_i - V_u)/(D_{\text{plasma,480}} - D_{\text{plasma,0}}) \]

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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