Regulation of metabolism: the rest-to-work transition in skeletal muscle

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Wilson DF. Regulation of metabolism: the rest-to-work transition in skeletal muscle. Am J Physiol Endocrinol Metab 309: E793–E801, 2015. First published September 22, 2015; doi:10.1152/ajpendo.00355.2015.—Mitochondrial oxidative phosphorylation is programmed to set and maintain metabolic homeostasis, and understanding that program is essential for an integrated view of cellular and tissue metabolism. The behavior predicted by a mechanism-based model for oxidative phosphorylation is compared with 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 (equivalent of 13.4 ml O2·100 g tissue−1 min−1 or 6 μmol O2·g tissue−1 min−1) or 0.3 mM ATP/s. 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 [NADH]/[NAD+] mitochondrial content, and 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 a decrease in [CrP] and an 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.

muscle work; metabolic control; metabolic homeostasis; rest-to-work transition; skeletal muscle

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 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][inorganic phosphate] ([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 105 M−1 (49, 50, 55), ABOUT 5 × 1010 GREATER THAN THE EQUILIBRIUM VALUE OF 2 × 10−6 M−1 (17, 34), AND MAINTAINS THAT VALUE (AVERAGE OVER TIME) WITHIN NARROW LIMITS.

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, PO2, pH, AND LEVEL OF REDUCTION OF CYTOCHROME C (51, 52, 55). THE RATE EXPRESSION FOR CYTOCHROME C OXIDASE CAN THEN BE EXTENDED TO OVERALL OXIDATIVE PHOSPHORYLATION, WITH THE FIRST TWO SITES OF OXIDATIVE PHOSPHORYLATION ADDED (WITHOUT THE pH DEPENDENCE OF ATP HYDROLYSIS): NADH + 2 P + 2 ADP + 2 ATP + 2 cyt c + (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. However, a more rigorous and demanding test of the model 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 study 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, which is 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 FADH2 for oxidative phosphorylation, the flux of ADP and Pi for synthesis of ATP, and delivery of O2, all of which are required 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 [Pi] in the energy state ([ATP]/[ADP][Pi]), and 3) the intramitochondrial [NAD+/NADH], 4) intracellular oxygen pressure (PO2), and 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 MATERIALS AND METHODS

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 oxidase 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 Eq. 15A of the published rate expression (55); this error was reported in Ref. 40 and corrected in Ref. 56. The constants $k_4a$ and $k_4b$ 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, its program in MatLab (www.mathworks.com), and the appropriate fitting constants can be accessed through the following URL: http://www.med.upenn.edu/biociobiop/faculty/wilson/index.html. Relative to the previously published program, the absolute temperature has been increased from 298 to 309°K to more closely approximate tissue temperatures. In addition, the ΔG for ATP hydrolysis at pH 7.1 has been slightly increased (from 7.3 to 7.5 kcal/mol), and the equilibrium constant for the creatine phosphokinase reaction slightly decreased (from 200 to 142) based on a rereading of the papers by Lawson and Veech (34) and Golding and Golding (17).

The behavior predicted by the model includes several metabolic parameters, notably mitochondrial content, intramitochondrial [NAD+]/[NADH], [ATP], [ADP], [Pi], 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, [P], and PO2. 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 −0.31 V 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 ~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 study 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 as, etc.) are bound together as units with defined molecular relationships. To calculate the metabolic behavior predicted for tissues with different mitochondrial content, the calculations need to be made for $1 \times 10^{-6}$ M cytochrome a (2 $\times 10^{-5}$ 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 compared directly 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 were processed for comparison with the experimentally measured behavior and graphed using Origin (www.originlab.com).

RESULTS

Effect of mitochondrial content on the metabolic changes in resting muscle following initiation of work. Figure 1, A and B, shows 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, 16, and 32 μM, whereas the intramitochondrial [NAD+]/[NADH], creatine pool, and PO2 are held constant at 0.1, 46 mM, and 25 torr, respectively. A PO2 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, <0.01 mM/s (47). For calculating the predicted changes, work was simulated by imposing a rate of ATP consumption of either 0.6 mM/s, equivalent to 13.4 ml O2/100 g tissue · min−1 and 6 μmol O2/g · min−1 (Fig. 1A), or 0.3 mM ATPs (Fig. 1B). These rates of ATP utilization are similar to those reported for work at −60 and 30% of maximum in untrained humans (26, 36, 44).

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. 1, A and B) increases slowly at first but accelerates to a maximum and then decreases 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/s (Fig. 1A).
than for 0.3 mM/s (Fig. 1B). The metabolic basis for the lag period and the conditions for which it is observed are discussed further 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 mM cytochrome c with an \([\text{NAD}^+/\text{NADH}]\) of 1 and 16 mM cytochrome c with an \([\text{NAD}^+/\text{NADH}]\) of 0.1. The predicted behaviors have been fitted to the equation: \(y = y_0 + Ae^{-kt}\). 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 the 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 Fig. 2B, when the rate of oxygen consumption
The greater decrease in [CrP]/[Cr] is needed to compensate for the lower [Pi] so that the rate of oxygen consumption in the working steady states is 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, 10, 20, 27, 49, 50, 53, 54). 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 \(\mu\)M, and 46 mM, respectively, the same as for Figs. 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 \(-3\) 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

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 that 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 phosphokinase 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 Fig. 1, A 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 [CrP]/[Cr] has to decrease further for the same increase in the rate of ATP synthesis. For a mitochondrial content of 16 \(\mu\)M, a work rate of 0.6 mM ATP/s and a creatine pool of 46 mM [CrP]/[Cr] in the working steady state are \(-1.1\) (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 that the rate of oxygen consumption in the working steady states is equal.

Dependence of the metabolic changes during the rest-to-work transition on the intramitochondrial [NAD\(^+\)]/[NADH] ratio. 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 Po\(_2\) at 25 torr, and the cyt c concentration at 16 \(\mu\)M. The predicted rates of ATP synthesis are plotted on the abissa against [CrP] (bottom axis) and [Pi] (top 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]. The increase in intramitochondrial [NAD\(^+\)]/[NADH] lowers the [CrP] for any specific rate of ATP synthesis. For a rate of 0.6 mM ATP/s, 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. 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 one-sixth of the rate of ATP synthesis, as 6 ATP are synthesized for each O\(_2\) consumed.
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 Fig. 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 to 31.6 mM, as might occur with a slight increase in resting ATP consumption, is shown in Fig. 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 PO2.** The metabolic changes during the rest-to-work transition have been calculated for PO2 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/s, respectively (Fig. 6). 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 the decrease in PO2 so that the time before reaching the working steady state is increased. The [CrP] in the working steady state is also dependent on PO2, decreasing from ~24 mM to <20 mM as the PO2 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 et al. (6) reported that rat hindlimb muscle contains 13.3 nmol cytochrome c/g wet wt of tissue, and this increased to 28.7 nmol/g wet wt with endurance training. Schollmeyer and Klingenberg (45) reported a value of 11.5 nmol/g wet wt in rat skeletal muscle, whereas Dudley et al. (7) reported that dog red gastrocnemius contained 19.5 nmol/g wet wt, and this increased to 26.3 nmol/g wet wt with training. The content of the enzymes of human skeletal muscle is reported to be ~70% of that in rat skeletal muscle (57), suggesting that the cytochrome c concentration in the skeletal muscle of untrained humans is closer to 8 μM than 16 μM, with the concentration increasing to ~24 μM with maximal training (26, 36). However, there is a large variability in the oxidative capacity of human muscle 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 Figs. 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 (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 [NAD+]/[NADH], also affect the length of the lag period.

Kindig and colleagues (29–31) and Poole and colleagues (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, an 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 is needed. However, the potential for a lag period is an intrinsic property of the regulation of oxidative phosphorylation under in vivo conditions.

Hogan (21), Holloszy (26), and Kindig et al. (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 the start of contractions were 13 ± 3 and 28 ± 5 s, respectively, for the first contraction cycle. These decreased to 5 ± 2 and 18 ± 4 s, 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 s and the time to 50% increase in respiratory rate is about 20 s for a work rate of 0.3 mM ATP/s. The longer lag period for the first cycle is consistent with J) insufficient activation of mitochondrial dehydrogenases such as pyruvate dehydrogenase, resulting in the intramitochondrial NAD couple becoming more oxidized and/or 2) 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 inhibition 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 the stimulations beginning. 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 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 and the change in [CrP] from rest to the working steady state from 40 to 30% and lowered the lactate concentra-

tration 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 with 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. 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 has 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 to 18.6 mM, and the time constant increases from 19 to 34 s. Experimental measurements of [CrP] in the working steady state have been reported for different PO2 in the inspired gas (22, 23, 32, 33). Hollis et al. (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 to 27.8 ± 4.3 s. This is similar to the reports of Haseler and colleagues (18, 19) in that 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 s, 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 workloads are imposed. The equilibrium constant for the creatine phosphokinase reaction is ~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 [P], the other substrate required for synthesis of ATP. ATP is hydrolyzed at the myofibrils, and the ADP and P produce have to diffuse back to the mitochondria 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 \textsuperscript{-1}·min \textsuperscript{-1}, and this translates into more than 0.7 mM ATP/s. The absence of a direct role in regulating oxidative phosphorylation. 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] 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 model, which shows similar linear correlations, does not attribute any direct effect of creatine phosphate on oxidative phosphorylation. The model, which shows similar linear correlations, does not attribute any direct effect of creatine phosphate on oxidative phosphorylation. The model, which shows similar linear correlations, does not attribute any direct effect of creatine phosphate on oxidative phosphorylation. The model, which shows similar linear correlations, does not attribute any direct effect of creatine phosphate on oxidative phosphorylation.
 tion 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 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.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
D.F.W. conception and design of research; D.F.W. performed experiments; D.F.W. analyzed data; D.F.W. interpreted results of experiments; D.F.W. prepared figures; D.F.W. drafted manuscript; D.F.W. edited and revised manuscript; D.F.W. approved final version of manuscript.

REFERENCES