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TRAINING-INDUCED ACCELERATION OF OXYGEN UPTAKE KINETICS IN SKELETAL MUSCLE: THE UNDERLYING MECHANISMS

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It is well known that the oxygen uptake kinetics during rest-to-work transition (\dot{V}_{O_2} on-kinetics) in trained subjects is significantly faster than in untrained individuals. It was recently postulated that the main system variable that determines the transition time ($t_{1/2}$) of the \dot{V}_{O_2} on-kinetics in skeletal muscle, at a given moderate ATP usage/work intensity, and under the assumption that creatine kinase reaction works near thermodynamic equilibrium, is the absolute (in mM) decrease in [PCr] during rest-to-work transition. Therefore we postulate that the training-induced acceleration of the \dot{V}_{O_2} on-kinetics is a marker of an improvement of absolute metabolic stability in skeletal muscles.

The most frequently postulated factor responsible for enhancement of muscle metabolic stability is the training-induced increase in mitochondrial proteins. However, the mechanism proposed by Gollnick and Saltin (1982) can improve absolute metabolic stability only if training leads to a decrease in resting $[\text{ADP}_{\text{free}}]$. This effect is not observed in many examples of training causing an acceleration of the \dot{V}_{O_2} on-kinetics, especially in early stages of training. Additionally, this mechanism cannot account for the significant training-induced increase in the relative (expressed in % or as multiples of the resting values) metabolic stability at low work intensities, condition in which oxidative phosphorylation is not saturated with $[\text{ADP}_{\text{free}}]$. Finally, it was reported that in the early stage of training, acceleration in the \dot{V}_{O_2} on-kinetics and enhancement of muscle metabolic stability may precede adaptive responses in mitochondrial enzymes activities or mitochondria content.

We postulate that the training-induced acceleration in the \dot{V}_{O_2} on-kinetics and the improvement of the metabolite stability during moderate intensity exercise in the early stage of training is mostly caused by an intensification of the “parallel activation” of ATP consumption and ATP supply pathways. A further acceleration in \dot{V}_{O_2} on-kinetics, resulting from prolonged periods of training, may be caused by a further and more pronounced improvement in the muscles’ absolute metabolic stability, caused by an intensification of the “parallel activation” as well as by an increase in mitochondrial proteins.

Key words: *bioenergetics, metabolic stability, oxidative phosphorylation, oxygen uptake kinetics, parallel activation/simultaneous regulation*

MUSCLE ENERGY SUPPLY AT THE ONSET OF EXERCISE

The rest-to-work transition or an increase in generated power output requires a rapid adjustment in ATP supply. Nobel Prize laureate August Krogh and his co-worker Johannes Lindhard (1) were the first to observe, in 1913, that during the transition from rest to work, pulmonary oxygen uptake does not rise instantly, and reaches a steady-state only after 2-3 minutes. During the first seconds or the first tens of seconds of moderate-intensity exercise the most important and immediately available source of energy is phosphocreatine (PCr) splitting through the creatine kinase (CK) reaction, with only a minor contribution by anaerobic glycolysis (see *e.g.* 2 - 6). At higher power output the contribution of anaerobic glycolysis to ATP production at the onset of the exercise increases (see *e.g.* 7, 8). Although the ATP supply from oxidative phosphorylation starts almost immediately after the onset of muscle contraction (9), the “acceleration” of this process is rather slow, and, as mentioned above, \dot{V}_{O_2} uptake (\dot{V}_{O_2}) requires usually about 2-3 minutes to reach a steady state during moderate intensity exercise (for review see 10, 11). During heavy-intensity exercise (*i.e.* – above the lactate threshold: LT), no steady state in the oxygen uptake is reached, but a progressive increase in \dot{V}_{O_2} (slow component of \dot{V}_{O_2} kinetics) takes place (see 10, 11). The \dot{V}_{O_2} slow component is usually considered to be associated with muscle fatigue and reduced exercise tolerance.

OXYGEN UPTAKE KINETICS AT THE ONSET OF EXERCISE

The rate of the increase in \dot{V}_{O_2} during the rest-to-work transition was originally described as a mono-exponential process (12 - 14). Further development of this approach resulted in more complex models of description of the \dot{V}_{O_2} on- and off-kinetics (15, 16). Currently, during the exercise of low and moderate intensity (*i.e.* below LT), two phases in the \dot{V}_{O_2} on-kinetics are recognized and characterised: the “cardiodynamic” phase, also called phase I; and the “primary” component, also called phase II. During heavy exercise intensity, an additional third phase, called the “slow component” of \dot{V}_{O_2} on-kinetics (or phase III), is present (for overview see 10, 11, 15).

Although a complete characterization of \dot{V}_{O_2} on-kinetics involves various time delays and amplitudes of response for the relevant phases (see 15), the most relevant parameters describing the rate of increase in \dot{V}_{O_2} are the time constant of the primary component (τ_p) or the half time of the overall response ($t_{1/2}$). The τ_p represents the time to reach $[1 - 1/e] \times 100\% = 63\%$ of the final response in \dot{V}_{O_2} during phase II of the rest-to-work transition. On the other hand, the $t_{1/2}$ indicates the time to reach 50% of the final response in \dot{V}_{O_2} during the rest-to-work transition (see 17).

When analyzing literature data on \dot{V}_{O_2} on-kinetics one has to realize the different meanings of parameters of \dot{V}_{O_2} on-kinetics measured in the working muscle (see *e.g.* 9, 18, 19) and in the lungs (pulmonary \dot{V}_{O_2}), although it has been demonstrated that τ_p value characterising the primary component of the pulmonary \dot{V}_{O_2} on-kinetics reflects rather closely the kinetics of \dot{V}_{O_2} determined across exercising muscles (τ_m) (18).

τ_p can significantly vary in healthy humans, between 20 and 60 seconds (20). Generally, τ_p in humans is inversely correlated with maximal oxygen uptake ($\dot{V}_{O_{2max}}$) (see also 20). The lowest values of τ_p , amounting to about 10 s have been reported in well-trained individuals (21 - 23). On the other hand the longest values, often exceeding 70 s, have been observed in patients, *e.g.* those suffering from cardio-pulmonary insufficiency (see *e.g.* 24, 25). Although faster \dot{V}_{O_2} kinetics in physiological conditions is associated with a high physical capacity, and less substrate level phosphorylation, it was recently postulated that a faster \dot{V}_{O_2} kinetics at the onset of exercise is not necessarily associated with an improved muscle function (for discussion see below and ref. 26, 27).

For a long time, two main factors, (1) oxygen delivery to the working muscles and (2) the metabolic properties of the muscles, have been discussed as possible determinants of \dot{V}_{O_2} on-kinetics (for review see 6, 28 - 29). In the recent years a substantial amount of evidence has been provided showing that in “normal conditions” (*e.g.* normoxia, no limitations to O_2 delivery, absence of pathology), at low or moderate exercise intensities the rate of \dot{V}_{O_2} increase at the onset of exercise is mainly determined by local factors within the working muscle cells, and not by O_2 delivery (see *e.g.* 9, 30 - 34). However, during transitions to heavy or maximal exercise, an enhanced oxygen delivery to the working muscle may accelerate the \dot{V}_{O_2} on-kinetics (for review see *e.g.* 6, 32). In the isolated *in situ* dog gastrocnemius preparation, abolishment of delays in convective O_2 delivery to skeletal muscle did not affect skeletal muscle \dot{V}_{O_2} kinetics during transitions to contractions of submaximal metabolic intensity (30), whereas the same experimental intervention determined a slightly but significantly faster \dot{V}_{O_2} kinetics during transitions to contractions corresponding to $\dot{V}_{O_{2max}}$ (35). These observations suggest that muscle blood flow, and therefore convective O_2 delivery, could be one important determinant of \dot{V}_{O_2} kinetics only during severe exercise. A similar scenario could be applied to the effects of training as well. Krustup *et al.* (36) recently observed that intense interval training elevates muscle \dot{V}_{O_2} , blood flow and vascular conductance in the initial phase of exercise at high, but not at low, intensities.

Recently, by utilizing a computer model of oxidative phosphorylation in mammalian skeletal muscles, we have suggested that the main factor which determines the transition time $t_{1/2}$ of \dot{V}_{O_2} on-kinetics during exercise, at a given level of ATP utilization (exercise intensity) and under the assumption that the creatine kinase reaction works near thermodynamic equilibrium, is the absolute (in mM) amount of [PCr] that has to be transformed into [Cr] during the rest-to-

work transition (27). This hypothesis agrees well with the experimental results by Philips *et al.* (see (37), *Fig. 4* therein).

TRAINING-INDUCED ACCELERATION OF \dot{V}_{O_2} KINETICS

It was originally reported by Whipp and Wasserman (38) that during the rest-to-work transition to the same power output, the \dot{V}_{O_2} on-kinetics in a well-trained subject is much faster than in a poorly-trained individual (see *Fig. 1* therein). This finding was soon confirmed by others (4, 39, 40). Moreover, Powers *et al.* (41) reported that in highly trained individuals with similar training habits the \dot{V}_{O_2} adjustment at the onset of work at 50% \dot{V}_{O_2} was more rapid in those with a higher $\dot{V}_{O_2\max}$.

Hickson *et al.* (42) were probably the first to demonstrate that, in previously untrained individuals, a rather short (10 weeks) but strenuous program of endurance training, involving running and cycling, resulted in a significant acceleration of the \dot{V}_{O_2} on-kinetics both at the same absolute and the same relative work rates. This finding was confirmed in another study of the same group (43). A training-induced acceleration of \dot{V}_{O_2} on-kinetics was also reported in previously trained athletes (44). Within a 8-week training period, involving 5 sessions per week lasting from 40 to 55 minutes, τ_p of pulmonary \dot{V}_{O_2} kinetics during cycling at moderate intensity was reduced by about 5 s (from 29.2 s to 24.4 s) after 4 weeks of training. Subsequent four weeks of training resulted in a further shortening the τ_p , to 21.9 s (see *Table 2* in (44)).

Interesting observations were made by Cerretelli *et al.* (4), who showed that in trained muscles, compared to untrained muscles, a faster \dot{V}_{O_2} on-kinetics is associated with a lower contribution of energy from anaerobic glycolysis at the onset of exercise (see *Fig. 4* therein). This experimental finding was subsequently confirmed by theoretical studies, showing that an increase in glycolytic ATP supply slows down the \dot{V}_{O_2} on-kinetics (45). Moreover, it has been demonstrated (4) that the training-induced acceleration of \dot{V}_{O_2} on-kinetics, observed in the trained legs of runners and in the trained arms of kayakers, as well as of swimmers, was limited to the specifically trained muscles (see *Figs. 1, 2 and 3* therein).

Further studies in this field have shown that the mechanism(s) responsible for the shortening of the \dot{V}_{O_2} on-kinetics is/are significantly activated already in early stages of endurance training. For example, Phillips *et al.* (37) have reported that as early as after 4 sessions of training, involving 2 hours of cycling at 60% $\dot{V}_{O_2\max}$, the τ_p of \dot{V}_{O_2} kinetics was reduced from 37.2 to 34.9 s; after 9 days of training the τ_p amounted to 32.5 s. At the end of training, *i.e.* after 30 days, the τ_p was 28.3 s. In another study, conducted on previously untrained 50-yr old subjects, a significant acceleration of the \dot{V}_{O_2} on-kinetics was reported as soon as after two weeks of an endurance/fitness training program (46). These authors have reported a clear tendency towards shortening of the τ_p (from the pre-training value of 46.9

s to 38.1 s) after only one week of training. After 15 days of training, a significant ($p < 0.05$) shortening of the τ_p to 34.4 s was reached. From the second week of training up to the end of the 3rd month of training, no significant changes in τ_p were found.

Recently, we have found that as few as 4 sessions of a maximal isometric strength training, including in total only 3 minutes and 20 seconds of maximal voluntary contractions (MVC), performed within one week (and resulting in a 15% increase of MVC) were sufficient to significantly accelerate the \dot{V}_{O_2} on- and off-kinetics during heavy-intensity exercise (Zoladz *et al.* 2006 – unpublished data).

The above presented data show that the training-induced acceleration of \dot{V}_{O_2} on-kinetics is a very rapid response, faster than the training-induced increase in $\dot{V}_{O_2 \max}$ (37, 46), and occurs earlier than increases in muscle mitochondria protein content (37).

MUSCLE METABOLIC STABILITY IN VARIOUS TYPES OF MUSCLE FIBRES

Good “metabolic stability” during rest-to-work transition in skeletal muscle means less decrease in [PCr] and in the cytosolic phosphorylation potential (ΔG_p), as well as less increase in $[P_i]$, $[ADP_{\text{free}}]$, $[AMP_{\text{free}}]$ and $[IMP_{\text{free}}]$ for a given increase in oxygen consumption/work intensity.

In order to analyze the relationship between metabolic stability and \dot{V}_{O_2} kinetics and the effect of mitochondria volume/activity it is very important to define more strictly the concept of metabolic stability. In particular, “absolute” metabolic stability should be clearly distinguished from “relative” metabolic stability. The absolute stability of, say, $[ADP_{\text{free}}]$ refers to absolute changes in $[ADP_{\text{free}}]$ (in μM), while relative stability refers to relative (in %, or expressed as multiples of the resting values) changes in $[ADP_{\text{free}}]$. These two types of metabolic stability are not equivalent to each other. Let us consider two hypothetical cases. In the first case $[ADP_{\text{free}}]$ increases during rest-to-work transition from 10 μM to 50 μM before training, and from 5 μM to 25 μM after training. Therefore, relative stability remains unchanged (a 5-fold increase), while absolute stability is 2-fold increased (increase in $[ADP_{\text{free}}]$ by 40 μM before training and by 20 μM after training). In the second case $[ADP_{\text{free}}]$ increases during rest-to-work transition from 10 μM to 50 μM before training and from 10 μM to 25 μM after training. In this case both the relative stability (2.5-fold instead of 5-fold increase in $[ADP_{\text{free}}]$) as well as the absolute stability ($[ADP_{\text{free}}]$ increases by 15 μM instead of by 40 μM) are improved. The distinction between these two types of metabolic stability is very important, because absolute metabolic (especially in terms of [PCr]) stability is relevant for the τ_p of pulmonary \dot{V}_{O_2} kinetics (as mentioned above), whereas relative metabolic (especially in terms of $[ADP_{\text{free}}]$) stability refers to the “phenomenological” (that is, observed *in vivo*, being a consequence of not only the mechanistic $\dot{V}_{O_2}/[ADP_{\text{free}}]$ dependence, observed *e.g.* in isolated mitochondria, but also of direct activation of oxidative

phosphorylation – see below) reaction order of oxidative phosphorylation (slope of the $\dot{V}_{O_2}/[ADP_{free}]$ relationship) and therefore to the mechanisms of the regulation of oxidative phosphorylation and of training-induced adaptation of this process (47). Namely, if the negative feedback via $[ADP_{free}]$ is the only mechanism of the regulation of oxidative phosphorylation in response to a varying energy demand, then the phenomenological reaction order may be maximally 1 (for hyperbolic, Michaelis-Menten mechanistic $\dot{V}_{O_2}/[ADP_{free}]$ dependence) (a, say, 3-fold increase in $[ADP_{free}]$ can be accompanied by a maximally 3-fold increase in \dot{V}_{O_2}). If, on the other hand, the phenomenological reaction order is much greater than 1 (a 3-fold in $[ADP_{free}]$ is accompanied by a, say, 10-fold increase in \dot{V}_{O_2}), other mechanisms must contribute to the regulation of oxidative phosphorylation. Additionally, if the phenomenological order (slope) of the $\dot{V}_{O_2}/[ADP_{free}]$ dependence increases as a result of muscle training, the contribution of other mechanisms of the regulation of oxidative phosphorylation must increase. The phenomenological reaction order is directly related to the relative metabolic stability, but not to the absolute metabolic stability. On the other hand, it should also be acknowledged that in most cases absolute and relative metabolite stabilities change in the same direction (that is, they both increase or decrease).

It is well documented that slow-twitch oxidative muscle fibers are characterized, during rest-to-work transitions, by a higher (absolute and relative) metabolic stability, compared to fast-twitch glycolytic muscle fibres (for review see 7, 48). This effect was observed both in animal muscles (49) as well as in human muscles (50). Moreover, it was shown that in endurance trained subjects the metabolic stability of calf muscle, determined by means of the ^{31}P NMR spectroscopy, is much better than in untrained subjects (51).

Important data regarding metabolic stability during exercise were provided by experiments in which metabolic changes in predominantly slow muscle (soleus) and in predominantly fast muscle (gastrocnemius) during calf exercise were determined by means of ^{31}P NMR spectroscopy (50). This study demonstrated that in soleus muscle an increase in the ATP turnover rate up to 40% of maximum was accompanied by almost no changes in the $[ADP_{free}]$, and by much smaller changes in $[PCr]$ and $[P_i]$ when compared to the gastrocnemius muscle (see Fig. 2 therein). In terms of metabolic stability endurance trained skeletal muscle resembles heart muscle, in which $[ATP]$, $[PCr]$, $[P_i]$ and $[ADP_{free}]$ remain constant even during a 5-fold increase in \dot{V}_{O_2} (see 52, 53).

The above discussed data show that the regulation of oxidative phosphorylation *in vivo* is more complex than that based exclusively on simple feedback control loops, with $[ADP_{free}]$ and $[P_i]$ as the main controllers of ATP production by oxidative phosphorylation (taking place *e.g.* in isolated mitochondria) (54, 55). This mandates a re-evaluation of our understanding of the physiological mechanisms underlying the adaptations to physical training (for overview of this point see also (56)).

TRAINING-INDUCED IMPROVEMENT OF MUSCLE METABOLIC STABILITY

It is well known that endurance training leads to a transformation of fast myosin heavy chains (MyHC) isoforms into slower MyHC isoforms (see *e.g.* 57, 58, 59), as well as to a transformation of fast glycolytic muscle fibers into slow oxidative muscle fibers. Since slow oxidative muscle fibres possess a higher (absolute and relative) metabolic stability than fast glycolytic muscle fibres, it derives that endurance training should result in an improvement of skeletal muscles' metabolic stability during exercise.

As far as we know, the first evidence for a training-induced improvement in skeletal muscles' metabolic stability during exercise in humans was presented by Karlsson *et al.* (60). These authors showed that 3 months of endurance training resulted in a less pronounced decrease in muscle PCr concentration and in an attenuated increase in muscle lactate concentration during cycling exercise at the same absolute power output. Further studies in this area, involving animal model preparations, confirmed this finding. Constable *et al.* (61) showed that, in rats, a few weeks of endurance training (running on the treadmill) resulted in a higher [PCr] and lower $[P_i]$, $[ADP_{free}]$, and $[AMP_{free}]$ concentrations in muscles, for the same contractile activity, compared to untrained rats. Clark III *et al.* (62) using ^{31}P - NMR spectroscopy showed that electrically induced conditioning of canine latissimus dorsi resulted in a much lower decrease in [PCr] concentration and greater maximal tension development, compared to untrained muscle during identical stimulation conditions (see *Fig. 7* therein).

It is also well documented that muscle metabolic adaptations to endurance training include an increase in mitochondrial enzymes involved in the oxidation of carbohydrates and fatty acids (63 - 65), as well as an increase in the size and number of mitochondria (mitochondrial volume density) (66, 67). It was reported that training in humans, as well as in other mammals, can increase muscles mitochondrial content, usually by between 30 to 100% within about 4-6 weeks (68).

It has been postulated that training-induced increase in mitochondria content/mitochondrial proteins would increase by itself muscle metabolic stability (see *e.g.* 64, 69, 70), allowing a given respiratory rate to be achieved in the presence of smaller disturbances in intermediate metabolite concentrations. Indeed, it was shown that the training-induced increase in mitochondrial density, accompanied by a decrease in resting $[ADP_{free}]$, led to an increase in both absolute and relative metabolic stability (71). In our opinion, however, the improvement of the absolute and relative metabolic stability observed in many experiments cannot be satisfactorily explained by the mechanism postulated by Gollnick and Saltin (69). In particular, it cannot explain the cases in which training improves metabolic stability, but does not decrease resting $[ADP_{free}]$, as well as the cases in which a significant increase in both absolute and relative metabolic stability at low work intensities is observed.

In order to be able to stimulate significantly \dot{V}_{O_2} during rest-to-work transition, $[ADP_{free}]$ at rest must be well below the K_m (Michaelis-Menten) constant of oxidative phosphorylation for $[ADP_{free}]$. When mitochondrial oxidative phosphorylation is not significantly saturated with $[ADP_{free}]$, an increase in mitochondria content itself can significantly improve absolute muscle metabolic stability, but not the relative metabolic stability. When other parameters are kept constant, a training-induced increase in the activity (and therefore in the maximal velocity V_{max}) of oxidative phosphorylation will result in an increase in the resting phosphorylation potential and $[PCr]$, and in a decrease in resting $[ADP_{free}]$ and $[P_i]$. Also during exercise the same \dot{V}_{O_2} will be accomplished at lower $[ADP_{free}]$. Additionally, a smaller increase in the absolute (in μM) $[ADP_{free}]$ concentration may cause the same relative (expressed as a multiple of the resting value) stimulation of \dot{V}_{O_2} . This is because at low resting $[ADP_{free}]$ the same relative increase in $[ADP_{free}]$ corresponds to a smaller absolute increase in $[ADP_{free}]$ (see above). However, because at low (much below K_m) $[ADP_{free}]$ concentrations the $\dot{V}_{O_2}/[ADP_{free}]$ relationship remains approximately first order (a, say, 3-fold increase in $[ADP_{free}]$ causes an about 3-fold increase in \dot{V}_{O_2}) regardless of the amount/activity of mitochondria, the same relative increase in \dot{V}_{O_2} must be accompanied by a similar relative increase in $[ADP_{free}]$. On the other hand, when resting $[ADP_{free}]$ is low, oxidative phosphorylation becomes saturated with ADP at higher \dot{V}_{O_2} and therefore the phenomenological $\dot{V}_{O_2}/[ADP_{free}]$ relationship at high work intensities becomes steeper than in the case when resting $[ADP_{free}]$ is high (69). If resting $[ADP_{free}]$ is not affected by muscle training, the increase in mitochondria content has no impact on either absolute or relative metabolic stability. For this reason, the training-induced change in the resting $[ADP_{free}]$ is extremely important for the hypothesis that an increase in mitochondria content improves metabolic stability.

It was indeed observed in some experimental studies that training decreases resting $[ADP_{free}]$ (60, 71). However, in other experimental studies (in some of which a training-induced improvement in metabolite concentrations was observed) training/conditioning caused either no changes in resting $[ADP_{free}]$ (61, 72 - 74), or even an increase in the concentration of this metabolite at rest (62, 75).

Additionally, it seems that a short-term (lasting from a few days to one month) training, leading to a significant improvement of metabolic stability, but not causing any detectable increase in the amount and activity of mitochondrial proteins, does not affect resting $[ADP_{free}]$ (61, 73, 74). In some cases after a short training improving metabolic stability even an increase in resting $[ADP_{free}]$ was encountered (62, 76). On the other hand, relatively long-lasting training (conducted to reach a steady-state of muscle adaptations) increases mitochondria content and lowers resting $[ADP_{free}]$ (71). This suggests that some other mechanism, which does not decrease resting $[ADP_{free}]$ and is not related to the increase in the amount of mitochondria, is responsible for the improvement of metabolic stability in early stages of training. Even muscle training that increases

the amount of mitochondria does not necessarily decrease the resting $[\text{ADP}_{\text{free}}]$. The latter may take place if changes in some other components of the system (for instance increases in resting ATP usage or proton leak) compensate for the increase in mitochondrial proteins. In such a case the increase in mitochondria amount can not account for the increase in the apparent sensitivity of \dot{V}_{O_2} to $[\text{ADP}_{\text{free}}]$ (increase in the relative $[\text{ADP}_{\text{free}}]$ stability) (see above).

It was also observed (62) that even at low work intensities, during which $[\text{ADP}_{\text{free}}]$ can be expected to be much below K_m , muscle conditioning causes a significant increase in both relative and absolute metabolic stability. This finding cannot be explained by the increase in mitochondria content, as discussed above. Finally, in intact skeletal muscle *in vivo* the phenomenological $\dot{V}_{\text{O}_2}/[\text{ADP}_{\text{free}}]$ relationship is usually much steeper than first-order, and therefore cannot be explained by the hyperbolic (Michaelis-Menten) kinetics observed in isolated mitochondria (7, 77).

Generally, if the training-induced increase in mitochondria volume/activity is associated with a decrease in resting $[\text{ADP}_{\text{free}}]$, at low work intensities it could account for some improvement of absolute $[\text{ADP}_{\text{free}}]$ and $[\text{PCr}]/[\text{Cr}]$ stability, and it could lead to a shortening of the τ_p of \dot{V}_{O_2} kinetics, but it could not account for a significant improvement of relative $[\text{ADP}_{\text{free}}]$ and $[\text{PCr}]/[\text{Cr}]$ stability. However, the latter effect could be expected at higher work intensities, during which oxidative phosphorylation becomes saturated with $[\text{ADP}_{\text{free}}]$ (according to the mechanism proposed by Gollnick and Saltin (69)). On the other hand, if, for some reasons, a training-induced increase in mitochondrial volume/activity does not lead to a decrease in resting $[\text{ADP}_{\text{free}}]$, it will not cause a faster \dot{V}_{O_2} kinetics and an improvement of (either absolute or relative) metabolic stability. Additionally, the mitochondria amount does not increase significantly in the early stages of training, and therefore it can not be responsible for the early improvement of metabolic stability. These limitations do not apply to the training-induced increase in the intensity of the parallel activation of ATP demand and ATP supply (see below), which can improve the absolute and relative $[\text{ADP}_{\text{free}}]$ and $[\text{PCr}]/[\text{Cr}]$ stability, can determine a faster \dot{V}_{O_2} kinetics without changing resting $[\text{ADP}_{\text{free}}]$ (27, 47), and is likely to take place in the early stages of exercise.

Burelle and Hochachka (78) observed a training-induced decrease in the half-saturation constant of oxidative phosphorylation for ADP, while Zoll *et al.* (79) encountered the opposite effect. However, in the skinned fibres preparation which was utilized by these authors significant ADP gradients are likely to take place, and therefore these experimental results are difficult to interpret. Moreover, an increase in K_m of oxidative phosphorylation for ADP would not lead to an increase in the “regulatory space” (potential increase in \dot{V}_{O_2} caused by an increase in $[\text{ADP}_{\text{free}}]$) as proposed by Zoll *et al.* (79), but simply to a proportional increase in resting $[\text{ADP}_{\text{free}}]$ (the ratio of K_m to resting $[\text{ADP}_{\text{free}}]$ would remain constant). Jeneson *et al.* (80) postulated that the mechanistic $\dot{V}_{\text{O}_2}/[\text{ADP}_{\text{free}}]$ dependence in isolated mitochondria and intact skeletal muscle is not hyperbolic, but at least

second order. However, even a steep but constant mechanistic $\dot{V}_{O_2}/[ADP_{free}]$ dependence can not account for the training-induced increase in $[ADP_{free}]$ stability (increase in the steepness of the phenomenological $\dot{V}_{O_2}/[ADP_{free}]$ relationship). (The mechanistic $\dot{V}_{O_2}/[ADP_{free}]$ dependence is due to the activation of oxidative phosphorylation by ADP, *e.g.* in isolated mitochondria, whereas the phenomenological $\dot{V}_{O_2}/[ADP_{free}]$ relationship *in vivo* results not only from the mechanistic $\dot{V}_{O_2}/[ADP_{free}]$ dependence, but also from other regulatory mechanisms, *e.g.* parallel activation – see below). Furthermore, as it was discussed previously (47, 77), such a kinetics yields several predictions which contradict experimental data (for instance it would dictate a sigmoidal \dot{V}_{O_2} on-kinetics).

Green *et al.* (72) reported a significant improvement in muscle metabolic stability after a short-term training programme involving 2 h of daily exercise at 59% of peak \dot{V}_{O_2} , repeated for 10-12 consecutive days, despite the absence of an increase in mitochondrial enzymes activities. These findings were confirmed by another study by the same group (76), showing a significant improvement in metabolic stability during cycling exercise in human muscles (see *Table 3* and *5* therein) after only a 5-7 days of endurance training, despite the absence of an increase in mitochondrial enzymes activities. Similarly, Phillips *et al.* (37) reported a significant improvement of muscle metabolic stability after only 5 days of training, before any increase in the maximal activity of mitochondrial enzymes. Moreover, as early as after a single, extended session of heavy exercise, an improvement in muscle metabolic stability (especially lower $[ADP_{free}]$ and $[AMP_{free}]$, see (74) *Fig. 3* therein) during cycling at 60 and 75% of pre-training $\dot{V}_{O_{2max}}$ was reported. Interestingly, Phillips *et al.* (37) reported a significant improvement in muscle metabolic stability, accompanied by an acceleration of the \dot{V}_{O_2} on-kinetics in humans, just after four sessions of endurance training, before any detectable increase in muscle mitochondrial enzymes activities. On the other hand, it has been reported that the training-induced increase in mitochondrial enzymes activity in humans occurs as early as within 7-10 days of endurance training (81) or within about two weeks of sprint interval training (82, 83). Thus, although, as discussed above, in some experimental conditions an increase in mitochondrial enzymes activity can be found in early stage of training (see 81 - 83), however it was also reported that the training-induced increase in muscle metabolic stability can precede increase in the muscle maximal mitochondrial enzymes activities (37, 72, 76).

Some conclusions can be taken from the above presented data and discussion. Firstly, the training-induced increase in mitochondrial volume/activity can increase the relative metabolic (especially $[ADP_{free}]$ and $[PCr]/[Cr]$) stability only at higher work intensities (in which oxidative phosphorylation becomes saturated with ADP). On the other hand, it is known that a significant increase in relative metabolite stability can occur also at low work intensities. Secondly, increases in mitochondrial volume/activity can increase both the absolute and relative

metabolic stability only if an increase in mitochondria volume/activity is associated with a decrease in resting $[\text{ADP}_{\text{free}}]$, whereas no decrease (or even an increase) in resting $[\text{ADP}_{\text{free}}]$ is seen in the early stages of exercise. Thirdly, training/conditioning of muscles in its very early stage can induce some adaptive responses that improve muscle metabolic stability and shorten the τ_p of \dot{V}_{O_2} kinetics independently from an increase in mitochondrial proteins.

A quick improvement of (absolute and relative) $[\text{ADP}_{\text{free}}]$ and $[\text{PCr}]/[\text{Cr}]$ stability at a constant resting $[\text{ADP}_{\text{free}}]$, as well as a faster \dot{V}_{O_2} kinetics could be achieved by a training-induced intensification of parallel activation of ATP usage and ATP production (see 27, 47). This mechanism does not have to involve the synthesis of significant amounts of proteins (genetic level regulation), which on the other hand is needed when the mitochondrial amount increases.

SIMULTANEOUS REGULATION/PARALLEL ACTIVATION OF ATP CONSUMPTION AND ATP PRODUCTION

The concept of “simultaneous regulation/parallel activation” of ATP consumption and ATP production is based on the assumption that some external cytosolic signal/mechanism (*e.g.* calcium ions and/or another, still not discovered factor) directly activates both the production and hydrolysis of ATP during muscle contraction, allowing to maintain relatively stable concentrations of $[\text{ATP}]$, $[\text{ADP}_{\text{free}}]$ and $[\text{P}_i]$ while increasing the turnover of these intermediates (for overview see 7, 48, 84, 85). The concept of simultaneous regulation was introduced by Hochachka and co-workers (see 7, 48, 84). The term parallel activation was introduced by Korzeniewski (85), but it is presently referred by different authors to rather different regulatory mechanisms. The discovery of the activation by calcium ions of three rate-controlling TCA cycle dehydrogenases prompted several authors (see *e.g.* 86) to postulate that both NADH supply (substrate dehydrogenation) and ATP usage are directly activated by calcium. Hochachka and co-workers postulated within their simultaneous regulation concept (see 7, 48, 84) that some “latent enzymes” within the ATP-producing block (the authors did not specified which ones) are directly activated during muscle contraction (7, 48). Balaban and co-workers (87, 88) proposed that ATP synthase is directly activated by calcium ions in parallel with the activation of ATP usage and NADH supply. Finally, Korzeniewski postulated that ATP usage, NADH supply and all oxidative phosphorylation complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P_i carrier) must be directly activated in order to account for different kinetic properties of oxidative phosphorylation in intact tissues (the so-called “each-step-activation” mechanism) (77, 85, 89). It was proposed (90) that the activating factor could be represented by the frequency of calcium oscillations (for discussion of this point see also (91).

It was also postulated (48, 77) that parallel activation (each-step-activation) would be highest in intact heart *in vivo*, in which [ATP], [PCr], [P_i] and [ADP_{free}] remain constant even during a 5-fold increase in \dot{V}_{O_2} (see 52, 53), intermediate in oxidative skeletal muscles (type I muscle fibres) and low in glycolytic skeletal muscles (type II muscle fibers), in which changes in metabolite concentrations are the highest (see also, 49). Therefore, it seems likely that the training-induced transformation of the fatigue-sensitive type II muscle fibers into the fatigue-resistant type I could be accompanied by an intensification of the parallel activation/simultaneous regulation (47). It was demonstrated that (27, 47) that this mechanism could account for both a significant improvement of the (absolute and relative) [ADP_{free}] and [PCr]/[Cr] stability, and to an at least two-fold decrease in τ_p of \dot{V}_{O_2} kinetics even if resting [ADP_{free}] remains unchanged.

It is worth to mention that in the most fatigue-resistant muscle (the heart), in which parallel activation seems to be highest and metabolite concentrations are most stable during work transitions, the \dot{V}_{O_2} on-kinetics is very quick: $t_{1/2}$ equals 4-8 (-12)s under physiological conditions (92, 93), and anyway seems to be slowed down by oxygen diffusion limitations (92) This kinetics is significantly slower in skeletal muscle (20), in which parallel activation seems to be smaller and quite significant changes in metabolite concentrations during rest-to-work transition take place.

We conclude that the improved metabolic stability after training is due, for the most part, to an enhanced parallel activation of ATP supply and ATP usage, and to a lesser extent, in cases in which muscle training causes a decrease in resting [ADP_{free}], to an increase in mitochondrial content.

O₂ DEFICIT, METABOLIC STABILITY AND EXERCISE TOLERANCE

The O₂ deficit, proportional to the amount of energy which must be derived from substrate level phosphorylation during rest-to-work transition, is determined, for a given amplitude of the \dot{V}_{O_2} response (*i.e.* the difference between the baseline \dot{V}_{O_2} and the steady-state \dot{V}_{O_2}), by the τ for muscle \dot{V}_{O_2} on-kinetics (τ_m) (11). Since the steady-state \dot{V}_{O_2} during exercise at a given work intensity is only a little or not affected by training, the effects of training on the O₂ deficit are determined by the kinetic properties of the oxidative phosphorylation system. It is generally thought that a lower O₂ deficit has, by itself, positive effects on exercise tolerance, since it is associated with less PCr and glycogen depletion, less H⁺ accumulation in muscle and blood, *etc.* This concept, however, may be not necessarily true. The training induced acceleration of the \dot{V}_{O_2} on-kinetics during moderate exercise intensity is usually caused by factors beneficial for muscle performance (*i.e.* intensification of parallel activation and/or increase in mitochondrial proteins), and therefore the acceleration of \dot{V}_{O_2} on-kinetics and the decrease of the O₂ deficit are usually considered as a positive adaptive response to exercise. However, acceleration of

the \dot{V}_{O_2} on-kinetics may also be caused by some factors that may be neutral or even harmful for muscle performance (*e.g.* decrease or inhibition of the creatine kinase activity, decrease in the total creatine pool) (see *e.g.* 27, 94). Therefore, what really matters, in terms of the O_2 deficit and its relationship with exercise tolerance, may not be its absolute value, but the kinetic properties of the oxidative phosphorylation system underlying the \dot{V}_{O_2} on-kinetics (for review see 26).

We postulate that the training-induced acceleration of the \dot{V}_{O_2} on-kinetics, caused by factors (increase in parallel activation and mitochondria content) improving muscle metabolic stability, is accompanied by an improvement of exercise tolerance at a given power output of moderate intensity (*e.g.* longer time to exhaustion at 50% $\dot{V}_{O_{2\max}}$). This effect may be caused by lower disturbances in muscle metabolic stability (attenuated increase in $[ADP_{\text{free}}]$ and $[P_i]$) after training, leading to reduced rate of glycogen depletion as well as to an attenuation of the negative effects of $[ADP_{\text{free}}]$, $[P_i]$ and $[H^+]$ on muscle power generating capabilities (for overview see *e.g.* 69, 71, 95). These effects could be more significant than, or even independent from, the effects on O_2 deficit. Within this scenario, then, the lower O_2 deficit after training may be considered just an epiphenomenon of the increased metabolic stability. The latter, and not the O_2 deficit by itself, would be responsible for the improved exercise tolerance.

CONCLUSIONS

We postulate that \dot{V}_{O_2} on-kinetics is a marker of absolute metabolic stability in skeletal muscle at a given level of ATP turnover (power output). In the early stages of training, the training-induced acceleration in the \dot{V}_{O_2} on-kinetics during moderate exercise intensity, expressed by shortening of the τ_p , would be caused by an improvement in muscle metabolic stability, and would be independent from increases in mitochondrial proteins. The improvement in muscle metabolic stability during muscle training may be caused by an intensification of the simultaneous regulation/parallel activation (each-step activation) of ATP consumption and ATP supply pathways (for overview see *e.g.* 7, 47, 48, 77, 84, 85). A further acceleration in \dot{V}_{O_2} on-kinetics, resulting from prolonged training, may be caused by a further and more pronounced improvement in muscle metabolic stability, caused by an intensification of the simultaneous regulation/parallel activation, as well as by an increase in mitochondrial proteins (see also 47). However, the latter effect (the increase in mitochondrial proteins) would depend on a training-induced decrease in resting $[ADP_{\text{free}}]$. We postulate that the training induced acceleration of \dot{V}_{O_2} on-kinetics, being a marker of improvement of the absolute metabolic stability at a given level of ATP turnover, would be more closely related to an improvement of endurance capacity (time to exhaustion at *e.g.* 50% $\dot{V}_{O_{2\max}}$) than to an increase in whole body $\dot{V}_{O_{2\max}}$, since the

latter is considered to be predominantly limited not by muscle oxidative capacity but by oxygen delivery to the working muscles.

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