AJRCCM Articles in Press. Published on 19-May-2019 as 10.1164/rccm.201904-0899ED

Deconstructing hyperlactatemia in sepsis using $ScvO_2$ and base deficit

Matthew W. Semler, MD, MSc; Mervyn Singer, MD, FRCP

First Author:
Matthew W. Semler, MD, MSc
Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, C-1216 Medical Center North 1161 21st Ave S., Nashville, TN 37232-2650
Email: matthew.w.semler@vanderbilt.edu

Final and Corresponding Author:
Mervyn Singer, MD, FRCP
Bloomsbury Institute of Intensive Care Medicine, Division of Medicine, University College London, Cruciform Building, Gower St, WC1E 6BT London, UK.
Email: m.singer@ucl.ac.uk.

Sources of Funding: M.W.S. was supported in part by the NHLBI (K23HL143053). M.S. was supported in part by the MRC, Wellcome Trust, EU and NIHR.
Conflict of interest: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

For over half a century, clinicians and researchers have endeavored to understand the relationship between oxygen delivery and lactic acidosis (1,2). Or, as discussed later, perhaps more readily considered as "hyperlactatemia with or without acidemia". In health, pyruvate (the generally-acknowledged end-product of glycolysis) is metabolized by mitochondria to acetyl CoA to feed the tricarboxylic acid cycle. Excess pyruvate is reduced by lactate dehydrogenase to L-lactate. Notably, this reduction consumes a proton: pyruvate + NADH + H^+ ←→ lactate + NAD^+. Lactate is subsequently oxidized back to pyruvate, either locally or after transfer to organs that utilise lactate as a fuel source (e.g. liver, kidney, brain), or that convert it back to glucose (Cori cycle in the liver). In concert, these processes maintain normal blood lactate levels.

During sepsis, lactate levels frequently rise. Indeed, hyperlactatemia (a measurable surrogate for cellular/metabolic perturbations) is closely associated with sepsis prognosis and is now one of the criteria for septic shock (3). However, it remains challenging to determine clinically when a persistently elevated serum lactate level indicates ongoing inadequacy of oxygen delivery, or when the problem lies elsewhere. The brainstem response to give yet more fluid is often inappropriate and potentially injurious.

Hyperlactatemia during sepsis may result from anaerobic glycolysis. When whole-body oxygen delivery fails to meet cellular demands, tissues transition from mitochondrial aerobic respiration to less efficient ATP generation by glycolysis. This is most common at the time of initial patient presentation and, in many cases, resolves with administration of intravenous fluids ± vasoactive agents. However,

AJRCCM Articles in Press. Published on 19-May-2019 as 10.1164/rccm.201904-0899ED

other factors may also increase serum lactate levels in sepsis, including β_2-receptor stimulation from endogenous/exogenous catecholamines, impaired tissue oxygen extraction (mitochondrial ± microcirculatory dysfunction), liver dysfunction, and thiamine deficiency.

To aid the clinician in his/her decision-making, Gattinoni and colleagues in this issue of the *Journal* propose a conceptual model relating oxygen delivery and utilization, serum lactate concentration, and acidemia (4). They analyzed data from 1741 intensive care unit (ICU) patients enrolled into the Albumin Italian Outcome Sepsis (ALBIOS) trial using serum lactate, central venous oxygen saturation ($ScvO_2$) and blood gas measurements taken at study enrollment (5). Fundamentally, their proposed model frames two clinical questions:

1. Is an elevated lactate level due to inadequate oxygen delivery, and therefore potentially responsive to interventions that increase oxygen delivery?
2. How does an elevated serum lactate affect arterial pH and base excess?

Hyperlactatemia and central venous oxygen saturation

High values of $ScvO_2$ suggest either a systemic oxygen delivery in excess of oxygen demand, impaired cellular (mitochondrial) oxygen utilization, and/or microcirculatory shunting. Low $ScvO_2$ values imply inadequate oxygen delivery that fails to meet metabolic demands. Gattinoni and colleagues propose using $ScvO_2$ to personalize sepsis management, reserving interventions to increase oxygen delivery only to those patients with low $ScvO_2$ values. Of note, only 35% of patients in the ALBIOS trial had $ScvO_2$ values <70%. Other recent sepsis trials report similar $ScvO_2$ values after initial resuscitation (6).

This proposal is not inherently novel. The concept of early goal-directed therapy (EGDT) (7) and the Surviving Sepsis Campaign recommendations (8) both suggest a low $ScvO_2$ should trigger interventions to increase oxygen delivery (e.g. fluid, inotropes, blood). This concept has a strong physiologic rationale, but the devil is in the detail.

First, the patients in ALBIOS study and the three recent EGDT trials (6) were all enrolled *after* initial resuscitation. On first presentation, many will have impaired oxygen delivery and thus lower $ScvO_2$ values, and a higher likelihood of responding positively to empiric fluid administration. An important caveat is that a low $ScvO_2$ in sepsis does not automatically equate to hypovolemia. Cardiomyopathy can also contribute, and may be worsened by excessive fluid administration.

Second, many patients with sepsis-associated hyperlactatemia have $ScvO_2$ values falling within an indeterminate range; even patients with an elevated $ScvO_2$ may respond physiologically to fluid administration (9). Moreover, $ScvO_2$ is a 'global' (or, rather an 'upper-body') measure of oxygen supply-demand balance, and may miss imbalances in specific tissue beds (10).

Finally, the history of sepsis research is paved with physiologically-rational interventions that nonetheless failed to improve patient outcomes (11). The recent EGDT trials showed no benefit in targeting $ScvO_2$ even among the subset

AJRCCM Articles in Press. Published on 19-May-2019 as 10.1164/rccm.201904-0899ED

of patients with baseline values <70% (6). Interventions to increase oxygen delivery may carry unintended consequences outside the mechanistic pathway assessed by $ScvO_2$ measurement (12,13). Therefore, an $ScvO_2$-based strategy to personalize interventions for patients with sepsis-associated hyperlactatemia requires careful evaluation in clinical trials before any recommendation of standard-of-care implementation in clinical practice.

Hyperlactatemia and arterial pH

Applying strong ion theory, lactate is a strong anion and should thus be completely dissociated from hydrogen in plasma, generating an acidosis. However, some sepsis patients with hyperlactatemia have a concurrently decreased pH (acidemia) whereas others maintain a normal pH. This suggests mechanisms that enable relatively rapid respiratory or metabolic compensation. Gattinoni and colleagues found that the ability to maintain a normal pH despite elevated lactate was more closely correlated with renal function than respiratory compensation. They propose using an indirect measure of the accumulation of renally-excreted fixed acids in plasma - the "*alactic base excess*" – to assess the kidneys' ability to compensate for acid-base disturbances.

Standard base excess, defined as the amount of strong acid that must be added to each liter of oxygenated blood to return the pH to 7.40 at a $PaCO_2$ of 40 mmHg, quantifies the degree of metabolic acidosis or alkalosis independently of respiratory compensation. Contributors to base excess include lactate, strong ions such as sodium and chloride, albumin, and ions that accumulate in renal failure such as phosphate and sulfate (14). By adding lactate to standard base excess, the authors arrive at the alactic base excess which they assert quantifies *"the role of renal function on acid-base balance in sepsis."*

This suggestion is certainly interesting but requires further thought and investigation. Renal compensation for acid-base disturbances has traditionally been considered to be slower than respiratory compensation. Detailed data on urine output, stage of acute kidney injury (15), minute ventilation, and other physiologic measures would be required before the relative causal effects of kidney injury in compensating for an acidosis could be fully understood. Alactic base excess is not necessarily an explicit measure of renal function. For example, administration of 0.9% sodium chloride decreases base excess, even in the presence of stable renal function and lactate concentrations (16). The impact of concurrent liver dysfunction requires consideration; few such patients were in the ALBIOS database. Nonetheless, the concept of alactic base excess and the role of renal function in modifying acidemia warrant evaluation in future physiologic studies.

In summary, Gattinoni and colleagues are to be congratulated for advancing an ambitious conceptual model relating oxygen delivery, lactate generation, renal function, and acidemia in sepsis. We are eager to see future research to confirm and refine this model – and move us closer to the authors' vision of a more personalized approach to early hemodynamic management for sepsis.

AJRCCM Articles in Press. Published on 19-May-2019 as 10.1164/rccm.201904-0899ED

References:

1. Huckabee WE. Abnormal resting blood lactate. I. The significance of hyperlactatemia in hospitalized patients. *Am J Med* 1961;30:840–848
2. Weil MH, Afifi AA. Experimental and clinical studies on lactate and pyruvate as indicators of the severity of acute circulatory failure (shock). *Circulation* 1970;41:989–1001.
3. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent J-L, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016;315:801–810.
4. Gattinoni L, Vasques, F, Camporota L, Meessen J, Romitti F, Pasticci I, Duscio E, Vassalli F, Fomi L, Payen D, Cressoni M, Zanella A, Latini R, Quintel M, Marini J. Understanding Lactatemia in Human Sepsis: Potential Impact for Early Management. *Am J Respir Crit Care Med* [online ahead of print] 15 April 2019; https://www.atsjournals.org/doi/abs/10.1164/rccm.201812-2342OC.
5. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L, ALBIOS Study Investigators. Albumin replacement in patients with severe sepsis or septic shock. *N Engl J Med* 2014;370:1412–1421

6. PRISM Investigators, Rowan KM, Angus DC, Bailey M, Barnato AE, Bellomo R, Canter RR, Coats TJ, Delaney A, Gimbel E, Grieve RD, Harrison DA, Higgins AM, Howe B, Huang DT, Kellum JA, Mouncey PR, Music E, Peake SL, Pike F, Reade MC, Sadique MZ, Singer M, Yealy DM. Early, Goal-Directed Therapy for Septic Shock - A Patient-Level Meta-Analysis. *N Engl J Med* 2017;376:2223–2234.
7. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M, Early Goal-Directed Therapy Collaborative Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001;345:1368–1377.
8. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche J-D, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, *et al.* Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Crit Care Med* 2017;45:486–552
9. Velissaris D, Pierrakos C, Scolletta S, De Backer D, Vincent JL. High mixed venous oxygen saturation levels do not exclude fluid responsiveness in critically ill septic patients. *Crit Care* 2011;15:R177.

AJRCCM Articles in Press. Published on 19-May-2019 as 10.1164/rccm.201904-0899ED

10. Dyson A, Stidwill R, Taylor V, Singer M. Tissue oxygen monitoring in rodent models of shock. *Am J Physiol Heart Circ Physiol* 2007;293:H526-533.
11. Hayes MA, Timmins AC, Yau EH, Palazzo M, Hinds CJ, Watson D. Elevation of systemic oxygen delivery in the treatment of critically ill patients. *N Engl J Med* 1994;330:1717–1722
12. Semler MW, Self WH, Wanderer JP, Ehrenfeld JM, Wang L, Byrne DW, Stollings JL, Kumar AB, Hughes CG, Hernandez A, Guillamondegui OD, May AK, Weavind L, Casey JD, Siew ED, Shaw AD, Bernard GR, Rice TW, SMART Investigators and the Pragmatic Critical Care Research Group. Balanced Crystalloids versus Saline in Critically Ill Adults. *N Engl J Med* 2018;378:829–839.
13. Andreis DT, Singer M. Catecholamines for inflammatory shock: a Jekyll-and-Hyde conundrum. *Intensive Care Med* 2016;42:1387–97.
14. Story DA. Stewart Acid-Base: A Simplified Bedside Approach. *Anesth Analg* 2016;123:511–515.
15. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int* 2012; 2(Suppl):1-138
16. Scheingraber S, Rehm M, Sehmisch C, Finsterer U. Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. *Anesthesiology* 1999;90:1265–1270.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Understanding lactatemia in human sepsis: potential impact for early management

Luciano Gattinoni[1], MD, FRCP, Francesco Vasques[2], MD, Luigi Camporota[2], MD, PhD, Jennifer Meessen[3], MSc, Federica Romitti[1], MD, Iacopo Pasticci[1], MD, Eleonora Duscio[1], MD, Francesco Vassalli[1], MD, Lui G. Forni[4], MD, Didier Payen[5], MD, Massimo Cressoni[6], MD, Alberto Zanella[7,8], MD, Roberto Latini[3], MD, Michael Quintel[1], MD, John J. Marini[9], MD

Authors' affiliations:

1 Department of Anaesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Germany

2 Department of Adult Critical Care, Guy's and St Thomas' NHS Foundation Trust, Health Centre for Human and Applied Physiological Sciences, King's College London, London, UK

3 Department of Cardiovascular Research, IRCCS - Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy.

4 Department of Intensive Care Medicine, Royal Surrey County Hospital NHS Foundation Trust, and Faculty of Health Sciences University of Surrey, Guildford, UK

5 Department of Anaesthesiology and Critical Care, Lariboisière University Hospital, Assistance Publique-Hôpitaux de Paris, University Paris 7 Diderot, Paris, France.

6 Dipartimento di Scienze della Salute, Università degli Studi di Milano Bicocca, Milan, Italy.

7 Anaesthesia and Critical Care, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

8 Department of Anaesthesia, Critical Care and Emergency, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

9 Regions Hospital and University of Minnesota, St. Paul, Minnesota, United States

Corresponding author:

Prof. Luciano Gattinoni, University of Göttingen

Department of Anaesthesiology

Robert-Koch-Straße, 40

37075 Göttingen – Germany

E-Mail: gattinoniluciano@gmail.com; Tel: +49-(0)551-394519

Authors' contribution:

Conception and design: Luciano Gattinoni, Michael Quintel, John J Marini

Data Collection: Massimo Cressoni, Alberto Zanella, Eleonora Duscio, Iacopo Pasticci

Analysis and interpretation: Francesco Vasques, Roberto Latini, Jennifer Meessen, Federica Romitti, Lui Forni, Didier Payen

Drafting the manuscript for important intellectual content: Luciano Gattinoni, FrancescoVasques, John J Marini, Luigi Camporota, Francesco Vassalli

Funding: institutional

Competing interests: The authors have declared that no competing interest exists.

Running head: lactatemia in sepsis

Descriptor number: 4.12 Sepsis/Multiple Organ Failure

Total word count of manuscript: 3349

Total word count of abstract: 250

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

ABSTRACT

Rationale: Hyperlactatemia in sepsis may derive from a prevalent impairment of oxygen supply/demand and/or oxygen utilization. Discriminating between these two mechanisms may be relevant for the early fluid resuscitation strategy.

Objectives: Understanding the relationship between central venous oxygen saturation (ScvO2), lactate and base excess to better determine the origin of lactate.

Methods: Post-hoc analysis of baseline variables of 1741 septic patients enrolled in a multicentre trial (ALBIOS). Variables were analysed as a function of sextiles of lactate concentration and sextiles of ScvO2. We defined the 'alactic base excess', as the sum of lactate and standard base excess.

Measurements: Organ dysfunction severity scores, physiological variables of hepatic, metabolic, cardiac and renal function and 90-day mortality.

Main results: ScvO2 was lower than 70% only in 35% of patients. Mortality, organ dysfunction scores, lactate were highest in the first and sixth sextiles of ScvO2. Although lactate level related strongly to mortality, it was associated with acidemia only when kidney function was impaired (creatinine >2 mg/dL), as rapidly detected by a negative alactic base excess. In contrast, positive values of alactic base excess were associated with a relative reduction of fluid balance.

Conclusions: Hyperlactatemia is powerfully correlated with severity of sepsis and, in established sepsis, is caused more frequently by impaired tissue oxygen utilization, rather than by impaired oxygen transport. Concomitant acidemia was only observed in the presence of renal dysfunction, as rapidly detected by alactic base excess. The current strategy of fluid resuscitation could be modified according to the origin of excess lactate.

Key words: sepsis, lactic acidosis, venous oxygen saturation, base excess

Total word count of abstract: 250

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

INTRODUCTION

Hyperlactatemia has historically been associated with adverse outcomes in critically ill patients (1) and still represents the strongest outcome indicator in sepsis. Hyperlactatemia, however, may originate from a variety of causes, such as a deficit in oxygen delivery (tissue hypoxia) or impaired oxygen extraction (2), peripheral shunting (3), stress (4) and increased adrenergic stimulation (5). It is not clear, however, in what proportions these different events may occur in septic patients, and how their relative importance could be clinically discriminated. Furthermore, hyperlactatemia may occur in presence or absence of acidemia, and the reasons for this variability are still unclear. Finally, but very importantly, we wonder if better recognition of the mechanism of hyperlactatemia and acidemia would influence the treatment of patients with sepsis and positively alter their outcome. To address these questions, we propose a unifying interpretation of the pathophysiology of lactate in sepsis. This approach broadens one previously suggested (6, 7), and still accepted by many: lactate elevation arises from tissue hypoxia that originates primarily from a deficit in oxygen transport. This common clinical perception often motivates adherence to the current resuscitation approach of aggressive and indiscriminate non-selective administration of fluid. Our expanded interpretation is based on accepted principles that are well known and documented in isolation but have never been grouped together and presented as a unified model. We applied this unifying conceptual interpretation to a large dataset of septic patients, derived from the ALBIOS study (8), using the baseline data collected before randomisation. We hypothesized that, after accounting for potentially relevant confounders, hyperlactatemia is present both at high and low values of $ScvO_2$ and that the presence or absence of kidney injury will determine the final effect of plasma lactate concentration on pH.

METHODS

Patients

This study is a secondary analysis of the ALBIOS study (8), a multicentre randomized controlled trial conducted between 2008 and 2012 in 100 Italian intensive care units that compared the effects of 20% albumin and crystalloids versus crystalloids alone in severe sepsis and septic shock. In the present study, we included 1741/1818 patients for whom both serum lactate and central venous oxygen saturation ($ScvO_2$) measurements were available at baseline (see electronic supplement, Figure E1). Measurement were collected at baseline (within 24 h from the diagnosis of sepsis) after randomization and before the albumin administration. We do not know the volume or composition of fluids given to the patients in the emergency room and/or in ICU before the randomization. Therefore, our analysis refers to the subsequent phase of sepsis management.

Study design

We analysed baseline clinical, physiological and hemodynamic variables as functions of lactate concentration, $ScvO_2$ levels and alactic base excess (see below). These variables were grouped into sextiles that included similar numbers of patients (more than 250 each).

Measured variables

Clinical

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

We recorded: sequential organ failure assessment score (SOFA) (9); simplified acute physiology score II (SAPS-II) (10), 90-day mortality, bilirubin, glucose, creatinine, albumin, platelets and leukocytes count, percentage of patients fulfilling the Sepsis-2 definition (as defined by the ALBIOS study entry criteria (8)) or Sepsis-3 criteria (i.e., vasopressor requirement to maintain MAP ≥ 65 mmHg with lactate levels > 2 mmol/L) (11, 12); and proportion of patients requiring renal replacement therapy (RRT).

Physiologic

Fraction of inspired oxygen, arterial and venous partial pressures of oxygen and carbon dioxide, arterial and venous pH, arterial base excess, sodium, chloride and potassium, diuresis and fluid balance in the first six hours after admission.

Hemodynamic

Central venous and mean arterial pressures, heart rate, use and dosing of epinephrine, norepinephrine, central venous oxygen saturation, arterio-venous difference in oxygen content, veno-arterial difference of CO_2 partial pressures and haemoglobin concentration.

Computed variables

We computed the standard base excess (BE) as:

$$Standard\ BE\ (^{mmol}/_{L}) = \left(HCO3^{-}\ (mmol/L) - 24.8\ ^{mmol}/_{L}\right) + 16.2\ ^{mmol}/_{L} * (pH - 7.4)$$

We used standard base excess rather than actual as better representative of the buffer base status of the extracellular fluid(13). See Online supplement data for details.

To better understand the relationship between hyperlactatemia and acidemia, we introduce the concept of "alactic base excess" (alactic BE), which helps in the rapid discrimination between metabolic acidosis secondary to lactate accumulation from that caused by an increase in fixed acids (unmeasured strong anions):

$$Alactic\ BE(mmol/L) = standard\ base\ excess(mmol/L) + lactate(mmol/L)$$

This variable focuses on the role of fixed acids other than lactate in the sepsis scenario (Fixed acids refer to the acids, e.g, phosphates and sulfates, which cannot be eliminated through the lungs).

Statistics

Patient characteristics are reported as mean ± standard deviation. Lactate and $ScvO_2$ were divided into sextiles. The division into sextiles was arbitrarily decided in order to provide reasonable resolution power of the model, while maintaining adequate patient number in each quantile (circa 250). In this way, the results are more easily understandable than splitting the independent variables according to restricted cubic splines, which provide, however, similar results. (See Online data supplement for details.) Comparison of continuous variables among groups was made through one-way ANOVA test with Tukey's honest significant difference test for pair-wise comparisons. Dichotomous variables were compared using the Chi-square test. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using R and Graphpad Prism Software.

Study approval

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

The protocol of the original ALBIOS study and the informed-consent process were approved by the ethics committee at each participating institution. Written informed consent or deferred consent was obtained from each patient.

RESULTS

Lactate and clinical variables

As shown in Figure 1, mortality and SOFA score progressively increased across the sextiles of lactate. In contrast, the central venous oxygen saturation ($ScvO_2$) remained remarkably similar ($ScvO_2$ ~ 72%) throughout the first five sextiles of lactate (i.e., lactate levels ranging from 0.1 to 5.6 mmol/L) and slightly, but significantly, decreased to 69.7% in the highest lactate sextile (i.e., lactate levels from 5.6 to 27 mmol/L). Similarly, pH remained similar and in the normal range within the first five sextiles of lactate and decreased significantly to a mean (±SD) of 7.31 (± 0.14) only in the terminal lactate sextile. Most of the other measured clinical, physiologic and hemodynamic variables deteriorated with increasing lactate levels, as reported in Table E1. Briefly, central venous pressure (p=0.01), heart rate (p<0.001), norepinephrine requirements, mean arterial pressure (p<0.001), diuresis and fluid balance deteriorated progressively (p<0.001); PaO_2 and $PaCO_2$ values were similar across all lactate sextiles. There was no obvious relationship between alactic BE and lactate level.

$ScvO_2$, lactate and tissue hypoxia

As shown in Figure 2, the $ScvO_2$, as recorded in all the septic patients without exclusions, ranged from 24% to 98% (median 73%, IQR 67%-79%). As shown in Figure 3, a number of clinical variables – including lactate, SOFA score and mortality– showed a U-shaped relationship with the $ScvO_2$. Indeed, the worst values of these variables were observed at the lowest and highest levels of $ScvO_2$. Among the other clinical, hemodynamic and laboratory variables grouped by sextiles of increasing $ScvO_2$ (see Table E2), creatinine and renal replacement therapy (RRT) displayed U-shaped behaviour, central venous oxygen pressure ($PcvO_2$), PaO_2, $PaCO_2$ and $PcvCO_2$ progressively increased ($p<0.001$); pH ($p<0.003$) and base excess ($p<0.001$) significantly decreased, while the arterio-venous difference in oxygen content showed a three-fold decrease (from 6 to 2 ml/dl) from the first to the sixth $ScvO_2$ sextile ($p<0.001$). Conversely, central venous pressure, heart rate and vasoactive drugs requirements were similar for all sextiles of $ScvO_2$. The average value of mean arterial pressure remained above 70 mmHg, showing no clear relationship with the $ScvO_2$ level.

Lactate, acidosis and alactic base excess

According to the physico-chemical approach of Stewart to the acid-base equilibrium (14), an increase in lactate - a strong negative ion - leads to a decrease in the strong ion difference, which finally results in metabolic acidosis and acidemia. Therefore, if the pH is not corrected by compensatory mechanisms, the lactate "per se" always produces acidemia. Actually, out of 1017 patients with lactate greater than 2 mmol/L, 57% had normal pH (greater than 7.35, see also Figure 1, panel D and Table E1). To better understand this lack of a consistent correlation between hyperlactatemia and acidemia, we introduce the concept of "alactic base excess" (alactic BE). This variable equals the amount of strong acids, other than lactate, which are present in the plasma in abnormal concentrations. The alactic BE was related to kidney

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

function (Figure 4), as indicated by its relationship to creatinine levels, urine output and use of renal replacement therapy (RRT). Accordingly, with worsening renal function, the concentration of fixed acids -other than lactate - increased in the plasma. This dysfunction led to worsening acidemia, as reflected in more negative values of alactic BE. Conversely, an alactic BE near 0 suggested that acidemia was fully explained by the lactate, as no other acids were present in excess, while a positive alactic BE suggested either that the kidney fully compensated for metabolic acidosis or that additional mechanisms contributed to metabolic alkalosis (e.g., diuretic usage, contraction of the extracellular volume). Actually, the alactic base excess was strongly associated to the fluid balance (see Figure E5). In Table E3, we summarize the most relevant clinical and physiological variables as functions of sextiles of alactic BE.

A comprehensive synthesis of the results

In Figure 5 we present an integrated view of our results. As shown, hyperlactatemia was increased quite independently from VO_2/DO_2 (Figure E6a); in contrast the VO_2/DO_2, viewed as an independent variable, strictly determines the ScvO2 levels: low when the oxygen transport is low (high VO_2/DO_2) and high when oxygen utilization is impaired (low VO_2/DO_2). The physiologically sound $ScvO_2$-VO_2/DO_2 relationship, unfortunately, is biased by mathematical coupling, which prevents a rigorous analysis of possible confounders. In addition, it is worth emphasizing that $ScvO_2$ might not be representative of the whole body average oxygen venous saturation, even though it is a broadly accepted surrogate of the mixed SvO_2(15). The second independent variable is renal function, upon which we hypothesize that acidemia should primarily depends. Among several variables, we found that

PCO_2, SOFA without its renal component and mean arterial pressure acted as real confounders both on creatinine and pH. Including these variables in a multiple linear regression model, the creatinine remained the variable most strongly independently related to the pH. (See online supplement data for complete analysis).

DISCUSSION

Over recent decades there has been a growing evidence that lactate in sepsis and shock state may increase for several reasons other than tissue hypoxia. (16) The possible heterogenous sources of lactate in septic shock, however, have rarely been quantified. Alegria et al. (17) in a retrospective analysis of 90 patients with septic shock, found that 70 patients presented elevated lactate in association with signs of hypoperfusion (including $ScvO_2$ < 70%). In our analysis on 1741 patients, after admission in ICU, we found that only 35% of the patients had a $ScvO_2$ < 70%, while 65% had high lactate coexisting with normal or increased $ScvO_2$. This finding suggests that high lactate levels, as observed in an ICU setting after initial fluid resuscitation made in the emergency department, are due to a macrocirculatory oxygen transport defect only in a minority of cases. Furthermore, we found that hyperlactatemia in this setting is reliably associated with acidemia only if renal dysfunction is simultaneously present. Finally, the estimation of the alactic BE is a useful tool by which the degree of renal compensation of the acid-base disorder can be rapidly determined.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Lactate and tissue hypoxia

Despite its limitations, $ScvO_2$ is one of the best surrogates for the assessment of tissue oxygen availability (i.e., the relationship between oxygen delivery and demand) and is widely used in clinical practice. We found that, on admission, only ~35% of our patients had $ScvO_2$ lower than 70%. This finding is consistent with what has been observed in most large clinical trials performed for sepsis (18-20). Although, admittedly, $ScvO_2$ is an imperfect indicator of the cellular oxygen environment, it is reasonable to associate extreme values of $ScvO_2$ either to a predominant oxygen *transport* insufficiency (low $ScvO_2$) or to a predominant oxygen *utilization* impairment (high $ScvO_2$). These two extremes of $ScvO_2$ are indeed associated with the highest lactate levels, renal dysfunction, disease severity and mortality, so that $ScvO_2$ has a U-shaped relationship with these characteristics. This interpretation is supported by other findings: the highest arterio-venous oxygen content difference and the greatest veno-arterial difference in PCO_2 were found in the first $ScvO_2$ sextile (24-62%). At the opposite extreme, the presence of hyperlactatemia at the most elevated $ScvO_2$ levels (78-98%) strongly suggests mechanisms other than an oxygen transport deficit. In sepsis, elevated lactate levels with high $ScvO_2$ may be explained by a variety of mechanisms ranging from the lack of pyruvate decarboxylation due to thiamine deficiency (21-24) to the impairment of the electron transport chain due to dysfunctional structure of the respiratory mitochondrial enzymes – induced, for example, by nitric oxide (25) or oxygen radicals (26). Another possible explanation for this association, though physiologically indistinguishable from the aforementioned mechanisms, entails the dysregulation of the microcirculation leading to peripheral shunting (3, 27).

Lactate and metabolic acidosis

An increase in the concentration of lactate results in metabolic acidosis (i.e., a process leading to an excess of negative strong ions) (14, 28). However, acidemia, i.e., an abnormally high proton concentration (low pH), is not necessarily present if other processes simultaneously promote a compensatory decrease in negative strong ions, with consequent widening of strong ion difference and restoration of pH towards normality. The kidney has a pivotal role in correcting for the excess of lactate. Indeed, given that $PaCO_2$ in our population was similar across lactate sextiles, the compensatory mechanisms – when present – were mainly due to an offsetting increase in the strong ion difference by the kidney.

To better understand the relation between hyperlactatemia and acidemia, we have introduced the concept of alactic BE, which helps to quickly discriminate between metabolic acidosis secondary to lactate from metabolic acidosis caused, for example, by an accumulation of fixed acids (unmeasured strong anions). The role of the renal function on the acid-base balance in sepsis is explicitly quantified by the alactic BE.

The association of a negative alactic BE with creatinine >2 mg/dl, indicates that fixed acids other than lactate are retained in the plasma, meaning that the kidney is no longer able to compensate for the lactic acidosis because of an associated renal dysfunction. An alactic BE of zero (observed at creatinine ~ 2 mg/dl) suggests that the kidney is still able to clear fixed acids but cannot fully "compensate" for the acidosis induced by lactate.

A positive alactic BE (generally with a creatinine <2 mg/dl), suggests the presence of metabolic alkalosis, usually due to diuretics or volume contraction (29). In summary, although

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

an abnormally high lactate – *per se* – nearly always indicates acidosis of some severity, the degree of associated acidemia depends upon renal ability to compensate. The concept of alactic BE is a simple, novel, and potentially useful method to immediately detect and track these phenomena over time. The classical base excess, obviously, includes all the information given by the alactic BE. However, alactic BE has more practical diagnostic and therapeutic potential. Indeed, a negative alactic BE, observed in these septic patients, alerts the physician to that fact that the renal function is impaired (unable to compensate for an excess of negative strong ions), whereas a positive alactic BE may indicate an additional process leading to metabolic alkalosis, e.g., excess use of diuretics and volume contraction. It should be noted that the alactic BE clearly differs from the anion gap (i.e., (sodium + potassium) – (chloride + bicarbonate), as this latter variable does not distinguish between lactate and other fixed acids. If the lactate is added to the anion gap computation, the alactic BE differs because it refers to a standard PCO_2 and pH, while the anion gap does not. The weak correlation between alactic BE and anion gap (adjusted R-squared: 0.113) is reported in Supplement (Figure E6).

Possible model of sepsis pathophysiology

We believe that the controversial lactate shuttle theory (30), broadly applied in other settings of lactate generation, fits well with the bulk of our findings. That construct considers lactate the normal product of glycolysis (see Supplement for details and description). Indeed, the model emphasizes the importance of lactate as a central key of glucose metabolism, instead of considering it, as historically proposed (1), as the result of anaerobiosis. According to this

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

model, all molecules of glucose entering the cytoplasm are metabolized into lactate, which is finally oxidized to CO_2 and water. If lactate production exceeds oxidative capacity (e.g., excessive β-adrenergic stimulation, thiamine deficiency, respiratory chain impairment, lack of oxygen), excess lactate is transported out of the cells, usually in association with a proton (30). The decrease in pH due to the increase in plasma lactate is sensed by the kidney, which decreases the urinary SID (31) to restore the normal plasma pH.

The plasma concentration of lactate reaches a plateau if the rate of lactate production in the non-functioning metabolic units equals the rate of lactate oxidation by the metabolically active functioning units. Most organs, primarily the liver, may "clear" circulating lactate (i.e. completely oxidize lactate) and the rate of oxidation in the functioning metabolic units increases with the lactate input. Indeed, a strong relationship has been shown between exogenous lactate input and its oxidation in patients during dialysis (32), and the same phenomenon has been observed in experimental animal models (33). Therefore, we may hypothesize that in sepsis, the lactate oxidation capability of the functioning metabolic units (Figure E10) increases with the increased availability of lactate (34) (see online supplement Figures E8 and E11). Interestingly, other metabolites that normally are oxidized by the Krebs' cycle within the mitochondria, e.g., non-esterified fatty acids, behave in sepsis as does lactate: increased levels promote higher rates of oxidation. (35)

Clinical implications

Our findings may help account for the ability of lactate to predict the severity and outcome of septic patients. Indeed, we showed that whatever the prevalent mechanism underlying the

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

deterioration in organ function in sepsis (i.e., impairment in the oxygen transport or oxygen utilization), the end result is an increase in the production of lactates and a decrease in their oxidation, leading to hyperlactatemia. However, despite this apparent similarity in outcome, a better understanding of the primary mechanism of hyperlactatemia – as we suggest in this model – might guide a more targeted and less indiscriminate approach to the management of sepsis. In strictly, following the management guidance currently advocated, all overtly septic patients would receive similar amount of fluids, regardless of their SvO_2. (36)

Actually, a deficit in the oxygen transport, as suggested by low $ScvO_2$ may justify a therapeutic approach aiming at increasing it, such as early goal directed therapy (37), and, even better, correcting, if possible, the precise cause of oxygen transport impairment. In contrast, at high $ScvO_2$ (impaired oxygen utilization) the same therapeutic approach may appear - at best – ineffective, as suggested by recent randomized controlled trials (18-20). We may rationally wonder whether, in such cases, the mandated use of a fixed amount of fluid has a sound pathophysiologic rationale, and whether this approach is devoid of adverse consequences, as suggested by studies reporting positive fluid balance, renal dysfunction and worse outcome after aggressive fluid replacement in sepsis. (38, 39)

We suggest that patients might be first stratified on the basis of $ScvO_2$ to understand the origin of lactate production, and then on the basis of the alactic BE to better understand organ (i.e., kidney) perfusion and volemia. Changes in this simple parameter over time may facilitate early restoration of appropriate fluid balance and/or prompt the use of renal replacement therapy.

Conclusions

Our results indicate that in septic patients: 1) Lactate is a powerful marker of illness severity; 2) Abnormal lactate levels, in established sepsis, appear to be generated primarily by impaired oxygen transport in the minority of cases whereas, in the majority, high lactate more likely results from impaired tissue oxygen utilization; 3) The degree of acidemia or alkalemia depends primarily on renal function; the alactic BE offers a potentially useful way to estimate renal capability of handling the disturbance to acid-base equilibrium. A clear recognition of the mechanisms underlying lactate elevation should result in an improved therapeutic approach for the individual, particularly regarding the aggressiveness of fluid administration.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Acknowledgments: The authors are grateful to Dr. Peter Suter for his fruitful review and critical evaluation of the present manuscript. This work was made possible by the generous donation of Ilse Liselotte Munz to the Department of Anesthesiology of Göttingen.

REFERENCES

1. Broder G, Weil MH. Excess Lactate: An Index of Reversibility of Shock in Human Patients. *Science* 1964; 143: 1457-1459.
2. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 2002; 360: 219-223.
3. Ince C, Mik EG. Microcirculatory and mitochondrial hypoxia in sepsis, shock, and resuscitation. *J Appl Physiol (1985)* 2016; 120: 226-235.
4. Hamanaka RB, Chandel NS. Cell biology. Warburg effect and redox balance. *Science* 2011; 334: 1219-1220.
5. James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet* 1999; 354: 505-508.
6. Valenza F, Aletti G, Fossali T, Chevallard G, Sacconi F, Irace M, Gattinoni L. Lactate as a marker of energy failure in critically ill patients: hypothesis. *Crit Care* 2005; 9: 588-593.
7. Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. *Crit Care* 2014; 18: 503.
8. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L, Investigators AS. Albumin replacement in patients with severe sepsis or septic shock. *The New England journal of medicine* 2014; 370: 1412-1421.
9. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive care medicine* 1996; 22: 707-710.
10. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *Jama* 1993; 270: 2957-2963.
11. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, Angus DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama* 2016; 315: 801-810.
12. Vasques F, Duscio E, Romitti F, Pasticci I, Caironi P, Meessen J, Latini R, Cressoni M, Camporota L, Pesenti A, Fumagalli R, Quintel M, Gattinoni L. Septic shock-3 vs 2: an analysis of the ALBIOS study. *Crit Care* 2018; 22: 237.
13. Berend K. Diagnostic Use of Base Excess in Acid-Base Disorders. *The New England journal of medicine* 2018; 378: 1419-1428.
14. Stewart PA. Stewart's textbook of acid-base. 2009.
15. Reinhart K, Kuhn HJ, Hartog C, Bredle DL. Continuous central venous and pulmonary artery oxygen saturation monitoring in the critically ill. *Intensive Care Med* 2004; 30: 1572-1578.
16. Levy B. Lactate and shock state: the metabolic view. *Curr Opin Crit Care* 2006; 12: 315-321.
17. Alegria L, Vera M, Dreyse J, Castro R, Carpio D, Henriquez C, Gajardo D, Bravo S, Araneda F, Kattan E, Torres P, Ospina-Tascon G, Teboul JL, Bakker J, Hernandez G. A hypoperfusion context may aid to interpret hyperlactatemia in sepsis-3 septic shock patients: a proof-of-concept study. *Ann Intensive Care* 2017; 7: 29.
18. ARISE Investigators, ANZICS Clinical Trials Group, Peake SL, Delaney A, Bailey M, Bellomo R, Cameron PA, Cooper DJ, Higgins AM, Holdgate A, Howe BD, Webb SA, Williams P. Goal-directed resuscitation for patients with early septic shock. *The New England journal of medicine* 2014; 371: 1496-1506.
19. Pro CI, Yealy DM, Kellum JA, Huang DT, Barnato AE, Weissfeld LA, Pike F, Terndrup T, Wang HE, Hou PC, LoVecchio F, Filbin MR, Shapiro NI, Angus DC. A randomized trial of protocol-based care for early septic shock. *The New England journal of medicine* 2014; 370: 1683-1693.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

20. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, Jahan R, Harvey SE, Bell D, Bion JF, Coats TJ, Singer M, Young JD, Rowan KM, Pro MTI. Trial of early, goal-directed resuscitation for septic shock. *The New England journal of medicine* 2015; 372: 1301-1311.
21. Donnino MW, Carney E, Cocchi MN, Barbash I, Chase M, Joyce N, Chou PP, Ngo L. Thiamine deficiency in critically ill patients with sepsis. *Journal of critical care* 2010; 25: 576-581.
22. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. *Chest* 2017; 151: 1229-1238.
23. Donnino MW, Andersen LW, Chase M, Berg KM, Tidswell M, Giberson T, Wolfe R, Moskowitz A, Smithline H, Ngo L, Cocchi MN, Center for Resuscitation Science Research G. Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study. *Critical care medicine* 2016; 44: 360-367.
24. Woolum JA, Abner EL, Kelly A, Thompson Bastin ML, Morris PE, Flannery AH. Effect of Thiamine Administration on Lactate Clearance and Mortality in Patients With Septic Shock. *Critical care medicine* 2018; 46: 1747-1752.
25. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. *Biochimica et biophysica acta* 2001; 1504: 46-57.
26. Turrens JF. Mitochondrial formation of reactive oxygen species. *The Journal of physiology* 2003; 552: 335-344.
27. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Critical care medicine* 2013; 41: 791-799.
28. Kishen R, Honore PM, Jacobs R, Joannes-Boyau O, De Waele E, De Regt J, Van Gorp V, Boer W, Spapen H. Facing acid-base disorders in the third millennium - the Stewart approach revisited. *Int J Nephrol Renovasc Dis* 2014; 7: 209-217.
29. Rose BD PT. Clinical physiology of acid-base and electrolyte disorders. McGraw-Hill; 2001.
30. Brooks GA. The Science and Translation of Lactate Shuttle Theory. *Cell Metab* 2018; 27: 757-785.
31. Gattinoni L, Carlesso E, Cadringher P, Caironi P. Strong ion difference in urine: new perspectives in acid-base assessment. *Critical care* 2006; 10: 137.
32. Wright DA, Forni LG, Carr P, Treacher DF, Hilton PJ. Use of continuous haemofiltration to assess the rate of lactate metabolism in acute renal failure. *Clinical science* 1996; 90: 507-510.
33. Zanella A, Patroniti N, Isgro S, Albertini M, Costanzi M, Pirrone F, Scaravilli V, Vergnano B, Pesenti A. Blood acidification enhances carbon dioxide removal of membrane lung: an experimental study. *Intensive Care Med* 2009; 35: 1484-1487.
34. Levy B, Gibot S, Franck P, Cravoisy A, Bollaert PE. Relation between muscle Na+K+ ATPase activity and raised lactate concentrations in septic shock: a prospective study. *Lancet* 2005; 365: 871-875.
35. Samra JS, Summers LK, Frayn KN. Sepsis and fat metabolism. *The British journal of surgery* 1996; 83: 1186-1196.
36. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Crit Care Med* 2017; 45: 486-552.

37. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M, Early Goal-Directed Therapy Collaborative G. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *The New England journal of medicine* 2001; 345: 1368-1377.
38. Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL, Sepsis Occurrence in Acutely Ill Patients I. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. *Critical care* 2008; 12: R74.
39. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, Lang T, Brent B, Evans JA, Tibenderana JK, Crawley J, Russell EC, Levin M, Babiker AG, Gibb DM, Group FT. Mortality after fluid bolus in African children with severe infection. *The New England journal of medicine* 2011; 364: 2483-2495.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Figures legend

Figure 1 90-day mortality (A), SOFA (B), $ScvO_2$ (C) and arterial pH (D) as a function of lactate sextiles at baseline (ICU admission). Data are presented as mean ± standard error. Lactate sextiles range: 1 (0.1-1.2 $mmol \cdot L^{-1}$); 2 (1.2-1.8 $mmol \cdot L^{-1}$); 3 (1.8-2.5 $mmol \cdot L^{-1}$); 4 (2.5-3.5 $mmol \cdot L^{-1}$); 5 (3.5-5.6 $mmol \cdot L^{-1}$); 6 (5.6-27 $mmol \cdot L^{-1}$). Level of statistical significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. The level of significance represented in panel A refers to the chi-square test, while for panels B, C and D it refers to pairwise comparison in ANOVA model. Only significant comparisons are displayed.

Figure 2 Observed frequency of distribution of the central venous oxygen saturation at baseline measured in the whole population at ICU admission. As shown, only a minority of patients presented a $ScvO_2$ consistent with oxygen transport deficit. Note, however, that the extreme values of $ScvO_2$ (1 patient < 25% and 3 patients > 95%) are likely artefactual.

Figure 3 Lactate (A), SOFA (B) and mortality (C) as a function of central venous oxygen saturation ($ScvO_2$) sextiles at baseline (ICU admission). Data are presented as mean ± standard error.

Figure 4 Creatinine (A), simplified strong ion difference [$(Na^{+}+K^{+})-Cl^{-}$] (B), diuresis (C) and RRT as a function of alactic BE sextiles at baseline (ICU admission). Data are presented as mean ± standard error.

Figure 5 Lactate metabolic pathways and kidney response. The arrows direction indicated increase (↑) or decrease (↓) of the given variable. See text and supplement for further details.

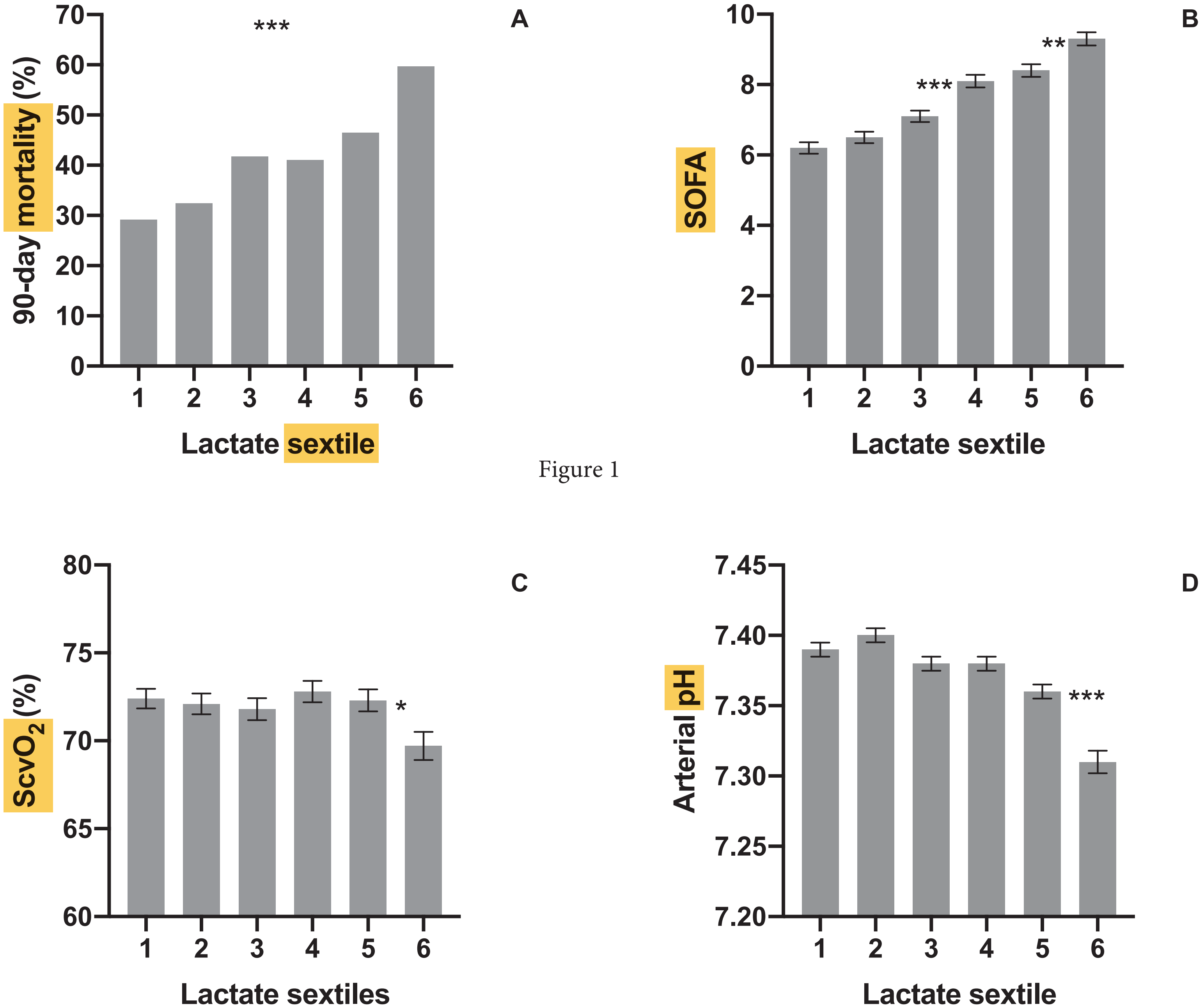

A
***
90-day mortality (%)
70
60
50
40
30
20
10
0
1 2 3 4 5 6
Lactate sextile
B
SOFA
10
8
6
4
2
0
***
**
Lactate sextile
C
ScvO2 (%)
80
75
70
65
60
*
Lactate sextiles
D
Arterial pH
7.45
7.40
7.35
7.30
7.25
7.20
***
Lactate sextile

Figure 1

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

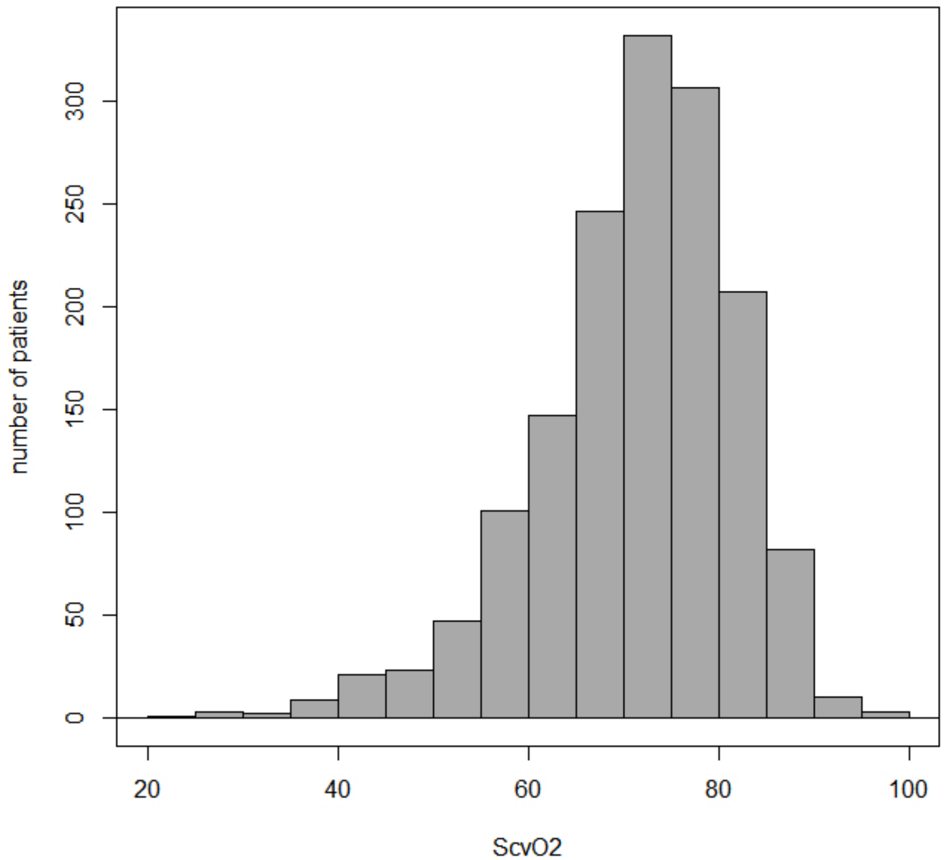


Figure 2 Observed frequency of distribution of the central venous oxygen saturation at baseline measured in the whole population at ICU admission. As shown, only a minority of patients presented a ScvO2 consistent with oxygen transport deficit. Note, however, that the extreme values of ScvO2 (1 patient < 25% and 3 patients > 95%) are likely artefactual.

237x237mm (72 x 72 DPI)

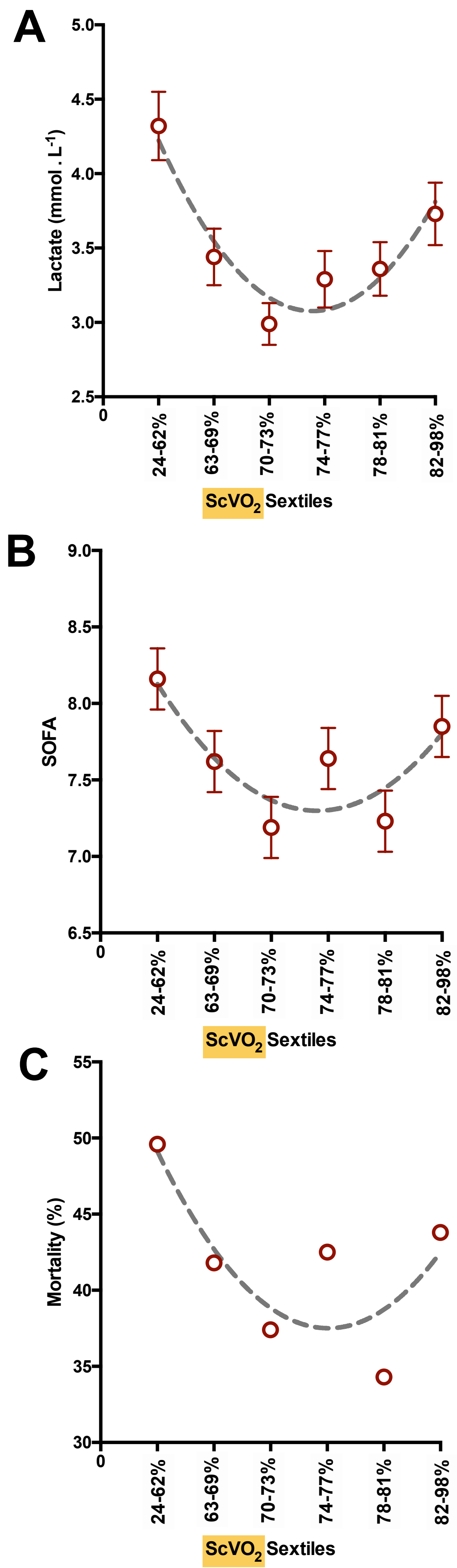

A
5.0
4.5
4.0
3.5
3.0
2.5
0
Lactate (mmol . L-1)
24-62%
63-69%
70-73%
74-77%
78-81%
82-98%
ScVO2 Sextiles
B
9.0
8.5
8.0
7.5
7.0
6.5
0
SOFA
24-62%
63-69%
70-73%
74-77%
78-81%
82-98%
ScVO2 Sextiles
C
55
50
45
40
35
30
0
Mortality (%)
24-62%
63-69%
70-73%
74-77%
78-81%
82-98%
ScVO2 Sextiles

Figure 3

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

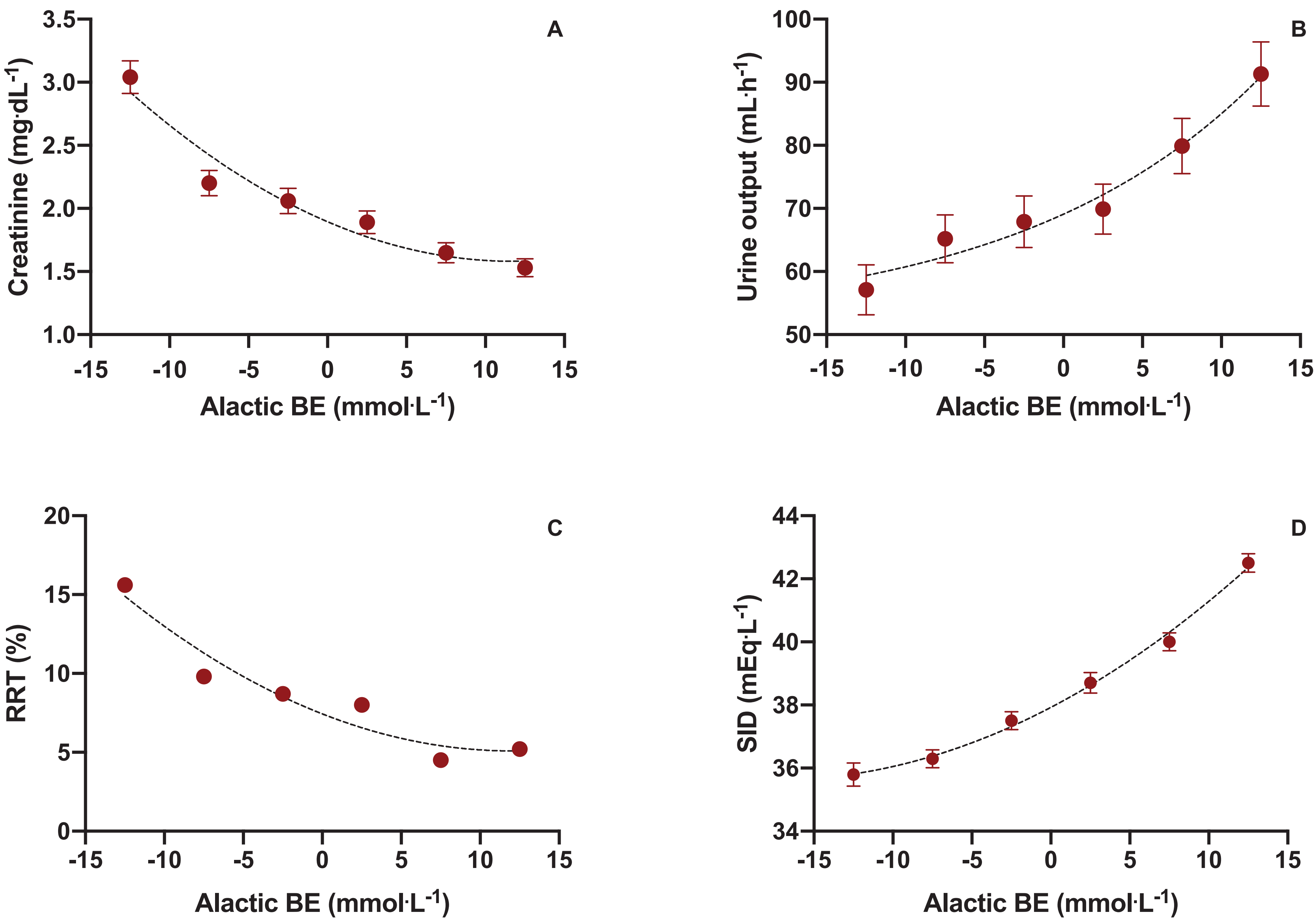
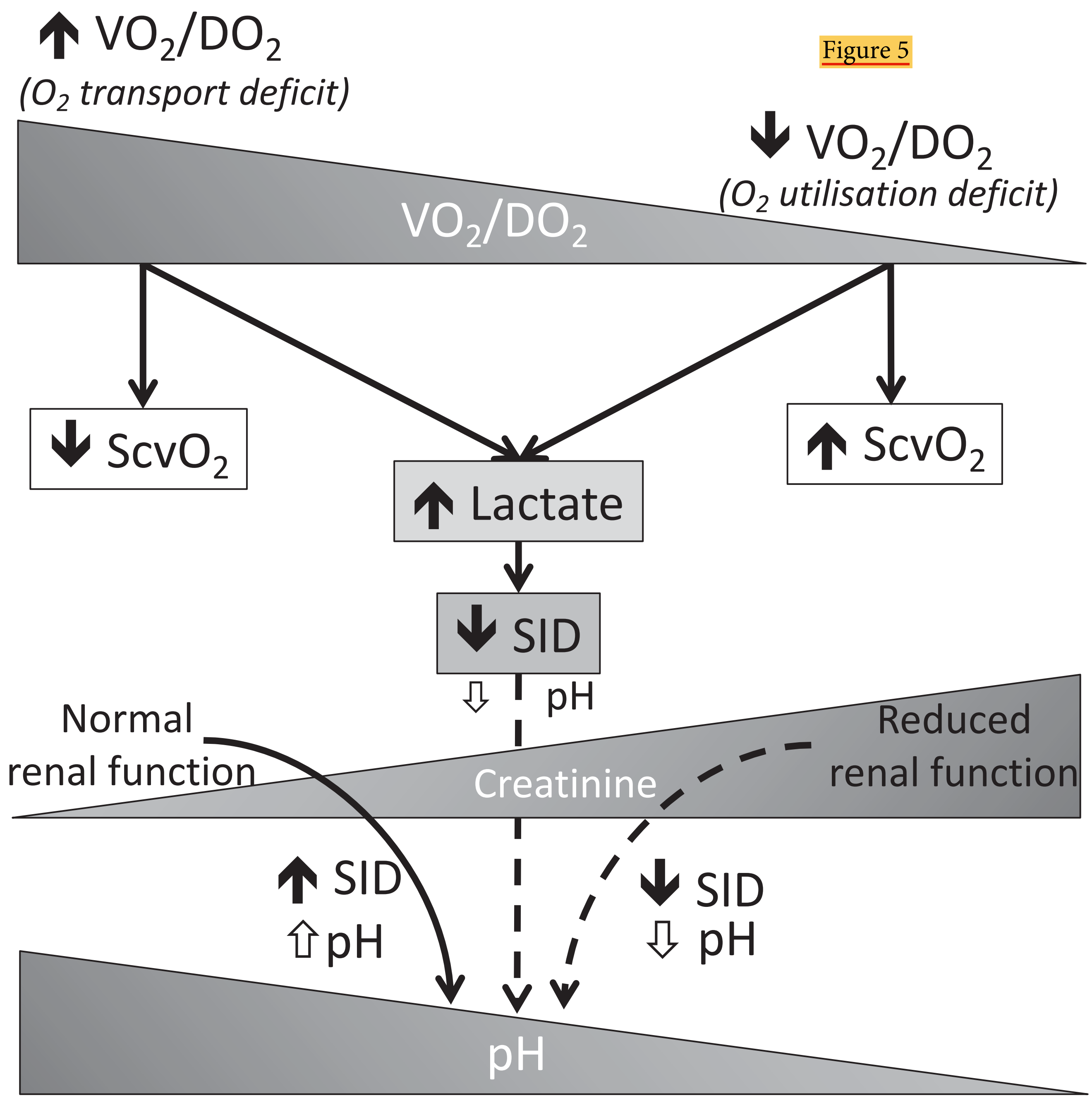


Figure 4

Figure 5



↑ VO2/DO2
(O2 transport deficit)
↓ VO2/DO2
(O2 utilisation deficit)
VO2/DO2
↓ ScvO2
↑ Lactate
↑ ScvO2
↓ SID
⇩ pH
Normal
renal function
Creatinine
Reduced
renal function
↑ SID
⇧ pH
↓ SID
⇩ pH
pH

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

ONLINE DATA SUPPLEMENT

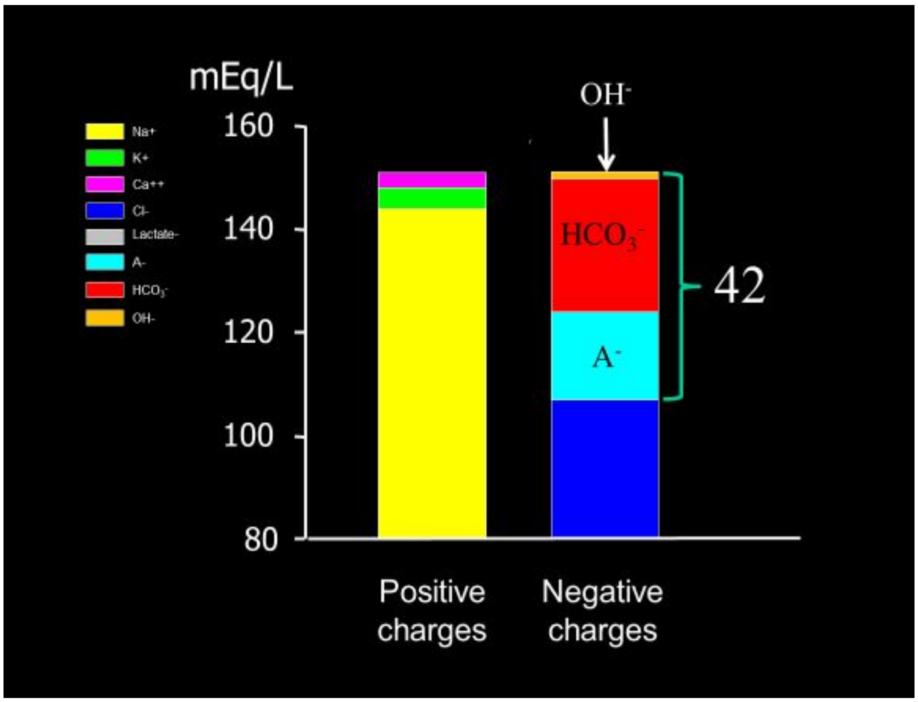
Understanding lactatemia in human sepsis: potential impact for early management

Luciano Gattinoni, MD, FRCP, Francesco Vasques, MD, Luigi Camporota, MD, PhD, , Jennifer Meessen, MSc, Federica Romitti, MD, Iacopo Pasticci, MD, Eleonora Duscio, MD, Francesco Vassalli, MD, Lui G. Forni, MD, Didier Payen, MD, Massimo Cressoni, MD, Alberto Zanella, MD, Roberto Latini, MD, Michael Quintel, MD, John J. Marini, MD

Additional materials and methods

Computed variables: base excess

Briefly, as shown in the figure below, in the plasma in equilibrium with the remaining extracellular space, the sum of all the electrical charges is equal to zero.



It must be noted that some elements present in the plasma are always electrically charged, as the sodium with its positive charge (Na^+, due to the loss of an electron) and chloride with its negative charge (Cl^-, due to the acquisition of one electron). At pH compatible with life, these electrolytes are always completely dissociated. i.e: they do not exist as NaOH or HCl but always as Na^+ and OH^- and Cl^- and H^+. The always dissociated elements are respectively called “strong bases” “strong acids”.

In contrast, some elements in the plasma, in the range of physiological pH, may appear electrically charged (dissociated as the strong elements) or neutral (undissociated, without electrical charge). The tendency of these elements (called also weak acids) to be present in the undissociated or dissociated form (at a given pH) is defined by the pKa, according to the classical Henderson – Hasselbalch equation.

$$pH = pK + \log\left(\frac{dissociated}{undissociated}\right)$$

These elements, are the HCO_3^-/CO_2 (pKa = 6.1), Albumin⁻/Albumin and $H_2PO_4^-/HPO_4^{2-}$ (pKa = 6.8). The sum of their dissociated forms has been called “buffer base” by Singer and Hastings in 1948. The

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

difference of charge due to the different concentration of strong bases and strong acids (primarily Na^+ and Cl^-) is called Strong Ion Difference (SID) and normally equivalent to 42 mmol/l (i.e., an excess of positive charges). To maintain the electroneutrality these charges must be neutralized by an equal amount of negative charges which are provided by the dissociated forms of the buffers, (i.e HCO_3^-, $Albumin^-$, $H_2PO_4^-$). Therefore, the buffer base is equal to SID and amounts in normal conditions to 42 mmol/l.

If an abnormal strong acid (e.g., lactic acid, dissociated in lactate + H^+) is added to the plasma the strong ion difference decreases accordingly. As an example, if 10 mmol/l of lactate are added to the plasma, the SID will decrease from 42 to 32 mmol/l. This will reduce the buffer concentration from 42 to 32 mmol/l. Indeed, a part of HCO3- will become CO_2 + H_2O, the $albumin^-$ will become albumin and the HPO_4^{--} will become H_2PO4^-.

The Base Excess, introduced by Siggaard – Andersen is nothing else than the difference between the amount of buffers actually present and the normal amount of buffers present at pH 7.40 (42 mmol/l).

$$Base\ Excess = actual\ Buffer\ Base - ideal\ Buffer\ Base$$

In our example,

$$Base\ Excess\ \left(-10\ ^{mmol}/_{l}\right) = actual\ Buffer\ Base\ \left(32\ ^{mmol}/_{l}\right) - ideal\ Buffer\ Base\ \left(42\ ^{mmol}/_{l}\right)$$

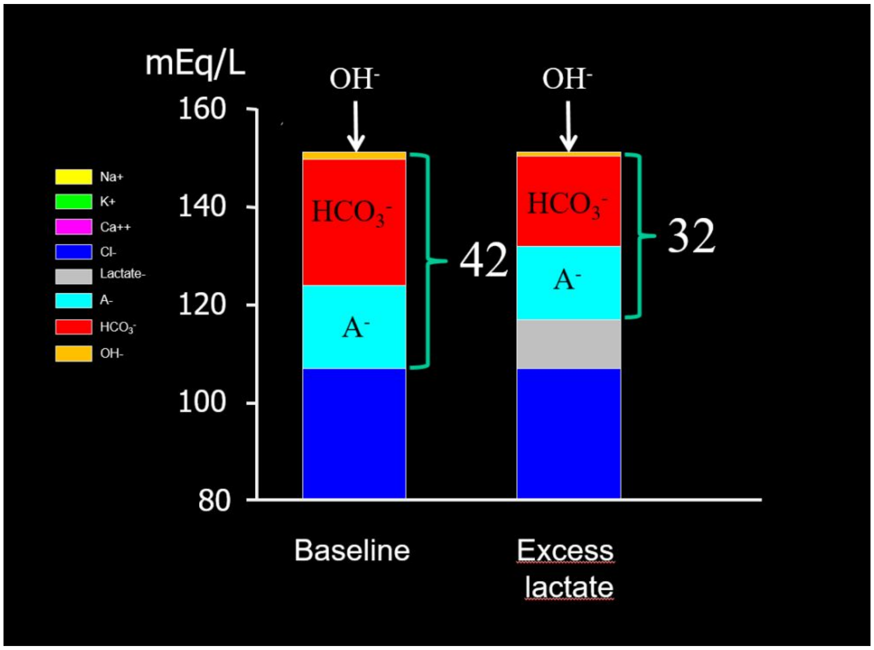
Normal metabolism produces about 100 mmol of strong organic acids per day: lactic acid, sulfuric acid and phosphoric acid which are present in the plasma as lactate, sulfate (SO_4^{2-}) and phosphate ($H_2PO_4^-$). The acids which cannot be eliminated by the lungs (Cl^-, SO_4^{2-}, H_2PO4^-) are called "fixed acids" or "non-volatile acids", and must be excreted daily by the kidney in order to maintain a constant acid-base status. Lactate, as well as ketoacids and fatty acids, is regulated by the metabolism. In normal conditions, the production and elimination of acids is in equilibrium in order to maintain the Base Excess = 0. Base Excess becomes negative only when SID is decreased. Several formulas have been proposed to compute the actual BE, but their discussion is outside the scope of this manuscript.

In this paper we split the Base Excess in two components: one includes the change of SID exclusively due to lactate (the lactic base excess) and the other the decrease of SID caused by the abnormal presence of acids other than lactate (organic acids) which usually increase in the course of Acute Kidney Injury (AKI). By definition the lactic Base Excess is equal to the lactate concentration. As the total BE is equal to the sum of lactic and alactic base excess, the alactic base excess will be:

$$Alactic\ BE = BE + [lactate]$$

For example, if the BE is 0 mmol/l and lactate is 2 mmol/l, the alactic BE will be 2 mmol/l. In this condition, we deduce from the BE that the SID is normal. The positive alactic BE tells us that this is possible thanks to the kidney that has compensated 2mmol/l of lactate by eliminating 2mmol/l of strong negative ions. Therefore, according to the so called "Stewart's approach", the SID is normal and the pH is normal.

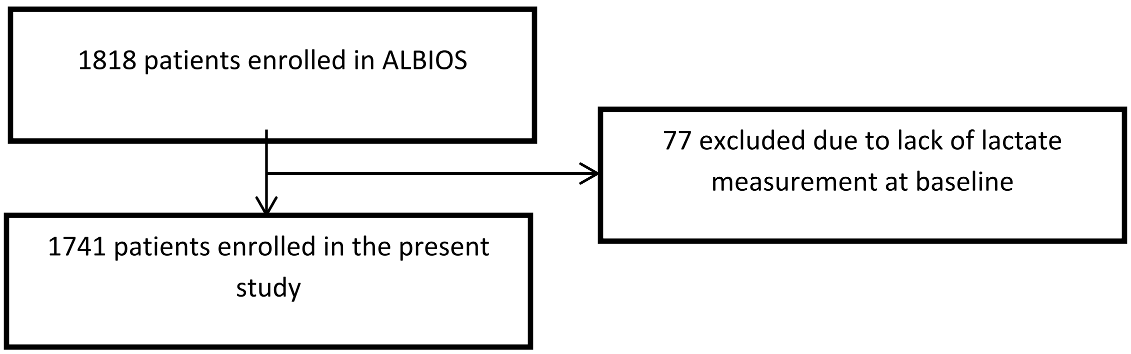
Instead, if BE equals -2 mmol/l and lactate is 2 mmol/l, the alactic BE will be 0 mmol/l. In this condition, we deduce from the BE that the SID is decreased by 2 mmol/l and from alactic BE that this happens because the kidney has not compensated for 2 mmol/l of lactate. Therefore, according to the Stewart approach the SID is reduced and the pH falls and acidaemia ensues.



Immediate effect of adding 10 mmol/l of lactate to a normal plasma. As shown, the lactate "steal" space to the buffers pairs (HCO_3^-/CO_2 and A^-/AH, which includes albumin and phosphates). Note that the total content of CO_2 will not change, as the bicarbonate is transformed in dissolved CO_2 (PCO_2) in a ratio 1:1.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

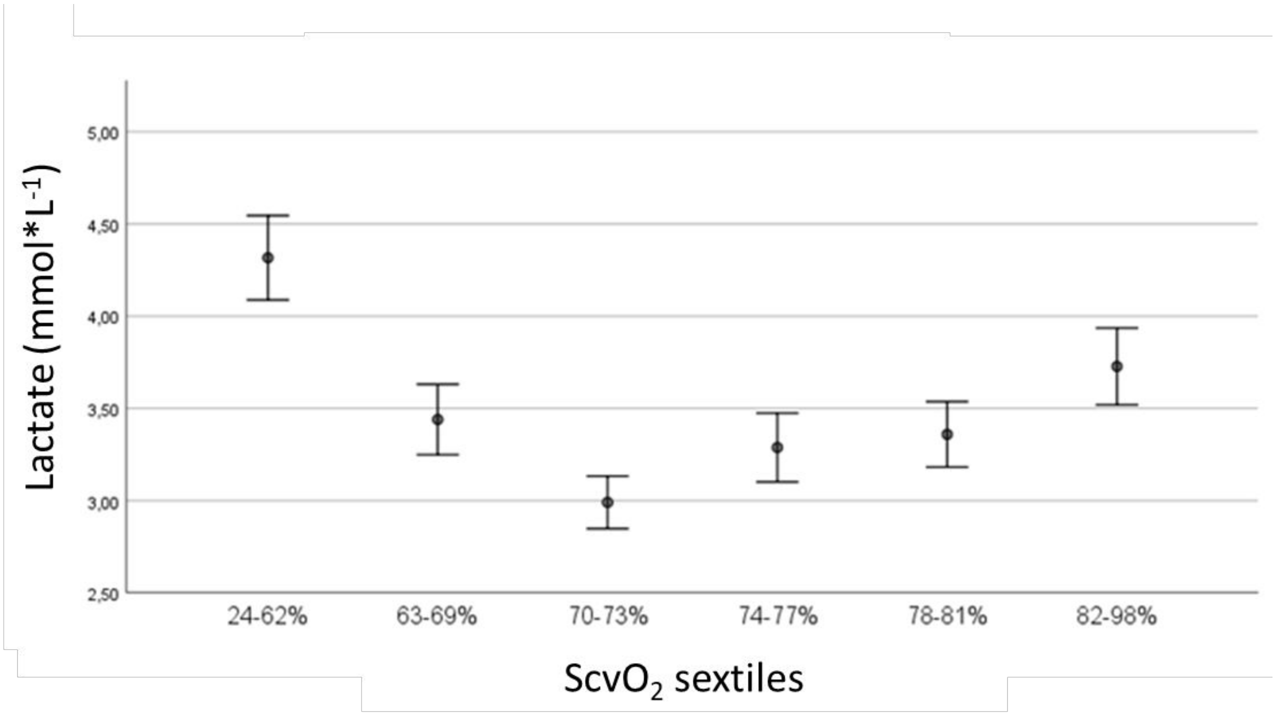
Figure E1 Flowchart of patient selection from ALBIOS database. (E1)



Statistics

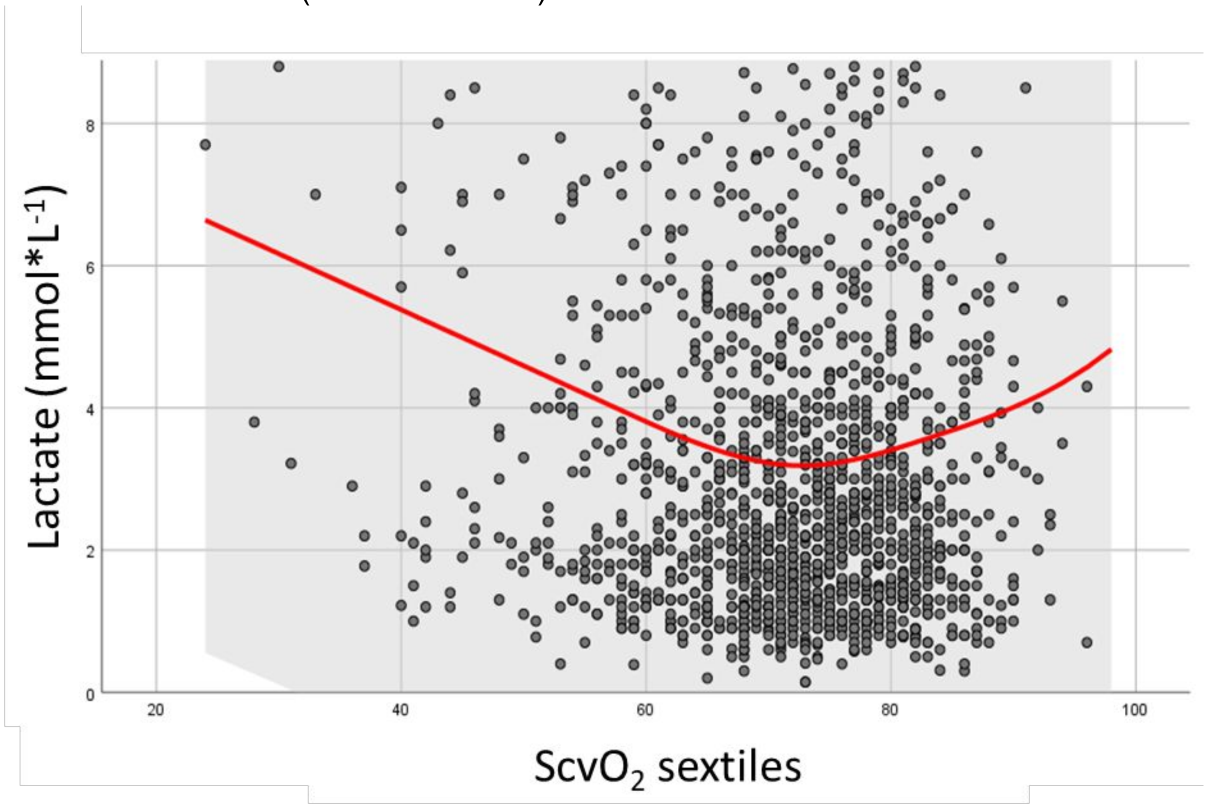
Patient characteristics are reported as mean ± standard deviation. Lactate and $ScvO_2$ were divided into sextiles. The division into sextiles was arbitrarily decided in order to provide reasonable resolution power of the model, while maintaining adequate patient numbers in each quantile. In this way, the results are more easily understandable than splitting the independent variables, according to restricted cubic splines, which provided, in fact, similar results. Here below we report an example of sextiles and restricted cubic splines, when analysing the lactate-$ScvO_2$ relationship.

Figure E2. Relationship between lactate and $SCVO_2$ sextiles. Normal scatterplot as in the paper.



AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Figure E3. Restricted cubic splines analysis inserting 3 knots to describe the relationship of baseline lactate and ScvO2 (zoomed version).



Additional results

Table E1 Clinical, physiological and hemodynamic variables per sextiles of arterial lactate

	1		2		3		4		5		6	P (ANOVA)
n	295		296		304		263		278		287	
Lactate (mmol/L)	0.9±0.24	***	1.53±0.17	***	2.12±0.2	***	3±0.29	***	4.42±0.59	***	9.01±3.42	**<0.001**
range	0.1 – 1.2		1.2 – 1.8		1.8 – 2.5		2.5 – 3.5		3.5 – 5.6		5.6 – 27.0	
SOFA	6.18±2.75		6.52±2.73		7.15±2.76	***	8.14±2.92		8.38±2.88	**	9.3±3.09	**<0.001**
SAPS 2	41.4±15.8		43.2±13.4		45.7±14.3	**	50.3±16.5		53.8±15.6	***	61.2±16.9	**<0.001**
Glucose (mg/dl)	136±44		147±56		146±61		154±73		146±72		144±88	0.059
Creatinine (mg/dl)	1.92±1.79		1.72±1.58		2.13±1.76		2.13±1.48		2.19±1.72		2.31±1.58	**<0.001**
Bilirubin (µmol/L)	1.04±1.24		1.23±1.87		1.35±1.54		1.82±2.23		1.77±3.01		2.02±2.57	**<0.001**
Albumin (g/dl)	25.4±6.1		25±6.4		24.1±5.9		24.1±5.7		23.6±6.7		22.7±6.3	**<0.001**
Platelets (x 10^3/L)	220±123		216±133		193±129		172±118		176±130	*	145±123	**<0.001**
Leukocytes (x 10^3/L)	13.4±7.9		14.3±8.5		13.7±9.3		13.7±10.7		12.7±12		12.7±12.2	0.311
Sepsis 2 (%)	49		52		57.5		66.4		78		77.4	**<0.001**
Sepsis 3 (%)	0		0		33.3		66.4		78		77.4	**<0.001**
CVVH (6h) (%)	3.8		4.7		3.0		5.3		9.7		12.5	**<0.001**
Mortality 90-day (%)	29.2		32.5		41.8		41.1		46.5		59.7	**<0.001**
FiO_2 (%)	57±19		59±17		58±19		58±20		61±21		61±22	**<0.001**
PaO_2 (mmHg)	109±48		107±46		113±53		107±48		114±58		114±58	0.322
PvO_2 (mmHg)	41.5±8.2		41.4±8.5		42±9.5		42.4±9		42.2±9		42.9±10.8	0.485
$PaCO_2$ (mmHg)	40.1±10.2		40.5±11.6		39.4±10.7		38.3±9.4		38.5±10.2		36.4±12.4	0.322
$PvCO_2$ (mmHg)	47±10.1		47.3±11.7		47±11.7		45.3±10.1		46.1±11		44.4±12.7	**0.026**
pHa	7.39±0.09		7.4±0.09		7.38±0.09		7.38±0.09		7.36±0.09	***	7.31±0.14	**<0.001**
pHv	7.35±0.08		7.35±0.08		7.33±0.11		7.33±0.08	*	7.31±0.1	***	7.25±0.15	**<0.001**
Bicarbonate	23.8±5.2		24.5±4.6	**	23.2±5		22.2±4.5		21.4±4.8	***	18.5±5.5	**<0.001**
BE (mEq/L)	-1.16±5.86		-0.28±5.2	**	-1.91±5.7		-3.02±5.11		-4.06±5.59	***	-7.73±6.89	**<0.001**
alactic BE (mEq/L)	-0.26±5.9	*	1.25±5.19		0.25±5.72		-0.01±5.14		0.36±5.55		1.28±6.32	**0.002**
Na^+ (mEq/L)	139.5±6.3		139.8±6.3		140.1±6.5		139.6±7		140.3±5.8		140.5±6.6	0.387
Cl^- (mEq/L)	106.3±6.4		105.3±6.5		106±7		105.5±6.9		105.6±6.2		104.6±6.9	0.053
K^+ (mEq/L)	4.13±0.65		4.07±0.65		4.13±0.77		4.13±0.81		4.11±0.77		4.1±0.8	0.907
Fluid balance (6h) (L)	0.63±1.00		0.79±1.18		0.95±1.29		1.13±1.36		1.33±1.37	***	1.90±2.04	**<0.001**
Diuresis (mL/h)	84.6±75.8		79.8±74		73.5±64.1		73.2±68.3		62.4±70.7		57.8±76.5	**<0.001**
CVP (mmHg)	9.6±40.6		9.6±4.6		9.9±4.6		9.3±4.4		10.3±4.9		10.7±5.4	**0.01**
HR (bpm)	97.6±20.4		100.5±21.6		103.9±18.4		106.1±20.8	*	111.2±20.7		113.9±19.9	**<0.001**
MAP (mmHg)	77.9±15.1		76.4±15.1		76±14.7		72.5±15.6		69.3±14.6		67.5±14	**<0.001**
Epinephrine (%)	1.7		2.6		3.9		2.3		9.9		13.2	**<0.001**
Epinephrine (mcg/kg/min)	0.048±0.004		0.22±0.44		0.1±0.07		0.1±0.06		0.16±0.14		0.22±0.21	0.185
Norepinephrine (mcg/kg/min)	0.24±0.26		0.28±0.26		0.29±0.29		0.32±0.37		0.34±0.37		0.43±0.46	**<0.001**
ScvO2 (%)	72.4±9		72.1±9.6		71.8±10.3		72.8±9.4		72.3±9.9	*	69.7±12.8	**0.011**
ΔO_2 (a-v) mL/dL	3.69±1.39		3.83±1.67		3.89±1.5		3.78±1.41		3.86±1.63		4.06±2.02	0.182

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

ΔPCO_2 (v-a) mmHg	6.8±4.4		7.1±4.5		6.9±4.5		7.5±5.6		7.9±5.8		7.7±5.3	0.079
Hb (g/dL)	10.6±1.9		11±1.9		11±2		11.2±2.0		11.2±2.1		10.9±1.1	**0.006**

Data are presented as mean±SD. SOFA, sequential organ failure assessment score; SAPS II, simplified acute physiology score II; RRT, renal replacement therapy; BE, base excess; CVP, central venous pressure; HR, heart rate; MAP, Mean arterial pressure, $\Delta(a\text{-}v)O_2$, arterio-venous difference in oxygen content; $\Delta(v\text{-}a)PCO_2$, veno-arterial difference in PCO_2; Hb, haemoglobin. Statistical significance levels: * p<0.05; ** p<0.01; *** p<0.001; one-way ANOVA with pairwise comparison

Table E2 Clinical, physiologic and hemodynamic variables as functions of $ScvO_2$ sextiles

	1		**2**		**3**		**4**		**5**		**6**	P (ANOVA)
n	258		279		265		256		236		246	
$ScvO_2$ (%)	54.6±7.5	***	66.5±2	***	71.5±1.1	***	75.5±1.1	***	79.4±1.1	***	85.1±3.1	
Range	**24-62**		**63-69**		**70-73**		**74-77**		**78-81**		**82-98**	
Lactate (mmol/L)	4.32±3.67	*	3.44±3.19		2.99±2.32		3.29±2.98		3.36±2.73		3.73±3.26	**<0.001**
SOFA score	8.16±3.1		7.62±2.95		7.19±3		7.64±2.97		7.23±3.02		7.85±3.05	**0.002**
SAPS-II score	53±17.6		50.4±18		47.1±14.9		48.7±16.8		46.7±16.2		49±16.9	**<0.001**
Glucose (mg/dl)	143±57		143±72		149±68		147±73		142±64		146±68	0.823
Creatinine (mg/dl)	2.31±1.93		2.25±1.82		1.92±1.68		2.07±1.55		1.81±1.42		2.11±1.65	**0.006**
Bilirubin (µmol/L)	1.7±2.97		1.39±1.91		1.32±1.65		1.52±1.71		1.53±2.14		1.47±1.74	0.400
Albumin (g/dl)	24.18±6.16		24.62±6.11		23.77±6.23		23.34±6.04		24.34±6.76		24.06±6.3	0.276
Platelet (x 10^3/L)	176±121		186±117		189±135		198±135		186±125		178±120	0.433
Leukocytes (x 10^3/L)	13.4±10		13.1±10.4		12.7±8.7		13.6±9.2		13.6±11.5		12.9±9.6	0.892
Sepsis-2 (%)	62.4		59.1		58.9		64.5		65.7		73.2	**0.009**
Sepsis-3	47.3		38.4		36.6		44.1		44.1		48	**0.04**
RRT 6h (%)	14.5		9		6.4		7.3		8		9.5	**0.04**
Mortality 90-day (%)	49.6		41.8		37.4		42.5		34.3		43.8	**0.01**
FiO_2 (%)	64±22	*	58±19		59±19		57±18		57±19	*	63±20	**<0.001**
PaO_2 (mmHg)	91.5±41.1		99.4±42.4		107±52		114±47		119±48	***	140±68	**<0.001**
SaO_2	93.7±6	***	95.3±4		96±3		96.3±3		96.8±2.7		97.2±2.6	**<0.001**
PvO_2 (mmHg)	32.8±6	***	38.2±6.2		39.7±5.3	***	43.8±6.2	**	46±6.2	***	53.1±9.8	**<0.001**
$PaCO_2$ (mmHg)	37.1±10.7		37.2±9.8		39±9.4		38.7±9.4		39.6±11.1	*	42.5±13.6	**<0.001**
$PvCO_2$ (mmHg)	45.8±11.4		44.9±10.4		45.7±9.7		45.9±10.3		46±11.4	*	49.1±14.	**<0.001**

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

											2	
pHa	7.37±0.12		7.37±0.1		7.38±0.1		7.37±0.11		7.38±0.08	**	7.34±0.11	**0.001**
pHv	7.32±0.13		7.31±0.12		7.34±0.1		7.32±0.11		7.33±0.85	*	7.3±0.11	**0.003**
Bicarbonate	21.6±5.9		21.4±5.4	*	23±5.2		22.1±4.8		23±5.4		22.7±5.2	**0.001**
BE (mEq/L)	-3.58±7.2		-3.82±6.24	*	-2.14±6.2		-3.21±5.92		-2.2±5.9		-3.02 ± 6.01	**0.007**
alactic BE (mEq/L)	0.73±5.9		-0.38±5.64		0.86±5.79		0.1±5.17		1.15±5.38		0.7±6.04	**0.03**
Na^+ (mEq/L)	140±7		140±6		140±6		139±6		140±6		140±6	0.438
Cl^- (mEq/L)	105±7		105±6		105±6		106±6		106±7		106±7	0.512
K^+ (mEq/L)	4.15±0.7		4.08±0.74		4.04±0.76		4.2±0.72		4.03±0.66		4.18±0.81	**0.027**
Fluid balance (6h)	1276±1591		1268±1519		1027±1298		987±1358		1059±1215		1120±1503	0.08
Diuresis (ml/h)	67±72		64±69		75±74		75±69		76±81		82±82	0.08
CVP (mmHg)	10.5±5.2		9.8±5.1		10.2±4.6		9.7±4.7		9.3±4.7		9.9±4.2	0.103
HR (bpm)	109±21		104±23		105±21		105±21		106±21		107±19	0.118
MAP (mmHg)	71.1±15.8		70.5±15.6	**	75±15.4		74.1±14.4		73.7±14.7		73.8±14	**0.002**
Epinephrine (%)	8.5		5.7		4.2		3.5		2.5		7.3	**0.021**
Epinephrine (mcg/kg/min)	0.167±0.204		0.126±0.144		0.071±0.03		0.147±0.124		0.152±0.1		0.212±0.161	0.28
Norepinephrine (mcg/kg/min)	0.32±0.36		0.29±0.3		0.27±0.27		0.33±0.39		0.31±0.33		0.31±0.33	0.751
Oxygen extraction ratio	0.41±0.08	***	0.3±0.04	***	0.25±0.03	***	0.21±0.03	***	0.18±0.03	***	0.12 ±0.04	<0.001
$\Delta(a\text{-}v)O_2$ (ml/dl)	6.01±1.74	***	4.48±1.03	***	3.91±0.9	***	3.45±0.67	***	2.89±0.67	***	2.13±0.77	**<0.001**
$\Delta(v\text{-}a)PCO_2$ (mmHg)	8.82±5.38		7.68±5		6.73±4.11		7.33±5.13		6.44±4.28		6.74±5.89	**<0.001**
$\Delta(v\text{-}a)PCO_2 : \Delta(a\text{-}v)O_2$	1.60±1.42		2.87±17.80		1.77±1.30		2.27±2.01		2.46±2.38	*	5.17±13.07	**<0.001**
Hb (g/dL)	10.7±2.1		10.8±1.9		10.9±1.9		11.3±2.0		11.1±2		11.1±2.0	**0.013**

Data are presented as mean ± standard deviation. SOFA, sequential organ failure assessment score; SAPS II, simplified acute physiology score II; SaO_2, haemoglobin oxygen saturation, RRT, renal replacement therapy; BE, base excess; CVP, central venous pressure; HR, heart rate; MAP, Mean arterial pressure; oxygen extraction ratio, $(CaO_2-CvO_2)/CaO_2$, Δ(a-v)O2, arterio-venous difference in oxygen content; Δ(v-a)PCO2, veno-arterial difference in PCO2; Hb, haemoglobin. Statistical significance levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; one-way ANOVA with pairwise comparison).

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Table E3 Clinical, physiologic and hemodynamic variables as functions of alactic base excess sextiles.

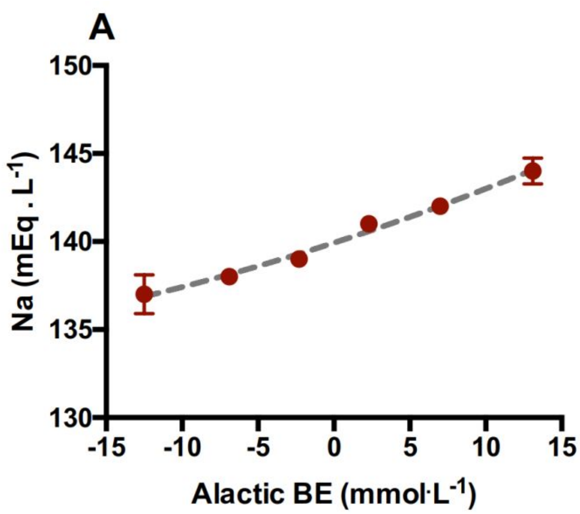
	1		2		3		4		5		6	P (ANOVA)
n	289		286		287		287		287		287	
alactic BE (mEq/L)	-7.74±2.8	***	-3.21±0.81	***	-0.85±0.65	***	1.42±0.69	***	4.15±0.91	***	9.16±3.24	
Range	-21.34 – -4.64		-4.64 – -1.99		-1.98 – 0.30		0.31 – 2.72		2.74 – 5.78		5.78 – 25.6	
SOFA score	8.3±2.85		8±2.85		7.5±3.28		7.57±3.02		6.95±3.02		7.13±3.08	**<0.001**
SAPS II score	54.7±17.6	***	49.1±16		49.9±18.1		48.4±15.8		45.2±15.9		47.7±16.3	**<0.001**
Glucose (mg/dl)	155±95		141±58		146±67		143±53.8		142±57		146±62	0.145
Creatinine (mg/dl)	3.04±2.18	***	2.20±1.63		2.06±1.65		1.89±1.42		1.65±1.29		1.53±1.08	**<0.001**
Bilirubin (µmol/L)	1.46±2.12		1.51±1.74		1.39±1.83		1.74±2.57		1.43±2.18		1.51±1.64	0.406
Albumin (g/dl)	23.5±6.3		24±6.6		24.3±6.1		24.1±6.3		24.6±5.9		24.6±6.2	0.387
Platelets (x 10^3/L)	186.9±123.9		177.8±126		193±132.4		185.4±123.6		192.7±135.5		190.8±132	0.722
Leukocytes (x 10^3/L)	13.7±10.2		13.2±10.5		13.15±10.3		13.4±10.4		13.4±10.3		13.3±9.2	0.991
Sepsis 2 (%)	65.7		66.8		65.5		65.9		56.4		56.1	**0.004**
Sepsis 3 (%)	40.1		41.3		40.8		44.9		41.1		41.1	**0.02**
Mechanical Vent. (%)	72		72		80.1		82.2		85.4		87.1	**<0.001**
RRT (6h) (%)	15.6		9.8		8.7		8		4.5		5.2	**<0.001**
Mortality 90d (%)	45		40.2		38.3		42.5		37.6		47.7	**0.046**
FiO_2 (%)	58.4±21.1		58.5±20.2		59.8±20		59.8±20		58.2±17.5	*	63.1±20	**0.027**
PaO_2 (mmHg)	133±57		110±48		112±52		112±49.5		106±52		111±54	0.62
$PcvO_2$ (mmHg)	43.3±10.7		42.5±8.9		42.3±9.3		42.3±		41±8.8		41±9.1	**0.03**
$PaCO_2$ (mmHg)	34.1±11.7	*	36.9±10.9		38.3±9.3		39.6±11.9		40.5±8.3	***	44.0±10.11	**<0.001**
$PcvCO_2$ (mmHg)	42.5±12		44.7±11.4		45.7±9.8		46.5±12.7		47.8±9.7		50.4±10.5	**<0.001**
pHa	7.27±7.12	***	7.34±0.08	*	7.37±0.08		7.39±0.1	**	7.41±0.07	**	7.44±0.07	**<0.001**
pHv	7.22±0.12	***	7.30±0.08		7.31±0.11	*	7.34±0.09		7.36±0.07	*	7.39±0.08	**<0.001**

BE (mEq/L)	-11.21±4.46	***	-6.31±2.5	***	-4.05±2.65	***	-2±3.29	***	0.6±2.92	***	5.1±4.7	**<0.001**
Na^+ (mEq/L)	137.8±7.2		138.3±6.1		139.3±6.2		140.8±6.1		140.6±5.6	***	142.8±6	**<0.001**
Cl^- (mEq/L)	106.2±7.8		106.2±6.5		105.8±6.8		106.1±6.7		104.7±6		104.2±6	**<0.001**
K^+ (mEq/L)	4.3±0.8		4.18±0.8		4.1±0.71		3.99±0.72		4.05±0.64		4.02±0.69	**<0.001**
Fluid balance (6h) (mL)	1622±2074	*	1243±1218		1009±1283		1003±1248		1028±1335		836±1321	**<0.001**
Diuresis (ml/h)	57±67		65±64		68±69		70±67		80±74		91±86	**<0.001**
CVP (mmHg)	10.5±5.6		9.6±5		10.2±4.6		10.1±4.5		9.5±4.6		9.5±4.3	**0.04**
HR (bpm)	107±20		106±21		107±22		103±21		105±21		104±21	0.102
MAP (mmHg)	71.1±16.9		71.7±15.4		73.3±13.9		73.9±14.5		74.5±15.8		75.5±15	**<0.001**
Epinephrine (%)	4.2		5.2		5.9		4.9		4.2		9.4	**0.063**
Epinephrine (mcg/kg/min)	0.33±0.34		016±0.13		0.14±0.09		0.14±0.11		0.16±0.2		0.14±0.21	0.117
Norepinephrine (mcg/kg/min)	0.37±0.37		0.34±0.37		0.33±0.36		0.33±0.37		0.27±0.39		0.3±0.25	0.222
Lactate (mmol/L)	3.48±3.43		3.1±2.4		3.21±2.59		3.42±3.19		3.55±2.77		4.07±3.8	**0.004**
$ScvO_2$ (%)	71±11		71±9.4		71.4±11		73±10		72.1±9.6		72.1±10.1	0.293
$\Delta(v\text{-}a)PCO_2$ (mmHg)	8.3±6.4		7.8±4.7		7±4.5		7±4.7		7.3±5.5		6.6±4	**0.001**
$\Delta(a\text{-}v)O_2$ (ml/dL)	3.84±2		4.03±1.6		4±1.6		3.7±1.45		3.7±1.4		3.8±1.5	0.144

Data are presented as mean±SD. SOFA, sequential organ failure assessment score; SAPS II, simplified acute physiology score II; RRT, renal replacement therapy; BE, base excess; CVP, central venous pressure; HR, heart rate; MAP, mean arterial pressure; $ScvO_2$, central venous oxygen saturation; $\Delta(a\text{-}v)O_2$, arterio-venous difference in oxygen content; $\Delta(v\text{-}a)PCO_2$, veno-arterial difference in PCO_2; Hb, haemoglobin. Statistical significance levels: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

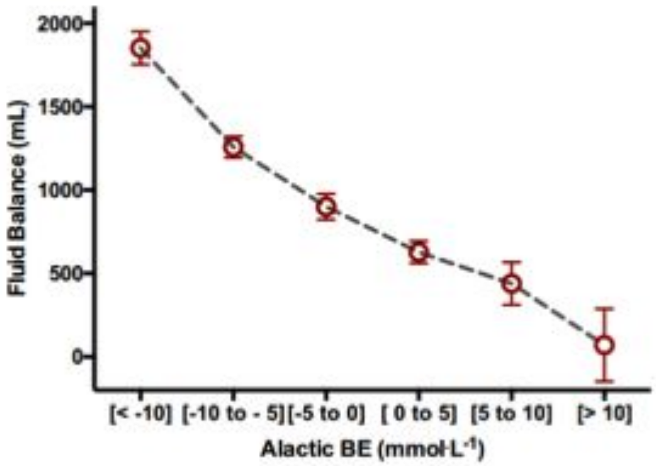
Figure E4 Plasma sodium (**A**) and chloride (**B**) as a function of alactic base excess. Data are presented as mean and standard error.



As shown in Table 3 in the main text, the sextiles of increasing alactic base excess (from positive to negative), are associated with changes of several variables. We believe that the various associations are best explained by considering the alactic base excess as primarily determined by the kidney function and, possibly, by the intravascular volume status. Oxygen transport and tissue oxygenation do not appear associated with the alactic base excess. Of note, the lactate levels are remarkably similar in the first five sextiles and slightly increased in the last sextile. Indeed, as shown in the table, the arterio-venous difference in oxygen content was similar throughout the sextiles, as was the $ScvO_2$.

In contrast, the alactic base excess was directly related with creatinine levels, decreased RRT use, and increased urine output. The negative alactic base excess seems, therefore, well explained by impaired kidney function (negative values), while the positive values could indicate primarily a contraction alkalotic process (i.e., volume depletion). Most of the other significant variable changes are the changes that can be predicted when the system status shifts from acidosis to alkalosis, e.g., the decreased deltaPCO_2, the blood gas variables and the strong ion difference components. Interestingly, the highest mortality rates were observed in the sextile at the extremes of alactic base excess (i.e., the first and the sixth sextiles: <-4.64 or >5.78 mEq/L), It should also considered that a more positive alactic base excess was associated with more frequent use of mechanical ventilation, the highest FiO_2, the highest $PvCO_2$, all characteristics compatible with greater impairment of the respiratory system.

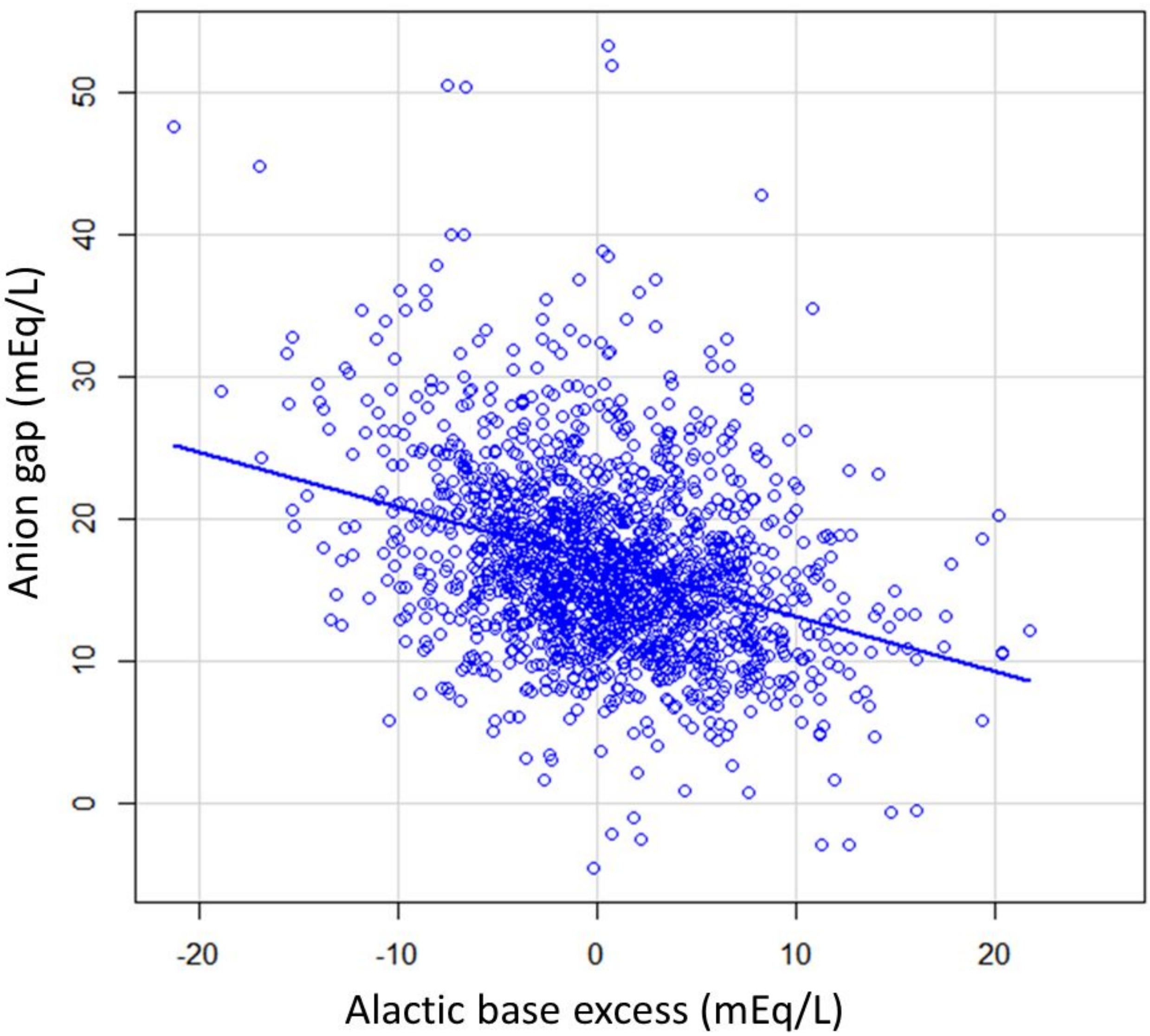
Figure E5 Relationship between the alactic base excess and fluid balance. Data are presented as mean and standard error.



As the alactic base excess becomes progressively more positive, the fluid balance declines. By definition of alactic base excess, this relationship is independent of the lactate concentration. The progressive increase of alactic base excess (more positive) may reflect the phenomenon otherwise called *contraction alkalosis*, whereas its decrease at higher fluid balance fits with the phenomenon otherwise called *dilution acidosis*

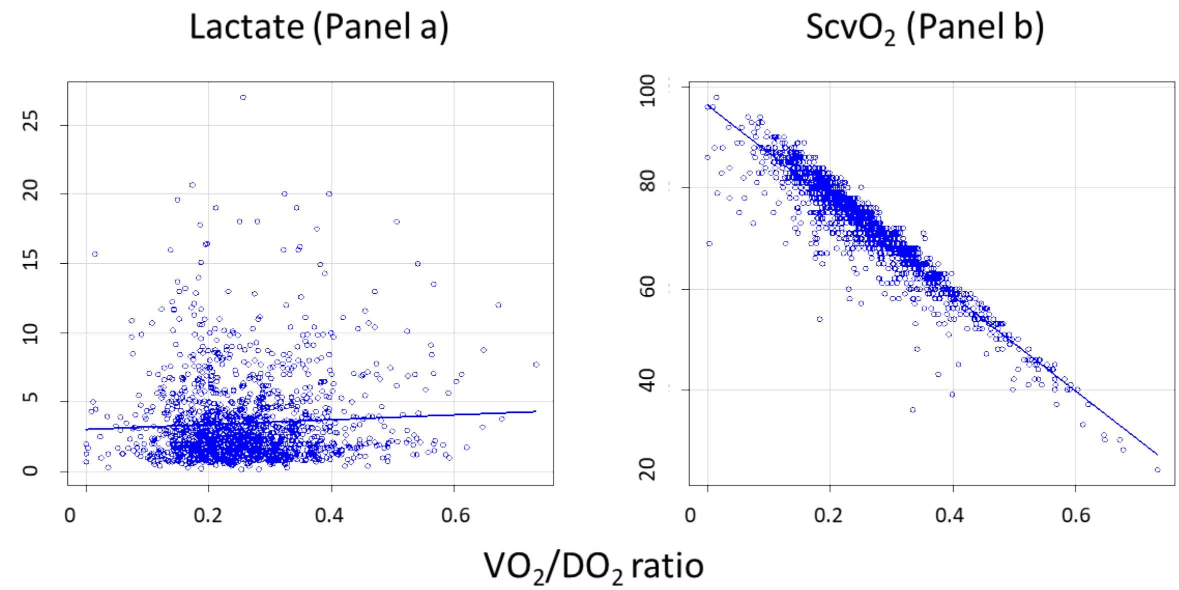
AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Figure E6 Relationship between alactic base excess and anion gap



Anion gap [(Na^+ + K^+) – ($HCO3^-$ + Cl^-)] as a function alactic base excess. As shown, the relationship is significant (adjusted R-squared 0.113, p value < 0.001), but in the individual patient the same alactic base excess may correspond to a large range of anion gap.

Figure E7 Relationship between VO_2/DO_2 ratio (as a surrogate of oxygen transport/ tissue oxygen availability), lactate (a) and $ScvO_2$ (b)



As shown in this graph, lactate appears weakly dependent on VO_2/DO_2 ratio, which reflects the oxygen transport (adjusted R squared 0.003, p 0.02), while conversely, the $ScvO_2$ was strongly related to the VO_2/DO_2 ratio (adjusted R squared: 0.88, $p < 0.001$). Despite the mathematical coupling, the physiological relationship is helpful to illustrate their relationship in this model.

Relationship between creatinine, as a surrogate of renal function, and pH

As potential confounders that may act independently both on the independent (renal function) and dependent (pH) variables, we chose, on clinical basis, the following: arterial PCO_2, arterial PO_2, lactate, SOFA, excluding the renal component, and, as perfusion associated variables, arterio-venous O_2 difference, fluid balance and mean arterial. We tested first independently these variables versus creatinine and pH, aiming to identify the ones significantly associated with both (real confounders).

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Multiple regression for creatinine				
term	estimate	std.error	statistic	p.value
(Intercept)	2.888	0.356	8.108	< 0.001
PaO_2	-0.000	0.001	-0.347	0.7
$PaCO_2$	-0.014	0.004	-3.492	< 0.001
Lactate	0.022	0.016	1.338	0.18
A-V O_2 difference	0.0118	0.027	0.441	0.65
Mean arterial pressure	-0.011	0.003	-3,627	<0.001
Fluid balance	0.000	0.000	1.796	0.07
SOFA (without renal SOFA)	0.057	0.018	3.147	0.001
Residual standard error: 1.622 on 1459 degrees of freedom (274 observations deleted due to missingness) Multiple R-squared: 0.04331, Adjusted R-squared: 0.03872 F-statistic: 9.436 on 7 and 1459 DF, p-value: <0.0001				

Table E4 $PaCO_2$, mean arterial pressure and SOFA without renal component were independently associated with the creatinine level.

Multiple regression for pH (without creatinine)				
term	estimate	std.error	statistic	p.value
(Intercept)	7.554	0.016	449.305	< 0.001
PaO_2	-0.000	0.000	-0.029	0.97
$PaCO_2$	-0.004	0.000	-24.399	< 0.001
Lactate	-0.009	0.001	-12.764	< 0.001
A-V O_2 difference	0.002	0.001	2.131	0.03
Mean arterial pressure	0.000	0.000	4.419	< 0.001
Fluid balance	-0.000	0.000	-6.722	< 0.001

SOFA (without renal SOFA)	-0.001	0.000	-2.162	0.0307
Residual standard error: 0.07653 on 1458 degrees of freedom (275 observations deleted due to missingness) Multiple R-squared: 0.4011, Adjusted R-squared: 0.3983 F-statistic: 139.5 on 7 and 1458 DF, p-value: < 0.001				

Table E5. $PaCO_2$, lactate, mean arterial pressure, fluid balance and SOFA (without the renal component) were all independently associated with the pH level.

Therefore, the confounders (variables independently associated both to creatinine and pH) were PCO2, mean arterial pressure and SOFA without its renal component. These confounders were fitted into a model where the pH was the outcome variable and the creatinine inserted as an explanatory variable.

Multiple regression for pH-creatinine relationship				
term	estimate	std.error	statistic	p.value
(Intercept)	7,541	0,015	503.945	< 0.001
Creatinine	-0,015	0,001	-12,067	< 0.001
$PaCO_2$	-0,004	0,000	-23,546	< 0.001
SOFA (without renal SOFA)	-0,004	0,000	-5,770	< 0.001
Mean arterial pressure	0,001	0,000	6,499	< 0.001
Residual standard error: 0.08453 on 1673 degrees of freedom (63 observations deleted due to missingness) Multiple R-squared: 0.3317, Adjusted R-squared: 0.3301 F-statistic: 207.6 on 4 and 1673 DF, p-value: < 0.001				

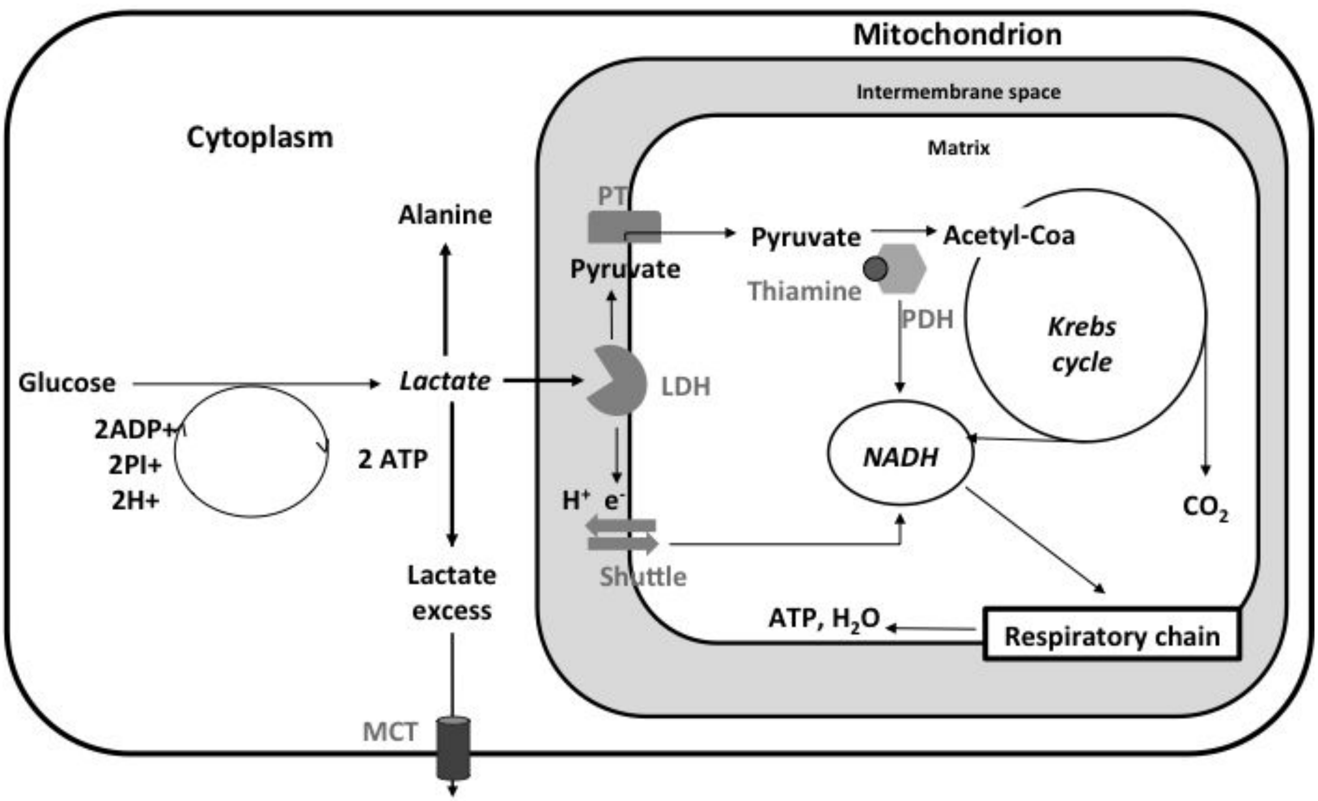
Table E6. Creatinine is significantly and independently associated with pH.

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Additional discussion

Lactate shuttle theory and proposed model

Figure E8 Lactate metabolic pathway.



LDH, lactic dehydrogenase; PDH, pyruvate dehydrogenase; PT, pyruvate transfer; MCT, monocarboxylate transporter.

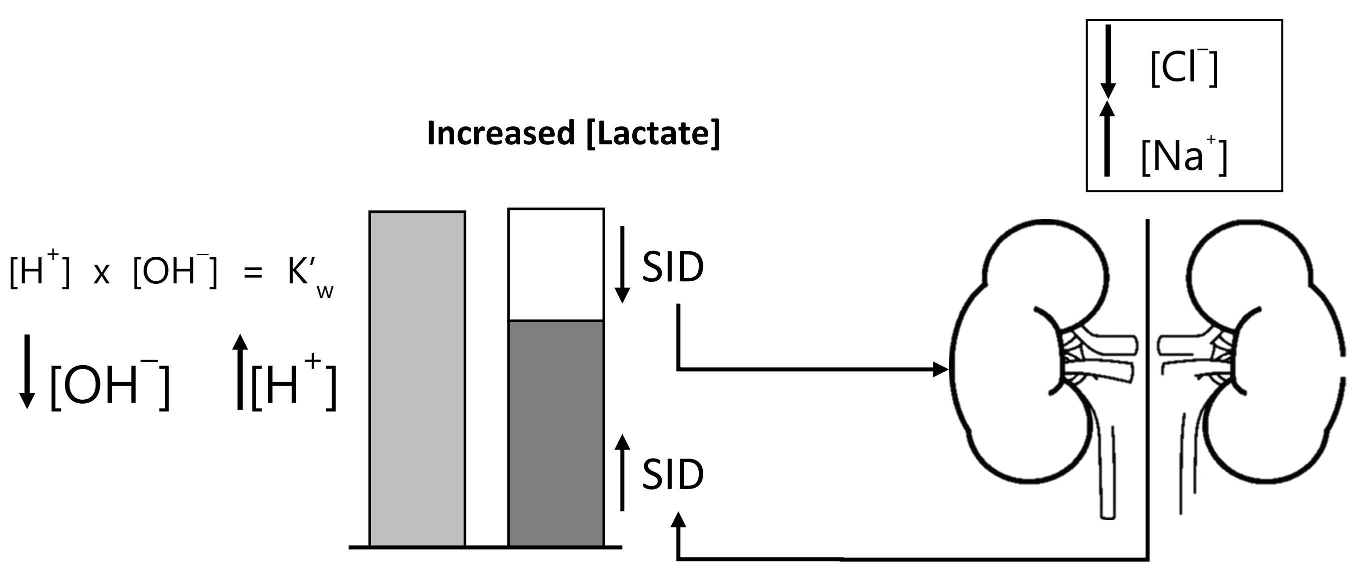
The key point of the lactate shuttle theory is that lactate is the normal "end product" of glycolysis, which occurs in the cytoplasm, under aerobic conditions and not the waste product of anaerobic metabolism (E2). Accordingly, both under aerobic and anaerobic conditions, the glycolysis of 1 mole of glucose produces 2 moles of lactate and 2 moles of ATP. The 2 moles of ATP are immediately hydrolyzed into 2 moles of ADP, phosphate and H^+ for energy cell requirements, and immediately regenerated via the energy provided by glycolysis. Therefore. under normal conditions. no free protons are released into the cytoplasm. (E3). The lactate enters the intermembrane space, where it is converted into pyruvate through the LDH anchored to the inner mitochondrial membrane. The pyruvate enters the matrix through a pyruvate translocase. Inside the matrix the pyruvate is decarboxylated via pyruvate dehydrogenase and enters the Krebs cycle as acetyl-CoA. The protons and the electrons released during the conversion from lactate to pyruvate enter the inner mitochondrial membrane via the malate-aspartate and glycerol-phosphate shuttle Several details are here omitted, however, it is worth noting that the most of energy is concentrated in the NADH, which feeds the respiratory chain after splitting protons and electrons.Note also that the intracellular ATP is present at high concentrations through continuous energy supply to maintain an "ATP/ADP disequilibrium"(E4). It is also important to note that in normal conditions (i.e. in aerobiosis), the lactate-pyruvate ratio in cytoplasm is largely in favor of lactate (E5).

The lactate produced in the cytoplasm has 3 possible pathways: 1) conversion to alanine; 2) entering the mitochondrial intermembrane space; 3) release from the cellular into the extracellular compartment. Carried from this extracellular site by the circulation, lactate may re-enter cells of other organs, either as a fuel to produce energy, as a substrate for gluconeogenesis or as a molecule for signaling. The lactate which enters the intermembrane space of mitochondria is converted by the membrane-linked lactic dehydrogenase into pyruvate, associated with NADH and H^+. The pyruvate, after thiamine-dependent decarboxylation under the activity of hypoxia-sensitive pyruvate dehydrogenase enzyme in the matrix (E6), enters the Krebs cycle as acetyl-CoA, while the $H^{+,}$ after transfer from the intermembrane space to the matrix via the malate-aspartate and glycerol phosphate shuttles, enters the respiratory chain. The end products of the process are CO_2, water and energy, which is used to maintain high ATP concentration and turnover (E4). According to this model, it is evident that there is only one basic mechanism by which excess lactate is generated and this is when the lactate oxidation capacity is exceeded. Indeed, increased glycolytic flux, thiamine deficiency, respiratory chain impairment or low tissue concentration of oxygen – the terminal acceptor of electrons and hydrogen ions – all may produce excess lactate if the oxidation capabilities are overwhelmed.

In hyperlactatemia, one has to consider: 1) its effects on acid-base equilibrium; 2) the lactate kinetic and why lactates increase only up to a certain level, which varies from patient to patient, after which it tends to remain constant.

Excess lactate and regulation by the kidney

The basic mechanisms for the interaction between kidney function and lactate are represented in eFigure 5.



AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

Figure E9. If, for any reason, the lactate production exceeds the oxidative capacity, the lactate is transported out of the cells, usually in association with a proton(2). Over the years, the origins of protons have been attributed to the production of lactic acid by glycolysis, ATP hydrolysis or, according to the Stewart´s approach, to water dissociation, which provides an almost infinite proton reservoir (55 M/L). Regardless of the origin of the protons, lactate shifts from the intracellular to the extracellular compartment via the monocarboxylate transporter (MCT), which simultaneously extrudes lactate and protons in a 1:1 ratio.

As the lactate concentration increases in the plasma, the strong ion difference, which is equal to the “buffer base”, decreases by an equimolar amount. For example. 10 mmol/L of lactate decreases the SID and the “buffer base” by 10 mEq. The “buffer base” is the sum of bicarbonate ($HCO3^-$), the dissociated form of proteins (A^-), phosphates and the hydroxyl ions (OH^-). After the strong negative ion lactate has been added, each of these components decreases proportionally to its physical characteristics, constituting a total decrease equal to the added lactate. The bicarbonate, taking one proton becomes CO_2+H_2O; the A^- becomes undissociated AH; and the OH^-, taking one proton. becomes H_2O. As the water dissociation equilibrium is constant, any decrease of OH^- is associated to a proportional increase of H^+, i.e., decreased pH.

Therefore, lactate accumulation, as such, should always cause acidemia and negative base excess (the base excess is the difference between the actual and the ideal buffer base or, if one prefers, the difference between the actual and the ideal SID). However, the immediate reaction of the functioning kidney to acidemia is to eliminate or reabsorb electrolytes to increase the plasmatic SID. This usually happens by increasing Cl^- excretion associated with newly generated ammonium and a possible increase in sodium reabsorption. In this study, this mechanism appears to accounts for the relationship we found between lactate, pH and base excess.

Peripheral shunt, lactate excess and lactate oxidation capabilities

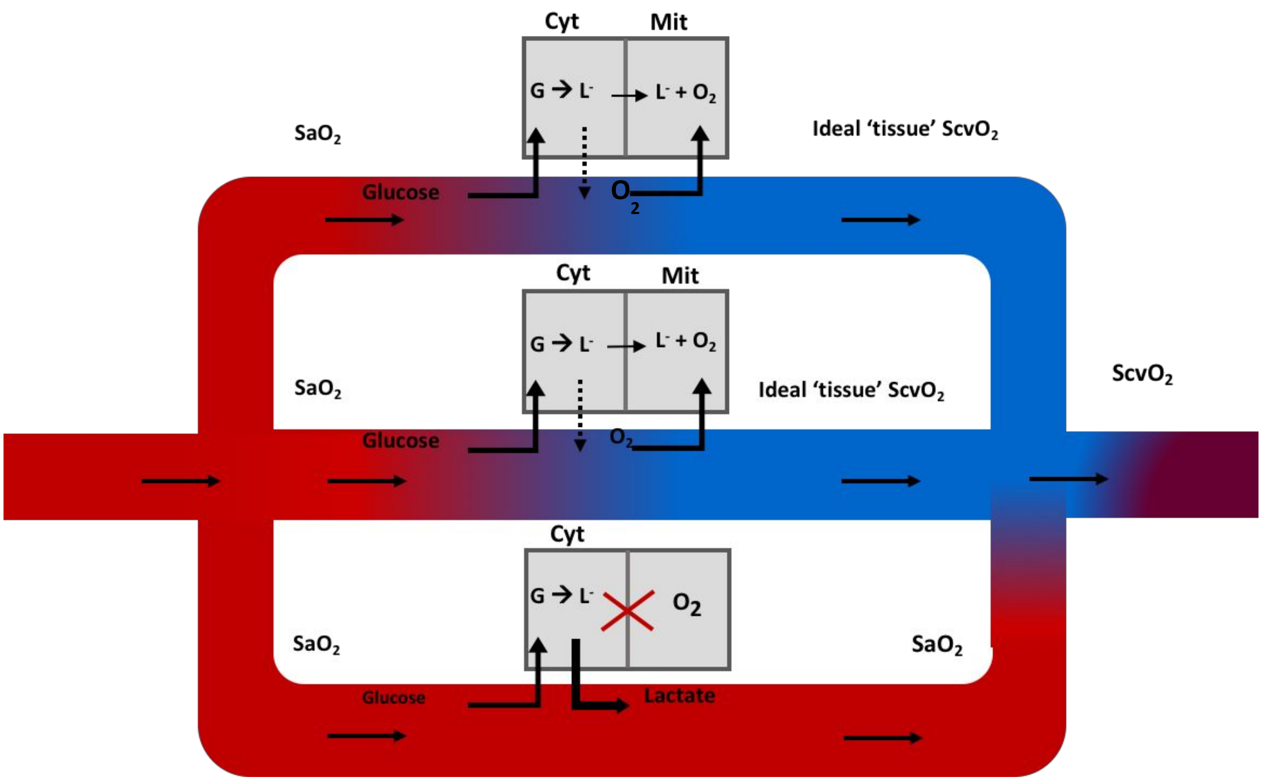


Figure E10 We depict a possible model of peripheral shunt, analogous to the reverse Riley's model of pulmonary shunt (E7). The model considers all the oxygen consuming units as part of two compartments: one functioning and the second non-functioning. i.e. the oxygen tension of the blood that flows through the non-functioning compartment remains unchanged and is in equilibrium with the surrounding tissues. In the example shown in the figure above, the peripheral shunt would be 33%. Indeed, two arms are normally perfused and undergo normal metabolic activity while the lowest arm is the shunted one; here the blood flow maintains its inlet oxygen tension, as oxygen is not consumed, and lactate is produced in the cytoplasm by the glycolysis. Analogously to Riley's model, if we assume as ideal $ScvO_2$ the surrogate of tissue oxygenation surrounding the normally perfused and metabolically active units, the peripheral shunt will be equal to:

$$\frac{Q_{sp}}{Q} = \frac{S_{CV}O_2 - S_VO_{2id}}{S_aO_2 - S_VO_{2id}}$$

Here, Q_{sp}/Q is the peripheral shunt fraction, $ScvO_2$ is the central venous blood oxygen saturation, SvO_{2id} is the ideal saturation of venous blood coming from oxygen-consuming tissue, and SaO_2 is the arterial oxygen saturation.

This model cannot discriminate between a true peripheral shunt and the lack of oxygen utilization and it is impossible to "measure" the ideal "tissue" SvO_2. In addition, we do not know what is the destiny of the excess lactate generated in the shunted arm during sepsis. If lactate would not re-enter in other functioning units, the lactate level would increase progressively. However, it is a common clinical observation that lactate increases up to a certain level, different from patient to patient, after which, in a relatively short time, a plateau is maintained. In eFigure 7, we show how in the six lactate sextiles the lactate levels are maintained unmodified for a long period of time (days).

AJRCCM Articles in Press. Published on 15-April-2019 as 10.1164/rccm.201812-2342OC

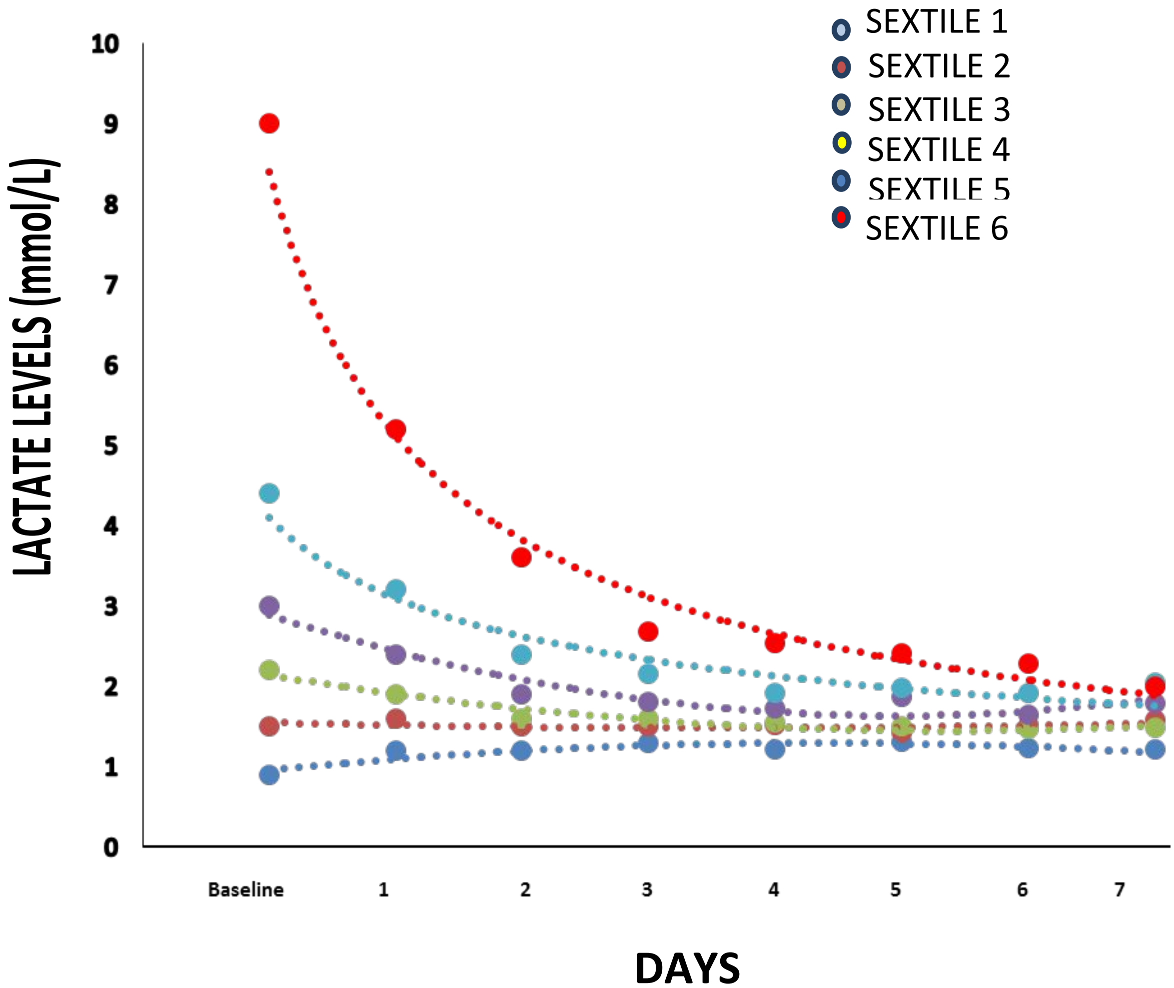


Figure E11. To maintain a constant plateau value, the rate of lactate production equals that of lactate oxidation. In normal conditions, the capability of lactate oxidation must be equal to the entirety of glucose metabolism. We would understand much better the whole picture if we knew the relationship between lactate production and lactate plateau in sepsis, as well as the rate of approach to equilibrium. Actually, in patients with renal failure it has been shown a strong relationship between the exogenous lactate input (mmol/min) given through the dialysis fluid and the plateau which is reached in 1-2 hours (E8). The greater the input, the greater the oxidation rate until the plateau is reached when the oxidation rate equals the lactate input. The same phenomenon was observed in experimental animals (E9). Therefore, we may hypothesize that the oxidation capability of the remaining functioning metabolic unit increases with the increased availability of lactate (i.e. substrate) and that the lactate level at which plateau is maintained is inversely related to the number of functioning units (i.e. it is directly related to what we call "peripheral shunt"). Interestingly, other metabolites, which are normally oxidized in the Krebs cycle, as the non-esterified fatty acids, behaves in sepsis as lactate: increased levels and higher rate of oxidation (E10). This extremely complicated picture can be summarized as follows:

- Excess lactate is redistributed to other working units (i.e. not shunted);
- The excess lactate is oxidized in the functioning metabolic units at a rate proportionate to the lactate level;
- At plateau, the rate of oxidation equals the rate of production in the non-functioning units;
- The plateau of the excess lactate should be proportional to the peripheral shunt;
- Oxygen consumption and energy production continue unmodified until the oxidation capability sharply decreases and the remaining units cannot accommodate the excess lactate;
- The acid-base status, in presence of excess lactate depends on kidney function.

References

E1. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L, Investigators AS. Albumin replacement in patients with severe sepsis or septic shock. *The New England journal of medicine* 2014; 370: 1412-1421.

E2. Brooks GA. The Science and Translation of Lactate Shuttle Theory. *Cell metabolism* 2018; 27: 757-785.

E3. Hochachka PW, Mommsen TP. Protons and anaerobiosis. *Science* 1983; 219: 1391-1397.

E4. David G. Nicholls SJF. Bioenergetics 4. 2013.

E5. Kane DA. Lactate oxidation at the mitochondria: a lactate-malate-aspartate shuttle at work. *Frontiers in neuroscience* 2014; 8: 366.

E6. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell metabolism* 2006; 3: 177-185.

E7. Riley RL, Cournand A. Ideal alveolar air and the analysis of ventilation-perfusion relationships in the lungs. *Journal of applied physiology* 1949; 1: 825-847.

E8. Wright DA, Forni LG, Carr P, Treacher DF, Hilton PJ. Use of continuous haemofiltration to assess the rate of lactate metabolism in acute renal failure. *Clinical science* 1996; 90: 507-510.

E9. Zanella A, Patroniti N, Isgro S, Albertini M, Costanzi M, Pirrone F, Scaravilli V, Vergnano B, Pesenti A. Blood acidification enhances carbon dioxide removal of membrane lung: an experimental study. *Intensive care medicine* 2009; 35: 1484-1487.

E10. Samra JS, Summers LK, Frayn KN. Sepsis and fat metabolism. *The British journal of surgery* 1996; 83: 1186-1196.