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Evaporation of free water causes concentrational alkalosis in vitro

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Summary
Background The development of metabolic alkalosis was described recently in patients with hypernatremia. However, the causes for this remain unknown. The current study serves to clarify whether metabolic alkalosis develops in vitro after removal of free water from plasma and whether this can be predicted by a mathematical model.

Materials and methods Ten serum samples of healthy humans were dehydrated by 29 % by vacuum centrifugation corresponding to an increase of the contained concentrations by 41 %. Constant partial pressure of carbon dioxide at 40 mmHg was simulated by mathematical correction of pH [pH(40)]. Metabolic acid–base state was assessed by Gilfix' base excess subsets. Changes of acid–base state were predicted by the physical–chemical model according to Watson.

Results Evaporation increased serum sodium from 141 (140–142) to 200 (197–203) mmol/L, i.e., severe hypernatremia developed. Acid–base analyses before and after serum concentration showed metabolic alkalosis with alkalemia: pH(40): 7.43 (7.41 to 7.45) vs 7.53 (7.51 to 7.55), $p=0.0051$; base excess: 1.9 (0.7 to 3.6) vs 10.0 (8.2 to 11.8), $p=0.0051$; base excess of free water: 0.0 (−0.2 to 0.3) vs 17.7 (16.8 to 18.6), $p=0.0051$. The acidifying effects of evaporation, including hyperalbuminemic acidosis, were beneath the alkalinizing ones. Measured and predicted acid–base changes due to serum evaporation agreed well.

Conclusions Evaporation of water from serum causes concentrational alkalosis in vitro, with good agreement between measured and predicted acid–base values. At least part of the metabolic alkalosis accompanying hypernatremia is independent of renal function.

Keywords Acid–base · Alkalosis · Dehydration · Hypernatremia

Short summary: The occurrence of metabolic alkalosis has been described recently in critically ill patients developing hypernatremia. To clarify whether a pure dehydration as cause of hypernatremia contributes to the development of metabolic alkalosis, we dehydrated serum samples of 10 healthy volunteers. Acid–base analysis before and after dehydration showed development of marked metabolic alkalosis by 29 % dehydration.

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Evaporation von freiem Wasser führt zur Entwicklung einer Konzentrationsalkalose in vitro

Zusammenfassung
Grundlagen Die Entstehung einer metabolen Alkalose wurde bei Patienten mit Hypernatriämie beschrieben, die Ursachen hierfür sind unklar. Die aktuelle Studie dient

der Klärung, ob eine metabole Alkalose in vitro nach Evaporation freien Wassers entsteht und ob diese durch ein mathematisches Modell berechnet werden kann.

Methodik Zehn Serumproben gesunder Probanden wurden um 29 % durch Vakuumzentrifugation dehydriert einer Zunahme der enthaltenen Konzentrationen von 41 % entsprechend. Ein konstantes pCO_2 von 40 mmHg wurde mittels einer mathematischen Korrektur des pH simuliert. Der metabole Säure-Basen Haushalt wurde anhand der Base Excess Subgruppen von Gilfix analysiert. Die Veränderungen des Säure-Basen Haushalts wurden anhand des physikalisch-chemischen Modells von Watson errechnet.

Ergebnisse Serum Natrium stieg von 141 (140–142) auf 200 (197–203) mmol/L, einer schweren Hypernatriämie entsprechend. Die Säure-Basen Analyse zeigte vor und nach Konzentration eine metabole Alkalose mit Alkalämie: (pH(40) 7,43 (7,41–7,45) vs 7,53 (7,51–7,55), p=0,0051; base excess 1,9 (0,7–3,6) vs 10,0 (8,2–11,8), p=0,0051; base excess of free water 0,0 (−0,2–0,3) vs 17,7 (16,8–18,6)), p=0,0051. Der azidifizierende Effekt der durch die Konzentration entstandenen Hyperalbuminämie lag unter dem alkalinisierenden Gesamteffekt. Die gemessenen Säure-Basenveränderungen korrelierten gut mit den mathematisch vorhergesagten.

Schlussfolgerungen Evaporation von freiem Wasser führt zu einer Konzentrationsalkalose in vitro mit gutem Übereinstimmen zwischen gemessenen und mathematisch errechneten Werten. Zumindest ein Teil der im Rahmen einer Hypernatriämie beobachteten metabolen Alkalose scheint von der Nierenfunktion unabhängig zu sein.

Schlüsselwörter Säure-Basen Haushalt · Alkalose · Dehydration · Hypernatriämie

Introduction

Hypernatremia develops in approximately 10 % of critically ill patients and is associated with increased morbidity and mortality [1–3]. Recently, we described that rising serum sodium (Na) levels during the development of hypernatremia in critically ill patients are associated with a concurrent development of metabolic alkalosis [4]. In the patient collective studied, hypernatremia was primarily caused by infusion of hypertonic solutions and, only in small part, by water loss [4, 5]. However, the pathophysiologic mechanisms responsible for this "hypernatremic alkalosis" are unclear. On the one hand, the classical concept of "contraction alkalosis" might apply, which explains metabolic alkalosis as a consequence of hypovolemia with subsequent increased renal bicarbonate retention [6]. On the other hand, Luke and Galla [7] recently suggested that the disorder should better be named "chloride depletion alkalosis," as it develops due to an increase in the Na-to-chloride (Cl) ratio and may also occur without hypovolemia. Both pathophysiologic explanations of metabolic alkalosis ("contraction alkalosis" and "chloride depletion alkalosis") involve various renal mechanisms, including increased proton secretion and augmented tubular reabsorption of bicarbonate.

In contrast to these explanations, metabolic alkalosis during hypernatremia may develop due to plain loss of free water independent of any renal mechanisms. The suspected mechanism is the analogous opposite to dilutional acidosis: during the development of hypernatremia, a loss of free water from blood increases the concentration of bicarbonate, which in turn leads to an increase in pH, as long as partial pressure of carbon dioxide (pCO_2) remains unchanged [8]. This mechanism would be a good explanation for the metabolic alkalosis observed during hypernatremia, as rising serum Na concentrations indicate diminished free water content of the plasma. However, so far, it has not been shown that metabolic alkalosis can really be induced in human plasma independent of renal function.

In this study, we aimed to clarify whether concentrational alkalosis can develop in vitro due to removal of free water from plasma and whether this can be predicted by a mathematical acid–base model.

Patients, materials, and methods

Blood sampling

After informed consent, blood samples were obtained by venipuncture from two of the investigators (Georg-Christian Funk and Daniel Doberer) through a 21-gauge needle into Vacutainer® tubes (Greiner bio-one, Graz, Austria). The policy of the institutional review board is to allow the use of investigators' blood for experimental purposes. After 10 min, serum was obtained from the whole blood by centrifugation at 2,000 *g* for 15 min at 4 °C. The serum was then divided into 10 aliquots of 3 mL each; 1 mL was used for baseline analysis, and 2 mL was transferred into 2-mL tubes (Eppendorf, Wesseling-Berzdorf, Germany) and used for the evaporation trial.

Evaporation trial

The physical process of water removal from serum will be referred to as "evaporation." Due to the in vitro character of the experiment, it is independent of the in vivo volemic state. Evaporation was quantified by the percentage reduction of baseline volume. For example, 25 % evaporation implies that 25 % of the baseline water has been evaporated). Consecutively, the concentrations of the contained ions and proteins increased by 33 % [9]. For example, if the baseline concentration of serum Na is 140 mmol/1,000 mL, a 25 % evaporation (=removal of 25 % of the contained water) induces a serum Na of 140 m mol/750 mL=140 × 1.33/1,000 mL=187 mmol/1,000 mL.

Evaporation was achieved by means of a centrifuge concentrator (SpeedVac Concentrator SVC100H, SAVANT, Farmingdale, NY). Samples were centrifuged at

800 rpm for 20 min. The desired degree of reduction of baseline volume by 25 % (i.e., 250 µL) was estimated visually. The actual degree of evaporation determined by the mean increases of the concentrations of Na, K, Cl, albumin, and total protein in each sample was 29 % (i.e., an increase of the concentrations by 41 %).

Measurements

All acid–base and laboratory analyses were performed before and after the evaporation trial. Acid–base analysis was performed twice from each sample, and the mean result was used for further analysis.

pH and pCO_2 were measured with a blood gas analyzer (ABL 725, Radiometer®, Copenhagen, Denmark). Serum samples were analyzed by standard potentiometric and photometric methods using a fully automated analyzer (Olympus AU5400 Chemistry Analyzer, Olympus Corporation, Hamburg, Germany) and appendant reagents. Concentrations of electrolytes ($[Na^+]$, $[K^+]$, $[Cl^-]$) were measured by indirect potentiometry with ion-selective electrodes, which have a linear (i.e., reliable) measuring range for $[Na^+]$, $[K^+]$, and $[Cl^-]$ of 50–200, 1–10, and 50–200 mmol/L, respectively. Concentration of inorganic phosphate $[Pi^-]$ was measured by a photometric UV test (molybdate reaction) with a reliable measuring range between 0.32 and 6.40 mmol/L. Concentration of albumin ([albumin]) was measured by a photometric color test (bromocresol green) with a reliable measuring range between 10 and 60 g/L. Concentration of total protein ([total protein]) was measured by a photometric color test (biuret reaction) with a reliable measuring range between 30 and 120 g/L. All measurements were performed at 37 °C.

Acid–base analysis

HCO_3^- was calculated from the measured pH and pCO_2 using the Henderson–Hasselbalch equation [10]. To avoid the influence of respiratory acid–base disorders, we used pH(40) in all analyses. pH(40) was obtained by a formula correcting the measured pH at a given pCO_2 for a pCO_2 of 40 mmHg [11]. Deviations of pH(40) from the normal range, therefore, reflect only metabolic acid–base disorders.

Base excess (BE) and pH(40) were calculated according to formulae by Siggaard-Andersen [10].

$$\mathrm{BE} = 0.5 \times \left(\frac{8a' - 0.919}{a'}\right) + 0.5 \times \sqrt{\left(\frac{0.919 - 8a'}{a'}\right)^2 - 4 \times \frac{24.47 - c\mathrm{HCO_3^-}(5.33)}{a'}}$$

$$a' = 4.04 \times 10^{-3} + 4.25 \times 10^{-4} c\mathrm{tHb}$$

$$c\mathrm{HCO_3^-}(5.33) = 0.23 \times 5.33 \times 10^{\left[\frac{(\mathrm{pH(st)} - 6.161)}{0.9524}\right]}$$

$$\mathrm{pH(st)} = \mathrm{pH} + \log\left(\frac{5.33}{p\mathrm{CO_2}}\right) \times \left(\frac{\mathrm{pH(Hb)} - \mathrm{pH}}{\log p\mathrm{CO_2(Hb)} - \log(7.5006\ p\mathrm{CO_2})}\right)$$

$$\mathrm{pH(Hb)} = 4.06 \times 10^{-2} c\mathrm{tHb} + 5.98 - 1.92 \times 10^{(-0.16169 c\mathrm{tHb})}$$

$$\log p\mathrm{CO_2(Hb)} = -1.7674 \times 10^{-2} c\mathrm{tHb} + 3.4046 + 2.12 \times 10^{(-0.15158 c\mathrm{tHb})}$$

The strong ion difference (SID), i.e., the difference between the sum of the strong cations and the strong anions, was calculated by a computer program using Stewart's acid–base model as modified by Watson [12].

Modified BE subsets according to Gilfix were calculated to compare the effects of electrolytes, albumin, and unmeasured anions on the metabolic acid–base state as assessed by BE (all values in mmol/L, if not otherwise indicated) [13]. Empirical normal values of the BE subsets are 0 ± 2 mmol/L.

a. Na^+ as the regulated variable that controls the extracellular fluid volume is used to assess the effect of free water removal or addition to serum. $[Na^+]_{normal}$ was 140 mmol/L.
   - BE attributable to the free water effect (BE_{fw}) = $0.3 \times ([Na^+]_{measured} - [Na^+]_{normal})$.
   - The constant 0.3 derives from $BE_{fw} = 42 \times ([Na^+] - 140)/140$, with $[Na^+]_{normal} = 140$ mmol/L.
   - Elevated BE_{fw} indicates concentrational alkalosis. Decreased BE_{fw} indicates dilutional acidosis.

b. Hyperchloremia and hypochloremia are associated with hyperchloremic acidosis and hypochloremic alkalosis, respectively. First, the effect of changes in Cl^- can be obtained by correcting Cl^- for changes in free water ($[Cl^-]_{normal} = 103$ mmol/L):
   - $[Cl]_{corrected} = [Cl]_{observed} \times ([Na^+]_{normal}/[Na^+]_{observed})$.
   - Second, BE attributable to Cl (BE_{Cl}) = $[Cl^-]_{normal} - [Cl^-]_{corrected}$.
   - Elevated BE_{Cl} indicates hypochloremic alkalosis. Decreased BE_{Cl} indicates hyperchloremic acidosis.

c. Albumin is a weak non-volatile acid. Thus, hypoalbuminemia is a lack of acid and results in hypoalbuminemic alkalosis. The BE effect attributable to albumin (BE_{Alb}) can be calculated ($albumin_{normal} = 44.7$ g/L):
   - $BE_{Alb} = (0.148 \times pH - 0.818) \times (albumin_{normal} - albumin_{observed})$.
   - The two constants in the equation account for albumin being a weak acid, i.e., possessing variable charges dependent on pH (7).
   - Elevated BE_{Alb} indicates hypoalbuminemic alkalosis. Decreased BE_{Alb} indicates hyperalbuminemic acidosis.

d. Any change in BE, not caused by changes in free water, Cl, or albumin, is attributed to other anions. These comprise lactate and Pi as well as unmeasured anions, e.g., sulfate. This can be quantified as:

- BE attributable to other anions (BE_{OA}) = BE − (BE_{fw} + BE_{Cl} + BE_{Alb}).
- In summary, BE comprises BE_{fw}, BE_{Cl}, Be_{Alb}, and BE_{OA}:
- $BE = BE_{fw} + BE_{Cl} + BE_{Alb} + BE_{OA}$.

Prediction of acid–base changes following the evaporation trial

The evaporation trial was mathematically simulated to study whether measured acid-base changes after evaporation agree with the ones predicted by physical chemistry. For simulation, baseline concentrations of SID, albumin, and Pi were multiplied by the percentage increase of concentrations as assessed by the median change of Na, K, Cl, albumin, and total protein. This factor was 1.41 for an evaporation of 29 %. The predicted pH after the evaporation trial was then calculated by Watson's formula [12]. The predicted BE and HCO_3^- were calculated from the predicted pH. The predicted BE subsets were calculated analogously.

Statistics

Measured concentrations of acid-base parameters, electrolytes, weak acids, and total protein before and after the evaporation trial were compared by Wilcoxon's matched pairs test. Correlation was measured using Spearman's $r(R_s)$. Significance was accepted at a value of $p < 0.05$. Data are presented as median and first to third quartiles. Statistical analyses were performed by SPSS version 15.0, and figures were drawn using STATISTICA version 6.

Results

Acid–base status before and after evaporation

As expected, all concentrations of electrolytes and proteins were increased after the evaporation trial. The main cation in plasma, Na, increased from 141 to 200 mmol/L after evaporation, whereas the main anion, Cl, increased from 102 to 149 mmol/L ($p = 0.0051$). Albumin concentration increased from 44.6 to 62.3 g/L ($p = 0.0077$). Details for all electrolytes and proteins are given in Table 1. The mean pCO_2 before evaporation was 39.4 ± 4.2 mmHg. Evaporation caused concentrational alkalosis by an increase of pH from 7.428 before evaporation to 7.532 ($p = 0.0051$) after evaporation, and bicarbonate concentration increased from 26 to 33.5 mmol/L ($p = 0.0051$). Analysis of BE subsets revealed concentrational alkalosis as the cause of the alkalosis. Hyperalbuminemic acidosis counteracted concentrational alkalosis. The effects of hyperchloremic acidosis and acidosis due to other anions, including phosphate and lactate, were minor. The net acidifying consequences of evaporation were beneath the alkalinizing ones, resulting in the observed net alkalosis. Acid-base parameters before and after the evaporation trial are given in Table 2.

Measured vs predicted acid–base changes after the evaporation trial

Measured and predicted acid-base changes correlated well. However, measured BE Cl deviated substantially from the predicted value. They are shown in Table 2 and in Fig. 1. The predicted median increase of pH(40) after the evaporation trial by 41 % was 0.107 and agreed well with the median measured increase of pH(40) of 0.104.

Discussion

In the present study, the removal of water from human plasma in vitro led to both dehydration and concentrational alkalosis. The observed alkalinization was predictable by a physical–chemical acid-base model. We conclude that a part of the alkalosis observed during hypernatremia is independent of renal function.

To our knowledge, this is the first study reporting the effects of pure water removal from human plasma on acid-base state. Recently, we reported that rising serum Na levels during the development of hypernatremia lead to a concurrent alkalinization resulting in alkalemia in critically ill patients [4]. In the present study, our intention was to clarify whether pure removal of free water, resembling dehydration in humans, would result in the

Table 1 Effect of evaporation of water from human serum on measured concentrations of electrolytes and proteins

N = 10	Measured before evaporation	Measured after evaporation	*p* Level for measured before vs measured after	Predicted after evaporation
[Na^+]	141 (140–142)	200 (197–203)	0.0051*	199 (198–200)
[K^+]	3.72 (3.6–3.9)	5.30 (5.10–5.55)	0.0051*	5.25 (5.08–5.50)
[Cl^-]	102 (102–104)	149 (146–150)	0.0051*	144 (144–146)
[Pi^-]	1.4 (1.2–1.4)	1.8 (1.7–1.9)	0.0077*	1.9 (1.7–2.0)
[Albumin] (g/L)	44.6 (44.6–45.2)	62.3 (61.6–63.4)	0.0077*	62.9 (62.9–63.7)
[Total protein] (g/L)	68.6 (68.2–69.4)	95.2 (93.4–97.6)	0.0077*	96.7 (96.2–97.7)

A total of 29 % of the baseline water was evaporated from the samples. Concentrations are expressed in mmol/L unless otherwise indicated. Data (median and first to third quartiles) are compared by Wilcoxon's matched pairs test
*Significant difference

Table 2 Measured serum acid–base parameters before and after the evaporation trial and predicted acid–base parameters after the evaporation trial

	Measured values before evaporation $N=10$	Measured values after evaporation $N=10$	p Level Measured values before vs after evaporation	Predicted values after evaporation $N=10$	Correlation coefficients between measured and predicted values after evaporation
pH(40)	7.428 (7.411 to 7.452)	7.532 (7.512 to 7.554)	0.0051*	7.535 (7.527 to 7.562)	0.99, $p<0.0001$
BE	1.9 (0.7 to 3.6)	10.0 (8.2 to 11.8)	0.0051*	10.2 (9.5 to 12.5)	0.99, $p<0.0001$
$[HCO_3^-]$	26.0 (24.9 to 27.5)	33.5 (31.8 to 35.3)	0.0051*	33.7 (33.0 to 36.0)	0.99, $p<0.0001$
SID	39.2 (38.6 to 39.7)	53.7 (52.4 to 55.2)	0.0077*	55.3 (54.4 to 56.0)	0.99, $p<0.0001$
BE_{fw}	0.0 (−0.2 to 0.3)	17.7 (16.8 to 18.6)	0.0051*	17.3 (17.1 to 17.8)	0.97, $p<0.0001$
BE_{Cl}	0.6 (−0.5 to 1.7)	−1.8 (−2.3 to −1.2)	0.0051*	0.6 (−0.5 to 1.7)	0.58, $p<0.0079$
BE_{Alb}	0.0 (−0.1 to 0.0)	−5.2 (−5.5 to −5.0)	0.0077*	−5.4 (−5.7 to −5.4)	0.94, $p<0.0001$
BE_{OA}	1.4 (0.7 to 2.1)	−0.5 (−1.0 to −0.2)	0.0077*	−0.9 (−2.3 to −0.8)	0.82, $p<0.0001$

Data are given as medians and first to third quartiles and compared by Wilcoxon's matched pairs test. All concentrations are expressed in mmol/L except pH(40)
pH(40) pH corrected for $pCO_2 = 40$ mmHg, *BE* base excess, *$[HCO_3^-]$* bicarbonate concentration, *SID* strong ion difference, *BE_{fw}* BE attributable to the free water effect, *BE_{Cl}* BE attributable to chloride, *BE_{Alb}* BE attributable to albumin, *BE_{OA}* BE attributable to other anions
*Significant difference

development of concentrational alkalosis. Specifically, the observed alkalinization was independent of renal function or dysfunction. However, in the living organism, a combination of renal and non-renal mechanisms is likely to cause the alkalosis observed during hypernatremia.

With regard to the chemical mechanism of the "concentrational alkalosis" observed in our experiment, we suspect that the loss of free water increases the concentration of bicarbonate, which in turn leads to an increase in pH if pCO_2 remains unchanged. This explanation is in line with standard chemistry and is in analogy to the explanation of dilutional acidosis [8, 14].

Another explanation for the mechanism of concentrational alkalosis is provided by the acid–base concept according to Stewart, proposing that the increase of the SID induces metabolic alkalosis [15]. However, this explanation disregards some chemical issues, and the analogous explanation of dilutional acidosis has been subject to critique [8]. Specifically, the increase of the SID is merely a marker of alkalinization, but not its chemical cause.

The methodology of our study is comparable with a study by Haskins et al. [16], who performed a hydration (120 % of baseline) and dehydration (80 % of baseline) procedure with goat plasma. However, their results are different compared with our study with human plasma: whereas Haskins et al. only detected an increase in bicarbonate concentration of 11 % by evaporation of 20 % of the plasma volume, we found an increase of 26 % by evaporation of 29 %. Also, in their study, BE (and bicarbonate) only slightly increased after evaporation, whereas it increased by a factor of five in the present study. The reason for this difference is unclear, but might be explained by a different method to achieve pure water removal.

Our present results bring some light into the mechanisms leading to alkalemia as described in hypernatremia and dehydration, although various other factors such as the administered infusion solutions, medications, and nutrition solutions most probably play a major role in hypernatremic alkalosis [4].

Some methodological issues with regard to the evaporation trial should be addressed. Only a small degree of water evaporation was achieved. This is due to the limits of reliable measurement of the high concentrations of electrolytes and albumin. Even at the given degree of evaporation, albumin and Na reach the upper range of reliable measurement. It can be assumed that the measured values can be used for analysis; however, further evaporation would require dilution of the samples by water to obtain reliable measurements. This would imply the effect of two opposing chemical processes (i.e., dilution and concentration), and errors of measurement would inevitably accumulate. The actual degree of evaporation was determined by the mean increase of the concentrations of Na, K, Cl, albumin, and total protein. The variation of the actual increases of concentrations of each parameter was 4 % only. pCO_2 was mathematically kept constant in this study, as some of the pCO_2 was lost during the evaporation trial and inter-sample variations of pCO_2 were observed. This simplification allowed us to describe changes in pH that are merely attributable to metabolic derangements and has been used before in an analogous experiment with animal plasma [16]. A closed system, which does allow for keeping pCO_2 constant, would be preferable but was not feasible for technical reasons at our institution. However, as mentioned earlier in the text, this should not influence our results on the "metabolic" effects of dehydration.

The rather low correlation between measured and predicted SID can be attributed to the accumulation of errors of measurement because three parameters (pH, albumin, and Pi) are used to calculate SID [17]. The same applies to BE_{Cl}, as under ideal conditions, BE_{Cl} would remain completely unchanged following evaporation of water. Due to unavoidable minimal measurement errors, measured BE_{Cl} deviates from the expected one. However, compared with the other BE subsets, the effect of the

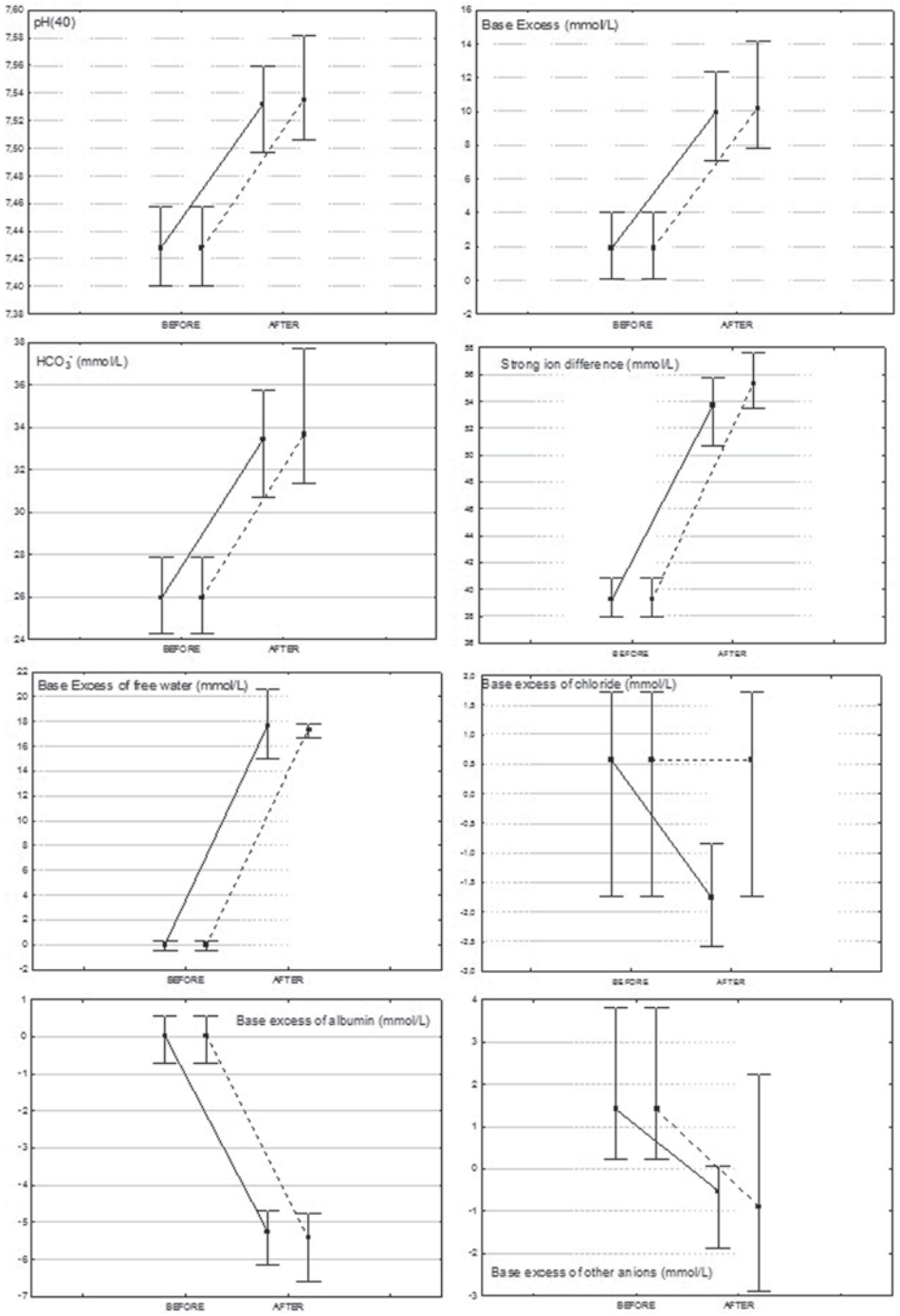

Fig. 1 Acid–base parameters before and after the evaporation trial (medians and first to third quartiles). *Full lines* indicate measured values; *dashed lines* indicate predicted values; *pH(40)* indicates pH corrected for pCO_2=40 mmHg

measured BE_{Cl} was minimal (~ −2.5 mmol/L). BE subsets for the acid–base effects of lactate and Pi are not provided in the Gilfix model, and therefore, their acidifying effects were not separately quantified. However, these effects are minor (~2 mmol/L) and are included in BE_{OA}.

We used human plasma exclusively to concentrate our experiment on the physical–chemical effects of evaporation of free water on acid–base state. In a functioning human organism, cells and tissues as well as kidney, lung, and liver function would influence acid–base state. Results from an experiment using whole blood might be more representative when applying the findings on patients' physiology; however, for reasons mentioned earlier in the text, they would also be very limited due to the lack of tissue and organ function.

In conclusion, evaporation of water from human serum causes concentrational alkalosis in vitro, with good agreement between measured and predicted acid–base values.

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Conflict of interest
Gregor Lindner, Daniel Doberer, Christoph Schwarz, Bruno Schneeweiss, and Georg-Christian Funk declare that they have no conflict of interest.

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