Lactate metabolism of contracting dog skeletal muscle in situ¹

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STAINSBY, WENDELL N., AND HUGH G. WELCH. Lactate metabolism of contracting dog skeletal muscle in situ. Am. J. Physiol. 211(1): 177-183. 1966.—These experiments were designed to quantitate the lactate production by in situ skeletal muscle at various steady work rates. The gastrocnemius-plantaris muscle group and the lower leg (foot excluded) of the dog were used. Venous outflow and arterial and venous concentrations of lactate and oxygen were measured. The muscle contractions were maximal single twitches in response to supramaximal stimuli applied to the distal stump of the cut sciatic nerve. Work rate was varied by changing the twitch rate over a range of 0.5-10 twitches/sec. Oxygen uptake increased with increasing twitch rate reaching 30 times resting at 3/sec. Increasing the twitch rate beyond 3/sec did not further increase oxygen uptake. Resting muscle usually produced a small amount of lactate. After the contractions began, lactate production increased transiently and then decreased as the contractions continued. About half the muscle preparations studied showed lactate uptake, which occurred at all twitch rates studied after 10-60 min of contractions.

lactate uptake; skeletal muscle metabolism; muscle lactate

PART OF THE ENERGY SUPPLY for the performance of work by skeletal muscle, particularly at higher work rates, is generally considered to be derived from the breakdown of glucose or glycogen to lactate (1, 8-11, 15). This study was initiated to quantitate lactate production during twitch contractions by in situ mammalian skeletal muscle with intact circulation.

METHODS

Forty-eight mongrel dogs of 8-22 kg body wt were used in these experiments. They were anesthetized with either Dial and urethan (Ciba, Summit, N. J.), 0.6 ml/kg (allobarbital, 100 mg/ml; urethan, 400 mg/ml; and monoethylurea, 400 mg/ml), or pentobarbital, 30 mg/kg.

A tracheotomy tube was inserted and the right femoral artery was cannulated for sampling arterial blood and monitoring arterial blood pressure. A thermoregulated heating pad was put over the thorax, and an incandescent lamp was directed at the legs and lower abdomen to maintain both rectal and muscle surface temperature at or near 37 C.

Two muscle preparations were used. In half the experiments the left gastrocnemius-plantaris muscle group was used. The venous outflow from this muscle group was isolated as described previously (18) by tying all vascular connections to the left popliteal vein that did not come directly from the muscle group and all venous connections to the muscle group that did not connect directly to the popliteal vein. In about half the experiments on this muscle group preparation the Achilles tendon was cut close to the calcaneus. A clamp connected to the end of the tendon was in turn hooked to an isometric myograph for monitoring tension developed during each twitch while the muscle was contracting. A 1-inch length of the sciatic nerve was isolated near the muscle. The central end was doubly ligated and cut between the ties. The distal stump was stimulated to produce the muscle contractions.

In the other experiments the left lower leg was used. A 3-inch longitudinal incision was made through the skin of the medial aspect of the midthigh directly over the femoral artery and vein. A length of the sartorius muscle was isolated, ligated at each end, and the center 1½ inch removed, thereby exposing the femoral artery and vein. The artery was not disturbed. The vein was cleared for cannulation and the femoral nerve was divided. The sciatic nerve was isolated through an incision over the sciatic notch about halfway between the base of the tail and popliteal fossa. The central end of the exposed length was doubly ligated and cut between ties. The muscles of the lower leg were made to contract by stimulating the distal stump of the nerve. Foot flow was excluded from this preparation by a tight ligature of rubber tubing around the ankle. It was not possible to monitor the muscle contractions with a myograph.

Single stimuli were used in both preparations. Most preparations were stimulated at only one frequency

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throughout each experiment. Different preparations were exposed to frequencies of 0.5 impulse/sec–10 impulses/sec. Each preparation was pretreated to assure supramaximal stimulation; i.e., further increase in voltage did not increase twitch tension development by the single-muscle group. In the lower leg this evaluation was necessarily subjective and was based on the apparent contraction of the muscles involved.

In each preparation the vein draining the area examined was cannulated with the largest polyethylene cannula that would fit. The cannula was connected by Tygon tubing to a rotameter for recording venous outflow. The cannula was connected by saline-soaked gauze and a plastic sheet to reduce drying and evaporative cooling. Coagulation of the blood was prevented in most experiments by intravenous heparin or at s-hr intervals. In the remaining experiments intravenous heparin was used as the anticoagulant.

Exposed tissues of both preparations were covered with saline-soaked gauze and a plastic sheet to reduce drying and evaporative cooling. Coagulation of the blood was prevented in most experiments by intravenous Mepesulfate (sodium sulfated polygalacturonic acid methyl ester methyl glycoside, Hoffmann-La Roche, Nutley, N. J.) 100 mg/kg body wt. Additional doses of 100–300 mg were given if any fibrin appeared in the rotemeters or at ½-hr intervals. In the remaining experiments intravenous heparin was used as the anticoagulant, 90 mg/kg body wt with additional 20–40 mg doses when needed or at ½-hr intervals.

Arterial blood samples were taken from the right femoral artery. Venous blood samples were drawn through a length of small polyethylene tubing threaded through the wall of the venous outflow tubing to the tip of the cannula in the vein. The sampling catheters were flushed prior to drawing each blood sample. The arterial and venous samples were usually taken simultaneously as pairs into appropriately sized syringes, the dead spaces of which had been filled previously with 0.9% sodium chloride solution. Since the dead space of the various syringes used was relatively small and the calculations utilized arteriovenous differences, no correction was made for dilution of the samples by the dead-space volume.

For oxygen analysis 0.6 ml arterial and venous blood samples were drawn into 1-ml tuberculin syringes. These samples were analyzed for oxygen content spectrophotometrically (17). In each experiment at least two 8-ml blood samples were drawn into 10-ml syringes and analyzed for oxygen content both manometrically (20) and spectrophotometrically as a check on the spectrophotometric method.

Arterial and venous blood lactate concentrations were measured in 1-ml samples in the first 12 experiments by the method of Barker and Summerson (2) with the analyses being done in duplicate. In the remainder of the experiments single analysis of each sample was done by the enzymatic method of Horn and Bruns (7). The latter method was modified slightly after it was discovered that the optical density plateau was reached in less than the prescribed hour and was stable for only 30–40 min. In order to ensure that the optical density readings for the samples were made during the stable portion of the plateau the standards and samples were prepared in the same order as they were to be analyzed in the spectrophotometer. Then the blank and the 50 mg/100 ml lactate standard were placed in the cuvette holder of a Beckman DU spectrophotometer. This standard was read against the blank at 5-min intervals until constant optical density readings were observed. Then all optical densities of the samples and other standards were read and recorded. Finally the 50 mg/100 ml standard was read again to ensure that the plateau had persisted until all the samples were read.

Repeated analyses of one blood sample gave a mean lactate concentration of 75.1 mg/100 ml and a standard deviation of 1.6 mg/100 ml, whereas repeated analyses of another sample gave a mean of 20.0 mg/100 ml and a standard deviation of 0.4 mg/100 ml. Since the variability of the analyses was related to the concentration of lactate in the sample, the precision of the method is best described by a coefficient of variation, \( s/\bar{x} \), in this case giving a value of 2 for both of the above tests. Such data indicate that the precision of the method is good. Because of the good precision only a single analysis of each sample was done to allow more samples in each experiment. One or two of about 40 lactate values were 10–20% higher or lower than the preceding and succeeding samples. These samples were reanalyzed and a few were shown to have been the result of analytical errors. We were unable to determine the cause of the remaining odd data, which may have been the result of some mistake in sampling or may have been correct values. They were accepted as correct and were included in the data.
The Fick relationship was used to calculate the uptake or production of the metabolites as follows:

**oxygen uptake:**

\[ V_{O_2} = \frac{(C_{AO_2} - C_{VO_2})}{W} Q \]

**lactate production:**

\[ L = \frac{(C_{V_l} - C_{A_l})}{W} Q \]

where \( C_{AO_2} \) and \( C_{VO_2} \) = concentrations of oxygen (ml/ml blood) in arterial and venous blood, respectively; \( C_{A_l} \) and \( C_{V_l} \) = concentrations of lactate (mg/ml blood) in arterial and venous blood, respectively; \( Q \) = blood flow rate (ml/min); \( W \) = wet weight of the muscle (g) (measurable for the single-muscle group only).

The basic protocol was essentially the same in all experiments. After the electrodes had been tested, blood samples were drawn at intervals for measurement of lactate and oxygen for 10 min while the muscle preparation was at rest. Then the contractions were started at the desired twitch rate and continued until the end of the experiment, 40-60 min. In 14 of the experiments the twitch rate was changed once or twice during the contraction period to permit examination of two or three twitch rates in a single-muscle preparation.

**RESULTS**

The results obtained in the six experiments using Dial anesthesia on the single-muscle group preparations while they were contracting at 1 twitch/sec are shown in Fig. 1. Five of the six muscle groups showed lactate production while resting. When the contractions began these five preparations showed an increase in lactate production followed by a decrease. In two of these five experiments lactate production continued until the end of the experiment, whereas in the other three the lactate production decreased to zero and lactate uptake began. In the one odd experiment lactate appeared to be taken up at rest and there was an increase in lactate uptake during the contractions. The quantitative differences among the individual experiments were fairly large.

One of the experiments that showed lactate uptake is presented in more detail in Fig. 2. Although the pattern of lactate uptake is not necessarily typical of the mean of all experiments presented in this report, the magnitude of the blood flows at rest and during contraction and the blood lactate concentrations are close to the average values of all the experiments. Accordingly, the data are presented in detail in Table 1.

![Graph](http://ajplegacy.physiology.org/)

**Fig. 2.** Single-muscle group preparation L-17, Dial anesthesia: arterial and venous oxygen concentrations, arterial (\( C_{AO_2} \)) and venous (\( C_{VO_2} \)) lactate concentrations, blood flow (\( Q \)), oxygen uptake (\( V_{O_2} \)), and lactate production (\( L \)) before and during contractions at 1 twitch/sec. Vol % = ml/100 ml blood; mg % = mg/100 ml blood.

Although no rigorous attempt has been made to prove the statistical validity of each point on every curve of lactate production, some simple calculations may serve to illustrate the magnitude of uptake or production necessary to be considered significant. If we accept the .01 level of confidence and the variation in the lactate analysis described earlier, at lactate concentrations of 30 mg/100 ml, the average of all arterial and venous analyses in the experiments, the arteriovenous difference becomes significant when it is greater than 1.6 mg/100 ml. Using the average blood flow of the experiments in Fig. 1 as a multiplier, 15 ml/min at rest and 35 ml/min during contractions, and the average muscle weight, 60 g, then the lactate uptake or production is significant when it is greater than 4.0 \( \mu g/g \) per min at rest and 9.3 \( \mu g/g \) per min during contractions.

In the experiments presented below on the lower leg
TABLE 1. Protocol of sample experiment, L-17 (Fig. 2)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Elapsed Time, min</th>
<th>Blood Flow, ml/min</th>
<th>Blood Lactate Cvl, mg/100 ml</th>
<th>Cvl, mg/100 ml</th>
<th>v-a, mg/100 ml</th>
<th>L, mg/min</th>
<th>L, μg/g per min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>18.5</td>
<td>23.0±0.5</td>
<td>26.8±0.5</td>
<td>3.8</td>
<td>0.70</td>
<td>11.9</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>18.5</td>
<td>29.6±0.5</td>
<td>29.4±0.6</td>
<td>4.8</td>
<td>0.88</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>17.0</td>
<td>24.0±0.5</td>
<td>20.6±0.6</td>
<td>3.8</td>
<td>0.65</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Contraction started immediately after drawing sample 3

Muscle weight, 39 g. *Values are ± 20.

preparation (Figs. 3 and 4) the resting blood flow averaged 25 ml/min and the flow during contractions averaged 50 ml/min. Lactate uptake or production would be significant when it is greater than 0.4 mg/min at rest and 0.8 mg/min during contractions.

The results from the lower leg preparation with Dial anesthesia were similar to those obtained with the single-muscle group. The first 30 min of the six experiments shown in Fig. 3A and the first 30 min of the five experiments in Fig. 3C are comparable to Fig. 1. The patterns of lactate production and uptake during contractions at 1 twitch/sec are like those of the single-muscle group preparation. There was usually lactate production at rest, and when the contractions began, lactate production tended to increase and then decrease. Half the experiments reached zero lactate production or lactate uptake by 30 min, when the experimental procedure was altered. Differences between individual experiments are also evident in the lower leg preparation. Since the muscle of the lower leg preparation weighed about 100 g, the lactate production and uptake rates are similar to those of the isolated muscle preparation.

With pentobarbital anesthesia, Fig. 3B, the four lower leg preparations studied tended to take up lactate when resting. The transient increase in lactate production did not appear when samples were taken at 10-min intervals. However, in six of seven experiments on the single-muscle group during pentobarbital anesthesia, which are not shown, and in which lactate samples were drawn at 30-sec intervals for 20 min after the start of contractions, it was observed that lactate production increased immediately but by 10 min it had reached approximately zero. The seventh experiment showed lactate uptake at rest and throughout the contraction period. Apparently the transient period of lactate production at the beginning of contractions at 1 twitch/sec was of shorter duration when pentobarbital anesthesia was used than when Dial was used. After the muscles had been contracting for 20-30 min, the behavior of lactate seemed to be about the same regardless of the anesthetic used.

Increasing the twitch rate above 1/set did not alter the basic pattern of lactate production. Figure 4 shows the five experiments done at a single-twitch rate during pentobarbital anesthesia. The main differences between the lactate production patterns of the lower twitch rates (0.5, 1, 2 twitches/sec) and the higher twitch rates (3-10 twitches/sec) were a) the maximum rate of lactate production was higher at the higher twitch rates, and b) the duration of the transient lactate production was longer. Surprisingly, all five preparations were taking up lactate by the end of each experiment.

In 14 experiments (7 on the lower leg and 7 on the single muscle group) two or three twitch rates were examined during the contraction period. In six of these experiments, shown in Fig. 3A, the twitch rate was changed suddenly from 1 twitch/sec to 2 twitches/sec. The lactate production tended to increase transiently after the onset of the 2-twitch/sec contractions. When one of the lower twitch rates was succeeded by one of the higher twitch rates the second transient production of lactate was larger, being similar to those in Fig. 4. When a higher twitch rate was followed by a lower twitch rate there was no apparent effect on lactate production.

The experiments in Fig. 3B and Fig. 4, which were done with pentobarbital anesthesia, usually showed lactate uptake while the muscles were resting. In view of the frequent observation of lactate uptake during muscle contractions, the possibility came to mind that the lactate uptake observed during the control period may have been an aftereffect of testing the electrodes and the concomitant muscle contractions just prior to the start of the experimental period. Three test experiments using pentobarbital anesthesia were done in which muscle lactate production was measured before and after testing the electrodes. All three experiments showed lactate production prior to electrode testing (seven or eight twitch contractions) and lactate uptake after electrode testing, which continued until the end of the experiment 20 min later, even though there was no further stimulation of the muscle. Apparently the observed lactate uptake during the resting period was an artifact related to testing the electrodes. No attempt was made to study this response during Dial anesthesia.

One of the possibilities that could account for the observation of little lactate production or lactate uptake by these muscles is that the metabolism of these muscles is limited by substrate supply rather than by oxygen supply. Since part of the substrate supply of these muscles is from endogenous glycogen, it was thought that infusing epinephrine, as one of its effects, increase phosphorylase activity in the muscles and thereby increase the availability of glucose to the glycolytic cycle (5, 6, 19).

In five experiments under Dial anesthesia 0.4 μg/kg body wt per min of epinephrine was infused intravenously. The infusions were begun after the muscles had been contracting at 1 twitch/sec for 30 min and continued at a constant infusion rate for an additional 30 min. The contractions were continued without inter-
rupture until the end of the infusion. The data from these five experiments are presented in Fig. 3C. In one of the five experiments there was no evident response to the epinephrine infusion; lactate uptake began soon after the infusion was started and continued to increase until the end of the experiment. Four of the experiments showed increased lactate production after the infusion began, which tended to decrease toward the end of the experiment. Apparently the infusion of epinephrine increased the propensity for lactate production.

Oxygen uptake was measured in all the experiments on the single-muscle group preparation and was monitored in the lower leg preparation by drawing arterial and venous blood samples during the control period and at 10 and 40 min after the contractions began. The pattern of oxygen uptake typical of the single-muscle group contracting once per second is shown in Fig. 2. In this as in the other experiments at 1 twitch/sec, after the contractions began, oxygen uptake increased to 15 times the average resting rate, then decreased slightly during the last 10 to 15 min of the experiment. At 3 twitches/sec oxygen uptake reached a maximum of 30-40 times resting by 5-10 min after the contractions began; and then decreased by the end of the experiment to nearly the same level as was observed at 1 twitch/sec. For frequencies above 3 twitches/sec an oxygen uptake pattern similar to 3 twitches/sec was observed with the immediate rise to 30-40 times the resting value followed by a decrease to approximately 15 times the resting value.

During the studies using the single-muscle group preparation, in which the tendon from the muscle group was connected to an isometric myograph, the tension developed during each contraction was measured. At 0.5 and 1 twitch/sec the tension developed during each twitch reached 6-8 kg (the initial resting tension was about 200 g) and was maintained for the duration of the contractions. Twitch tension was not maintained at the higher twitch rates. These experiments were not intended as a careful study of this relationship, but the data seem to indicate a progressive decrease in twitch tension as the twitch rate increased. The extreme observed was for 10 twitches/sec where the tension of 6-8 kg, developed at the beginning of the experiment, fell to approximately 4.5 kg after 15 min of contractions, where it remained until the end of the experiment.

**DISCUSSION**

The observation of lactate uptake by many of the muscle preparations at all twitch rates studied is indeed an interesting observation. However, the amount of lactate taken up was variable and it was not seen in every experiment. The trend in the experiments was toward approximately zero lactate production as the duration of the contractions continued. The main concern in this discussion is relative to the cause of the low lactate production as the contractions continued, especially at the higher twitch rates. Although there was fairly brisk lactate production at the higher twitch rates immedi-
The average response to is included, A-A.

Astrand et al. (1). They report low values of blood lactate after prolonged maximal work. They suggest that there is a different kind of fatigue which limits performance depending on the duration of severe exercise. We would suggest a common limiting factor in their experiments and those reported here.

The observation that oxygen uptake does not continue to rise when the frequency of contractions is increased beyond a critical value has been reported previously by Merker et al. (12). These authors believed that oxygen uptake could not be elevated further because the blood flow and hence the supply of oxygen had reached a maximum value. In the experiments reported here the highest twitch rate at which oxygen uptake was maintained at least fairly constant was 1 twitch/sec. The maximal oxygen uptake was observed at 3 twitches/sec but this level was not maintained. At twitch rates higher than 1/sec when the oxygen uptake decreased as the contractions continued, the tension developed during each contraction also decreased. It certainly seems that something was limiting oxygen uptake and muscle performance. If oxygen supply were the limiting factor surely lactate would have been produced in large quantities (8, 10).

The absence of large lactate production and the observation of lactate uptake in many experiments appear to preclude the possibility that, under the circumstances of these experiments, oxygen was the rate-limiting factor.

Another possibility that has been suggested is that the contractile mechanism, perhaps as a result of some change in its immediate environment during the contractions, is not able to utilize energy beyond a certain rate and that this rate decreases as a part of fatigue (14). If the ability of the contractile mechanism to utilize energy is below the capability of the blood to supply oxygen and that of the metabolic machinery to utilize the oxygen and produce energy, then oxygen is not likely to limit metabolism. Perhaps the diffusion of energy-carrying intermediate substances to and from sites of utilization and production limits the maximum metabolic rate. The possibility also exists that the metabolic machinery is not able to supply energy as fast as the contractile machinery can use it. Besides oxygen, substrate, both fat and carbohydrate, is a large input to the metabolic machinery. If, for example, the carbohydrate supply from exogenous glucose and endogenous glycogen is not adequate to meet demand, then the maximum metabolic rate may be limited without production of lactate (3, 4). The increased tendency for the muscles to produce lactate during epinephrine infusion implies support of this hypothesis. Unfortunately, epinephrine has multiple effects and, as a result, these experiments cannot be accepted as proof of the hypothesis.

The transient production of lactate when the contractions were begun or when the twitch rate was increased suddenly from a lower twitch rate to a higher one may result from at least two factors. This lactate production may stem from aerobic glycolysis as does resting lactate production (19), or oxygen supply may be inadequate transiently and lactate formed as a result of hypoxia. Perhaps both these mechanisms are involved.

The propensity for lactate production appeared to be greater with Dial anesthesia than with pentobarbital anesthesia. This difference may have come from inequalities in depth of anesthesia, but we have no evidence in this regard. We know of no other reasons for these anesthetic agents to alter the results.

REFERENCES


