Muscle Studies by ^{31}P MRS

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^{31}P Magnetic resonance spectroscopy (^{31}P MRS) yields information about skeletal muscle metabolism in vivo which is hard to obtain in other ways. The ability of muscle to alter ATP turnover allows us to probe energy metabolism and cellular pH homeostasis dynamically by analyzing the kinetics of pH, [PCr], [Pi], and [ADP] during exercise and recovery. Quantitative interpretation depends on understanding the relevant physiology, and exploiting three principles: first, the creatine kinase (CK) equilibrium ensures that any imbalance between ATP supply (by anaerobic glycolysis and by oxidative metabolism) and ATP demand (by basal processes, plus that related to contraction) is balanced by net breakdown or resynthesis of PCr; second, any imbalance between H^+ production (by anaerobic glycolysis, and by PCr synthesis) and H^+ disposal (consumption by PCr breakdown, and H^+ efflux) results in a pH change moderated by cytosolic buffers; and third, the CK system acts as comparator of ATP supply and demand with an important role in the feedback regulation of ATP production. Here, we review the application of these interpretative principles to exercise/recovery studies of various kinds, and some other uses of ^{31}P MRS data in muscle.

Keywords: metabolism, ATP turnover, bioenergetics, pH, glycogenolysis, mitochondria, noninvasive methods, ^{31}P magnetic resonance spectroscopy, skeletal muscle

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Introduction

^{31}P MRS can detect and quantify a limited set of metabolites in skeletal muscle, which nevertheless occupy a central place in ATP turnover and cellular pH homeostasis. If correctly interpreted, these measurements allow useful inferences about processes that are otherwise difficult to access in noninvasive ways suited to multiple measurements and clinical studies.

Some Background

Metabolism and Physiology

The essential metabolic background has been recently reviewed,[1] and is summarized in Figure 1. Three important physiological principles underpin the interpretation of ^{31}P MRS data.

ATP Turnover. The first of these principles is that the ATP supply must equal ATP demand. In muscle, as in other oxidative tissues, ATP can be produced in two ways: anaerobically by glycolysis, mainly from glycogen, to lactate (at a flux we call L); and more efficiently (at flux Q), by oxidation in the tricarboxylic acid cycle of acetyl coenzyme A (derived either from glycolysis or β-oxidation of fatty acids) and subsequent oxidation of reducing equivalents via the mitochondrial electron transport chain.

On the demand side, ATP is used (at flux U) by a variety of ion pumps including the sarcolemmal Na^+/K^+-ATPase and the sarcoplasmic Ca^{2+}-ATPase, and by the force-generating myosin ATPase. It is useful to distinguish a basal component (U_B) independent of force-generation from the component which increases with mechanical output, much of which is due to the myosin ATPase.[5] It is generally assumed that basal ATP demand is the same during exercise and recovery as at rest,[1] and as resting glycolysis is negligible, we can identify U_B with the resting oxidative ATP synthesis rate Q_B. The proportionality constant relating ATP turnover to mechanical output (which we will call M, meaning force or power appropriately scaled for muscle cross-sectional area) is defined as contractile cost (C), and its reciprocal as contractile efficiency.

ATP demand can switch on very quickly, while glycolytic and oxidative ATP generation takes time to respond. Catastrophic ATP depletion is avoided by the 'temporal buffering' action of the creatine kinase (CK) system. To put it in simple terms, CK catalyzes this reaction: $PCr + ADP \leftrightarrow ATP + Cr$. In skeletal muscle, CK is always near-equilibrium and works such that any temporary mismatch between ATP demand and supply is met by a change in [PCr], and not [ATP]. When ATP hydrolysis ($ATP \rightarrow ADP + Pi$) is thus matched by PCr breakdown ($PCr + ADP \rightarrow ATP + Cr$), the result is the apparent 'splitting' of $PCr \rightarrow Cr + Pi$ (sometimes wrongly called PCr hydrolysis, a reaction that does not actually occur). The involvement of H^+ in this, omitted here for clarity, is important for cellular pH homeostasis, as we shall see in the section titled 'Cellular pH Homeostasis'.

Thus, ATP demand is equal to the sum of glycolytic ATP synthesis, oxidative ATP synthesis, and PCr breakdown: algebraically,

$$U = L + Q + -\delta[\mathrm{PCr}]/\delta t \quad (1)$$

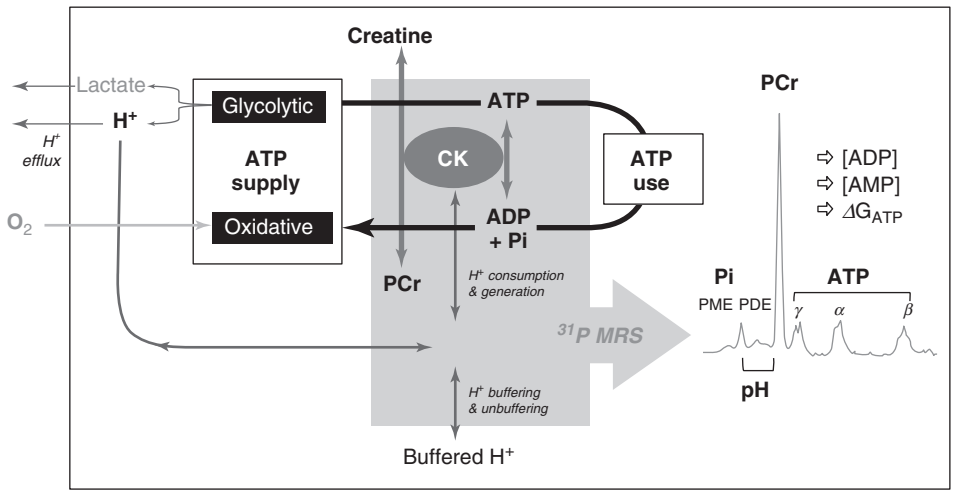

Figure 1. Muscle energy metabolism as seen by ^{31}P magnetic resonance spectroscopy. Muscle energy metabolism is simplified here to ATP supply and demand, buffered by the creatine kinase (CK) reaction that equilibrates phosphocreatine (PCr), creatine (Cr), ATP, ADP, and H^+; ADP and inorganic phosphate (Pi) are products of ATP hydrolysis, and also substrates for ATP synthesis by oxidative metabolism and anaerobic glycolysis; any temporary mismatch between ATP supply and demand is buffered by PCr breakdown (equation (1) in main text). In terms of cellular pH homeostasis, glycolytic production of lactate is accompanied by H^+, and both can leave the cell; H^+ are buffered in the cytosol when pH falls, and 'unbuffered' when pH rises; H^+ are consumed when PCr falls and generated when PCr rises (equation (7)). In terms of metabolic regulation, ATP turnover is largely demand-driven, and CK-related metabolites play an important role as feedback regulators of ATP supply. The shaded block represents what ^{31}P MRS can detect: the illustrative resting-muscle ^{31}P MRS spectrum shows the peaks of phosphomonoester (PME), Pi, phosphodiester (PDE), and PCr, and the γ (doublet), α (doublet), and β (triplet) peaks of ATP. We can calculate cytosolic pH (from the chemical shift difference between Pi and PCr), the free concentrations of ADP (equation (3) in the main text) and AMP (equation (4)), the free energy of ATP hydrolysis (ΔG_{ATP}; equation (5)), and free [Mg^{2+}] (from the chemical shift of β-ATP[2]). In resting muscle PME comprises largely[3] the hexose monophosphates glucose 6-phosphate (~80%), fructose 6-phosphate (~15%) and glucose 1-phosphate; inosine monophosphate can also contribute post exercise when there has been loss of ATP. Muscle PDE comprises metabolites of phospholipid breakdown. Typical published values in normal resting muscle[4] are [PCr] 33 mmol (l cytosolic water)$^{-1}$, more economically expressed as 33 mM, [Pi] 4 mM, pH 7.0, [ADP] 14 μM, ΔG_{ATP} −63 kJ mol^{-1}, [TCr] 43 mM and [ATP] 8 mM.

(where δ refers both to small measurable increments and to differentials derived from fits). The principles are the same during recovery from exercise: as ATP synthesis is now overwhelmingly oxidative (so $L \approx 0$) and directed toward PCr resynthesis (so $U \approx 0$),

$$Q \approx \delta[\mathrm{PCr}]/\delta t \qquad (2)$$

Relationships among Metabolites. The relationships imposed by the CK equilibrium are important not only in metabolic control, but also in interpreting ^{31}P MRS data. Acute changes in [PCr] are matched by opposite changes in creatine (Cr) so that total Cr ([TCr] = [Cr] + [PCr]) remains constant, and also in Pi so that [PCr] + [Pi] remains (approximately) constant. ADP concentration, whose importance in metabolic regulation shall be discussed in the sections titled 'Feedback Regulation of ATP Turnover' and 'Mitochondrial Function Studies in Recovery from Exercise', can be estimated as

$$[\mathrm{ADP}] = \{([\mathrm{TCr}]/[\mathrm{PCr}]) - 1\}[\mathrm{ATP}]/(K_{CK}[H^+]) \qquad (3)$$

where $K_{CK} = 1.66 \times 10^9$ l mol^{-1}. This calculation uses either a measured or (more commonly) assumed [TCr], and a [PCr] either measured by calibrated ^{31}P MRS or (more commonly) calculated from a fully relaxed or saturation-corrected PCr/ATP and an assumed [ATP].[4] It can be seen from equation (3) that [ADP] tends to increase when [PCr] falls or pH rises; however, it is important not to mistake this algebraic truism for a causal mechanism.[1]

Free [AMP] can be estimated from the adenylate kinase equilibrium, as

$$[\mathrm{AMP}] = K_{AK}[\mathrm{ADP}]^2/[\mathrm{ATP}] \qquad (4)$$

where $K_{AK} = 1.12$. That this has been little used in ^{31}P MRS experiments is perhaps a missed opportunity, given the importance of AMP-activated protein kinase in metabolic regulation.

The free energy of ATP hydrolysis, whose importance to mitochondrial function we mention in the section titled 'Mitochondrial Function Studies in Recovery from Exercise', can be estimated as

$$\Delta G_{ATP} = \Delta G^0{}_{ATP} + RT \ln([\mathrm{ADP}][\mathrm{Pi}]/[\mathrm{ATP}]) \qquad (5)$$

where $\Delta G^0{}_{ATP} = 32$ kJ mol^{-1} and RT (gas constant × absolute temperature) = 2.57 kJ mol^{-1}. More complex expressions are available for equations (3)–(5), taking more detailed account of ionization states and metal binding.[3] Obviously, a much more complicated picture of cellular energy metabolism could be constructed, involving details not only of the metabolic machinery of ATP production and use,[6] but also compartmentation and spatiochemical interactions involving subcellular components such as mitochondrial CK.[7] However, their implications for the practical interpretation of ^{31}P MRS data remain unclear.

Cellular pH Homeostasis. The second of the principles that underpin the interpretation of ^{31}P MRS data can be thought

of as a supply and demand relationship for 'protons' (H^+).[3,8] Anaerobic glycolytic ATP synthesis produces equimolar amounts of lactate and H^+ with a stoichiometry of 2/3 H^+ per ATP from glycogen (cf. 1 H^+ per ATP from glucose, which is less relevant for ^{31}P MRS experiments).[9] H^+ production tends to lower cytosolic pH, but this is mitigated by three processes. First, PCr splitting consumes H^+ at a rate of $\gamma\delta[\mathrm{PCr}]/\delta t$, where γ is a negative stoichiometric coefficient arising from the pH-dependent difference in charge (strictly, in 'bound H^+') between Pi and PCr. Second, as pH falls, the buffers of the cytosol (whose total buffer capacity we call β_T) buffer H^+ at a rate of $-\beta_T\delta\mathrm{pH}/\delta t$. It is convenient to regard β_T as the sum of the buffer capacity due to Pi, calculable as

$$\beta_{\mathrm{Pi}} \approx 2.3[\mathrm{Pi}]/\{[1 + 10^{(\mathrm{pH}-\mathrm{pK})}][1 + 10^{(\mathrm{pK}-\mathrm{pH})}]\} \qquad (6)$$

where pK = 6.8, and a non-Pi buffer capacity β_{NP}. The third mitigating process is that when pH falls, various pH-dependent processes of net H^+ efflux (whose fluxes we lump together and call E) tend to restore it.[10] Thus, H^+ buffering is equal to glycolytic H^+ production less H^+ consumption and H^+ efflux:

$$-\beta_T\delta\mathrm{pH}/\delta t = (2/3)L - \gamma\delta[\mathrm{PCr}]/\delta t - E \qquad (7)$$

In early exercise the alkalinizing effect of PCr breakdown often temporarily outweighs the acidifying effect of glycolytic H^+, in which case H^+ is 'unbuffered' at a rate of $\beta_T\delta\mathrm{pH}/\delta t$ as pH rises.[11] This is also the case throughout exercise when glycolysis is impaired or absent, as in McArdle's disease, and the alkalinizing effect of PCr breakdown is largely unopposed:[12] equation (7) remains valid, but in the special-case form $\beta_T\delta\mathrm{pH}/\delta t \approx \gamma\delta[\mathrm{PCr}]/\delta t$.

The same principles apply during postexercise pH recovery: now ATP synthesis is overwhelmingly oxidative and thus essentially pH-neutral; resynthesizing PCr 'generates' H^+ at a rate of $-\gamma\delta[\mathrm{PCr}]/\delta t$, which tends to lower cell pH further, but despite this H^+ efflux restores pH to resting values. Thus, H^+ efflux is equal to H^+ generation plus H^+ unbuffering:[13]

$$E = -\gamma\delta[\mathrm{PCr}]/\delta t + \beta_T\delta\mathrm{pH}/\delta t \qquad (8)$$

In early recovery, the acidifying effect of PCr resynthesis often temporarily outweighs H^+ efflux, in which case H^+ is buffered at a rate of $-\beta_T\delta\mathrm{pH}/\delta t$ during a transient postexercise pH fall: however, equation (8) remains valid. The total H^+ lost from the cell in recovery is in effect that part of the glycolytic H^+ produced during exercise which was buffered and consumed in the cytosol as pH and PCr decreased:

$$\begin{aligned}\int E\mathrm{d}t \text{ in recovery} &= \int\{-\gamma\delta[\mathrm{PCr}]/\delta t + \beta_T\delta\mathrm{pH}/\delta t\}\mathrm{d}t \text{ in recovery} \\ &\approx \int\{\gamma\delta[\mathrm{PCr}]/\delta t - \beta_T\delta\mathrm{pH}/\delta t\}\mathrm{d}t \text{ in exercise} \qquad (9)\end{aligned}$$

Note that the dependence of both γ and β_T on pH, and of β_T also on [Pi] (and therefore on [PCr]), makes these time-integrals path-dependent.

Feedback Regulation of ATP Turnover. The third principle that underpins the interpretation of ^{31}P MRS data relates to metabolic regulation. For much of the dynamic range, until fatigue supervenes, control of ATP supply is dominated by ATP demand.[14] For oxidative ATP synthesis, attention has long been focused on closed-loop negative feedback by CK-related metabolites. The general idea[1] is that oxidative ATP synthesis is some function (f) of a feedback signal (X)

$$Q = f(X) \qquad (10)$$

where X is related to the fall in [PCr] which results from any tendency toward shortfall in ATP supply. ADP, Cr, and Pi have all been considered as contributors to X, although the precise model adopted does not much affect the practical interpretation of ^{31}P MRS data. This general model appears to be compatible with detailed computational simulation of oxidative phosphorylation and related processes.[6] The possibility of an additional direct ('open loop', 'feed-forward', or 'parallel') activation of oxidative ATP synthesis, independent of closed-loop feedback,[15] is discussed elsewhere.[1] We return to this briefly in the section titled 'Mitochondrial Function Studies in Recovery from Exercise'.

Comparatively little is known about the control of glycolytic ATP synthesis in muscle in vivo. Increases in glycogen phosphorylase flux in exercise seem to depend on both the conversion of phosphorylase b to phosphorylase a, mediated by Ca^{2+}- activation of phosphorylase b kinase (which is formally an open-loop mechanism), and the increase in the cosubstrate Pi as a consequence of PCr splitting (formally closed-loop). Closed-loop factors such as AMP, Pi, and ADP are also important influences on downstream glycolytic enzymes. However, the evidence is somewhat ambiguous. A dominant open-loop element is suggested by the abrupt switch-off at the end of exercise (despite the elevation of ADP and Pi),[16,17] and the delayed switch-on at the start of exercise.[18] However, computational modeling of glycolysis in closed oxygen-free incubation accounts well for experimental data in vitro without recourse to open-loop influences.[19]

Some Practical and Experimental Considerations

MRS Aspects. Much ^{31}P MRS muscle work has used pulse-and-acquire methods and physical location by surface coil. Single-voxel localization has, until recent 7 T developments,[20] required an unacceptable trade-off with time resolution, and even more so when using spectroscopic imaging methods,[21] although this can be improved with the use of gated multiply-repetitive methodologies[22] or specialized imaging sequences.[23]

To quantify ^{31}P MRS spectra, signal intensity estimates derived by an appropriate fitting method are either treated in relative terms or calibrated to yield absolute concentrations. There is surprisingly poor agreement about these in healthy human muscle.[4] A common approach is to collect a fully relaxed resting spectrum to establish baseline Pi/ATP and PCr/ATP and then use a shorter repetition time for rest-exercise-recovery acquisition. For some kinetic analyses, only relative changes in PCr are important, and resting muscle PCr/ATP and Pi/ATP are often interpretable on the assumption that [ATP] does

not change. For calculation of [ADP], [AMP], and ΔG_{ATP} (equations (3)–(5)), assumptions about [ATP] and [TCr] are unavoidable unless they have been measured by other means – they do not 'cancel out'.

Practical Aspects of ^{31}P MRS Muscle Studies. Most human ^{31}P MRS studies are of quadriceps (including vastus lateralis and rectus femoris), calf muscle (soleus and gastrocnemius), or plantar flexors (tibialis anterior), which are all accessible at magnetic isocenter in whole-body scanners, and can be easily exercised.[24] Studies of upper limb muscles (finger flexors and first dorsal interosseus) were formerly popular using smaller magnets into which an arm could be inserted. Animal studies are typically of rat or mouse gastrocnemius muscle.[25]

Muscle offers the advantage that responses can be studied to changes in ATP turnover, generally induced by voluntary exercise in human studies and nerve stimulation in animal work. This offers an opportunity to estimate several net metabolic fluxes from measurements of concentration kinetics,[1] as we discuss in the sections titled 'Resting Muscle under Cuff Ischemia' and 'Exercise Manipulations'. We shall also mention magnetization transfer (MT) measurements of exchange fluxes, which have a rather different interpretation.

Much of the published work has used purpose-built exercise equipment and a wide variety of exercise protocols: in humans, most commonly isometric or concentric (shortening) exercise, sometimes with a poorly defined component of eccentric (lengthening) exercise. Incremental (ramp) protocols have been used for their convenience in assessing a range of intensities in a single session, but are more complicated to analyze. Animal protocols are usually isometric.[25]

Exercise physiologists distinguish levels of exercise intensity based on responses of circulating lactate and measured oxygen consumption ($\dot{V}O_2$):[26] in *moderate* exercise below the lactate threshold (LT), $\dot{V}O_2$ quickly reaches a steady state; in *heavy* exercise between LT and 'critical power' (CP), $\dot{V}O_2$ shows a 'slow component', an increase beyond the expected steady state; in *very heavy* exercise above CP, the $\dot{V}O_2$ slow component and lactate increases until the limit of tolerance; in *severe* exercise, the metabolic rate exceeds maximal aerobic capacity ($\dot{V}O_{2max}$) from the start. Most ^{31}P MRS studies, particularly those of oxidative ATP metabolism, use *moderate* or *heavy* exercise, often not tightly defined, as higher intensities are difficult to manage in the scanner. The lack of agreement on exercise equipment and protocols means that there are few agreed 'reference ranges' for dynamic MR parameters.

Combining ^{31}P MRS with other modalities, although useful, is technically challenging. Specialized interleaved sequences have been used to combine ^{31}P MRS with ^{1}H MRS measurements of lactate[27] or deoxymyoglobin. Combination of ^{31}P MRS with simultaneous whole-body $\dot{V}O_2$ is possible,[28] but it is simpler to repeat identical exercise in and out of the scanner.[29] Combination with invasive measurements of muscle $\dot{V}O_2$ would be valuable, but very difficult. As an indirect approach to this, the quantitative comparison of published data obtained by different modalities has been used to highlight the interpretative difficulties of MT measurements (see the section titled 'Magnetization Transfer Methods'),[30] and to suggest that most MR exercise studies to date have operated at relatively low-power outputs that may have missed interesting features of mitochondrial regulation.[1]

Resting Muscle

Muscle's dynamic responses are conditioned by the resting state from which they take origin, and some resting measurements are themselves interpretable in terms of disease or physiological change. We begin, therefore, by reviewing how ^{31}P MRS-detectable metabolites are regulated in resting muscle.

pH and Metabolite Measurements in Normally perfused Muscle at Rest

At rest (for which we use the subscript B, for 'basal' state), ATP is supplied almost exclusively by oxidation of fatty acids in the fasting state and glucose postprandially. Any metabolite that functions as a mitochondrial feedback signal (equation (10)) should presumably be at a value appropriate to basal ATP demand, in accordance with

$$Q_B = f(X_B) \tag{11}$$

This probably applies to [ADP], which then defines [Cr]/[PCr] (equation (3), with causation running 'left to right'). It is probably also true of [Pi], which along with [ADP] defines ΔG_{ATP} (equation (5)); however, resting cell [Pi] is also dependent (by pump-and-leak principles) on sarcolemmal Na^+-dependent Pi uptake. Total cell creatine ([TCr]) depends similarly on sarcolemmal Na^+-dependent Cr uptake. Resting cell pH is set by processes of H^+ efflux, dominated by the Na^+/H^+-antiporter, being the pH at which net H^+ efflux is near-zero. Lastly, [ATP] is set by the balance of adenine nucleotide synthesis and breakdown.[1]

We do not know whether observed resting ^{31}P MRS abnormalities are quantitatively consistent with equation (11). The high [Pi] and low [PCr] in, for example, mitochondrial myopathies appear to make sense in these terms.[31] However, we usually do not know how particular disease or other states affect basal ATP turnover Q_B (a study of the aged mouse[32] is an exception, showing increased [ADP] despite decreased Q_B); we usually lack measured [TCr], which makes calculated [ADP] and ΔG_{ATP} potentially misleading; and we have no agreed models of how ADP and Pi contribute to the relevant feedback signal X.

Other aspects of the resting muscle spectrum are of interest. The PME and PDE components are empirical markers of disease activity in muscular dystrophies[33] and show some metabolically interesting correlations.[34] It has recently been suggested that the small intramitochondrial Pi pool is detectable in resting muscle, which may offer a new biomarker of oxidative function.[35]

Magnetization Transfer Methods

In MT, magnetic labeling is used to measure exchange flux. For practical reasons, this is usually applied to resting muscle and there have been two main applications.

First, CK activity can readily be measured as PCr-ATP exchange.[36] But what does it mean? In general, exchange

flux depends on enzyme activity and the concentrations of substrates and products: for CK, the rate expression is known[37] and the relevant metabolites can be measured, calculated, or assumed. However, the physiological importance of CK is simply that is near equilibrium (see the section titled 'ATP Turnover'). The measured exchange flux is therefore probably best considered a noncausal marker of pathology.

Second, Pi-ATP exchange measured by MT in resting muscle has been taken to reflect largely oxidative ATP synthesis, on the assumptions that this is unidirectional (so that exchange flux ≈ net flux) and that other contributions [notably that mediated by the near-equilibrium glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK)] are small. However, observed Pi-ATP exchange is much faster than oxidative ATP synthesis in resting muscle, so one or both assumptions must be wrong.[30] An earlier proposal that it can be used as a measure of mitochondrial capacity (as defined in the section titled 'Mitochondrial Function Studies in Recovery from Exercise') also fails because of the clear distinction, in demand-driven cellular ATP turnover, between a rate and a capacity. However, some interesting correlations between Pi-ATP exchange and measures of resting ATP turnover and mitochondrial capacity[38] remain unexplained.[30]

It has been suggested[39] that some anomalous features of MT experiments in muscle imply an element of metabolic compartmentation which might call into question some of the principles of interpretation described here, particularly in relation to calculated [ADP].

Resting Muscle under Cuff Ischemia

In what follows we shall be considering perturbations, mainly of ATP turnover (Table 1); however, we begin with a manipulation of resting muscle: cuff ischemia, which cuts off vascular O_2 delivery and H^+ efflux.

Once residual oxidative ATP synthesis has depleted muscle O_2 content, basal ATP demand (U_B) is met first by PCr breakdown, which can be measured directly

$$U_B \approx -\delta[\mathrm{PCr}]/\delta t \tag{12}$$

and then increasingly also by glycogenolysis

$$U_B = L - \delta[\mathrm{PCr}]/\delta t \tag{13}$$

which can be estimated from pH and PCr changes using an assumed or measured buffering capacity:

$$L = (3/2)(\gamma\delta[\mathrm{PCr}]/\delta t - \beta_T \delta \mathrm{pH}/\delta t) \tag{14}$$

It is generally assumed that what equations (12) and (13) measure is a valid estimate of the resting rate of oxidative ATP synthesis before ischemia, Q_B; in other words, that replacement in ischemia of oxidative by nonoxidative ATP supply does not alter ATP demand. Published data on resting ATP turnover are summarized elsewhere.[30]

There is an interesting application of combined ^{31}P MRS and appropriately calibrated NIRS measurements in resting ischemia: dividing the measured rate of ATP turnover (equation (13)) by the initial rate of fall of muscle O_2 content yields an estimate of the P:O ratio, a measure of mitochondrial coupling.[32]

Exercise Manipulations

From now on we consider exercising muscle, which we shall approach as a series of special cases. For present purposes we shall ignore[1] the basal component Q_B.

Initial Exercise

Regardless of exercise intensity, it is worth distinguishing 'initial exercise', the slightly ill-defined phase before significant glycolytic or oxidative ATP synthesis, when ATP is supplied almost entirely by PCr breakdown

$$U \approx -\delta[\mathrm{PCr}]/\delta t \tag{15}$$

The initial-exercise rate of PCr breakdown (for which we use the subscript 0) is often conveniently measured from an exponential fit

$$-(\delta[\mathrm{PCr}]/\delta t)_0 = k_{exer}(-\Delta[\mathrm{PCr}]_{SS}) \tag{16}$$

where k_{exer} is the exponential rate constant of PCr and $-\Delta[\mathrm{PCr}]_{SS}$ is the fitted steady-state fall.

If a suitable measure of mechanical work is available, contractile cost can be estimated from this as

$$C = \delta U/\delta M \approx U/M \tag{17}$$

Contractile cost measured in this way is an interesting but relatively under-studied physiological property.[40,41]

If, sufficiently early in exercise, ATP is supplied solely by PCr breakdown, buffer capacity can be estimated from the transient pH rise as[11]

$$\beta_T = \gamma\delta[\mathrm{PCr}]/\delta \mathrm{pH} \tag{18}$$

from which the non-Pi buffer capacity β_{NP} can be obtained by subtracting β_{Pi} (equation (6)). Contributions to $\beta_{NP,}$ which like all buffer capacities is expected to be pH-dependent (broadly, increasing as pH falls below resting) are dominated by protein-bound histidine residues. Values of β_{NP} measured in this way have not been systematically reviewed, but range from 10 to 30 slykes at resting pH.[3,11]

Ischemic Exercise

In exercise under cuff ischemia, there is (after initial O_2 depletion) no oxidative ATP synthesis, and no H^+ efflux, and no steady state is possible in what is now a closed system. The situation resembles ischemic resting muscle (equation (12)), but with increased ATP demand (U). If we estimate U from initial exercise (equation (15)) and assume constant contractile cost, the resulting estimate of glycogenolysis

$$L \approx U - \delta[\mathrm{PCr}]/\delta t \tag{19}$$

and can be used to estimate cytosolic buffer capacity

$$\beta_T = \{\delta(\gamma[\mathrm{PCr}]) - \delta[\mathrm{lactate}]\}/\delta \mathrm{pH} \tag{20}$$

Table 1. How the principles of analysing ^{31}P MRS data apply to some particular cases

Case	Section of this review	ATP use	ATP synthesis	PCr change and effect	pH change and effect	H^+ efflux
Resting muscle	3.1	Basal	Oxidative	None	None	Negligible: tiny H^+ load
Ischemic resting muscle	3.3	Basal	Glycolytic[a]	PCr breakdown: consumes H^+	pH fall: buffers H^+	None: no washout
Initial exercise	4.1	Basal and contractile	Oxidative	PCr breakdown: consumes H^+	pH rise: 'unbuffers' H^+	Negligible: tiny H^+ load
Ischemic exercise	4.2	Basal and contractile	Glycolytic	PCr breakdown: consumes H^+	Increasing pH fall: buffers H^+	None: no washout
Moderate exercise	4.3	Basal and contractile	Oxidative	None at steady-state	Little rise or fall	Negligible: tiny H^+ load
Postexercise recovery	4.4	Basal	Oxidative	PCr synthesis: generates H^+	pH rises[b] back to resting: unbuffers H^+	Responsible for pH recovery
Heavy and severe exercise	4.5	Basal and contractile	Glycolytic and oxidative	PCr breakdown: consumes H^+ until (if) steady state is reached[c]	pH fall: buffers H^+ until (if) steady state is reached.	Matches glycolytic H^+ production, completely if steady state is reached

[a]Some oxidative ATP is produced until O_2 is used up.
[b]pH typically falls transiently in very early recovery before recovering back to basal.
[c]The 'slow component' represents change in 'steady-state' PCr.

where $\delta[\text{lactate}]/\delta t = (2/3)L$, from equation (19).[3] Alternatively we could assume buffer capacity, and use the resulting estimate of glycolytic rate (cf. equation (14)) to obtain an estimate of total ATP turnover (cf. equation (13)) which could be used to assess contractile cost throughout exercise. Note that the 6-carbon flux through glycogen phosphorylase can be calculated as half the rate of glycolytic H^+ production, plus the rate of increase (if any) of PME, which reflects mainly hexose monophosphates (HMPs).[3]

Measuring muscle lactate directly by ^{1}H MRS is possible but challenging. Interleaved or parallel-experiment combinations of ^{31}P MRS and ^{1}H MRS lactate measurement offer the potential of avoiding these assumptions, for example, estimating buffer capacity using equation (20) with measured lactate,[27] rather than that inferred by ATP-turnover analysis of ^{31}P MRS data (equation (19)).

'Oxidative' Exercise

In physiological terms, this means moderate intensity exercise, below the LT, where glycolysis can for present purposes be neglected. Equation (1) now becomes

$$U \approx Q - \delta[\text{PCr}]/\delta t \tag{21}$$

The kinetics of PCr are mono-exponential (as in equation (16)), which implies a linear steady-state relationship between oxidative ATP synthesis and [PCr]

$$-\delta Q/\delta[\text{PCr}] \approx -Q/\Delta[\text{PCr}] = k_{\text{exer}} \tag{22}$$

and thus, also similar mono-exponential kinetics of oxidative ATP synthesis. In principle, k_{exer} reflects mitochondrial function like the rate constant of PCr resynthesis in recovery (k_{reco}) which we discuss in the section titled 'Mitochondrial Function Studies in Recovery from Exercise'; these are the on- and off-kinetics of a first-order system. There is a close parallel between the analysis of PCr kinetics and of pulmonary $\dot{V}O_2$.[26]

A simple interpretation of ^{31}P MRS measurements in steady-state exercise (for which we use the subscript SS) below the LT, takes mechanical output as a surrogate for oxidative ATP synthesis

$$U_{\text{SS}} \approx Q_{\text{SS}} = f(X_{\text{SS}}) \tag{23}$$

Other things being equal, impaired mitochondrial function means bigger steady-state changes in putative mitochondrial regulators or their correlates (e.g., [PCr], [Pi], and [ADP]), in other words bigger X for the same Q. In practice, this argument may be complicated by possible differences in relative work output, contractile costs, and glycolytic contribution to ATP turnover.

Recovery from Exercise

We have mentioned the special features of recovery in the section titled 'Some Background' (equations (2) and (7)). There are two main aspects to consider.

Mitochondrial Function Studies in Recovery from Exercise. This has recently been reviewed in detail elsewhere.[1] PCr resynthesis is a measure of the rate (strictly, the suprabasal rate) of oxidative ATP synthesis (equation (2)) and given its mono-exponential kinetics when pH change during exercise is small (in the off-phase of 'purely oxidative' exercise considered in the section titled '"Oxidative" Exercise')

$$\delta[\text{PCr}]/\delta t = k_{\text{reco}}(-\Delta[\text{PCr}]) \tag{24}$$

where k_{reco} is the rate constant of PCr in recovery and $-\Delta[PCr]$ is the fall below basal. Even where PCr recovery follows more complicated kinetics after acidifying exercise, the earlier phase is often usefully close to mono-exponential. A useful special case is the initial rate of PCr recovery, which is generally assumed[1] to be a measure of end-exercise (subscript E) oxidative ATP synthesis

$$Q_E \approx k_{reco}(-\Delta[PCr]_E) \quad (25)$$

Muscle 'mitochondrial capacity' can be thought of as a maximum rate of mitochondrial ATP synthesis under some actual or notional maximal activation. There are three main ways to assess this:[1] by measuring the machinery of oxidative ATP synthesis (muscle content of mitochondrial or mitochondrial components); by measuring $\dot{V}O_2$ in maximal exercise (either whole-body or locally by invasive methods); or the one we are concerned with here, by making inferences from ^{31}P MRS data from what is, for practical reasons, usually submaximal exercise. There are several approaches to such '^{31}P MRS-based measures of mitochondrial function' (MMMF), each implicitly or explicitly assuming a model of the regulation of oxidative ATP synthesis, and it is useful here to take a general approach.[1] The basic assumption is equation (10), and Q_{MAX} ('mitochondrial capacity') is the extrapolated maximal rate

$$Q_{MAX} = f(X_{MAX}) \quad (26)$$

where X_{MAX} is the notional value of X at some maximal stimulation and/or mechanical output. We will consider briefly three specific approaches.[1]

The linear analysis mentioned in the section titled '"Oxidative" Exercise' for 'oxidative' exercise predicts that postexercise PCr recovery kinetics will be mono-exponential with a rate constant k_{reco} proportional to mitochondrial capacity. Thus, k_{reco} is a 'relative' MMMF (and the time constant $1/k_{reco}$ and half-time $\ln(2)/k_{reco}$ are inverse relative MMMFs). To obtain an 'absolute' MMMF from this analysis, one approach is to extrapolate the definitional first-order linear relationship between $\delta[PCr]/\delta t$ and $-\Delta[PCr]$ to notional complete PCr depletion. This amounts to taking $-\Delta[PCr]$ as X in equation (26), so that

$$Q_{MAX} = k_{reco}[PCr]_B \approx k_{reco}[TCr] \quad (27)$$

where $[PCr]_B$ is resting [PCr].

In literal terms such extrapolation is unphysiological, as intense exercise lowers cell pH, which slows PCr recovery (i.e., decreases k_{reco}) for reasons which can be explained solely in terms of the interactions of pH with the CK equilibrium[1] if it is assumed that the dominant feedback signal X is [ADP], acting analogously to mitochondrial incubation ex vivo. Good evidence for this assumption is the often-observed approximately hyperbolic relationship between [ADP] and oxidative ATP synthesis rate measured from recovery (equation (24)) or its surrogates such as aerobic-exercise workrate (equation (23)). Considerations of dynamic range mandate that this relationship is more exactly sigmoid than purely hyperbolic, and it is is debated to what extent this can be explained computationally.[1] Empirically, though, extrapolating PCr resynthesis rate to 'infinite' [ADP] ('zero' [PCr]) gives an estimate of mitochondrial capacity which is largely independent of end-exercise pH

$$Q_{MAX} = (\delta[PCr]/\delta t)_E\{1 + (Km/[ADP]_E)^n\} \quad (28)$$

where $[ADP]_E$ is end-exercise [ADP], Km is the apparent [ADP] for half-maximal flux, and n is the Hill coefficient relating to sigmoidicity. Coupled with the simplest assumption of linearly pH-dependent H^+ efflux (see equation (30)) this ADP-feedback model can account semiquantitatively for many of the relationships between PCr and ADP recovery and end-exercise pH.[1]

The same is true of the alternative 'nonequilibrium thermodynamic' model,[42] which assigns causal primacy to the sigmoid relationship between oxidative ATP synthesis and ΔG_{ATP}, and which can also be used to extrapolate an estimate of mitochondrial capacity[1]

$$Q_{MAX} = -(\delta[PCr]/\delta t)_E\{a.\exp[(\Delta G_E - c)/(RT)] - 1\} / \{a.\exp(\Delta G_E - c)/(RT) + b\} \quad (29)$$

where a is an empirical constant; b, a constant relating to thermodynamic reversibility; c, a 'static head' (the ΔG_{ATP} at which the flux theoretically is zero); and ΔG_E, an end-exercise ΔG_{ATP}. The near-linear slope ($\delta Q/\delta \Delta G_{ATP}$) of the middle part of this sigmoid relationship, defined on an electrical analogy as 'mitochondrial conductance', can also be used as a relative MMMF, and a simple analysis based on the ADP-model[43] equates it approximately to $6Q_{MAX}/RT$. An additional relative MMMF is the ADP recovery time constant (or the halftime, an inverse MMMF).[44]

The constraints of the CK equilibrium mean that all of these MMMF correlate reasonably closely (when the effects of pH on PCr recovery are accounted for)[45] and they all respond broadly as expected to physiological manipulations and pathophysiological situations.[1] For example, MMMF increase with suitable aerobic training, and in cross-sectional studies are usually reported as declining with age. MMMF are decreased as expected in mitochondrial myopathy,[31] and in diseases impairing vascular O_2 delivery such as peripheral vascular disease[46] and congenital heart disease. In other diseases (cardiac, renal, and pulmonary), decreased MMMF are likely multifactorial, with impaired O_2 delivery and O_2 diffusion and loss of mitochondria and mitochondrial components playing a role. Decreased MMMF in McArdle's disease[12] presumably reflect impaired glycogenolytic supply of pyruvate for oxidation rather than mitochondrial dysfunction as such, pointing up the need for caution in interpretation.

Comparison with other measures of oxidative function such as $\dot{V}O_2$ during maximal exercise is complicated by a number of factors including the choice of correct P:O for cross-calculation; the lack of agreement between the absolute maximal values which emerge from the different models, and the different influences of systemic cardiac-pulmonary-vascular limitations on maximal $\dot{V}O_2$ by muscle groups of different sizes and in different kinds of exercise.[1] Resolution of these issues will take major advances in systems physiology.

In practice, the best way to assess muscle mitochondrial function by ^{31}P MRS is probably to use moderate exercise which

causes little change in pH, and take the PCr recovery rate constant as a relative MMMF. Strictly speaking, MMMF depend on the intactness of the whole cardiovascular/respiratory/muscle O_2 delivery and usage system, whose overall performance can also be assessed by whole body $\dot{V}O_{2max}$ or $\dot{V}O_2$ kinetics. However, typical 'MR' exercise is unlikely to be significantly limited by cardiopulmonary function, and so MMMF reflect mainly 'intramuscular' properties: the numbers and function of skeletal muscle mitochondria, and the adequacy of microvascular O_2 delivery.

It has been argued that some anomalous features of recovery (e.g., transient undershoot of ADP below resting values[44]), among other things, are evidence of parallel activation (i.e., open-loop control) of mitochondrial ATP synthesis.[1] The implications are potentially important: if there were significant parallel activation in exercise, PCr recovery kinetics could be dominated by its intrinsic off-kinetics rather than 'fixed' mitochondrial properties, and it could no longer be simply assumed that the end-of exercise and the immediate initial-recovery rates of oxidative ATP synthesis are the same.

Proton Efflux in Recovery. As we have seen, pH recovers despite H^+ generation from PCr resynthesis because H^+ leaves the cell. Equation (8) shows how H^+ efflux rate can be calculated from pH and PCr recovery kinetics, given a measured or assumed buffer capacity. This can be a tricky calculation, highly sensitive to spectrum-to-spectrum pH variation, especially when Pi becomes very small. H^+ efflux thus calculated increases with fall in pH below basal ($-\Delta$pH), both in initial-recovery comparisons between studies, and during the recovery time-course. We could take this relationship as, very approximately, linear

$$-\delta E/\delta \mathrm{pH} \approx \lambda = -E/\Delta \mathrm{pH} \qquad (30)$$

where λ is an empirical slope. A more realistic assumption would be a hyperbolic relationship, possibly time-dependent,[13] and in any case H^+ efflux depends also on extracellular pH, to which ^{31}P MRS gives no access. H^+ efflux thus calculated shows some other expected properties, being decreased by inhibitors of the Na^+/H^+-antiporter[10] and in vascular disease,[47] and increased in an apparently compensatory manner in mitochondrial myopathy.[31]

High-intensity Exercise

Here, glycolytic ATP synthesis cannot be neglected. In supra-LT exercise which has reached steady state,

$$U_{SS} = Q_{SS} + L_{SS} \qquad (31)$$

where Q_E from recovery (equation (24)) is probably a reasonable approximation to Q_{SS}, and

$$L_{SS} \approx (3/2)E_{SS} \qquad (32)$$

where E_E from recovery (equation (8)) is probably a reasonable approximation to E_{SS}.

Slow components of $\dot{V}O_2$ have attracted much physiological interest, and a similar phenomenon can be detected in PCr (which of course complicates the notion of steady state).[48] Possible causes of this reduced work efficiency during heavy exercise are a progressive increase in the contraction cost or a decrease in effective mitochondrial capacity or P:O, or (as recent work[29] combining ^{31}P MRS and $\dot{V}O_2$ suggests) both.

The general equations (1) and (7) apply throughout high-intensity exercise. The start and end of exercise (i.e., the beginning of the on-transient and the off-transient) are the two points where (given the assumptions reviewed here) ^{31}P MRS kinetic data can give reliable estimates of metabolic fluxes: initial ATP turnover from equation (16), and initial-recovery oxidative ATP synthesis (equation (24)) and H^+ efflux (equation (8)), which it seems reasonable (at least in the absence of proven open-loop effects) to take as estimates of the end-exercise values. To see how these might be used, consider supra-LT exercise that has not reached steady state. How could we estimate ATP turnover? In equation (1), the PCr changes in exercise are of course directly measurable. We have an estimate of Q_E from recovery (equation (24)), and to estimate how this changes during exercise we might use $Q = f(X)$ assuming a constant Q_{MAX} obtained from analysis of recovery (any of equations (27–29)). But how could we estimate glycolytic rate? Rearranging equation (7)

$$L = (3/2)(\gamma\delta[\mathrm{PCr}]/\delta t - \beta_T \delta \mathrm{pH}/\delta t + E) \qquad (33)$$

The PCr and pH changes are directly measurable. We have an estimate of E_E from recovery (equation (8)), and to estimate how this changes during exercise we might take $E = \lambda(-\Delta \mathrm{pH})$ assuming λ obtained from analysis of recovery (equation (30)). This would allow us to calculate $C = U/M$ throughout exercise.

An alternative would be to assume a constant contractile cost. We could obtain initial ATP turnover from equation (16), and take this (corrected if necessary for changing mechanical output) as U through exercise in order to estimate glycolytic ATP synthesis by rearranging equation (1) as

$$L \approx U - Q + \delta[\mathrm{PCr}]/\delta t \qquad (34)$$

without any assumptions about H^+ buffering or efflux, but instead estimating $Q = f(X)$ assuming a constant Q_{MAX} from analysis of recovery (any of equations (27)–(29)). This would enable us to calculate H^+ efflux during exercise as that part of glycolytic H^+ production not accounted for by cytosolic buffering or PCr breakdown

$$E = (2/3)L - \gamma\delta[\mathrm{PCr}]/\delta t + \beta_T \delta \mathrm{pH}/\delta t \qquad (35)$$

This estimated E for the end of exercise could then be tested for equality with the initial-recovery value E from equation (8), as this analysis presupposes.

Exercise of this kind – not very severe in the physiologist's sense, but enough for pH change indicative of substantial glycolytic contribution – has often been used in ^{31}P MRS studies, proving useful in detecting metabolic differences and responses in disease and therapy.[41] Some use has been made of the kind of analysis outlined in this section. However, is it not entirely clear how reliable the assumptions are across a range of exercise conditions and physiological states.

Other Uses of ^{31}P MRS Data

Some attention has been paid to 'split Pi' (i.e., the presence of discrete components of Pi at different pH) as a marker of different fiber types.[28] It is difficult to combine this observation and approach with calculations which depend on the assumption that MRS detects a 'fair sample' of cells that behave in fundamentally similar ways during a given protocol. Another use of measurements of pH, ADP, and Pi relates to their causal role in mechanisms of cellular fatigue.[49]

^{31}P MRS has not proved useful in studying the classical biochemical issue of fuel selection. The differences between carbohydrate and fatty acid oxidation are relatively subtle changes in ATP yield which are not accessible to ^{31}P MRS, and practical considerations preclude the long-duration exercise necessary for substantial transition from glycogen to fatty acid oxidation. In principle, the balance between glycolytic and oxidative ATP synthesis is 'visible' to ^{31}P MRS in supra-LT exercise, with effects on the balance of pH and PCr changes, and thus on [ADP]; however, in the absence of detailed computational models of both processes inferences can only be indirect.

Conclusion: What We Do and Do Not Know

Examination of the detailed evidence, particularly comparison with results of invasive methods, supports the generally accepted picture reviewed here. Initial-exercise PCr and pH changes can be used to measure ATP turnover (hence contractile cost) and cytosolic buffer capacity, PCr exercise or recovery kinetics report mitochondrial function, and pH recovery yields information about H^+ efflux. More complex considerations underpin the abnormalities detectable in higher intensity or more elaborate (e.g., incremental) exercise protocols.

Several methodological limitations have been discussed earlier. There are still disagreements about stoichiometric factors and the best exercise protocols. Muscles of considerable clinical interest (e.g., diaphragm) remain inaccessible. Estimates of buffer capacity and H^+ efflux are technically difficult because of their dependence on precise pH measurements. Changes in contractile cost have not often been defined rigorously or measured precisely. There is in general a lack of direct validation, largely because of the technical difficulty of combining MRS with invasive measures, and of interleaving ^{31}P MRS with other MR measures. With a few exceptions[50] there is a lack of really detailed datasets covering wide ranges of exercise conditions to define the scope of the approximations and regulatory relationships described here. All MRS methods have problems with cellular-scale heterogeneity, and so far technical limitations have constrained spectroscopic imaging approaches to larger scale heterogeneity.[21] Validated computational models of glycolytic and oxidative ATP synthesis, and of cardiopulmonary and vascular function, and of H^+ efflux, would place physiological interpretation on a more solid foundation. Higher field working and novel pulse sequences[20] have some potential to avoid the limitations discussed here.

Abbreviations

CK	creatine kinase
Cr	creatine
MT	magnetization transfer
LT	lactate threshold
CP	critical power
TCr	Total cell creatine
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
PGK	phosphoglycerate kinase
HMP	hexose monophosphate
MMMF	MRS-based measures of mitochondrial function

Symbols

β_T, β_{Pi}, and β_{NP}	cytosolic total, Pi, and non-Pi buffer capacity
C	contractile efficiency
E	H^+ efflux rate
γ	a negative stoichiometric coefficient
k_{exer}, k_{reco}	exponential PCr rate constants in exercise, recovery
λ	apparent linear pH-dependence of E
L	glycolytic ATP synthesis rate
$-\Delta$[PCr], $-\Delta$pH	fall in [PCr], pH below basal
Q	oxidative ATP synthesis rate
Q_B	basal ATP turnover rate (oxidative)
Q_{MAX}	estimated maximum Q ('mitochondrial capacity')
U	total ATP usage rate
$\dot{V}O_2$	O_2 consumption rate
$\dot{V}O_{2max}$	maximum $\dot{V}O_2$
X	generalized ATP turnover feedback signal
M	mechanical output

Subscripts

B	resting muscle or non-contraction-related
0	initial exercise
E	end of exercise
MAX	maximal exercise
SS	steady-state exercise

Values

K_{CK}	1.66×10^9 l mol^{-1}
K_{AK}	1.12
ΔG^0_{ATP}	32 kJ mol^{-1}
RT	2.57 kJ mol^{-1} (see also legend to Figure 1)

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Biographical Sketch

Graham J. Kemp. b in 1956; Oxford BA (1977), BM, BCh (1980), DM (1994); FRCPath (2004). Clinical training (1980–1984), clinical research fellow, Sheffield and Oxford (1984–1996). University of Liverpool (from 1996), currently Professor (from 2011). About 230 publications. Main research interests are MRI/MRS in clinical and physiological research.

Related Articles

Phosphorus-31 NMR; Quantitation in In Vivo MRS

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