Relation between muscle Na^+K^+ ATPase activity and raised lactate concentrations in septic shock: a prospective study

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Summary

Background **Hyperlactataemia during septic shock is often viewed as evidence of tissue hypoxia. However, this blood disorder is not usually correlated with indicators of perfusion or diminished with increased oxygen delivery. Muscles can generate lactate under aerobic conditions in a process linking glycolytic ATP supply to stimulation of Na^+K^+ ATPase. Using in-vivo microdialysis, we tested whether inhibition of Na^+K^+ ATPase can reduce muscle lactate.**

Methods **In 14 patients with septic shock, two microdialysis probes were inserted into the quadriceps muscles and infused with lactate-free Ringer's solution in the absence or presence of 10^{-7} mol/L ouabain, a specific inhibitor of Na^+K^+ ATPase. We measured lactate and pyruvate concentrations in both the dialysate fluid and arterial blood samples.**

Findings **All patients had increased blood lactate concentrations (mean 4·0 mmol/L; SD 2·1). Lactate and pyruvate concentrations were consistently higher in muscle than in arteries during the study period, with a mean positive gradient of 1·98 mmol/L (SD 0·2; p=0·001) and 230 μmol/L (30; p=0·01), respectively. Ouabain infusion stopped over production of muscle lactate and pyruvate (p=0·0001). Muscle lactate to pyruvate ratios remained unchanged during ouabain infusion with no differences between blood and muscle.**

Interpretation **Skeletal muscle could be a leading source of lactate formation as a result of exaggerated aerobic glycolysis through Na^+K^+ ATPase stimulation during septic shock. Lactate clearance as an end-point of resuscitation could therefore prove useful.**

Relevance to clinical practice **In patients with septic shock, a high lactate concentration should be interpreted as a marker of disease, portending a bad outcome. The presence of hyperlactataemia in resuscitated septic patients should not be taken as proof of oxygen debt needing increases in systemic or regional oxygen transport to supranormal values. Lactate, instead of being regarded only as a marker of hypoxia, might be an important metabolic signal.**

Introduction

Raised blood **lactate** concentrations during shock states are often viewed as evidence of tissue hypoxia, with values being proportional to the defect in oxidative metabolism.[1] However, many tissues generate pyruvate and lactate under aerobic conditions (aerobic **glycolysis**) in a process linking glycolytic ATP supply to the activity of membrane ion pumps such as **Na^+K^+ ATPase**.[2] Indeed, aerobic glycolysis and glycogenolysis occur not only in resting, well oxygenated skeletal muscles, but also during experimental haemorrhagic shock and experimental sepsis, and are closely linked to stimulation of active sarcolemmal Na^+K^+ ATPase transport due to epinephrine release.[3–5]

Hyperlactataemia and lactic acidosis are not synonymous. Lactic acidosis refers to a cellular metabolic process characterised by rises in blood lactate (>5 mmol/L) and decreases in blood pH (<7·25). During cellular hypoxia, hydrolysis of ATP leads to accumulation of H^+ ions in the cytosol and subsequent acidosis. On the other hand, under aerobic conditions the H^+ ions produced by hydrolysis of ATP are recycled during metabolism of glucose and therefore the process is non-acidifying. During septic shock, the distinction between hypoxic or non-hypoxic production of lactate is critical for understanding cellular responses to injury and for interpretation of lactate concentration during resuscitation.[6] This knowledge could lead to the abandonment of potentially harmful actions aimed at increasing oxygen delivery to the tissue.[7]

We propose that increased epinephrine release in septic shock stimulates sarcolemmal Na^+K^+ ATPase and greatly accelerates aerobic glycolysis and Na^+K^+ ATPase–coupled lactate production in skeletal muscle. Consequently, most of the increase in blood lactate would thus be unrelated to poor perfusion and unlikely to respond to a rise in oxygen delivery. To our knowledge, there is no human study that has addressed whether the reported increase in lactate concentrations in septic shock is associated with a rise in muscle Na^+K^+ ATPase activity. We used microdialysis technology to assess whether specific inhibition of Na^+K^+ ATPase by ouabain is able to prevent production of lactate in muscle in patients with septic shock.

Methods

Patients

Our institutional review board approved this study, and patients or their relatives provided written informed consent before enrolment. 14 consecutive patients with septic shock, defined according to the International Sepsis Definitions Conference,[8] were prospectively enrolled within 12 h of the occurrence of shock. All

Lactate
Mainly derived from pyruvate through the action of the enzyme lactate dehydrogenase, located in the inner mitochondrial membrane, according to the following equation: Pyruvate + NADH $+H^+ \leftrightarrow$ lactate + NAD^+.

Glycolysis
Oxidation of glucose to either pyruvate or lactate. Glycolysis proper is completely anaerobic. The global reaction of glycolysis is: Glucose + 2 NAD^+ + 2 ADP + 2 Pi → 2 NADH + 2 pyruvate + 2 ATP + 2 H_2O + 4 H^+

Na^+K^+ ATPase
Pump system located in the sarcolemma, which requires a continuous source of ATP to function. For every ATP molecule used, three Na^+ ions are pumped out of the cell and 2 K^+ ions are pumped into the cell.

patients were treated according to international recommendations with broad-spectrum antibiotics, low-dose hydrocortisone in case of non-response to a short corticotropin test, activated protein C in the absence of exclusion criteria, and fluid infusion. If hypotension persisted despite fluid resuscitation, an infusion of epinephrine or norepinephrine was started. Patients were monitored with an arterial catheter (radial or femoral site) and a pulmonary artery catheter with continuous measurement of cardiac index. We used mixed venous oxygen saturation (SvO_2) (Baxter Edwards Critical-Care, Irvine, CA, USA) and a gastric tonometer (Tonocap, Datex, Helsinki, Finland) to assess adequacy of gastric mucosal perfusion. Inspiratory fraction of oxygen (FiO_2) was adjusted to accomplish an oxygen arterial saturation of more than 92%.

Microdialysis technique

Two microdialysis catheters (CMA 60, CMA/Microdialysis, Stockholm, Sweden) were inserted into the quadriceps femoris muscle on each side. The probes were connected to a microinjection pump. One probe (control) was continuously infused with lactate-free Ringer's solution, whereas the other was infused with the same solution to which 10^{-7} mol/L ouabain was added, a specific inhibitor of Na^+K^+ ATPase (Meda, Germany). Ouabain itself has no effect on either lactate metabolism or blood flow in muscle.[9] The length of the microdialysis membrane (30 mm) together with a very slow perfusion flow rate (0·3 μl/min) guarantee 100% recovery (ie, uptake of molecules from the interstitial space) for molecules up to 20 kDa, thus providing true tissue concentrations.[10,11] An hour equilibration period was allowed after insertion of the probe. After initial verification that microdialysis metabolites did not differ between the two probes, ouabain was introduced into one probe. After a second 1-h equilibration period measurements were done hourly for 24 h.

Muscle tissue partial pressure of oxygen

We monitored partial pressure of oxygen (PO_2) in muscle tissue, adjusted for temperature, in five patients using the Licox sensor system (Integra Neuroscience, Plainsboro, NJ, USA) inserted into the quadriceps femoris muscle. Three patients were infused with epinephrine and two with norepinephrine. We calibrated the sensors according to the manufacturer's specifications before insertion. Adequate reactivity of the oxygen sensor element of the Licox probe was tested by briefly increasing forced inspiratory oxygen (FiO_2) concentrations to 100% after insertion of the sensor and by recording the subsequent rise in tissue oxygen tension. We recorded all values online with ICU pilot software (Microdialysis AB, Solna, Sweden), documented in 1-min intervals and entered into a database for further analysis. Normal values in volunteers at rest varied from 15 to 30 mm Hg.

Measurements

We measured lactate and pyruvate concentrations hourly in both the dialysate fluid and arterial blood using a CMA 600 analyser (Microdialysis AB, Solna, Sweden). Arterial pressure, cardiac output, venous oxygen saturation, and the partial pressure of carbon dioxide (PCO_2) gap—difference between gastric mucosal PCO_2 and arterial PCO_2—were also recorded. Normal muscle values for lactate and pyruvate are 1–2 mmol/L and 100–200 μmol/L.

Statistical analysis

We analysed differences between catheters as well as between catheters and blood at each time point using the *t* test (data were normally distributed as assessed by the Kolmogorov-Smirnov test). The change in measurements over time was calculated for each catheter and for blood. We calculated and analysed changes in lactate to pyruvate ratios over time using the repeated measures analysis of variance test (ANOVA). Analysis was completed with Statview software (Abacus Concepts, version 5.0), and a two-tailed p value of less than 0·05 was deemed significant.

Role of the funding source

This work was supported by a grant from Société de Réanimation de Langue Française. The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 14 patients with septic shock, ten survived the septic episode. Mean age was 65 years (SD 12). All patients were mechanically ventilated and ten had bicarbonate haemofiltration. Eight were infused with epinephrine and six with norepinephrine. Since the same analyses were done for both groups, pooled data are presented. A hyperdynamic state, as shown by a high mean cardiac index of 4·0 L/min per m^2 body-surface area (SD 0·4), was present in all patients at inclusion. Mean SvO_2 was 76% (6). All patients had raised blood lactate concentrations at enrolment (4·0 mmol/L, SD 2·1). Mean lactate to pyruvate ratio was 27 (8), mean arterial pH was 7·29 (0·02), and PCO_2 gap was 14 mm Hg (4). An infectious microorganism was recorded in all patients (five with *Escherichia coli*, five with *Streptococcus pneumoniae*, three with *Staphylococcus aureus*, and one with *Pseudomonas aeruginosa*). Mean arterial pressure (75 mmHg; 8), heart rate (110 beats/min; 15), cardiac index, SvO_2, and PCO_2 gap remained constant throughout the study.

Epinephrine and norepinephrine doses decreased from 0·9 μg/kg per min (0·1) and 1·0 μg/kg per min (0·1) at 0 h, to 0·4 μg/kg per min (0·1) and 0·5 μg/kg

per min (0·1) at 24 h, respectively. Blood lactate fell within the 24 h study period (p=0·02) (figure). Muscle lactate concentrations were always higher than arterial lactate concentrations during the study, with a mean positive gradient of 1·98 mmol/L (SD 0·2, p=0·001) (figure). Ouabain infusion totally abolished the gradient between muscle and arterial lactate concentrations (p=0·0001)(figure). Similarly, an initial positive pyruvate musculoarterial gradient of 230 μmol/L (30) (p=0·001) was also abolished by ouabain. Lactate to pyruvate ratios were similar in both blood and muscle (p=0·28) and remained unchanged during ouabain infusion. Muscle PO_2 measured in the last five patients remained unchanged during the study. The lowest mean value was 36 mm Hg (4) and the highest was 44 mm Hg (7).

Discussion

All patients had increased blood lactate concentrations, and lactate and pyruvate concentrations were consistently higher in muscle than in arteries during the study period. Selective inhibition of Na^+K^+ ATPase with ouabain infusion stopped over-production of muscle lactate and pyruvate.

Hypoxic-anaerobic metabolic production of lactate can be global (eg, hypovolaemia, cardiac failure) or focal (eg, bowel ischaemia). A non-hypoxic increase in lactate concentration can result from impaired lactate clearance (liver dysfunction), a dysfunction of pyruvate dehydrogenase (the enzyme that regulates the rate of pyruvate use by the tricarboxylic acid cycle), or an increased muscular protein degradation causing aminoacid conversion to pyruvate. Nevertheless, evidence seems to implicate accelerated aerobic glycolysis, a definite state when the rate of glucose metabolism exceeds the oxidative capacity of the mitochondria. Accelerated aerobic glycolysis is induced by an endogenous or exogenous catecholamine and an inflammatory state. Gore and colleagues[12] showed that pyruvate production and oxidation are enhanced in septic patients. The rise in pyruvate concentration will ultimately drive lactate production by a mass effect. Another possible cause of non-hypoxic hyperlactataemia in sepsis could be the inhibition of mitochondrial respiration.[13]

Microdialysis is a unique method for assessing local production of substances such as lactate and pyruvate.[14] Moreover, it allows analysis of local effects of pharmacological agents added to the perfusate, thus avoiding confounding systemic effects. The technique uses the insertion of a semi-permeable membrane continuously infused with dialysis solution in a specific tissue (brain, muscle, adipose tissue). At the tissue level, the solutes present in the interstitium freely diffuse into the catheter according to their concentration gradient. At a very low perfusion flow rate (0·3 μL/min), the gradient between muscular interstitium and arterialised blood concentration indicates whether muscle produces or uses a specific substrate.[11] Microdialysis can also be used to study the effects of biologically active substances on muscle metabolism. The tissue can be exposed to a high concentration through the microdialysis device without causing any systemic effect.

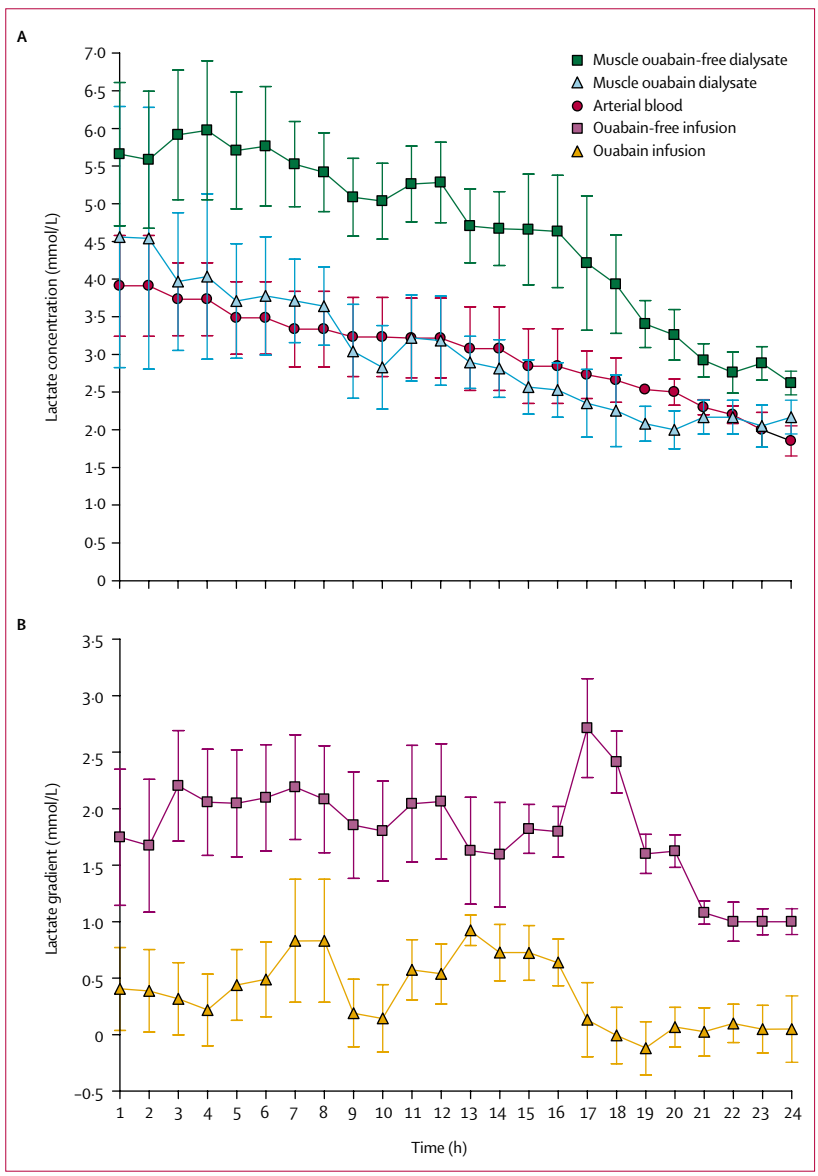

Figure: **Lactate concentrations (A) and gradients (B) in 14 patients with septic shock in 24 h of study**
Data are mean and SD (vertical bars). Gradient=difference between dialysate lactate and arterial blood lactate.

In our study, lactate concentrations were consistently higher in muscle than in blood, which contrasts sharply with concentrations reported in volunteers in whom the gradient between muscle and arterial blood was very low and did not change with ouabain. Our finding lends support to the notion of muscle lactate production during septic shock.[15] As skeletal muscles account for roughly 40% of total body-cell mass, it could represent a major source of lactate during sepsis. Epinephrine-

induced hyperlactataemia under fully aerobic conditions has been recorded in healthy volunteers at rest and during exercise.[16,17] During shock, plasma epinephrine concentrations are raised, originating from an exaggeration in endogenous release and from the use of pharmacological infusion.[18] Physiologically, epinephrine enhances glycogenolysis with a net increase in pyruvate production. This mechanism is mediated via stimulation of muscle and hepatic phosphorylase and inhibition of glycogen synthetase. Experimental data lend support to the fact that epinephrine induces hyperlactataemia by binding to muscle adrenergic β_2 receptors and raising AMP production.[19] Such an increase leads to the coordinated stimulation of both Na^+K^+ ATPase activity and glycogenolysis.[20] Activation of Na^+K^+ ATPase generates ADP, thereby raising phosphofructokinase activity, accelerating aerobic glycolysis, and thus increasing lactate concentration.

Glycolytic flux in the cytoplasm is functionally compartmentalised. There are two glycolytic pathways with separate sets of glycolytic pathway enzymes. The enzymes of one of these pathways have been shown to be associated with Na^+K^+ ATPase activity. The glucose units traversing the other cytosolic glycolytic pathway are partly channelled to oxidative metabolism. Glycogenolysis and acceleration of aerobic glycolysis provide ATP to sustain Na^+K^+ ATPase activity in cells with intact oxidative capacity, including skeletal muscle. Sair and colleagues[21] reported an increased PO_2 in muscles in septic shock patients compared with controls, thus arguing against the notion of muscle hypoxia. Our results, although for only few patients, accord with those of Sair and colleagues.

In our study, ouabain, a specific inhibitor of Na^+K^+ ATPase, substantially decreased muscular lactate, underlining the role of this enzyme in lactate formation. Lactate efflux from the intracellular space results from one of three pathways: (1) free diffusion of undissociated acid; (2) exchange for another anion such as Cl^- or HCO_3^-; and (3) H^+ linked carrier mechanism, which is pH dependent. Barron and co-workers[22] showed that ouabain, in decreasing Na^+K^+ ATPase activity, reduced both glycolysis and the intracellular concentration of H^+ and indirectly decreased monocarboxylate B-H+ transporter activity (the enzyme responsible for lactate and pyruvate efflux) resulting in accumulation of intracellular lactate. Thus the size of the decrease in lactate by ouabain might be affected by a combined action on lactate formation and lactate intracellular efflux.

The basis for aerobic lactate production during septic shock is not straightforward.[23] Epinephrine-induced lactate production serves as the main metabolic precursor for the liver by the Cori cycle.[24] This mechanism underscores the pivotal role of lactate in aerobic energy metabolism.[25] Indeed, the lactate shuttle theory suggests that aerobic glycolysis confers increased metabolic flexibility by allowing tissues to share a common source for oxygenation or **gluconeogenesis**.[2] Raised blood lactate concentrations reported in shock states could therefore represent a key protective metabolic event, favouring lactate rather than glucose oxidation in tissues in which oxygen is available, and preserving glucose for non-aerobic ATP resynthesis through glycolysis in tissues in which oxidative metabolism is scarce.[26] The lactate to pyruvate ratio indicates the cytoplasmic accumulation of reducing equivalents.[25] The high lactate to pyruvate ratio in our study could be associated with a large inter-organ flux of reducing equivalents (lactate, NADH) for ATP synthesis. Therefore, this seemingly high and unnecessary rate of aerobic glycolysis under epinephrine stimulation could provide some help to organs, such as the heart, wounded tissue, or brain, to sustain specific processes that need a high rate of cytoplasmic ATP. For example, in the brain, lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia,[27] and lactate improves cardiac function in haemorrhagic shock.[28] Thus instead of being regarded as a marker of hypoxia or severity in critically ill patients, lactate could be a metabolite used as a substrate for specific purposes.[25]

Our study does not establish a direct link between increased Na^+K^+ ATPase activity and epinephrine. We did not measure plasma catecholamine since previous studies showed that epinephrine and norepinephrine concentrations were greatly raised in septic shock. Nevertheless, the link between epinephrine and increased Na^+K^+ ATPase activity is well established in laboratory animal models and in man.[29]

Two unresolved issues need to be addressed: (1) whether increased Na^+K^+ ATPase activity is a specific hallmark of sepsis or a general occurrence in shock, and (2) the effects of systemic blockade of epinephrine-induced hyperlactataemia. Experimental studies seem to show that epinephrine-induced hyperlactataemia is present even in states of low cardiac output, such as haemorrhagic shock. In burn-injured patients, lactate concentration can be lowered by β blockade with propanolol.[4] We are studying acute and long-term laboratory models of sepsis to address these questions.

Our results elucidate on the mechanism of lactate generation during septic shock revealing skeletal muscle as a leading source of production through exaggerated aerobic glycolysis rather than tissue hypoxia. In the setting of sepsis, a high lactate concentration should be interpreted as a marker of disease, one that portends a bad outcome. Therefore, the presence of hyperlactataemia in patients with septic shock should not be taken as proof of oxygen debt requiring increases in systemic or regional oxygen transport to supranormal values. Our findings could offer new implications for the usefulness of lactate clearance as an end-point of resuscitation.[30]

Gluconeogenesis
Synthesis of glucose from non-carbohydrate precursors, such as pyruvate, aminoacids, and glycerol. Takes place largely in the liver and serves to maintain blood glucose under conditions of starvation or intense exercise.

Contributors
B Levy and S Gibot designed the study; B Levy coordinated the study; B Levy, S Gibot, and A Cravoisy were responsible for patient screening, enrolment, and follow–up; P Franck did blood sampling and all laboratory studies; B Levy, S Gibot, and PE Bollaert analysed the data and wrote the manuscript; and all authors contributed to the intellectual content of the manuscript.

Conflict of interest statement
We declare that we have no conflict of interest.

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