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

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Wilson DF. Regulation of metabolism: the work-to-rest transition in skeletal muscle. Am J Physiol Endocrinol Metab 310: E633–E642, 2016.—The behavior of oxidative phosphorylation predicted by a model for the mechanism and kinetics of cytochrome c oxidase is compared with the experimentally observed behavior during the work-to-rest transition in skeletal muscle. For both experiment and model, when work stops, the increase in creatine phosphate and decrease in creatine and inorganic phosphate concentrations ([CrP], [Cr], and [Pi]) begin immediately. The rate of change for each is maximal and then progressively slows as the increasing energy state ([ATP]/[ADP][Pi]) suppresses the rate of oxidative phosphorylation. The time courses can be reasonably fitted to single exponential curves with similar time constants. The energy state in the working and resting steady states at constant P O2 are dependent on the intramitochondrial [NAD+] /[NADH], mitochondrial content, and size of the creatine pool ([CrP] + [Cr]). The rate of change in [CrP] is linearly correlated with [CrP] and with [Pi] and [Cr]. The time constant for [CrP] increase in the resting and working steady states, and the rate of decrease in oxygen consumption are similarly dependent on the P O2 in the inspired gas (experimental) or tissue P O2 (model). Myoglobin strongly buffers intracellular P O2 below ~15 torr, truncating the low end of the oxygen distribution in the tissue and suppressing intra- and intermyocytic oxygen gradients. The predictions of the model are consistent with the experimental data throughout the work/rest transition, providing valuable insights into the regulation of cellular and tissue metabolism.

energy metabolism; oxidative phosphorylation; metabolic control; metabolic homeostasis; exercise; oxygen consumption; respiration

MITOCOHRDIAL OXIDATIVE PHOSPHORYLATION is programmed to set and maintain metabolic homeostasis (55–57, 62). As a result, oxidative phosphorylation provides robust, precise, and real-time control of metabolism, responding to rapid changes in energy requirements as needed to return metabolism to the homeostatic set point. A precise metabolic set point combined with a robust system for maintaining that set point provides cross-platform coordination of metabolism and allows cellular differentiation within an organism and development of complex structures. A model describing control of oxidative phosphorylation has been developed by combination of the rate expression for the mechanism of oxygen reduction by cytochrome c oxidase (42, 58, 59, 62, 63) with near equilibrium of the first two sites of oxidative phosphorylation (55–57). This model predicts behavior consistent with the steady-state energy status and respiratory rates for a wide range of cells and tissues (55–57, 62). In order to gain insights into the dynamics of metabolic control and to more rigorously test the model, the predicted behavior is compared with that observed experimentally for physiologically important transients in energy metabolism. The larger the changes in metabolic rate, the more rigorously the predictive reliability of the model can be tested. To be considered successful, the behavior predicted by the model should not only be consistent with available experimental measurements but also predict behavior that has not yet been measured but can be experimentally verified. The largest physiologically observed changes in metabolism, and those for which the most experimental data are available, occur when skeletal muscle begins or stops working. These involve increase or decrease in the rate of ATP utilization that can exceed 100-fold, providing a very demanding test for the model. The physiological changes in metabolic rate in other tissues are typically less than in muscle, but current evidence indicates that the internal program of oxidative phosphorylation is the same for all tissues (55–57, 62). The differences among tissues are in the context of the “tissue-specific” parameters, the content of mitochondria, size of the creatine phosphate (CrP) pool, and dehydrogenases that provide the reducing equivalents.

In an earlier paper, the predictions of the model were shown to be consistent with experimental measurements of the metabolic changes that occur when resting muscles begin working (56). A priori, it might be expected that the rest-to-work and work-to-rest transitions would be mirror images, the same process but one the reverse of the other, as long as the work did not significantly fatigue the muscle. In this paper, fatigue will be defined as “tiredness resulting from physical exertion” and assumed to be due to progressive changes not fully compensated over time. Moreover, since only metabolism is discussed, the work rate is assumed to be the rate of ATP utilization (in mM/s) and not mechanical work. When muscle starts to work, the metabolic transition is driven by the increased rate of ATP utilization. Metabolism responds to the decreasing energy state by increasing ATP production until it again equals the rate of consumption and a new working steady state is achieved, but at a lower energy state. When muscle stops working, the rate of ATP consumption suddenly decreases relative to the rate of production, and metabolism responds to the decreasing energy state by decreasing the rate of production until it is again equal to the rate of consumption. Experimental measurements often show significantly different behavior for the rest-to-work and work-to-rest transitions. Although some of the reported differences can be attributed to muscle fatigue, differences have been observed even for short submaximal work efforts where fatigue is not a significant issue. When work is initiated, for example, oxygen consumption often shows a lag period (delay), during which the rate of increase starts slowly and goes through a period of accelerating rate before the maximum rate of increase is attained (4, 15–17, 22, 43, 44, 54). This contrasts with the work-to-rest transition where the decrease in oxygen consumption and increase in [CrP] begin immediately when work ceases. In the present paper, the behavior predicted by the model is compared with experimental measurements of the

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metabolic changes that occur when muscles stop working. The metabolic parameters that regulate the rate of mitochondrial oxidative phosphorylation (8–10, 21, 25, 40, 55–62) include: 1) the content of mitochondrial respiratory enzymes; 2) the energy state ([ATP]/[ADP][Pi]), with the Cr-CrP pool playing an important support role in muscle and brain; 3) the intramitochondrial [NAD\(^+\)]/[NADH]; and 4) the intracellular PO\(_2\). The present paper shows that the predictions of the model for oxidative phosphorylation are consistent with the available data for the work-to-rest transition. In the model, the contribution of each metabolic parameter in determining the rate and extent of the metabolic changes is readily quantified. As a result, predictions by the model provide insight into the quantitative relationships among the regulatory parameters that are very difficult to work out experimentally.

**EXPERIMENTAL METHODS AND MATERIALS**

The steady-state rate expression for the mechanistic model for mitochondrial mitochondrial cytochrome c oxidase has been derived and shown to fit the kinetics of the reaction in isolated mitochondria (58, 59, 62, 63). The rate expression for the cytochrome c oxidase (site 3 of oxidative phosphorylation) has been extended by adding the first two sites:

\[
\text{NADH} + 2c^3c^+ + 2\text{ADP} + 2\text{Pi} = \text{NAD}^+ + \text{H}^+ + 2c^{2+} + 2\text{ATP}
\]

(Eq. 1)

Equation 1 has been shown to be fully reversible and near equilibrium (8–11, 18, 21, 40, 55, 56, 57). Combining the equilibrium expression for Eq. 1 with the rate expression for cytochrome c oxidase results in a rate expression appropriate for oxidative phosphorylation:

\[
\text{NADH} + 1/2\text{O}_2 + 3\text{ADP} + 3\text{Pi} \rightarrow \text{NAD}^+ + \text{H}^+ + 3\text{ATP} + \text{H}_2\text{O}
\]

(2)

where the NAD couple is that of the intramitochondrial space, the cofactor for the NAD-linked dehydrogenases of the citric acid cycle, fatty acid oxidation, and amino acid catabolism. The derivation of the steady-state rate expressions for cytochrome c oxidase and for oxidative phosphorylation as well as the programs for those rate expressions for MatLab (www.mathworks.com) can be found at ULR:

http://www.med.upenn.edu/bioiobiop/faculty/wilson/index.html.

The behavior predicted by the model has been calculated using MatLab and processed for comparison with the experimentally measured metabolite levels and graphed using Origin (www.originlab.com).

To compare the behavior predicted by the rate expression with experimental data for tissue metabolism, it is important to know and use the appropriate tissue-specific parameters, which include the mitochondrial content, ATP, ADP, Pi, CrP, Cr, and tissue PO\(_2\). Metabolite measurements from the literature have been used where possible, but in many cases missing parameters have been “filled in” using data from other papers. The “filled-in” values most often needed were the mitochondrial content, total [Cr], [Pi], and PO\(_2\). It should be noted that the tissue-specific parameters vary widely among muscle fiber types and where possible this should be taken into account. In the present paper, however, most of the data are from muscles with a mixture of fiber types and fit was to the data for the whole muscle. Although it would be interesting and informative to compare fiber types, this was considered outside of what could be discussed in this paper. The concentrations of ADP and AMP presented in this paper are for the free ADP and AMP, not total cellular concentrations, calculated assuming near equilibrium of the adenylate kinase and creatine phosphokinase reactions (13, 31). The PO\(_2\) for oxygen binding to myoglobin in vivo is assumed to be 5 mM (3.6 torr). When the dependence on regulatory parameters other than PO\(_2\) was calculated, the PO\(_2\) in tissue was assumed to be 25 torr, a value considered representation of the mean value in the interstitial space of normal muscle.

It should be kept in mind that mitochondria are particulate enzyme complexes and the internal components (cytochrome c, cytochrome a\(_x\), etc.) are bound together with defined stoichiometry. When calculating the predicted metabolic behavior for tissues with different mitochondrial content, the calculations are made for 1 × 10\(^{-6}\) M cytochrome a (2 × 10\(^{-6}\) M cytochrome c), the conditions for which the model was fitted 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 compared with the experimental measurements in tissues.

**RESULTS**

The effect of mitochondrial content on the changes in metabolism that occur when muscle stops working and metabolism transitions to a resting state. Figure 1, A and B, shows the predicted changes in [CrP] when muscle working at a moderate rate stops working. The calculations were made for cytochrome c concentrations of 8 and 16 μM while the intramitochondrial [NAD\(^+\)]/[NADH] ratio, total [Cr], and PO\(_2\) are held constant at 0.33, 46 mM, and 25 torr, respectively. In resting muscle, the basal rate of ATP utilization is assumed to be low, less than 0.01 mM ATP/s (53), and in the working steady state this increased to 0.6 mM ATP/s (Fig. 1A) or 0.3 mM ATP/s (Fig. 1B). An ATP consumption rate of 0.6 mM/s is ~6 μmol O\(_2\)/100 g tissue **·min**\(^{-1}\), a work rate ~60% of the maximal oxygen consumption rate in human muscle (sedentary). The work-to-rest transition was initiated by stopping consumption of ATP. The [CrP] in the working steady state is higher for higher mitochondrial content. For a work rate of 0.6 mM ATP/s the [CrP] levels for 8 and 16 μM cytochrome c are 15.9 and 20.8 mM, respectively, whereas for a work rate of 0.3 mM ATP/s they are 21.6 and 24.6 mM, respectively. When work stops, the increase in [CrP] begins immediately and then slows as the increasing energy state suppresses the rate of ATP synthesis by oxidative phosphorylation.

Experimental measurements of the increase in [CrP] with time after the muscles stop work are often reported as the time constant calculated by fit to a single exponential. The increase in [CrP] with time after work stops as predicted by the model is readily approximated by a single exponential (see Fig. 1). The experimentally measured rate of respiration (rate of ATP synthesis) has also been reported to be linearly correlated with [CrP]. As shown in Fig. 2, the rate of ATP synthesis (oxygen consumption) predicted by the model is linearly correlated with [CrP] over a wide range of rates, the slope of the straight line increasing in proportion to the increase in concentration of cytochrome c. Synthesis of CrP results in a stoichiometric decrease in [Pi] and [Cr]. As a result, decreases in [Cr] and [Pi] that occur also fit a single exponential with the same time constant as that for increase in [CrP]. Their concentrations, like [CrP], are linearly correlated with the rate of ATP synthesis. As an illustration, the [Pi] is plotted with the axis label in Fig. 2, top. The axis has been adjusted such that the points for [Pi] are superimposed onto those for [CrP].

**Dependence of metabolism during work-to-rest transition on the size of the creatine pool (Cr + CrP).** The creatine phosphokinase reaction and the [CrP]/[Cr] ratio are important to
Dependence of metabolic steady states and rate of changes that occur on the intramitochondrial [NAD^+]/[NADH] ratio when work stops. The intramitochondrial [NAD^+]/[NADH] plays an important role in regulating oxidative phosphorylation (8–11, 21, 40, 55–57, 60). Figure 4, A and B, shows the predicted dependence of the rate of ATP synthesis on [CrP] and [Pi] at intramitochondrial [NAD^+]/[NADH] ratios of 0.033, 0.1, 0.33, 1, and 3. The P_O2, cytochrome c concentration, and Cr pools were 25 torr, 16 µM, and 46 mM, respectively. The [CrP] at each rate of ATP synthesis is higher as the intramitochondrial NAD pool becomes more reduced. An ATP synthesis rate of 0.3 mM/s is achieved at [CrP] concentrations of about 28.5, 27, 22.4, and 19.5 mM when the [NAD^+]/[NADH] ratio is 0.033, 0.1, 0.33, 1, and 3, respectively. These ratios have been selected to encompass the ratios reported for muscles in vivo (14, 21, 40) as adjusted to the intramitochondrial pH of 7.5 (11). The values for the [CrP] are appropriate only for the a Cr pool of 46 mM, because the rate of ATP synthesis is dependent on the adenylate energy state, not [CrP]. The values of the [CrP]/[Cr][Pi] are 192, 142, 99, and 65 M⁻¹, respectively, equivalent to energy state ([ATP]/[ADP][Pi]) values of 2.7 × 10⁴, 2.0 × 10⁴, 1.4 × 10⁴, and 9.2 × 10³ M⁻¹. The dependence of the rate of ATP synthesis on the adenylate energy state is independent of the size of the creatine pool.

Figure 4B presents a 10× expansion of the y-axis of the curves in Fig. 4A, showing that the linear correlation between the rate of ATP synthesis and [CrP] is observed only well above the resting rate and there is a pronounced nonlinearity as the resting steady state is approached. The rate of ATP consumption in skeletal muscle at rest is low, typically less than 1% of the maximal rate, or a cytochrome c turnover of ~0.5/s.

Fig. 1. Changes in metabolism when muscle stops working, as calculated for different concentrations of cytochrome c (cyt c; mitochondrial content). Calculations were made for a total creatine (creatine [Cr] + creatine phosphate [CrP]) concentration of 46 mM, intramitochondrial [NAD^+]/[NADH] of 0.33, and a P_O2 of 25 torr. The behavior of cytochrome c concentrations of 8 and 16 µM are presented for muscle working at 0.6 mM ATP/s (A) and 0.3 mM ATP/s (B). Levels of [CrP] in the working steady state increase with higher content of mitochondria and with decrease in work rate. [CrP] begins to rise immediately when work stops. Rate of increase slows due to increasing energy state with an asymptotic approach to resting steady-state levels. Increases in [CrP] in A have been fitted to a single exponential (y = y₀ + Ae⁻kt). Calculated data points are in larger, open symbols; fitted exponential in smaller, solid symbols. The constants yielding the best fit to a single exponential are given in Table 2 for both A and B.

Fig. 2. Correlation of the predicted increase in [CrP] and decrease in inorganic phosphate ([Pi]) with the decrease in the rate of ATP synthesis. The predicted increase in [CrP] and decrease in [Pi] following work stoppage are shown for cytochrome c concentrations of 8, 16, and 32 µM. Conditions used are the same as those for Fig. 1A: a Cr pool of 46 mM, an [NAD^+]/[NADH] of 0.33, and a P_O2 of 25 torr. The [CrP] scale is shown on the bottom abscissa; that for [Pi] is shown on the top abscissa. Scales have been adjusted so that individual points superimpose. There is a nearly linear correlation between [CrP] and [Pi] and rate of ATP synthesis with the slope increasing with increasing cyt c concentration.

Figures 3 shows the effect of decreasing the Cr pool from 46 to 23 mM while holding the other variables the same as for Fig. 1, except that the intramitochondrial [NAD^+]/[NADH] is 0.1 instead of 0.33. In each case, the increase in [CrP] and decrease in oxygen consumption begin as soon as the work stops. In the working steady state, the [Pi] is lower for the smaller Cr pool, so the [CrP]/[Cr] in the working steady state is lower. As for the 46 mM Cr pool, there is a linear correlation between [CrP], [Pi], and [Cr] and the rate of ATP synthesis over a wide range of rates.
The combination of a quite reduced intramitochondrial NAD couple ([NAD⁺]/[NADH] near 0.1) and the low rate of ATP utilization results in the [CrP]/[Cr] ratio being high in the resting steady state.

PO₂ dependence on metabolic changes that occur when muscle stops working. The work to rest metabolic changes have been calculated for PO₂ values of 50, 25, 10, and 5 torr while holding the Cr pool, intramitochondrial [NAD⁺]/[NADH], cytochrome c concentration, and work rate at 23 mM, 0.2, 16 μM, and 0.6 mM ATP/s, respectively (Fig. 5). The initial [CrP] (in the working steady state) is lower for lower oxygen pressures. When work ends, [CrP] begins to increase with time. The initial rate of increase is determined by the rate of ATP utilization/synthesis in the working steady state and is not dependent on oxygen pressure. As [CrP] increases, the rate of ATP synthesis by oxidative phosphorylation progressively decreases, and the increase in [CrP] slows until the resting steady state is attained. For PO₂ values of 50, 25, 10, and 5 torr, the approximate [CrP] values in the working steady state (Cr pool of 23 mM) are 11, 10.4, 8.9, and 7.7 mM. The differences in rate of increase in [CrP] when work stops and in the level of [CrP] in the working steady state are expressions of the oxygen dependence of oxidative phosphorylation.

Role of myoglobin in maintaining local oxygen pressure and sustaining oxidative phosphorylation. Human oxidative skeletal muscle contains 0.3–0.5 mM myoglobin (1, 64). This is sufficient to substantially increase the oxygen available for oxidative phosphorylation (expressed as nmol·ml⁻¹·torr⁻¹) at oxygen pressures below ~30 torr. Figure 6 shows that as PO₂ decreases, the oxygen supplied by oxymyoglobin is approximately equal to that dissolved in the cellular medium. With continuing fall in PO₂, the oxygen provided by oxymyoglobin (nmol·ml⁻¹·torr⁻¹) progressively increases, reaching 5, 10, 20, and 30 times that in the absence of myoglobin at PO₂ values of 50, 25, 10, and 5 torr, respectively (Fig. 6).

Fig. 3. Dependence of metabolism during the work-to-rest transition on the size of the Cr pool. [CrP] and oxygen consumption rates were calculated for muscle in which the Cr pool was 23 mM instead of the 46 mM used in the previous figures. Intramitochondrial [NAD⁺]/[NADH], PO₂, and cyt c concentrations were 0.1, 25 torr, and 16 μM, respectively. Two different work rates (0.6 and 0.3 mM ATP/s) are presented. The increase in [CrP] and decrease in the rate of ATP production occur over a shorter time scale than for a Cr pool of 46 mM. The decrease in [Pi] for a pool of 23 mM is less than for a pool of 46 mM, so the [CrP]/[Cr] in the working steady state is lower.

Fig. 4. Dependence of the rest-to-work metabolic transition on intramitochondrial [NAD⁺]/[NADH]. The intramitochondrial NAD pool is the primary source of the reducing equivalents used by oxidative phosphorylation to synthesize ATP. The influence of intramitochondrial [NAD⁺]/[NADH] on rest-to-work transition was calculated for values of 0.1, 0.3, 1, and 3 while holding the Cr pool at 46 mM, PO₂ at 25 torr, and cyt c concentration at 16 μM. Predicted rates of ATP synthesis are plotted against [CrP]. As [CrP] increases from working steady state to ~34 mM in the resting state, the rate of ATP synthesis initially falls linearly with increase in [CrP] and decrease in [Pi]. There is a strong curvature at low rates, however, as [CrP] and [Pi] asymptotically approach resting steady-state values. This is more obvious in B, where the y-axis has been expanded 10× to show the approach to the resting steady state in more detail. Decreasing intramitochondrial [NAD⁺]/[NADH] decreases [CrP] for any specific rate of ATP synthesis. For a rate of 0.6 mM ATP/s, for example, [CrP] in the working steady state is 24, 21.6, 19.2, and 16.4 mM for [NAD⁺]/[NADH] values of 0.1, 0.3, 1, and 3, respectively.
ATP synthesis after stopping work were independent of PO2. Working steady-concentration, and work rate were 23 mM, 0.1, 16/H9262 M, and 0.6 mM ATP/s, respectively. The initial rate of increase in [CrP] and decrease in the rate of ATP synthesis after stopping work were independent of PO2. Working steady-state [CrP] is, however, lower for lower values of PO2, 7.8, 8.9, 10.3, and 11 mM for PO2 values of 5, 10, 25, and 50 μM, respectively. The time required to reach resting steady state increases markedly with decrease in PO2.

19, 11, 6, and 3 torr, respectively. The increase in oxygen that can be used per torr decrease in PO2 means that, in working muscle, the low oxygen part of the oxygen distribution remains significantly higher than it would in the absence of myoglobin. The presence of an oxygen buffer (myoglobin) decreases both local (intramyocyte) and regional (intermyocyte) differences in PO2. Buffering of PO2 by myoglobin suppresses the appearance of regions with oxygen pressures less the ~5 torr needed to maintain the energy state and is critically important to muscle function, particularly at near maximal work rates.

DISCUSSION

The model is expected to be consistent with cellular energy metabolism throughout the regulatory range up to near, but not including, the maximal rate. From an evolutionary standpoint, the maximal rate is the design limit, presumably because the “cost” of further increase was greater than the evolutionary advantage. As the maximal rate is approached, the rate becomes dependent on limitations in oxygen delivery and other factors not directly involved in regulation of oxidative phosphorylation. These are not included in the model, so the model is not able to predict behavior near the maximal rate. For this reason, in this paper the presentation is limited to metabolic rates of up to 60–80% of the maximal rates. The dynamic range covered by the model is large, from rest to rates 80 times the resting rate. This encompasses most experimental exercise protocols except those designed to determine the maximal rates for individual muscles. Similarly, the model does not predict any of the progressive changes observed with sustained or very hard work, such as glycogen depletion or lactate accumulation.

Comparison of experimental data with behavior predicted by the model. When skeletal muscle stops working, the time dependence of the resulting increase in [CrP], both experimen-

tally observed (Table 1) and predicted by the model (Table 2), can be fitted reasonably well by a single exponential. Table 1 shows the reported time constants for the increase in [CrP] with time after work stops, while Table 2 shows the time constants predicted by the model for cytochrome c contents of 8 and 16 μM and [NAD+]/[NADH] ratio of 0.33 and a PO2 of 25 torr. The predicted increases are not single exponentials, because the value of the time constant obtained by fit to a single exponential increases with decreasing working steady state. The deviation from a single exponential is, however, small enough that the noise in experimental data is sufficient to obscure it. The time constants calculated for the experimental data and for the model are in agreement within the uncertainty in the measurements of mitochondrial content and other tissue-specific metabolic parameters. Decrease in the rate of oxygen consumption is linearly correlated with the increase in [CrP] for both experiment (3, 12, 19, 26, 34, 36, 37, 39, 48, 50, 51) and model. It should be noted that fit of the increase in [CrP] with time by a single exponential indicates there is a linear correlation between [CrP] and rate of ATP synthesis (and rate of respiration). The creatine kinase reaction, however, is not involved in the regulation of oxidative phosphorylation except through equilibration with the adenine nucleotide pool. The model predicts that the [CrP] in the working steady state at constant PO2, work rate, and intramitochondrial [NAD+] /[NADH] increases with increasing mitochondrial content. This is consistent with the experimental observations in different muscle types (5, 29) and with endurance training of muscles (6, 7, 27, 35). The rate of oxidative phosphorylation (rate of ATP synthesis and rate of respiration) increases with decreasing energy state. The rate of ATP production for any specific metabolic condition is proportional to the mitochondrial content, so increasing the mitochondrial content twofold decreases the required cytochrome c turnover number by one-half. The transition from working steady state to the resting steady state

Fig. 6. Amount of oxygen in muscle that can be used for each decrease of 1 torr in oxygen pressure. Muscle was assumed to have myoglobin content of 0.5 mM and PO2 for binding oxygen of 3.6 torr. Solubility of oxygen in cells was assumed to be 1.4 mmol·ml⁻¹·torr⁻¹ (no hemoglobin, □). Oxygen released from oxymyoglobin becomes ~1.4 mmol·ml⁻¹·torr⁻¹ at 30 torr, and this increases rapidly as PO2 falls below 30 torr (+ myoglobin, ■).
occurs more quickly with increasing mitochondrial content, consistent with experimental observations in different fiber types and when one compares sedentary and trained animals (6, 7, 35, 38). In rats, the cytochrome c content of skeletal muscle is \( \sim 12 \text{ nmol/g wet wt} \) (7, 49), and in human muscle it is reported to be \( \sim 5.8 \text{ nmol/g wet wt} \) in upper leg muscle (2) or \( \sim 70\% \) of that in rat skeletal muscle (65). This suggests that in sedentary humans the cytochrome c level is near 8 \( \mu \text{M} \), with 16 \( \mu \text{M} \) being more appropriate for trained individuals. Humans have a wider range of muscle oxygen consumption rates than rats, and when one compares sedentary individuals with elite athletes the differences in mitochondrial content might be expected to be greater than for sedentary vs. trained rats. If the mitochondrial content is included when calculating the predictions for the model, they are consistent with the available experimental data.

The dependence of muscle metabolism on local oxygen pressure. Measurements of the rate of oxygen consumption by cells typically show that the rate is maintained until the \( \text{PO}_2 \) falls below \( \sim 5 \text{ torr} \), with the rate decreasing to 50% below 1 torr (32, 45). The model predicts that if the energy state and reduction of the intramitochondrial NAD couple are held constant, the respiratory rate begins to decrease at a \( \text{PO}_2 \) above 35 torr and to decrease to 50% of maximal rate by \( \sim 15 \text{ torr} \). In vivo, however, the energy state and intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) are regulatory variables. The function of oxidative phosphorylation is to synthesize ATP at the rate that it is being consumed, and the reactions that consume ATP (ion transport, mechanical work, molecular synthesis, etc.) are generally not very, if at all, dependent on the oxygen pressure. As \( \text{PO}_2 \) decreases, the respiratory rate begins to fall only when the decrease in energy state and intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\) no longer sustain the rate of ATP synthesis. The changes in energy state and \([\text{NAD}^+] / [\text{NADH}]\) needed to decrease the \( P_50 \) for oxygen from 15 torr to less than 1 torr can be calculated. Figure 7 shows the increase in reduction of the intramitochondrial NAD couple, as a percentage of NADH, predicted to hold the respiratory rate constant as the \( \text{PO}_2 \) decreases from 35 torr to less than 5 torr when the energy state is constant. For a cytochrome c turnover number of 4/s, the increase in NADH is from \( \sim 70\% \) to 95\%, an increase of 25\%. This increase is sufficient to keep the rate of ATP synthesis constant as the \( \text{PO}_2 \) decreases from 50 torr down to 5 torr (decreasing the \( P_50 \) to \(<1 \text{ torr} \)). The dependence is on the \([\text{NAD}^+] / [\text{NADH}]\), not \([\text{NADH}]\), so, as the reduction of the NAD couple at 50 torr increases to above 50\%, the increase in NADH needed to hold the rate constant is smaller. For a cytochrome c turnover of 8/s, for example, the increase is from 84 to 94\%.

Decrease in energy state can also maintain the rate of ATP synthesis as \( \text{PO}_2 \) decreases. The decrease in \([\text{CrP}]\), at constant intramitochondrial \([\text{NAD}^+] / [\text{NADH}]\), that would hold the respiratory rate constant from 50 torr down to 5 torr is shown in Fig. 8. The calculations are for a \( \text{Cr} \) pool of 46 mM, an \([\text{NAD}^+] / [\text{NADH}]\) of 0.2, and cytochrome c TNs of 3, 5, and 10/s. The \([\text{CrP}]\) in the working steady states at a \( \text{PO}_2 \) of 50 torr are 30.5, 29.8, and 28.5 mM, respectively, and as the \( \text{PO}_2 \) decreases to 5 torr, \([\text{CrP}]\) decreases to 28, 26.9, and 24 mM, respectively. The decreases in \([\text{CrP}]\) are 8, 9.7, and 16%, respectively. The oxygen dependence of oxidative phosphorylation predicted by the model is consistent with the \( P_50 \) for oxygen measured in cells and tissues. Isolated mitochondria are damaged during isolation, and this introduces an energy dependent “leak” reaction that confounds both experimental design and data interpretation. In vivo, both the intramitochondrial

### Table 1. Measured time constants for increase in [CrP] and decrease in [Pi] in human muscle that were measured when exercise was stopped

<table>
<thead>
<tr>
<th>Source</th>
<th>Muscle Group</th>
<th>( N )</th>
<th>pH</th>
<th>TCrP-Off</th>
<th>TPi-Off</th>
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<tr>
<td>McCann et al. 1995 (36)</td>
<td>Wrist flexor</td>
<td>4</td>
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<td>22.5</td>
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<td>Plantor flexors</td>
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<td>6.99</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>McCreary et al. 1996 (37)</td>
<td>Wrist flexion</td>
<td>&quot;*&quot;</td>
<td>pH adj</td>
<td>33.3</td>
<td>28.6</td>
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<td>Plantor flexion</td>
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<td>7.08</td>
<td>25.0 ( \pm ) 2.7</td>
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<td>Plantor flexion</td>
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<td>7.01</td>
<td>31.5 ( \pm ) 8.5</td>
<td></td>
</tr>
<tr>
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<td>Quadriceps</td>
<td>9</td>
<td></td>
<td>20.9 ( \pm ) 2.9</td>
<td></td>
</tr>
<tr>
<td>Vanhatalo et al. 2011 (30)</td>
<td>Quadriceps</td>
<td>9</td>
<td>7.0</td>
<td>23 ( \pm ) 6</td>
<td></td>
</tr>
<tr>
<td>Vanhatalo et al. 2014 (51)</td>
<td>Quadriceps</td>
<td>8</td>
<td>7.11</td>
<td>24 ( \pm ) 4</td>
<td></td>
</tr>
<tr>
<td>Fulford et al. 2013 (12)</td>
<td>Quadriceps</td>
<td>8</td>
<td>7.03</td>
<td>25.4 ( \pm ) 5.0</td>
<td></td>
</tr>
</tbody>
</table>

Data are all for exercise work rates and periods of time for which the muscle was not significantly acidified. This was done to avoid the effects of muscle fatigue and altered tissue pH. TCrP-off and TPi-off are the time constants in seconds for the increase in [CrP] and decrease in [Pi] obtained by fit of the measurements to a single exponential.

Table 2. Time course for increase in [CrP] predicted by the model, as fitted to a single exponential

<table>
<thead>
<tr>
<th>yo (mM)</th>
<th>A</th>
<th>T (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 mM ATP/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt c = 8 ( \mu \text{M} )</td>
<td>28.6</td>
<td>-12.6</td>
</tr>
<tr>
<td>Cyt c = 16 ( \mu \text{M} )</td>
<td>29.3</td>
<td>-7.5</td>
</tr>
<tr>
<td>0.3 mM ATP/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt c = 8 ( \mu \text{M} )</td>
<td>28.7</td>
<td>-7.1</td>
</tr>
<tr>
<td>Cyt c = 16 ( \mu \text{M} )</td>
<td>29.6</td>
<td>-4.6</td>
</tr>
<tr>
<td>0.15 mM ATP/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyt c = 8 ( \mu \text{M} )</td>
<td>29.2</td>
<td>-4.2</td>
</tr>
<tr>
<td>Cyt c = 16 ( \mu \text{M} )</td>
<td>29.8</td>
<td>-2.9</td>
</tr>
</tbody>
</table>

Predicted behavior was calculated for a creatine pool, cytochrome c (Cyt c) concentration, \( \text{PO}_2 \), and \([\text{NAD}^+] / [\text{NADH}]\) of 46 mM, 8 or 16 \( \mu \text{M} \), 25 torr, and 0.33, respectively. The concentration of inorganic phosphate ([Pi]) was assumed to be 3 mM when the [CrP] was 34 mM. Data for work rates of 0.6, 0.3, and 0.15 mM ATP/s were fitted to the equation \( y = yo + A e^{-t/T} \) using Origin. Values in the table are the best fit (least squares) to a single exponential. The increase in the time constant (T) as the steady-state work rate decreases shows that the increase in [CrP] is not exponential. The deviation from exponential is small enough, however, that in experiments it would be “hidden” by the noise.
In the absence of myoglobin and at a PO2 of 20 torr, the phosphorylation, where a further fall in PO2 compromises energy for the decrease in P O2 in order to hold the rate of ATP turnover (rate of ATP synthesis) constant at 2, 6, and 8 s
chondrial NAD couple needs to become in order to hold the rate of cyt c turnover (rate of ATP synthesis) constant at 2, 6, and 8 s−1 while PO2 was decreased from 50 to 5 torr. [CrP], [Cr], and [Pi] was held constant at 28, 18, and 9 mM, respectively, for an energy state of 2.4 × 10^4 M−1. Values for [NADH] are shown as %total NAD and NADH. These turnover numbers are similar to those seen in unstimulated cultured cells or isolated hepatocytes. At constant energy state, the intramitochondrial NAD couple has to be more reduced to support higher rates of ATP synthesis.

Role of myoglobin in muscle metabolism. It is clear that myoglobin plays an important positive role(s) in muscle function or it would not be so widely conserved in evolution. Myoglobin is not present in tissues such as kidney, brain, and liver, although these tissues have high metabolic rates and their content of mitochondria is similar to that of skeletal muscle. Thus, advantages proposed for myoglobin need to be specific to muscle. Oxygen buffering by myoglobin is well matched to the oxygen requirements of oxidative phosphorylation. Half-saturation of oxygen binding by myoglobin results in strong buffering of the PO2 in the critical range for oxidative phosphorylation, where a further fall in PO2 compromises energy metabolism. The presence of myoglobin is much more important to muscle than to other tissues because of the rapid changes in rate of oxygen consumption (ATP utilization), the high rates reached, and the compression that occurs during strong muscular contractions, especially sustained tetanic contractions, which can suppress blood flow and result in transient periods of ischemia.

One additional function of myoglobin is to store oxygen. The myoglobin content of muscle in diving mammals, such as the sperm whale, is ~10 times higher than in human muscle (1, 41). In the absence of myoglobin and at a PO2 of 20 torr, the dissolved oxygen is just 28 nmol/ml, equivalent to 0.168 mM ATP. In diving mammals, with ~5 mM myoglobin, oxymyoglobin in muscle provides a store of oxygen that is 200 times that of the dissolved oxygen (equivalent to 30 mM ATP). This is a substantial increase, particularly when account is taken of the large volume of muscle, only part of which is used for diving. The oxygen from muscles that are less active during the dive reenters the blood and is transported to the working muscles. This is consistent with oxygen bound to myoglobin playing an important role in making possible the long dive times use for feeding (41). This increase in myoglobin content is an evolutionary adaptation in which the levels for myoglobin in muscle increased in response to a specific environmental challenge.

It has been suggested that myoglobin is present in order to facilitate oxygen diffusion (64). This view assumes that oxygen delivery to the mitochondria in muscle is diffusion limited and that diffusion of myoglobin provides a parallel pathway for oxygen to reach the mitochondria. Theoretical calculations indicate that myoglobin diffusion may increase the oxygen flux by as much as a factor of 4 (64). If delivery of oxygen to the mitochondria were diffusion limited, however, this would eliminate the role of myoglobin as an oxygen buffer, replacing a large benefit of myoglobin with a much smaller one. Diffusion limitation of the delivery of oxygen to the mitochondria may be a significant factor in determining the maximal work rate of muscle but is not a factor under normal working conditions.

Summarizing for the role of myoglobin. The oxidative skeletal muscle of land animals has 0.3 to 0.5 mM myoglobin. This is sufficient for it to act as an oxygen buffer that 1) truncates the low end of the oxygen distribution in working muscle to keep the local PO2 values from falling into the region where the function of oxidative phosphorylation is severely compromised, and 2) flattens distribution of oxygen within and among myocytes (52), helping to coordinate their energy states for optimal work efficiency. It is also a store of oxygen that helps
to delay failure of muscles during the ischemic episodes induced by tetanic contractions. These functions of myoglobin are important for optimal performance of oxidative red muscle, and myoglobin content of muscle correlates with mitochondrial content, high in oxidative red muscle and very low in glycolytic white muscle. These functions are not needed for other tissues, providing the evolutionary bases for the presence of myoglobin in muscles.

Role of glycogen and lactate production in muscle. Glycogen is capable of providing substantial amounts of ATP in addition to that formed by oxidative phosphorylation, 1.5 ATP per lactate produced. If the concentration of lactate in the muscle tissue becomes too high, however, the resulting acidosis can limit muscle function and, in extreme cases, cause tissue damage. When the pO2 is above about 5 torr and the work rate is not too high, i.e., oxidative phosphorylation can maintain the [CrP]/[Cr] above about 0.5, glycogenolysis is regulated to provide sufficient pyruvate for oxidative phosphorylation (the citric acid cycle) plus a small excess that appears as lactate. In normoxia this lactate production is small, when the energy state decreases to less than 50% of the control value (55–57). The sensitivity and range of control are also extraordinary. In muscle with a constant [NAD+/NADH], for example, the rate of ATP synthesis increases about 20 fold when [CrP] decreases only 24%, and the increases in [ADP] and [Pi] are only 90% and 150%, respectively. Robust cross platform control of metabolism allowed individual cells to differentiate and form groups (tissues) with specific functions and that had to work together for the benefit of the whole. It is reasonable to suggest that successful integration of the oxidative phosphorylation into metabolism contributed significantly to the “Cambrian explosion”, a relatively short period of time about 540 million years ago during which most of the current animal phyla developed. Importantly, mitochondrial oxidative phosphorylation is associated with the formation of specialized tissues in both animals and plants. The timing of the appearance of differentiation in plants is not as well documented as for animals, but occurred later. In plants, differentiation may have not progressed until chloroplasts were formed and photosynthesis could be controlled separately, but in coordination with, oxidative phosphorylation.

In summary. In experiments, where both the energy state and [NAD+]/[NADH] are expected to change, the increase in intramitochondrial [NADH] and decrease in [CrP] are difficult to measure accurately even under ideal conditions. It has not yet been possible to measure all of the regulatory parameters for oxidative phosphorylation, pO2, energy state (either [ATP], [ADP], and [Pi] or [CrP], [Cr], and [Pi]), intramitochondrial [NAD+]/[NADH], cytochrome content, and respiratory rate in the same experiment. The model developed earlier (55, 56, 59, 62, 63) has been shown to be consistent with the wide range of available experimental data, including the rapid and large metabolic transitions from rest to work (56) and work to rest (this paper). As such, the model is a robust mathematical representation of the regulation of oxidative phosphorylation, and therefore of energy metabolism in eukaryotic cells. This opens a window through which to “see” how the individual parameters work together to form an integrated and precise control system. The model therefore provides important insight into how metabolic homeostasis is set and maintained. The precision with which the program in oxidative phosphorylation sets the metabolic energy state in eukaryotes is remarkable, as is its ability to maintain homeostasis while allowing for large swings in metabolic rate. It is easy to see how this precise, robust, across platform control of metabolism could provide the basis for cellular differentiation and the development of complex organisms.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
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.

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