There is no single answer to the question ‘How is oxidative phosphorylation controlled (or regulated)?’. Control varies with conditions, and is shared between at least two different reactions under all conditions. However, it is possible to provide a simple quantitative description of the control using control analysis. Because of the kinetics of all three blocks of reactions involved, oxidation and phosphorylation are controlled differently. This has important consequences for the regulation of the rate and of the efficiency of oxidative phosphorylation.

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Control of oxidative phosphorylation in muscle
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Introduction
Metabolic control in skeletal muscle accommodates a wide dynamic range of energy demand from at rest, through during exercise to in recovery. During exercise both the anaerobic and the aerobic pathways are activated, while in recovery, aerobic ATP production is dominant. In skeletal muscle, phosphorus-containing metabolites are the most important regulators of the synthesis of ATP by the mitochondrion. There remains disagreement about the precise mechanism of the control of oxidative phosphorylation in vivo [1]. Several mechanisms may be involved, including control by P₃, ADP, phosphorylation potential and by substrate availability. It is unlikely that a single mechanism regulates ATP production under all conditions.

Information about the control of mitochondrial function in skeletal muscle in vivo can be obtained from the relationship between the rate of mitochondrial oxidation and the intracellular concentrations of phosphorus metabolites, although the analysis is complicated by the constraints that are imposed by the creatine-kinase equilibrium [2]. We and others have used phosphorus (³¹P) n.m.r. of skeletal muscle in humans and in animals to analyse the concentrations of ATP, ADP, phosphocreatine (PCr), P₃ and H⁺, at rest, during exercise at different workloads and in recovery from exercise (for a review see [3]).

We approached the problem of the control in skeletal muscle in several ways: (1) by studying the

Abbreviation used: PCr, phosphocreatine.
control of PCr resynthesis during recovery from exercise in human skeletal muscle [2], including the consideration of processes that generate and that remove H+ from the muscle cell [4]; (2) by formulating a theoretical model for the interplay of mitochondrial ATP synthesis and H+ efflux in recovery from exercise [5]; (3) by measuring the flux of PCr resynthesis during recovery from exercise in human skeletal muscle [2], including the removal of H+ from the muscle cell [4]; (2) by formulating a theoretical model for the interplay of mitochondrial ATP synthesis and H+ efflux in recovery from exercise in human skeletal muscle [2], including the removal of H+ from the muscle cell [4].

**Relationship between PCr resynthesis and metabolite concentrations**

The analysis of the dependence of mitochondrial oxidation rate on phosphorus-metabolite concentrations in muscle is complicated by the near-equilibrium processes catalyzed by creatine kinase which ensures that PCr, ADP, and intracellular pH are related by the expression [2]:

\[
|\text{ADP}| / |\text{ATP}| = \left( \frac{|\text{total creatine}| / |\text{PCr}|} - 1 \right) (1 / K_m) \times 10^{11}
\]

In addition, during exercise and recovery, [PCr + P_i] is approximately constant. These constraints impose correlations on metabolite concentrations: for example, if pH is constant, then [P_i]/[PCr] is proportional to [ADP], the free energy of ATP hydrolysis is proportional to [PCr], and the contributions of [ADP], [PCr], [creatinine], [P_i] and phosphorylation potential to the overall mitochondrial driving function cannot be distinguished [7]. This is the reason that it has not been possible to reconcile the model based on hyperbolic ADP control with those based on linear driving function. If the pH is allowed to vary, the additional degree of freedom should enable the dependence on ADP and on PCr concentrations to be dissociated. We have shown that, under such conditions in human muscle, the recovery kinetics of ADP and of PCr show that mitochondrial ATP synthesis has a hyperbolic dependence on [ADP], but remains approximately linear with respect to [P_i], [PCr] and the free energy of ATP hydrolysis [2]. The first of these is consistent with the kinetic control of the adenine-nucleotide translocase by [ADP] [8, 9], and is in contrast with some of the reported work on exercising animal muscle [10, 11]. This can explain all the other correlations, although other regulators cannot be excluded. The \( K_m \) for ADP control is 30 \( \mu \text{mol}/(\text{l of cell water}) \) and the apparent maximal rate for mitochondrial ATP synthesis \( (r_{max}) \) is approx. 40 mmol per l of cell water per min [2].

**Model for the control of ATP synthesis**

Based on the observations above, we developed a model that substantially reproduces the metabolic response in recovery of a wide range of PCr and of pH values and that can be used to quantitatively assess the changes that are observed in diseased muscle [5].

The main postulates of the model are: (1) ATP production is controlled by [ADP], according to a hyperbolic function with a \( K_m \) and a \( r_{max} \) [2]. (2) ATP production during recovery is used to supply a resting demand and to resynthesize PCr. (3) The creatinine kinase reaction is at equilibrium. (4) The synthesis of PCr produces protons, which is dependent on the pH [4]. (5) The proton efflux is proportional to the cellular acidification [4]. (6) The cell has a constant buffering capacity.

The model is run by entering the initial conditions of [PCr] and of pH, calculating [ADP] and hence the ATP production over a short time interval, determining the associated proton load and efflux, and thus arriving at new values for the next time increment.

The calculated recovery curves reproduce the main features of the observed \(^{31}\text{P} \) n.m.r. studies of normal muscle, namely: (1) PCr recovery is slowed when the pH is low. (2) pH recovery shows an initial acidification when the proton load from PCr resynthesis exceeds the proton extrusion. (3) ADP recovery is rapid, and is not much changed by the metabolic state at the end of exercise. By altering \( r_{max} \) and \( K_m \), the model reproduces the changes observed in mitochondrial disease.

**Direct measurement of ATP synthesis in rat muscle**

We used \(^{31}\text{P} \) n.m.r. saturation-transfer techniques to measure the flux between ATP and P, during steady-state isometric muscle contraction in the rat hind limb \textit{in vivo}, over a range of workloads [6]. Steady-state contraction was obtained by supramaximal sciatic-nerve stimulation. Increasing the stimulation pulse width from 10 to 90 ms, at a pulse frequency of 1 Hz, or increasing the frequency of a 10 ms pulse 0.5–2 Hz resulted in an increase in the flux that was an approximately linear function of the increase in the tension–time integral. Comparison with the fluxes calculated from previous measurements of oxygen consumption or of PCr...
breakdown during a tetanus, indicated that the measured flux is due predominantly to the activity of the mitochondrial $F_0F_1$ ATP synthase. The ATP synthesis rate showed an approximately linear dependence on the free ADP concentration in the muscle, up to an ADP concentration of about 90 $\mu$M, implying that the mitochondrial ATP generation in the muscle is controlled by the free ADP concentration, with an apparent $K_m$ of at least 30 $\mu$M.

**Mitochondrial disease**

In the patients with known severe mitochondrial myopathy, the rate of PCr synthesis is decreased throughout recovery, but there is some evidence that defective mitochondrial function can be partially compensated by an increase in [P] or [ADP] during exercise and recovery [2]. The scope for increasing [P] (by PCR hydrolysis) during exercise is limited by the resting [PCR], which is, in any case, low in these patients. However, [ADP] is considerably increased as a result of the PCR depletion which is enhanced by the partial failure of the muscle to acidify during exercise, which may be the result of an adaptive increase in the proton flux [4]. In spite of this, in severe disease PCR resynthesis is still low, apparently because of decreased maximum mitochondrial capacity. In some patients there appears also to be an increased $K_m$ for ADP, as the relationship between the rate of PCR synthesis and [ADP] is nearly linear [2]. The model described above accounts fully for these observations.

**Conclusions**

The $^3$P n.m.r. analysis of recovery data indicates the important control function of ADP for oxidative phosphorylation. Through the creatine-kinase-catalysed equilibrium, the concentration of ADP is pH-dependent and the control is therefore also linked to glycogenolysis and to lactate production, as well as to proton removal.

The extension of the analysis and $^3$P n.m.r. measurements to exercising human muscle leads to quantitative estimates of buffer capacity, the rate of proton efflux and of the relative contributions to ATP production of oxidation and of anaerobic glycogenolysis [4].

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