Lactate metabolism in resting and contracting canine skeletal muscle with elevated lactate concentration

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Kelley, Kevin M., Jason J. Hamann, Christine Navarre, and L. Bruce Gladden. Lactate metabolism in resting and contracting canine skeletal muscle with elevated lactate concentration. J Appl Physiol 93: 865–872, 2002.—This study was undertaken to quantitatively account for the metabolic disposal of lactate in skeletal muscle exposed to an elevated lactate concentration during rest and mild-intensity contractions. The gastrocnemius plantaris muscle group (GP) was isolated in situ in seven anesthetized dogs. In two experiments, the muscles were perfused with an artificial perfusate with a blood lactate concentration of ~9 mM while normal blood gas/pH status was maintained with [U-14C]lactate included to follow lactate metabolism. Lactate uptake and metabolic disposal were measured during two consecutive 40-min periods, during which the muscles rested or contracted at 1.25 Hz. Oxygen consumption averaged 10.1 ± 2.0 μmol·100 g~1·min~1 (2.26 ± 0.45 ml·kg~1·min~1) at rest and 143.3 ± 16.2 μmol·100 g~1·min~1 (32.1 ± 3.63 ml·kg~1·min~1) during contractions. Lactate uptake was positive during both conditions, increasing from 10.5 μmol·100 g~1·min~1 at rest to 25.0 μmol·100 g~1·min~1 during contractions. Oxidation and glycogen synthesis represented minor pathways for lactate disposal during rest at only 6 and 15%, respectively, of the [14C]lactate removed by the muscle. The majority of the [14C]lactate removed by the muscle at rest was recovered in the muscle extracts, suggesting that quiescent muscle serves as a site of passive storage for lactate carbon during high-lactate conditions. During contractions, oxidation was the dominant means for lactate disposal at >80% of the [14C]lactate removed by the muscle. These results suggest that oxidation is a limited means for lactate disposal in resting canine GP exposed to elevated lactate concentrations due to the muscle’s low resting metabolic rate.

resting metabolic rate; [14C]lactate; lactate oxidation; glycogenogenesis

INTENSE EXERCISE RESULTS in increased muscle and blood lactate concentrations ([La]s), and the disposal of this lactate load is an important part of recovery from exercise. Sites contributing to lactate elimination during and after heavy exercise include cardiac muscle (23), inactive skeletal muscle (2, 3, 6, 38, 40, 45, 51), actively contracting skeletal muscle (50, 51), previously active skeletal muscle (12, 33, 43), and the splanchnic bed (1). Inactive muscle plays an important role in lactate removal during contraction of other muscle groups (38, 40, 45); however, the relative importance of the various pathways of metabolic disposal for lactate elimination remains the subject of debate, and it has even been suggested that the role of resting skeletal muscle in lactate uptake is entirely as a passive sink (13, 45).

During recovery from exhaustive exercise, pathways for lactate elimination in skeletal muscle include glycogenogenesis (5, 7, 12, 33, 43, 48), oxidation to CO2 and H2O (11, 12, 34), and transamination to alanine with subsequent incorporation into proteins (12, 48). In general, mammalian muscles are believed to dispose of lactate primarily by oxidation during and after exercise (11, 12, 19–21, 34). Although this may adequately address the ultimate metabolic fate of lactate in muscle, the role of resting muscle in lactate metabolism during high lactate conditions remains the subject of debate.

Radioisotope research in humans has demonstrated a relationship between metabolic rate and lactate oxidation during rest and exercise (9, 10, 42). However, this effect of metabolic rate has received more attention for exercise than resting conditions. It is quite possible that the resting metabolic rates (RMRs) of different muscles play an important role in determining the predominant route of metabolic disposal of lactate by quiescent muscle during exercise and recovery. The canine gastrocnemius plantaris muscle preparation in situ (GP) presents an attractive model to examine the role of RMR. GP is composed of 55% slow-twitch, fatigue-resistant fibers and 45% fast-twitch, fatigue-resistant fibers and is considered a highly oxidative muscle (41) with peak oxygen consumption (VO2 peak) values exceeding 230 ml O2·kg~1·min~1 (4, 37). However, regardless of its highly oxidative characteristics, it often displays a low RMR (2.2 ml O2·kg~1·min~1) (28) compared with the RMRs reported for the muscles of other mammalian

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species, such as humans (4.1 ml O$_2$·kg$^{-1}$·min$^{-1}$) (42), rabbits (6.7–9.0 ml O$_2$·kg$^{-1}$·min$^{-1}$) (44), and rats (16.9 ml O$_2$·kg$^{-1}$·min$^{-1}$) (48).

Previous investigations of lactate uptake at elevated [La] at various metabolic rates (16, 28) have been unable to account for a large amount of net substrate uptake by the muscle through the pathways of oxidation and glycogen synthesis alone. Despite the fact that several studies have endeavored a quantitative analysis of the metabolic end points of lactate through the pathways of oxidation and glycogen synthesis alone. The nerve was stimulated by supramaximal square pulses of 3.0- to 5.0-V amplitude and 0.2-mas duration (Grass S48 stimulator) isolated from ground by a stimulus isolator (Grass SIU8TB). Outputs from the load cell, flowmeter, and pressure transducer were recorded continuously on a strip chart recorder (NarcoTrace 40, Narco Biosystems) throughout the experiments.

Before each experiment, the muscle was set to optimal length ($L_o$) by progressively lengthening the muscle as it was stimulated at a rate of 0.2 Hz, until a peak in developed tension (total tension minus resting tension) was reached. Once the muscle had returned to a resting steady state, resting blood flow to the intact muscle was measured for 1 min and recorded. The muscle was then stimulated to contract at the same rate described in Contractions so that muscle blood flow for the intact contracting muscle could be determined. After the blood flow measurements, the muscle was allowed to recover for 35 min, permitting a return to resting conditions before artificial perfusion began.

**Perfusion Medium**

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (46) and fresh, thoroughly washed bovine erythrocytes yielding a final hemoglobin concentration (Hb) of 13.2 ± 0.3 g/dl and a hematocrit of 35–40%. The perfusate contained 25 mM sodium bicarbonate, 1.0 g/dl bovine serum albumin (fraction V, Sigma Chemical), 5 mM glucose, 2.5 mM calcium chloride, and 6 g/dl dextran (low fraction, EK18894, Eastman Kodak) as a colloidal agent. The nonerythrocyte portion of the perfusate was pushed through a filter stack [1-μm pore-size glass filter, followed by 0.8-, 0.45-, and 0.22-μm cellulose acetate filters (MSI)] to remove any large particles from the solution, and stored overnight at 2°C.

On the day preceding each experiment, fresh bovine blood was collected via jugular catheter into 4-liter blood collection bags containing acid-citrate dextrose anticoagulant solution. This blood was transported to the laboratory on ice and centrifuged (2,500 revolutions/min; Sorvall RT6000B). After aspiration of the plasma from the centrifuge tubes, the packed cells were washed four times with an equal or greater volume of cold normal saline previously bubbled with 100% O$_2$. Erythrocytes were then poured through glass beads (4-mm diameter) to remove any remaining clotting factors, washed five to six additional times with cold O$_2$-saturated saline, and stored as packed cells overnight at 2°C.

On the morning of the experiments, stored erythrocytes were given two final washes in cold O$_2$-saturated saline. The prefiltred nonerythrocyte portion of the perfusate containing dextran was bubbled with 95% O$_2$-5% CO$_2$ and placed in a water bath to warm the perfusate to 37°C. Before initiation of the pump-controlled perfusion (see Muscle Perfusion below), a 42 μCi bolus of [U-$^{14}$C]lactate was added to the experimental perfusate to approximate a lactate-specific activity (LSA) of 10,000 disintegrations/min (dpm)/mmol. Unlabeled sodium lactate, sodium pyruvate, and glucose were also added to elevate the experimental perfusate substrate concentrations to the following nominal values (all in mM): 10 lactate, 1 pyruvate, and 5 glucose. The pH of the perfusate was adjusted to 7.35 at 37°C. A separate portion of the perfusate was prepared to perfuse the muscle before the experiments (unlabeled perfusate). This portion of the perfusate was identical to the experimental perfusate except that it had a [La] of 1 mM (approximating the [La] of arterial blood at rest) and did not include any [$^{14}$C]lactate. Erythro-
cytes were added to both perfusate solutions and equilibrated to experimental conditions shortly before the onset of artificial perfusion.

Muscle Perfusion

A peristaltic pump (Gilson Minipuls 3) was used to withdraw perfusate from its reservoir and pass the perfusate through an in-line bubble trap en route to the muscle. The perfusate reservoir, bubble trap, and all tubing were maintained at 37°C within a heated plastic canopy enclosing the lower half of the dog and the perfusion apparatus. The perfusate was mixed throughout the experiment by a suspended stir bar within the reservoir to ensure homogeneity and to prevent the red cells from settling during the perfusion.

After preparation of the muscle and the artificial perfusates, the popliteal artery was cannulated, and arterial flow from the reservoir of unlabeled perfusate was initiated immediately. The animal was then euthanized with an overdose of pentobarbital sodium by way of the jugular cannula. The time between ligation of the arterial supply to the muscle and establishment of perfusate flow to the muscle was as brief as possible (typically 30–90 s). A pressure transducer (model RP-1500, Narco Biosystems) was connected to a T valve in the arterial line supplying the GP at the same level as the muscle to monitor perfusion pressure. After the initiation of artificial perfusion, warm saline-soaked gauze was placed over the muscle, and a heating lamp was directed over the hindlimb. Plastic wrap was placed over the gauze to minimize evaporative heat loss.

With perfusate flow initiated, and \( L_v \) of the muscle determined, the muscle was allowed to rest for 30 min while perfused by the unlabeled perfusate. This period allowed stabilization of blood flow and pressure after the switch from spontaneous perfusion with the animal’s own blood to perfusion with the artificial perfusate. After this 30-min resting perfusion, the perfusate was switched to experimental perfusate for 15 min to allow for washout of the unlabeled perfusate and equilibration with the elevated [La] and radioactive label.

Experimental Protocols

Rest. These measurements were used to evaluate muscle metabolism at an elevated [La] (−9 mM) at a RMR. During the resting phase, blood flow was adjusted to maintain blood pressure at ~100 mmHg and/or match the resting blood flow from the muscle before arterial cannulation. Preliminary experiments confirmed that muscle metabolism was in a steady state with regard to \( V_{\text{O}_2} \) consumption (\( V_{\text{O}_2} \)) and lactate exchange after 20 min of perfusion. The rest protocol lasted 40 min.

Contractions. Immediately after the resting measurements, the muscle was stimulated (0.2-ms duration, 4–6 V) to eliciting twitch contractions at 1.25 Hz for a period of 40 min. Pilot experiments had demonstrated that this stimulation protocol would raise the metabolic rate of the muscle to ~20% of \( V_{\text{O}_2} \) peak, an easily sustainable submaximal exercise intensity. At the onset of contractions in this series, blood flow was increased to a level that 1) minimized fatigue and maintained blood pressure at ~100 mmHg, and/or 2) was similar to the intact muscle’s contracting blood flow before perfusion. Again, a steady state with regard to \( V_{\text{O}_2} \) and lactate exchange was achieved by 20 min.

Perfusate and Muscle Sampling

Arterial samples were taken directly from the perfusate reservoir, whereas venous samples were taken anaerobically from a T valve in the venous cannula exiting the muscle. Simultaneous arterial and venous (A-V) samples were collected into 3-ml syringes every 10 min, beginning at the initiation of each protocol. These samples were analyzed for blood gases, pH, [Hb], and percent saturation (% sat). From 20 to 40 min, samples were taken every 5 min. Samples were analyzed for blood metabolites and tracer determinations in addition to the blood gas measurements monitored during the first 20 min. Blood flow rates were determined after each sample by a timed collection of venous perfusate. Three muscle biopsies were taken during each trial: 1) a biopsy from the resting muscle before cannulation and initiation of perfusion; 2) a biopsy from the resting muscle between rest and contractions; and 3) a biopsy from the contracting muscle at the end of the contractions period. Each biopsy was immediately submerged in liquid nitrogen and subsequently stored at −80°C. Mean muscle biopsy weight was 108.3 ± 10.7 mg.

Analytical Procedures

Perfusate for metabolite analysis was ejected directly into preweighed test tubes containing chilled 10% perchloric acid (HClO₄), then vortexed and refrigerated. After blood density was determined and each sample test tube was reweighed, blood volume for each tube was calculated. Samples were then centrifuged, partially neutralized with potassium hydroxide (pH ~2.5), vortexed, and stored at 5°C. The neutralized supernatant was then used for subsequent determinations of lactate (32), pyruvate (18), alanine (54), glucose (53), and for use in ion-exchange chromatography. Pyruvate analysis was performed within 48 h of sample collection.

Ion-exchange chromatography was employed to isolate labeled compounds and to determine the LSA (36, 48). Control samples (labeled lactate, pyruvate, and alanine) were run concurrently with each group of experimental samples to determine the completeness of separation. The lactate, pyruvate, and amino acid eluants were collected and assayed for metabolite concentrations and radioactivity via liquid scintillation counting (Wallac 1414). \(^{11} \text{CO}_2\) was determined by using the method of Chan and Dehaye (14) immediately after the conclusion of each experiment. Arterial samples were run alongside venous samples to correct for background.

For blood gas (P \( \text{O}_2 \), P\( \text{CO}_2 \), and pH) determinations, A-V samples were anaerobically drawn and analyzed immediately (IL 1304). [Hb] and percent saturation were measured immediately with a CO-oximeter (IL 282) set for bovine blood. Total \( \text{O}_2 \) concentration was calculated from P\( \text{O}_2 \), [Hb], and percent saturation (see Calculations).

Frozen muscle samples were divided into two portions. One portion was freeze dried and used for determination of muscle lactate and gross radioactive content. The freeze-dried portion was homogenized in cold saline and then deproteinized with 10% HClO₄. After centrifugation, aliquots were partially neutralized with potassium hydroxide (2 N). Aliquots were then assayed for lactate and labeled compounds (as described above). The second (non-freeze-dried) portion of the muscle was used to assay the glycogen concentration and radioactivity according to the methods of Chan and Exton (15). Water content in the perfused muscles was determined by surgically extracting the entire GP after each experiment and then weighing the muscle before and after drying in an oven.

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**Calculations**

\[ \text{V}_{O_2} \left( \mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1} \right) \] and net substrate balance \((\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1})\) were calculated as the product of A-V difference (mM) and flow rate (ml·kg\(^{-1}\)·min\(^{-1}\)). Oxygen concentration \((O_2)\) in the blood was calculated as:

\[
[O_2] = \left[ \{(1.39 \text{ ml} \ O_2/\text{g} \ Ha) \times (\text{g} \ Ha/100 \text{ ml})\} \times (\% \text{Sat}) \right] + \left[ (0.003 \text{ ml} \ O_2/100 \text{ ml blood})/(\text{Torr} \ P_{O_2}) \right] \times (P_{O_2})
\]

Total net carbon uptake (\(\mu \text{mol} \ C_3 \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)) was calculated as the sum of the net pyruvate uptake, net lactate uptake, and twice the net glucose uptake. \([14C]\)lactate removal (dpm·100 g\(^{-1}\)·min\(^{-1}\)) was calculated as the product of the A-V \([14C]\)lactate difference (dpm/ml) and the flow rate. Tracer estimated removal (\(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)) was calculated as the \([14C]\)lactate removal divided by the venous LSA (dpm/\(\mu \text{mol}\)). Rate of \(14^\text{CO}_2\) release (dpm·100 g\(^{-1}\)·min\(^{-1}\)) was calculated as the product of \(\text{A-V} \ 14^\text{CO}_2\) difference (dpm/ml) and the flow rate. Apparent oxidation (\(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)) was calculated as the \(14^\text{CO}_2\) release divided by the venous LSA (dpm/\(\mu \text{mol}\)). Apparent glycogen synthesis \((\mu \text{mol} \ C_6 \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1})\) from \([14C]\)lactate was calculated as the incorporation of \([14C]\)lactate into glycogen (dpm·100 g\(^{-1}\)·min\(^{-1}\)) divided by the venous LSA X 2.

**Statistical Analysis**

A paired \(t\)-test was used to compare resting and contracting protocols. Pre-, intermediate, and postexercise muscle biopsy data and steady-state metabolite data were assessed by using a one-way repeated-measures ANOVA. The Student-Newman-Keuls post hoc procedure was used when a significant \(F\) ratio was observed. Statistical significance was accepted at \(P < 0.05\). Results are reported as means \(\pm SE\).

**RESULTS**

**General Characteristics**

The average mass of the GP used in these experiments was 41.8 \(\pm 2.3\) g. The small muscle size, typical in beagles, required lower total blood flow rates and, therefore, less artificial perfusate and radioactive lactate during the experiments. Arterial perfusate blood gas and acid-base values varied only slightly throughout the two protocols and were typical of previous experiments with this muscle preparation (26, 28, 29). Overall averages for rest and contractions, respectively, were as follows: arterial \(O_2\) concentration = 18.87 \(\pm 0.04\) and 18.76 \(\pm 0.04\) ml \(O_2/100\) ml blood; arterial \(P_{CO_2}\) = 35.1 \(\pm 0.8\) and 33.6 \(\pm 0.7\) Torr; arterial \(pH\) = 7.39 \(\pm 0.01\) and 7.40 \(\pm 0.01\); arterial bicarbonate concentration = 20.6 \(\pm 0.2\) and 20.2 \(\pm 0.2\) mM.

As noted in METHODS, blood flow was controlled by a perfusion pump and was adjusted to match intact resting and contracting blood flows while maintaining perfusion pressure at or below 100 mmHg. As a result, average resting blood flow was 16.3 \(\pm 2.2\) ml·100 g\(^{-1}\)·min\(^{-1}\) with a mean pressure of 79.8 \(\pm 8.8\) mmHg. During contractions, mean blood flow and perfusion pressure increased to 50.0 \(\pm 3.7\) ml·100 g\(^{-1}\)·min\(^{-1}\) and 100.7 \(\pm 9.7\) mmHg, respectively. The metabolic steady states achieved during the final 20 min of each experimental protocol are evident in the minimal changes in \(V_{O_2}\) from 20 to 40 min (see Fig. 1). During rest, \(V_{O_2}\) varied by <5% during the final 20 min and averaged 2.3 \(\pm 0.4\) ml·kg\(^{-1}\)·min\(^{-1}\) (10.1 \(\pm 2.0\) \(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)). \(V_{O_2}\) increased more than 10-fold during contractions to 32.1 \(\pm 3.6\) ml·kg\(^{-1}\)·min\(^{-1}\) (143.3 \(\pm 16.2\) \(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)) and varied by <7% during the steady-state phase.

Mean tension required to stretch the muscles to their \(L_o\) was 52.8 \(\pm 4.5\) g. Peak developed force during contractions was 158.4 \(\pm 12.7\) g with declines in force production of 18.6 \(\pm 2.3\) % during the first 5 min of contractions and a total of 29.2 \(\pm 2.8\)% during the entire contraction period. The majority of the fatigue occurred during the first 10 min of contractions; hence fatigue was minimal during the 20 min steady-state measurement period.

The addition of lactate to the perfusate elevated arterial perfusate [La] to average values of 8.6 \(\pm 0.1\) and 8.9 \(\pm 0.1\) mM for rest and contractions, respectively. At these perfusate [La]s, the muscles exhibited net uptake of lactate and pyruvate and net output of alanine during steady-state conditions, as shown in Table 1. During the 20-min steady-state measurement phase during both protocols, lactate uptake did not vary significantly (Fig. 2). Net lactate uptake (\(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\)) was more than two times greater during contractions than at rest. Furthermore, \([14C]\)lactate uptake increased in proportion to the unlabeled lactate uptake (1,470 \(\pm 200\) to 3,690 \(\pm 510\) dpm·100 g\(^{-1}\)·min\(^{-1}\)) in contractions compared with rest. Tracer-estimated lactate removal increased proportionally as well (18.6 \(\pm 3.3\) and 46.2 \(\pm 5.2\) \(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\) for rest and contractions, respectively). Apparent oxidation of lactate increased 30-fold during contractions from 1.1 \(\pm 0.4\) to 36.4 \(\pm 5.4\) \(\mu \text{mol} \cdot 100 \text{~g}^{-1} \cdot \text{min}^{-1}\). The arterial lactate-to-pyruvate ratio in both protocols was maintained at 12.5, within the target range of 10–15. Arterial perfusate glucose concentration was similar during both protocols at 5.3 \(\pm 0.1\) and 5.1 \(\pm 0.1\) mM.
Table 1. Net substrate exchange during rest and contractions

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rest, μmol·100 g⁻¹·min⁻¹</th>
<th>Contractions, μmol·100 g⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>10.5 ± 1.5</td>
<td>25.0 ± 3.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.8 ± 1.0</td>
<td>15.5 ± 3.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>-0.8 ± 0.2</td>
<td>-1.4 ± 0.3</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.9 ± 0.5</td>
<td>6.3 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of n = 7 dogs. Negative values indicate net releases.

with mean values for net glucose uptake (μmol · 100 g⁻¹·min⁻¹) increasing fourfold during contractions compared with rest. Total net carbon uptake averaged 19.9 ± 3.5 μmol C3·100 g⁻¹·min⁻¹ at rest and increased to 62.3 ± 9.0 μmol C3·100 g⁻¹·min⁻¹ during contractions.

Muscle [La] increased significantly during both conditions. During the rest protocol, muscle lactate increased from a preperfusion [La] of 2.3 ± 0.4 to 4.7 ± 0.5 mmol/kg wet wt. Muscle lactate continued to increase during contractions to a final concentration of 8.2 ± 1.2 mmol/kg wet wt. In addition, muscle glycogen increased significantly during rest from 30.8 ± 5.1 to 39.6 ± 7.1 mmol glucosyl units/kg wet wt. Muscle glycogen then decreased significantly during contractions to 31.5 ± 6.9 mmol glucosyl units/kg wet wt. The apparent synthesis of glycogen from [14C]lactate during rest was 30.8 ± 9.4 μmol C6·100 g⁻¹·40 min⁻¹ and did not differ significantly from the calculated value for glycogen synthesis of 21.9 ± 7.9 μmol C6·100 g⁻¹·40 min⁻¹. During the rest protocol, the apparent glycogen synthesis occurred during a period of net glycogen synthesis. As shown in Table 2, ~14% of the [14C]lactate taken up by the GP at rest was incorporated into glycogen and ~6% was recovered as 14CO2 in the venous perfusate. The majority of the 14C label taken up by the resting muscle was recovered in the muscle tissue extracts. By comparison, during contractions, the majority of the label was recovered as 14CO2, whereas 14C recovery in muscle glycogen, amino acids, and muscle extract all decreased. Only small amounts of 14C were recovered in the muscle extracts after contractions. Therefore, the majority of the [14C]lactate recovered at rest was in muscle metabolites, whereas oxidation to 14CO2 represented the primary fate of [14C]lactate during contractions. Total [14C] recovery relative to [14C]lactate extraction averaged 80.9 ± 13.1% at rest and 91.4 ± 5.9% during contractions.

The venous effluent LSA (normalized to an arterial LSA of 10,000 dpm/μmol) was significantly higher during contractions than during rest (9,651 ± 91 vs. 9,424 ± 60 dpm/μmol). No significant differences in venous LSA were detected across time during the steady state in either protocol.

GP muscle water content after the experiments averaged 75.6 ± 0.8%, a value similar to those previously reported (26, 28).

**DISCUSSION**

**Rest**

The most significant finding of this study is that oxidation is a limited pathway for lactate utilization in resting canine GP at elevated [La]s. This was surprising in the context of previous research on muscle in other species, especially given the oxidative nature of this muscle preparation. Previous radioisotope studies of lactate metabolism in intact rats (20), dogs (34), and men (42), and across muscle groups in rats (48), dogs (17, 31), and men (10) have reported oxidation to be a major route of lactate disposal at rest (27). In contrast to these investigations, apparent oxidation accounted for only 6% of the total net carbon removal by the resting canine GP at 8.6 mM lactate.

If the resting VO2 (10.1 μmol·100 g⁻¹·min⁻¹) were exclusively devoted to lactate oxidation, the muscle could oxidize no more than 3.4 μmol La·100 g⁻¹·min⁻¹ (for calculations, see Ref. 26), constituting 32% of the lactate taken up by the muscle and 18% of the tracer-estimated removal of lactate. Previous research has demonstrated that, as the arterial [La] rises, the fractional contribution of oxidation to removal declines (44). This likely reflects the fact that skeletal muscle's RMR is unaffected by lactate delivery. As resting muscle lactate uptake increases with increasing blood [La]s (22, 28, 34, 44), the increased lactate uptake at 8.6 mM

![Fig. 2. Net lactate (La) uptake during the 20-min steady-state measurement periods at rest (●) and during contractions (○) (n = 7). La uptake was always significantly greater during contractions than at rest. However, there were no significant changes in net La uptake over time within the steady state of either metabolic condition.](http://jap.physiology.org/)

![Table 2. [14C]lactate recovery into products as a percentage of the total [14C]lactate extracted by the muscle](http://jap.physiology.org/)
[La] will “dilute” the lactate oxidation when expressed as a percentage of lactate taken up by the muscle.

In addition, previous research has clearly demonstrated a relationship between skeletal muscle fiber types and means of lactate disposal (44). Pagliassotti and Donovan (44) reported that a mixed fiber-type muscle group in the rabbit hindlimb oxidized 39% of the lactate taken up at 8 mM arterial [La] with a resting VO$_2$ of 40 µmol·100 g$^{-1}$·min$^{-1}$. The mixed fiber-type canine GP (55% slow-twitch and 45% fast-twitch fatigue resistant) (41) has a lower RMR than the rabbit muscle (10 vs. 38 µmol·100 g$^{-1}$·min$^{-1}$) and would be capable of oxidizing no more than 25% of its lactate uptake. A general trend of increased oxidation with increasing RMR is evident, at least when arterial [La]s are elevated. Mazzeo et al. (42) previously demonstrated that lactate disposal rates and lactate oxidation rates in humans are significantly correlated with VO$_2$, with greater disposal and oxidation at higher metabolic rates.

The majority (55%) of the [14C]lactate removed by the GP at rest in our experiments was not accounted for by oxidation but was instead recovered in the muscle tissue extracts. This is similar to previous reports in quiescent muscle at elevated [La]s (16, 28). Because inactive muscle takes up lactate in response to increasing [La] with no concomitant increase in RMR, recruitment of alternative removal pathways is required. Unlike previous reports of skeletal muscle lactate metabolism at [La]s of >10 mM (43), the additional removal of lactate was not primarily accomplished via glycogen synthesis (~14%). Alamine release has also been reported as an important means of lactate disposal in resting muscle as intracellular pyruvate levels are increased (39, 44). However, labeled alanine output by the muscle at rest was also small (4%) as was pyruvate output (2%). Limited muscle biopsy size prevented specific identification of the label recovered in the muscle extracts. A recent report by Sumida and Donovan (52) examined lactate metabolism in resting rat hindlimb muscle at 10 mM [La]$_{14}$CO$_2$ efflux was similarly low (9.6%), and muscle biopsies contained 23.5% of the [14C]lactate with 11% recovered as lactate, 3.8% as pyruvate, and 8.7% as amino acids. Chin et al. (16) suggested that as much as 60–72% of the net lactate uptake by resting rat hindlimb perfused with 11 mM lactate might be involved in metabolic cycling in the glycolytic-glyconeogenic pathways or triacylglycerol-free fatty acid substrate cycling. Recent experiments utilizing $^{13}$C NMR spectroscopy to follow $^{13}$C-enriched lactate in the perfused rat hindlimb support the involvement of lactate in a variety of metabolic pathways (8). Bertocci and Lujan (8) reported lactate’s entry and exit from the citric acid cycle via nonoxidative pathways in the rat hindlimb exposed to 5 mM [La]. They indicated that lactate might be involved in anaplerotic entry to citric acid cycle intermediates as suggested by Gollnick (30). One of the more important reactions in tricarboxylic acid cycle pool expansion is the alanine amino-transferase reaction, an equilibrium reaction. Thus increases in intracellular [La] and, therefore, pyruvate concentration could result in an increase in TCA cycle intermediates by way of mass action. Although this anaplerotic activity has been primarily viewed as an exercise phenomenon (24, 25, 47), it is likely to also occur in quiescent muscle with rising intracellular [La]s. Application of $^{13}$C NMR spectroscopy techniques in the canine GP are warranted to accurately account for lactate metabolism in the muscle.

Previous studies have examined lactate uptake by inactive forearm muscles in human subjects at [La]s elevated by exercise and have reported “passive” uptake of lactate with little evidence that the lactate had been metabolized (13, 45). If active lactate disposal in skeletal muscle was, for the purposes of this discussion, limited exclusively to oxidation and glycogen synthesis, then the residence of lactate in muscle, the accumulation of lactate as metabolic intermediates in various pathways, and the conversion of lactate to pyruvate and alanine in skeletal muscle (and their subsequent release and/or accumulation) could collectively be considered a “passive lactate sink.” The tissue is passive in the sense that the lactate is neither oxidized nor stored as a fuel. The tissue acts as a sink because lactate carbons entering the muscle are not immediately metabolically disposed. In this way, skeletal muscle may act as a storage medium for the lactate until the lactate is either slowly oxidized by the resting muscle or released back into the blood as circulating [La]s fall back to normal. The second role may be an overlooked component of the lactate shuttle hypothesis, whereby resting muscle tissue may provide an intermediate location for lactate until blood lactate levels fall closer to baseline values.

Finally, although we found oxidation to be a limited means for lactate disposal in canine GP, this does not necessarily contradict the numerous reports from whole body studies of lactate metabolism that suggest that oxidation is the primary means of lactate disposal in the resting muscle. As circulating lactate levels decline in postexercise recovery, the lactate taken up by quiescent muscles exposed to high [La]s may be slowly oxidized over time in those tissues or released back into circulation so that the majority of the lactate taken up by resting muscle is ultimately consumed by oxidative metabolism. In summary, our examination of lactate metabolism in resting skeletal muscle during a period of blood [La] elevation demonstrates that the capability of skeletal muscle for lactate oxidation is inhibited by a low RMR. This potential limitation of lactate disposal in resting skeletal muscle has not been clearly identified in any previous studies.

**Contractions**

As demonstrated in previous research with this preparation at elevated [La]s (26, 28), net lactate uptake by skeletal muscle increased as metabolic rate increased, and tracer-estimated lactate removal rose to a similar degree. Oxidation was the primary means of lactate disposal (~83%) in contracting canine GP. This
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is similar to reported values for lactate oxidation during moderate-intensity exercise in whole dogs (19, 34), rats (20), and humans (35, 42). There were also very minor recoveries of \( ^{14}\text{C} \) in glycolgen, alanine, and pyruvate, none of which exceeded 2.9% of the total lactate disposal by the muscle.

A much smaller proportion (<5%) of the \([^{14}\text{C}]\)lactate taken up by the muscle was recovered in muscle extracts after contractions. The difference between recovery of \([^{14}\text{C}]\) in muscle extracts after rest and contractions likely reflects the greatly increased metabolic rate during contractions. Unlike resting conditions where a low RMR may be limiting lactate oxidation, the >10-fold increase in metabolic rate during contractions permits the muscle to readily oxidize lactate. The increased turnover rate of lactate within the muscle could forestall \([^{14}\text{C}]\)lactate accumulation. The increase in muscle [La] was likely the result of increased endogenous lactate production combined with increased lactate uptake. Increased glycogenolysis during contractions is consistent with the decrease in muscle glycogen concentrations.

The large increase in metabolic rate and the accompanying increase in lactate turnover may account for decreases seen in \(^{14}\text{C} \) recovery in pyruvate and amino acids during contractions. Without an excess of intracellular \([^{14}\text{C}]\)lactate and a low RMR limiting disposal, intracellular pyruvate pools are metabolized at the same rate as lactate, leaving little excess pyruvate for amino acid synthesis, net pyruvate output, or incorporation into other metabolic pathways. Similarly, the higher \( \dot{V}O_2 \) and increased lactate oxidation may supersede incorporation of lactate into alternative pathways of lactate removal.

Although the values for lactate oxidation during contractions are in complete agreement with previous results, methodological concerns dictate that the numerical values for oxidation during the contraction protocol be interpreted with a degree of caution. The recovery of such a large percentage of the \([^{14}\text{C}]\)lactate taken up by the muscle at rest was not expected. Although it is likely that the muscle cells would have preferentially disposed of the labeled lactate and glycogen already present in the cells during the initial 20 min of the exercise bout, it is possible that some of the labeled carbon recovered as \(^{14}\text{CO}_2 \) originated from \([^{14}\text{C}]\)-labeled moieties residing in the muscle before the exercise bout began. Because the contractions resulted in net glycogen breakdown and a substantial decrease in \(^{14}\text{C} \) recovered in the muscle extracts, the sources of \(^{14}\text{CO}_2 \) produced during contractions might not be exclusively exogenous lactate. It is possible that the specific activity of \([^{14}\text{C}]\)lactate in the intracellular space was different from that in the extracellular space. Hence, it is difficult to precisely estimate the contribution of the oxidative pathway for lactate metabolism during contractions. Despite these concerns, the values for lactate oxidation during contractions are in agreement with previous results.

In summary, the results of the present study along with additional evidence from prior studies (8, 13, 16, 45) suggest that the role of resting skeletal muscle in lactate uptake at elevated arterial [La] may be one of passive storage with limited lactate metabolism. Although the canine GP is a highly oxidative muscle preparation with \( \dot{V}O_2 \) values exceeding 230 ml·kg\(^{-1} \)·min\(^{-1} \) (37), the relatively low RMR of this preparation limits the muscle's ability to dispose of lactate via oxidation. Previous research has demonstrated that, as arterial concentrations rise, the fractional contribution of oxidation to removal declines (44). In contrast, when \( \dot{V}O_2 \) was elevated by >10-fold during mild-intensity contractions, lactate was readily oxidized by the muscle. We believe that these experiments point to a simple but important point concerning lactate metabolism: If the RMR of a muscle is low, the rate of ultimate disposal of lactate in that muscle will be low unless it is highly suited to glyconeogenesis from lactate. What is the implication for the role of resting, oxidative muscle during recovery from intense exercise with elevated [La]? Most likely, resting skeletal muscle with a low metabolic rate serves as a storage site for lactate until it is slowly oxidized or released back into the blood as blood [La] declines.

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