Aerobic fitness level typical of elite athletes is not associated with even faster VO₂ kinetics during cycling exercise

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Abstract
The aim of this study was to address the question if the VO₂ kinetics is further improved as the aerobic training status increases from trained to elite level athletes. Maximal oxygen uptake (VO₂max), work-rate associated to VO₂max (IVO₂max) and VO₂ kinetics of moderate (Mod) and maximal exercise (Max) were determined in fifty-five subjects. Then, they were assigned into three groups: low (LF), intermediate (IF) and high (HF) aerobic fitness level. In average, the VO₂max of LF, IF and HF groups were, respectively, 36.0 ± 3.1, 51.1 ± 4.5 and 68.1 ± 3.9 ml·kg⁻¹·min⁻¹ (p ≤ 0.05 among each other). VO₂ kinetics mean response time of both exercise intensities were significantly faster (p ≤ 0.05) in HF (Mod, 27.5 ± 5.5 s; Max, 32.6 ± 8.3 s) and IF (Mod, 25.0 ± 3.1 s; Max, 42.6 ± 10.4 s) when compared to LF (Mod, 35.7 ± 7.9 s; Max: 57.8 ± 17.8 s). We can conclude that VO₂ kinetics is improved as the fitness level is increased from low to intermediate but not further improved as the aerobic fitness level increases from intermediate to high.

Key words: Oxidative metabolism, VO₂ kinetics, aerobic training.

Introduction
Pulmonary gas-exchange kinetics has been used to gain insight into oxidative energy release within active muscle during transitions to a higher metabolic rate (i.e., rest-exercise transition). The speed at which oxidative energy metabolism increases towards the new energy requirement imposed by exercise is the main determinant of oxygen deficit, and hence the cell reliance on non-oxidative ATP turnover (Özyener et al., 2003). From a homeostatic view, the VO₂ kinetics analysis is particularly insightful in pathological conditions where exercise tolerance may be compromised by limited aerobic energy turnover due to impaired O₂ transport and utilization with consequent higher internal milieu disturbance (conditions such as aging and cardio-respiratory diseases are associated with slow VO₂ kinetics response) (for a review, see Xu and Rhodes, 1999). On the other hand, health adults may present wide inter-individual differences in VO₂ kinetics speed (expressed as time constant or mean response time - MRT), but apparently non-related to sub-maximal exercise tolerance. Rather than focusing on the consequences of either quite slow or fast VO₂ kinetics values on exercise tolerance, VO₂ kinetics analysis in several pathological and physiological conditions altering O₂ transport and metabolism has been thought to be valuable, since such conditions may provide insights into the regulation of aerobic energy release in response to an abrupt challenge of cell energy homeostasis (i.e., transition to a higher metabolic rate) (Grassi et al., 1998; Grassi, 2001; MacDonald et al., 1997).

In the search for the culprit of such initial incompetence of aerobic energy release in providing ATP resynthesis at a rate that lately is achieved during square-wave exercise, many studies have attempted to manipulate the rate-limiting steps involved in O₂ transport and utilization (Grassi et al., 1998; MacDonald et al., 1997). From these several animal and humans experimental models, mainly two hypotheses regarding the rate-limiting step (O₂ delivery vs. O₂ utilization) have been addressed (Grassi, 2001; Hughson et al., 2001). Currently, it is reasonable to believe that the rate-limiting step of VO₂ kinetics depends on exercise intensity.

The leading findings suggest that the VO₂ kinetics of moderate-intensity exercise (below lactate threshold) is limited peripherally by O₂ utilization and that at higher exercise intensities, the O₂ delivery begins to exert limitation on VO₂ kinetics (Grassi, 2001; Hughson et al., 2001). The well known physiological adaptations induced by endurance exercise are likely the most extreme means to overcome rate-limiting steps determining VO₂ kinetics across exercise intensities. However, its impact on VO₂ kinetics speeding may vary as the operating rate-limiting step does across exercise intensities (Krustrup et al., 2004). Unfortunately, this interaction between aerobic fitness level and the rate-limiting steps of VO₂ kinetics over the wide range of exercise intensities has never been addressed, since the direct assessment of required variables face methodological issues such as invasiveness.

Far from directly answering the question above, there are some studies showing the association between aerobic fitness level and VO₂ kinetics response, which seem worth in providing details for future mechanistic studies. These studies have described that the VO₂ kinetics is improved as the aerobic fitness level increases (i.e. moving from sedentary/active to trained aerobic status) up to a point where VO₂ kinetics is not further improved by increasing aerobic fitness (Phillips et al., 1995; Carter et al., 2000). Such response could suggest a possible upper limit for VO₂ kinetics to improve. However, these studies have evaluated this association in individuals whose aerobic fitness level was well bellow the values possible to achieve with years of competitive endurance training (i.e. VO₂max > 65 ml·kg⁻¹·min⁻¹). In this scenario it is still unknown if aerobic fitness level typical of elite athletes are associated with even faster VO₂ kinetics than that presented by trained individuals. Based on previous works (Carter et al., 2000; Phillips et al., 1995), we hypothesize...
that VO₂ kinetics stop improving as aerobic fitness level still does so. Along in this issue, the exercise intensity at which VO₂ kinetics is analyzed may show different influences of aerobic fitness level on VO₂ kinetics, since the intensity specific rate-limiting step of VO₂ kinetics could undergo differential adaptation to endurance training (Krstrup et al., 2004).

The aim of the present study is to address the question if the VO₂ kinetics is further improved as the aerobic training status (aerobic fitness level) increases from trained to elite level athletes. Additionally we evaluated this response in two exercise intensities (moderate-intensity exercise and maximal-intensity exercise) where the likely rate-limiting step is different from each other.

**Methods**

**Subjects**

Among sixty-seven subjects tested (sedentary subjects, recreational and national level cyclists volunteered for this study), fifty-five individuals matching the criterions of one of our groups were selected to participate in this study. These subjects were assigned into six groups (three aerobic fitness levels X two exercise intensities) based on their aerobic fitness level (VO₂max) and exercise intensity performed (maximal intensity – Max or moderate intensity – Mod). The different levels of aerobic fitness were named as follow: Low aerobic fitness level (LF), intermediate aerobic fitness level (IF) and high aerobic fitness level (HF). The number of individuals in each of the 6 groups (N) is described in Table 1. All subjects gave informed consent and the protocol was approved by the university’s ethics committee. The subjects were asked to avoid exercise training during the last 2 days before each test and to report to the laboratory at least 3 h after the last meal. Table 1 shows the subject’s characteristics of each group.

**Experimental design**

The subjects performed 1) an incremental test to determine the maximal oxygen uptake (VO₂max) and the intensity associated with the achievement of VO₂max (iVO₂max); 2) constant work-rate cycling exercises to exhaustion at the intensity corresponding to 50% (moderate-intensity exercise - Mod) or 100% (maximal-intensity exercise – Max) of VO₂max to determine the time constant of oxygen uptake response (MRT - mean response time). Thereafter, those individuals matching the VO₂max range values of one of the three aerobic fitness level groups (LF – below 40 ml·kg·min⁻¹; IF – between 43 and 60 ml·kg·min⁻¹; HF – above 63 ml·kg·min⁻¹) were selected. All tests were performed at the same time of day in a climate-controlled (21-22°C) laboratory. Subjects performed only one test on any given day and the tests were at least 48 h apart from each other but completed within the period of 2 weeks.

**Procedures**

The tests were conducted on a mechanically braked cycle ergometer (Monark 828E, Stockholm, Sweden) with the pedal frequency maintained constant at 70 rpm (Ciclocomputer CatEye, Japan). Throughout the tests, gas-exchange variables were measured breath-by-breath using a portable gas analyzer system (Cosmed K4b², Rome, Italy). These analyzers have previously been validated over a wide range of exercise intensities (McLaughlin et al, 2001). Before each test, the O₂ and CO₂ analysis systems were calibrated using ambient air and a gas of known O₂ and CO₂ concentration according to the manufacturer’s instructions, while the K4b² turbine flowmeter was calibrated using a 3-1 syringe (Cosmed K4b², Rome, Italy).

Subjects performed incremental exercise to volitional exhaustion to determine VO₂max and iVO₂max at the cycle ergometer. The test started at 70 W with increases in power output of 35 W every 3 min. Each subject was encouraged to give a maximum effort and the blood was sampled at the point of exhaustion. Breath-by-breath VO₂ was smoothed using a five-step average filter, and then reduced to 15 s stationary averages for incremental test (Data Management Software, Cosmed, Rome, Italy) to reduce the noise. The VO₂max was defined as the highest 15 s VO₂ value reached during the incremental test. All subjects fulfilled at least two of the following three criteria for VO₂max: 1) respiratory exchange ratio (R) greater than 1.1, 2) a blood lactate concentration greater than 8 mM, and 3) peak HR at least equal to 90% of the age-predicted maximal (Taylor et al., 1955). The iVO₂max was defined as the minimal power output at which VO₂max occurred (Billat and Koralsztein, 1996).

The subjects subsequently performed a constant work rate test (at 50% VO₂max – Mod exercise or at 100% VO₂max – Max exercise) to determine VO₂ kinetics parameters. The subjects were instructed to perform the required intensity until the exhaustion (cadence < 65 rpm for 10 consecutive sec or voluntary exhaustion) at Max exercise or for 6 min at Mod exercise. At the start of cycling exercise, the subjects cycled against zero resistance, until a consecutive sec or voluntary exhaustion) at Max exercise. The tests were conducted on a mechanically braked cycle ergometer (Monark 828E, Stockholm, Sweden) with the pedal frequency maintained constant at 70 rpm (Ciclocomputer CatEye, Japan). Throughout the tests, gas-exchange variables were measured breath-by-breath using a portable gas analyzer system (Cosmed K4b², Rome, Italy). Earlobe capillary blood samples (25 µl) were collected into a glass tube and were analyzed for lactate concentration using an automated analyzer (YSI 2300, Ohio, USA).

**Table 1. Means values (±SD) of the subjects characteristics.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mod</th>
<th>Max</th>
<th>Body Mass (Kg)</th>
<th>Mod</th>
<th>Max</th>
<th>Height (m)</th>
<th>Mod</th>
<th>Max</th>
<th>Age (Years)</th>
<th>Mod</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>9</td>
<td>10</td>
<td>82.0 (11.1)</td>
<td>74.0 (15.5)</td>
<td>1.78 (0.07)</td>
<td>1.74 (0.05)</td>
<td>21.9 (4.6)</td>
<td>27.7 (4.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>8</td>
<td>9</td>
<td>76.9 (7.4)</td>
<td>67.7 (8.3)</td>
<td>1.78 (0.05)</td>
<td>1.71 (0.07)</td>
<td>21.1 (3.5)</td>
<td>23.6 (3.2) **</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>9</td>
<td>10</td>
<td>65.3 (6.9)</td>
<td>64.3 (2.7)</td>
<td>1.75 (0.05)</td>
<td>1.74 (0.06)</td>
<td>21.8 (2.3)</td>
<td>24.3 (4.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mod, moderate exercise. Max, maximal exercise. LF, low aerobic fitness. IF, intermediate aerobic fitness. HF, high aerobic fitness.

* significantly different from LF at the same intensity. † significantly different from all other groups.
Table 2. Mean values (± SD) of the indexes of aerobic fitness level and VO2 kinetics time constant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>VO2max (mL·Kg⁻¹·min⁻¹)</th>
<th>iVO2max (W·Kg⁻¹)</th>
<th>iVO2max (W)</th>
<th>MRT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mod</td>
<td>Max</td>
<td>Mod</td>
<td>Max</td>
</tr>
<tr>
<td>LF</td>
<td>36.2 (2.9)</td>
<td>35.8 (3.5)</td>
<td>2.3 (2.2)</td>
<td>2.6 (3.3)</td>
</tr>
<tr>
<td>IF</td>
<td>51.3 (4.8) *</td>
<td>50.9 (4.0) *</td>
<td>3.7 (4.6) *</td>
<td>3.9 (6.6) *</td>
</tr>
<tr>
<td>HF</td>
<td>67.4 (3.8) ab</td>
<td>68.7 (4.1) ab</td>
<td>5.4 (5.1) ab</td>
<td>5.3 (4.1) ab</td>
</tr>
</tbody>
</table>

Mod, moderate exercise. Max, maximal exercise. LF, low aerobic fitness. IF, intermediate aerobic fitness. HF, high aerobic fitness. VO2max, maximal pulmonary oxygen uptake; iVO2max, work-rate associated to VO2max. MRT, mean response time of oxygen uptake kinetics.

* significantly different from LF at the same intensity. ab significantly different from IF at the same intensity.

VO2 kinetics curve fitting

The VO2 kinetics during constant work-rate was modeled with a mono-exponential function by an iterative non-linear regression process from the Microcal Origin 6.0 (Northampton, MA, USA) using the following equation (Lamarra et al., 1987):

\[ VO_2(t) = VO_{2b} + A \times (1 - e^{-t/\tau}) \]

Where: \( VO_2(t) \) represents oxygen uptake at time (t), \( VO_{2b} \) is the baseline value of \( VO_2 \) at rest, \( A \) is the amplitude of the increase in \( VO_2 \) above the baseline value and \( \tau \) is the mean response time - MRT (defined as the time required to attain 63% of \( A \)). In order to increase the confidence of estimated parameters of the equation (Lamarra et al., 1987), \( VO_{2b} \) and \( A \) were constrained at the fitting window, respectively, as the mean value at the minute before constant work-rate exercise onset and as the difference between \( VO_{2b} \) and the end exercise \( VO_2 \) value.

Statistical analysis

Data are presented as mean ± SD. The effects of aerobic fitness on VO2 kinetics were tested using one-way ANOVA, with Tukey’s post-hoc tests where appropriate. ANOVA two-way was applied to compare all other variables across groups (exercise intensity vs. fitness level). Pearson product moment coefficients were used to assess the significance of relationships between VO2 kinetics and VO2max. Statistical significance was set at \( p \leq 0.05 \).

Results

Table 2 contains the indexes of aerobic fitness level obtained from progressive intensity exercise (VO2max, iVO2max relative to body mass and absolute iVO2max) and the mean response time (MRT) that describes VO2 kinetics during Mod and Max exercises. All indexes of aerobic fitness were significantly different (\( p \leq 0.05 \)) between the three levels of aerobic fitness. MRT of the IF and HF groups were significantly faster than MRT of LF group, however, there was no difference between IF and HF groups for this variable (\( p > 0.05 \)).

Figure 1 and 2 show the coefficient of correlation between MRT of, respectively, Mod (\( r = -0.51; p \leq 0.05 \)) and Max (\( r = -0.63; p \leq 0.05 \)) exercises and VO2max.

Although these coefficients were statistically significant, VO2max explains little of MRT variability at both exercise intensities (Mod, \( R^2 = 26\% \); Max, \( R^2 = 40\% \)).

Figure 3 and 4 show representative VO2 data and its curve fitting of each fitness levels, respectively, at moderate and maximal exercise. The individual MRT and VO2max values of these subjects, whose data are shown, are placed on each figure panel.

Discussion

To our knowledge, this is the first study evaluating the effects of aerobic fitness level on VO2 kinetics of subjects in the high-end aerobic power range (VO2max). The faster MRT along with the increase in aerobic fitness
level from low to intermediate is in agreement with several reports describing such adaptation in the low-end range of aerobic fitness level (Fukuoka et al., 2002; Phillips et al., 1995; Yoshida et al., 1992). Our main results showing unchanged MRT of Mod and Max exercise as the fitness level increases from intermediate to high are somehow in line with previous short-term longitudinal data obtained from less fit subjects (Carter et al., 2000; Phillips et al., 1995). Our current findings add to previous data by describing that exercise adaptation leading individuals to the high-end of aerobic fitness level range (VO\(_2\)max near of above 65 ml·kg·min\(^{-1}\)) is not able to further improve VO\(_2\) kinetics during both, Mod and Max intensity exercise.

The subjects included in this study were assigned to one of the three groups based on aerobic fitness level (VO\(_2\)max). Regarding our purpose, the composition of the high fit group is especially critical, since it must represent the high-end of aerobic fitness level. The indexes often used to describe aerobic fitness and exercise performance (VO\(_2\)max and iVO\(_2\)max) (Table 2) seem to match the level typical of elite athletes (Caputo and Denadai, 2004; Jeukendrup et al., 2000).

From a time course standpoint, improvements in sub-maximal VO\(_2\) kinetics of low fit individuals seem to occur very early (within a week) after exercise training program onset, even anticipating changes in other indexes of aerobic fitness such as VO\(_2\)max and lactate threshold (Phillips et al., 1995). Although this time course response for maximal exercise VO\(_2\) kinetics is still unknown, it seems that as the exercise intensity increases (intensity of exercise test), the effects of aerobic training on these parameters are more likely to occur (Carter et al., 2000; Krustrup et al., 2004). Collectively, these studies cited above show a good frame of the initial response of VO\(_2\) kinetics, where aerobic fitness level and exercise intensity seem to play a role in determining VO\(_2\) kinetics changes in response to exercise training.

Within the limitation of our cross-sectional experimental design, the VO\(_2\) kinetics of maximal exercise seems to follow the same pattern of that of sub-maximal response across the three levels of aerobic fitness studied. This similar pattern between these two exercise intensities is not obvious, since VO\(_2\) kinetics during high-intensity exercise has been described to present early improvements in response to training interventions along with no concomitant changes in moderate exercise VO\(_2\) kinetics (Krustrup et al., 2004). Such disparity has been understood as a phenomenon mainly related to the difference in exercise intensity, since a reduced reliance on non-oxidative energy source may improve cell homeostasis and exercise tolerance during, particularly, high-intensity exercise (Jones et al., 2003). Our data suggest that above a certain level of aerobic fitness, this disparity between exercise intensities may no longer exist.

Phillips et al. (1995) have demonstrated that low fit adult subjects have their VO\(_2\) kinetics improved by exercise training. Along with this result, middle age subjects also present similar improvements in VO\(_2\) kinetics early after training program onset (Fukuoka et al., 2002). Interestingly, Carter et al. (2000) studying active college students (VO\(_2\)max ~55 ml·kg·min\(^{-1}\)) did not find any improvement in moderate exercise VO\(_2\) kinetics after 6 weeks of exercise training, although other indexes of aerobic fitness were improved (lactate threshold and VO\(_2\)max). It seems that these findings rise two questions: Does VO\(_2\) kinetics only improve initially while aerobic fitness is rather low? And, will longer and severe training regime further improve VO\(_2\) kinetics?

Addressing the later question, our assessment of elite-level athletes seems to provide evidence that VO\(_2\) kinetics stop improving above a given level of aerobic fitness.
Oxygen uptake kinetics and aerobic fitness

Figure 3. Representative VO$_2$ data and the curve fitting of each group at moderate exercise. From up to bottom, respectively low, intermediate and high fitness subjects.

fitness during both, maximal and moderate exercise. This result is in accordance with previous time course study of VO$_2$ kinetics response to exercise training and the similarity of MRT reported here (Table 2) and by other studies involving highly fit subjects (Koppo et al., 2004; Kilding et al., 2007). Hence, this value of MRT may represent the fastest speed that VO$_2$ kinetics can be.

Some studies have reported significant relationship between VO$_2$max and VO$_2$ kinetics speed (Powers et al., 1985; Gurd et al., 2005). In spite of significant correlation coefficients between VO$_2$max and MRT at both exercise intensities when all the groups are joined together (Figure 1 and 2), their $R^2$ are low and the difference in VO$_2$max along with no associated difference in MRT between the intermediate and high aerobic fitness groups (Table 2), suggest a loose association between these variables. The significant correlations are probable due to the heterogeneity and the inclusion of low fit subjects whose VO$_2$ kinetics seems still sensitive to changes in aerobic fitness level.
Conclusion

In conclusion, we have demonstrated that VO$_2$ kinetics is improved when the fitness level is increased from low to intermediate but not further improved as the aerobic fitness level increases from intermediate to a high level typical of elite athletes. In addition, such response seems to be independent of exercise intensity (moderate vs. maximal).

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References


Key points
- Currently, it is reasonable to believe that the rate-limiting step of VO2 kinetics depends on exercise intensity.
- The well known physiological adaptations induced by endurance training are likely the most extreme means to overcome rate-limiting steps determining VO2 kinetics across exercise intensities.
- However, exercise adaptation leading individuals to the high-end of aerobic fitness level range (VO2max > 65 ml.kg.min-) is not able to further improve VO2 kinetics during both, moderate and maximal intensity exercise.

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