

T. Langer  
E. Carlesso  
A. Protti  
M. Monti  
B. Comini  
L. Zani  
D. T. Andreis  
G. E. Iapichino  
D. Dondossola  
P. Caironi  
S. Gatti  
L. Gattinoni

## In vivo conditioning of acid–base equilibrium by crystalloid solutions: an experimental study on pigs

Received: 24 August 2011  
Accepted: 24 November 2011  
Published online: 25 January 2012  
© Copyright jointly held by Springer and ESICM 2012

### Electronic supplementary material

The online version of this article (doi:10.1007/s00134-011-2455-2) contains supplementary material, which is available to authorized users.

T. Langer · E. Carlesso · A. Protti ·  
M. Monti · B. Comini · L. Zani ·  
D. T. Andreis · G. E. Iapichino ·  
P. Caironi · L. Gattinoni (✉)  
Dipartimento di Anestesiologia,  
Terapia Intensiva e Scienze  
Dermatologiche, Fondazione IRCCS Ca'  
Granda Ospedale Maggiore Policlinico  
di Milano, Università degli Studi,  
Via Francesco Sforza, 35  
20122 Milano, Italy  
e-mail: gattinon@policlinico.mi.it  
Tel.: +39-02-55033232  
Fax: +39-02-55033230

D. Dondossola · S. Gatti  
Centro di Ricerche Chirurgiche Precliniche,  
Fondazione IRCCS Ca' Granda Ospedale  
Maggiore Policlinico di Milano,  
Milan, Italy

P. Caironi · L. Gattinoni  
Dipartimento di Anestesia,  
Rianimazione (Intensiva e Subintensiva) e  
Terapia del Dolore, Fondazione IRCCS Ca'  
Granda Ospedale Maggiore Policlinico di,  
Milano, Italy

**Abstract** *Purpose:* Large infusion of crystalloids may induce acid-base alterations according to their strong ion difference ([SID]). We wanted to prove in vivo, at constant PCO<sub>2</sub>, that if the [SID] of the infused crystalloid is equal to baseline plasma bicarbonate, the arterial pH remains unchanged, if lower it decreases, and if higher it increases. *Methods:* In 12 pigs, anesthetized and mechanically ventilated at PCO<sub>2</sub> ≈ 40 mmHg, 2.2 l of crystalloids with a [SID] similar to (lactated Ringer's 28.3 mEq/l), lower than (normal saline 0 mEq/l), and greater than (rehydrating III 55 mEq/l) baseline bicarbonate (29.22 ± 2.72 mEq/l) were infused for 120 min in randomized sequence. Four hours of wash-out were allowed between the infusions. Every 30 min up to minute 120 we measured blood gases, plasma electrolytes, urinary volume, pH, and electrolytes. Albumin, hemoglobin, and phosphates were measured at

time 0 and 120 min. *Results:* Lactated Ringer's maintained arterial pH unchanged (from 7.47 ± 0.06 to 7.47 ± 0.03) despite a plasma dilution around 12%. Normal saline caused a reduction in pH (from 7.49 ± 0.03 to 7.42 ± 0.04) and rehydrating III induced an increase in pH (from 7.46 ± 0.05 to 7.49 ± 0.04). The kidney reacted to the infusion, minimizing the acid-base alterations, by increasing/decreasing the urinary anion gap, primarily by changing sodium and chloride concentrations. Lower urine volume after normal saline infusion was possibly due to its greater osmolarity and chloride concentration as compared to the other solutions. *Conclusions:* Results support the hypothesis that at constant PCO<sub>2</sub>, pH changes are predictable from the difference between the [SID] of the infused solution and baseline plasma bicarbonate concentration.

**Keywords** Crystalloid solutions · Acid-base equilibrium · Stewart's approach · Volume resuscitation · Animal study

## Introduction

Fluid resuscitation with large amounts of crystalloids is usual in critically ill patients, and normal saline is the most widely used solution for this purpose. It has been observed that large amounts of normal saline induce metabolic acidosis [1–3]. According to misinterpreted Stewart’s model [4, 5], this metabolic acidosis is due to a reduction in the plasma strong ion difference ([SID]). However, to fully understand the relationship among the dilution, kind of solution, and acid-base equilibrium, all three independent variables of Stewart’s model must be considered together, not only [SID], but also  $PCO_2$  and the total amount of weak acids  $[A_{TOT}]$  [6]. Normal pH is assured by an adequate proportion of these components, whose individual variation may have opposite effects on pH. As an example, a decrease in  $PCO_2$  and  $A_{TOT}$  causes alkalosis, while a decrease in [SID] causes acidosis. Therefore, a correct application of Stewart’s model provides the same results as the classical bicarbonate/base excess approach [6, 7]. However, we believe that Stewart’s approach has the great advantage to fully integrate the electrolyte and the acid-base equilibrium.

Whatever crystalloid solution not containing weak anions, before the infusion, has  $PCO_2$ ,  $[A_{TOT}]$ , and [SID] values equal to zero. Crystalloids containing organic ions, such as lactated Ringer’s, develop a positive [SID] only after the associated organic ions (such as lactate and acetate) are metabolized [8, 9]. Therefore, when a crystalloid solution is added to normal plasma ( $PCO_2$  40 mmHg,  $[A_{TOT}]$  17 mmol/l, SID 42 mEq/l, pH 7.4) in a closed system, the independent variables are equally diluted, and pH remains constant—for example, a 100% dilution of normal plasma leads to  $PCO_2$  of nearly 20 mmHg,  $A_{TOT}$  of 8.5 mmol/l, [SID] of 21 mEq/l, and pH of 7.4. When  $PCO_2$  increases from 20 to 40 mmHg, opening the closed system to gases, pH decreases, with  $[A_{TOT}]$  and [SID] being unchanged [8]. Of course, by manipulating also the actual [SID] of the infused solution, the pH will change accordingly.

Provided the same mechanisms operate in vivo, we may expect that a proper combination of  $PCO_2$ , [SID], and  $A_{TOT}$  could manipulate the acid-base equilibrium toward acidosis, alkalosis, or unchanged pH. Actually, it was shown that no acidosis developed in normal animals when the diluting solution had a [SID] of nearly 24 mEq/l [9]. We expanded this observation, showing in vitro that plasma pH after crystalloid dilution at constant  $PCO_2$  depends on the relationship between infused [SID] ( $[SID]_{inf}$ ) and the baseline plasma bicarbonate concentration ( $[HCO_3^-]_{baseline}$ ) (see the Online Data Supplement for the mathematical derivation). At constant  $PCO_2$ , when  $[SID]_{inf} - [HCO_3^-]_{baseline} = 0$  no change in pH occurs, whatever the degree of dilution. When  $[SID]_{inf} - [HCO_3^-]_{baseline} > 0$  pH increases, while it decreases when  $[SID]_{inf} - [HCO_3^-]_{baseline} < 0$  [7].

As these rules were identified in vitro, we aimed to confirm them in vivo, considering the animals as a “black box.” In these experiments, we describe the acid-base equilibrium of the black box for a given input (crystalloid infusion) and output (urine volume and electrolyte composition). If the rules identified in vitro apply in vivo, changes in the acid-base equilibrium following the infusion of whatever crystalloid solution in subjects undergoing volume replacement will be easily predictable also in clinical practice.

## Materials and methods

The study was approved by the Institutional Review Board and conducted in accordance with international recommendations [10]. Twelve healthy female pigs weighing  $22 \pm 3$  kg were anesthetized and mechanically ventilated (Engstrom Carestation, GE Healthcare) with PEEP 5 cm  $H_2O$ , tidal volume 10 ml/kg and respiratory rate adjusted to maintain  $PCO_2$  constant throughout the experiment by capnography. Arterial, central venous, and bladder catheters were surgically placed [11]. Two hours of stabilization preceded the beginning of the protocol.

### Study protocol

In each pig, normal saline (Baxter spa, Roma, Italy), lactated Ringer’s (Fresenius Kabi Italia spa, Verona, Italy), and rehydrating III (Monico spa, Venezia/Mestre, Italy) were infused (see Table 1 for compositions). Six different sequences of dilution were possible, so pigs were randomly assigned to a sequence of dilutions to assure a

**Table 1** Characteristics of the crystalloid solutions

	Rehydrating III (RIII)	Lactated Ringer’s (LR)	Normal saline (NS)
$[Na^+]$ (mEq/l)	140.0	130.9	154.0
$[Cl^-]$ (mEq/l)	103.0	111.7	154.0
$[K^+]$ (mEq/l)	10.0	5.4	0.0
$[Ca^{2+}]$ (mEq/l)	5.0	3.7	0.0
$[Mg^{2+}]$ (mEq/l)	3.0	0.0	0.0
$[Lac^-]$ (mEq/l)	0.0	28.3	0.0
$[Acetate^-]$ (mEq/l)	47	0	0
$[Citrate^-]$ (mEq/l)	8	0	0
[SID] (mEq/l)	55.0	28.3	0.0
Osmolarity (mOsm/l)	307.0	278.0	308.0

All concentrations are expressed as mEq/l. Osmolarity is expressed as mOsm/l

$Na^+$  sodium concentration,  $Cl^-$  chloride concentration,  $K^+$  potassium concentration,  $Ca^{2+}$  calcium concentration,  $Mg^{2+}$  magnesium concentration,  $Lac^-$  lactate concentration,  $Acetate^-$  acetate concentration,  $Citrate^-$  citrate concentration,  $SID$  strong ion difference,  $Osmolarity$  declared osmolarity

balanced distribution (see Fig. 1 in the Online Data Supplement for study design). Each experimental set consisted of the 2-h infusion of the assigned crystalloid solution, in the amount of 10% of baseline body weight, followed by a 4-h 'wash-out' period, with the same fluid being infused at a rate of 3 ml/h to keep the vein open. Thereafter, a new experimental set began or, in the case of the last study period, the animal was killed with the injection of a bolus of 40 mEq of potassium.

#### Data collection

During the 2-h study period, data were collected at baseline and every 30 min. Thereafter, during the wash-out period, data were recorded every 2 h (minute 240 and 360). For each point, data on arterial blood gases, electrolytes, and acid-base variables (ABL800, Radiometer GmbH, Germany) were recorded. Urinary output measurement and analysis of urinary pH ( $pH_U$ ) and urinary electrolytes (sodium  $[Na^+]_U$ , potassium  $[K^+]_U$ , chloride  $[Cl^-]_U$ , and ammonium  $[NH_4^+]_U$ ) were performed by the urinary analyzer K.IN.G<sup>®</sup> (collaboration of Orvim and Kardia, Milan, Italy) [12] and synchronized with blood data collection. For each dilution, samples for measurement of hemoglobin, albumin, and phosphates concentrations were sent to the central laboratory at time 0, 120, 240 and 360 min.

#### Definitions

Fluid balance was computed as  $\sum$  Infused fluid –  $\sum$  Urinary output. Hemoglobin-derived ( $DF_{Hb}$ ) and albumin-derived dilution ( $DF_{Alb}$ ) were computed as previously described [13] and used to estimate plasma dilution. The plasma [SID] and urinary anion gap  $[AG]_U$  were computed using standard formulas. The concentration of dissociated weak acids  $[A^-]$  was calculated from plasma albumin and phosphates concentration as previously described [14], and the corresponding  $[A_{TOT}]$  was computed rearranging the dissociation equation for weak acids. Effective plasma osmolarity was calculated with the standard formula from sodium and glucose (see the Online Data Supplement for further details and equations).

#### Statistical analysis

Data are presented as mean  $\pm$  standard deviation. Two-way ANOVA RM was used to test the effects of different crystalloids and time and Bonferroni's  $t$  test for multiple comparison. A rank transformation was used in the case of variables that were not normally distributed and did not pass the equal variance test. Infusion and wash-out (plus baseline) were tested separately. Linear regression was used to assess the level of agreement between variables.

**Table 2** Baseline plasma and urinary acid-base variables

PCO <sub>2</sub> (mmHg)	40.4 $\pm$ 2.7
[SID] (mEq/l)	40.43 $\pm$ 2.14
[A <sub>TOT</sub> ] (mmol/l)	13.75 $\pm$ 1.73
[Alb] (g/l)	23.4 $\pm$ 4
[Phosph] (mmol/l)	2.67 $\pm$ 0.36
[BE] (mmol/l)	5.47 $\pm$ 2.78
pHa	7.47 $\pm$ 0.04
[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	29.22 $\pm$ 2.72
[Na <sup>+</sup> ] (mEq/l)	138 $\pm$ 2.73
[Cl <sup>-</sup> ] (mEq/l)	103.08 $\pm$ 3.32
[K <sup>+</sup> ] (mEq/l)	3.78 $\pm$ 0.34
[Ca <sup>2+</sup> ] (mEq/l)	2.60 $\pm$ 0.22
[Lac <sup>-</sup> ] (mEq/l)	0.88 $\pm$ 0.25
Osmolarity (mOsm/l)	281.1 $\pm$ 5.1
pH <sub>U</sub>	6.49 $\pm$ 0.6
[Na <sup>+</sup> ] <sub>U</sub> (mEq/l)	58.0 $\pm$ 23.2
[Cl <sup>-</sup> ] <sub>U</sub> (mEq/l)	127.8 $\pm$ 68.5
[K <sup>+</sup> ] <sub>U</sub> (mEq/l)	46.1 $\pm$ 17.4
[NH <sub>4</sub> <sup>+</sup> ] <sub>U</sub> (mEq/l)	16.0 $\pm$ 13.1
[AG] <sub>U</sub> (mEq/l)	-23.6 $\pm$ 57.8

All values are expressed as mean  $\pm$  standard deviation. PCO<sub>2</sub> partial pressure of carbon dioxide, SID strong ion difference, A<sub>TOT</sub> plasma concentration of non-carbonic weak acids, Alb albumin concentration, Phosph phosphate concentration, BE base excess, pHa arterial pH, HCO<sub>3</sub><sup>-</sup> bicarbonate concentration, Na<sup>+</sup> sodium concentration, Cl<sup>-</sup> chloride concentration, K<sup>+</sup> potassium concentration, Ca<sup>2+</sup> calcium concentration, Lac<sup>-</sup> lactate concentration, Osmolarity effective plasma osmolarity, [pH]<sub>U</sub> urinary pH, [Na<sup>+</sup>]<sub>U</sub> urinary sodium concentration, [Cl<sup>-</sup>]<sub>U</sub> urinary chloride concentration, [K<sup>+</sup>]<sub>U</sub> urinary potassium concentration, [NH<sub>4</sub><sup>+</sup>]<sub>U</sub> urinary ammonium concentration, [AG]<sub>U</sub> urinary anion gap

Paired  $t$  test was used to compare  $DF_{Hb}$  and  $DF_{Alb}$ . Statistical significance was defined as  $p < 0.05$ . Analysis was performed with SAS v.9.2. (SAS, Cary, NC) and Sigmaplot v.11.2 (Systat Software Inc., San Jose, CA).

## Results

### Baseline characteristics

Baseline acid-base variables and electrolytes in plasma and urine are summarized in Table 2. Of note, before any intervention all pigs presented slight metabolic alkalosis and hypoalbuminemia. However, all values are consistent with previously reported data on acid-base variables in healthy piglets weighing between 20 and 25 kg, which, compared to humans, present higher pH (7.48  $\pm$  0.038), similar PCO<sub>2</sub> (40  $\pm$  2.8 mmHg), higher bicarbonate concentrations (29  $\pm$  2.2 mEq/l), and lower albumin concentrations (25.4  $\pm$  2.08 g/l) [15].

### Effective dilution

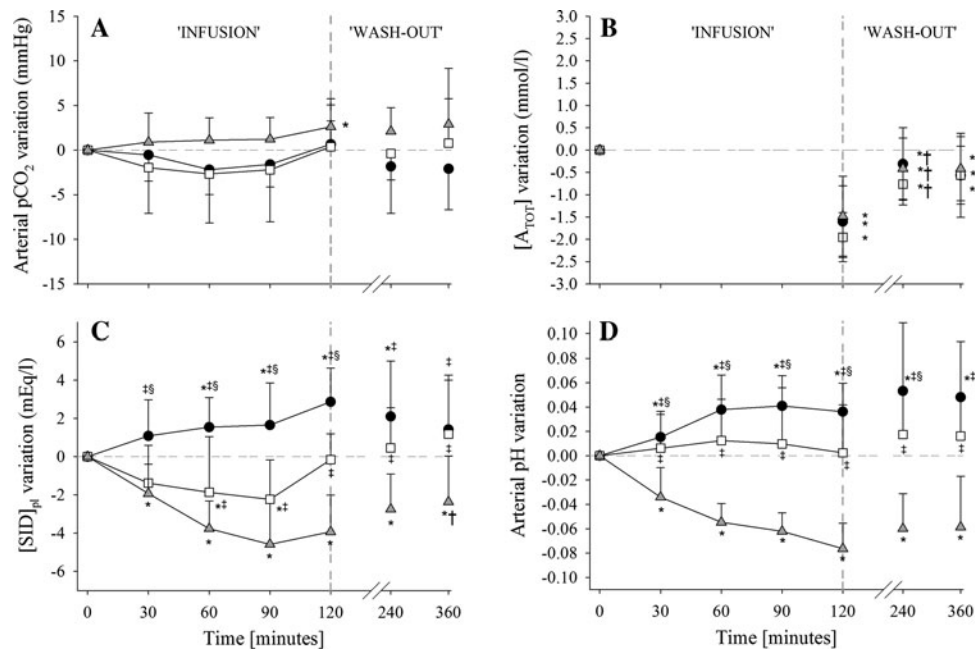
Table 3 summarizes the principal data concerning infused fluids, urinary output, water balance, and consequent

**Table 3** Dilution variables at time 120

	Rehydrating III (RIII)	Lactated Ringer's (LR)	Normal saline (NS)
Infused fluid (ml)	2,213 ± 329	2,213 ± 329	2,213 ± 329
Urinary output (ml)	907 ± 254	754 ± 190	644 ± 251*
Fluid balance (ml)	1,306 ± 452	1,459 ± 333	1,569 ± 347*
Hb-derived plasma dilution (%)	12.20 ± 9.47	15.53 ± 6.10	10.92 ± 11.06
Albumin-derived plasma dilution (%)	11.00 ± 7.67	14.66 ± 5.27	12.61 ± 9.49

The table summarizes dilution parameters at the end of the infusion (120 min). Fluid balance was defined as infused fluid minus urinary output. See the Online Data Supplement for the Hb- and albumin-derived dilution explanation

\*  $p < 0.05$  compared to rehydrating III



**Fig. 1** Plasma variations in independent variables induced by the infusion of crystalloid solutions at different [SID]s (**a, b, c**) and the consequent variations in plasma pH (**d**). Grey triangles represent variations induced by the infusion of normal saline. White squares represent variations induced by the infusion of lactated Ringer's solution. Black dots represent variations induced by the infusion of rehydrating III. All data are expressed as mean ± standard deviation. [A<sub>TOT</sub>] represents the plasma concentration of non-

carbonic weak acids; [A<sub>TOT</sub>] was measured at baseline, at the end of the infusion, and at time 240 and 360 min; [SID]<sub>pl</sub> represents the plasma strong ion difference. \* $p < 0.05$ : Compared to baseline value of the same group. † $p < 0.05$ : Compared to time 120 min of the same group; § $p < 0.05$ : Rehydrating III or lactated Ringer's compared to normal saline. § $p < 0.05$  Rehydrating III compared to lactated Ringer's. See text for additional explanations

effective plasma dilution at the end of the infusion period (time 120 min), according to the infused crystalloid. The mean hemoglobin and albumin-derived plasma dilution coefficients were not significantly different ( $12.9 \pm 9.1\%$  and  $12.8 \pm 7.6\%$ , respectively) and did not differ significantly among the crystalloids used. Of note, however, the urinary output was significantly lower and fluid balance more positive after the infusion of normal saline, compared to lactated Ringer's and rehydrating III infusion.

#### Plasma variables

Figure 1 shows the time course of the independent variables and the resulting pH as a function of the three crystalloid solutions. As shown in panel A, PCO<sub>2</sub> was maintained constant throughout the experiments, while [A<sub>TOT</sub>] (panel B) was equally diluted by the three solutions. In contrast the time course of plasma [SID] variations (panel C) was significantly different when infusing solutions with different SIDs. These changes in

**Table 4** Variations in plasma acid-base variables, electrolytes, and osmolarity

Time (min)	0	30	60	90	120	240	360
<b>Rehydrating III</b>							
d[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	0.00 ± 0.00	0.73 ± 1.25	1.19 ± 1.20	1.75 ± 1.67	3.23 ± 1.71	2.51 ± 2.43	2.01 ± 2.30
P <sub>TIME</sub>			**	***	***	***	*** †
P <sub>INF</sub>		+++ §	+++ §	+++ §§§	+++ §§§	+++ §	+++
dBE (mmol/l)	0.00 ± 0.00	0.84 ± 0.99	1.60 ± 1.27	2.13 ± 1.64	3.28 ± 1.56	2.94 ± 2.52	2.46 ± 2.28
P <sub>TIME</sub>			***	***	***	***	***
P <sub>INF</sub>		+++§	+++ §§§	+++ §§§	+++ §§§	+++§	+++
d[Na <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	0.67 ± 1.07	1.33 ± 1.72	1.83 ± 1.75	1.92 ± 2.11	0.83 ± 2.59	0.25 ± 1.66
P <sub>TIME</sub>			**	***	***		+++†
P <sub>INF</sub>		‡	‡‡	‡‡‡	‡‡‡	‡	‡
d[Cl <sup>-</sup> ] (mEq/l)	0.00 ± 0.00	-0.42 ± 2.28	-0.25 ± 2.45	0.08 ± 2.78	-1.08 ± 2.61	-1.58 ± 4.44	-1.50 ± 3.12
P <sub>TIME</sub>		+++	+++ §	+++ §§	+++	+++	+++
P <sub>INF</sub>							
d[K <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	0.04 ± 0.14	0.03 ± 0.23	-0.04 ± 0.28	-0.05 ± 0.37	-0.20 ± 0.36	-0.23 ± 0.56
P <sub>TIME</sub>		+++	+++	+++	+++	§§	§§
P <sub>INF</sub>							
d[Lactate <sup>-</sup> ] (mEq/l)	0.00 ± 0.00	0.00 ± 0.12	0.03 ± 0.16	0.01 ± 0.15	0.07 ± 0.12	0.02 ± 0.20	-0.05 ± 0.21
P <sub>TIME</sub>		+++ §§§	+++ §§§	+++ §§§	+++ §§§	+++ §	
P <sub>INF</sub>							
dOsm (mOsm/l)	0.00 ± 0.00	1.19 ± 1.99	2.49 ± 3.12	3.43 ± 3.14	3.65 ± 3.91	1.38 ± 4.71	0.34 ± 3.06
P <sub>TIME</sub>		**	**	**	**	†	+++†
P <sub>INF</sub>		‡‡	‡‡	‡‡	‡‡	‡‡	‡
<b>Lactated Ringer's</b>							
d[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	0.00 ± 0.00	-0.86 ± 1.51	-0.83 ± 2.00	-0.69 ± 1.01	0.64 ± 1.11	1.07 ± 1.35	1.52 ± 2.54
P <sub>TIME</sub>			+++	+++	+++	+++	**
P <sub>INF</sub>							+++
dBE (mmol/l)	0.00 ± 0.00	-0.60 ± 1.10	-0.60 ± 1.85	-0.41 ± 1.04	0.62 ± 1.16	1.14 ± 1.55	1.44 ± 2.10
P <sub>TIME</sub>		‡‡	+++	+++	+++	*	**
P <sub>INF</sub>						+++	+++
d[Na <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	0.33 ± 1.16	1.00 ± 2.09	1.17 ± 1.80	1.08 ± 1.78	0.00 ± 2.30	-0.82 ± 2.36
P <sub>TIME</sub>			*	*	*		†
P <sub>INF</sub>		‡‡	+++	+++	+++	+++	+++
d[Cl <sup>-</sup> ] (mEq/l)	0.00 ± 0.00	0.92 ± 2.47	2.00 ± 4.22	2.50 ± 2.54	0.67 ± 2.35	-0.17 ± 2.59	-1.73 ± 3.80
P <sub>TIME</sub>		‡‡	+++	+++	+++	+++	+++
P <sub>INF</sub>							‡‡
d[K <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	-0.12 ± 0.27	-0.13 ± 0.42	-0.11 ± 0.32	0.04 ± 0.25	0.25 ± 0.27	0.27 ± 0.41
P <sub>TIME</sub>		‡	+++	‡‡	‡‡	*	
P <sub>INF</sub>						‡	
d[Lactate <sup>-</sup> ] (mEq/l)	0.00 ± 0.00	0.68 ± 0.22	0.74 ± 0.22	0.79 ± 0.31	0.68 ± 0.41	-0.08 ± 0.11	-0.06 ± 0.13
P <sub>TIME</sub>		***	***	***	***	*** †††	* †††
P <sub>INF</sub>		+++	+++	+++	+++		
dOsm (mOsm/l)	0.00 ± 0.00	0.35 ± 2.17	1.71 ± 3.71	2.20 ± 3.45	2.35 ± 3.44	-0.04 ± 4.52	-1.86 ± 4.58
P <sub>TIME</sub>		**	**	**	**		†††
P <sub>INF</sub>		+++	+++	+++	+++	+++	‡‡
<b>Normal saline</b>							
d[HCO <sub>3</sub> <sup>-</sup> ] (mmol/l)	0.00 ± 0.00	-1.83 ± 1.20	-3.06 ± 1.20	-3.44 ± 1.60	-3.49 ± 1.61	-2.68 ± 1.29	-2.25 ± 1.83
P <sub>TIME</sub>		***	***	***	***	***	*** †
P <sub>INF</sub>							
dBE (mmol/l)	0.00 ± 0.00	-2.09 ± 0.93	-3.48 ± 0.99	-3.93 ± 1.36	-4.20 ± 1.30	-3.19 ± 1.29	-2.88 ± 1.30
P <sub>TIME</sub>		***	***	***	***	***	*** †
P <sub>INF</sub>							
d[Na <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	2.17 ± 0.84	3.25 ± 1.06	4.17 ± 1.59	4.33 ± 1.92	2.92 ± 1.24	2.08 ± 2.19
P <sub>TIME</sub>		***	***	***	***	***	*** ††
P <sub>INF</sub>							
d[Cl <sup>-</sup> ] (mEq/l)	0.00 ± 0.00	3.75 ± 1.77	6.67 ± 1.37	8.50 ± 3.12	8.08 ± 2.61	5.75 ± 2.22	4.58 ± 3.18
P <sub>TIME</sub>		***	***	***	***	*** ††	*** †††
P <sub>INF</sub>							
d[K <sup>+</sup> ] (mEq/l)	0.00 ± 0.00	-0.43 ± 0.13	-0.54 ± 0.17	-0.48 ± 0.19	-0.45 ± 0.34	-0.13 ± 0.30	-0.02 ± 0.28
P <sub>TIME</sub>		***	***	***	***	††	†††
P <sub>INF</sub>							

**Table 4** continued

Time (min)	0	30	60	90	120	240	360
$d[\text{Lactate}^-]$ (mEq/l)	$0.00 \pm 0.00$	$-0.12 \pm 0.07$	$-0.19 \pm 0.08$	$-0.20 \pm 0.13$	$-0.20 \pm 0.14$	$-0.17 \pm 0.14$	$-0.14 \pm 0.22$
$P_{\text{TIME}}$		***	***	***	***	***	***
$P_{\text{INF}}$							
$d\text{Osm}$ (mOsm/l)	$0.00 \pm 0.00$	$3.81 \pm 1.51$	$6.10 \pm 1.81$	$7.81 \pm 2.73$	$8.29 \pm 3.54$	$5.48 \pm 2.54$	$3.69 \pm 4.60$
$P_{\text{TIME}}$		***	***	***	***	***	*** ††
$P_{\text{INF}}$							

The table summarizes variations in plasma acid-base variables, electrolytes, and osmolarity induced by the infusion of the crystalloid solutions. All data are expressed as mean  $\pm$  standard deviation

$d[\text{HCO}_3^-]$  variations in bicarbonate concentration,  $d\text{BE}$  variations in base excess,  $d[\text{Na}^+]$  variations in sodium concentration,  $d[\text{Cl}^-]$  variations in chloride concentration,  $d[\text{K}^+]$  variations in potassium concentration,  $d[\text{Lactate}^-]$  variations in lactate concentration,  $d\text{Osm}$  variations in effective osmolarity

\*  $p < 0.05$  versus baseline, \*\*  $p < 0.01$  versus baseline, \*\*\*  $p < 0.001$  versus baseline, †  $p < 0.05$  versus time 120, ††  $p < 0.01$  versus time 120, †††  $p < 0.001$  versus time 120, ‡  $p < 0.05$  versus normal saline, ‡‡  $p < 0.01$  versus normal saline, ‡‡‡  $p < 0.001$  versus normal saline, §  $p < 0.05$  versus lactated Ringer's, §§  $p < 0.01$  versus lactated Ringer's, §§§  $p < 0.001$  versus lactated Ringer's.  $P_{\text{TIME}}$  defines statistics over time, i.e., compared to baseline value or to time 120.  $P_{\text{INF}}$  defines statistics among different types of crystalloids

independent variables ( $[\text{SID}]$  and  $[\text{ATOT}]$ ) induced the variations in pH represented in panel D. As shown, while the infusion of normal saline and rehydrating III caused significant variations in pH (reduction and increase, respectively), no variations were observed during the infusion of lactated Ringer's solution. Of note, the difference between baseline  $[\text{HCO}_3^-]$  and  $[\text{SID}]_{\text{inf}}$  expressed in mEq/l was  $+26.4 \pm 2.5$  for rehydrating III,  $-29 \pm 2.7$  for normal saline, and  $+1.05 \pm 2.35$  for lactated Ringer's solution.

#### Plasma electrolytes and osmolarity

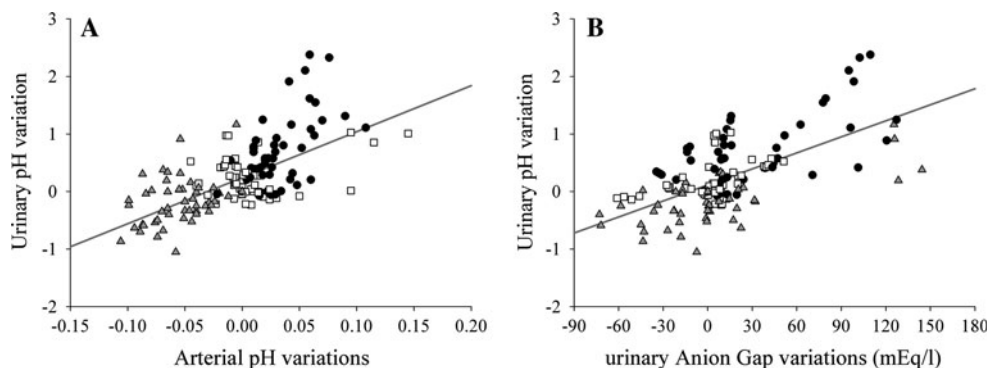
The time course of the individual electrolytes as well as of osmolarity variations is reported in Table 4. As shown, compared to normal saline, rehydrating III and lactated Ringer's differed in the time course of  $[\text{Na}^+]$  (lower increase),  $[\text{Cl}^-]$  (lower increase),  $[\text{K}^+]$  (significantly

decreased in normal saline), and lactate. Of note, the increase in effective osmolarity was remarkably greater with normal saline compared to the other two solutions.

#### Urinary output

As shown in Table 3, urinary output at the end of dilution significantly differed among the solutions, being significantly lower with normal saline. However, after the wash-out period (4 h), the urinary volume was similar among the three groups ( $1,366 \pm 288$  ml for rehydrating III,  $1,336 \pm 316$  ml for lactated Ringer's, and  $1,263 \pm 265$  ml for normal saline).

In Fig. 2a we report the urinary pH changes as a function of arterial pH changes recorded at each infusion time and for each crystalloid solution. As shown, the urinary pH variations roughly paralleled the arterial pH variations, suggesting that the kidney reacted to the



**Fig. 2** Figure represents, at each experimental point during the infusion, the relationship between the urinary pH variations and the arterial pH variations (panel A, intercept = 0.24, slope = 7.99,  $r^2 = 0.39$ ,  $p < 0.0001$ ), and the relationship between urinary pH variations and the urinary anion gap (panel B, intercept = 0.11, slope =  $9.29e-3$ ,  $r^2 = 0.41$ ,  $p < 0.0001$ ) during the infusion of

different crystalloid solutions. Grey triangles represent variations induced by the infusion of normal saline. White squares represent variations induced by the infusion of lactated Ringer's. Black dots represent variations induced by the infusion of rehydrating III. Grey solid lines represent linear regressions. See text for additional explanations



acid-base plasma perturbations by trying to minimize them. This was likely obtained by readjusting the urinary [SID], one of the determinants of urinary pH. Actually, as shown in Fig. 2b, the changes in the urinary anion gap ( $[\text{Na}^+]_{\text{U}} + [\text{K}^+]_{\text{U}} - [\text{Cl}^-]_{\text{U}}$ ), a surrogate for urinary [SID], were more negative during normal saline infusion, unchanged during lactated Ringer's infusion, and increased during rehydrating III infusion.

Pooling together all the experimental points, we found that the small variations in plasma [SID] were weakly but significantly correlated with the larger variations of the urinary anion gap ( $r^2 = 0.07$ ,  $p = 0.0002$ ), which in turn were significantly associated both with  $[\text{Na}^+]_{\text{U}}$  ( $r^2 = 0.22$ ,  $p < 0.0001$ ) and  $[\text{Cl}^-]_{\text{U}}$  ( $r^2 = 0.17$ ,  $p < 0.0001$ ) changes (see Figures 2, 3, and 4 in the Online Data Supplement). In contrast,  $[\text{K}^+]_{\text{U}}$  changes were unrelated to urinary anion gap changes, but were directly related to the potassium loaded during the infusions (see Figures 5, 6, 7 and Table 1 in the Online Data Supplement).

## Discussion

The findings in this study are consistent with the hypothesis that in intact healthy animals at constant  $\text{PCO}_2$  the plasma pH can be predictably increased, decreased, or maintained unchanged, according to the difference between the baseline plasma  $[\text{HCO}_3^-]$  and the strong ion difference of the crystalloid solution infused. The theoretical background has been published previously [7] and is summarized in the Online Data Supplement. In vitro experiments with human plasma are consistent with the rules identified in theory, and, in the present study, we found that an intact animal behaves as the simple plasma model.

In fact, in the current work, three different solutions with  $[\text{SID}]_{\text{inf}}$  greater than, similar to, or lower than the baseline plasma  $[\text{HCO}_3^-]$  were infused in each pig. Actually, the baseline  $[\text{HCO}_3^-]$  was around 29 mEq/l. When diluting with lactated Ringer's whose [SID] was similar to the baseline  $[\text{HCO}_3^-]$  ( $[\text{SID}]_{\text{inf}} = 28.3$  mEq/l and  $[\text{HCO}_3^-] = 29.35 \pm 2.35$  mmol/l), no significant changes in pH were observed. In contrast, dilution with the other two solutions having  $[\text{SID}]_{\text{inf}}$  greater (rehydrating III,  $[\text{SID}]_{\text{inf}} = 55$  mEq/l) or smaller (normal saline,  $\text{SID}_{\text{inf}} = 0$  mEq/l) than baseline  $[\text{HCO}_3^-]$  determined a significant increase and reduction in pH, respectively, as observed in vitro.

The plasma dilution we achieved, according to the reduction of the albumin and hemoglobin concentration averaged 12%, a value that is consistent with the amount of fluid retained during the infusion period. In this study we did not measure or estimate the dilution of the different compartments, such as the interstitium and

cellular space. However, the fact that the recorded changes in plasma pH were consistent with the ones observed during the dilution of isolated plasma suggests that also in vivo (regardless of the movements among the intravascular, interstitial, and intracellular space), the general rule found in vitro may be true, i.e., plasma changes during crystalloid infusion depend upon the difference between infused [SID] and baseline  $[\text{HCO}_3^-]$ . Our findings imply therefore that the body can be considered as a "black box" where, whatever the exchanges among plasma, interstitial fluid and cells are, the acid-base status can be easily predicted for any given input and output. While input conditions are easily computed (the kind of solution used and rate of delivery), the output has to be calculated from the urine volume and its electrolyte composition, at least of the electrolytes that are more relevant in determining the plasma [SID].

In this study we found that the response of the kidney to a given crystalloid solution is, in general, to preserve homeostasis. As far as the acid-base equilibrium is concerned, the kidney reacts by decreasing the urinary [SID], as inferred from the urinary anion gap, when infusing a solution causing acidosis and increasing it when infusing a solution causing alkalosis. This is a normal kidney "compensatory" response to plasma acid-base alterations [16, 17].

However, beyond the [SID] differences, the infused solution presented several other differences that could be relevant in determining urine volume and its composition, such as differences in osmolarity, potassium, and chloride load. While the volume receptors were likely equally stimulated by an identical volume load, the osmoreceptors were probably differently activated by the three solutions. In fact, after metabolism of the organic ions, lactated Ringer's and rehydrating III had an osmolarity that was about 50 mOsm/l lower than that of normal saline. The fact that lactate was nearly fully metabolized during lactated Ringer's infusion was documented by plasma lactate variations lower than 1 mmol/l (see Table 4). Therefore, the lower osmolarity of lactated Ringer's and rehydrating III via ADH suppression could have contributed to the greater urine volume caused by these infusions compared to the infusion of normal saline. Another possible factor influencing the urine volume and the electrolyte composition is the potassium load. Hypokalemia can cause a downregulation of aldosterone, with potassium-sparing effects and an increase of sodium plus water in the urine, whereas hyperkalemia can produce the opposite effects. Actually, in our experiments, we found that during normal saline infusion the plasma level of potassium was significantly reduced as well as its urinary concentration. In contrast, the infusion of lactated Ringer's and rehydrating III was associated with a significant increase in potassium excretion in proportion to the potassium load. Finally, the higher chloride concentration present in normal saline, per se, may have led to

variations in glomerular filtration rates/renal arteriolar diameters [18, 19] with a consequent decrease in urinary volume. In summary, different mechanisms are likely to operate (sometimes in opposite directions) during the infusion, and all of them may have contributed to the urine pattern we recorded in these experiments.

We conclude that in healthy pigs with normal kidney function, the acid-base response to the infusion of solutions with different [SID]s is highly predictable and that the kidney response is specific for each solution, acting to restore the baseline conditions. If these findings could be applied to human beings, the response to large volume replacement of modifying plasma pH could be predicted. Although these experiments were conducted in healthy animals, the physicochemical basis of pH changes during crystalloid infusion should be the same in patients. It is however reasonable that the effects of crystalloid infusions on acid-base concentrations may be amplified in

some conditions that are typical for critical illness, e.g., hypovolemia and renal failure [20–22]. Indeed, the physiological triggers for water and sodium elimination are not immediately activated in the first case, and in the case of renal failure, the kidney response to volume and electrolyte load is inadequate for the maintenance of homeostasis.

The call for a reasoned and rational use of crystalloid solutions is therefore straightforward, especially for critically ill patients.

**Acknowledgments** We are grateful to Fabio Ambrosetti for his technical support, to GE Healthcare for providing mechanical ventilators, to A. De Mori S.p.A., Italy, who kindly provided the blood gas analyzer, and to Roberto Castenetto for technical support with the KING analyzer. This study was supported in part by an Italian grant provided by Fondazione Fiera di Milano for Translational and Competitive Research (2007, Luciano Gattinoni).

## References

- Williams EL, Hildebrand KL, McCormick SA, Bedel MJ (1999) The effect of intravenous lactated Ringer's solution versus 0.9% sodium chloride solution on serum osmolality in human volunteers. *Anesth Analg* 88:999–1003
- Scheingraber S, Rehm M, Sehmisch C, Finsterer U (1999) Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. *Anesthesiology* 90:1265–1270
- O'Malley CM, Frumento RJ, Hardy MA, Benvenisty AI, Brentjens TE, Mercer JS, Nett-Guerrero E (2005) A randomized, double-blind comparison of lactated Ringer's solution and 0.9% NaCl during renal transplantation. *Anesth Analg* 100:1518–1524, table
- Fencel V, Leith DE (1993) Stewart's quantitative acid-base chemistry: applications in biology and medicine. *Respir Physiol* 91:1–16
- Stewart PA (1983) Modern quantitative acid-base chemistry. *Can J Physiol Pharmacol* 61:1444–1461
- Doberer D, Funk GC, Kirchner K, Schneeweiss B (2009) A critique of Stewart's approach: the chemical mechanism of dilutional acidosis. *Intensive Care Med* 35:2173–2180
- Carlesso E, Maiocchi G, Tallarini F, Polli F, Valenza F, Cadringer P, Gattinoni L (2010) The rule regulating pH changes during crystalloid infusion. *Intensive Care Med* 37:461–468
- Gattinoni L, Carlesso E, Maiocchi G, Polli F, Cadringer P (2009) Dilutional acidosis: where do the protons come from? *Intensive Care Med* 35:2033–2043
- Morgan TJ, Venkatesh B, Hall J (2004) Crystalloid strong ion difference determines metabolic acid-base change during acute normovolaemic haemodilution. *Intensive Care Med* 30:1432–1437
- Institute of Laboratory Animal Resources ColSNRC (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington
- Protti A, Cressoni M, Santini A, Langer T, Mietto C, Febres D, Chierichetti M, Coppola S, Conte G, Gatti S, Leopardi O, Masson S, Lombardi L, Lazzarini M, Rampoldi E, Cadringer P, Gattinoni L (2011) Lung stress and strain during mechanical ventilation: any safe threshold? *Am J Respir Crit Care Med* 183:1354–1362
- Caironi P, Langer T, Taccone P, Bruzzone P, De CS, Vagginelli F, Caspani L, Marengi C, Gattinoni L (2010) Kidney instant monitoring (K.IN.G): a new analyzer to monitor kidney function. *Minerva Anestesiol* 76:316–324
- Ewaldsson CA, Hahn RG (2005) Kinetics and extravascular retention of acetated ringer's solution during isoflurane or propofol anesthesia for thyroid surgery. *Anesthesiology* 103:460–469
- Fencel V, Jabor A, Kazda A, Figge J (2000) Diagnosis of metabolic acid-base disturbances in critically ill patients. *Am J Respir Crit Care Med* 162:2246–2251
- Hannon JP, Bossone CA, Wade CE (1990) Normal physiological values for conscious pigs used in biomedical research. *Lab Anim Sci* 40:293–298
- Gennari FJ, Goldstein MB, Schwartz WB (1972) The nature of the renal adaptation to chronic hypocapnia. *J Clin Invest* 51:1722–1730
- Polak A, Haynie GD, Hays RM, Schwartz WB (1961) Effects of chronic hypercapnia on electrolyte and acid-base equilibrium. I. Adaptation. *J Clin Invest* 40:1223–1237
- Hansen PB, Jensen BL, Skott O (1998) Chloride regulates afferent arteriolar contraction in response to depolarization. *Hypertension* 32:1066–1070
- Wilcox CS (1983) Regulation of renal blood flow by plasma chloride. *J Clin Invest* 71:726–735
- Bagshaw SM, George C, Dinu I, Bellomo R (2008) A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 23:1203–1210
- Bagshaw SM, Lapinsky S, Dial S, Arabi Y, Dodek P, Wood G, Ellis P, Guzman J, Marshall J, Parrillo JE, Skrobik Y, Kumar A (2009) Acute kidney injury in septic shock: clinical outcomes and impact of duration of hypotension prior to initiation of antimicrobial therapy. *Intensive Care Med* 35:871–881
- Joannidis M, Metnitz B, Bauer P, Schusterschitz N, Moreno R, Druml W, Metnitz PG (2009) Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. *Intensive Care Med* 35:1692–1702