Commentary

Krebs cycle anions in metabolic acidosis

Francis G Bowling[1] and Thomas J Morgan[2]

[1]Director of Biochemical Diseases, Mater Children's Hospital and Professor of Medical Biochemistry, School of Molecular and Microbial Sciences, University of Queensland, Brisbane, Australia
[2]Senior Specialist, Adult Intensive Care Units, Mater Health Services, Brisbane, Australia

Corresponding author: Thomas J Morgan, thomas.morgan@mater.org.au

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Abstract

For many years it has been apparent from estimates of the anion gap and the strong ion gap that anions of unknown identity can be generated in sepsis and shock states. Evidence is emerging that at least some of these are intermediates of the citric acid cycle. The exact source of this disturbance remains unclear, because a great many metabolic blocks and bottlenecks can disturb the anaplerotic and cataplerotic pathways that enter and leave the cycle. These mechanisms require clarification with the use of tools such as gas chromatography–mass spectrometry.

In this issue of *Critical Care* a familiar acid–base conundrum is addressed [1]. It has long been suspected that the list of endogenous anions that can cause metabolic acidosis in sepsis and shock states is far from complete. Scanning tools such as the anion gap [2] and more recently the strong ion gap [3] have signalled this probability for years [4-6]. However, tools based on electrical neutrality provide no clues to their identity. To give a recent example, Kaplan and Kellum detected marked elevations in the strong ion gap (mean value 10.8 mEq/L) in plasma from patients with major vascular injuries, elevations that were closely correlated with mortality [7]. The authors could only speculate on the identity of the hidden anionic charges, because not even β-hydroxybutyrate concentrations could be analysed in this retrospective study.

However, they were able to add one piece to the puzzle. The fact that sampling preceded resuscitation eliminated any role for administered resuscitation fluids. Of course, saline was never a potential culprit, despite its known propensity to cause metabolic acidosis. The mechanism here is simple narrowing of the concentration difference between extracellular sodium and chloride, reducing strong ion difference [8]. The anion gap will tend to fall rather than rise, primarily as a result of albumin dilution, and there should be no change in the strong ion gap. However, the so-called 'balanced' fluids contain strong organic anions such as lactate, gluconate and acetate, which require metabolic processing on administration. In situations of metabolic stress, their delayed disappearance could increase the anion gap and particularly the strong ion gap, at least transiently. This is certainly true in cardiopulmonary bypass [9], and potentially so in sepsis and shock states. Similarly, colloids containing gelatin, with its properties as a non-volatile weak acid, are known to elevate the strong ion gap [10], this time by contributing an unmeasured component to the buffer base.

Now Forni and colleagues report on a series of carefully conducted plasma assays from patients with various types of metabolic acidosis, as well as healthy controls [1]. They took pains to minimise continuing metabolic activity, using centrifugation and ultrafiltration to remove all cellular remnants. In lactic acidosis, ketoacidosis and in acidosis when the anion gap was elevated by unclear mechanisms, they found significant increases in intermediates of the citric acid (Krebs) cycle. This did not occur in normal anion gap acidosis. The raised anion gap groups displayed increases across the board in isocitrate, α-ketoglutarate and malate. Citrate was elevated only in lactic acidosis, whereas succinate was increased in lactic acidosis and acidosis of unknown origin. Surprisingly, there were increases in D-lactate in all types of metabolic acidosis, anion gap or otherwise.

The authors found that these anions in aggregate were sufficient to make a significant contribution to the anion gap. They deemed it unlikely that the acidaemia itself was responsible for the accumulated Krebs cycle intermediates, although we are not told the comparative severities of the acidaemia in the various groups. Their data are of interest and raise a number of questions.

First, why was there an accumulation of D-lactate? This molecule is normally generated by bacterial metabolism in the gut. Was there splanchnic hypoperfusion and increased gut permeability in these presumably very unwell individuals [11],

with or without accompanying enteric bacterial overgrowth? More fundamentally, we need to know that the D-lactate elevations were not simply an artefact. For example, if L-lactate was measured by an enzymatic method and D-lactate was subsequently derived from the total lactate concentration determined by another method such as mass spectrometry, an opportunity for analytical error would have existed. A systematic underestimation of L-lactate would lead to an overestimate of D-lactate, the error being in proportion to the total lactate concentration. Along these lines it is noteworthy that the highest D-lactate concentrations were seen in the lactic acidosis group.

Second, as for the Krebs intermediates, we need to ask what was disturbing the delicate interaction between the anaplerotic and cataplerotic processes that normally keep each station of the citric acid cycle replenished but not overloaded [12]. The authors postulate that the increases were driven by anaplerosis secondary to accelerated amino acid catabolism. The usual end product of amino acid oxidation is the formation of ketone bodies, although it is true that these substrates can also feed into the Krebs cycle. Such a hypothesis can be tested by direct measurement of plasma amino acids.

There are other possibilities, although none completely satisfying. For example, the Krebs and urea cycles are intimately linked and cross-regulated through the aspartate arginino-succinate shunt. Within the liver two enzymes, glutamine synthase and glutamate dehydrogenase, regulate the urea cycle and the production of ammonium. These enzymes are pH dependent. During acidaemia glutamine synthase predominates, so that the urea cycle is inhibited and the intermediate arginino-succinate anion is depleted. This particular hypothesis can be tested by the measurement of ammonium levels, which would be expected to accumulate.

In contrast, gas chromatography–mass spectrometry might have identified other organic acids present, because there are a host of metabolic intermediates that can affect the citric acid cycle on accumulation. For example, if the D-lactate release was truly a biomarker for enteric disruption and bacterial overgrowth as we have hypothesised, a functional B_{12} deficiency not revealed by total B_{12} assays could have resulted [13]. This would cause 3-methylcitrate to accumulate, along with other direct inhibitors of the Krebs cycle. A disturbance along these lines could explain the reduced ratio of citrate to isocitrate commented on by the authors, as well as the accumulation of the other intermediates.

Other hypotheses can be made. All are mere speculation at this point, and need to be tested. As is so often the case, answering one question has triggered a host of new ones.

Competing interests

The author(s) declare that they have no competing interests.

References

1. Forni LG, McKinnon W, Lord GA, Treacher DF, Peron J-M, Hilton PJ: **Circulating anions usually associated with the Krebs cycle in patients with metabolic acidosis.** *Crit Care* 2005, **9:**R591-R595.
2. Emmet M, Narins RG: **Clinical use of the anion gap.** *Medicine* 1977, **56:**38-54.
3. Kellum JA, Kramer DJ, Pinsky MR: **Strong ion gap: a methodology for exploring unexplained anions.** *J Crit Care* 1995, **10:**51-55.
4. Rackow EC, Mecher C, Astiz ME, Goldstein C, McKee D, Weil MH: **Unmeasured anion during severe sepsis with metabolic acidosis.** *Circulatory Shock* 1990, **30:**107-115.
5. Hatherill M, Waggie Z, Purves L, Reynolds L, Argent A: **Correction of the anion gap for albumin in order to detect occult tissue anions in shock.** *Arch Dis Child* 2002, **87:**526-529.
6. Kellum JA, Bellomo R, Kramer DJ, Pinsky MR: **Splanchnic buffering of metabolic acid during early endotoxemia.** *J Crit Care* 1997, **12:**7-12.
7. Kaplan LJ, Kellum JA: **Initial pH, base deficit, lactate, anion gap, strong ion difference, and strong ion gap predict outcome from major vascular injury.** *Crit Care Med* 2004, **32:**1120-1124.
8. Morgan TJ: **The meaning of acid-base abnormalities in ICU Part III: Effects of fluid administration.** *Crit Care* 2005, **9:**204-211.
9. Liskaser FJ, Bellomo R, Hayhoe M, Story D, Poustie S, Smith B, Letis A, Bennett M: **Role of pump prime in the etiology and pathogenesis of cardiopulmonary bypass-associated acidosis.** *Anesthesiology* 2000, **93:**1170-1173.
10. Hayhoe M, Bellomo R, Liu G, McNicol L, Buxton B: **The aetiology and pathogenesis of cardiopulmonary bypass-associated metabolic acidosis using polygeline pump prime.** *Intensive Care Med* 1999, **25:**680-685.
11. Sun ZQ, Fu XB, Zhang R, Lu Y, Deng Q, Jiang XG, Sheng ZY: **Relationship between plasma D(–)-lactate and intestinal damage after severe injuries in rats.** *World J Gastroenterol* 2001, **7:**555-558.
12. Owen OE, Kalhan SC, Hanson RW: **The key role of anaplerosis and cataplerosis for citric acid cycle function.** *J Biol Chem* 2002, **277:**30409-30412.
13. Herrmann W, Obeid R, Schorr H, Geisel J: **Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk.** *Clin Chem Lab Med* 2003, **41:**1478-1488.