Leukocyte Glycolysis and Lactate Output in Animal Sepsis and Ex Vivo Human Blood

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Lactate is released in large quantity from sites of sepsis and inflammation. We asked whether the increased lactate production found in sepsis can be explained by the augmented glycolysis of inflammatory cells. The glycolytic metabolism of rat peritoneal leukocytes was measured following cecal ligation and perforation (CLP) or sham laparotomy. CLP augmented glucose uptake, the pentose phosphate pathway, and glucose oxidation. Lactate output increased from 1.03 ± 0.05 to 1.20 ± 0.05 fmol · cell^{-1} · min^{-1} ($P < .001$). Total lactate output of peritoneal lavage fluid increased from 7.94 ± 2.59 to 28.12 ± 5.60 nmol L · min^{-1} ($P < .005$). The effect of lipopolysaccharide (LPS) on the lactate output of whole blood from 31 critically ill patients was measured. Leukocyte lactate production was calculated by multiple linear regression analysis. Following exposure to LPS, human leukocyte lactate output increased from 0.20 ± 0.09 to 1.22 ± 0.14 fmol · cell^{-1} · min^{-1} ($P < .001$). This rate of production is so high that it suggests that the lactate output of different tissue beds in sepsis may be affected by their different cell populations and state of activation. This study supports the hypothesis that lactate may be more a product of inflammation than a marker of tissue hypoxia in sepsis.

RECENT STUDIES HAVE SHOWN an elevation of lactate turnover in septic patients under apparently normoxic conditions.[1] This calls into question the long-standing assumption that lactic acidosis is due to tissue hypoxia in trauma and sepsis.[2]

In studies of regional lactate production in patients with acute respiratory distress syndrome, lactate is released in large quantities from the lung apparently in proportion to the degree of lung dysfunction.[3-6] In an animal model of early endotoxin sepsis, there were large lactate fluxes from tissues not normally considered to be hypoxic. Moreover, in these animals, the increase in blood lactate was associated with elevated oxygen uptake rather than the inverse as would be expected if it were an indicator of tissue oxygen debt.[7] The production of lactate in sepsis would seem to arise from sites of inflammation within the body, rather than areas of poor perfusion.[5,8] A similar pattern is observed if glucose uptake in sepsis is separately mapped.[9]

Inflammatory cells and especially leukocytes can be active producers of lactate. In the resting normoxic normoglycemic state, it has been suggested that approximately 80% of all glucose metabolized in these cells is converted to lactate. This nonoxidative metabolism accounts for nearly 50% of the resting cells' energy needs[10] and even more following septic stimulation.[11]

The hypothesis of this study is that the increased lactate production observed in sepsis can be explained by the augmented glycolytic metabolism of inflammatory cells, and this augmented metabolism can be mimicked by stimulation of resting whole blood leukocytes with lipopolysaccharide (LPS). In other words, it is proposed that lactate is a product of inflammation in sepsis rather than a sign of impaired tissue perfusion as commonly thought.

MATERIALS AND METHODS

Animals

Female inbred Wistar rats weighing 175 to 245 g were maintained in an environment with a 12-hour light/dark cycle and a temperature of 20°C. Until the day of the operation, they were allowed free access to pellet chow and water. Following laparotomy, they were fasted and allowed free access to water only.

Design of Experiment

Before both the laparatomy and killing, animals were weighed and blood was taken by tail snip for glucose and insulin determinations. Anesthesia was then induced by inhalation of ether. On the first day, this was followed by either a sham laparotomy or cecal ligation and puncture (CLP). Twenty-four hours later, the anesthetized rats were exsanguinated via cardiac puncture. Peritoneal leukocytes were harvested by lavage with 10 mL chilled Hanks balanced salt solution ([HBSS] GIBCO BRL, Life Technologies, Paisley, Scotland) with heparin 100 U/mL added.

CLP

Polymicrobial sepsis was induced using the CLP model described by Wichterman et al[12] and Baker et al.[13] In brief, under anesthesia, a 2-cm midline abdominal incision was made to expose the cecum. The cecum was isolated and ligated with 4-0 Ethilon (Ethicon, Somerville, NJ). It was then punctured twice with a 21-gauge needle and gently compressed to extrude the fecal contents through the puncture holes. Upon returning the bowel to the abdomen, the midline incision was closed in layers with 4-0 ethilon for the abdominal wall and staples for the skin. Sham-operated controls underwent the same surgical procedure (ie, laparotomy, manipulation, cecal isolation, and closure), but the cecum was neither ligated nor punctured.

Preparation of Peritoneal Leukocytes

The peritoneal lavage fluid was centrifuged for 5 minutes at 375 × *g* to prepare a cell pellet. Red blood cells were lysed by resuspension in 2 mL distilled water for 30 seconds, and lysis was terminated by addition of 2 mL 1.8% NaCl. The remaining leukocytes were then again centrifuged (10 minutes at 425 × *g*), washed, and eventually resuspended in 10 mL sterile HBSS, with a sample of the cell suspension kept for a differential cell count. Cell viability (≥95%) was confirmed with trypan blue, and the cells were counted with a hemocytometer. An aliquot of cell suspension was then centrifuged, and the resulting cell pellet was resuspended in a HEPES- and bicarbonate-buffered medium

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0026-0495/99/4806-0017$10.00/0

containing bovine serum albumin (1 mg/mL) to attain a concentration of 4×10^6 cells/mL for metabolic studies.

Preparation of Blood Leukocytes

Leukocytes from heparinized blood were prepared by dextran separation. Blood samples (5 to 10 mL) were mixed with an equal volume of 5% dextran (molecular weight, 250,000) in 0.9% sterile saline. This was left for 30 minutes at 37°C in a water bath to allow cell sedimentation. The supernatant was aspirated and centrifuged at 425 × *g* for 5 minutes, with the cell pellet resuspended in sterile HBSS and red blood cells lysed as already described. A differential cell count was performed, cell viability was confirmed with trypan blue, and the cells were counted with a hemocytometer. An aliquot of cells were then resuspended in the HEPES- and bicarbonate-buffered medium at a concentration of 4×10^6 cells/mL for metabolic studies.

Metabolic Studies

The methods used in the metabolic studies have been previously reported.[14-16] Briefly, the utilization of D-[5-^{3}H]glucose and oxidation of either D-[6-^{14}C]glucose or D-[1-^{14}C]glucose at 7.0 mmol/L D-glucose were measured simultaneously in samples of 1.6×10^5 cells incubated for 120 minutes at 37°C in a final volume of 80 μL in the presence or absence of LPS (*E. coli* 026:B6 with a 50% lethal dose of 12.5 mg/kg body weight in the standardized white mice assay; Difco Laboratories, Detroit, MI). The sealed samples were gassed with a mixture of O_2/CO_2 (19:1 vol/vol) for the first 5 minutes of incubation. The production of ^{14}C-labeled acidic metabolites and amino acids was also measured in these experiments. The output of unlabeled lactate was studied separately with aliquots of 2.4×10^5 cells incubated in a final volume of 120 μL of the same medium and under comparable conditions. After a brief centrifugation (3 minutes at 5,000 × *g*), a sample (100 μL) of the supernatant was incubated for 15 minutes at 85°C and further treated as described elsewhere[17] for lactate measurement.

Protein Estimation

Two samples of 100 μL counted cell suspension were centrifuged and resuspended in 200 μL protein-free medium. This was then centrifuged and resuspended in 250 μL distilled water before sonication, and the homogenate was assayed for protein content by the method of Lowry et al[18] using bovine serum albumin as the standard.

Ex Vivo Whole-Blood Studies

An unselected group of consecutive patients admitted to the Intensive Care Unit at Erasmus Hospital (Brussels, Belgium) were studied. Patients were eligible if they were already monitored invasively with an intra-arterial catheter and were clinically stable at the time of sampling, there was a concurrent sample for a full blood cell count available, and the patient or their next of kin provided consent. The study was approved by the Ethics Committee of Erasmus Hospital.

Eight milliliters of heparinized blood was taken via the arterial line and divided into two sterile 10-mL syringes with an equal volume of air. To each was added 200 μL sterile saline or 0.1 mg/mL LPS in sterile saline. The syringes were capped and incubated in a water bath at 37°C. Immediately after the addition of LPS or saline and at 20- to 30-minute intervals thereafter for up to 180 minutes, the lactate level was measured in a blood gas analyzer (865 blood gas analyzer; Chiron Diagnostics, Medfield, MA).[19] From these data, the rate of whole-blood lactate output was calculated from the slope of the linear regression line (Fig 1). Clinical information, data for APACHE II[20] and Sepsis-Related Organ Failure Assessment (SOFA)[21] scoring, and the day's full blood cell count were obtained concurrently.

LPS was chosen as the septic stimulus in these whole-blood studies because it is known to elicit identical alterations in cellular activation and glucose metabolism as whole bacteria[22,23] but in a more predictable dosage.[24]

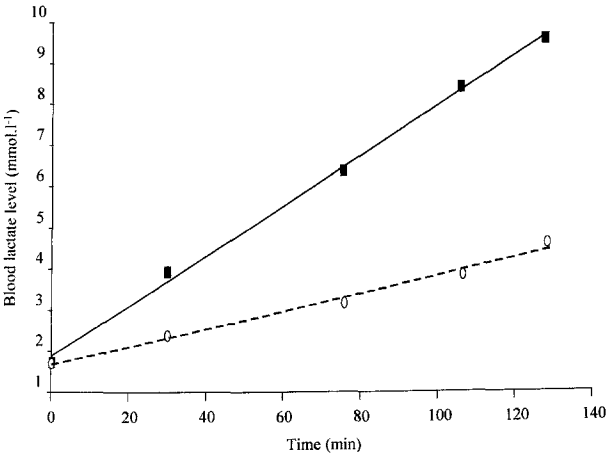

Fig 1. Typical lactate concentration curve used to calculate whole-blood lactate production. (○) Control samples; (■) LPS-stimulated samples. The respective rates of lactate production are 21.4 and 60.0 μmol · L^{-1} · min^{-1}.

Statistical Analysis

The data were analyzed using Excel 5.0 (Microsoft, Redmond, WA). with the Astute statistical program (University of Leeds, UK) in a Windows 3.11 network platform (Microsoft). Linear lines of regression were calculated by the least-mean-squares technique. Multiple regression analysis of calculated lactate production against categorical variables (namely red blood cell count, white blood cell count, and platelet count) was performed by a multiple logistic regression procedure. The respective regression coefficients were taken as the rate of lactate production for each cell type. Parametric data are expressed as the mean ± SEM together with the number of independent measurements (n) and are compared using a paired or unpaired *t* test where appropriate. Nonparametric results are expressed as the median ± interquartile range. A P value less than .05 was considered significant.

RESULTS

Animal Study

Both CLP and control rats lost weight over the 24-hour period preceding death, with a paired value for the decrease of 11 ± 1 g (n = 22) and 15 ± 1 g (n = 11) in CLP and control animals, respectively (Table 1). In the former group, two of 24 animals died within 24 hours after surgery. Plasma D-glucose and insulin concentrations were lower at death versus 24 hours prior, in both CLP and control rats (Table 1). However, as a rule, these differences failed to achieve statistical significance.

The protein content of cells collected from the peritoneal cavity was not significantly different between CLP rats and control animals. However, they differed by a higher number of cells ($P < .02$), higher percentage of polymorphonuclear cells ($P < .001$), and lower percentage of macrophages ($P < .05$) in CLP versus sham rats. The presence of bacteria was identified in the peritoneal material collected from CLP rats, but not from sham rats. In the blood, the total number of white blood cells was markedly ($P < .005$) decreased in CLP rats, but the percentage of either polymorphonuclear cells or macrophages appeared unaltered.

Table 1. Metabolic, Hormonal, and Cytometric Data in Sham and CLP Rats

Parameter	Sham	CLP
Body weight (g)		
Before surgery	215 ± 6 (11)	213 ± 6 (22)
At death	200 ± 5 (11)	202 ± 6 (22)
Paired change	15 ± 1 (11)	11 ± 1 (22)
Plasma glucose (mmol/L)		
Before surgery	7.66 ± 0.28 (11)	7.61 ± 0.14 (12)
At death	6.67 ± 0.36 (10)	7.12 ± 0.36 (12)
Paired change	1.15 ± 0.29 (10)	0.49 ± 0.35 (12)
Plasma insulin (μU/mL)		
Before surgery	30.2 ± 5.1 (11)	31.8 ± 5.5 (12)
At death	23.4 ± 4.0 (10)	26.0 ± 5.0 (6)
Paired change	6.0 ± 7.4 (10)	13.6 ± 7.8 (6)
Peritoneal cells		
No. ($\times 10^6$)	7.7 ± 2.5 (6)	23.4 ± 4.6* (7)
Polymorphonuclear cells (%)	37.0 ± 5.5 (6)	82.6 ± 3.1* (7)
Macrophages (%)	31.2 ± 5.6 (6)	16.6 ± 3.2* (7)
Protein content (ng/10^3 cells)	83.0 ± 9.9 (10)	99.6 ± 11.7 (13)
Blood cells		
No. ($\times 10^3$/μL)	4.23 ± 0.28 (4)	0.54 ± 0.22* (2)
Polymorphonuclear cells (%)	20.2 ± 4.1 (4)	50.6 ± 24.3 (3)
Macrophages (%)	5.2 ± 0.8 (4)	17.2 ± 8.4 (3)

*$P < .05$ *v* sham.

In studies of cellular metabolism, peritoneal leukocytes harvested from rats following CLP showed an increased ($P < .05$ or less) basal and glucose-stimulated lactate output, D-[5-^{3}H]glucose utilization, D-[1-^{14}C]glucose conversion to $^{14}CO_2$ and ^{14}C-labeled acidic metabolites, and D-[6-^{14}C]glucose conversion to radioactive acidic metabolites (Table 2). The fractional contribution of the pentose phosphate pathway to the generation of CO_2 and D-glyceraldehyde-3-phosphate was also increased,

Table 2. Metabolic Data in Cells Collected From the Peritoneal Cavity in Sham and CLP Rats

Parameter	Sham	CLP
D-[1-^{14}C]glucose oxidation*	3.40 ± 0.29 (24)	8.81 ± 0.59 (25)
D-[1-^{14}C]glucose conversion to acidic metabolites*	56.55 ± 4.91 (25)	74.45 ± 4.36 (25)
D-[1-^{14}C]glucose conversion to amino acids*	0.87 ± 0.07 (25)	0.94 ± 0.07 (25)
D-[5-^{3}H]glucose utilization*	118.3 ± 10.1 (25)	147.4 ± 7.2 (22)
D-[1-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (%)	2.89 ± 0.06 (24)	5.85 ± 0.19 (22)
D-[6-^{14}C]glucose oxidation*	0.26 ± 0.01 (25)	0.17 ± 0.01 (24)
D-[6-^{14}C]glucose conversion to acidic metabolites*	64.13 ± 4.74 (25)	79.53 ± 4.48 (25)
D-[6-^{14}C]glucose conversion to amino acids*	0.69 ± 0.04 (25)	0.80 ± 0.08 (25)
D-[5-^{3}H]glucose utilization*	120.4 ± 9.7 (24)	147.4 ± 6.9 (25)
D-[6-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (%)	0.23 ± 0.01 (24)	0.12 ± 0.01 (25)
Pentose shunt (% of D-glucose utilization)	0.90 ± 0.02 (24)	1.99 ± 0.07 (22)
Lactate output*		
No glucose	16.4 ± 1.3 (25)	45.8 ± 2.2 (25)
7.0 mmol/L D-glucose	61.9 ± 2.7 (25)	72.1 ± 3.1 (25)

*Results expressed as pmol D-glucose equivalent/10^3 cells/120 min.

as was the paired ratio between D-[1-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization. CLP failed to affect significantly the generation of ^{14}C-labeled amino acids from either D-[1-^{14}C]glucose or D-[6-^{14}C]glucose. It decreased the oxidation of D-[6-^{14}C]glucose, the paired ratio between such oxidation and D-[5-^{3}H]glucose utilization, and the glucose-induced increment in lactate output above basal ($P < .001$ in all cases).

In cells exposed to D-glucose (7.0 mmol/L), lactate output was comparable to the net generation of acidic metabolites from either D-[1-^{14}C]glucose or D-[6-^{14}C]glucose. This suggests that glucose suppressed the production of lactate from endogenous nutrients. Since glycogen could not be detected in the cells (<20 pmol glucose residue/10^3 cells), the basal output of lactate probably corresponded mainly to the catabolism of endogenous amino acids.

During incubation in the presence of 7.0 mmol/L D-glucose, LPS (1.0 μg/mL) failed to affect significantly the metabolic variables measured in peritoneal cells (Table 3). Likewise, LPS failed to affect significantly the production of lactate by circulating leukocytes isolated from either control or CLP rats and incubated in the presence of 7.0 mmol/L D-glucose. The results recorded in the presence of LPS were 100.3% ± 5.8% (n = 18) of the mean corresponding control value (ie, 79.1 ± 6.3 pmol/10^3 cells/120 min).

Human Study

Clinical data for 31 patients studied and their median blood cell count and lactate measurements are presented in Table 4. The linear correlation between whole-blood lactate production and white blood cell count was significant only following stimulation with LPS at a concentration of 5 μg/mL (Fig 2). Calculation by multiple linear regression analysis of the individual cell type contribution to whole-blood lactate production is shown in Table 5. The mean resting white blood cell lactate production was 0.20 fmol/cell/min, and this increased over sixfold to 1.22 fmol/cell/min following stimulation with LPS. The relative contribution of white blood cells to the lactate production of whole blood in the critically ill also increased following incubation in LPS from 13% to 47%. The other sources of lactate production in whole blood were erythrocytes and platelets, both of which show little change following incubation with LPS.

DISCUSSION

An elevated lactate level in a critically ill patient is a reliable predictor of outcome,[26,27] and its change over time is a useful guide to the patient's response to treatment.[28,29] The pathophysiology of lactate metabolism in the critically ill is poorly understood, but is widely thought to represent inadequate tissue perfusion and disordered cellular oxygenation.[30,31] In apparent conflict with this is a steady stream of research suggesting that lactic acidosis in sepsis is associated with a specific metabolic defect. Acute functional derangements of both the phosphofructokinase and pyruvate dehydrogenase enzyme complexes in muscle and liver have been implicated.[32,33]

The degree to which an elevated lactate level represents tissue hypoxia has been challenged recently.[2-5] Direct measurement of tissue oxygen tension in animal models of sepsis has failed to demonstrate a physiologically important change in

Table 3. Effect of LPS (1.0 μg/mL) on Metabolic Variables in Cells Collected From the Peritoneal Cavity in Sham and CLP Rats

	LPS (μg/mL)			
	Sham		CLP	
Parameter	Nil	1.0	Nil	1.0
D-[1-^{14}C]glucose oxidation*	1.87 ± 0.33 (8)	1.58 ± 0.32 (7)	4.39 ± 0.36 (15)	4.31 ± 0.36 (15)
D-[1-^{14}C]glucose conversion to acidic metabolites*	38.12 ± 7.88 (8)	43.33 ± 7.71 (8)	47.91 ± 4.01 (15)	58.61 ± 4.30 (15)
D-[1-^{14}C]glucose conversion to amino acids*	0.70 ± 0.19 (8)	0.93 ± 0.20 (8)	0.57 ± 0.06 (15)	0.85 ± 0.07 (15)
D-[5-^{3}H]glucose utilization*	95.2 ± 20.3 (7)	91.0 ± 18.0 (8)	107.9 ± 7.0 (15)	124.8 ± 9.3 (15)
D-[1-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (%)	2.16 ± 0.07 (8)	1.96 ± 0.06 (7)	4.01 ± 0.11 (15)	4.25 ± 0.35 (15)
D-[6-^{14}C]glucose oxidation*	0.23 ± 0.04 (8)	0.12 ± 0.04 (8)	0.11 ± 0.01 (15)	0.10 ± 0.01 (15)
D-[6-^{14}C]glucose conversion to acidic metabolites*	50.93 ± 9.45 (8)	49.80 ± 9.22 (8)	59.03 ± 4.09 (15)	62.11 ± 3.70 (15)
D-[6-^{14}C]glucose conversion to amino acids*	1.01 ± 0.19 (8)	0.90 ± 0.26 (8)	0.97 ± 0.09 (15)	0.80 ± 0.08 (15)
D-[5-^{3}H]glucose utilization*	102.0 ± 18.2 (7)	98.4 ± 19.4 (8)	113.2 ± 7.3 (15)	125.9 ± 8.4 (15)
D-[6-^{14}C]glucose oxidation/D-[5-^{3}H]glucose utilization (%)	0.24 ± 0.01 (8)	0.10 ± 0.02 (8)	0.10 ± 0.01 (15)	0.09 ± 0.01 (15)
Pentose shunt (% of D-glucose utilization)	0.65 ± 0.02 (7)	0.63 ± 0.02 (7)	1.34 ± 0.04 (15)	1.16 ± 0.06 (15)
Lactate output at 7.0 mmol/L D-glucose*	33.1 ± 3.2 (12)	33.7 ± 2.8 (16)	47.3 ± 2.5 (24)	45.9 ± 3.3 (24)

*Results expressed as pmol D-glucose equivalent/10^3 cells/120 min.

cellular oxygen availability.[34,35] The lactate to pyruvate ratio, a marker of the redox status of cells and, by inference, the adequacy of cellular oxygenation, is normal in an animal model of sepsis.[36] Furthermore, increasing oxygen delivery by volume resuscitation of septic animals with elevated lactate is not associated with a clear reduction in the lactate level.[37] On a population basis, lactate levels in critically ill and septic patients fail to show a significant correlation with oxygen delivery, oxygen consumption, or mixed venous oxygen saturation.[26,38]

In studies of regional metabolism, lactate is released in large quantity by sites of multiple organ failure,[5] burns,[8] and infection.[39] In patients with acute respiratory distress syndrome, Brown et al[6] have documented lactate flux from the lung of the order of 0.8 mmol · min^{-1} · m^{-2}. Not only is this so large as to be equivalent to the total body lactate metabolism in a healthy individual, but the actual amount seems related to the severity of lung injury.[4,6] This release of lactate seems coherent not with hypoxia but with cytokine release, suggesting a link with local inflammation.[5] If glucose[9] and lactate[7] metabolism are separately mapped in animal models of early sepsis, the sites of highest turnover are found in areas associated with inflammation and inflammatory cell populations such as the spleen, lung, and intestine. In the case of the intestine, this lactate flux is unrelated to blood flow and oxygen delivery, and seems to be as marked from the whole perfused intestine as from the mucosa alone.[40,41]

Table 4. Clinical Characteristics of the Patients Studied

Characteristic	Value
No. of subjects (female/male)	31 (5/26)
Medical/surgical (n)	17/14
Age, yr (mean ± SEM)	59.9 ± 3.1
APACHE II score (mean ± SEM)	16.2 ± 1.1
SOFA score (mean ± SEM)	7.7 ± 0.7
Baseline lactate (mmol · L^{-1})	2.09 (1.59-2.80)
Hemoglobin (g · dL^{-1})	9.3 (8.5-10.7)
Red blood cell count (×10^6 · mm^{-3})	3.14 (2.80-3.53)
White blood cell count (×10^3 · mm^{-3})	11.6 (7.1-16.1)
Platelet count (×10^3 · mm^{-3})	155 (72-245)
Resting whole-blood lactate production (μmol · L^{-1} · min^{-1})	15.5 (14.2-19.3)
Stimulated whole-blood lactate production (μmol · L^{-1} · min^{-1})	27.6 (22.4-37.7)

NOTE. Data are the median (interquartile range) unless otherwise indicated.

Given that the sites of glucose utilization and lactate production in sepsis correlate with inflammation and sites of inflammatory cell activity, the implication is that inflammatory cells themselves could be an important source of lactate in sepsis. Phagocytic cells such as polymorphonuclear cells and macrophages exhibit a high rate of glucose utilization, some of which occurs via the pentose phosphate cycle. This cycle is important for the generation of NADPH and pentose sugars from glucose.[42] In phagocytic cells, NADPH is the source of energy for the generation of oxygen free radicals and their bactericidal oxidative burst.[43]

The present data reveal three major changes in the metabolism of peritoneal cells in the CLP model of polymicrobial sepsis. First, the basal production of lactate, presumably attributable to the catabolism of endogenous amino acids, was much higher in CLP versus sham rats. This suggests an increased energy expenditure in the leukocytes of CLP animals. Second, and consistent with such a suggestion, CLP increased the overall utilization of exogenous D-glucose, coinciding with a higher production of ^{14}C-labeled acidic metabolites by cells exposed to D-[1-^{14}C]glucose or D-[6-^{14}C]glucose and a higher output of lactate from cells exposed to unlabeled D-glucose. Finally, CLP increased the fractional contribution of the pentose shunt to the generation of CO_2 and D-glyceraldehyde-3-phosphate. As a matter of fact, both the absolute value for D-[1-^{14}C]glucose oxidation and the paired ratio between such oxidation and D-[5-^{3}H]glucose utilization were increased in CLP rats, while the opposite situation prevailed in the case of D-[6-^{14}C]glucose oxidation.

In this set of experiments, leukocytes obtained from peritoneal lavage in both sham and CLP rats have a very high rate of glycolysis, of which less than 1% was oxidized to CO_2 in the Krebs cycle. Indeed, in phagocytic cells, glycolysis and the Krebs cycle would seem to be uncoupled, with the principal

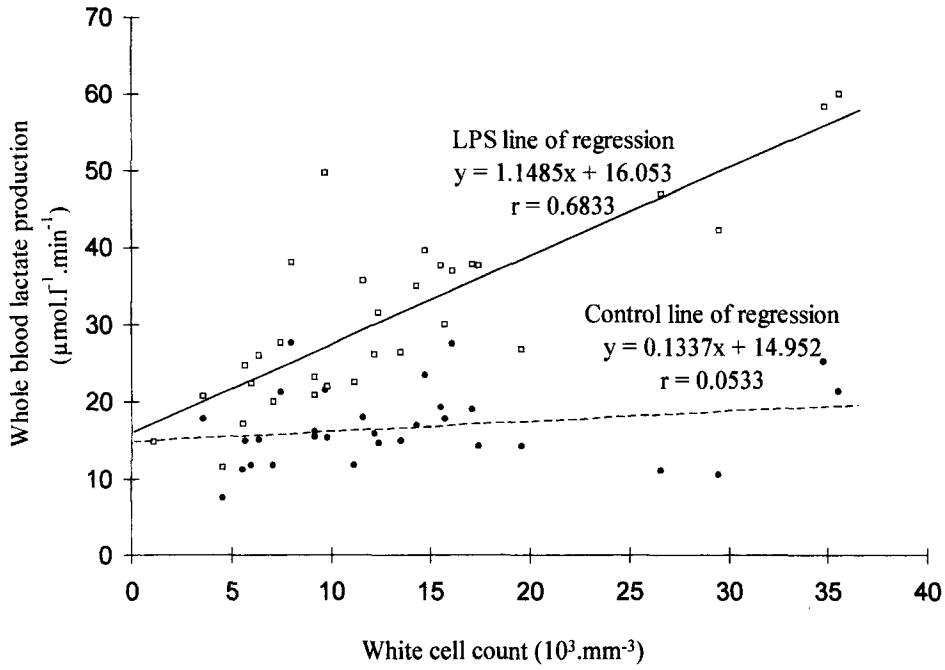

Fig 2. Correlation between whole-blood lactate production and white blood cell count. (●) Control values; (□) LPS-stimulated values.

sources of carbon residues for oxidation being glutamine and free fatty acids.[44,45]

Although such metabolic differences may reflect, at least in part, a change in the respective percentage of distinct cell types (eg, polymorphonuclear cells *v* macrophages) in material collected from the peritoneal cavity, CLP dramatically augmented the production of lactate by cells present in the peritoneal cavity. Thus, taking into account the number of such cells and the results recorded at a near-physiologic concentration of D-glucose, the total output of lactate increased dramatically in CLP rats (from 0.95 ± 0.31 to 3.37 ± 0.68 µmol/120 min, $P < .005$).

Lactate in these cells is the predominant end product of glycolysis. This is produced by leukocytes in CLP rats at approximately 1.20 fmol · cell^{-1} · min^{-1} or 12.0 mmol · kg-protein^{-1} · min^{-1}, and is similar to other published studies on lactate production in polymorphonuclear leukocytes.[46,47] These findings are coherent with our studies of ex vivo human blood. The resting human blood leukocytes from our unselected group of critically ill patients were relatively quiescent, with a lactate production rate of only 0.20 fmol · cell^{-1} · min^{-1}, but showed increased lactate production to 1.22 fmol · cell^{-1} · min^{-1} following incubation with LPS.

Lactate metabolism may be seen not as the involuntary product of inadequate oxygenation but rather as an intermediary metabolite of normal glycolysis. Of the pyruvate produced from glycolysis in healthy subjects, almost 50% is not metabolized immediately, but passes via the blood lactate pool as the Cori cycle of intermediary metabolism.[48] The reasons for this are presumably to share the metabolic load of oxidative work, with the clearest example being heavy exercise. Nevertheless, in a resting individual, lactate metabolism is between 10 and 15 µmol · kg^{-1} · min^{-1}, ie, 0.7 to 1.0 mmol/min in a 70-kg adult.[48,49] In studies on stable, recovering intensive-care patients, Wright et al[50] have documented a total lactate turnover rate little changed from this level, at about 0.8 mmol/min.

During sepsis, glucose turnover is dramatically increased from 10 to 20 to 35 to 45 µmol · kg^{-1} · min^{-1} in human studies.[51,52] Pyruvate oxidation is increased, but not as much as production, resulting in most of the extra turnover passing into the blood lactate pool at about 20 to 40 µmol · kg^{-1} · min^{-1}.[51,52] Again, in a 70-kg critically ill adult, this agrees well with a measured total body turnover of lactate of between 1.4 and 2.5 mmol/min.[1,53] If this excess lactate production were solely due to occult cellular hypoxia, given the known maximal fluxes of lactate from the intestine (300 µmol · kg^{-1} · min^{-1}) and resting

Table 5. Different Blood Cell Type Contribution to Whole-Blood Lactate Production (mean ± SEM)

Parameter	Coefficient (fmol · 10^{-3} cells · min^{-1})	Statistical Significance *P*	Mean Cell Volume (fL)	Proportion of Median Whole-Blood Lactate Production (%)
Resting lactate production				
Red blood cells	4.5 ± 1.3	.0023	89.1 ± 0.7	80
Leukocytes	195 ± 89	.0378	195.2 ± 0.4*	13
Platelets	8.5 ± 4.8	.0851	9.16 ± 0.29	7
LPS-stimulated lactate production				
Red blood cells	4.7 ± 2.1	.0354	89.1 ± 0.7	49
Leukocytes	1,223 ± 143	<.0001	195.2 ± 0.4*	47
Platelets	−0.5 ± 7.7	.9483	9.16 ± 0.29	<5

*Given the volumes of different cell types as in Schmid-Schönbein et al.[25]

muscle (10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) in hypotensive septic animal models,[54,55] then this would imply the presence of occult cellular hypoxia in a large tissue mass of between 3 and 100 kg in a 70-kg adult.

In contrast, on a weight-for-weight basis, leukocytes are the most potent source of lactate in the body, outpacing other cell types by orders of magnitude (Table 6). Traditionally, these cells have been overlooked in terms of their contribution to total lactate metabolism because they were believed to be too small in number and mass to play a significant role. However, supplementary to cells at the site of inflammation, polymorphonuclear cells have a blood phase ($\sim 7.5 \times 10^9$ cells/L) and a much larger storage pool in the bone marrow, estimated at about 4.7×10^9 cells/kg body weight.[59] Given the known average size and density of polymorphonuclear cells of 200 fL[25] and 1.07 kg/L,[58] respectively, their total mass in a 70-kg adult can be calculated. Thus, in addition to the cells already at the site of inflammation, the total polymorphonuclear cell mass is at least 90 g. Tissue macrophages are largely of unknown numbers, but Kupffer cells are estimated to form 2.5% of the cell mass in the liver, and the same may also be true of other macrophage-rich tissues such as the intestine and lung.[22] If these cells are also similarly active, then the source of the excess lactate production in sepsis could indeed be acute inflammatory cells (polymorphonuclear cells and macrophages). In essence, as little as 100 g active human leukocytes at the site of sepsis could produce a significant tissue lactate flux (0.67 mmol/min). This is similar to the amount arising from the lungs of patients with acute respiratory distress syndrome.[4]

Future studies of regional lactate metabolism in sepsis will need to account for the presence and number of inflammatory cells in tissues and vessels of the organs studied. The liver, for example, may contain only 2.5% Kupffer cells by weight, but these cells account for nearly 50% of total liver glycolytic metabolism in sepsis.[22] An even greater potential for error exists in studies of tissues with a low lactate fluxes such as red blood cells and muscle, where a 0.5% contamination of leukocytes by mass could double the apparent tissue lactate production.

Table 6. Reported Lactate Production of Various Tissues During Sepsis

Tissue	Lactate Production ($\mu mol \cdot min^{-1} \cdot kg^{-1}$ wet weight)	Reference
Leukocytes	6,690	Our data, Jemelin et al,[46] Elliot et al[47]*
Intestine	300	Vallet et al,[54] Curtis et al[55]
Resting muscle	10	Vray et al[56]
Red blood cells	30	Hotchkiss et al[57]

*Assuming a mean cell volume of 195 fL[25] and specific gravity of 1.07.[58]

Inflammatory cell lactate metabolism may also contribute to the increase in lactate during the early postoperative phase following liver transplantation[60] or prolonged hypothermic cardiopulmonary bypass,[61] which is usually unrelated to global parameters of tissue oxygenation. Other observers have already noted an association between this and both reperfusion injury and later graft failure,[60] further implicating leukocyte involvement.

In conclusion, we have documented a high rate of glucose utilization and lactate output in leukocytes both at the site of sepsis in an animal model and in human blood leukocytes after exposure to LPS. This rate of production is so high that it suggests the lactate output of different tissue beds in sepsis may be affected by their different cell populations and state of activation. This study supports the hypothesis that lactate may be more a product of inflammation than a marker of tissue hypoxia in sepsis.

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