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

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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 O_2 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 $\dot{V}O_2$ 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 $\dot{V}O_2$, which occurs 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, O_2 uptake ($\dot{V}O_2$) 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 "$\dot{V}O_2$ kinetics." The issue of the limiting, or regulating, factor(s) for $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 O_2 delivery to muscle fibers ("O_2 delivery limitation" hypothesis) (26,28,49). It is indeed well known that, upon a step increase in workload, variables related to O_2 delivery (e.g., heart rate [HR], cardiac output [$\dot{Q}$], muscle blood flow [$\dot{Q}$m]) 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 $\dot{V}O_2$ kinetics, and to present some of the mechanisms or factors possibly involved.

WHICH IS FASTER TO ADJUST? O_2 DELIVERY OR O_2 UPTAKE?

For some time the approach to the problem has been to define whether the adjustment of O_2 delivery (usually estimated on the basis of HR or $\dot{Q}$) was indeed faster than that of O_2 utilization (usually inferred from the kinetics of pulmonary $\dot{V}O_2$) (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, $\dot{Q}$, pulmonary $\dot{V}O_2$) were quite "distant" from the relevant ones, that is, $\dot{Q}$m and muscle $\dot{V}O_2$. This problem was at least partially overcome by some studies that determined the kinetics of O_2 delivery and $\dot{V}O_2$ 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 O_2 delivery exceed increases in $\dot{V}O_2$, 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.

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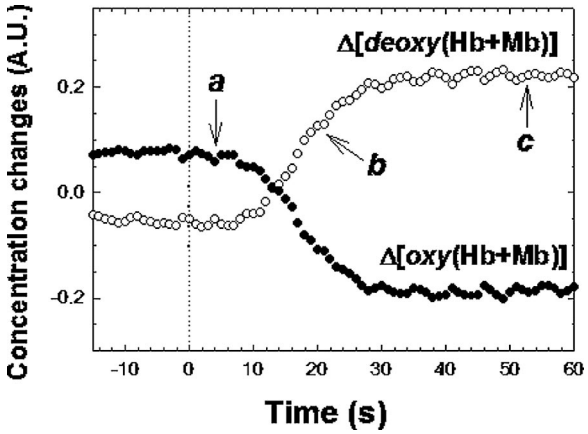

FIGURE 1—Time courses of changes of deoxygenated Hb + myoglobin (Mb) concentration (Δ[*deoxy*(Hb+Mb)]), as well as of oxygenated Hb + Mb concentration (Δ[*oxy*(Hb+Mb)]), obtained by NIRS in the vastus lateralis of a typical human subject during the transition from unloaded pedaling to constant-load exercise below the ventilatory threshold. Data are expressed in arbitrary units (A.U.). Δ[*deoxy*(Hb+Mb)] is usually taken as a muscle oxygenation index, and an increase in this variable indicates an increased O_2 extraction (see 20). *a*, *b*, *c* indicate different phases of the response. At the transition (time 0, vertical broken line), for approximately 10 s both variables remained unmodified (phase *a*) compared to the unloaded pedaling baseline. After this initial period, Δ[*oxy*(Hb+Mb)] decreased and Δ[*deoxy*(Hb+Mb)] increased (phase *b*) and subsequently reached in approximately 60 s a steady-state level (phase *c*). (Taken with permission from: Grassi, B., S. Pogliaghi, S. Rampichini, V. Quaresima, M. Ferrari, C. Marconi, and P. Cerretelli. Muscle oxygenation and gas exchange kinetics during cycling exercise on- transition in humans. *J. Appl. Physiol.* 95: 149–158, 2003)

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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ (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 $\dot{Q}m$ (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 red blood cells velocity and flux (synonymous with $\dot{Q}m$) 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 $\dot{V}O_2$ 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 $\dot{V}O_2$. 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, $\dot{V}O_2$ 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 "biphasic" 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 $\dot{V}O_2$ does not allow to exclude, *per se*, that an enhanced O_2 delivery could, in theory, lead to a faster $\dot{V}O_2$ 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 $\dot{V}O_2$ kinetics, experiments showing that a significantly faster than normal or an enhanced O_2 delivery is (or is not) associated with faster than normal $\dot{V}O_2$ 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-

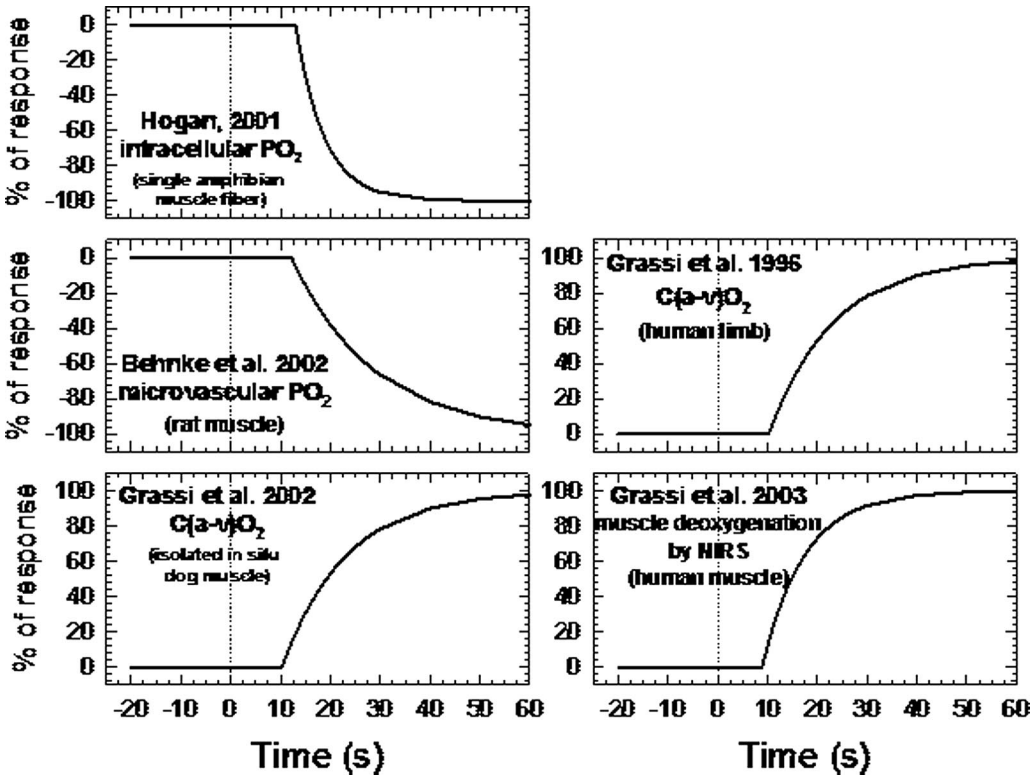

FIGURE 2—Kinetics of different variables related to O_2 extraction upon a step increase in metabolic demand, as determined in studies conducted by utilizing different techniques and experimental models (3,17,20,21,25). Note that an increased O_2 extraction is reflected by an increased muscle deoxygenation by NIRS, by an increased $C(a-v)O_2$, but by a decreased microvascular or intracellular PO_2. The equation representing a monoexponential increase of the variables was the following: $y(t) = y_{BAS} + A\,[1 - e^{-(t - TD)/\tau}]$, whereas the equation representing a monoexponential decrease of the variables was the following: $y(t) = y_{BAS} - A\,[1 - e^{-(t - TD)/\tau}]$. Mean values of time delay (TD) and time-constant (τ) obtained in the various studies were utilized for the Figure. In the equations, y_{BAS} indicates the baseline value and A the amplitude between y_{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_{BAS} was set equal to 0 and A was set equal to 100 for the variables that increased during the transition [$C(a-v)O_2$, muscle deoxygenation], or to −100 for the variables that decreased during the transition (intracellular PO_2 and microvascular PO_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 $\dot{V}O_2$ 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)).

fornia, San Diego) and L. Bruce Gladden (Auburn University). 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 $\dot{V}O_2$ kinetics by Piiper et al. (40). These authors determined $\dot{Q}m$ and muscle $\dot{V}O_2$ kinetics, but did not experimentally manipulate O_2 delivery to the working muscle. In our studies, the first approach was to eliminate all delays in the adjustment of convective O_2 delivery to muscle, by pump-perfusing it at a constantly elevated $\dot{Q}m$, at rest and throughout the transition, as well as by the concurrent administration of a vasodilatory drug. These interventions did not speed the $\dot{V}O_2$ kinetics during transitions to approximately 60% of $\dot{V}O_2$peak (15), and determined only a relatively minor speeding during transitions to approximately 100% of $\dot{V}O_2$peak (18). In further experiments we enhanced peripheral O_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_2. Also in this case, we described no effects on $\dot{V}O_2$ 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_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)?

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 $\dot{V}O_2$ 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 $\dot{V}O_2$. 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ and PCr responses and less blood lactate accumulation. The lower amplitude of the $\dot{V}O_2$ response determined a lower O_2 deficit even in the presence of unchanged $\dot{V}O_2$ kinetics. For the same power output, a reduced amplitude of the $\dot{V}O_2$ 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 $\dot{V}O_2$ and no significant differences for substrate level phosphorylation. Closing the circle, then, the increased metabolic efficiency and the reduced amplitudes of the $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ more ATP can be generated and a higher force can be sustained (17), or, conversely, for the same power output less $\dot{V}O_2$ is needed to sustain ATP needs (43). In our study energy expenditure ($\dot{V}O_2$ + 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 inotropic 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ in the electron transport chain, specifically at the cytochrome *c* oxidase level (6). Through its combined effects of vasodilation and $\dot{V}O_2$ 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 $\dot{V}O_2$; 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 $\dot{V}O_2$ kinetics, both during heavy (33) and moderate-intensity exercise (34). Slightly but significantly faster pulmonary $\dot{V}O_2$ kinetics after L-NAME were also described in humans during transitions to moderate- (29) and heavy-intensity (30) exercise. A faster muscle $\dot{V}O_2$ 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

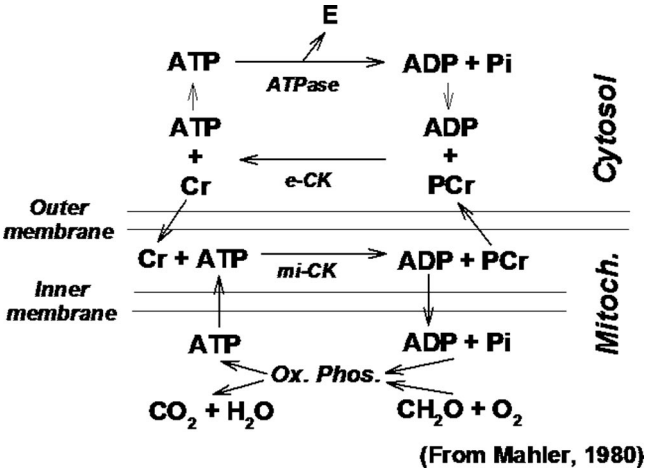

FIGURE 3—Schematic representation of the mechanism through which PCr hydrolysis in the cytoplasm could be functionally coupled to oxidative phosphorylation in the mitochondrion (modified from (50)). See text for futher details. E, free energy; e-CK, extramitochondrial isoform of CK; mi-CK, mitochondrial 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.)

indeed more than one role, and seems strictly related to the control of mitochondrial respiration (22). Some decades ago, the papers by Piiper et al. (40) and by di Prampero and Margaria (11) described an inverse linear relationship between [PCr] (square brackets denote concentration) and muscle $\dot{V}O_2$ in steady-state conditions, thereby suggesting some regulatory link between the two variables. A regulatory role of PCr hydrolysis on $\dot{V}O_2$ kinetics was clearly hypothesized by Margaria et al. (38): "...the oxidative processes in muscles are dictated by the concentration at a given time of the high energy compounds when split." Further evidence in favor of this concept has been recently provided by the observation of a close correlation between the kinetics of phase II pulmonary $\dot{V}O_2$ increase and [PCr] decrease in humans performing constant-load quadriceps exercise, both in the absence (42) and in the presence (44) of the $\dot{V}O_2$ slow component (13).

The concept of a regulatory role of PCr or of the products of PCr hydrolysis on oxidative phosphorylation, through the so-called "Cr–PCr shuttle," derives from the original work by Bessman and Fonyo (5), and has received both theoretical and experimental evidence (7,36,39,45,50). As schematically presented in Figure 3, during muscle contraction [ATP] changes in the cytosol are prevented by the activity of the extramitochondrial isoform of creatine kinase (e-CK), thanks to which the energy contained in the high-energy phosphate bond of PCr is utilized to resynthetize ATP. The outer mitochondrial membrane is permeable to Cr, which enters the mitochondrion. The resulting increase in [Cr] in the mitochondrial intermembrane space leads to ADP production through the activity of the mitochondrial isoform of CK (mi-CK), which is functionally coupled to oxidative phosphorylation through its close proximity to an ATP-ADP translocator on the inner mitochondrial membrane. Thus, the nascent ADP reaches the mitochondrial matrix to be reconverted to ATP thanks to the 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 resynthetizing PCr, which can leave the mitochondrion and reconstitute cytoplasmic PCr levels. This rather complex series of reactions couples $\dot{V}O_2$, 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 iodoacatemide (31), or in isolated rabbit hearts after blocking glycolysis by iodoacetic acid (24). A more rapid $\dot{V}O_2$ 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 O_2 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 $\dot{V}O_2$ 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 (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 $\dot{V}O_2$, which occurs in mitochondria, with PCr hydrolysis occurring in the cytoplasm.(9)

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REFERENCES

1. BANGSBO, J., M. J. GIBALA, P. KRUSTRUP, J. GONZALEZ-ALONSO, and B. SALTIN. Enhanced pyruvate dehydrogenase activity does not affect muscle O_2 uptake at onset of intense exercise in humans. *Am.J. Physiol.* 282:R273–R280, 2002.
2. BANGSBO, J., P. KRUSTRUP, J. GONZALEZ-ALONSO, R. BOUSHEL, and B. SALTIN. Muscle oxygen kinetics at onset of intense dynamic exercise in humans. *Am. J. Physiol.* 279:R899–R906, 2000.
3. BEHNKE, B. J., C. A. KINDIG, T. I. MUSCH, W. L. SEXTON, and D. C. POOLE. Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. *J. Physiol.* 593:927–934, 2002.
4. BERZIN, R. M., C. WOLFE, M. KWASMAN, D. C. LAU, K. TANAKA, P. KHORRAMI, G. N. HENDERSON, T. DE MARCO, and K. CHATTERIE. Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate. *J. Am. Coll. Cardiol.* 23:1617–1624, 1994.
5. BESSMAN, SP, and A. FONYO. The possible role of the mitochondrial bound creatine kinase in the regulation of mitochondrial respiration. *Bioch. Biophys. Res. Comm.* 22:597–602, 1966.
6. BROWN, GC. Nitric oxide as a competitive inhibitor of oxygen consumption in the mitochondrial respiratory chain. *Acta Physiol. Scand.* 168:667–674, 2000.
7. CERRETELLI, P, and P. E. DI PRAMPERO. Gas exchange in exercise. In: *Handbook of Physiology*, Section 3, The Respiratory System, vol. IV, Gas Exchange, Fahri L.E. and S. M. Tenney, (Eds.). Bethesda: American Physiological Society, 1987, p. 297–339.
8. CERRETELLI, P., D. PENDERGAST, W. C. PAGANELLI, D. W. RENNIE. Effects of specific muscle training on $\dot{V}O_2$ on-response and early blood lactate. *J. Appl. Physiol.* 47:761–769, 1979.
9. CERRETELLI, P., D. W. RENNIE, and D. R. PENDERGAST. Kinetics of metabolic transients during exercise. In *Exercise Bioenergetics and Gas Exchange*, Cerretelli and P. B. J. Whipp (Eds.). Amsterdam: Elsevier, 1980, p. 187–209.
10. DI PRAMPERO PE. Energetics of muscular exercise. *Rev. Physiol. Biochem. Pharmacol.* 89:143–222, 1981.
11. DI PRAMPERO P. E. and R. Margaria. Relationship between O_2 consumption, high energy phosphates and the kinetics of the O_2 debt in exercise. *Pflüg.Arch.* 304:11–19, 1968.
12. FRANDSEN, U., J. BANGSBO, M. SANDER, L. HOFFNER, A. BETAK, B. SALTIN, and Y. HELLSTEN. Exercise-induced hyperaemia and leg oxygen uptake are not altered during effective inhibition of nitric oxide synthase with N^G-nitro-L-arginine methyl ester in humans. *J. Physiol.* 531:257–264, 2001.
13. GAESSER, GA, and DC POOLE. The slow component of oxygen uptake kinetics in humans. In: *Exercise and Sport Sciences Reviews*, Vol. 24, Holloszy (Ed.). J. O. Baltimore: Williams & Wilkins, 1996, p. 35–71.
14. GRASSI. B. Skeletal muscle $\dot{V}O_2$ on-kinetics: set by O_2 delivery or by O_2 utilization? New insights into an old issue. *Med. Sci. Sports Exerc.* 32:108–116, 2000.
15. GRASSI, B., L. B. GLADDEN, M. SAMAJA, C. M. STARY, and M. C. HOGAN. Faster adjustment of $\dot{V}O_2$ delivery does not affect O_2 on-kinetics in isolated in situ canine muscle. *J. Appl. Physiol.* 85:1394–1403, 1998.
16. GRASSI, B., L. B. GLADDEN, C. M. STARY, P. D. WAGNER, and M. C. HOGAN. Peripheral $\dot{V}O_2$ diffusion does not affect O_2 on-kinetics in isolated in situ canine muscle. *J. Appl. Physiol.* 85:1404–1412, 1998.
17. GRASSI, B., M. C. HOGAN, P. L. GREENHAFF, J. J. HAMANN, K. M. KELLEY, W. G. ASCHENBACH, D. CONSTANTIN-TEODOSIU, and L. B. GLADDEN. $\dot{V}O_2$ on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *J. Physiol.* 538:195–207, 2002.
18. GRASSI, B., M. C. HOGAN, K. M. KELLEY, W. G. ASCHENBACH, J. J. HAMANN, R. K. EVANS, R. E. PATTILLO, and L. B. GLADDEN. Role of convective $\dot{V}O_2$ delivery in determining O_2 on-kinetics in canine muscle contracting at peak $\dot{V}O_2$. *J. Appl. Physiol.* 89:1293–1301, 2000.
19. GRASSI, B., M.C. HOGAN, K.M. KELLEY, R.A. HOWLETT, R.E. PATTILLO, and L.B. GLADDEN. Effects of L-NAME during metabolic transitions in isolated canine muscle. *Med. Sci. Sports Exerc.* 35:S227, 2003 (Abstract).
20. GRASSI, B., S. POGLIAGHI, S. RAMPICHINI, V. QUARESIMA, M. FERRARI, C. MARCONI, and P. CERRETELLI. Muscle oxygenation and gas exchange kinetics during cycling exercise on- transition in humans. *J. Appl. Physiol.* 95:149–158, 2003.
21. GRASSI, B., D. C. POOLE, R. S. RICHARDSON, D. R. KNIGHT, B. K. ERICKSON, and P. D. WAGNER. Muscle O_2 uptake kinetics in humans: implications for metabolic control. *J. Appl. Physiol.* 80: 988–998, 1996.
22. GREENHAFF. PL. The creatine-phosphocreatine system: there's more than one song in its repertoire. *J. Physiol.* 537:657, 2001.
23. GUSTAFSON, LA and H. G. M. J. VAN BEEK. Activation time of myocardial oxidative phosphorylation in creatine kinase and adenylate kinase knockout mice. *Am. J. Physiol.* 282:H2259–H2264, 2002.
24. HARRISON, G. J., M. H. VAN WIJHE, B. DE GROOT, F. J. DIJK, L. A. GUSTAFSON and J, H. G. M. VAN BEEK. Glycolytic buffering affects cardiac bioenergetic signaling and contractile reserve similar to creatine kinase. *Am. J. Physiol.* 285:H883–H890, 2003.
25. HOGAN, MC. Fall in intracellular PO_2 at the onset of contractions in *Xenopus* single skeletal muscle fibers. *J. Appl. Physiol.* 90: 1871–1876, 2001.
26. HUGHSON, RL. Exploring cardiorespiratory control mechanisms through gas exchange dynamics. *Med. Sci. Sports Exerc.* 22:72–79, 1990.
27. HUGHSON, R. L., J. K. SHOEMAKER, M. E. TSCHAKOVSKY, and J. M. KOWALCHUCK. Dependence of muscle $\dot{V}O_2$ on blood flow dynamics at the onset of forearm exercise. *J. Appl. Physiol.* 81:1619–1626, 1996.
28. HUGHSON, R. L., M. E. TSCHAKOWSKY, and M. E. Houston. Regulation of oxygen consumption at the onset of exercise. *Exerc. Sport Sci. Rev.* 29:129–133, 2001.
29. JONES, A. M., D. P. WILKERSON, K. KOPPO, S. WILMSHURST, and I. T. CAMPBELL. Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary $\dot{V}O_2$ kinetics in the transition to moderate intensity exercise in man. *J. Physiol.* 552:265–272, 2003.
30. JONES, A. M., D. P. WILKERSON, S. WILMSHURST, and I. T. CAMPBELL. Influence of L-NAME on pulmonary O_2 uptake kinetics during heavy-intensity cycle exercise. *J. Appl. Physiol.* 96:1033–1038, 2004.
31. KINDIG, C.A., R.A. HOWLETT, C.M. STARY, B. and WALSH, M.C. HOGAN. Effects of acute creatine kinase inhibition on metabolism and tension development in isolated single myocytes. *J. Appl. Physiol.* Aug 27 [Epub ahead of print]
32. KINDIG, C. A., K. M. KELLEY, R. A. HOWLETT, C. M. STARY, and M. C. HOGAN. Assessment of O_2 uptake dynamics in isolated single skeletal myocytes. *J. Appl. Physiol.* 94:353–357, 2003.
33. KINDIG, C. A., P. MCDONOUGH, H. H. ERICKSON, and D. C. POOLE. Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse. *J. Appl. Physiol.* 91:891–896, 2001.
34. KINDIG, C. A., P. MCDONOUGH, H. H. ERICKSON, and D. C. POOLE. Nitric oxide synthase inhibition speeds oxygen uptake kinetics in horses during moderate domain running. *Respir. Physiol. Neurobiol.* 132:169–178, 2002.
35. KINDIG, C. A., T. E. RICHARDSON, and D. C. POOLE. Skeletal muscle capillary hemodynamics from rest to contractions: implications for oxygen transfer. *J. Appl. Physiol.* 92:2513–2520, 2002.
36. KORZENIEWSKI, B, and J. A. Zoladz. Factors determining the oxygen consumption rate ($\dot{V}O_2$) on-kinetics in skeletal muscles. *Biochem. J.* 379:703–710, 2004.
37. LAUGHLIN, M. H., R. J. KORTHUIS, D. J. DUNCKER, and RJ BACHE. Control of blood flow to cardiac and skeletal muscle during exercise. In: *Handbook of Physiology*. Section 12. Exercise: Regulation and Integration of Multiple Systems, Rowell and L. B. J. T. SHEPHERD (EDS.). NEW YORK AND OXFORD: OXFORD UNIVERSITY PRESS, 1996, P. 705–769.
38. MARGARIA, R., F. MANGILI, F. CUTTICA, and P. CERRETELLI. The kinetics of the oxygen consumption at the onset of muscular exercise in man. *Ergonomics.* 8:49–54, 1965.
39. MEYER, RA. A linear model of muscle respiration explains mono-

exponential phosphocreatine changes. *Am. J. Physiol.* 254:C548–C553, 1988.
40. PIIPER, J P. E di Prampero, and P. Cerretelli. Oxygen debt and high energy phosphates in gastrocnemius muscle of the dog. *Am. J. Physiol.* 215:523–531, 1968.
41. RA˚DEGRAN, G, and B. SALTIN. Muscle blood flow at onset of dynamic exercise in humans. *Am. J. Physiol.* 274:H314–H322, 1998.
42. ROSSITER, H. B., S. A. WARD, V. L. DOYLE, F. A. HOWE, J. R. GRIFFITHS, and B. J. WHIPP. Inferences from pulmonary O_2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *J. Physiol.* 518:921–932, 1999.
43. ROSSITER, H. B., S. A. WARD, F. A. HOWE, D. M. WOOD, J. M. KOWALCHUCK, J. R. GRIFFITHS, and B. J. WHIPP. Effects of dichloroacetate on $\dot{V}O_2$ and intramuscular ^{31}P metabolite kinetics during high-intensity exercise in humans. *J. Appl. Physiol.* 95:105–1115, 2003.
44. ROSSITER, H. B., S. A. WARD, J. M. KOWALCHUCK, F. A. HOWE, J. R. GRIFFITHS, and B. J. WHIPP. Effects of prior exercise on oxygen uptake and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. *J. Physiol.* 537:291–303, 2001.
45. SAKS, V.A., and R. VENTURA-CLAPIER. Cellular Bioenergetics: Role of coupled creatine kinases. Kluwer Academic Publishers, Dordrecht, The Netherlands, 1994,p. 1–346.
46. STACPOOLE, PW. The pharmacology of dichloroacetate. *Metabolism.* 38:1124–1144, 1989.
47. TIMMONS, J. A., T. GUSTAFSSON, C. J. SUNDBERG, E. JANSSON, and P. L. GREENHAFF. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am. J. Physiol.* 274:E377–E380, 1998.
48. TIMMONS, J. A., S. M. POUCHER, D. CONSTANTIN-TEODOSIU, I. A. MACDONALD, and P. L. GREENHAFF. Metabolic responses from rest to steady state determine contractile function in ischemic skeletal muscle. *Am. J. Physiol.* 273:E233–E238, 1997.
49. TSCHAKOVSKY, ME, and R. L. HUGHSON. Interaction of factors determining oxygen uptake at the onset of exercise. *J. Appl. Physiol.* 86:1101–1113, 1999.
50. WHIPP, B.J. and M. MAHLER. Dynamics of pulmonary gas exchange during exercise. In Pulmonary Gas Exchange, Vol. II, J.B. West (Ed.). Academic, New York, NY, USA ,1980, p. 33–96.