Regulation of Metabolism: the Rest to Work Transition in Skeletal Muscle

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ABSTRACT

Mitochondrial oxidative phosphorylation is programmed to set and maintain metabolic homeostasis and understanding that program is essential to an integrated view of cellular and tissue metabolism. The behavior predicted by a mechanism based model for oxidative phosphorylation is compared to that experimentally measured for skeletal muscle when work is initiated. For the model, initiation of work is simulated by imposing a rate of ATP utilization of either 0.6 mM ATP/sec (equivalent of 13.4 ml O₂/100g tissue/min or 6 µmole O₂/g tissue/min), or 0.3 mM ATP/sec. Creatine phosphate ([CrP]) decrease, both experimentally measured and predicted by the model, can be fit to a single exponential. Increase in ATP synthesis begins immediately, but can show a “lag period” during which the rate accelerates. The length of the lag period is similar for both experiment and model: in the model, the lag depends on intramitochondrial [NAD⁺]/[NADH], mitochondrial content, size of the creatine pool ([CrP] + [Cr]), as well as the resting [CrP]/[Cr]. For in vivo conditions, increase in oxygen consumption may be linearly correlated with decrease in [CrP], and with increase in inorganic phosphate ([Pi]), and [Cr]. The decrease in [CrP], resting and working steady state [CrP], and the increase in oxygen consumption are dependent on the pO₂ in the inspired gas (experimental) or tissue pO₂ (model). The metabolic behavior predicted by the model is consistent with available experimental measurements in muscle upon initiation of work, with the model providing valuable insight into how metabolic homeostasis is set and maintained. (247 words)

Key words: muscle work, metabolic control, metabolic homeostasis, rest to work transition, skeletal muscle
INTRODUCTION

In cells, metabolism changes continuously and rapidly in response to alterations in the cellular environment or intracellular and extracellular functions and this can involve large changes in metabolite levels and enzymatic activity. These metabolic responses can occur within seconds, and include all aspects of metabolism; ion transport, mechanical work, protein synthesis etc. Integrated metabolism, however, counters these metabolic displacements and on a time average basis operates near a particular set point (metabolic homeostasis) and this set point is very similar for all eukaryotic cells. Real time control of metabolism and maintaining metabolic homeostasis requires a metabolic unit that is programmed with that set point and is connected to the rest of metabolism through a network of regulatory pathways through which the set point is maintained (49, 50, 55). In eukaryotic cells, mitochondrial oxidative phosphorylation is programmed with a set point for the energy state ([ATP]/[ADP][Pi]) (49-56). The energy state has bidirectional communication with all of the metabolic pathways in the cell through the energy level (free energy of hydrolysis of ATP) and the individual concentrations of ATP, ADP, and Pi. The concentrations of ATP, ADP, and Pi and of the many other small metabolites that are coupled to their concentrations (notably AMP) transmit, in real time and to all parts of the cell, the information used to maintain metabolic homeostasis. The intrinsic program of oxidative phosphorylation sets the energy state near $10^5$ M$^{-1}$ (49, 50, 55), about $5 \times 10^{10}$ greater than the equilibrium value of $2 \times 10^{-6}$ M$^{-1}$ (17, 34) and maintains that value (average over time) within narrow limits.

In order to better understand how oxidative phosphorylation is able to set and maintain metabolic homeostasis, it is necessary to understand the mechanism(s) by which oxidative phosphorylation is regulated and to construct a model that quantifies the dependence on each of the regulatory parameters. The rate of oxidative phosphorylation is determined by the flux through cytochrome c oxidase, so a mechanism based model has been built for the cytochrome c oxidase (55, 56). The steady state rate expression derived for that model fits the observed dependences on energy state, pO$_2$, pH and level of reduction of cytochrome c (51, 52, 55). The rate expression for cytochrome c oxidase can then been extended to overall oxidative phosphorylation adding the first two sites of oxidative phosphorylation (without the pH dependence of ATP hydrolysis):
NADH + 2 Pi + 2 ADP + 2 cyt c\(^{3+}\) = NAD\(^{+}\) + H\(^{+}\) + 2 ATP + 2 cyt c\(^{2+}\) \hspace{1cm} (1)

The reactions of the first two sites are readily reversible and near equilibrium (8-11, 16, 20, 39, 49, 50, 54), and the result is the steady state rate expression for all of oxidative phosphorylation (49, 50, 55). This rate expression has been shown to predict metabolic energy states very similar to those observed experimentally in several types of cells and tissues (49, 50). In addition, the predicted rates of respiration agree with those observed experimentally for tissues in which the data are available. A more rigorous and demanding test of the model, however, is whether the behavior predicted by the model is also consistent with experimental measurements made during periods of rapid, physiologically relevant, change. To answer this question, in the present paper the behavior predicted by the model is compared with experimental measurements of the rest to work transition in muscle. Skeletal muscle was chosen because the rate of ATP consumption can increase by 100 fold or more during the transition from resting to maximal work and there have been many measurements of the metabolic changes that occur during this transition, in part due to its importance in exercise physiology. During the rest to work transition many aspects of metabolism need to be coordinated. This includes the catabolic pathways that provide NADH and FADH for oxidative phosphorylation, the flux of ADP and Pi for synthesis of ATP, and delivery of O\(_2\), all of which are required in order for oxidative phosphorylation to synthesize the ATP consumed to do work. The key parameters affecting the metabolic transitions between rest and work include: 1. the content of mitochondrial respiratory enzymes, expressed as the concentration of cytochrome oxidase and/or cytochrome c. 2. the concentrations of ATP, ADP ([ADP]), and inorganic phosphate ([Pi]) as expressed in the energy state ([ATP]/[ADP][Pi]). 3. the intramitochondrial [NAD\(^{+}\)]/[NADH]. 4. intracellular oxygen pressure (pO\(_2\)). 4. the total creatine pool ([CrP] + [Cr]).

The predictions of the model are shown to be consistent with the experimentally measured metabolic changes that occur when work is initiated in resting skeletal muscle (the rest to work transition). The regulatory parameters that determine the rate and extent of the metabolic changes during transition from rest to work are quantified.
EXPERIMENTAL METHODS AND MATERIALS

Our model for the cytochrome c oxidase reaction is based on the chemistry of oxygen, the thermodynamics of the system, and the properties of cytochrome c oxidase (55). The steady state rate expression for the model has been derived and fitted to the dependence of the reaction rate on pO₂, energy state, pH, and cytochrome c reduction as measured in suspensions of isolated mitochondria. The fit retained the basic chemical and thermodynamic properties of the mechanism of oxygen reduction by cytochrome c oxidase and the values for the rate and equilibrium constants are consistent with independent measurements of those parameters, where measurements are available. The rate expression was fitted to the behavior of cytochrome c oxidase in suspensions of rat liver mitochondria, but it is also consistent with the behavior of other types of mitochondria (49, 50) as well as for paracoccus denitrificans (8).

It should be noted that there is an error in equation A15 of the published rate expression (see 40, 56): the constants k₄a and k₄b were interchanged prior to fitting to the data. Correction of the rate expression and then refitting to the data resulted in small changes in the rate constants (56). The fit to the data is the same whether the original equation is used with its table of fitting constants or the corrected equation with its table of fitting constants. Additional information, including derivation of the steady state rate expression for cytochrome c oxidase reaction, its program in MatLab (www.mathworks.com), and the appropriate fitting constants can be accessed through URL: http://www.med.upenn.edu/biociop/faculty/wilson/index.html.

Relative to the previously published program, the absolute temperature has been increased from 298ºK to 309ºK to more closely approximate tissue temperatures. In addition, the ΔG for ATP hydrolysis at pH 7.1 has been slightly increased (from -7.3 Kcal/mole to -7.5 Kcal/mole) and the equilibrium constant for the creatine phosphokinase reaction slightly decreased (from 200 to 142) based on rereading of the papers by Veech and coworkers (34) and by Golding and Golding (17).

The behavior predicted by the model includes several metabolic parameters, notably mitochondrial content, intramitochondrial [NAD⁺]/[NADH], [ATP], [ADP], [Pi], [CrP], [Cr], and pO₂. It is not possible to measure all of these parameters in the same experiment, and measurements in one paper had to be “filled in” using data from other papers. The "fill in" values most often needed were the mitochondrial content and the intramitochondrial
[NAD\(^+\)]=[NADH] (for which measurement is rarely attempted), total creatine concentration, [Pi], and pO\(_2\). In the present communication, the intramitochondrial [NAD\(^+\)]=[NADH] calculated for the free concentrations is assumed to be between 0.1 and 3. At pH 7.1 this corresponds to a redox potential of -0.315 to -0.350 V. Measurements in muscle made by assuming equilibrium of the glutamate dehydrogenase reaction and calculated for pH 7.0, indicate that the potential is near -0.31V for isolated glucose perfused rat heart (20, 39) and human skeletal muscle (12). In the perfused rat heart this becomes more negative, i.e. the NAD pool becomes more reduced, when the heart is perfused with its physiological substrate, fatty acids. Calculations based on an intramitochondrial pH of 7.5 (16) would be more negative by about 15 mV, so for muscle oxidizing fatty acids a range of potentials for the intramitochondrial NAD couple is between -0.315 and -0.350 V. The calculations of the model are based on redox potential, with the metabolite ratio used to calculate the appropriate potential. The concentrations of ADP and AMP presented in this paper are for the free ADP and AMP, not the total cellular concentrations. The adenylate kinase and creatine phosphokinase reactions are assumed to be near equilibrium.

Mitochondria are particulate enzyme complexes and the internal components (cytochrome c, cytochrome a\(_3\), etc) are bound together as units with defined molecular relationships. In order to calculate the metabolic behavior predicted for tissues with different mitochondrial content, the calculations need to be made for 1 x 10\(^{-6}\) M cytochrome a (2 x 10\(^{-6}\) M cytochrome c), the conditions for which the model was fit to the data for suspensions of mitochondria. The calculated rates can then be scaled linearly with mitochondrial content i.e. doubling the mitochondrial content doubles the calculated rate. This allows the effect of mitochondrial content on tissue metabolism to be calculated and directly compared with the experimentally measured values.

The steady state rate expression programmed in MatLab has been used to calculate the behavior predicted by the model and the calculated values was processed for comparison with the experimentally measured behavior and graphed using Origin (www.originlab.com).

RESULTS

The effect of mitochondrial content on the metabolic changes in resting muscle following initiation of work. Figures 1A and B show the change in creatine phosphate concentration
([CrP]) that occurs when work is initiated in resting skeletal muscle. The calculations were made for cytochrome c concentrations of 8 µM, 16 µM, and 32 µM while the intramitochondrial 
[NAD\(^+\)]/[NADH], creatine pool, and pO\(_2\) are held constant at 0.1, 46 mM, and 25 torr, 
respectively. A pO\(_2\) of 25 torr was chosen as a reasonable "physiological" tissue oxygen 
pressure. It is less than the mean value in resting skeletal muscle, brain, and kidney but close to 
the mean values in heart and liver. For the resting steady state, the values for [CrP] and [Pi] 
were assumed to be 34 and 3 mM respectively. This corresponds to a resting [CrP]/[Cr] ratio of 
2.8. In resting skeletal muscle, the basal rate of ATP utilization is very low, less than 0.01 
mM/sec (47). For calculating the predicted changes, work was simulated by imposing a rate of 
ATP consumption of either 0.6 mM/sec, equivalent to 13.4 ml O\(_2\)/100g tissue/min or 6 µmole 
O\(_2\)/g/min, (Fig. 1A) or 0.3 mM ATP/sec (Fig. 1B). These rates of ATP utilization are similar to 
those reported for work at about 60% and 30% of maximum in untrained humans (26,44,36).

When work is initiated, the [CrP] begins to fall linearly in time. As [CrP] falls, however, 
the decreasing energy state progressively increases the rate of synthesis of ATP by oxidative 
phosphorylation until ATP production is again equal to the rate of consumption and a working 
steady state is attained. Upon initiation of work, the rate of ATP synthesis (oxygen 
consumption) (Fig. 1A, B) increases, slowly at first but accelerating to a maximum and then 
decreasing again until the new working steady state is attained. The time that passes before a 
near maximal rate of increase in the rate of ATP synthesis is due to a period during which the 
rate of oxidative phosphorylation is low but accelerating. Everything else being equal, this "lag 
period" is shorter for higher work rates i.e. it is shorter when ATP is being consumed at 0.6 
mM/sec (Fig. 1A) than for 0.3 mM/sec (Fig. 1B). The metabolic basis for the lag period and the 
conditions for which it is observed, are further discussed below.

The measured decrease in [CrP] following initiation of work is often reported as the time 
constant calculated by fit of the data to a single exponential. Figure 2A, shows the predicted 
change in [CrP] with time for two different conditions; 8 µM cytochrome c with an 
[NAD\(^+\)]/[NADH] of 1, and 16 µM cytochrome c with an [NAD\(^+\)]/[NADH] of 0.1. The predicted 
behaviors have been fitted to the equation: 
y = yo + Ae\(^{-k/t}\). For each condition, the predicted 
behavior is reasonably well fitted by a single exponential, although close examination reveals 
there are systematic deviations. The conditions for which most of the available experimental
data were collected are such that fit to a single exponential is reasonably good, but this does not
mean that the metabolic behavior is necessarily an exponential function.

Another experimental observation is that the rate of oxygen consumption is
approximately linearly correlated with [CrP]. As shown in Figure 2B, when the rate of oxygen
consumption predicted by the model is graphed against [CrP], the two show a linear correlation
over a wide range of metabolic rates. The slope of the straight line increases with increasing
concentration of cytochrome c. The linear correlation is approximate, however, and holds only
for limited ranges of metabolic conditions. Since hydrolysis of creatine phosphate results in a
stoichiometric increase in [Pi] and [Cr], the increases in [Cr] and [Pi] can also be fitted with
single exponentials with the same time constant as for the decrease in [CrP]. Their
concentrations, like those of creatine phosphate, show an extensive range over which there is a
linear correlation with the rate of oxygen consumption.

**Dependence of the metabolic changes that occur upon initiation of work on the size of the creatine pool.** The creatine phosphokinas reaction, the [CrP]/[Cr] ratio, and the size of the creatine pool are important to muscle function for several reasons. Figure 3 shows the effect of decreasing the creatine pool to 23 mM while holding the other variables the same as for Figures 1A and B. With the smaller creatine pools everything happens more quickly and for the same work rate and cytochrome concentration the lag period in the rate of ATP synthesis (and oxygen consumption) is shorter. The initial linear region in the fall in [CrP] is still apparent, but there are differences between the predicted responses for high (46 mM) and low (23 mM) creatine pools. The increase in [Pi] due to hydrolysis of creatine phosphate, for example, is smaller for the lower creatine pool so the [CrP]/[Cr] has to decrease further for the same increase in the rate of ATP synthesis. For a mitochondrial content of 16 µM, a work rate of 0.6 mM ATP/sec, and a creatine pool of 46 mM, [CrP]/[Cr] in the working steady state is approximately 1.1 (24 mM CrP, 13 mM Pi). For the creatine pool of 23 mM, the [CrP]/[Cr] falls to 0.84 (10.5 mM CrP, 9 mM Pi). The greater decrease in [CrP]/[Cr] is needed to compensate for the lower [Pi] so the rate of oxygen consumption in the working steady states are equal.

**Dependence of the metabolic changes during the rest to work transition on the intramitochondrial [NAD⁺]/[NADH] ratio.** One of the important parameters that determines the behavior of oxidative phosphorylation is the intramitochondrial [NAD⁺]/[NADH] ratio (9,
Figure 4 shows the dependence of the rate of ATP synthesis on [CrP] and [Pi] for intramitochondrial [NAD\(^+\)]/[NADH] ratios of 0.1, 0.3, 1, and 3. The pO\(_2\), cytochrome c concentration, and creatine pools are 25 torr, 16 µM, and 46 mM, respectively, the same as for Figures 1 and 3. The linear regions in the decrease in [CrP] occur at lower [CrP] as the NAD pool becomes more oxidized (higher [NAD\(^+\)]/[NADH]). Increasing intramitochondrial [NAD\(^+\)]/[NADH] also results in a decrease in the [CrP] in the working steady state. This is consistent with more oxidizing conditions both limiting the maximal work rate and increasing the susceptibility to fatigue. The lag period in the increase in ATP synthesis, assuming the same resting [CrP] of 34 mM (point A), is also longer, as the [CrP] at the elbow decreases about 3 mM for each 10 fold increase in [NAD\(^+\)]/[NADH]. The lag period is determined by the resting [CrP] relative to the [CrP] at the "elbow" in the graph, i.e. it is the time required for the work rate to decrease [CrP] from 34 mM to the elbow. Once the elbow is reached, the rate of synthesis of ATP increases rapidly with further decrease in [CrP].

The reason for the lag period is more readily seen in Figure 5 where the rates of ATP synthesis (oxygen consumption) for each level of reduction of the intramitochondrial NAD are plotted against the [CrP]. Decrease in resting [CrP] either through a small increase in basal metabolic rate or incomplete recovery between work periods can completely eliminate the lag period. Decreasing the resting [CrP] from 34 mM to 31.6 mM, as might occur with a slight increase in resting ATP consumption, is shown in Figure 5 and the lag period is completely eliminated. The lag period is sensitive to the resting conditions and for experiments in vivo the value would be expected to be quite variable.

**Dependence on the metabolic transition from rest to work on pO\(_2\).** The metabolic changes during the rest to work transition have been calculated for pO\(_2\) values of 50, 25, 10, and 5 torr while holding the creatine pool, intramitochondrial [NAD\(^+\)]/[NADH], cytochrome c concentration, and work rate at 46 mM, 0.1, 16 µM, and 0.6 mM ATP/sec, respectively. When work is initiated, [CrP] begins to fall linearly with time. The initial rate of decrease is determined by the work rate, and is independent of oxygen pressure. The time constant for fit of the decrease in [CrP] to a single exponential gets longer with decrease in pO\(_2\) so the time before reaching the working steady state is increased. The [CrP] in the working steady state is also dependent on pO\(_2\), decreasing from about 24 mM to less than 20 mM as the pO\(_2\) is decreased from 50 to 5 torr. The lag period before the maximal rate of ATP production is not very
dependent on pO2 but the maximal rate of increase falls and the time required to reach the resting steady state increases with decreasing pO2.

DISCUSSION

Many experimental measurements of the rest to work transition in muscle have been made in humans where the cytochrome content of the muscles was not measured. Davies and coworkers (6) reported that rat hind limb muscle contains 13.3 nmole cytochrome c/g wet weight of tissue and this increased to 28.7 nmole/g wet weight with endurance training. Schollmeyer and Klingenberg (45) reported a value of 11.5 nmole/g wet weight in rat skeletal muscle, while Dudley (7) reported dog red gastrocnemius contained 19.5 nmole/g wet weight and this increased to 26.3 nmole/g wet weight with training. The content of the enzymes of human skeletal muscle is reported to be approximately 70% of that in rat skeletal muscle (57), suggesting that the cytochrome c concentration in the skeletal muscle of untrained humans is nearer 8 µM than 16 µM, with the concentration increasing to about 24 µM with maximal training (26, 36). There is a large variability in the oxidative capacity of human muscle, however, and almost certainly a similar large variability in cytochrome content.

The presence of an experimentally measured lag period (delay) before oxygen consumption increases following initiation of work has been reported/discussed by many researchers in different types of skeletal muscle and muscle preparations (1-3, 13-15, 21, 29-31, 41-44, 48). The kinetics and regulation of oxidative phosphorylation can, under physiological conditions, show a lag period with a form and extent consistent with the experimental measurements. The experimental conditions for which a lag will, or will not, be observed can be predicted by the model as seen in Figures 2B and 3-5. The predictions of the model are consistent with published measurements, but the experimental data are not sufficiently well defined for quantitative comparison with model predictions. In the model, the lag period is the time required for the work induced ATP consumption to lower the [CrP] from that in the resting steady state to the “elbow” in plots of the rate of ATP synthesis against [CrP] (Figs. 2B and 4). A delay is no longer observed if the resting [CrP] is near the elbow for any reason (see Fig 5). This means that, in experiments, no lag period would be observed if the "resting" metabolic rate
is slightly increased for any reason. Other metabolic parameters, notably the intramitochondrial 
\([\text{NAD}^+]/[\text{NADH}]\), also affect the length of the lag period.

Poole, Hogan, and colleagues (29-31, 41, 42) made the very physiologically important 
observation that for circulation intact preparations of rat spinotrapezius and dog gastrocnemius 
the capillary red cell flux increases rapidly (within the first contraction/relaxation cycle) when 
work is initiated. They suggested that this increase in oxygen delivery "masked" an increase in 
oxygen consumption and ATP synthesis, and the increases began with no delay. Certainly 
increase in oxygen delivery could decrease the rate of fall in oxygen pressure in the tissue, but 
the ATP being synthesized would also suppress the rate at which the concentration of creatine 
phosphate falls, and this is not apparent in the data. In general, the discussions have assumed 
that the lag could not be due to oxidative phosphorylation and therefore some other explanation 
was needed. The potential for a lag period is, however, an intrinsic property of the regulation of 
oxidative phosphorylation under *in vivo* conditions.

Hogan and coworkers (21, 26, 31) reported that, for isolated muscle fibers from Xenopus, 
the lag period and the time for oxygen to decrease to 50% of the full transition after start of 
contractions were 13 ± 3 sec and 28 ± 5 sec respectively for the first contraction cycle. These 
decreased to 5 ± 2 sec and 18 ± 4 sec, respectively, for the second and later cycles. The values 
for the second and later cycles are not different from those predicted by the model for a 
[CrP]/[Cr] of 2.8, where the lag period is about 5 sec and the time to 50% increase in respiratory 
rate is about 20 sec for a work rate of 0.3 mM ATP/sec. The longer lag period for the first cycle 
is consistent with: A. insufficient activation of mitochondrial dehydrogenases, such as pyruvate 
dehydrogenase, resulting in the intramitochondrial NAD couple becoming more oxidized and/or 
B. the [CrP] being lower prior to the second and later cycles than for the first cycle due to the 
long time period required for complete recovery. Kindig et al (31) reported that in isolated 
Xenopus muscle fibers there was no lag period in the increase in oxygen consumption after acute 
hindrance of the creatine phosphokinase reaction, consistent with prediction by the model.

It would be interesting to see measurements for the Xenopus muscle fibers equilibrated 
with different oxygen pressures prior to beginning the stimulations. The model predicts that the 
lag period and the rate of increase in the rate of oxygen consumption are dependent on pO2. This 
might provide another method for determining the dependence of muscle metabolism on pO2 and
another test of the ability of the model to accurately simulate the regulation of mitochondrial 
oxidative phosphorylation in vivo.

When work is initiated the dehydrogenases, particularly pyruvate dehydrogenase, need to 
be activated in order to provide NADH for the increase in oxidative phosphorylation. Grassi et 
al (14) determined the effect of dichloroacetate (DCA), an activator of pyruvate dehydrogenase, 
pretreatment in self perfused dog muscle when work was imposed by electrical stimulation. 
DCA treatment decreased the lag period from 5.7 ± 0.4 to 4.7 ± 0.5 sec, the decrease in the 
change in [CrP] from rest to the working steady state from 40% to 30%, and lowered the lactate 
concentration in both the resting and working steady states. Although the individual values were 
not significantly different, in the model this is consistent with DCA causing a small (< 2 fold) 
decrease in the intramitochondrial [NAD⁺]/[NADH] compared to control. The more reduced 
NAD couple would increase the energy state (increases [CrP]) in the working state. The higher 
energy state would lower [AMP] and thereby suppress lactate production and the more reduced 
NAD couple would decrease the lag period in ATP synthesis (oxygen consumption) (Fig. 4).

Measurements in humans show that the decrease in [CrP] begins as soon as exercise 
starts and the time course of the decrease is often presented as the fit to a single exponential. In 
order to compare the experimental measurements with the behavior predicted by the model, the 
later also been fitted to a single exponential. As shown in the figures, the predicted behavior is 
not really a single exponential function so exponents obtained are somewhat dependent on the 
portion of the curve analyzed. The deviation from exponential is not obvious in the fit to 
experimental data because: A. the metabolic conditions in vivo are such that the deviations from 
a single exponential are small, and B. noise in the collected data is typically larger than the 
systematic deviations. As a result the systematic deviations do not significantly affect the fit 
criteria.

The behavior predicted by the model for cytochrome c concentrations of 8, 16, and 32 
µM and pO₂ values of 50, 25, 10 and 5 torr have been fitted to a single exponential and the time 
constants presented in Table 1. The time constants calculated for the model and cytochrome c 
concentrations of 8 to 16 µM and a pO₂ of 25 torr are similar to those reported for measurements 
in humans (Table 2). The model predicts that for 16 µM cytochrome c the intracellular pO₂ 
decreases from 50 to 5 torr the [CrP] in the working steady state (y₀) decreases from 25.8 mM to
18.6 mM and the time constant increases from 19 to 34 sec. Experimental measurements of 
[CrP] in the working steady state have been reported for different pO2 in the inspired gas (22, 23, 
32, 33). Hollis and coworkers (25) reported that changing from normal (20.9% O2) to hypoxic 
(14.5% O2) inspired gas resulted in the time constant for recovery of [CrP] increasing from 20.9 
± 2.9 sec to 27.8 ± 4.3 sec. This is similar to the report of Haseler et al (18, 19) that for inspired 
gases with O2 content of 10, 21, and 100% oxygen resulted in recovery times of 33.5 ± 4.1, 25.0 
± 2.7, and 20.0 ± 1.8 sec, respectively. The cellular oxygen pressures were not measured and 
would, in any case, consist of a distribution of oxygen pressures. The similarities between the 
calculated and experimental time constants and in their increase with decreasing pO2 support the 
model.

The creatine phosphokinase reaction and the creatine pool are important to muscle 
function, particularly when high work loads are imposed. The equilibrium constant for the 
creatine phosphokinase reaction is approximately 150, and the [CrP]/[Cr] in resting muscle is 
about 2.5, so the [ATP]/[ADP] is 375. The [ATP] in many muscles is about 10 mM, so in 
resting muscle the [ADP] is near 30 µM. This [ADP] is only about 1% that of [Pi], the other 
substrate required for synthesis of ATP. ATP is hydrolyzed at the myofibrils and the ADP and 
Pi produced have to diffuse back to the mitochondria in order to be used for ATP synthesis. At a 
moderate work rate, one for which [CrP] decreases to 67% of the resting value, [ADP] increases 
to 67 µM and this not high enough for its diffusion to support the nearly 50 fold increase in ATP 
cycling. Creatine, with a 300 times higher concentration (23 mM), raises the effective diffusion 
flux of ADP to match those of ATP and Pi. Another important role of the creatine 
phosphokinase reaction is to suppress the large changes in [ADP], and therefore in adenosine 
monophosphate (AMP), that would otherwise occur when work is initiated in muscle. For many 
skeletal muscles, the maximal work rate is accompanied by oxygen consumption rates in excess 
of 15 ml O2/100 g tissue/min and this translates into more than 0.7 mM ATP/sec. In the absence 
of the creatine phosphokinase reaction, start of work would result in production of 0.7 mM ADP 
in the first sec or about 20 times the resting concentration in the first sec. In the presence of 
creatine phosphokinase and 34 mM creatine phosphate, 0.7 mM CrP is hydrolyzed instead. As a 
result, there is no significant change in [ATP] and [ADP] increases by only 8%, to 32 µM. The 
large rise in [Pi] results in the [ADP] levels in the working steady state being much lower than 
would be the case in the absence of the creatine phosphokinase reaction. More important is the
effect on the concentration of AMP, an important regulator of metabolism. Without the increase in [Pi] to suppress the increase in [ADP], initiation of work would result in an immediate and very large rise in [AMP] through the adenylate kinase reaction.

Observation of a linear correlation between [CrP] and oxygen consumption has generated much discussion over the role of CrP in regulating oxidative phosphorylation. This is only a correlation, however, and does not indicate a causal relationship. [CrP] \textit{per se} does not have a significant role in regulating oxidative phosphorylation. The increase in oxygen consumption is, for example, also linearly correlated with [Cr] and [Pi]. Inhibiting creatine phosphokinase (31), using gene knockout animals (43), and other approaches have all failed to find a direct role for the creatine phosphokinase reaction in regulating oxidative phosphorylation. The model, which shows similar linear correlations, does not attribute any direct effect of creatine phosphate on oxidative phosphorylation. The linear correlations observed experimentally arise from a combination of the large size of the creatine pool compared to the adenine nucleotide pool and the fact that oxidative phosphorylation \textit{in vivo} operates where the creatine buffer capacity is maximum; i.e. [CrP]/[Cr] ratios from 0.8 to 2.8.

The absence of a direct role in regulating oxidative phosphorylation does not diminish the importance of the creatine phosphate reaction to muscle function (see 50, 53). The roles of the creatine phosphokinase reaction in buffering of the energy state, enhancing effective ADP diffusion flux, moderating the change in ADP and AMP concentrations, and increasing the role of Pi in regulating oxidative phosphorylation, are all critically important to the ability of cellular metabolism to respond to rapid, large changes in ATP utilization.

The rate and extent of the metabolic changes when work is initiated that are predicted by the model are remarkably consistent with the available experimental data for skeletal muscle. This includes the values for the [CrP]/[Cr] under resting conditions and working steady state, the time course for the metabolite changes, and the relationship of the respiratory rate to the energy state. The time course for the decrease in [CrP] when work is initiated is reasonably fitted by a single exponential (1, 4, 12-24, 26, 28-31, 44, 46) for both the experimental data and the behavior predicted by the model. The experimentally measured increase in oxygen consumption when muscle work is initiated is reported to be linearly correlated with the decrease in [CrP] and this is predicted by the model for metabolic conditions similar to those \textit{in vivo}. The model also
shows that increasing the mitochondrial content results in less decrease of the [CrP] at each working steady state and a more rapid transition between the resting and working steady states, consistent with observations comparing muscles from sedentary and trained rats (5-7, 26) and for different muscle types (29). It is important to keep in mind that there are uncertainties in the mitochondrial content of the tissues, the size of the creatine pool, the work rate, pO₂, etc and that the model is based on mitochondrial measurements made at room temperature. On the other hand, the predictions by the model are consistent with the available experimental measurements, providing solid justification for using the model for experimental design and data interpretation. The consistency between predictions of the model and experimental measurement suggests that the model is an accurate representation of the mechanism of cytochrome c oxidase and of regulation of oxidative phosphorylation in vivo.
REFERENCES


FIGURE LEGENDS

Figures 1A, B. The calculated dependence of metabolism during the rest to work transition on the concentration of cytochrome c (mitochondrial content) of the tissue. The calculations were made for a total creatine (creatine plus creatine phosphate) concentration of 46 mM, and intramitochondrial \([\text{NAD}^+]/[\text{NADH}]\) of 0.1, and a pO\(_2\) of 25 torr, and cytochrome c concentrations of 8, 16, and 32 \(\mu\text{M}\). The \([\text{CrP}]\) begins to fall linearly when the work is initiated by imposing an ATP consumption rate of 0.6 mM/sec (Fig. 1A) or 0.3 mM/sec (Fig. 1B). The rate of decrease slows due to the increasing rate of ATP synthesis until a new steady state is approached in which production of ATP has increased until it is equal to consumption. The increase in the rate of ATP synthesis (O\(_2\) consumption) begins immediately but at an accelerating rate. The result is a “lag period” which is about 5 seconds for the imposed work rate of 0.6 mM ATP/sec (Fig. 1A) and slightly longer for a work rate of 0.3 mM/sec (Fig. 1B). The working steady state that is approached is at a lower \([\text{CrP}]\) for the higher work rate.

Figures 2A, B. Comparing the predicted behavior with experimental measurements showing empirical fit of the decrease in \([\text{CrP}]\) with time to a single exponential (2A) and of linear correlation between \([\text{CrP}]\) and the rate of oxygen consumption (2B). Figure 2A shows the predicted decrease in \([\text{CrP}]\) with time is presented for two conditions that cover much of the range relevant to metabolism in vivo. An 8 \(\mu\text{M}\) concentration of cytochrome c with an intramitochondrial \([\text{NAD}^+]/[\text{NADH}]\) of 1, and a cytochrome c concentration of 16 \(\mu\text{M}\) with an intramitochondrial \([\text{NAD}^+]/[\text{NADH}]\) of 1. For both 2A and 2B the creatine pool was 46 mM, the work rate 0.6 mM ATP/sec, and the pO\(_2\) 25 torr. The predicted values are shown using large filled in symbols while the fit to a single exponential is shown with smaller open symbols. The fit was to the equation \(y = y_0 + Ae^{-kt}\). For 8 \(\mu\text{M}\) cytochrome c the values for \(y_0\), A, and the time constant are 13.7 mM, 20.8, and 26.2 sec, respectively while for a cytochrome c concentration of 16 \(\mu\text{M}\) they are 24.5 mM, 9.8, and 11.5 sec, respectively. Systematic deviations are present, but they are small and would be masked by the noise level in experimental data.

In Figure 2B the rate of oxygen consumption is plotted against \([\text{CrP}]\) for cytochrome c concentrations of 8, 16, and 32 \(\mu\text{M}\). The creatine pool, intramitochondrial \([\text{NAD}^+]/[\text{NADH}]\), and pO\(_2\) were 46 mM, 0.1, and 25 torr, respectively. Point A is for a resting steady state with 34 mM \([\text{CrP}]\) and point B is the \([\text{CrP}]\) level at which the rate of oxygen consumption begins to
increase rapidly as [CrP] falls. The time required for [CrP] to decrease from A to B causes a “lag period” in the increase in ATP synthesis (increase in oxygen consumption) when work is begun.

**Figure 3. Dependence of metabolism during the rest to work transition on the size of the creatine pool.** The [CrP] and oxygen consumption rates were calculated for muscle in which the creatine pool was 23 mM instead of the 46 mM used in the previous figures. The intramitochondrial [NAD⁺]/[NADH], pO₂, and cytochrome c concentrations were 0.1, 25 torr, 16 µM, respectively. Two different work rates (0.6 and 0.3 mM ATP/sec) are presented. The decrease in [CrP] and increase in the rate of oxygen consumption occur over a shorter time scale than for a creatine pool of 46 mM, and the lag period before the steep rise in oxygen consumption is shorter. The increase in inorganic phosphate concentration for a pool of 23 mM is less than for a pool of 46 mM, however, so the [CrP]/[Cr] in the working steady state is lower.

**Figure 4. The dependence of the rest to work metabolic transition on intramitochondrial [NAD⁺]/[NADH].** The influence of the intramitochondrial [NAD⁺]/[NADH] on the rest to work transition has been calculated by assuming values of 0.1, 0.3, 1 and 3 while holding the creatine pool at 46 mM, the pO₂ at 25 torr, cytochrome c concentration at 16 µM. The predicted rates of ATP synthesis are plotted on the absissa against [CrP] (lower axis) and [Pi] (upper axis). As the value of [CrP] decreases from 34 mM the rate of ATP synthesis increases, first slowly but then more quickly, until it enters the region where the increase is linearly correlated with decrease in [CrP] and increase in [Pi]. Increase in intramitochondrial [NAD⁺]/[NADH] lowers the [CrP] for any specific rate of ATP synthesis. For a rate of 0.6 mM ATP/sec, for example, the [CrP] levels in the working steady state are 24, 21.6, 19.2, and 16.4 for [NAD⁺]/[NADH] values of 0.1, 0.3, 1, and 3, respectively. The point A is marked as resting steady state [CrP] of 34 mM. The time required for [CrP] to fall to the "elbow" in each curve corresponds to the lag period, and this increases markedly as the [NAD⁺]/[NADH] increases. The rate of oxygen consumption is 1/6 of the rate of ATP synthesis as 6 ATP are synthesized for each O₂ consumed.

**Figure 5. Dependence of the lag period before the rapid rise in the rate of oxidative phosphorylation on the [CrP] level in the resting state.** One of the important determinants of the lag period before the rapid rise in oxygen consumption is the energy state when work is
initiated (see Fig. 2B). In this figure the predicted behavior is presented for [CrP] in the resting steady state being either 34 (□,■) or 31 mM (○,●): creatine pool 46 mM; pO₂, 25 torr; cytochrome c concentration, 16 mM; work rate 0.6 mM ATP/sec; [NAD⁺]/[NADH], 0.1. These two conditions correspond to resting [CrP]/[Cr] ratios of 2.8 and 2.07. The lag period of about 5 sec for a resting [CrP] of 34 mM is completely absent when the resting [CrP] is 31 mM.

Figure 6. The effect of oxygen pressure on metabolism during the rest to work transition. The changes during the rest to work transition were calculated for pO₂ values of 5, 10, 25, and 50 torr, all with the same creatine pool, intramitochondrial [NAD⁺]/[NADH], cytochrome c concentration, and work rate, 46 mM, 0.1, 16 µM, and 0.6 mM ATP/sec, respectively. The initial decrease in [CrP] after initiation of work is the same and linear for all values of pO₂, but with decreasing oxygen pressure the [CrP] approached for the working steady state progressively decreases. The lag period before the rapid increase in the rate of oxygen consumption is similar for all of the oxygen pressures, perhaps slightly increasing as the pO₂ decreases, but this depends on how the lag period is calculated. The most easily observed effect of lowering pO₂ is that [CrP] in the working steady state decreases as the pO₂ decreases.
Table 1. The time constants obtained by fitting the predicted decrease in [CrP] to a single exponential decay.

<table>
<thead>
<tr>
<th></th>
<th>0.6 mM ATP/sec</th>
<th>0.6 mM ATP/sec</th>
<th>0.3 mM ATP/sec</th>
<th>0.3 mM ATP/sec</th>
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<tr>
<td></td>
<td>[NAD⁺]/[NADH]=0.1</td>
<td>8 µM cytochrome c</td>
<td>16 µM cytochrome c</td>
<td>[NAD⁺]/[NADH]=1</td>
</tr>
<tr>
<td>pO₂</td>
<td>y₀ (mM)</td>
<td>A</td>
<td>T (sec)</td>
<td>y₀ (mM)</td>
</tr>
<tr>
<td>50 torr</td>
<td>20.0</td>
<td>12.9</td>
<td>18</td>
<td>24.5</td>
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<td>25 torr</td>
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<td>14.5</td>
<td>19.4</td>
<td>23.4</td>
</tr>
<tr>
<td>10 torr</td>
<td>15.3</td>
<td>17.9</td>
<td>22.9</td>
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<tr>
<td>5 torr</td>
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<td>21.6</td>
<td>27.7</td>
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<tr>
<td>50 torr</td>
<td>13.4</td>
<td>19.9</td>
<td>24.4</td>
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<tr>
<td>25 torr</td>
<td>11.1</td>
<td>22.2</td>
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<td>16.4</td>
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<tr>
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<td>30.3</td>
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<tr>
<td>5 torr</td>
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<tr>
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<td>44</td>
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<td>28.5</td>
<td>77.7</td>
<td>9.6</td>
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</table>

The conditions used for calculating the rate at each oxygen pressure were: total creatine, 46 mM; intramitochondrial [NAD⁺]/[NADH], 0.1 or 1.0; work rate 0.6 or 0.3 mM ATP/sec. The equation fitted was: \( y = y₀ + A e^{-x/t} \).
Table 2. The experimentally measured rest to work decrease in [CrP] and increase in inorganic phosphate reported as the time constants obtained by fit to a single exponential.

<table>
<thead>
<tr>
<th>Source</th>
<th>Muscle group</th>
<th>O₂ cons -on</th>
<th>[CrP]-on</th>
<th>[Pi]-on</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCann et al (37)</td>
<td>Wrist Flexor</td>
<td>33</td>
<td>38</td>
<td></td>
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<tr>
<td>Marsh et al (35)</td>
<td>Wrist Flexor</td>
<td>33.5</td>
<td>32.1</td>
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<tr>
<td>Barstow et al (1)</td>
<td>Plantor flexors</td>
<td>26.3</td>
<td>30.7</td>
<td></td>
</tr>
<tr>
<td>Binzoni et al. (4)</td>
<td>Plantor flexors</td>
<td>23.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rossiter et al (44)</td>
<td>Quadriiceps</td>
<td>35 ± 13.5</td>
<td>33 ± 12</td>
<td></td>
</tr>
<tr>
<td>Grassi et al. (13)</td>
<td>Dog gastrocnemus</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grassi et al, (13)</td>
<td>Dog gastrocnemus</td>
<td>25</td>
<td></td>
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</tr>
</tbody>
</table>

The data were selected to be moderate exercise of short duration in order to avoid evidence of fatigue and, in so far as possible, significant change in tissue pH. The time constants measured for the rise in inorganic phosphate [Pi] are included. The [Pi] increase is almost exclusively due to hydrolysis of creatine phosphate and the time constant should be experimentally indistinguishable from that for the decrease in [CrP].