Delayed Metabolic Activation of Oxidative Phosphorylation in Skeletal Muscle at Exercise Onset

BRUNO GRASSI
Department of Science and Biomedical Technologies Università degli Studi di Milano, Milano, ITALY

ABSTRACT
GRASSI, B. Delayed Metabolic Activation of Oxidative Phosphorylation in Skeletal Muscle at Exercise Onset. Med. Sci. Sports Exerc., Vol. 37, No. 9, 1567–1573, 2005. In “normal” conditions (e.g., normoxia, absence of pathological conditions) convective and diffusive O2 delivery to skeletal muscle fibers do not seem to represent important determinants for the kinetics of adjustment of oxidative phosphorylation following increases in metabolic demand. Whereas a limiting role by PDH has not been experimentally confirmed, inhibition of mitochondrial respiration by NO could be partially responsible for the delayed activation of oxidative phosphorylation at exercise onset. The main determinants of muscle VO2 kinetics, however, likely reside in the intricate interplay between the various mechanisms of energy provision at exercise onset. By acting as high-capacitance energy buffers, PCr hydrolysis and anaerobic glycolysis would delay or attenuate the increase in [ADP] within the cell following rapid increases in ATP demand, thereby “buffering” a more rapid activation of oxidative phosphorylation. The “PCr–Cr shuttle” concept of a regulatory role of PCr or of the products of PCr hydrolysis on oxidative phosphorylation provides a mechanism that couples VO2 kinetics occurring in mitochondria, to PCr hydrolysis occurring in the cytoplasm. Key Words: GAS EXCHANGE KINETICS, OXIDATIVE METABOLISM, METABOLIC REGULATION, BIOENERGETICS

Upon a step increase in power output, O2 uptake (VO2) increase is slower than the step increase in ATP utilization. The finite rate of adjustment of oxidative phosphorylation to sudden increases in energy demand is often termed “VO2 kinetics.” The issue of the limiting, or regulating, factor(s) for VO2 kinetics has been a matter of debate and controversy for many years, mainly between those in favor of the concept that the finite kinetics of VO2 adjustment to workload increases is attributable to delayed metabolic activation, or to an intrinsic slowness, of intracellular oxidative metabolism to adjust to the new metabolic requirement (“metabolic limitation,” or “oxidative inertia” hypothesis) (8,11,38,50), and those who suggest that an important limiting factor resides in the finite kinetics of O2 delivery to muscle fibers (“O2 delivery limitation” hypothesis) (26,28,49). It is indeed well known that, upon a step increase in workload, variables related to O2 delivery (e.g., heart rate [HR], cardiac output [Q], muscle blood flow [Qm]) adjust to the new requirements according to finite kinetics, slower than the increase in metabolic demand, and could therefore represent a determinant of the relatively slow rate of increase of oxidative phosphorylation at exercise onset. The purpose of the present paper is to review experimental evidence pointing to a delayed metabolic activation as the main limiting factor for VO2 kinetics, and to present some of the mechanisms or factors possibly involved.

WHICH IS FASTER TO ADJUST? O2 DELIVERY OR O2 UPTAKE?

For some time the approach to the problem has been to define whether the adjustment of O2 delivery (usually estimated on the basis of HR or Q) was indeed faster than that of O2 utilization (usually inferred from the kinetics of pulmonary VO2) (8,26). This approach, besides providing only indirect evidence in favor or against the hypotheses outlined above, was troubled by the fact that, in humans, for methodological reasons, the investigated variables (HR, Q, pulmonary VO2) were quite “distant” from the relevant ones, that is, Qm and muscle VO2. This problem was at least partially overcome by some studies that determined the kinetics of O2 delivery and VO2 in humans at the level of exercising limbs (2,21,27). A common finding from all these studies is that, during the first seconds of exercise, increases in O2 delivery exceed increases in VO2, whereas for the ensuing part of the transition the results are less unequivocal and may lead to different interpretations. In these studies, however, measurements were carried out across exercising limbs, so that transit delays from the sites of gas exchange to the measurements sites confounded the overall picture, as demonstrated by Bangsbo et al. (2), who estimated such delays by dye injection into the arterial circulation.
Recently, another series of studies “got inside the muscle,” during metabolic transitions, by utilizing different techniques, such as the intravascular phosphorescence quenching technique for the determination of microvascular PO$_2$ (3) or near-infrared spectroscopy (NIRS) for the determination of tissue oxygenation (20). A common denominator among these different techniques, lies in the fact that the determined variables allow to evaluate the balance (or the lack thereof) between O$_2$ delivery and VO$_2$ in the area of interest, being therefore conceptually similar to O$_2$ extraction, or to arterio-venous O$_2$ concentration difference [C(a-v)O$_2$]. Increased microvascular PO$_2$, or an increased oxygenation, would indicate a faster adjustment of O$_2$ delivery versus that of VO$_2$ in the initial phase of the transition, thereby providing indirect evidence against the “O$_2$ delivery limitation” hypothesis. Conversely, decreased microvascular PO$_2$ or oxygenation, suggesting faster adjustment of VO$_2$ versus that of O$_2$ delivery, would provide indirect evidence in favor of the “O$_2$ delivery limitation” hypothesis. The results of these studies, however, suggested unchanged (or only slightly decreased) O$_2$ extraction for several seconds after an increase in work rate, reflecting a tight coupling, during that period, between O$_2$ delivery and VO$_2$ (3,20). Interpretation of these findings is not straightforward, as discussed below (see “Is limited O$_2$ availability the culprit?”). Some data obtained by NIRS by Grassi et al. (20) are shown in Figure 1. The immediate and pronounced increase in Qm (associated with vasodilation) at the onset of exercise is well-known (37,41). Kindig et al. (35) demonstrated by an optically gated intravital microscopy method that, in the rat spinotrapezius muscle, capillary blood cells velocity and flux (synonymous with Qm) increase without discernible delay within the first contraction-relaxation cycle (1 s), and achieve an apparent steady state within 30–45 s. The studies mentioned above (3,20) suggest that such rapid and pronounced increase in O$_2$ delivery at the transition allows VO$_2$ to increase even in the presence of an unchanged O$_2$ extraction. Only after this initial delay, an increased O$_2$ extraction at the muscle level would contribute, together with the ongoing O$_2$ delivery increase, to the further increase in VO$_2$. The O$_2$ extraction pattern suggested by the above-mentioned studies (3,20) is very similar to that indicated by the C(a-v)O$_2$ data obtained across exercising legs in humans (21) (see also Fig. 2 in (14)) and across the isolated in situ dog gastrocnemius preparation (17).

It appears of interest that a similar time course was also described by Hogan (25) for intracellular PO$_2$ (determined by phosphorescence quenching) in an isolated amphibian muscle fiber model. In the model O$_2$ is uniformly made available in the medium surrounding the cell, and PO$_2$ is the result of the balance between O$_2$ availability and O$_2$ utilization at the intracellular level. At contraction onset a monoexponential decrease in intracellular PO$_2$ was preceded by about 10 s in which the variable remained constant (25). Considering that, in the same experimental model, VO$_2$ increases with a very short time delay (about 2 s) at contraction onset (32), the finding of a 10-s delay before the start of PO$_2$ decrease within the cell suggests that there is plenty of O$_2$ available at the mitochondrial level, in these cells, during the first seconds of contractions.

Thus, upon a step increase in metabolic demand, a “bi- phasic” response of O$_2$ extraction (i.e., no change for a few seconds, followed by a monoexponential increase to reach a new steady state) seems to be the rule across experimental models ranging from exercising humans to single amphibian fibers (3,17,20,21,25) (Fig. 2).

**IS LIMITED O$_2$ AVAILABILITY THE CULPRIT?**

Going back to the main issue of the present paper, the tight coupling between the increased O$_2$ delivery and the increased VO$_2$ does not allow to exclude, per se, that an enhanced O$_2$ delivery could, in theory, lead to a faster VO$_2$ response. Moreover, the experiments mentioned above do not allow to make much inferences, in terms of limiting factors, for the ensuing phases of the transition, that is, beyond the initial 10 s. To demonstrate whether O$_2$ delivery/availability does (or does not) represent a significant limiting factor for VO$_2$ kinetics, experiments showing that a significantly faster than normal or an enhanced O$_2$ delivery is (or is not) associated with faster than normal VO$_2$ kinetics were needed.

This issue was approached by a series of studies conducted by our group on the isolated dog gastrocnemius preparation in situ. Experiments were carried out in the laboratories of Drs. Peter D. Wagner and Michael C. Hogan (University of Cali-
Advantages and limitations of this model have been discussed in detail previously (15–18, 20, 21). Although animal models allow more aggressive experimental interventions and invasive measurements, caution is obviously necessary when extrapolating the obtained results to humans. The same experimental model had been utilized to study muscle V\textsubscript{O2} kinetics by Piiper et al. (40). These authors determined Q\textsubscript{m} and muscle V\textsubscript{O2} kinetics, but did not experimentally manipulate O\textsubscript{2} delivery to the working muscle. In our studies, the first approach was to eliminate all delays in the adjustment of convective O\textsubscript{2} delivery to muscle, by pump-perfusing it at a constantly elevated Q\textsubscript{m}, at rest and throughout the transition, as well as by the concurrent administration of a vasodilatory drug. These interventions did not speed the V\textsubscript{O2} kinetics during transitions to approximately 60% of V\textsubscript{O2}\text{peak} (15), and determined only a relatively minor speeding during transitions to approximately 100% of V\textsubscript{O2}\text{peak} (18). In further experiments we enhanced peripheral O\textsubscript{2} diffusion, by hyperoxic breathing and by the administration of a drug (RSR13, Allos Therapeutics), which shifted to the right the oxyhemoglobin (Hb) dissociation curve, thereby reducing the affinity of Hb for O\textsubscript{2}. Also in this case, we described no effects on V\textsubscript{O2} kinetics during transitions to submaximal loads (16). Taken together, these studies provide evidence that, in “normal” conditions (e.g., normoxia, absence of pathological conditions, see also (14, 28)), convective and diffusive O\textsubscript{2} delivery to skeletal muscle fibers do not represent important determinants of the kinetics of adjustment of oxidative phosphorylation following increases in metabolic demand.

### POSSIBLE DETERMINANTS OF THE INERTIA OF OXIDATIVE PHOSPHORYLATION

Thus, which could be the cause(s) responsible for the relative slowness of oxidative phosphorylation to adjust to a higher metabolic requirement, compared to the other mechanisms of energy provision within skeletal muscle cells, that is, phosphocreatine [PCr] hydrolysis and anaerobic glycolysis (10)?

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**FIGURE 2**—Kinetics of different variables related to O\textsubscript{2} extraction upon a step increase in metabolic demand, as determined in studies conducted by utilizing different techniques and experimental models (3, 17, 20, 21, 25). Note that an increased O\textsubscript{2} extraction is reflected by an increased muscle deoxygenation by NIRS, by an increased C(a-v)O\textsubscript{2}, but by a decreased microvascular or intracellular PO\textsubscript{2}. The equation representing a monoeponential increase of the variables was the following: \(y(t) = y_{\text{BAS}} + A [1 - e^{-t/\tau}]\), whereas the equation representing a monoexponential decrease of the variables was the following: \(y(t) = y_{\text{BAS}} - A [1 - e^{t/\tau}]\). Mean values of time delay (TD) and time-constant (\(\tau\)) obtained in the various studies were utilized for the Figure. In the equations, \(y_{\text{BAS}}\) indicates the baseline value and \(A\) the amplitude between \(y_{\text{BAS}}\) and the asymptotic value. In order to facilitate visual comparison of kinetics of variables that, in the original studies, were expressed by utilizing their respective units, data are presented as a percentage of the total response; that is to say, \(y_{\text{BAS}}\) was set equal to 0 and \(A\) was set equal to 100 for the variables that increased during the transition [C(a-v)O\textsubscript{2}, muscle deoxygenation], or to \(-100\) for the variables that decreased during the transition (intracellular PO\textsubscript{2} and microvascular PO\textsubscript{2}). The vertical broken lines (time = 0) indicate the time at which the metabolic demand was increased. All variables show a quite similar “biphasic” response, that is, an early phase, lasting about 10 s, in which no significant change vs the baseline value is observed, followed by the monoexponential increase (or decrease) to the asymptotic value. See text for further details. (The original data from Grassi et al. (21) were “corrected” to take into account the estimated “dead space” volume of blood from venules to the site of blood gas sampling.) (Taken with permission from: B. Grassi, Limitation of skeletal muscle V\textsubscript{O2} on-kinetics by inertia of cellular respiration. In: A. M. Jones and D. C. Poole (Eds.): Oxygen Uptake Kinetics in Health and Disease. Routledge, London, UK, 2005 (p. 212–229)).
Pyruvate dehydrogenase. There are several possible rate-limiting reactions within the complex oxidative pathways, and some studies had pointed to acetyl group availability within mitochondria and to the activation of pyruvate dehydrogenase (PDH). Experiments conducted in ischemic dog muscles (48) and in humans during transitions from rest to submaximal exercise (47) pointed to PDH as one of the sites where the delayed metabolic activation of oxidative phosphorylation may occur. These authors observed, following activation of PDH and stockpiling of acetyl groups at rest by administration of the drug dichloroacetate (DCA) (46), less muscle phosphocreatine (PCr) degradation, less lactate accumulation and less fatigue during submaximal contractions. They hypothesized that the attenuation of anaerobic energy production during the transition, that is, the lower $O_2$ deficit (the amount of energy that at the onset of constant-load exercise must derive from energy sources different from oxidative phosphorylation, (10)), could be explained by a faster adjustment of oxidative phosphorylation. In none of these studies, however, was VO$_2$ kinetics determined. We tested the hypothesis in the isolated dog gastrocnemius in situ. PDH activation was obtained by DCA, and the investigated transition was from rest to approximately 60–70% of the muscle peak VO$_2$. DCA infusion resulted in a significant activation of PDH, but it did not significantly affect “anaerobic” energy provision (PCr hydrolysis, muscle lactate accumulation and the calculated substrate level phosphorylation) and VO$_2$ kinetics (17). Thus, in this experimental model, PDH activation status did not seem to be responsible for the delayed metabolic activation of oxidative phosphorylation. Similar conclusions were drawn by another study conducted on humans, in which the authors determined VO$_2$ kinetics across the exercising limb during leg extension exercises (1). Rossiter et al. (43) confirmed that PDH activation by DCA does not determine, in humans, faster pulmonary VO$_2$ kinetics, nor a faster kinetics of PCr hydrolysis. These authors, however, after the administration of DCA observed for the same workload a lower amplitude of the VO$_2$ and PCr responses and less blood lactate accumulation. The lower amplitude of the VO$_2$ response determined a lower $O_2$ deficit even in the presence of unchanged VO$_2$ kinetics. For the same power output, a reduced amplitude of the VO$_2$ and PCr responses and less blood lactate accumulation suggest an improved metabolic efficiency. Interestingly, an increased metabolic efficiency after DCA was also observed by our group (17) in the dog gastrocnemius: in the presence of less muscle fatigue (higher force production) after DCA, we indeed observed unchanged VO$_2$ and no significant differences for substrate level phosphorylation. Closing the circle, then, the increased metabolic efficiency and the reduced amplitudes of the VO$_2$ and PCr responses after DCA could explain, at least in part, the PCr “sparing” described by Timmons et al. (47,48), with no need to hypothesize a faster VO$_2$ kinetics. A higher metabolic efficiency after DCA could be explained, at least in part, by a preferential utilization of carbohydrate energy sources induced by the drug. The energy made available at the muscle level per unit of $O_2$ consumed is indeed about 11% higher when pyruvate is oxidized, compared to when free fatty acids are utilized (10). Thus, when carbohydrates are preferentially utilized as energy fuels (vs fats), for the same VO$_2$ more ATP can be generated and a higher force can be sustained (17), or, conversely, for the same power output less VO$_2$ is needed to sustain ATP needs (43). In our study energy expenditure (VO$_2$ + substrate level phosphorylation) per unit of force was 13% lower in DCA than in C; because it appears difficult to imagine a complete shift from lipid to glucidic energy sources induced by the drug, it is likely that other factor(s) was/were involved in the increase in metabolic efficiency with DCA. In any case, the increased metabolic efficiency could represent one of the main effects of DCA administration. It is known that DCA exerts a direct isotropic effect on the heart, presumably by facilitating oxidative metabolism of carbohydrates over fats (46). DCA has been utilized to support myocardial contractility during heart failure (4). Because myocardial VO$_2$ and coronary blood flow were not affected by DCA (4), a decrease of $O_2$ requirements to oxidize pyruvate, compared to fatty acids, would provide a reasonable mechanism by which the drug could cause the observed increase in myocardial efficiency.

Inhibition of mitochondrial respiration by nitric oxide. The delayed metabolic activation of skeletal muscle oxidative phosphorylation at exercise onset might be related, at least in part, to a regulatory role of nitric oxide (NO) on mitochondrial respiration. Among a myriad of functions, which comprehend vasodilation, NO competitively inhibits VO$_2$ in the electron transport chain, specifically at the cytochrome $c$ oxidase level (6). Through its combined effects of vasodilation and VO$_2$ inhibition, NO may serve as part of a feedback mechanism aimed at increasing O$_2$ delivery and reducing the reliance on O$_2$ extraction to meet the increase in muscle VO$_2$: by this mechanism, NO would work in the direction of maintaining higher intramyocyte PO$_2$ levels during exercise (33). Inhibition of NO synthases by the administration of the arginine analog L-NAME (12) to exercising horses determined indeed a slightly but significantly faster pulmonary VO$_2$ kinetics, both during heavy (33) and moderate-intensity exercise (34). Slightly but significantly faster pulmonary VO$_2$ kinetics after L-NAME were also described in humans during transitions to moderate- (29) and heavy-intensity (30) exercise. A faster muscle VO$_2$ kinetics after L-NAME administration, on the other hand, was not observed in a recent study conducted by our group in the isolated dog gastrocnemius in situ preparation (see preliminary data in (19)). Thus, although inhibition of mitochondrial respiration by NO could be partially responsible for the delayed metabolic activation of oxidative phosphorylation at exercise onset, the issue needs clarification and warrants further investigations.

Regulatory role of PCr hydrolysis. At exercise onset PCr hydrolysis and anaerobic glycolysis act to provide a temporal buffer for the initial ATP demand. This could slow the onset of oxidative phosphorylation by delaying key energetic controlling signal(s) between sites of ATP hydrolysis and mitochondria. The creatine (Cr)–PCr system plays
FIGURE 3—Schematic representation of the mechanism through which PCr hydrolysis in the cytoplasm could be functionally coupled to oxidative phosphorylation in the mitochondrion (modified from (50)). See text for further details. E, free energy; e-CK, extramitochondrial isoform of CK; mi-CK, mitochondrial isoform of CK. (Taken with permission from: Grassi, B. Oxygen uptake kinetics: old and recent lessons from experiments on isolated muscle in situ. Eur. J. Appl. Physiol. 90: 242–249, 2003.)

energy derived from oxidative phosphorylation. Then, closing the cycle, the ATP produced by oxidative phosphorylation is transported to the mitochondrial intermembrane space by the translocase and reacts with Cr, thereby resynthesizing PCr, which can leave the mitochondrion and reconstitute cytoplasmic PCr levels. This rather complex series of reactions couples VO₂, which occurs in mitochondria, with PCr hydrolysis occurring in the cytoplasm.

Within this scenario, PCr hydrolysis would act as a high-capacity temporal buffer, that would delay or attenuate the increase in [ADP] within the cell following rapid increases in ATP demand, thereby “buffering” a more rapid activation of oxidative phosphorylation (31,39). A similar role of temporal buffering of [ADP] increases at exercise onset would be played by ATP provision by anaerobic glycolysis, as proposed by Cerretelli et al. (8) some decades ago. The intricate interplay between the various mechanisms of energy provision at exercise onset suggests that pharmacological interventions aimed at blocking PCr hydrolysis and/or glycolysis could speed the rate of adjustment of oxidative phosphorylation. This seems indeed to be the case, as demonstrated by studies conducted on isolated amphibian myocytes after blocking CK and PCr hydrolysis by iodoacetamide (31), or in isolated rabbit hearts after blocking glycolysis by iodoacetic acid (24). A more rapid VO₂ response to an elevation in metabolic demand has also been demonstrated in cardiac muscle of CK-knockout mice (23).

CONCLUSIONS

In "normal" conditions (e.g., normoxia, absence of pathological conditions, see also (14,28)) convective and diffusive O₂ delivery to skeletal muscle fibers do not seem to represent important determinants for the kinetics of adjustment of oxidative phosphorylation following increases in metabolic demand (15,16,18). Whereas a limiting role by PDH has not been experimentally confirmed (1,17,43), inhibition of mitochondrial respiration by NO could be partially responsible for the delayed activation of oxidative phosphorylation at exercise onset (29,30,33,34). The main determinants of muscle VO₂ kinetics, however, likely reside in the intricate interplay between the various mechanisms of energy provision at exercise onset. By acting as high-capacity energy buffers, PCr hydrolysis and anaerobic glycolysis would delay or attenuate the increase in [ADP] within the cell following rapid increases in ATP demand, thereby buffering a more rapid activation of oxidative phosphorylation (8,31,39). The "PCr–Cr shuttle" concept of a regulatory role of PCr or of the products of PCr hydrolysis on oxidative phosphorylation (5,7,36,39,45,50) provides a mechanism that couples VO₂, which occurs in mitochondria, with PCr hydrolysis occurring in the cytoplasm. (9)
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