Anaerobic Lactate Production Is Associated With Decreased Microcirculatory Blood Flow and Decreased Mitochondrial Respiration Following Cardiovascular Surgery With Cardiopulmonary Bypass

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OBJECTIVES: Quantify the relationship between perioperative anaerobic lactate production, microcirculatory blood flow, and mitochondrial respiration in patients after cardiovascular surgery with cardiopulmonary bypass.

DESIGN: Serial measurements of lactate-pyruvate ratio (LPR), microcirculatory blood flow, plasma tricarboxylic acid cycle cycle intermediates, and mitochondrial respiration were compared between patients with a normal peak lactate (≤ 2 mmol/L) and a high peak lactate (≥ 4 mmol/L) in the first 6 hours after surgery. Regression analysis was performed to quantify the relationship between clinically relevant hemodynamic variables, lactate, LPR, and microcirculatory blood flow.

SETTING: This was a single-center, prospective observational study conducted in an academic cardiovascular ICU.

PATIENTS: One hundred thirty-two patients undergoing elective cardiovascular surgery with cardiopulmonary bypass.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Patients with a high postoperative lactate were found to have a higher LPR compared with patients with a normal postoperative lactate (14.4 ± 2.5 vs. 11.7 ± 3.4; $p = 0.005$). Linear regression analysis found a significant, negative relationship between LPR and microcirculatory flow index ($r = -0.225$; $\beta = -0.037$; $p = 0.001$ and proportion of perfused vessels: $r = -0.17$; $\beta = -0.468$; $p = 0.009$). There was not a significant relationship between absolute plasma lactate and microcirculation variables. Last, mitochondrial complex I and complex II oxidative phosphorylation were reduced in patients with high postoperative lactate levels compared with patients with normal lactate (22.6 ± 6.2 vs. 14.5 ± 7.4 pmol O_2/s/10^6 cells; $p = 0.002$).

CONCLUSIONS: Increased anaerobic lactate production, estimated by LPR, has a negative relationship with microcirculatory blood flow after cardiovascular surgery. This relationship does not persist when measuring lactate alone. In addition, decreased mitochondrial respiration is associated with increased lactate after cardiovascular surgery. These findings suggest that high lactate levels after cardiovascular surgery, even in the setting of normal hemodynamics, are not simply a type B phenomenon as previously suggested.

KEYWORDS: cardiovascular surgery; lactic acidosis; microcirculation; mitochondrial respiration; resuscitation

Shock has been historically defined as the imbalance between oxygen supply and demand, but there is growing evidence that impairments in oxygen utilization contribute to organ dysfunction in shock states (1, 2).

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KEY POINTS

Question: What is the relationship between anaerobic lactate production and microcirculatory blood flow? Is there a difference in mitochondrial respiration between critically ill patients with high and normal lactate after cardiopulmonary bypass?

Findings: Lactate-pyruvate ratio has a weak to moderate negative relationship with microcirculatory flow index ($r = –0.225$; $\beta = –0.037$; $p = 0.001$ and proportion of perfused vessels: $r = –0.170$; $\beta = –0.468$; $p = 0.009$). Postoperative peak lactate greater than or equal to 4 mmol/L was associated with decreased mitochondrial complex I and complex II respiration (22.6 ± 6.2 vs. 14.5 ± 7.4 pmol O_2/s/10^6 cells; $p = 0.002$).

Meaning: Anaerobic lactate generation has an inverse relationship with microcirculatory blood flow after cardiovascular surgery. In patients with high postoperative lactate, microcirculatory blood flow and mitochondrial complex I and complex II respiration are decreased, indicating that even in the setting of normal hemodynamics, a high postoperative lactate may not be a type B phenomenon as previously suggested.

While interventions to augment macrocirculatory derangements can normalize hemodynamics, these interventions may not reverse abnormalities in microcirculatory blood flow or mitochondrial function. Current methods for assessing the balance between oxygen delivery (Do_2) and oxygen consumption (Vo_2) involve blood gas derived calculations and measuring biomarkers such as lactate. These methods have significant limitations, as they only provide an estimate of global oxygen extraction but are unable to detect perfusion-specific abnormalities in microcirculatory blood flow.

Clinical decisions are commonly influenced by changes in lactate immediately after surgery, but an incomplete understanding of postoperative lactate generation can lead to inappropriate resuscitative efforts. Previous research has suggested that lactic acidosis in patients with normal hemodynamics following cardiopulmonary bypass (CPB) is likely the result of a nonhypoxic cause (type B lactic acidosis) (3). Contrasting literature has suggested lactate levels greater than 2 mmol/L suggest occult tissue malperfusion, and when greater than 4 mmol/L, a strong predictor of overt shock and poor outcome (4–6). Decreases in mitochondrial respiration may also affect lactate production, as a reduction in adenosine triphosphate generation from oxidative phosphorylation may require increased reliance on other metabolic pathways, leading to increases in circulating lactate.

To better understand and interpret changes in lactate after cardiovascular surgery, additional research that concomitantly evaluates microcirculatory blood flow and oxygen utilization would be valuable (7, 8). The objective of this study was to quantify the relationship between perioperative lactate, microcirculatory blood flow, and mitochondrial respiration in patients after cardiovascular surgery with CPB.

MATERIALS AND METHODS

Study Design

This prospective, observational, cross-sectional substudy was approved by the University of Pennsylvania Institutional Review Board (No. 829765 on August 3, 2020), registered with ClinicalTrials.gov (NCT05330676), and performed in accordance with the ethical standards of the Helsinki Declaration of 1975. The full study protocol has been previously published, which includes a more detailed description of clinical, imaging, and laboratory collection time points (9).

Inclusion and Exclusion Criteria

Patients undergoing elective cardiovascular surgery requiring CPB were consented for study enrollment, pending availability of research personnel. Efforts were maintained to include a diverse patient sample that considered race, ethnicity, and gender. Patients were excluded if they were unable to tolerate sublingual microcirculation imaging, had an active hematologic malignancy, or a known mitochondrial disorder.

Intraoperative Care

All patients were monitored with an invasive arterial blood pressure catheter, five-lead electrocardiography, pulse oximetry, end-tidal capnography, pulmonary artery catheter, central venous pressure

catheter, and transesophageal echocardiography. Anesthesia was induced with IV fentanyl (up to 5 μg/kg), propofol (1–2 mg/kg), and vecuronium (0.1 mg/kg) to facilitate intubation. Anesthesia was maintained with inhaled isoflurane. All patients received invasive mechanical ventilation. Tidal volumes were set to 6–8 mL/kg of ideal body weight and positive end-expiratory pressure of 5–10 cm H_2O. Respiratory rate was adjusted maintain an end-tidal carbon dioxide measurement of 30–35 mm Hg. Before CPB, 300 international units (IU)/kg of heparin was administered to achieve an activated clotting time greater than 450 seconds. A bypass flow rate of 2.2–2.4 L/min/m^2 was maintained to achieve a Do_2 index greater than 260 mL/min/m^2. Mean arterial pressure (MAP) was kept greater than 60 mm Hg using IV phenylephrine as needed. After weaning from bypass, MAP was maintained greater than 60 mm Hg and cardiac index greater than 2.2 L/min/m^2 using fluid boluses, phenylephrine, and epinephrine infusions as needed. Postoperative cardiac function and filling status was optimized by transesophageal echocardiography at the end of the procedure. Systemic anticoagulation was reversed with protamine, using 1 mg for each 100 IU of heparin to restore activated clotting time to baseline values.

Sublingual Microcirculation Imaging

Microcirculation imaging was obtained from each patient using incident darkfield video microscopy (CytoCam; Braedius Medical BV, Huizen, The Netherlands) (10). A minimum of three videos of 5 seconds (100 frames) duration were recorded at each time point, with attention to quality factors in accordance with consensus standards (11). Each clip was de-identified and coded after enrollment was complete.

Images were only analyzed if the six-factor Massey quality score was less than 10 (12). Video sequences were manually analyzed by two blinded investigators (J.C.G., F.M.T.) using Automated Vascular Analysis software v.3.2 (Microvision Medical BV, Amsterdam, The Netherlands). Only microvessels less than or equal to 20 μm in diameter were included in the analysis. Microcirculatory flow index (MFI), microcirculatory heterogeneity index (MHI), total vessel density (TVD), proportion of perfused vessels (PPVs), and perfused vessel density (PVD) were quantified according to the current best practice guidelines for reporting microcirculatory variables (11).

Liquid Chromatography and Mass Spectroscopy

A 50-μL aliquot of plasma was spiked with 10 μL of isotopically labeled organic acid internal standards and derivatized with O-benzylhydroxylamine. This mixture was extracted with 500 μL of ethyl acetate, vortexed, and centrifuged at 18,100 × *g* for 5 minutes at 4°C. Fifty microliters of dried organic extract was reconstituted in high purity solvents before liquid chromatography and mass spectroscopy (LC/MS).

Derivatized organic acids were quantified on an Agilent 1290 Infinity UHPLC/6495B triple quadrupole mass spectrometer. A 12-minute linear gradient from 95% A (0.1% formic acid in water) to 95% B (0.1% formic acid in acetonitrile) on a Waters Acquity BEH C_{18} 2 × 100 mm, 1.7 μm column was used to separate organic acid derivatives. Multiple reaction monitoring was used to quantify a fragment ion of the parent ion of each derivatized organic acid with standard calibration curves.

Blood Samples and Peripheral Blood Mononuclear Cells Isolation

Blood gas samples were drawn into a commercial, preheparinized 1 mL syringe then immediately analyzed (ABL90 FLEX; Radiometer America, Brea, CA) at the time of microcirculation measurement. Peripheral blood mononuclear cells (PBMCs) were used as a surrogate to assess global mitochondrial function (13). Fifteen milliliters of whole blood were collected into K_2EDTA tubes before surgery, after ICU admission, and on postoperative day 1. PBMCs were isolated for respirometry using differential centrifugation with Leucosep tubes (Greiner Bio-One, Monroe, NC). Plasma specimens were stored at –80°C for future analysis.

Mitochondrial Respiration

A sample of PBMCs were obtained from the plasma buffy coat within 1-hour of blood draw and used to measure mitochondrial respiration as previously described (9, 14). Briefly, between 4 and 5×10^6 PBMC cells were suspended in a MiR05 buffer

followed by exposure to a substrate-uncoupler-inhibitor titration (SUIT) protocol using an Oroboros O2k FluoRespirometer (Oroboros Instruments, Innsbruck, Austria). Routine respiration, complex I (CI) and complex II (CII) linked oxydative phosphorylation, nonphosphorylating respiration (electron transfer system) activity, complex III, and complex IV (CIV)-linked respiration were recorded. All data were normalized to cell count. A representative mitochondrial respiration tracing is shown in **eFigure 1** (http://links.lww.com/CCM/H532).

Statistical Analysis

Sample Size. Sample size was calculated with G*Power, Version 3.1.9.4. For regression analysis, we estimated that we would need at least 118 subjects to show an effect size of 0.15 with an $\alpha = 0.05$, $\beta = 0.8$, using 10 independent predictors in our final model. For the mitochondrial respiration cohort, we estimated a 20% difference in mitochondrial respiration between postoperative controls and subjects with circulatory shock. We calculated that we would need to compare at least ten patients with and without shock to achieve adequate power to identify a statistical difference in mitochondrial respiration between groups (13, 15).

General Analysis. Patients were grouped by peak postoperative lactate as either high (≥ 4 mmol/L) or normal (≤ 2 mmol/L) based on previous literature that has identified lactate levels greater than or equal to 4 mmol/L being associated with poor outcomes after cardiovascular surgery (16, 17). Data normality was assessed with D'Agostino and Pearson testing. Continuous variables characterizing demographic data, microcirculation data, mitochondrial respiration measurements, and outcomes data were reported as means with SDswhen normally distributed or medians with interquartile ranges when not normally distributed. Categorical variables are represented as frequencies and proportions. The unpaired *t* test or Mann-Whitney *U* test was used to compare continuous variables. Repeated measure analysis of variance was used to compare changes in variables over time. To adjust for multiple comparisons, a post hoc pairwise Tukey-Kramer *t* test was performed when appropriate.

Regression Analysis. Our primary analysis examined the relationship between postoperative lactate, lactate-pyruvate ratio (LPR), and clinically relevant variables (including microcirculatory blood flow) after cardiovascular surgery. Simple regression analysis for plasma lactate and LPR was first performed using selected demographics (age, gender, comorbidities), intraoperative data (CPB time, aortic cross-clamp time, crystalloid administration, blood product administration), postoperative hemodynamic data (MAP, cardiac index, central venous pressure), blood gas-derived Vo_2 index and Do_2 index, catecholamine dose (epinephrine, norepinephrine), laboratory data (creatinine clearance, hemoglobin, WBC count), and sublingual microcirculation imaging measurements (MFI, MHI, PPV, TVD, and PVD).

To create our multivariable regression models, we began with a model that included all variables that had coefficients significant at the 0.20 level using simple regression. We then performed a stepwise model adjustment by removing variables with the highest *p* value until all variable coefficients were significant at the 0.10 level.

RESULTS

Patient Characteristics

From September 1, 2020, to July 5, 2022, 132 patients were enrolled in the MicroRESUS study. Twelve subjects were excluded after enrollment (eight patients had inadequate microcirculation imaging, three patients had their surgery rescheduled, and one patient declined to participate in the study after surgery). Demographic and baseline characteristics of subjects are listed in **Table 1**. Clinical data at ICU admission are listed in **Table 2**. A complete patient analysis flow diagram can be found in **eFigure 2** (http://links.lww.com/CCM/H532).

Correlation and Regression Analysis

Correlation, simple regression coefficients, and the adjusted multivariable model for postoperative lactate are shown in **Table 3**.

Plasma lactate was strongly correlated with plasma organic acids, including pyruvate ($r = 0.953$; $\beta = 0.061$; $p < 0.001$), succinate ($r = 0.482$; $\beta = 0.491$; $p < 0.001$), citrate ($r = 0.682$; $\beta = 0.38.13$; $p < 0.001$), and malate ($r = 0.795$, $\beta = 1.418$; $p < 0.001$). Lactate had a weak to moderate linear relationship with glucose, epinephrine dose, CBP time, aortic cross-clamp time, and creatinine clearance. Plasma lactate also had a moderate relationship with postoperative WBC count ($r = 0.289$;

TABLE 1.
Subject Demographics and Clinical Data

Patient Characteristics	Total ($n = 120$)	Peak Lactate ≤ 2 mmol/L ($n = 31$)	Peak Lactate ≥ 4 mmol/L ($n = 63$)	p
Age, yr	64 ± 11	65 ± 15	66 ± 9	0.49
Sex, male, %	83 (69)	19 (61)	47 (75)	0.43
Caucasian, *n* (%)	108 (90)	29 (93)	56 (89)	0.82
European System for Cardiac Operative Risk Evaluation II	2.66 ± 3.5	2.4 ± 2.6	2.9 ± 3.8	0.50
Operation, *n* (%)				
CABG	43 (35.8)	13 (42)	21 (33)	0.54
CABG + valve replacement/repair	16 (13.3)	4 (13)	9 (14)	0.99
Valve surgery only	55 (45.8)	13 (42)	29 (46)	0.74
Other	6 (5.0)	1 (3)	4 (6)	0.59
Comorbidities, *n* (%)				
Hypertension	89 (74)	23 (74)	46 (73)	0.99
Diabetes	35 (29)	11 (35)	13 (21)	0.40
Heart failure with reduced ejection fraction (left ventricular ejection fraction < 30%)	12 (10)	1 (3)	10 (16)	0.11
Chronic kidney disease	20 (20)	7 (23)	11 (17)	0.62
Operative data				
Cardiopulmonary bypass time, min	93 (73–126)	81 (64–113)	111 (84–143)	0.01
Cross-clamp time, min	71 (54–94)	64 (45–88)	79 (57–105)	0.05
Cell saver blood, mL	675 (450–675)	450 (113–675)	675 (450–900)	0.003
RBC transfusion, mL	0 (0–225)	0 (0–0)	0 (0–300)	0.46
Fresh frozen plasma transfusion, mL	0 (0–75)	0 (0–0)	0 (0–250)	0.27
Platelet transfusion, mL	0 (0–272)	0 (0–0)	0 (0–326)	0.04
IV crystalloid fluid, mL	1000 (600–1500)	1000 (700–1450)	1000 (500–1500)	0.99
Urine output, mL	1006 ± 891	869 ± 591	1023 ± 717	0.31

CABG = coronary artery bypass graft.
Values are expressed as mean ± SD or median (interquartile range), unless otherwise indicated.

β = 0.516; $p < 0.001$). A multivariable model including pyruvate, malate, glucose, epinephrine dose, capillary refill time, CBP time, and aortic cross clamp time predicted 93.8% of the variability in plasma lactate.

The correlation and simple regression analysis of LPR with clinical variables revealed similar relationships as found with lactate. However, unlike with absolute lactate level, microcirculatory flow variables (MFI, MHI, and PPV) had a significant relationship with LPR (**Table 4**). The multivariable model to predict LPR including malate, glucose, catecholamine dose, microcirculatory blood flow (MFI and PPV), and creatinine clearance predicted 26.1% of the variation in plasma LPR. Correlation matrixes with heatmaps for plasma lactate and LPR are included in **eFigures 3** and **4** (http://links.lww.com/CCM/H532).

Circulating Tricarboxylic Acid Cycle Intermediates

First, we performed a quality control check to ensure that lactate concentrations remained stable in plasma samples that were frozen at –80°C. We found a strong relationship between point-of-care lactate and lactate measured by LC/MS ($n = 476$; $R^2 = 0.61$; $p \leq 0.0001$) (**eFig. 5**, http://links.lww.com/CCM/H532). Changes in plasma LPR, citrate, and malate during the early postoperative period between normal and high lactate

TABLE 2.
Hemodynamic, Perfusion, and Laboratory Data at ICU Admission

Patient Characteristics	Preoperative Reference ($n = 120$)	Peak Lactate ≤ 2 mmol/L ($n = 31$)	Peak Lactate ≥ 4 mmol/L ($n = 63$)	p
Hemodynamic data				
Mean arterial pressure, mm Hg	95 ± 13	77 ± 14	73 ± 12	0.098
Central venous pressure, mm Hg	15 ± 6	10 ± 4	10 ± 4	0.941
Cardiac index, L/min/m^2	2.5 ± 0.8	2.6 ± 0.6	2.7 ± 0.7	0.485
Perfusion and laboratory data				
SvO_2, %	–	70 ± 9	72 ± 9	0.172
$Pvaco_2$, mm Hg	–	6 ± 3	5 ± 2	0.381
Capillary refill time, s	2.6 ± 0.7	5.0 ± 1.3	5.1 ± 1.3	0.690
Oxygen consumption index, mL/min/m^2	–	106.9 ± 29.4	108.7 ± 36.4	0.830
Oxygen delivery index, mL/min/m^2	–	388.4 ± 89.0	429.1 ± 126.8	0.171
Lactate-pyruvate ratio	11.5 ± 2.4	11.7 ± 3.4	14.4 ± 2.5	0.005
Microcirculatory flow index (AU)	2.8 ± 0.2	2.5 ± 0.4	2.3 ± 0.5	0.05
Microcirculatory heterogeneity index (AU)	0.2 ± 0.2	0.48 ± 0.39	0.65 ± 0.56	0.13
Proportion of perfused vessels (%)	94.5 ± 5.3	88.4 ± 9.2	85.8 ± 7.9	0.16
Perfused vessel density (mm/mm^2)	23.4 ± 5.5	23.0 ± 5.2	21.7 ± 4.4	0.20
Glucose, mg/dL	104 ± 28	143 ± 28	161 ± 29	0.007
Hemoglobin, g/dL	13.5 ± 2.2	11.3 ± 1.7	11.8 ± 2.2	0.260
WBC count, 10^9/L	6.8 ± 2.0	15.4 ± 5.4	19.7 ± 5.4	< 0.001
Patients with lactate-pyruvate ratio > 10 (%)	77 (64)	20 (64)	51 (81)	0.08
Vasoactive infusions				
Epinephrine, n, μg/kg/min	–	13, 0.03 (0.02–0.06)	60, 0.04 (0.02–0.05)	< 0.001
Norepinephrine, n, μg/kg/min	–	12, 0.03 (0.03–0.08)	47, 0.03 (0.02–0.05)	0.266

AUs = arbitrary units, $Pvaco_2$ = central venous-to-arterial carbon dioxide tension, SvO_2 = central venous oxygen saturation.
Patient baseline values listed as preoperative reference.
Values are expressed as mean ± SD or median (interquartile range), unless otherwise indicated. Dashes indicate no measured value.

groups and depicted in **Figure 1**. Changes in glucose, succinate, and α-ketoglutarate are included in **eFigure 6** (http://links.lww.com/CCM/H532).

Plasma pyruvate (0.34 ± 0.19 vs. 0.13 ± 0.06 mmol/L; $p < 0.0001$), citrate (207.05 ± 232.23 vs. 104.46 ± 62.44 μM; $p = 0.008$), and malate (8.53 ± 6.99 vs. 3.47 ± 2.40 μM; $p = 0.0001$) were higher in the high lactate group at ICU admission. Fumarate, succinate, and a-ketoglutarate were similar between groups. **eFigure 7** (http://links.lww.com/CCM/H532) depicts the tricarboxylic acid cycle (TCA) cycle and relative differences in intermediate organic acids at baseline and upon ICU admission between patients with a normal and high lactate.

Mitochondrial Respiration

Mitochondrial respiration was measured using PBMCs from a subgroup of 40 consecutive patients. Six subjects were excluded due to technical error (no measurable routine mitochondrial respiration). Five subjects were excluded from the final analysis because the peak lactate levels were between 2 and 4 mmol/L. In total, 29 subjects ($n = 11$ peak lactate ≤ 2, $n = 18$ with peak lactate ≥ 4) were included in the final analysis. Preoperative and postoperative mitochondrial respiration values are provided in **eTable 1** (http://links.lww.com/CCM/H532). Subjects with high postoperative lactate had lower CI and CII-linked

TABLE 3.
Relationship of Variables With Plasma Lactate During the First 4 Hours After Surgery

	Correlation	Simple Regression		Multivariable	
Variable	Pearson *R*	Coefficient	*p*	Coefficient	*p*
Intercept				−1.722	0.001
Pyruvate (mmol/L)	0.953	0.061	< 0.001	12.470	< 0.001
Succinate (μmol/L)	0.482	0.491	< 0.001		
Citrate (μmol/L)	0.682	38.130	< 0.001		
Malate (μmol/L)	0.795	1.418	< 0.001	0.084	< 0.001
Glucose (mg/dL)	0.197	1.812	0.002	0.006	0.009
Epinephrine (μg/kg/min)	−0.089	0.003	< 0.001	5.269	0.110
Norepinephrine (μg/kg/min)	0.407	0.000	0.711		
Central venous pressure (mm Hg)	−0.024	0.125	0.169		
Mean arterial pressure (mm Hg)	0.090	−0.254	0.237		
Cardiac index (L/min/m^2)	0.112	0.022	0.095		
Oxygen extraction ratio (%)	−0.021	−0.001	0.762		
Microcirculatory flow index	−0.073	−0.011	0.266		
Microcirculatory heterogeneity index	0.023	0.003	0.724		
Proportion of perfused vessels (%)	−0.044	−0.108	0.506		
Capillary refill time (s)	0.155	0.060	0.016	0.092	0.101
WBC count (10^9 cells/L)	0.289	0.516	< 0.001		
Cross-clamp time (min)	0.230	3.032	0.018	0.011	0.030
Cardiopulmonary bypass time (min)	0.246	4.019	0.010	−0.009	0.022
Creatinine clearance (mL/min)	−0.159	−2.104	0.015		

The multivariable model explained 93.8% of the variation in plasma lactate.

respiration compared with patients with normal lactate (22.6 ± 6.2 vs. 14.5 ± 7.4 pmol O_2/s/10^6 cells; p = 0.002). Compared with baseline, postoperative CIV-linked respiration was similar in the normal lactate group (31.7 ± 6.7 vs. 38.1 ± 13.4 pmol O_2/s/10^6 cells; p = 0.02) and lower in the high lactate group (27.6 ± 11.3 vs. 38.1 ± 13.4 pmol O_2/s/10^6 cells; $p < 0.0001$). Mean mitochondrial respiration values during sequential interrogation of different substrate and coupling states is shown in **Figure 2**.

DISCUSSION

Our study examined physiologic contributors to postoperative lactic acidosis in the early postoperative phase after cardiac surgery with cardiopulmonary bypass. This is the first study to integrate both direct microcirculatory imaging and functional mitochondrial testing in a large patient cohort to investigate both microcirculatory flow and metabolic causes of postoperative lactate production. Our findings indicate that microcirculatory blood flow has a significant, negative relationship with anaerobic lactate production when quantified using LPR. Second, we observed a strong, positive relationship between postoperative lactate and circulating TCA cycle intermediates, indicating mitochondrial CI and CII injury may be a significant contributor to increased postoperative lactate. Finally, we were able to establish the presence of mitochondrial CI and CII injury in patients with high postoperative lactate by measuring PBMC mitochondrial respiration.

First, microcirculatory alterations (specifically decreased MFI, PPV, and increased flow heterogeneity) have a significant, weak to moderate relationship with LPR but not absolute plasma lactate

TABLE 4.
Relationship of Variables With Plasma Lactate-Pyruvate Ratio During the First 4 Hours After Surgery

	Correlation	Simple Regression		Multivariable	
Variable	Pearson *R*	Coefficient	*p*	Coefficient	*p*
Intercept				8.385	< 0.001
Succinate (μmol/L)	0.264	0.308	< 0.001		
Citrate (μmol/L)	0.213	13.600	0.001		
Malate (μmol/L)	0.290	0.592	< 0.001	0.086	0.005
Glucose (mg/dL)	0.192	2.031	0.003	0.014	0.008
Epinephrine (μg/kg/min)	0.012	0.0030	< 0.001	17.860	0.014
Norepinephrine (μg/kg/min)	0.360	0.002	0.041	9.635	0.029
Central venous pressure (mm Hg)	0.132	0.262	0.012	0.068	0.069
Mean arterial pressure (mm Hg)	0.165	−0.458	0.062		
Cardiac index (L/min/m^2)	0.021	0.005	0.757		
Oxygen extraction ratio (%)	0.110	0.003	0.107		
Microcirculatory flow index	−0.225	−0.037	0.001	−1.664	0.001
Microcirculatory heterogeneity index	0.133	0.021	0.043		
Proportion of perfused vessels (%)	−0.170	−0.468	0.009	0.060	0.045
Capillary refill time (s)	0.164	0.072	0.011		
WBC count (10^9 cells/L)	0.088	0.184	0.346		
Cross-clamp time (min)	0.055	0.705	0.583		
Cardiopulmonary bypass time (min)	0.038	0.602	0.700		
Creatinine clearance (mL/min)	−0.188	−2.875	0.004	−0.007	0.086

The multivariable model explained 26.1% of the variation in plasma lactate-pyruvate ratio.

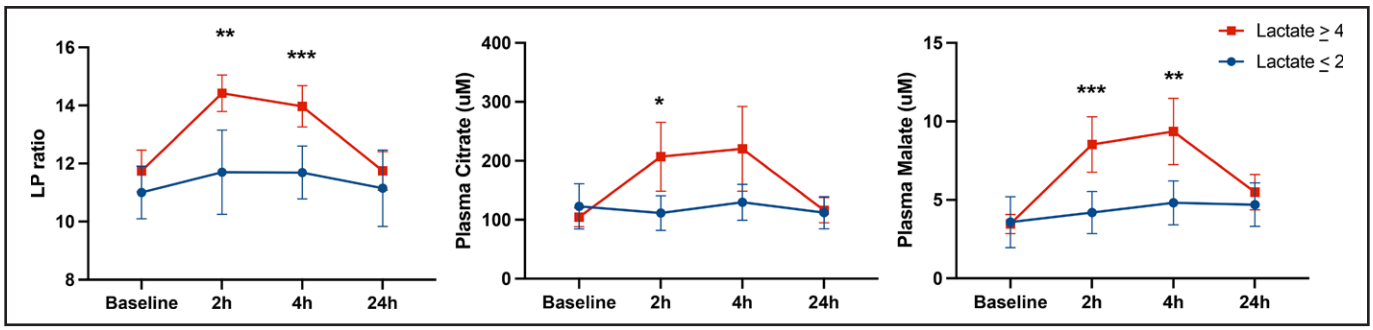

Figure 1. Change in plasma organic acids lactate-pyruvate (LP) ratio, citrate, and malate. *Red line* is representative of measurements in the high postoperative lactate group; *blue* represents values from the normal lactate group. Timepoints include preoperative baseline, 2 hr, 4 hr, and 24 hr after surgery (mean [95% CI]). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

concentration. By using LPR as a more sensitive marker for anaerobic metabolism, we were able to quantify the relationship between abnormal microcirculatory blood flow and type A lactic acidosis that is difficult to distinguish when measuring plasma lactate, as commonly performed in the clinical setting (18–20). Over 50% of our patients developed a severe lactic acidosis while having normal hemodynamics, which is similar to cardiovascular surgery patient cohorts in the literature (3, 21). The LPR in our high lactate group was 23% higher than in patients with a normal lactate, despite having

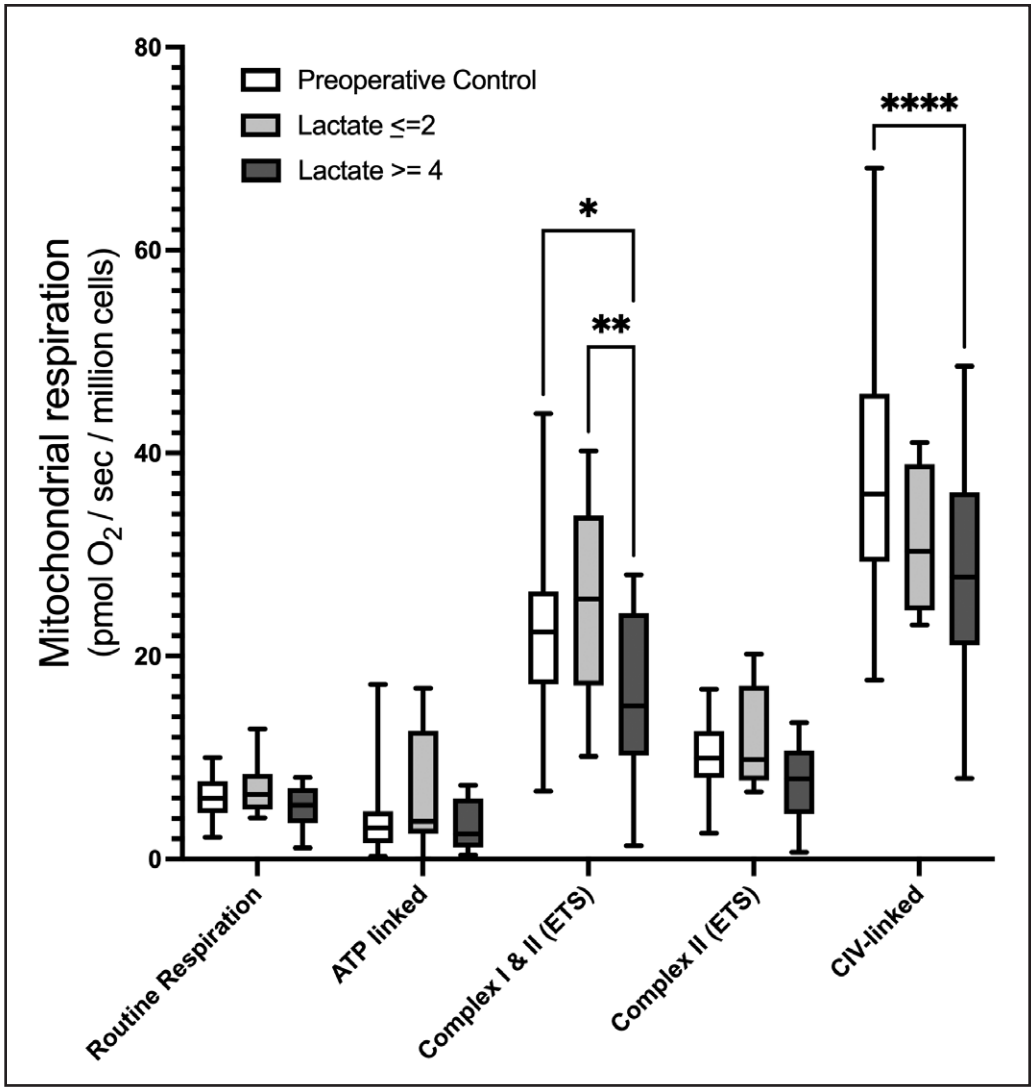

Figure 2. Mitochondrial respiration measured using high-resolution respirometer. Data are presented as oxygen flux (mean [95% CI]) which is directly proportional to oxygen consumption rate. Differences in mitochondrial respiration between baseline (preoperative control), normal, and high lactate patients at ICU admission. Mitochondrial respiration at complex I and II, and complex IV (CIV) were lower in patients with high lactate compared with preoperative baseline and with normal postoperative lactate. *< 0.05, **< 0.01, **** < 0.0001. ATP = adenosine triphosphate, ETS = electron transfer system.

similar global Vo_2, Do_2, and macrocirculatory indices. These results imply that regional disturbances in microcirculatory blood flow may cause occult tissue hypoxia not recognized by global measures of Do_2 and Vo_2. The nonsignificant relationship of the composite microcirculatory flow measure PVD with LPR may be due to a disproportionate change in PPV over capillary density. These findings are consistent with previous reports of acute decreases in the percentage of perfused capillaries affecting clinical outcomes after cardiac surgery, cardiogenic, and septic shock (22–24).

A reduction in the proportion of perfused capillaries was a distinguishing change in microcirculatory blood flow in patients with increased LPR. Postoperative leukocyte and platelet activation leading to capillary thrombosis, vascular endothelial activation, or glycocalyx degradation are all contributors to a reduction in capillary perfusion during shock (25, 26). The calculated regression coefficient for PPV in this study estimates that for each 1% decrease in nonperfused capillaries there is a 2-unit increase in LPR. This may account for why small decreases in microcirculatory flow better predict worse clinical outcomes in patient populations with hemorrhagic and septic shock, independent of lactate levels (27, 28).

The findings of our lactate regression analysis were expected. We did not find a relationship between absolute lactate and measured Vo_2, Do_2, or microcirculatory flow variables. Plasma glucose and epinephrine dose were weakly related to lactate. The lack of a relationship between microcirculatory flow and plasma lactate likely reflects the heterogeneous etiology of lactate production in this patient population, which includes changes in postoperative metabolism and iatrogenic adrenergic stimulation. Changes in skeletal muscle metabolism during CBP have previously been described using tissue microdialysis, which may account for the moderate, positive relationship with aortic cross clamp and bypass time (29). We did not identify any differences in postoperative liver injury that

might account for decreased lactate clearance. Our local practice is to use epinephrine as a primary inotrope after cardiovascular surgery, which is known to cause an increase lactate levels by β2 adrenergic stimulation (21). We did not analyze enzymatic processes such as lactate dehydrogenase activity, pyruvate dehydrogenase activity, and other metabolic pathways that may have contributed to postoperative lactate levels.

Our multivariable regression model for postoperative lactate was able to explain almost 94% of the variation in plasma lactate, but this was largely driven by the strong relationship between plasma lactate and circulating TCA intermediates. The strong, positive correlation with circulating succinate, citrate, and malate (Table 4) suggests either an impairment in mitochondrial respiration or an enzymatic deficiency within the TCA cycle. Increases in circulating TCA cycle intermediates over time (Fig. 1; and eFig. 7, http://links.lww.com/CCM/H532) suggest a metabolic bottleneck may occur, which leads to the removal of excess TCA cycle substrates that were unable to be used for oxidative phosphorylation (cataplerosis).

Finally, decreased mitochondrial respiration in patients with high lactate is an important exploratory finding. Abnormalities in mitochondrial function have been identified as a key contributor to energy balance, calcium balance, reactive oxygen species signaling, and regulation of cell death after cardiac surgery (30). Mitochondrial respiration abnormalities have also been linked to organ failure and death in patients with septic and hemorrhagic shock (15, 31). Using our SUIT protocol, we were able to identify a reduction in CI and CII-linked respiration in PBMCs, despite a normal oxygen availability (Pao_2). These findings are consistent with previous literature, which found complex I is particularly sensitive to ischemic injury and can occur after just 20 minutes of hypoxia (32). Mitochoncrial complex I injury may also reduce the oxidation of malate to oxaloacetate, decreasing the availabiilty of an important substrate for gluconeogenesis during periods of metabolic stress (33). Decreased PBMC mitochondrial health may also lead to cytokine release, immunosuppression, and cell damage that can result in significant organ injury (34).

We acknowledge our study has some limitations. First, this was a single-center study, which decreases the ability to generalize these results. Second, we only used one anatomic site for microcirculation imaging. The sublingual space is the most common site of microcirculation imaging with incident dark field microscopy and represents a well-established approach with consensus standards; however, we acknowledge that regional perfusion differences may vary outside of the sublingual capillary bed (11). Finally, our use of PBMCs for mitochondrial function should also be interpreted with caution, as they may not reflect mitochondrial activity in other organ systems.

CONCLUSIONS

Increased anaerobic lactate production, estimated by LPR, has a negative relationship with microcirculatory blood flow after cardiovascular surgery. This relationship does not persist when measuring lactate alone. In addition, decreased mitochondrial respiration is associated with increased lactate after cardiovascular surgery. These findings suggest that high lactate levels after cardiovascular surgery, even in the setting of normal hemodynamics, are not simply a type B phenomenon as previously suggested.

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All original data and materials are kept in a locally managed Research Electronic Data Capture database at the University of Pennsylvania. De-identified data will be made available online once accepted for publication. The limited dataset will be uploaded to the open access Zenodo database found here: 10.5281/zenodo.10075753.

This study was approved by the University of Pennsylvania's Institutional Review Board (No. 829765 on August 3, 2020) and informed consent was obtained before enrollment. All consent forms were copied in triplicate, one given to the subject, the second placed in the official medical record, the third kept in a secured location within Dr. Greenwood's office. All procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975.

As part of the informed consent document, all subjects have signed the study's informed consent form and agreed to allow their data to be published in this article.

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