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EXTRA O_2 CONSUMPTION ATTRIBUTABLE TO $NADH_2$ DURING MAXIMUM LACTATE OXIDATION IN THE HEART

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The lactate/pyruvate oxidation (Qo_2) ratio was 1.21 ± 0.04 for heart homogenates as compared to 0.92 ± 0.05 for white quadriceps muscle homogenates during state 3 respiration. The extra lactate Qo_2 could be accounted for by the oxidation of additional $NADH_2$ from lactate, assuming the oxidation of 12 H^+/lactate and 10 H^+/pyruvate. A high correlation of 0.92 was observed between extra lactate Qo_2 and activity of heart-type LDH isozyme. This finding and the mitochondrial location of heart-type isozyme (1) suggests the extra lactate Qo_2 in heart homogenates could represent the oxidation of $NADH_2$ formed from lactate by the mitochondria.

Kaplan (2) has suggested that tissues with a predominance of the heart form (H) of lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) might be able to oxidize lactate more readily than those with low H isozyme, but data to support this contention are not available. Several recent findings now make it possible to test this postulate. First, Baba and Sharma (1) have shown histochemically that the parent H_4 isozyme and the hybrid H_3M of lactate dehydrogenase (LDH) are associated with the outer and inner mitochondrial membranes, whereas the parent M_4 isozyme and the hybrid H_3M of LDH are associated primarily with the sarcoplasmic reticulum of heart (HT) and white skeletal muscle in the rat. Second, white quadriceps (WQ) muscle contains about 99% M-LDH isozyme (3) and can be isolated by visual inspection (4) as readily as HT which has about 78% H-LDH isozyme (5). Thus, if the H isozyme enhances lactate oxidation (Qo_2), it might be readily apparent in HT, but may not be in WQ muscle. This hypothesis was tested by comparing the lactate/pyruvate (L/P) Qo_2 ratios for HT and WQ homogenates from female rats during maximum state 3 respiration with excess ADP, P_i, malate, cytochrome c, and optimal substrate. The L/P Qo_2 ratio was used to normalize HT and WQ for differences in maximum

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pyruvate oxidation due to mitochondrial content, thereby facilitating the comparison of these tissues for any additional O_2 consumption attributable to lactate.

METHODS: Six female rats of a Wistar strain (Charles River) were 6 mo of age and had a mean body weight of 282 ± 7 g at sacrifice. Immediately after decapitation, the heart (HT) and the superficial white portion of the quadriceps muscle (WQ), which consists essentially of fast-twitch fibers of low oxidative but high glycolytic capacity (4), were rapidly excised, rinsed in cold saline, and placed on ice. The valves, large vessels, and atria were removed from the HT, and the ventricles were minced, mixed, and weighed. Homogenates of each tissue (1:10, w/v) were prepared in cold 175 mM KCl + 2 mM EDTA, pH 7.3, as recently described (6).

An aliquot of each homogenate was frozen for determination of total LDH activity with 0.33 mM pyruvate as previously described (7). The percent inhibition of LDH by 10 mM pyruvate was also determined so that the fraction of the activity attributable to the H and M isozymes could be computed according to the method of Bernstein and Everse (8). LDH isozyme bands were also evaluated by standard starch-gel electrophoretic techniques.

A Clark polarographic electrode (YSI) was used to measure the maximum oxidation of lactate and pyruvate. The electrode was calibrated with water saturated with room air (20.9% O_2) at 30°C, assuming the O_2 solubility in water at this temperature is 5.45 μliters O_2/mℓ water at a pO_2 of 158.8 mm Hg. The oxidation of lactate and pyruvate was determined in duplicate for HT and WQ homogenates during state 3 respiration with excess ADP, P_i, malate, and optimal substrate. Assay conditions were established such that doubling the amount of homogenate (0.005 to 0.01 g muscle) resulted in a proportional increase in O_2 consumption. The concentration of reagents in the reaction mixture for pyruvate Qo_2 were as previously given (6). The assay for lactate Qo_2 was the same as that for pyruvate, except 10 mM lactate replaced 10 mM pyruvate and 1.25 mM NAD^+ was added to the mixture (final concentrations). As recently demonstrated (6), exogenous cytochrome c (Sigma Type IIA) is required for maximum oxidation. Therefore, cytochrome c (final concentration of 0.075 mM) was added to the reaction vessel after ADP to determine the maximum lactate and pyruvate Qo_2. The cytochrome stimulated state 3 Qo_2 about 70 and 6% for HT and WQ homogenates, respectively, with no differences observed for lactate and pyruvate substrates. Respiration with cytochrome c was not evident in the absence of either substrate or tissue.

RESULTS: Table 1 illustrates that homogenates of HT oxidized lactate 7.3x and pyruvate 5.4x faster than those of WQ. Only HT exhibited a greater lactate relative to pyruvate oxidation (Table 1). The L/P Qo_2 ratio was 1.21 ± 0.04 for HT homogenates as compared to 0.92 ± 0.05 for WQ homogenates during maximum, state 3 respiration with excess ADP, P_i, cytochrome c, malate, and optimal substrate.

Total LDH activity, with pyruvate as substrate, was high for both tissues (Table 1), but HT exhibited markedly greater H-LDH activity of 470 ± 36 compared to 41 ± 2 μmoles/min/g muscle for WQ. The correlation between extra

TABLE 1. Maximum Lactate and Pyruvate Oxidation, LDH Activity, and %H Isozyme for Heart and White Quadriceps Muscle of Female Rats.

VARIABLE[a]	HEART	WHITE QUADRICEPS
Lactate Qo_2	17,622 ± 358[b]	2,425 ± 154
Pyruvate Qo_2	14,534 ± 869	2,683 ± 107
L/P Qo_2 Ratio	1.21 ± .04[c]	0.92 ± .05[d]
LDH Activity[e]	628 ± 44	817 ± 18
%H Isozyme	74.9 ± 2.1	5.0 ± 2.3

[a]Values represent the mean ± 1 SEM for 6 female rats. Qo_2 is expressed in units of µliters O_2/hr/g wet weight muscle.
[b]Heart lactate Qo_2 versus pyruvate Qo_2, $P < 0.05$
[c]L/P Qo_2 ratio is significantly greater than 1.0, $P < 0.05$.
[d]L/P Qo_2 ratio is not significantly less than 1.0, $P > 0.05$.
[e]LDH determined with 0.33 mM pyruvate; activity is expressed as µmoles NADH oxidized/min/g muscle at 30°C.

lactate Qo_2 and H isozyme activity for HT and WQ was equal to 0.92. The least-squares regression equation was $\Delta Qo_2 = 6.74(H) - 339$, where ΔQo_2 is the difference in lactate and pyruvate Qo_2 expressed as µliters O_2/hr/g muscle and H represents H-LDH activity in µmoles $NADH_2$ oxidized/min/g muscle. The negative intercept of -339 was not significantly different from zero ($P > 0.05$). The regression analysis suggests there is a proportionality between extra lactate Qo_2 and H isozyme content of muscle.

DISCUSSION: We have shown that only HT exhibited a greater lactate relative to pyruvate Qo_2. That homogenate Qo_2 is substrate-specific is supported by the following observations made in preliminary experiments. Lactate Qo_2 was entirely dependent on exogenous NAD^+. Endogenous respiration with ADP was low with either HT or WQ homogenate. The addition of lactate or pyruvate markedly increased the Qo_2 to a maximum which was between 10 and 15 fold above the endogenous rate. The addition of rotenone to block the entry of $NADH_2$ into the respiratory chain effectively inhibited state 3 respiration of lactate and pyruvate. We conclude our homogenate lactate and pyruvate Qo_2 activities re-

present the oxidation of these substrates, and therefore, it is reasonable to assume they are entirely mitochondrial processes.

The focus of the present study was to determine if extra lactate Qo_2 was associated with H-LDH activity of HT and WQ. That mitochondrial H-LDH might be involved is suggested by the high correlation of 0.92 observed between extra lactate Qo_2 and H isozyme activity; HT with high H activity exhibited extra lactate Qo_2, but WQ with low H activity showed lactate Qo_2 was equal that of pyruvate. Obviously these data do not establish causality, but they are consistent with the cellular location of the H and M isozyme in these muscles. Baba and Sharma (1) have shown the H isozyme is associated with the outer and inner mitochondrial membranes, whereas the M isozyme is associated primarily with the sarcoplasmic reticulum of HT and white muscle of the rat.

It seems reasonable to assume that the M and H isozymes have distinct metabolic roles which influence the oxidation of lactate and pyruvate (2). This might be achieved by virtue of the topographic specificity within the cell, the absolute activities in each compartment, and the kinetic properties of the LDH isozymes. Perhaps the former two possibilities are paramount, since it is uncertain whether the unique kinetic properties demonstrated on the purified LDH isozymes (9) relate to those of our homogenate system in state 3. Regardless, the difference in the L/P Qo_2 ratios between HT and WQ homogenates in state 3, where conditions are presumably optimized, could represent compartmentalized LDH isozyme function. The cytosolic M and the mitochondrial H isozymes could predominate in WQ and HT, respectively.

How might this compartmentalized isozyme function relate to maximum lactate and pyruvate Qo_2 in state 3? Let us examine the easier case of WQ, where lactate was oxidized at the same rate as pyruvate. If the cytosolic M isozyme reaction was paramount, then extra $NADH_2$ formed from lactate in the cytosol would not be oxidized by the respiratory chain, since it cannot penetrate the inner mitochondrial membrane (10). Thus, the L/P Qo_2 ratio of unity observed for WQ suggests the rate of uptake and oxidation of pyruvate was the same,

whether arising from pyruvate directly or from lactate. This interpretation implies the pyruvate concentration in our homogenate system was optimal for maximum Qo_2 with either substrate. Although the pyruvate concentration was not measured, several considerations support this contention. The unfavorable equilibrium constant of LDH in the direction of pyruvate formation would maintain $NADH_2$ and pyruvate concentrations low. Nevertheless, the steady state concentration could be sufficient to maintain maximum lactate Qo_2, since we observed the apparent K_m for pyruvate oxidation was also extremely low (about 0.01 mM). A limiting pyruvate concentration causing a submaximal Qo_2 with pyruvate as substrate can be excluded as a possibility because the 10 mM pyruvate added was far in excess and its removal by LDH likely was minimized by the omission of $NADH_2$ in the reaction mixture. Thus, with either substrate, the Qo_2 represented maximum uptake and oxidation of pyruvate in state 3 for WQ; cytosolic $NADH_2$ from lactate was not oxidized.

Lactate was oxidized 1.20x faster than pyruvate in HT homogenates. This finding could mean that the primary reaction was initiated at the site of the mitochondrial membranes, with the action of the H isozyme facilitating pyruvate uptake and/or $NADH_2$ transfer from lactate into the mitochondria by some undefined mechanism. The possibility that lactate diffuses into the mitochondria before reacting with the H isozyme implies a dependency on the mitochondrial pool of NAD^+. Such a process is discounted by the fact that lactate Qo_2 is completely dependent on exogenous NAD^+ (unpublished observation). If carbon flux through the pyruvate dehydrogenase (PDH) complex and tricarboxylic acid (TCA) cycle with lactate was equal to that with pyruvate in HT, as appears to occur for WQ, then production of $NADH_2$ by the PDH complex and TCA cycle and its oxidation by the respiratory chain also would be the same for both substrates. However, the LDH reaction produces an additional $NADH_2$/lactate. If this extra $NADH_2$ is oxidized, then the L/P Qo_2 ratio would be greater than unity. The expected L/P Qo_2 ratio would be 1.20 only if equal carbon fluxes occurred with lactate and pyruvate substrates, since the stiochiometry is 6

O_2/12 H^+ for lactate and 5 O_2/10 H^+ for pyruvate (11), thereby making the ratio of the O_2 rate equivalent for the utilization of these substrates 1.20. It follows that the L/P Qo_2 ratio can only be greater than 1.20 when both pyruvate and $NADH_2$ from lactate are oxidized at a faster rate than with pyruvate as substrate. We found the L/P Qo_2 ratio for HT to be exactly 1.20. This finding, viewed in the context of the preceding analysis, suggests the extra lactate Qo_2 of HT homogenates can be completely accounted for by the oxidation of additional $NADH_2$ formed from lactate by the mitochondrial H-LDH isozyme. Therefore, the assumption of equality of carbon flux for both substrates appears to hold; facilitated pyruvate transfer by the H isozyme would not appear to be involved in the extra lactate Qo_2 of HT homogenates. This conclusion is consistent with the finding that the L/P Qo_2 ratio was not significantly different from unity in WQ, since absence of H-LDH function to facilitate pyruvate flux and $NADH_2$ in this muscle would require the L/P Qo_2 ratio to be less than unity.

It must be reiterated that the findings of the present study do not establish causality. In this regard, experiments are needed to establish that extra lactate Qo_2 represents the oxidation of the additional $NADH_2$ formed from lactate as argued. Also, it should be shown with more definitive experiments that the extra lactate Qo_2 is a property of mitochondria which exists only when the compliment of H isozyme is intact. In addition, it must be shown that extra lactate Qo_2 does not represent $NADH_2$ oxidation of contaminating submitochondrial particles (12) or enzymes other than H-LDH, such as L-lactate cytochrome dehydrogenase (13), NAD-specific malic enzyme (14), microsomal $NADH_2$ cytochrome c oxidoreductase (15), or the cytosolic component of the α-glycerophosphate and/or malate-aspartate H^+ shuttle. Until these questions are resolved, it would be premature to speculate as to the metabolic significance of the relationship between extra lactate Qo_2 and H isozyme activity observed in the present study.

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REFERENCES:

1. Baba, N. and Sharma, H. M. (1971) J. Cell Biol. 51:621-635.
2. Kaplan, N. O. (1965) J. Cell Compara. Physiol. 66(Suppl. 1):1-10.
3. York, J. W., Oscai, L.B., and Penney, D. G. (1974) Biochem. Biophys. Res. Commu. 61:1387-1393.
4. Baldwin, K. M., Klinkerfuss, G. H., Terjung, R. L., Molé, P. A., and Holloszy, J. O. (1972) Am. J. Physiol. 222:373-378.
5. York, J. W., Penney, D. G., and Oscai, L. B. (1975) Biochem. Biophys. Acta. 381:22-27.
6. Van Handle, P. J., Sandel, W. R., and Molé, P. A. (1977) Biochem. Biophys. Res. Commu. 74:1213-1219.
7. Molé, P. A., Baldwin, K. M., Terjung, R. L., and Holloszy, J. O. (1973) Am. J. Physiol. 224:50-54.
8. Bernstein, L. H. and Everse, J. (1975) Methods in Enzymology. Vol. 41B, p. 47-52. Ed. by W. A. Wood. Academic Press, New York.
9. Vessel, E. S. (1965) Science 150:1590-1593.
10. Lehninger, A. L. (1951) J. Biol. Chem. 190:345-359.
11. Lehninger, A. L. (1970) Biochemistry, p. 338. Worth Publ., New York.
12. Hedman, R. (1965) Exper. Cell Res. 38:1-12.
13. Symons, R. H. and Buroyne, L. A. (1966) Methods in Enzymology, Vol. 9, p. 314-321. Ed. by W. A. Wood. Academic Press, New York.
14. Davis, E. J., Lin, R. C., and Chao, D. L. (1972) Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria. p. 211-238. Ed. by M. A. Mehlman and R. W. Hanson. Academic Press, New York.
15. Mackler, B. (1967) Methods in Enzymology, Vol. 10, p. 551-553. Ed. by R. W. Estabrook and M. E. Pullman. Academic Press, New York.