Plasma amino acids of the transsulfuration pathway and plasma lactate in septic patients

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Objectives. In sepsis, increasing plasma lactate, even if unrelated to hypoperfusion and hypoxia, is a cause of concern. Among the patterns associated with increasing lactate, several plasma amino acid (AA) abnormalities, more in particular those of sulfur AAs, have remained unexplored, and their assessment has been the purpose of our study.

Materials and Methods. A systematic and detailed analysis of 183 simultaneous determinations of plasma AA-grams and lactate, from 12 trauma surgery patients who had developed sepsis, was performed. Sepsis severity ranged from moderate to extreme illness. Correlations between changes in lactate and in AA levels were assessed by regression analysis.

Results. Increasing lactate was related to increasing alanine, proline, asparagine, tyrosine, cystathionine, histidine, glutamine, citrulline, methionine, phenylalanine and hydroxyproline (r from 0.62 to 0.36, p < 0.001 for all) and to decreasing taurine (r = -0.62, p < 0.001). Furthermore, increasing lactate was strongly related to increasing cystathionine/taurine ratio (r = 0.77, p < 0.001). These correlations were independent of the simultaneous relationship found between increasing lactate and decreasing mixed venous O2 tension.

Discussion. The overall findings and the correlation with the cystathionine/taurine ratio support the hypothesis that increasing lactate in sepsis may be paralleled by impaired hepatic AA transsulfuration. Because this may disable antioxidant protection by limiting glutathione and taurine availability, the metabolic perturbations associated with septic hyperlactatemia may include enhanced exposure to oxidative stress.

Key words: hepatic transsulfuration, oxidative stress, plasma amino acids, plasma lactate, sepsis

Introduction

High plasma lactate in sepsis may reflect hemodynamic instability and tissue hypoperfusion, determining hypoxia and anaerobic glycolysis. In hemodynamically stable patients, high lactate might be interpreted to reflect adaptive aerobic glycolysis to support cell membrane metabolism and integrity, and to provide a convenient energy shuttle for fueling the metabolism of heart, brain and of other important cells (1-9). However, in apparent contrast with these possibly adaptive roles, high lactate more often signals worsening of the septic illness, even in the presence of adequate perfusion and hemodynamic stability, and hyperlactatemia is only reversed by valid interventions to resolve sepsis. The apparent contrast may be explained by the circumstance that adaptive mechanisms grow in intensity (and consequently hyperlactatemia increases) as disease severity worsens (10).

In the clinical setting the quick identification of the cause of hyperlactatemia may be far from obvious. In general, cardiovascular causes of peripheral hypoperfusion with tissue ischemia, O2 debt and anaerobiosis are immediately addressed and treated if present. However in sepsis derangement of perfusion and O2 delivery commonly occur at the microvascular level (9, 11) even when the macroscopic evidence of hemodynamic adequacy might exclude perfusion disturbances. With regard to non-anaerobic, metabolic causes of septic hyperlactatemia, these have long been the object of pioneer studies (1) and their understanding has progressively improved, although many aspects still remain imperfectly known, as do the multiple implications of hyperlactatemia (10, 12) always taking into account that various causes of hyperlactatemia may obviously coexist.

Our study aimed at analyzing several incompletely assessed pathophysiologic aspects and correlations of hyperlactatemia. The correlation with various amino acid (AA) abnormalities has been evaluated in the past, however a detailed and systematic analysis for all AAs, more in particular for those involved in the transsulfuration pathway was never performed, at least to our knowledge, and this was our specific purpose. Indeed this pathway has a major role in maintaining antioxidant protection through glutathione and taurine production, and the correlations between the involved AAs and lactate may help assessing its adequacy in the presence of hyperlactatemia.

Materials and Methods

The study was based on the detailed retrospective analysis of 183 simultaneous and complete assays of plasma AAs, lactate ad glucose, which were performed after the develop-
Transsulfuration and lactate in sepsis

Table 1 legend. Patient data. Symbols: M, male; F, female; S, survived; D, died

Results

The data on plasma AAs are shown in Table 2. Plasma lactate was 1.57 ± 0.82 mmol/L (mean ± standard deviation; median 1.34, range 0.56 - 5.26) and glucose was 7.9 ± 2.3 mmol/L (median 7.2, range 4.7 - 16.5). Regression analysis showed strong direct correlations between lactate and proline, alanine and asparagine (r = 0.62, p < 0.001 for all), and an inverse correlation with taurine (r = -0.62, p < 0.001). Furthermore there were direct correlations with tyrosine, cystathionine, histidine, glutamine, citrulline, methionine, phenylalanine and hydroxyproline (r from 0.55 to 0.36, p < 0.001 for all) (Table 3). Other AAs were more weakly related (r < 0.30) or unrelated to lactate. Within this panel of correlations, a parallelism of changes in lactate and gluconeogenic AA flux (17-19) was well recognizable, except for the relationships found with the sulfur AAs taurine, cystathionine and methionine.

Therefore the correlations between lactate and the AAs involved in the transsulfuration pathway methionine (Met), cystathionine (Cysta), cyst(e)ine (Cys) and taurine (Tau) were assessed in greater detail, in particular on the basis of their precursor/by-product ratios. The strongest direct correlation was found between lactate and the Cysta/Tau ratio: lactate = 1.21 + 3.79 [Cysta/Tau]; r = 0.77, p < 0.001. Other ratios were less strongly related to lactate (Table 3) and the Met/Cysta ratio was unrelated to it. Of interest, in multiple regressions, increasing Cysta and Met and decreasing Tau showed strong direct correlations between lactate and proline, alanine and asparagine (r = 0.62, p < 0.001 for all), and an inverse correlation with taurine (r = -0.62, p < 0.001). Furthermore there were direct correlations with tyrosine, cystathionine, histidine, glutamine, citrulline, methionine, phenylalanine and hydroxyproline (r from 0.55 to 0.36, p < 0.001 for all) (Table 3). Other AAs were more weakly related (r < 0.30) or unrelated to lactate. Within this panel of correlations, a parallelism of changes in lactate and gluconeogenic AA flux (17-19) was well recognizable, except for the relationships found with the sulfur AAs taurine, cystathionine and methionine.

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metabolic decompensation, who reached a SOFA score of 16 and 13, respectively: at decompensation Cysta and lactate first increased, followed by increasing Met, while Tau decreased. At subsequent recovery Cysta and lactate first decreased, followed by decreasing Met, while Tau increased. It is worth mentioning that the sulfur AA ratios and lactate, in the whole group of patients, were unrelated to routine values of aminotransferases, bilirubin, prothrombin time and creatinine.

To simultaneously account for the potential impact of hypoperfusion (although no patient had hypotension or clinically relevant evidence of hypoperfusion at the time of the measurements) the correlations between the sulfur AA ratios and lactate were separately assessed for the measurements associated with a simultaneous determination of mixed venous O₂ tension (PvO₂) < 40 mmHg, or ≥ 40 mmHg. This was because low PvO₂ may reflect inadequate blood flow to tissues, with more likely exposure to cellular hypoxia and lactate production from anaerobic metabolism. The PvO₂ measurement was available in 104 cases, with a median value of 40.0 mmHg (mean 41.6 ± 5.5, range 23.0 - 57.8). The analysis showed that cases having PvO₂ < 40.0 had higher lactate for any given Cysta/Tau and other similar ratios, compared to cases having PvO₂ ≥ 40.0 (p < 0.01 for all). Moreover, the inclusion of PvO₂ as an additional independent variable in the regressions in Table 3 almost always had a highly significant effect on lactate, with PvO₂ being inversely related to it (that is, for any given AA, or AA ratio, lactate increased with decreasing PvO₂ ) (Table 4; p < 0.005 for all regressions and regression coefficients but that of PvO₂ in regression 4, having p = 0.046). Finally, there was no correlation between lactate and heart rate, blood pressure or blood base excess. Increasing lactate was associated with increasing blood glucose for any given exogenous glucose infusion rate (r = 0.30, p < 0.001). There was no evident correlation between the measured AAs and glucose except for alanine, which was positively related to it (r = 0.31, p < 0.001).

### Discussion

Main results. While several AA correlations reconfirmed the association of increasing lactate with increased gluconeogenetic flux from AAs, which is an already known pattern (1, 17-19), the correlations with the sulfur AAs were particularly interesting. The strong positive correlation found with the Cysta/Tau ratio, and the results on the individual sulfur AAs, suggested that increases in plasma lactate were paralleled by signs of impairment of hepatic AA transsulfuration. The transsulfuration pathway involves the conversion of Met to Cysta (through homocysteine plus serine), which is then converted to Cys, which finally is a substrate for obtaining Tau, or for glutathione or protein synthesis. The enzyme gamma-cystathionase is involved in the conversion of Cysta to Cys. Hence the increases in the precursor AAs Cysta and Met, with reduction in the by-product Tau, which in our study were associated with increasing lactate, may suggested dysfunction of this enzyme. This would be congruent with previous studies which addressed dysfunction of gamma-cystathionase in stress conditions, sepsis and AIDS (20-24). However, a correlation between increasing lactate and signs of altered sulfur AA metabolism such as that found in our study was never described earlier, at least to our knowledge.
Anaerobic elevation of lactate in sepsis. This may have multiple determinants and, more obviously, those which impair peripheral O₂ transport therefore inducing anaerobic glycolysis. In addition, however, microcirculatory disturbances (peripheral shunts or microthrombi diverting blood flow away from cells) or cytopathic inability to use O₂ may hamper (peripheral shunts or microthrombi diverting blood flow away from cells) and reducing lactate extraction even in the presence of apparently adequate peripheral O₂ transport, especially in sepsis (1, 9, 25).

A combination of these factors was likely reflected in our study by the higher lactate found in patients with PVO₂ < 40.0 mmHg compared to those having PVO₂ ≥ 40.0 (p < 0.001), for any given Cysta/Tau and other analogous ratios. It was also reflected by the significant inverse relationship found between PVO₂ and lactate when PVO₂ was added as an independent variable in the regressions in Table 3 (as shown in Table 4). In fact, with decreasing PVO₂, septic patients are more easily predisposed to marginal tissue O₂ extraction, with lactate production from anaerobic metabolism. Our data are in accordance with this well-known effect, while simultaneously showing a distinct correlation between altered sulfur AA metabolism and increasing lactate. Of note, the Cysta/Tau ratio and PVO₂ accounted together for 76% of the variability of lactate (Table 4, regression 13, multiple r = 0.87, r² = 0.76). The data in Table 4 also show the tendency for PVO₂ to maintain a coefficient of about -0.05, reflecting a mean increase in lactate of 0.05 mmol/L per mmHg decrease in PVO₂. Although it is obvious that multiple regressions and quantifications based on a limited number of measurements (n = 104) may not have very strong value, most of the described results were statistically highly significant.

Non-anaerobic elevation of lactate in sepsis. In the past this has been related to dysfunction of pyruvate dehydrogenase, of energy-producing pathways of cells, of hepatic lactate clearance, of cofactor thiamine (1, 5, 6, 9, 25, 26), and more in general to overproduction of pyruvate from accelerated glycolysis or AA catabolism (1, 5, 6, 9, 17), a phenomenon which is consistent with the general AA pattern observed in our study. However it has further been related to adaptive glycolysis in muscle to maintain cell membrane activity (2-6, 27) whilst other potentially adaptive functions of increasing lactate might include its role as a metabolic substrate for the heart, the brain, other tissues, and wound healing (1, 5-7, 9, 27).

Nevertheless high lactate, in the setting of hemodynamic stability and good oxygenation, cannot be dismissed as the sign of a relatively compensated condition (28). Indeed, our findings show a correlation between increasing lactate and previously unreported signs of liver dysfunction. Subclinical liver dysfunction is often present in sepsis (26, 29-33) and our data are congruent with an impairment of AA transsulfuration, which is a critical function of the liver. Its deterioration may enhance oxidative stress through insufficient glutathione and/or taurine disposal (20-24, 34, 35). This is in agreement with the simultaneous correlations found in our patients between increasing lactate and increasing proline, phenylalanine and tyrosine, because their elevations also reflect liver dysfunction (17, 18), and the enzymatic reactions involved in phenylalanine and tyrosine metabolism are very sensitive to enhanced oxidative stress (36).

All these newly described and peculiar abnormalities were observed in a setting where the general AA panel and the correlations between increasing alanine, glucose and lactate reconfirmed the already well-known features of septic metabolic dysregulation, glucose intolerance and enhanced Cori cycling and gluconeogenesis (1, 17).

Our observation that imbalance between lactate clearance and production correlated with signs of impaired AA transsulfuration was well defined and highly significant. However this is an apparently unexplored field, within the debates regarding antioxidant therapy in sepsis and critical illness (37, 38). Our original and hypothesis-generating data, if prospectively reconfirmed in a wider population of patients with various combinations of organ failures, may support the need for antioxidant treatment in septic patients with hyperlactatemia. This should overcome the limitations of our study, which were mainly related to its retrospective nature and the reduced number of patients; furthermore, although in our cases sulfur AA changes were unrelated to serum creatinine, the possible impact of renal dysfunction on sulfur AA patterns (34) in septic multiple organ failure deserves better characterization.

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### Conclusions

Our findings support the concept that increase in plasma lactate in sepsis, even in the presence of apparent hemodynamic stability, may be the hallmark of relevant metabolic
perturbation aggravated by disabiliy of antioxidant defense. This might implicate, beyond the obvious need to rapidly eradicate sepsis, also the need to implement supportive therapies with enhanced antioxidant protection.

References

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