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LACTATE OXIDATIVE CAPACITY IN DIFFERENT TYPES OF MUSCLE

by

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SUMMARY

The capacity of different types of muscle homogenates to oxidize lactate (2.0 to 10.0 mM) under state 3 conditions was determined in the context of their maximal pyruvate oxidative capacity. Both cardiac and fast-oxidative-glycogenolytic (FOG) muscle oxidized 10 mM lactate at approximately 100% of their respective capacities to maximally oxidize pyruvate (2.5 mM); whereas slow-oxidative (SO) and fast-glycogenolytic (FG) skeletal muscle oxidized lactate at 78% and 63% of their respective capacities for pyruvate. The lactate oxidation correlated with (a) pyruvate and NADH oxidation and (b) the gross lactate dehydrogenase heart type isozyme profile in the different muscle types. These results suggest that different muscle types possess quantitatively and qualitatively different capacities for metabolizing lactate.

Although blood lactate accumulation during exercise primarily results from skeletal muscle (low oxidative fibers) production (1,2,3), recent findings also suggest that both cardiac and skeletal muscle (high oxidative fibers) readily oxidize blood derived lactate during exercise (2,3,4,5). However, the optimal capacity for lactate oxidation in a given muscle type cannot be predicted, since there is a wide spectrum of both total LDH and LDH isozyme subunit (Hand M) activity in different types of muscle (6). Furthermore, since the equilibrium constant of the LDH reaction normally lies markedly

abbreviations: Fast-oxidiative-glycogenolytic (FOG); slow-oxidative (SO); fast glycogenolytic (FG); lactate dehydrogenase (LDH); malate dehydrogenase (MDH); nictone adenine dinucleotide (NAD and NADH); LDH heart and skeletal isozyme (H and M).

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in favor of lactate formation (7), the potential for reversing the reaction is also dependent on the muscle's capacity to oxidize both pyruvate and NADH during state 3 metabolic conditions. Therefore, the objective of this study was to determine the capacity of different types of muscle homogenates to oxidize varying physiological concentrations of lactate in the context of their maximal capacity for pyruvate and NADH oxidation, total LDH activity, gross LDH isozyme profile, and cytoplasmic and mitochondrial levels of MDH activity.

METHODS

Tissue Preparation: Adult female rats of a Wistar strain, weighing approximately 300 grams, were housed in light and temperature controlled quarters and provided with Purina lab chow and water ad libitum. Three series of experiments were conducted consisting of: (1) heart muscle pyruvate and lactate oxidation; (2) skeletal muscle fiber-type pyruvate and lactate oxidation; and (3) measurements of LDH and of MDH activity in different types of muscle. For each series, the animals were killed by decapitation and exsangunated. In series 1 and 2, 400 mg portions of heart, red vastus (FOG), white vastus (FG) and soleus (SO) muscle were homogenized in 9.6 ml of 175 mM KCl, 2 mM EDTA, pH 7.4, and the homogenates were used immediately for substrate oxidation. In series 3, the different muscle types were processed as described above, and the samples were placed on ice and used for measurements of enzyme activity.

Assay Procedures: Oxygen uptake was measured in a Gilson differential respirometer at 30°C with air as the gas phase according to procedures described by Holloszy (8). Reaction mixtures consisted of the following in a final volume of 2 ml: 20 mM potassium phosphate, 20 mM KCl, 1.6 mM EDTA, 5 mM $MgCl_2$, 1 mM sodium malate, 2 mM ADP, 50 mM sucrose, homogenate equivalent to 40 mg and either sodium lactate or sodium pyruvate (2.5 to 10.0 mM), final pH 7.4. In lactate oxidation reactions, 2.0 mM NAD was also included. Reaction rates were carried out for 30 min in the presence and absence of the designated substrate. Basal rates without substrate (averaging less than 10% of the total activity for a given muscle) were subtracted from the rates with substrate. Also, reactions were carried out with 2.0 mM NADH substituted for the normal substrate in order to determine the capacity of the muscles to oxidize this cofactor separately.

Measurements of total LDH activity were performed at 30°C as described by Pesce et al. (9). Relative amounts of H and M isozymes in muscle homogenates were distinguished as described

by Wilson et al. as based on substrate inhibition (10). Total LDH activity consisting of the H subunit H_4, H_3M_1, H_2M_2, H_1M_3) was estimated on the basis of the total activity LDH activity determined in the present study and the total percentage of H subunits found in different types of rodent muscle as reported by Peter et al. (6). These estimates of total H activity were used for correlational purposes only (Fig. 2).

MDH activity was measured by following the rate of NADH oxidation as described by Holloszy et al (11). Mitochondrial and cytoplasmic MDH were distinguished as described by Shonk and Boxer (12).

RESULTS

Substrate oxidation - Maximal pyruvate oxidation rates for each muscle type occurred at 2.5 mM concentration (table 1). Increasing the lactate concentration from 2.5 to 10.0 mM resulted in rates of 63, 99, 80, and 104%, the capacity for pyruvate in FG, FOG, SO, and cardiac muscle, respectively. All homogenates also were capable of oxidizing NADH (table 1). These rates, although low, were in proportion to the overall oxidative capacity of the different muscle types. Also, they were consistent with the profile of cytoplasmic and mitochondrial MDH activity for the different muscle types (table 1). Figure 1 shows that lactate oxidation correlates highly with both the pyruvate and NADH oxidative capacity of the muscle types.

LDH Activity: As shown in table 2, total LDH activity, as measured at 1.0 mM pyruvate concentration, was highest in FG muscle. Levels in cardiac and in FOG muscle were similar averaging approximately 70% the level measured in FG muscle. However, soleus muscle contained less than 30% the total seen in FG vastus.

Both FG and FOG muscle were less sensitive to 10 mM pyruvate inhibition as compared to soleus and cardiac muscle (table 2). This indicates that the two fast-twitch types of muscle contain a lower percent of the H type subunit relative

Table 1. FG vastus, FOG vastus, soleus, and heart muscle MDH activity and capacity to oxidize pyruvate, lactate, and NADH.

		FG Vastus (5)	FOG Vastus (5)	Soleus (5)	Heart (5)
Pyruvate*	2.5 mM	3213 ± 310	8350 ± 126	5163 ± 185	17,326 ± 780
Lactate*	2.5 mM	1350+ ± 106	4625+ ± 196	2800+ ± 234	12,150+ ± 311
	10.0 mM	2025++ ± 402	8275++ ± 809	4125++ ± 312	18,175++ ± 339
NADH*	2.0 mM	103 ± 25	750 ± 70	450 ± 50	2,870 ± 402
MDH µmol/min/G Muscle	Cytoplasmic	285 ± 16	536 ± 26	576 ± 30	698 ± 31
	Mitochondrial	205 ± 12	536 ± 22	350 ± 21	1,285 ± 45

Values are mean ± SEM. Number of measurements is given in parenthesis. *-$\mu lO_2/g/hr$; +2.5 mM pyruvate vs. 2.5 mM lactate ($P<.05$); ++ - 2.5 mM lactate vs. 10 mM lactate ($P<.05$)

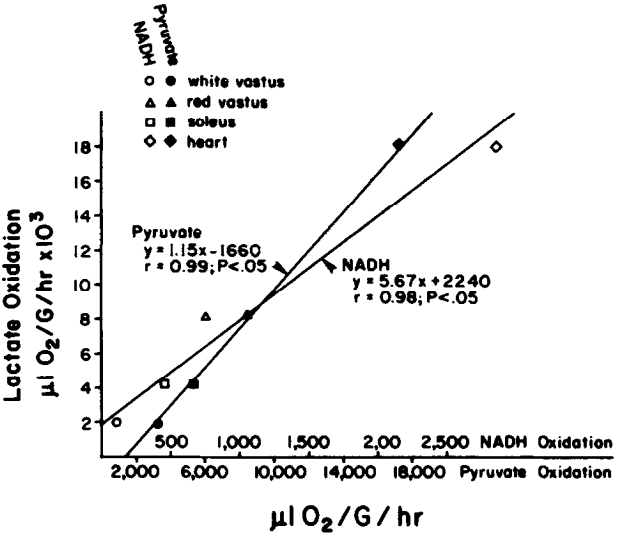

Figure 1: Correlations of pyruvate oxidation (2.5 mM) and of NADH oxidation (2.0 mM) with lactate oxidation (10 mM) in different types of skeletal muscle and in cardiac muscle. Averaged values for each parameter were plotted.

to total activity as compared to soleus and cardiac muscle. As shown in Fig. 2, total LDH activity, irrespective of isozyme content, did not correlate with lactate oxidation ($r = 0.112$).

Table 2. Total LDH activity and ratios of LDH activity at different concentrations of pyruvate in FG vastus, FOG vastus, soleus, and heart muscle.

Concentration	FG Vastus(6)	FOG Vastus(6)	Soleus(6)	Heart(6)
0.33 mM	801 ± 66	661 ± 45	327 ± 26	710 ± 52
1.0 mM	1065 ± 71	759 ± 76	327 ± 43	703 ± 56
10.0 mM	655 ± 82	345 ± 38	102 ± 7.0	198 ± 20
0.33/10.0 mM	1.30 ± 0.17	1.92 ± 0.12	3.27 ± 0.33	3.64 ± 0.24

Activity is expressed as μMol/g muscle/min; values are means ± SEM. Number of determinations is given in parenthesis.

However, lactate oxidation did correlate with the LDH isozyme profile reflecting estimated total H subunit activity in the different muscle types ($r = 0.96$; $p < .05$, Fig. 2).

DISCUSSION

The present findings, although representing experiments performed *in vitro*, provide direct evidence that all types of muscle can readily oxidize lactate. However, this capacity is both quantitatively and qualitatively different among the spectrum of muscle examined. For example, for a given level of mitochondrial respiration occurring in FOG and FG muscle, the same level of lactate might be expected to contribute a greater percent of the derived energy in the former as compared to the latter, because FOG muscle has a much greater relative capacity to convert lactate to pyruvate than FG muscle. Also, as in intact animal experiments (4), the capacity for lactate oxidation is concentration dependent, since lactate levels that would be normally present in blood during strenuous exercise (10 mM) were oxidized at a faster rate than levels normally seen at rest (2.5 mM).

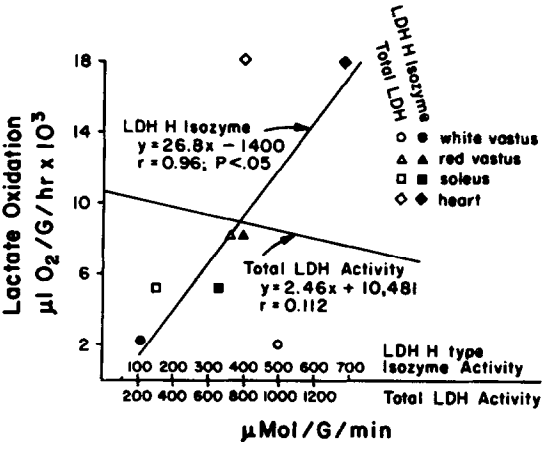

Figure 2: Correlations of total LDH activity and of the estimated total LDH H isozyme activity with lactate oxidation rates in different muscle types. Averaged values for each parameter were plotted.

Interestingly, lactate oxidation appears to be related, in part, to a given muscle's total amount of LDH H subunit activity. Stambaugh and Post (13) found that the H isozyme is more sensitive than the M isozyme to product inhibition by lactate. Neither type is sensitive to substrate inhibition by lactate in physiological concentrations. Moreover, the sensitivity of the H isozyme to substrate inhibition by pyruvate is never reached physiologically in muscle cells (13). Consequently, the role of the H subunit in muscle may serve to better facilitate lactate conversion to pyruvate under appropriate metabolic conditions. Although the equilibrium constant of the LDH reaction normally favors lactate formation (7), this reaction could easily be reversed in the high oxidative muscle types during exercise by (a) elevating cellular lactate via diffusion from blood and (b) turning on mitochondria via contraction to oxidize both pyruvate and NADH (table 1). These responses collectively would increase the

ratios of lactate/pyruvate and of NAD/NADH and thus favor lactate conversion to pyruvate for subsequent oxidation.

Consequently, in light of the wide metabolic spectrum existing in different types of muscle for glycogenolysis and oxidative metabolism, it seems likely that different types of skeletal muscle are simultaneously involved in lactate production (FG fibers) and utilization (cardiac and oxidative red fibers) for producing ATP to support muscle contraction.

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