

Hyperlactaemia Without Acidosis – An Investigation Using an *In Vitro* Model

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ABSTRACT

Objective: To use an *in vitro* dilutional blood model to simulate aerobic hyperlactaemia, and to question whether base excess and anion gap distinguish aerobic from anaerobic lactate production.

Methods: Cooled fresh blood was diluted (3:1) with nine different crystalloid solutions, each with a sodium concentration of 140 mmol/L but with strong ion difference values ranging from -5 mEq/L to 40 mEq/L due to varying concentrations of Cl⁻, HCO₃⁻ and lactate anions. Normocapnic pH and base excess values post-dilution were determined by gas equilibration. Strong ion difference and anion gap values were measured.

Results: There was close correlation between the normocapnic pH and both the diluent strong ion difference and the final strong ion difference of the diluted specimens ($R^2 = 0.96$ and 0.89 respectively). This was independent of lactate concentrations in diluent or in post-dilution plasma. Where lactate-containing crystalloid was added, base excess, normocapnic pH and anion gap were strongly correlated with the final plasma lactate concentrations ($R^2 \geq 0.99$). However, only at final lactate concentrations of approximately 10 – 15 mmol/L did values of base excess, normocapnic pH or anion gap indicate metabolic acidosis.

Conclusions: Hyperlactaemia from any source reduces strong ion difference and base excess and increases the anion gap, but values may remain in the normal range until hyperlactaemia is severe. Abnormal base excess and anion gap values do not distinguish aerobic from anaerobic lactate production. Normal values merely reflect low sensitivity to small lactate elevations (aerobic or otherwise). (**Critical Care and Resuscitation 1999; 1: 354-359**)

Key words: Hyperlactaemia, lactic acidosis, strong ion difference, dysoxia, blood dilution, aerobic glycolysis

Lactic acidosis is an acid-base disorder which alerts critical care practitioners to the possibility of tissue dysoxia.¹ It is characterised by the presence of a metabolic (non-respiratory) acidosis and an elevated anion gap, due to increased concentrations of the lactate anion.² When lactic acidosis is caused by bioenergetic failure, excessive endogenous lactate is generated as a result of anaerobic glycolysis.³ Protons are liberated in anaerobic tissues as a consequence of uncompensated ATP hydrolysis, titrating HCO₃⁻ and causing regional⁴ and sometimes venous⁵ hypercapnic acidosis.

There are occasions when hyperlactaemia develops without metabolic acidosis. For example, Hotchkiss and Karl drew attention to the occurrence in sepsis of mild to moderate hyperlactaemia (up to approximately 5 mmol/L) without metabolic acidosis and apparently without tissue dysoxia.³ They used evidence from a number of clinical studies and animal models where bioenergetic failure was generally excluded on the basis of normal lactate/pyruvate ratios, and occasionally on the basis of measured concentrations of high-energy phosphate compounds. The authors attributed this type

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of hyperlactaemia to 'accelerated aerobic glycolysis', also termed 'stress hyperlactaemia' by others.⁶ Hotchkiss and Karl opined that metabolic acidosis does not arise in this condition.³ A failure to elevate the anion gap was also mentioned as a defining characteristic, with both phenomena attributed to the absence of anaerobic ATP hydrolysis and associated proton release.

Other instances of mild hyperlactaemia without acidaemia include hyperventilation and exposure to β -adrenergic agonists.⁶ Similarly, hyperlactaemia can exist without tissue dysoxia, and sometimes without metabolic acidosis, when patients with hepatic and renal disease are exposed to large exogenous lactate loads. A common example is in critical illness when renal replacement therapy is initiated using dialysate and/or replacement solutions buffered with lactate. Although mild to moderate hyperlactaemia occurs regularly under these circumstances,^{7,8} metabolic acidosis may not be evident. In fact plasma lactate concentrations of around 4 – 5 mmol/L can coexist with base excess values of 4 – 5 mEq/L, indicative of a concurrent metabolic alkalosis. With more extreme elevations in plasma lactate concentrations, a metabolic acidosis usually appears.

Can hyperlactaemia develop without any tendency towards an associated metabolic acidosis or increase in the anion gap? According to the physico-chemical approach to acid-base, lactate is a strong anion.^{9,10} Acute hyperlactaemia taken in isolation reduces plasma strong ion difference (SID) by elevating strong anion concentrations, and a reduction in SID shifts the PCO_2/pH buffer relationship in the direction of metabolic acidosis.¹¹ If these physico-chemical concepts are correct, the rule applies equally to all types of hyperlactaemia, including the so-called 'stress hyperlactaemia' of sepsis and that of excessive exogenous lactate loading. If metabolic acidosis does not eventuate, other factors must be acting simultaneously to either prevent a significant reduction in SID or even to increase SID.

We devised a simple *in vitro* simulation of the effects of aerobic hyperlactaemia on acid-base balance. The simulation was designed primarily to investigate the mechanism whereby haemofiltration and replacement with lactate-buffered fluid can cause hyperlactaemia without metabolic acidosis in patients with varying ability to metabolise lactate. The model was also designed to provide information as to whether base excess and anion gap distinguish aerobic from anaerobic lactate production.

METHODS

Fresh whole blood was diluted (3:1 vol:vol) with nine different crystalloid solutions, each with $[\text{Na}^+] = 140$ mmol/L but with strong ion difference values

ranging from -5 mEq/L to 40 mEq/L due to varying concentrations of Cl^- , HCO_3^- and lactate anion.

In vitro blood dilutions

Solutions 1-3 were prepared as in Table 1, with calculated final strong ion concentrations as set out in Table 2. Venous blood was collected from one of the investigators (TJM) in a heparin-coated syringe and stored on ice. In separate 10mL syringes, 3mL aliquots of blood were mixed with varying combinations of Solutions 1-3 to a final volume of 4mL (Table 3). Nine separate blood dilutions were thus performed.

Table 1. Composition of diluent solutions

Solution*	Dialysate [†]	0.9% NaCl [‡] (mL)	0.45% NaCl [§] (mL)	8.4% NaHCO ₃ (mL)
1	100			
2		37	59	4
3		86.5	13.5	

* Final volume 100 mL

[†] Haemofiltration solution Formula No. 1 (Gambro, Sydney, Australia), [‡] 0.9% sodium chloride (Baxter, Australia), [§] 0.45% sodium chloride (Baxter Australia)

Table 2. Strong ion concentrations of diluent solutions

Diluent	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)	La ⁻ * (mEq/L)
1	140	1	101	45
2	140		100	
3	140		140	

* L-lactate anion

Table 3. Final blood dilutions (Dilutions 1-9)

Dilution	Blood (mL)	Solution 1 (mL)	Solution 2 (mL)	Solution 3 (mL)
1	3	1		
2	3	0.75	0.25	
3	3	0.5	0.5	
4	3	0.25	0.75	
5	3			1
6	3		0.25	0.75
7	3		0.5	0.5
8	3		0.75	0.25
9	3		1	

Gas equilibrations and measurements

A gas mixture containing 95% O₂ and 5% CO₂ (Carbogen, BOC Gases Australia, Ltd) was aspirated into each syringe containing cooled diluted blood specimens, and partial equilibration performed by agitation for two minutes. The gas was then expelled and blood gas analysis at 37°C, haemoglobin concentration and plasma lactate measurements were

performed (ABL-625, Radiometer, Copenhagen, Denmark). Two or three partial equilibrations with air were carried out subsequently in the same manner, until the PCO_2 at the final equilibration had fallen to less than 40 mm Hg. One further plasma lactate concentration was also measured, and the mean of the two values adopted as the final plasma lactate concentration ($[\text{La}^-]$). The plasma concentrations of Na^+ , K^+ , Cl^- and albumin were measured in each diluted specimen by automated multichannel analysis.

Gas equilibrations were performed on cooled blood primarily to suppress endogenous lactate production. In addition, equilibration of cooled blood with 5% CO_2 and subsequent blood gas analysis at 37°C produced initial PCO_2 values exceeding 40 mm Hg. Using subsequent equilibrations with air to serially reduce PCO_2 , it was then possible to define the log PCO_2/pH relationship spanning normocapnia, facilitating accurate calculation of the normocapnic pH. Although the sudden warming from a cooled state to 37°C within the analyser had the potential to cause bubbles, there was no detectable specimen inhomogeneity due to gas formation in these specimens.

By intent, all equilibrations resulted in a degree of hyperoxia, so that haemoglobin-oxygen saturations remained close to 100% throughout the period of blood gas data collection. This eliminated acid-base changes due to the appearance of reduced haemoglobin with its altered buffer capacity.¹²

Determination of pH at $\text{PCO}_2 = 40$ mm Hg (normocapnic pH), and calculation of base excess.

For each diluted specimen, the relationship between log PCO_2 and pH was determined by linear regression, and the normocapnic pH then calculated. Base excess values were determined for each specimen by substituting $\text{PCO}_2 = 40$ mm Hg, the corresponding normocapnic pH and the relevant mean haemoglobin concentration in the Van Slyke equation.¹³

Other calculations

The anion gap values for Dilutions 1 – 9 were determined as $[\text{Na}^+] - \{[\text{Cl}^-] + [\text{HCO}_3^-]\}$. The intrinsic SID of the final mixture of crystalloid diluent (prior to addition to blood) was determined for each admixture from the calculated final concentration of strong ions in the 1mL aliquot. The apparent SID (SID_a) of the blood specimens after dilution with crystalloid was determined as $\text{SID}_a = \{[\text{Na}^+] + [\text{K}^+]\} - \{[\text{Cl}^-] + [\text{La}^-]\}$

The relationships of normocapnic pH with the intrinsic SID of the diluting crystalloid and with the SID_a of the diluted blood specimens were determined

for all nine dilutions by linear regression. In the four dilutions where lactate-containing crystalloid had been added (Dilutions 1 - 4, using Dilution 9 as the baseline), the relationships of $[\text{La}^-]$ with normocapnic pH, base excess and anion gap were also derived by linear regression.

Calculations and statistics

A commercial software package was used (Excel 7.0 for Windows, Microsoft, Redmond, WA). Where relevant, data are reported as mean \pm SD.

RESULTS

The mean blood haemoglobin, plasma albumin and plasma sodium concentrations for the nine dilutions were all highly consistent, with values of 110 ± 6 g/L (29 measurements), 30 ± 2 g/L (9 measurements) and 141.7 ± 0.6 mmol/L (9 measurements) respectively. All log PCO_2/pH relationships were linear (all R^2 values were 1). The slopes and intercepts allowed simple calculation of normocapnic pH for each of the nine dilutions (these pH values are reported graphically in Figures 1 and 2).

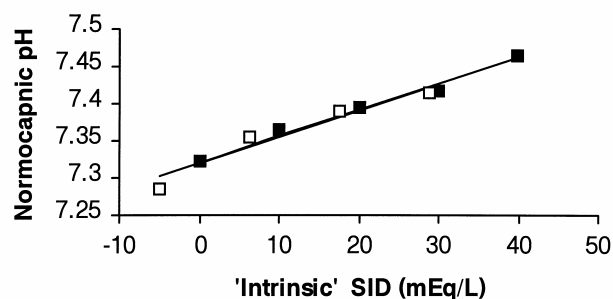


Figure 1. The relationship between the 'intrinsic' SID of the diluent fluids and the normocapnic pH of the diluted blood specimens. Open squares represent dilutions with lactate-containing diluent, and closed squares represent dilutions free of lactate. The regression line is illustrated. The equation of the regression is $\text{pH} = 0.004 \times \text{SID} + 7.32$.

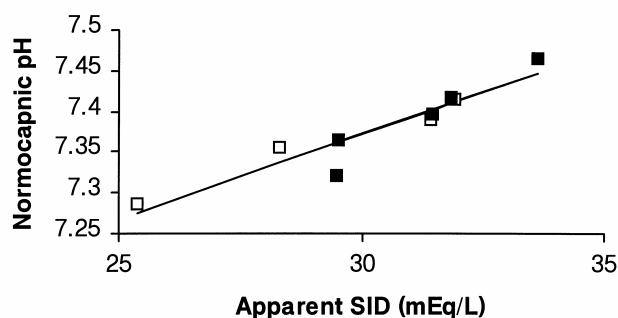


Figure 2. The relationship between the SID_a of the diluted blood specimens and the corresponding normocapnic pH values. Open and closed squares as in Figure 1. The equation of the regression is $\text{pH} = 0.021 \times \text{SID}_a + 6.75$.

There was close correlation between the normocapnic pH of all dilutions and both the intrinsic SID of the diluting crystalloid and the SIDa of the diluted specimens ($R^2 = 0.96$ and 0.89 respectively, Figures 1 and 2). This was true irrespective of whether lactate-containing crystalloid had been added (Figures 1 and 2).

The plasma lactate concentration in Dilution 1 was outside the quoted measurement range of the instrument (0-14.9 mmol/L). Since this dilution had been designed to produce a final plasma lactate concentration of slightly less than 15 mmol/L, this value was recorded as 15 mmol/L. In all specimens where lactate-containing crystalloid had been added (Dilutions 1 - 4, using Dilution 9 as baseline), base excess, normocapnic pH and anion gap were linearly related to the final plasma lactate concentrations ($R^2 \geq 0.99$, Figures 3 - 5). By inspection, only when final lactate concentrations exceeded approximately 10 mmol/L did values of base excess and normocapnic pH fall below their respective normal ranges (Figures 3 and 4). Similarly, only at a final lactate concentration close to 15 mmol/L did the anion gap exceed the laboratory reference range (Figure 5).

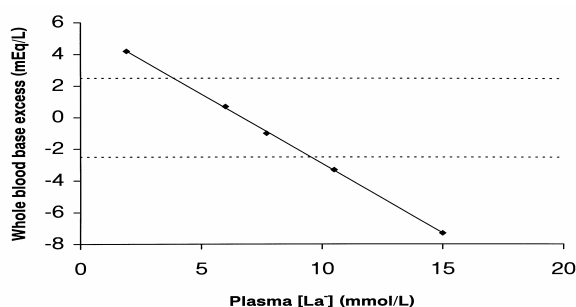


Figure 3. The relationship between plasma lactate concentrations and base excess values for blood specimens mixed with lactate-containing diluent (using Dilution 9 as baseline). Dotted horizontal lines denote the normal range for base excess. The equation of the regression is $\text{base excess} = 0.88 \times [\text{La}^-] + 5.88$.

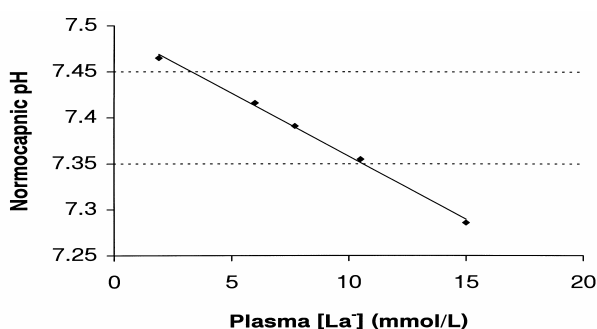


Figure 4. The relationship between plasma lactate concentrations and normocapnic pH values for blood specimens mixed with lactate-containing diluent (using Dilution 9 as baseline). Dotted horizontal lines denote the normal range for plasma pH. The equation of the regression is $\text{pH} = 0.137 \times [\text{La}^-] + 7.50$.

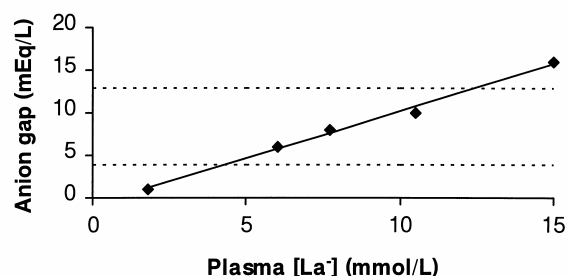


Figure 5. The relationship between plasma lactate concentrations and anion gap values for blood specimens mixed with lactate-containing diluent (using Dilution 9 as baseline). Dotted horizontal lines denote the laboratory reference range for anion gap. The equation of the regression is $\text{anion gap} = 1.1 \times [\text{La}^-] - 0.9$.

DISCUSSION

The simulation was originally designed to imitate aspects of continuous haemofiltration using lactate-buffered crystalloid as replacement fluid. The diluent component of the final plasma modeled that which originates from haemofiltration replacement solution. Graded combinations of Solutions 1 and 2 in the diluent represented a wide spectrum of metabolism and indirect conversion of the contained lactate to HCO_3^- . The 'intrinsic' SID of Solution 1, a lactate-buffered replacement solution in common use, is 40 mEq/L provided all the contained lactate is metabolised. (Calcium and magnesium ions are excluded from this calculation since they are weak ions with reversible pH-dependent binding to albumin and other proteins).¹⁴ Solution 2 was designed in such a way that it was lactate-free but with an identical SID at 40 mEq/L. Dilution 9 was thus a simulation of haemofiltration with complete metabolism of the infused lactate and total indirect conversion of lactate to HCO_3^- . However, the intrinsic SID of Solution 1 becomes -5 mEq/L if no lactate is metabolised. This scenario was mimicked by Dilution 1. Intermediate levels of lactate handling were represented by Dilutions 2 - 4. A second set of dilutions was devised to create a similar range of 'intrinsic' diluent SID values (Dilutions 5-9; 'intrinsic' diluent SID values 0 to 40 mEq/L). However, for these dilutions, alterations in SID were entirely due to changes in chloride rather than lactate concentrations.

The results support a basic tenet of the physical-chemical approach to acid-base, namely that the lactate anion acts like any other strong anion in its effect on acid-base balance. In the simulation, the important final determinant of plasma metabolic acid-base status was the SIDa, irrespective of whether the principle strong anion in the calculation was chloride alone or any combination of chloride and lactate (Figure 2). Non volatile weak acids $[\text{A}_{\text{TOT}}]$ in plasma (primarily albumin) and in whole blood (primarily haemoglobin)

were kept virtually constant by diluting at a fixed ratio of crystalloid to whole blood. The potential confounding influence of varying $[A_{TOT}]$ on the final metabolic acid-base balance was thus eliminated.⁹

The 'intrinsic' SID of the diluting crystalloid also strongly influenced the metabolic acid-base status of the diluted specimens, independently of the individual contributions of chloride and lactate (Figure 1). If lactate handling was simulated as normal (Dilution 9), the 'intrinsic' SID of haemofiltration replacement fluid was sufficiently large to cause a metabolic alkalosis. As plasma lactate concentrations rose with the simulated progressive inability to metabolise lactate (Dilutions 4,3,2 and 1 in sequence), base excess and normocapnic pH fell (Figures 3 and 4). This was due to a progressive reduction in SIDa. Only with simulated severe inability to metabolise lactate did metabolic acidosis begin to appear (Figures 3 and 4). These findings are consistent with our clinical observations on patients receiving continuous renal replacement therapy who have varying ability to metabolise lactate.

It is acknowledged that *in vitro* simulations never fully reflect *in vivo* events. The most important disparity is that acid-base changes *in vivo* are buffered in the total extracellular space rather than solely in blood. In the current simulation, a better approximation of *in vivo* conditions might have been obtained if the dilutions were performed at haemoglobin concentrations of 50 g/L, the approximate mean haemoglobin concentration of the extracellular space. However, even very dilute blood does not reproduce precisely the distributions and concentrations of proteins and strong ions in the extracellular compartment. A second concern is that a single dilution cannot fully represent the continuous process of fluid and electrolyte convection and replacement used in renal replacement therapy, and is even less successful at simulating diffusive dialytic mechanisms. Despite these *caveats*, *in vitro* simulations using whole blood can still illustrate broad principles.¹⁵ None of the weaknesses in the current simulation are considered sufficient to alter the broad thrust of the results, or to invalidate conclusions drawn from them in this paper.

What of the 'accelerated aerobic glycolysis' of Hotchkiss and Karl, who linked the absence of metabolic acidosis or elevation of the anion gap to a presumed lack of anaerobic ATP hydrolysis and associated proton release?³ Of relevance to their observations are the following factors. First, mild plasma hyperlactaemia (≤ 5 mmol/L) is a relatively small stimulus for metabolic acidosis, and can be masked by any concurrent tendency to a metabolic alkalosis (Figures 3 and 4). Second, such small rises in plasma lactate produce even smaller reductions in whole

blood base excess, as demonstrated by the slope of the linear regression (0.88) in Figure 3. This is because whole blood lactate concentrations are significantly less than plasma lactate concentrations,¹⁶ as dictated by plasma / erythrocyte lactate concentration gradients.¹⁷⁻¹⁹ For these two reasons, the sensitivity of base excess (the whole blood measure of metabolic acid-base status used in many relevant studies) is low for mild lactate elevations.

Finally, although hyperlactaemia strongly influences the anion gap (Figure 5), its value is likely to remain normal with small to moderate elevations in plasma lactate. In one clinical study, the anion gap remained normal in nearly 80% of cases of mild plasma hyperlactaemia (≤ 4.9 mmol/L).²⁰ This insensitivity is exacerbated by low plasma albumin concentrations as seen in our simulation and also in critical illness.²¹ Taking all these factors into account, it seems likely that the absence of metabolic acidosis or anion gap elevation in the mild hyperlactaemia of so-called 'accelerated aerobic glycolysis' is merely due to the low sensitivities of these tools in detecting small plasma lactate elevations.

Our data provide confirmation that, like any strong anion, hyperlactaemia reduces SID and base excess and increases the anion gap independently of any proton release from underlying dysoxia. This is logical since dysoxic proton release and consequent regional hypercapnia are exclusively tissue and venous phenomena. Provided respiratory homeostasis is intact, reductions in SID and base excess are the only manifestations in arterial blood – phenomena which are not exclusive to bioenergetic failure. Conversely, the absence of metabolic acidosis or of an elevated anion gap in mild hyperlactaemia allows no conclusions to be drawn about bioenergetic failure versus so called 'accelerated aerobic glycolysis'. Even a normal lactate/pyruvate ratio is a poor discriminator, since blood lactate/ pyruvate ratios may not accurately reflect mitochondrial redox potential and bioenergetic status.^{22,23} Rather than invoking 'accelerated aerobic glycolysis', we suggest that bioenergetic dysfunction cannot be ruled out in at least some cases of sepsis with mild hyperlactaemia, irrespective of the anion gap and base excess values or lactate/pyruvate ratios. However, if bioenergetic failure is present, it is unlikely to be due to tissue hypoxia.^{24,25} A more likely mechanism is mitochondrial dysoxia arising from cytochrome impairment induced by endotoxin, cytokines or nitric oxide.²⁶⁻²⁸

We conclude that hyperlactaemia reduces SID and base excess like any strong anion, and also increases the anion gap. However, values of these indices may remain in the normal range until very high lactate concentrations prevail, as in our simulation. Neverthe-

less, our data confirm that metabolic acidosis and anion gap elevations can occur without the proton release associated with anaerobic lactate production. Conversely normal values of these indices in mild hyperlactaemia merely reflect their low sensitivities to small lactate elevations – whether the hyperlactaemia is aerobic or anaerobic in origin.

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