Influence of Mitochondrial Content on the Sensitivity of Respiratory Control*

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This study evaluated the sensitivity of mitochondrial respiratory control as a function of tissue oxidative capacity. The mitochondrial content of rat skeletal muscle was increased by exercise training or decreased by hypothyroidism. Muscles of the lower hindlimb were stimulated to tetanically contract in situ for 3 min at one of four frequencies to elicit a 30-fold range of oxygen consumption rates. Freeze-clamped sections of fast-twitch red gastrocnemius muscle were extracted and analyzed for metabolite levels. The sensitivity of mitochondrial content increased by exercise training or decreased by hypothyroidism.

Increases in the sensitivity of mitochondrial respiratory control resulted in lower flux through the near-equilibrium energy exchange reactions of creatine kinase and myokinase such that calculated free concentrations of ADP and AMP were lower. Other energetically important reactions/pathways were also affected. Accumulation of lactate and the deamination of AMP to IMP were lower in tissues with higher mitochondrial content. In summary, changes in oxidative capacity and respiratory control sensitivity. The span of oxygen consumption rates. The smaller relative changes in cytosolic modulator(s) of oxidative phosphorylation would be required to elicit the same rate of oxygen consumption. The smaller relative changes in cytosolic modulator(s) associated with increased sensitivity of respiratory control should also have important implications for the control of other ATP-generating pathways. A change in respiratory control sensitivity with oxidative capacity is particularly important in skeletal muscle where mitochondrial content varies considerably between fiber types and adapts to the imposed energy demand.

Skeletal muscle is an especially appropriate tissue in which to examine the relationship between maximal oxidative capacity and respiratory control sensitivity. The span of oxygen consumption rates is very large, being able to increase 30 times that of rest, and can be precisely regulated by experimentally controlling the energy demand in a physiologically intact system. We have chosen to study rat fast-twitch red muscle because the muscle is relatively fatigue-resistant due to a high mitochondrial content and blood flow capacity.

We report here experiments designed to test the hypothesis that muscle mitochondrial content is a crucial determinant of the sensitivity of mitochondrial respiratory control and that differences in sensitivity have important consequences for the control of flux through adenylate kinase, creatine kinase, AMP deaminase, and lactate end point glycolysis.

MATERIALS AND METHODS

Animals—Male Sprague-Dawley rats were housed in a constant temperature room (20°C) with 12 h of light/day. Purina rat chow and water were provided ad libitum. One group of rats was exercise trained using a motor-driven treadmill by a previously described protocol; a second group was rendered hypothyroid by supplying 0.04% propylthiouracil in the drinking water; a third group served as sedentary controls.

Experimental Protocol—Animals were anesthetized by pentobarbital sodium (66 mg/kg, intraperitoneally), and a catheter (PE-50) for monitoring arterial pressure was inserted in the right carotid artery. The in situ stimulation procedure was exactly as described previously, with the gastrocnemius-plantaris-soleus muscle group stimulated to develop maximal isometric tension with trains of impulses (0.1-s square waves at 6 V at 100 Hz for 100-ms duration) via the tibial nerve at 7.5, 15, 30, or 45 tetani/min. After 3 min of stimulation the deep red portion of the lateral gastrocnemius (primarily fast-twitch red fibers, 15, 19) was freeze-clamped in tongs cooled in liquid nitrogen. This duration of stimulation was selected to permit sufficient time for changes in appropriate metabolites to proceed (21, 27). Tension production was monitored with a force transducer.
transducers attached to the Achilles tendon and arterial pressure was recorded continuously. The unstimulated contralateral muscle section served as the control. While the entire stimulated muscle mass is comprised of different fiber types, only the fast-twitch red muscle section was selected for study, because it has an extremely high mitochondrial content (16), a very high blood flow and oxygen delivery (17), and a very high capacity for aerobic work (15).

Analyses—Frozen muscle samples were extracted in cold, alkaline perchloric acid (22). Neutralized muscle extracts were assayed for ATP (23), ADP (24), AMP (24), creatine phosphate (23), creatine (23), F$_h$ (26) as modified (27), IMP (28), NH$_4$ (29), lactate (30), and pyruvate (26) by conventional enzymatic techniques. Metabolite concentrations were corrected in each experimental group for changes in water content that occur with contractions using total creatine (31). To assess differences in oxidative capacity among treatment groups, citrate synthase was assayed (32) as a mitochondrial marker enzyme and cytochrome c was extracted and measured spectrophotometrically (33). Citrate synthase and cytochrome c were determined in the unstimulated control muscle section.

Calculations—Since ADP and AMP are largely bound, free concentrations of these nucleotides have been calculated using the reactants and equilibrium constants of the near-equilibrium reactions catalyzed by creatine kinase and adenylate kinase, respectively. Free ADP (ADP$_f$) was calculated on the basis of measured concentrations of ATP, creatine phosphate, creatine, and the calculated H$^+$ concentration following Lawson and Veech (34). Muscle cell pH was estimated by the relationship established by Sahlin et al. (35) between pH and the sum of the lactate and pyruvate concentrations as described earlier (27). Likewise, free AMP was calculated using the calculated free ADP (36). Free P$_i$ (P$_{ii}$) in resting muscle was assumed to be 2.5 mM on the basis of $^{31}P$ NMR measurements in rat skeletal muscle (36, 37). In stimulated muscles P$_i$ has been calculated as the difference between the mean resting creatine phosphate concentration and the measured creatine phosphate concentration of the stimulated muscle plus 2.5 mM P$_i$ present in resting muscle. Increases in P$_i$ with stimulation have been shown to be stoichiometric with creatine phosphate breakdown (36). The phosphorylation state potential (ATP/ADP$_f$ X P$_{ii}$) was calculated using the measured ATP concentration and estimated free ADP and P$_i$ concentrations.

Muscle oxygen consumption was estimated from the oxygen cost of tetanic contractions obtained from recent experiments (15) where oxygen uptake was measured directly during identical contraction conditions as presently employed. In that study we found an oxygen cost of 0.26 $\mu$mol of oxygen/g of muscle for each tetanic contraction when corrected to initial tension (i.e. with no fatigue failure) (15). This oxygen cost per contraction times the contraction frequency is the oxygen consumption of muscle in the present experiment, when there is no contraction failure in the fast-twitch red muscle section. Evidence is provided (see "Results") to indicate that this condition is fulfilled for the critical stimulation conditions important to our conclusions.

Statistics—One-way analysis of variance was used to determine significant differences among means. Differences between means were tested by Tukey's $\nu$ ($p < 0.05$).

RESULTS

Muscle Mitochondrial Content—Exercise training increased muscle cytochrome c content by 30% and citrate synthase activity about 16% compared with values from sedentary animals, while the propylthiouracil-treated hypothyroid rats showed 40 and 25% declines, respectively (Table I).

Muscle Performance—Because of technical constraints it is not possible to measure the tension development of the specific fast-twitch red muscle section used for biochemical analyses. However, it is possible to assess the functional aspect of the mixed-fibered muscle mass that comprises the entire stimulated muscle group (Fig. 1). Tension development was well maintained (i.e. 85% of initial or greater) by most of the groups (9 of 12) during stimulation. Thus, most of the biochemical measurements were obtained during conditions where little or no fatigue was evident. The exceptions are the most severe stimulation condition of 45 tetani/min for the normal sedenany group (57% of initial tension) and the 30 and 45 tetani/min conditions for the propylthiouracil group (55 and 42% of initial tension, respectively). It should be emphasized that the muscle mass connected to the load cell is comprised of primarily (approximately 67%) fast-twitch white fibers (38). This is the fiber type that cannot sustain tension development during high frequency tetanic contractions (39). Thus, our tension records essentially represent contraction failure of the fast-twitch white muscle mass. Most importantly, however, tension failure did not progress beyond 33% of initial tension, the point beyond which, based on mass composition, failure of the remaining muscle mass (primarily fast-twitch red muscle) must occur. Thus, we have evidence that the fast-twitch red muscle fiber section did not exhibit contraction failure. This assures the applicability of our oxygen consumption calculations.

Muscle Metabolites—A clear pattern emerges regarding the management of the high energy phosphates, ATP and creatine phosphate, in fast-twitch red muscle having varying mitochondrial content. In the trained rats, depletion of ATP and CP is clearly less than that found in sedentary rats at every frequency of tetanic contractions while in propylthiouracil-treated animals it is greater (compare Tables II, III, and IV). This pattern has important implications for the calculated values of each of the putative modulators of oxidative phosphorylation considered here (ADP$_f$, ATP/ADP$_f$, and ATP/ADP$_f$ X P$_{ii}$), since the calculated free ADP and P$_i$ are directly dependent on the measured ATP and creatine phosphate concentrations. The calculation of ADP$_f$ depends explicitly on the equilibrium constant of the creatine kinase reaction which in turn depends on the assumed free Mg$^2+$ concentration of the muscle (34). Varying the assumed free Mg$^2+$ between 0 and 5 mM during recalculation had no influence on any of our conclusions.

DISCUSSION

Our experiments address two physiologically important questions. Does the maximal aerobic capacity (mitochondrial content) of a tissue influence the pattern of response of putative cytosolic modulators of mitochondrial respiration (ADP$_f$, ATP/ADP$_f$, and ATP/ADP$_f$ X P$_{ii}$) over a wide range of oxygen consumptions? If so, are there changes evident in other ATP-generating pathways within the cell consistent

<table>
<thead>
<tr>
<th>Table I</th>
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<tr>
<td>Effects of exercise training and propylthiouracil treatment on mitochondrial content of rat red gastrocnemius</td>
</tr>
</tbody>
</table>

<p>| Values are mean ± S.E. |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight</th>
<th>Mean arterial pressure</th>
<th>Initial muscle tension</th>
<th>Citrate synthase</th>
<th>Cytochrome c content</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>mm Hg</td>
<td>g</td>
<td>μmol·min·g$^{-1}$</td>
<td>nmol·g$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Trained</td>
<td>444 ± 11</td>
<td>130 ± 2</td>
<td>3764 ± 52</td>
<td>46.3 ± 2.3$^a$</td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>463 ± 15</td>
<td>127 ± 4</td>
<td>3735 ± 53</td>
<td>39.9 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Propylthiouracil</td>
<td>395 ± 4$^b$</td>
<td>104 ± 2$^b$</td>
<td>3450 ± 53</td>
<td>30.1 ± 0.8$^b$</td>
<td></td>
</tr>
</tbody>
</table>

$^a p < 0.0025$.  
$^b p < 0.005$ versus sedentary or trained.
Sensitivity of Mitochondrial Respiratory Control

Fig. 1. Tension production of the gastrocnemius-soleus-plantaris muscle group expressed as percent of initial tension. Muscles were stimulated to contract at 7.5, 15, 30, or 45 tetani/min. The groups represent low (hypothyroid; propylthiouracil (PTU)-treated), normal (sedentary), and high (exercise trained) mitochondri
dr muscle.

with this altered pattern of cytosolic modulator (e.g. ADP)? In physiologically intact systems, the tissue values of potential cytosolic modulators are always dependent variables; the concentra-
tions or ratios are dependent upon the rates of ATP utilization relative to production. From the perspective of mitochondrial respiration the ATP utilization rate is an independent variable matched, during steady-state aerobic respir-
ation, by the requisite oxygen consumption rate. Hence, we have plotted oxygen consumption as the independent variable with putative cytosolic modulators of mitochondrial respiration as dependent variables (Fig. 2). When these pu-
tative modulators of respiration are plotted as a function of muscle oxygen consumption (Fig. 2, Panels 1a, 2a, and 3a), differences in the pattern emerge as a function of mitochon-
drial content. We interpret the change in initial slopes to be a reflection of inherent differences in the sensitivity of cyto-
solic respiratory control within tissues with different oxidative capacities. The sensitivity of mitochondrial respiratory control increases, as shown by the lower initial slope, with a greater oxidative capacity. Evidence for this increased sensitivity is not dependent on the model of respiratory control as a similar pattern of altered slopes is observed with each putative modulator. Thus, the cytosolic signal required to drive respiration within a contracting muscle fiber during a given energy demand appears to be significantly modified by the muscle's mitochondrial content.

The differences in initial slopes evident in Fig. 2, Panels 1a, 2a, and 3a, are based, in part, on a precise knowledge of the oxygen consumption of the tissue mass. For technical reasons it is not possible to measure the oxygen consumption of only the deep lateral gastrocnemius fast-twitch red muscle section used in the present experiments. However, since the oxygen cost of tension development for rat fast-twitch muscle is known and found to be a constant (15), the oxygen con-
sumption of a given contraction condition can be established if tension development is well maintained. This appears to be the case in the present experiments, certainly for the condi-
tions used to describe the initial linear pattern of response, since total tension development of the entire mixed-fibered muscle was well maintained (85% of initial or greater). Any decrease in tension development must reasonably be attrib-
ted to the easily fatigued fast-twitch white muscle fibers (39) that were not included in this study. Further, the oxygen cost of muscle contractions in performing work is not altered by training (40). However, the oxygen cost of muscle contraction is reduced by hypothyroidism (41). Likewise, any energy contribution from lactate accumulation (estimated to be 1–
11% of total energy expenditure) would serve to lessen the estimated oxygen consumption. Thus, we have probably over-
estimated the oxygen consumption of the propylthiouracil group. This does not confound our conclusions, since our error would tend to underestimate the initial slope for the hypothy-
roid muscle. Thus, the actual difference in respiratory control sensitivity between muscles of low and high mitochondrial content is probably greater than illustrated in Fig. 2.

The improvement in respiratory control sensitivity as oxidative capacity increases suggests that, in tissue with an exceptionally high mitochondrial content, respiration may increase with little or no discernible change in these putative modulators (ADP, ATP/ADP, or ATP/ADP × Pi). The relatively low slope observed in our high mitochondria trained fast-twitch red muscle section became evident over a wide 30-
fold range of oxygen consumption. This expected low slope could explain, at least in part, the apparent constant creatine phosphate/ATP ratio observed over a relatively limited 5-fold range of energy expenditure in the in vivo working dog heart (42), which probably contains twice the mitochondrial content of the trained rat fast-twitch red muscle section. On the other hand, it is likely that respiratory control factors operating within the mitochondrion (e.g. redox state) represent the major influence controlling respiration in heart tissue (43, 44).

In the high mitochondria muscle (trained) the fairly linear relationship described between oxygen consumption rates and each putative modulator suggests that adenine nucleotide control of oxidative phosphorylation may be a sufficient regu-
lator of oxidative phosphorylation over the 30-fold span of respira-
tion rates required by these stimulation conditions. However, in both normal (sedentary) and low mitochondria (propylthiouracil-treated) muscle a marked nonlinearity is observed at higher rates of oxygen consumption. A similar nonlinearity has been observed in the relationship between the creatine phosphate/Pi ratio and energy expenditure over

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### Table II

Measured metabolites in red gastrocnemius of sedentary rats

<table>
<thead>
<tr>
<th>Stimulation conditions</th>
<th>n</th>
<th>ATP (µmol/g)</th>
<th>ADP (µmol/g)</th>
<th>AMP (µmol/g)</th>
<th>P; (µmol/g)</th>
<th>Creatine phosphate (µmol/g)</th>
<th>Creatine (µmol/g)</th>
<th>IMP (µmol/g)</th>
<th>NH₃ (µmol/g)</th>
<th>Lactate (µmol/g)</th>
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</thead>
<tbody>
<tr>
<td>Tetani/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>15</td>
<td>7.49 ± 0.08</td>
<td>0.884 ± 0.021</td>
<td>0.054 ± 0.004</td>
<td>9.98 ± 0.47</td>
<td>19.52 ± 0.53</td>
<td>14.62 ± 0.60</td>
<td>0.030 ± 0.004</td>
<td>0.38 ± 0.04</td>
<td>1.74 ± 0.22</td>
</tr>
<tr>
<td>7.5</td>
<td>4</td>
<td>7.81 ± 0.15</td>
<td>0.974 ± 0.067</td>
<td>0.054 ± 0.009</td>
<td>12.92 ± 1.01</td>
<td>17.11 ± 1.59</td>
<td>17.01 ± 1.59</td>
<td>0.032 ± 0.002</td>
<td>0.48 ± 0.07</td>
<td>3.28 ± 0.40</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>7.59 ± 0.24</td>
<td>1.067 ± 0.024</td>
<td>0.070 ± 0.016</td>
<td>15.86 ± 1.80</td>
<td>13.43 ± 1.81</td>
<td>20.70 ± 1.81</td>
<td>0.049 ± 0.012</td>
<td>0.60 ± 0.06</td>
<td>5.09 ± 1.84</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
<td>7.26 ± 0.22</td>
<td>0.926 ± 0.040</td>
<td>0.076 ± 0.005</td>
<td>18.14 ± 0.73</td>
<td>11.17 ± 0.89</td>
<td>22.94 ± 0.89</td>
<td>0.069 ± 0.013</td>
<td>0.70 ± 0.07</td>
<td>5.72 ± 0.65</td>
</tr>
<tr>
<td>45</td>
<td>3</td>
<td>6.80 ± 0.22</td>
<td>0.998 ± 0.014</td>
<td>0.033 ± 0.006</td>
<td>20.20 ± 0.50</td>
<td>9.94 ± 0.26</td>
<td>24.17 ± 0.98</td>
<td>0.559 ± 0.154</td>
<td>0.98 ± 0.08</td>
<td>5.74 ± 1.66</td>
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</tbody>
</table>
a broad range of exercise intensities with phosphorus-NMR spectroscopy (45). We would expect a similar nonlinear regional behavior in the high mitochondrial muscle, if a more intense stimulation pattern had been employed. This loss of linearity (Fig. 2, Panels 1a, 2a, and 3a) suggests that other regulatory factors participate in the control of respiration. One likely possibility is the mitochondrial redox state (7, 8, 43, 44, 46) which reflects the rate of supply of reducing equivalents to the electron transport chain relative to the rate of their oxidation. For instance, if the oxygen delivery to working muscle is less adequate, the mitochondrial redox state will become more reduced, thus stimulating respiration (46). This seems especially likely in the propylthiouracil-treated group, where delivery of oxygen might have been marginally adequate at the more intense stimulation frequencies; mean arterial pressure was significantly lower than in sedentary or trained rats (Table I). The upper limit of the apparent linear range of respiratory control by adenine nucleotides is noteworthy. This region may represent increments in energy requirement which are not easily met via oxidative phosphorylation. Lactate end point glycolysis (as evidenced by lactate accumulation) was highest beyond these points (Tables II, III, and IV).

During contractions, the same apparent rate of oxygen consumption is maintained in these tissues with different complements of mitochondria by alteration of the average rate of electron transport per respiratory chain. When the rate of oxygen consumption is normalized to the muscle cytochrome c content, differences in sensitivity to the putative modulators are no longer evident (Fig. 2, Panels 1b, 2b, and 3b), although the curves are still offset. Resting ATP/ADP and ATP/(ADP × P) are higher in the red gastrocnemius of the trained group and lower in the low mitochondria muscle as compared with the ratios measured in normal muscle. Similarly, ADP is highest in the low mitochondria muscle and lowest in the high mitochondria muscle. These differences persist with increasing rates of tetanic contractions and are due to the differences observed in muscle ATP and creatine phosphate/creatinine between tissues with different mitochondrial content.

Several interrelated consequences can be seen to arise from the differences in aerobic capacity. Because the concentrations of energy-rich phosphates are better maintained during contractions in muscle with a higher mitochondrial concentration, the net flux of energy transfer from creatine phosphate to ADP via creatine kinase is less; in addition, the concentration of ADP is lower, this reducing the net flux through adenylate kinase and lowering the concentration of AMP. Fig. 3 shows the calculated AMP concentrations for each treatment group with increasing rates of oxygen consumption. High mitochondria muscle shows the smallest and slowest rate of increase in AMP while low mitochondria muscle exhibits the largest and most rapid increase. An important result of the increase in AMP is an increase in flux through AMP deaminase, the first reaction of the purine nucleotide cycle, catalyzing the deamination of AMP to IMP + NH₃ (47). Since the apparent Kₘ for AMP deaminase (approximately 1 mM (48)) is well above the tissue concentration, the increase in AMP during contractions appears to be an important factor leading to AMP formation, especially in this fast-twitch red muscle section (27). While the AMP deamination reaction leads to adenine nucleotide depletion, it serves to limit the elevation in tissue AMP and ADP concentrations. This process is accelerated in muscle with a lower oxidative capacity as evidenced by the larger accumulations in muscle IMP (Fig. 3) and NH₃ (Tables II, III, and IV). Thus, the higher the muscle’s mitochondrial content, the more effective is the respiratory process in mitigating increases in ADP during contractions, while minimizing the flux through AMP deaminase.

The well characterized differences in lactate production and glycogen depletion between muscle differing in oxidative capacity can be explained, in part, by the present results. As has been demonstrated repeatedly (13), exercise training lessens the rate of lactate accumulation (Table III) compared with the untrained state (Table II). Conversely, decreasing the muscle mitochondrial content by hypothyroidism increases the rate of lactate accumulation (Table IV), a result that can be best explained by the greater increase in ADP and AMP concentrations, potent physiological modulators of enzymes catalyzing nonequilibrium reactions of glycolysis and glycogenolysis (49).

Endurance type exercise training induces a well characterized proliferation of skeletal muscle mitochondria in the working muscle. This adaptation includes increases in electron
FIG. 2. Panels 1a, 2a, and 3a, potential cytosolic modulators of mitochondrial respiration (ADP$_f$, ATP/ADP$_f$, and ATP/(ADP$_f$ × $F_o$), respectively), expressed as a function of estimated oxygen consumption in fast-twitch red gastrocnemius muscle in low (propylthiouracil (PTU)-treated), normal, and high (trained) mitochondria muscle. ADP$_f$ and $F_o$ were calculated as described under "Materials and Methods." Note the increase in slope obtained in muscle with lower mitochondrial content. Panels 1b, 2b, and 3b, same as left panels except the calculated values are expressed as a function of estimated oxygen consumption normalized per mole of muscle cytochrome c content. While the relationships are still offset, the slopes of the initial linear phases are not different.
transport chain components and enzymes of the tricarboxylic acid cycle and an enhanced capacity for \( \beta \)-oxidation (13). Underlying the improved performance of trained muscle is the preferential oxidation of lipid, in place of carbohydrate, a result, glycolysis would be stimulated less and fatty acid oxidation should provide a comparatively larger fraction of the reducing equivalents necessary for ATP production.

In conclusion, direct experimental evidence has been obtained supporting the previously untested hypothesis that variations in mitochondrial content will result in differences in the sensitivity of respiratory control. In addition, we show important consequences to increasing oxidative capacity as occurs in exercise training; fluxes through creatine kinase, adenylate kinase, AMP deaminase, and glycglycosis are less at submaximal workloads.

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REFERENCES