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Determination of plasma lactic acid concentration and specific activity using high-performance liquid chromatography

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ABSTRACT

Assessment of lactate metabolism is of particular interest during exercise and in disease states such as diabetes, shock, and absorptive abnormalities of short-chain fatty acids by the colon. We describe an analytical method that introduces radio-active tracers and high-performance liquid chromatography (HPLC) to simultaneously analyze concentrations and specific activities (SAs) of plasma lactate. The HPLC conditions included separation on a reversed-phase column (octadecylsilane) and an isocratic buffer (30% acetonitrile in water). [^{3}H]Acetate served as an internal standard. Lactate and acetate were extracted from plasma samples with diethyl ether following a pH adjustment to less than 1.0 and back-extracted into a hydrophilic phase with sodium carbonate (2 m*M*, pH > 10.0). Lactate is detected in the ultraviolet range (242 and 320 nm) by derivatization with α-bromoacetophenone. Control plasma samples were studied after an overnight fast for precision and analytical recovery. Calibration curves were linear in the range 0.18–6.0 m*M* ($r = 0.92$). The precision was 3% and the analytical recovery was 87%. The detection limit of the method was 36 pmol. Determination of lactate metabolism was performed in a patient with chronic congestive heart failure who was administered primed-continuous L-[U-^{14}C]lactate (10 μCi bolus and 0.3 μCi/min continuously) during a 60-min rest period. Mean arterial lactate concentration and SA were

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1.69 ± 0.2 m*M* and 253.8 ± 22 dpm/μmol, respectively. Systemic lactate turnover was 25.65 μmol/kg per min. Lactic acid systemic turnover, organ uptake and release rates can be accurately determined by isocratic HPLC.

INTRODUCTION

Lactate determination in biogical fluids has recently gained considerable interest. The currently favoured enzymatic methods for lactate determination are based on its reaction with β-NAD^+ in the presence of lactate dehydrogenase to produce pyruvate and NADH [1]. NADH concentration is then monitored colorimetrically (540 nm) [2], by UV detection [3,4] or fluorimetrically [5–10]. Several other analytical methods employ electrochemical detection or coupled enzymatic assay with high-performance liquid chromatographic (HPLC) separation [11–15]. HPLC has been applied to analyze carboxylic acids by cation-exchange [16–19] and reversed-phase columns [20]. The use of isotopic tracers with HPLC allows a detailed analysis of the rates and pathways of lactate turnover and oxidation during physical acitivity and recovery. However, none of the existing techniques simultaneously measure concentrations and specific activities (SAs).

Our method involves a diethyl ether extraction of short-chain fatty acids from plasma, excluding the proteins, followed by back-extraction of the acids into a hydrophilic phase with sodium carbonate. Lactate is then converted to its α-bromoacetophenone (α-BAP) ester to yield a derivative which can be separated by isocratic reversed-phase HPLC. It absorbs at the optimal UV wavelength of 242 nm and is reliably detected at 320 nm [21]. The addition of a suitable internal standard, *e.g.* [1-^{3}H]acetate, prior to extraction, permits simultaneous determination of plasma lactate concentration and SA.

The method enables lactate measurement over a wide range of concentrations and SAs, employing an isocratic HPLC separation system on an octadecylsilane (ODS)-bonded column and subsequent liquid scintillation spectrometry. One injected sample can be simultaneously measured for concentration and SA, by respectively collecting the separated fractions (lactate and acetate).

Lactate concentration is an index of tissue oxygen deprivation and is helpful in grading the severity of shock [22]. Blood lactate concentration increases during physical work [23]. In patients with chronic heart failure, and impaired cardiac output response to exercise, lactate kinetics may provide a useful and objective index of the severity of heart failure [24].

EXPERIMENTAL

Reagents and chemicals

HPLC-grade solvent acetonitrile (UV cut-off 190 nm, Scientific Products, Baxter Labs., Fair Lawn, NJ, USA) and scintillation liquid (Optima Gold, Packard)

were employed. L-[U-^{14}C]-Lactate and [1-^{3}H]acetate were obtained from Dupont-New England Nuclear (Boston, MA, USA). Sodium carbonate, α-BAP, 18-crown-6-ether (CrE) and propionic acid were obtained from Aldrich (Milwaukee, WI, USA). L-(+)-Lactic acid (lithium salt) was obtained from Sigma (St. Louis, MO, USA) and diethyl ether (purified for fat extraction) was obtained from Mallinkrodt. Plasma samples were derived from laboratory personnel (control) and a patient with chronic congestive heart failure. This patient with congestive heart failure also received infusion of L-[U-^{14}-C]lactate.

Equipment

HPLC was performed using a reversed-phase ODS-bonded (C_{18}) column (5 μm, 250 mm × 4.6 mm I.D., Beckman, San Ramon, CA, USA), a UV detector, two pumps, a recording integrator and a controller (Shimadzu, Beltville, MD, USA). The isocratic mobile phase (1.0 ml/min) consisted of acetonitrile–water (30:70, v/v). The two peaks of interest containing the radioactive lactate and acetate fractions were collected (LKB 2111 MultiRac fraction collector, Bromma, Sweden), and measured by liquid scintillation spectrometry (LKB 1219 liquid scintillation counter).

Subjects

The protocol was reviewed and approved by the Institutional Review Board of the Albert Einstein College of Medicine. Consent forms were obtained from all participants.

Experimental protocol

Plasma specimens from a patient with congestive heart failure and a normal subject were collected following an overnight fast. Control plasmas and standards (n = 21) were used to measure precision (coefficient of variation) and analytical recovery of lactate levels by the HPLC method described here. The recovery experiment included three groups of samples: (1) seven plasma samples (1.0 ml); (2) seven samples with 20 μl of standard, comprised of 100 mM lactate solution added to 1.0 ml of 2 mM sodium carbonate; (3) seven plasma samples of 1.0 ml with 20 μl of standard (100 mM lactate solution).

Administration of primed-continuous L-[U-^{14}C]lactate (10 μCi bolus and 0.3 μCi/min continuously) into the medial antecubital vein of a patient with congestive heart failure was made during a 60-min rest period. The labeled lactate, diluted in 0.9% saline, was sterilized by Millipore filtration. Arterial (radial artery) blood samples were obtained for measurement of the systemic lactate turnover. Administration and sample collection procedure for lactate systemic turnover has been previously described [25,26]. Steele's equation [27] for determining systemic lactate turnover during isotopic steady state conditions is:

lactate systemic turnover (μmol/kg per min) = F(dpm/min per kg)/SA_A (dpm/μmol)

where F = L-[U-^{14}C]lactate infusion rate and SA_A = specific activity, arterial.

Concentrations of plasma lactate were calculated from peak heights and [1-^{3}H]acetate counts (dpm) as the internal standard using the calibration curve. SAs were calculated using the lactate quantity in micromoles and [^{14}C]lactate counts (dpm) collected from plasma samples (corrected for initial levels with the internal standard). L-Lactate standard levels were confirmed using the YSI Model 23L lactate analyzer [15].

Sample processing

A 1-ml volume of plasma (stored at −70°C) was pipetted into extraction tubes. Aliquots of standard solutions of sodium lactate (range 180–6000 μM) were processed along with each set of plasma samples. A 50-μl volume of [1-^{3}H]acetate was added to each sample as an internal standard. The samples then were adjusted to low pH (< 1) with 2 M hydrochloric acid (100 μl), followed by the addition of 10 ml of diethyl ether, and shaken for 60 min. Centrifugation (2500 g) followed for 10 min, with subsequent transfer of the top (organic) layer. Back-extraction by the addition of 2 mM sodium carbonate (1.0 ml) and shaking for 5 min was followed by centrifugation for an additional 5 min. The top phase was discarded, and the low aqueous phase was dried in a Speed-Vac. Derivatization was carried out with 50 μl of α-BAP and CrE solution (25 and 33 mg/ml in acetone, respectively) for 15 min at 100°C after brief sonication. Then, 100 μl of 50 mM propionic acid in acetone were added to the sample, followed by heating for 5 min at 100°C. The sample was dried in a Speed-Vac, and resuspended in 250 μl of 30:70 (v/v) acetonitrile–water. The sample (240 μl) was subsequently injected onto the HPLC column.

HPLC conditions and radioactive spectrometry

The buffer consisted of 30% (v/v) acetonitrile in water running at a flow-rate of 1.0 ml/min, with spectrophotometric detection at 320 nm (human plasma samples). The effluent, consisting of peaks of interest, was collected, scintillant was added, and the radioactivity (dpm) determined by liquid scintillation spectrometry. The SAs of lactic acid were calculated by dividing the collected radioactivity by the absolute quantity (micromoles) in the lactate fraction. The ^{3}H and ^{14}C radioactivity were determined using an LKB liquid scintillation counter with dual-counting mode.

Statistical analysis

Data are expressed as the mean ± S.D. Calibration curve was analyzed by simple regression analysis.

RESULTS

The chromatographic peaks of lactate, β-hydroxybutyrate and acetate (reten-

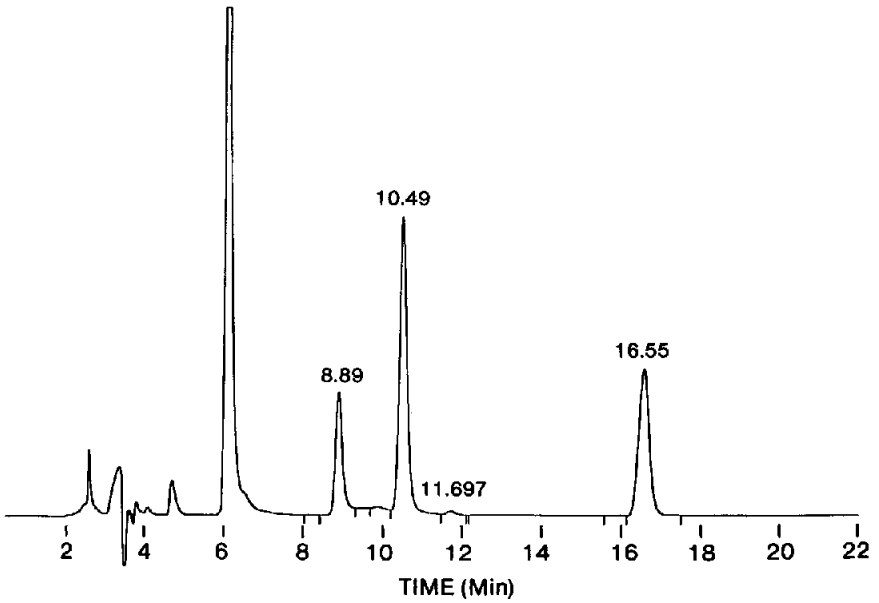

Fig. 1. Chromatogram of lactate, β-hydroxybutyrate and acetate (retention times are 8.89, 10.49 and 16.55 min, respectively).

TABLE I

CONTROL PLASMAS AND STANDARDS STUDIED FOR PRECISION AND ANALYTICAL RECOVERY OF LACTATE

Sample	Concentration (m*M*)		
	Plasma lactate (1 ml)	Lactate (20 μl, 100 m*M*) added to Na_2CO_3 (1 ml, 2 m*M*)	Plasma (1 ml) + lactate (20 μl, 100 m*M*)
1	1.43	1.934	2.917
2	1.544	2.011	3.063
3	1.523	1.911	2.976
4	1.494	1.969	2.946
5	1.515	1.873	2.883
6	1.569	1.914	2.997
7	1.457	1.907	3.136
Mean ± S.D.	1.504 ± 0.048	1.93 ± 0.045	2.988 ± 0.087
C.V. (%)	3.2	2.36	2.9

Analytical recovery 87%

TABLE II

ARTERIAL LACTATE LEVELS, SPECIFIC ACTIVITIES AND SYSTEMIC TURNOVER IN A RESTING CHRONIC CONGESTIVE HEART FAILURE PATIENT

Resting systemic lactate turnover is 25.65 μmol/kg per min. F = isotope infusion rate = 6510 dpm/kg per min. APC = arterial plasma concentration; SA_A = specific activity, arterial.

Time (min)	APC (mM)	SA_A (dpm/μmol)
20	1.862	255.038
25	1.977	220.042
30	1.665	241.052
35	1.487	280.619
40	1.485	272.524
Mean ± S.D.	1.695 ± 0.22	253.85 ± 24.36

tion times of 8.89, 10.49 and 16.55 min, respectively) are depicted in Fig. 1. The regression analysis showed a linear relationship: peak heights/[^{3}H]acetate (dpm) = y = 120.19 + 488.39 [lactate (mM)]; standard error of the slope = 20.84, r = 0.92, n = 102. This line is a more stringent measurement than an individual line of assay reproducibility. Comparison of all the standard curves generated an r of 0.92. Individual standard calibration curves for each set of experimental samples generated an r of >0.95. [1-^{3}H]Acetate served as the internal standard, showing an equivalent recovery (30–40%) as L-[U-^{14}C]lactate.

Overnight control fasting plasma lactate level was 1.5 ± 0.04 mM (mean ± S.D.) with a precision of 3%. Analytical recovery was 87% of the added lactate standard (Table I). The detection limit was 36 pmol, which was three times greater than the noise in the blank samples.

During administration of L-[U-^{14}C]lactate to a patient with congestive heart failure, isotopic steady state was reached after a period of 20 min. Arterial lactate levels, SAs and lactate systemic turnover are listed in Table II.

DISCUSSION

The measurement of lactate metabolism is of particular interest to exercise physiologists. Muscles have the capacity for glycolysis, and according to conditions, may either produce lactate or oxidize lactate to carbon dioxide. Though lactate production increases the available energy for contraction, it tends to suppress contractile and metabolic activity. High-level performance requires as much energy as possible, while limiting or delaying the development of accumulation of detrimental metabolic effects [28].

Blood lactate concentrations during various types of exercise do not provide

much information on the rates and pathways of lactate turnover and oxidation during physical activity and recovery. Radiolabelled isotopes, especially [^{14}C]glucose, have been widely used for the study of glucose metabolism. However, studies with [^{14}C]lactate are few [29].

Release and uptake of lactate can be studied *in vivo* by administration of radiolabeled lactate into the circulation with subsequent determination of the specific radioactivity of plasma L-(+)-lactate [30]. Different techniques have been used, including ion-exchange chromatography [31,32] and radioisotopic dilution analysis [33,34]. Methods involving the isolation of lactate from plasma by ion-exchange chromatography [26] and subsequent enzymatic analysis of the isolated fraction for lactate and liquid scintillation counting of radioactivity content are potentially error-prone [31,32,35].

Different HPLC approaches have been developed to measure lactate [20,21,36–39]. Owens and Robinson [20] have provided SA measurements which are needed to measure lactate kinetics. However, they lack quantitative results (precision). Simonides *et al.* [36] have previously measured lactate levels, but SAs were not determined. The determination of plasma lactate concentration and SA by HPLC on an ODS-bonded column, as shown in our study, is precise (3%) and sensitive (36 pmol). Small volumes of plasma are needed due to the sensitivity of the technique. The minimum plasma volume required is, however, limited by measurement of the tracer [^{14}C]lactate. The addition of a suitable internal standard prior to extraction permits concomitant determination of plasma lactate concentrations and SAs.

The data obtained for resting lactate turnover in the patient with congestive heart failure showed an increased level compared to normal values reported by Stanley *et al.* [26] These investigators used a method that split the plasma sample into two, measuring lactate levels and radioactivity separately, and therefore may not be as reliable as our method. The patient with congestive heart failure was 60 years old, while the normal population studied by Stanley *et al.* [26] was younger. Nevertheless, our data suggest that cardiovascular disease may alter lactate metabolism and promote greater insight into the disease process.

CONCLUSION

This HPLC assay has substantial practical advantages over gas chromatography and other HPLC or enzymatic methods, and could well be applied to assess changes in plasma levels and turnovers of lactate in different physiological and pathological states, such as exercise, diabetes, oxygen deprivation of tissues, shock states and gastrointestinal malabsorption.

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