# Aerobic fitness level typical of elite athletes is not associated with even faster VO<sub>2</sub> kinetics during cycling exercise

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# Abstract

The aim of this study was to address the question if the VO<sub>2</sub> kinetics is further improved as the aerobic training status increases from trained to elite level athletes. Maximal oxygen uptake (VO<sub>2</sub>max), work-rate associated to VO<sub>2</sub>max (IVO<sub>2</sub>max) and VO<sub>2</sub> 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 VO<sub>2</sub>max of LF, IF and HF groups were, respectively,  $36.0 \pm 3.1$ ,  $51.1 \pm 4.5$  and  $68.1 \pm$ 3.9 ml·kg·min<sup>-1</sup> ( $p \le 0.05$  among each other). VO<sub>2</sub> kinetics mean response time of both exercise intensities were significantly faster (p  $\le 0.05$ ) in HF (Mod, 27.5  $\pm$  5.5 s; Max, 32.6  $\pm$  8.3 s) and IF (Mod,  $25.0 \pm 3.1$  s; Max,  $42.6 \pm 10.4$  s) when compared to LF (Mod,  $35.7 \pm 7.9$  s; Max:  $57.8 \pm 17.8$  s). We can conclude that VO<sub>2</sub> 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, VO<sub>2</sub> 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., restexercise 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 nonoxidative ATP turnover (Özyener et al., 2003). From an homeostatic view, the VO<sub>2</sub> kinetics analysis is particularly insightful in pathological conditions where exercise tolerance may be compromised by limited aerobic energy turnover due to impaired O<sub>2</sub> transport and/ utilization with consequent higher internal milieu disturbance (conditions such as aging and cardio-respiratory diseases are associated with slow VO<sub>2</sub> kinetics response) (for a review, see Xu and Rhodes, 1999). On the other hand, health adults may present wide inter-individual differences in VO<sub>2</sub> kinetics speed (expressed as time constant or mean response time - MRT), but apparently non-related to submaximal exercise tolerance. Rather than focusing on the consequences of either quite slow or fast VO<sub>2</sub> kinetics values on exercise tolerance, VO2 kinetics analysis in several pathological and physiological conditions altering O<sub>2</sub> 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 O<sub>2</sub> 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-liming step (O<sub>2</sub> delivery vs. O<sub>2</sub> utilization) have been addressed (Grassi, 2001; Hughson et al., 2001). Currently, it is reasonable to believe that the rate-limiting step of VO<sub>2</sub> kinetics depends on exercise intensity.

The leading findings suggest that the VO<sub>2</sub> kinetics of moderate-intensity exercise (below lactate threshold) is limited peripherally by O<sub>2</sub> utilization and that at higher exercise intensities, the O2 delivery begins to exert limitation on VO<sub>2</sub> 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 VO<sub>2</sub> kinetics across exercise intensities. However, its impact on VO<sub>2</sub> 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 VO<sub>2</sub> 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 VO<sub>2</sub> kinetics response, which seem worth in providing details for future mechanistic studies. These studies have described that the VO<sub>2</sub> kinetics is improved as the aerobic fitness level increases (i.e. moving from sedentary/active to trained aerobic status) up to a point where  $VO_2$  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 VO<sub>2</sub> 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.  $VO_2max > 65 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ ). In this scenario it is still unknown if aerobic fitness level typical of elite athletes are associated with even faster VO<sub>2</sub> kinetics than that presented by trained individuals. Based on previous works (Carter et al., 2000; Phillips et al., 1995), we hypothesize

that VO<sub>2</sub> kinetics stop improving as aerobic fitness level still does so. Along in this issue, the exercise intensity at which VO<sub>2</sub> kinetics is analyzed may show different influences of aerobic fitness level on VO<sub>2</sub> kinetics, since the intensity specific rate-limiting step of VO<sub>2</sub> kinetics could undergo differential adaptation to endurance training (Krustrup et al., 2004).

The aim of the present study is to address the question if the  $VO_2$  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 (VO<sub>2</sub>max) 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 (VO<sub>2</sub>max) and the intensity associated with the achievement of VO<sub>2</sub>max (iVO<sub>2</sub>max); 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 VO<sub>2</sub>max to determine the time constant of oxygen uptake response (MRT - mean response time). Thereafter, those individuals matching the VO<sub>2</sub>max range values of one of the three aerobic fitness level groups (LF – below 40 ml·kg·min<sup>-1</sup>; IF – between 43 and 60 ml·kg·min<sup>-1</sup>; HF – above 63 ml·kg·min<sup>-1</sup>) were selected. All tests were performed at the same time of day in a climate-controlled (21-22°C) laboratory. Subjects per-

#### 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, gasexchange variables were measured breath-by-breath using a portable gas analyzer system (Cosmed K4b<sup>2</sup>, Rome, Italy). These analyzers have previously been validated over a wide range of exercise intensities (McLaughlin et al, 2001). Before each test, the O<sub>2</sub> and CO<sub>2</sub> analysis systems were calibrated using ambient air and a gas of known O<sub>2</sub> and CO<sub>2</sub> concentration according to the manufacturer's instructions, while the K4b<sup>2</sup> turbine flowmeter was calibrated using a 3-1 syringe (Cosmed K4b<sup>2</sup>, 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).

Subjects performed incremental exercise to volitional exhaustion to determine VO<sub>2</sub>max and iVO<sub>2</sub>max 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-bybreath VO<sub>2</sub> 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 VO<sub>2</sub>max was defined as the highest 15 s VO<sub>2</sub> value reached during the incremental test. All subjects fulfilled at least two of the following three criteria for  $VO_2max$ : 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 iVO<sub>2</sub>max was defined as the minimal power output at which VO<sub>2</sub>max occurred (Billat and Koralsztein, 1996). The subjects subsequently performed a constant work rate test (at 50% VO<sub>2</sub>max – Mod exercise or at 100% VO<sub>2</sub>max - Max exercise) to determine  $VO_2$  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 pedal cadence of 70 rpm was reached, at that point, the pre-selected work rate was imposed and timing began. Oxygen consumption data from constant work-rate tests were filtered for aberrant values (values not included in the interval of average  $\pm 4$  SD were excluded).

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

	N		Body Mass (Kg)		Heigh	t (m)	Age (Years)	
Groups	Mod	Max	Mod	Max	Mod	Max	Mod	Max
LF	9	10	82.0 (11.1)	74.0 (15.5)	1.78 (.07)	1.74 (.05)	21.9 (4.6)	27.7 (4.2)
IF	8	9	76.9 (7.4)	67.7 (8.3)	1.78 (.05)	1.71 (.07)	21.1 (3.5)	23.6 (3.2) <sup>a*</sup>
HF	9	10	65.3 (6.9) <sup>a</sup>	64.3 (2.7)	1.75 (.05)	1.74 (.06)	21.8 (2.3)	24.3 (4.9)

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. Freah values (± 5D) of the indexes of actoble intress level and v O <sub>2</sub> kinetics time constant.												
	VO <sub>2max</sub> (ml·Kg <sup>-1</sup> ·min <sup>-1</sup> )		iVO <sub>2max</sub> (W·Kg <sup>-1</sup> )		iVO <sub>2max</sub> (W)		MRT (s)					
Groups	Mod	Max	Mod	Max	Mod	Max	Mod	Max				
LF	36.2 (2.9)	35.8 (3.5)	2.3 (.2)	2.6 (.3)	191 (26)	194 (44)	35.7 (7.9)	57.8 (17.8)				
IF	51.3 (4.8) <sup>a</sup>	50.9 (4.6) <sup>a</sup>	3.7 (.8) <sup>a</sup>	3.9 (.6) <sup>a</sup>	284 (57) <sup>a</sup>	266 (51) <sup>a</sup>	25.0 (3.1) <sup>a</sup>	42.6 (10.4) <sup>a</sup>				
HF	67.4 (3.8) <sup>ab</sup>	68.7 (4.1) <sup>ab</sup>	5.4 (.5) <sup>ab</sup>	5.3 (.4) <sup>ab</sup>	356 (40) <sup>ab</sup>	342 (28) <sup>ab</sup>	27.5 (5.5) <sup>a</sup>	32.6 (8.3) <sup>a</sup>				

Table 2. Mean values (± SD) of the indexes of aerobic fitness level and VO<sub>2</sub> kinetics time constant.

Mod, moderate exercise. Max, maximal exercise. LF, low aerobic fitness. IF, intermediate aerobic fitness. HF, high aerobic fitness. VO<sub>2max</sub>, maximal pulmonary oxygen uptake; IVO<sub>2max</sub>, work-rate associated to VO<sub>2max</sub>. MRT, mean response time of oxygen uptake kinetics. <sup>a</sup> significantly different from LF at the same intensity. <sup>b</sup> significantly different from IF at the same intensity.

#### VO<sub>2</sub> kinetics curve fitting

The VO<sub>2</sub> kinetics during constant work-rate was modeled with a mono-exponential function by an iterative nonlinear regression process from the Microcal Origin 6.0 (Northampton, MA, USA) using the following equation (Lamarra et al., 1987):

$$VO_{2}(t) = VO_{2h} + A \times (1 - e^{-(t/\tau)})$$

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  $\pm$  SD. The effects of aerobic fitness on VO<sub>2</sub> 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 VO<sub>2</sub> kinetics and VO<sub>2</sub>max. Statistical significance was set at  $p \le 0.05$ .

# Results

Table 2 contains the indexes of aerobic fitness level obtained from progressive intensity exercise (VO<sub>2</sub>max, iVO<sub>2</sub>max relative to body mass and absolute iVO<sub>2</sub>max) and the mean response time (MRT) that describes VO<sub>2</sub> kinetics during Mod and Max exercises. All indexes of aerobic fitness were significantly different ( $p \le 0.05$ ) 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 VO<sub>2</sub>max. Although these coefficients were statistically significant, VO<sub>2</sub>max explains little of MRT variability at both exercise intensities (Mod, R<sup>2</sup> = 26%; Max, R<sup>2</sup> = 40%).

Figure 3 and 4 show representative VO<sub>2</sub> data and its curve fitting of each fitness levels, respectively, at moderate and maximal exercise. The individual MRT and VO<sub>2</sub>max 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  $VO_2$  kinetics of subjects in the high-end aerobic power range ( $VO_2$ max). The faster MRT along with the increase in aerobic fitness



Figure 1. Coefficient of correlation between maximal oxygen uptake (VO<sub>2</sub>max) and mean response time (MRT) of moderate exercise.



Figure 2. Coefficient of correlation between maximal oxygen uptake (VO<sub>2</sub>max) and mean response time (MRT) of maximal exercise.

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<sub>2</sub>max near of above 65 ml·kg·min<sup>-1</sup>) is not able to further improve VO<sub>2</sub> 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<sub>2</sub>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<sub>2</sub>max and iVO<sub>2</sub>max) (Table 2) seem to match the level typical of elite athletes (Caputo and Denadai, 2004; Jeukendrup et al., 2000).

From a time course stand-point, improvements in sub-maximal VO<sub>2</sub> 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<sub>2</sub>max and lactate threshold (Phillips et al., 1995). Although this time course response for maximal exercise VO<sub>2</sub> 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<sub>2</sub> kinetics, where aerobic fitness level and exercise intensity seem to play a role in determining VO<sub>2</sub> kinetics changes in response to exercise training.

Within the limitation of our cross-sectional experimental design, the VO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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 nonoxidative 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<sub>2</sub> kinetics improved by exercise training. Along with this result, middle age subjects also present similar improvements in VO<sub>2</sub> kinetics early after training program onset (Fukuoka et al., 2002). Interestingly, Carter et al. (2000) studying active college students (VO<sub>2</sub>max ~55 ml·kg·min<sup>-1</sup>) did not find any improvement in moderate exercise VO<sub>2</sub> kinetics after 6 weeks of exercise training, although other indexes of aerobic fitness were improved (lactate threshold and VO<sub>2</sub>max). It seems that these findings rise two questions: Does VO<sub>2</sub> kinetics only improve initially while aerobic fitness is rather low? And, will longer and severe training regime further improve VO<sub>2</sub> 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



Figure 3. Representative VO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> kinetics can be.

Some studies have reported significant relationship between VO<sub>2</sub>max and VO<sub>2</sub> kinetics speed (Powers et al., 1985; Gurd et al., 2005). In spite of significant correlation coefficients between  $VO_2max$  and MRT at both exercise intensities when all the groups are joined together (Figure 1 and 2), their R<sup>2</sup> are low and the difference in  $VO_2max$ 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.



Figure 4. Representative VO<sub>2</sub> data and the curve fitting of each group at maximal exercise. From up to bottom, respectively low, intermediate and high fitness subjects.

# 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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# **Key points**

- Currently, it is reasonable to believe that the ratelimiting step of VO<sub>2</sub> 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 VO<sub>2</sub> kinetics across exercise intensities.
- However, exercise adaptation leading individuals to the high-end of aerobic fitness level range (VO<sub>2</sub>max > 65 ml.kg.min<sup>-1</sup>) is not able to further improve VO<sub>2</sub> kinetics during both, moderate and maximal intensity exercise.

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