

# Age-related slow fundamental recovery $\dot{V}O_2$ kinetics during submaximal exercise

## Abstract

We studied, the fundamental ( $\Phi_2$ ) pulmonary oxygen uptake recovery ([off]- $\dot{V}O_2$  response) to submaximal exercise in terms of  $\dot{V}O_2$  kinetics ( $\tau^2$ , the time constant two). We assessed healthy male volunteers eight young (YG=8) and nine old (OG=9) if [off]  $\dot{V}O_2$  response to submaximal exercise show [off]-asymmetry in the  $\dot{V}O_2$  kinetics ( $\tau_{YG}^2 < \tau_{OG}^2$ ) from the YG vs OG characterised from the best exponential fitting models. Subjects (YG=25.2±2.9; OG= 71.0±4.3) completed an initial incremental ramp test (YG=25 W Amin-1, OG=15 W Amin-1) to volitional fatigue from which the ventilatory threshold ( $\dot{V}eT$ ) and work rates corresponding to 80% $\dot{V}eT$  (ModRel) and 120% $\dot{V}eT$  (HvyRel) were identified, and an “absolute” work rate of 50W (AbsMod) (submaximal exercise) was selected. The [off] step-transitions in work rate were initiated from a baseline without warning to the subject. Each [off]-transition lasted 6 min (entire [off]- $\dot{V}O_2$ ) and 4 to 6 transitions were performed at each sub maximal exercise intensity. The  $\dot{V}O_2$  [off]-response was measured breath-by-breath at one min baseline and throughout each transition. Data were interpolated to 1-s intervals and ensemble-averaged to yield a single response profile for each subject and intensity were filtered. The averaged response for each subject was fitted with a two- (2C for Mod), and three-component (3C for Hvy) exponential model by using entire [off]- $\dot{V}O_2$  fitting windows. Parameter estimates (i.e.,  $\tau_2$ ) were determined for each component. Our best statistically and/or physiologically fitting model showed significant slow  $\tau_2$  age-related ( $\tau_{OG} - \tau_{YG} = 19.8s$ ,  $t=7.3$ ,  $P<0.001$ ) independent of the level of exercise intensity. There was an age-related slow fundamental recovery exercise  $\dot{V}O_2$  kinetics (long  $\tau_2$ ) during submaximal exercise in old adult men.

**Keywords:**  $\dot{V}O_2$  uptake kinetics, [off]-phase two  $\dot{V}O_2$ , time constant, young and old men.

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## Introduction

Physical exercise requires the interaction of physiological control mechanisms to enable the blood-cardiovascular and respiratory systems to couple their functions to support the increased energy metabolism in terms of oxygen ( $O_2$ ) consumption ( $\dot{V}O_{2M}$ ) and carbon dioxide production ( $\dot{V}CO_{2M}$ ) of the contracting muscles.<sup>1</sup> The exercise recovery pulmonary  $\dot{V}O_2$  uptake ([off]- $\dot{V}O_2$ ) and their kinetics ([off]- $\dot{V}O_2$  kinetics) are currently characterized by empirical mathematical models that are a weighted sum of an offset and delayed exponentials.<sup>2-4</sup> Because of the different mechanisms in ATP regeneration have different effects on gas exchange<sup>5</sup> study of the pulmonary gas exchange ( $\dot{V}CO_2/\dot{V}O_2$ ) responses to exercise can reveal information regarding the kinetics of the relative contributions of aerobic respiration, phosphocreatine hydrolysis, and anaerobic glycolysis to the total bioenergetic response, it is specially important to study the  $\dot{V}O_2$  kinetics that reflects the skeletal muscle  $\dot{V}O_{2M}$  during physical exercise<sup>6-8</sup> ( $\dot{V}O_2$ , transient phase two  $\dot{V}O_2$ ). Moreover, during recovery from moderate and heavy exercise the estimated muscle capillary blood flow kinetics have been observed biphasic.<sup>6,7</sup> Exercise tests in which gas exchange is determined realistically evaluate the ability of these systems to promote their common major function, which is support of cellular respiration and allows the investigator to search for mechanisms to distinguish between a young adult and old human response characteristic of the ageing processes, grade the adequacy of the coupling mechanisms, and assess the effect of therapy on a diseased organ system.<sup>8</sup> The moderate-to-heavy aerobic exercise off-transient  $O_2$  response, shows an initial

rapid decline, similar to recovery from light exercise, named the off-transient phase one  $\dot{V}O_2$  response ([off]-  $\dot{V}O_2$ ) followed by a more gradual decline to baseline resting levels, the [off]-transient phase two  $\dot{V}O_2$  response ([off]-  $\dot{V}O_2$ ).<sup>1,2</sup> Evermore, the recovery kinetics may be able to reflect the exercise capacity of people and provide the prognostic information about mortality for particular disease group.<sup>9</sup>

The exercise transient ((on)  $\dot{V}O_2$  kinetics in terms of its time constant  $\tau$  ((on)-  $\dot{V}O_2\tau$ ) are slowed with aging (long  $\tau_2$  numeric value)<sup>10</sup> but whether the slowed (on)-  $\dot{V}O_2$  kinetics observed in older adults during whole body large muscle mass exercise, such as cycling is due to an inability to increase muscle blood flow and  $O_2$  delivery and/or a reduced capacity to utilize  $O_2$ , has not been clearly established.<sup>1,11</sup> Nevertheless, information on the  $\dot{V}O_2$  kinetics during end recovery submaximal exercise ([off]-transient  $\dot{V}O_2\tau$ ) in older adults was limited.<sup>10</sup> Thus, the two components (2C, 7P<sub>parameters</sub>) and the three component (3C, 10P<sub>parameters</sub>) exponential mathematical models<sup>11,12</sup> for moderate intensity exercise and heavy intensity exercise respectively were constructed. The 2C, 7P and the 3C, 10P models have been statistically and/or physiologically assessing were used to get the best fit data from the end recovery submaximal exercise [off]-transient  $\dot{V}O_2\tau$ .<sup>11,12</sup> Justification In the absence of a modeling evaluation and its kinetics of aging, of response of oxygen uptake of submaximal exercise, it was decided to perform this study using mathematical modeling and its kinetics to offer a new approach that could contribute to obtain a deeper insight into the mechanisms of recovery related to age. This can be useful in future research to compare kinetically between different conditions

such as growth, development and disease status and rehabilitation.<sup>13</sup> Thus, the purpose of this research was to compare the [off]-transient  $\Phi_2\text{VO}_2\tau$  of young men and older adults using the best exponential mathematical models of statistical and/or physiological adjustment such as the two component model (2C,7P) and three component model (3C,10P) for moderate intensity exercise and heavy intensity exercise, respectively.<sup>12,13</sup>

## Hypothesis

If aging affects the fundamental kinetics of submaximal exercise recovery then the duration of the transient recovery time [off]-transient  $\Phi_2\text{VO}_2\tau$  of submaximal exercise should be longer (slow  $\tau_2$ ) in older men compared to men young adults. If the hypothesis is corroborated, this would not affect the clinical method used by a physician and its collaborators, but it would have a kinetic approach, among other approaches, that reflects in a more objective way the status of the patients themselves diagnosing the degree of affect of their homeostasis and also allow the feedback of the physiotherapeutic methods of their rehabilitation. Of course there is also the possibility of going deeper into the causality of the kinetics of oxygen consumption in the promotion of health and physical activity and aging.

## Material and methods

### Subjects

The subjects in this study were eight healthy men with an average (mean±SD) age of 25.2±2.9 years (YG) and nine healthy elderly (OG) with an average age of 71.0±4.3 years. The data was obtained from the studies carried out under control conditions in our laboratory for several years. The subjects performed seated in a cycle ergometer exercise of both moderate intensity and intense intensity.

### Ethical approval

The Review Board for Research with Human Subjects provided ethical approval and each subject gave their informed consent.

### Testing procedures

The determination of the maximum pulmonary uptake of oxygen (VO<sub>2</sub>max) and the

O<sub>2</sub> of the ventilatory threshold (VeT) was carried out on a cycle ergometer with electric brake (Lode H-300-R Roxon Medi-Tech). The test was performed as a ramp function at a work rate that increased at a speed of 25W@min<sup>-1</sup> for the YG or 15 W@min<sup>-1</sup> for OG. The VeT was determined by visual inspection of data using the criteria outlined by Davis et al. (1979)<sup>14</sup> of a systematic increase in V<sub>E</sub>/VO<sub>2</sub> and in the end-tidal O<sub>2</sub> tension (PETO<sub>2</sub>) with no concomitant rise in V<sub>E</sub>/VCO<sub>2</sub> or a decrease in PETCO<sub>2</sub>. Constant load exercise tests were performed on subsequent laboratory visits. The exercise started with 6 minutes of cycling at no load (~15 W). The working speed was then increased as a stepped function to an intensity corresponding to a VO<sub>2</sub> of approximately 80% of VO<sub>2</sub> of VeT (ModRel, exercise of relative moderate relative intensity) or VO<sub>2</sub> of approximately 120% of VeT (HvyRel, exercise of intense relative intensity).<sup>13</sup> An “absolute” work rate of 50W (AbsMod, exercise of absolute moderate intensity) corresponding to approximately 62% of VO<sub>2</sub> of VeT was also selected. The subjects were exercised at the appropriate working speed for 6 min (transient response of VO<sub>2</sub>), after which and without prior notice to the subjects, the work speed was abruptly reduced and the subjects continued cycling without load for 6 min ([off]-transient response of VO<sub>2</sub>).<sup>13</sup>

## Data collection and analysis

Gas exchange was determined using previously reported methods.<sup>12,13</sup> Throughout the exercise, inspired and expired gas volumes were measured using a bidirectional turbine (VMM110, Alpha technologies) previously calibrated. Respirated gases were sampled continuously (1 mlAs<sup>-1</sup>) at the mouth and analysed for concentrations of O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> by mass spectrometry (MGR 9N, Airspec 2000) after calibration with precision-analysed gas mixtures. Changes in gas concentration were aligned with gas volumes by measuring the time delay for a bolus of gas to pass the turbine to the resulting changes in fractional gas concentrations as measured by the mass spectrometer. Breath-by-breath alveolar gas exchange was calculated using previously described algorithms<sup>15</sup> and data were interpolated to 1 s intervals and to improve the signal-noise ratio each subject performed a number of repetitions of the exercise protocol (constant-load exercise tests): 6-8 for Mod (2-4 transitions A visit<sup>-1</sup>) and 2-3 for HvyRel (one transition A visit<sup>-1</sup>). The interpolated data were then averaged for each individual to yield a single overlaid response from 50W, 80%VeT and 120%VeT data that was used for determining the kinetics of the VO<sub>2</sub> [off]-transient responses to submaximal exercise.

## Models

The data for VO<sub>2</sub> [off]-transients were constructed with our previously assessed best fitting models. For moderate-intensity exercise, an exponential model with the twocomponent and seven Parameters<sup>12,13</sup> were fitted to the data. For heavy-intensity exercise exponential model with threecomponent and 10 Parameters<sup>11,13</sup> were fitted to the data. The double empirical mathematical model of 2C is adjusted to a transient temporal course of the transient response curve [off]-O<sub>2</sub> from a resting baseline (A<sub>0</sub>) to a steady state, with consecutive transitory periods and exponentials of time with 7 parameters; 7P={A<sub>0</sub>, A<sub>1</sub>, δ1, τ1; A<sub>2</sub>, δ2, τ2}, expressed as 2C,7P.<sup>12,13</sup>

$$[\text{Off}]-\text{VO}_2(t)=A_0-A_1 \cdot (1-\exp(-(t-\delta_1)/\tau_1))-A_2 \cdot (1-\exp(-(t-\delta_2)/\tau_2));$$

also, the 3C empirical triple exponential mathematical model was performed, it fits a transient temporal course of the transient response curve [off] - VO<sub>2</sub> from a resting baseline (A<sub>0</sub>) to a steady state, with consecutive periods transient and exponential time with 10 parameters; 0P= {A<sub>0</sub>,A<sub>1</sub>,δ1,τ1; A<sub>2</sub>,δ2, τ2; A<sub>3</sub>,δ3,τ3}, expressed as 3C,10P.<sup>12,13</sup>

$$[\text{Off}]-\text{VO}_2(t)=A_0-A_1 \cdot (1-\exp(-(t-\delta_1)/\tau_1))-A_2 \cdot (1-\exp(-(t-\delta_2)/\tau_2))-A_3 \cdot (1-\exp(-(t-\delta_3)/\tau_3)),$$

where [Off] -VO<sub>2</sub> (t) is the rate of change of recovery VO<sub>2</sub> per unit of time (d [Off]-VO<sub>2</sub> Vdt<sup>-1</sup>) assuming δ=0; A: is the distance value [Off]-VO<sub>2</sub> (ml·min<sup>-1</sup>) from A<sub>0</sub> to [Off]-VO<sub>2</sub> required, or the difference between the baseline and the response [Off]-VO<sub>2</sub> final for the amplitudes of phase one (A<sub>1</sub>), phase two (A<sub>2</sub>) and phase three (A<sub>3</sub>) in ml. For example, A is the difference between the baseline A<sub>0</sub> ([Off] - VO<sub>2</sub>) of A<sub>0</sub> and the value of [Off] - VO<sub>2</sub> final of the entire response to the submaximal exercise. A<sub>0</sub> is the resting basal amplitude (units dependent on the variable analyzed); an A with an integer subscript > 0 is the gain of the response. A<sub>1</sub> is the gain in the model of a component or in phase one. A<sub>2</sub> is the gain in the two-component model or in phase two. A<sub>3</sub> is the gain in the three-component model or in phase three. 1-e (t/τ): is the negative exponential distribution.<sup>19,20</sup> e(t/τ) is the disappearance factor with the time constant τ. t is the time in which the transient response [Off]-VO<sub>2</sub> induced decays exponentially; when t=τ, which means the time required for the transient response of

[Off]-  $\text{VO}_2$  induced to decay away to part e-1 (0.3678) of its original value, and therefore,  $\tau=1-0.3678=0.63$  and  $e=2.718281=[(1+n-1)]$ , where  $n \geq 10$  and 'e' is proportional to 1.  $\tau$  is the kinetic parameter of time (time constant); is the time required to reach 63% of the final amplitude of the value of [Off] -  $\text{VO}_2$  or to approximate 37% of the value of [Off] -  $\text{VO}_2$  final of an exponential response from  $A_0$  to an asymptotic value.  $\tau$  is the time constant in seconds where  $\tau_1$  is the response time constant in the model of a component or in phase one,  $\tau_2$  is constant of response time in the two-component model or in phase two and  $\tau_3$  is the response time constant in the three-component model or in the three-phase.  $\delta$  is the time delay in seconds, related in each of the phases where  $\delta\delta_1$  is the delay of response time in a model of a component or in phase one,  $\delta_2$  is the delay of response time in the model of two components or in phase two and  $\delta_3$  is the response time delay in the three-component model or in phase three.

Data were modelled using these multi-component models mentioned above using non-linear least squares regression techniques,<sup>16</sup> and the best fit defined by the minimisation of the residual sum of squares. We used initial estimates of phases' (from one up to three) time delay:  $\Phi_1\text{VO}_2\delta$ , 0s;  $\Phi_2\text{VO}_2\delta$ , 20s;  $\Phi_3\text{VO}_2\delta_3$ , 180s; and from one up to three time constant:  $\Phi_1\text{VO}_2\tau$ , 5s;  $\Phi_2\text{VO}_2\tau$ , 30s;  $\Phi_3\text{VO}_2\tau_3$ , 180s. Usually, 100 iterations were run and the parameter estimates examined to allow further iterations with the estimates obtained. The models were run with  $\Phi_2\tau$  underestimated (e.g. 15s) or overestimated (e.g. 70s) to assure that the minimised residuals were not due to a localised minimised least squares residuals.<sup>17</sup> The 2C,7P model for the submaximal exercise (Mod and Hvy) [off]-transient  $\text{VO}_2$  fitted from one min baseline (BL1min)-end exercise to end recovery exercise (ERE) with two exponential equations differentiating  $\Phi_1$  and  $\Phi_2$  ( $2C,7P_{BL1\text{ to ERE}}$ ). The 3C,10P model for the heavy exercise (Hvy) [off]-transient  $\text{VO}_2$  fitted from one min baseline-end exercise to end recovery exercise with three exponential equations differentiating  $\Phi_1$ ,  $\Phi_2$  and  $\Phi_3$  ( $3C,10P_{BL1\text{ to ERE}}$ ).<sup>12,13</sup> The goodness of fit for each fitting model was assessed using the lowest residual sum of squares (RSS values) from a computerized nonlinear regression technique.<sup>16</sup>

$$F = \frac{(SS1 - SS2) / (df1 - df2)}{(SS2 / df2)}$$

where  $SS$  is the residual sum of squares of each fit,  $df$  is the number of degrees of freedom, the suffixes 1 and 2 refer to the models being compared where suffix 1 refers to the model with the fewest parameters. The RSS values were used for models that fit the same number of experimental data points.

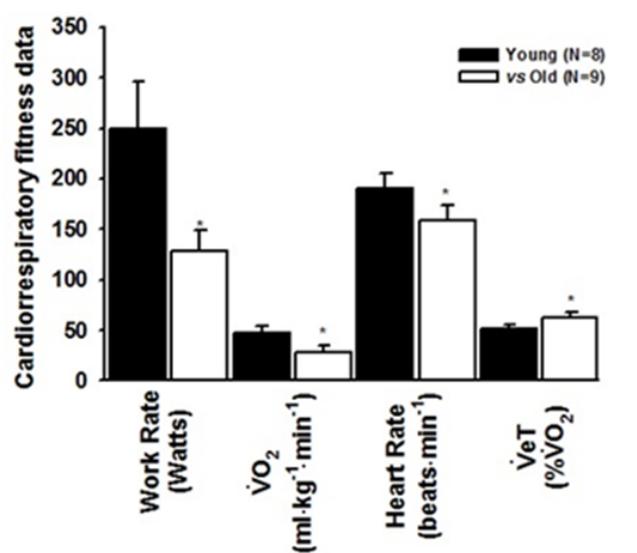
Amplitudes both from  $\Phi_2$  (the fundamental  $A_2$ ) and from  $\Phi_3$  ( $A_3$ ) were also expressed in terms of functional gain ( $G = \Delta\text{VO}_2 / \Delta\text{WorkRate}$ ) from models 2C,7P<sub>BL1 to ERE</sub> ( $G_{A2}$ ) and 3C,10PP<sub>BL1 to ERE</sub> ( $G_{A2}$  and  $G_{A3}$ ).<sup>18</sup> The kinetic analyses of  $\text{VO}_2$  transient response recovery from the submaximal exercise ([off]) was assessed in terms of the [off]-  $\text{VO}_2$  kinetics ( $\tau_2$ ).<sup>13</sup>

**Statistical analysis:** Estimated values of the  $\Phi_2\text{VO}_2$  (i.e.,  $\tau_2, \delta_2, A_2$ ) and from the different models used were compared, young versus old data group, using two-way analysis of variance all pair wise multiple comparison procedures (Holm-Sidak method) with repeated measures.<sup>19</sup> The Student's t-test was used to determine if the mean values of the two groups were significantly different.<sup>19</sup> The probability level of 0.05 was chosen as the criterion for acceptance of statistical significance.

## Results

### General physical characteristics, except age, were similar between YG and OG but there were age-related low cardiorespiratory fitness and age-related high ventilatory threshold

Old subjects compared young subjects were not significantly different in physical characteristics. However, in age (years) YG resulted ( $25.16 \pm 2.95$ ) low compared OG ( $71.02 \pm 4.73$ ) ( $t=24$ ,  $P \leq 0.001$ ), in height (cm) YG was ( $179.6 \pm 5.7$ ) was not significantly different to OG ( $174.1 \pm 5.5$ ), in total body mass (kg) YG was ( $79.2 \pm 9.3$ ) was not significantly different to OG ( $79.9 \pm 9.9$ ) and in body mass index ( $\text{kg} \cdot \text{m}^{-2}$ ) YG was ( $24.5 \pm 2.3$ ) was not significantly different to OG ( $26.4 \pm 3$ ). OG resulted significantly low in cardio-respiratory fitness compared the YG (except  $\dot{V}E_T$  that was conversely) (Figure 1).



**Figure 1** Groups' maximal cardiorespiratory fitness data from a ramp test.

\*Student t-test significant differences ( $P < 0.05$ ) between young and old subjects for work rate,  $t = 7.2$ ; pulmonary oxygen uptake ( $\text{VO}_2$ ),  $t = 6.0$ ; heart rate,  $t = 4.6$  and; ventilatory threshold ( $\text{VeT}$ ),  $t = 3.4$ .

### Age-related low submaximal work rate intensity exercise

The subjects [off]-transient pulmonary oxygen uptake response profiles to AbsMod (recovery from 50W); ModRel (80%  $\dot{V}E_T$ ): recovery from  $84.2 \pm 14$  W (YG) and recovery from  $36.6 \pm 11.3$  W (OG) and; HvyRel (120%  $\dot{V}E_T$ ) recovery from  $160.3 \pm 24$  W (YG) and recovery from  $90.0 \pm 16.5$  W (OG) square wave exercise is shown in Figure 2. As expected, analyses showed significant ( $P < 0.001$ ) aged-low ModRel ( $Y_{G_{\text{mean}}} - O_{G_{\text{mean}}} = 47.6$ W,  $t = 7$ ) and HvyRel ( $Y_{G_{\text{mean}}} - O_{G_{\text{mean}}} = 70.3$ W,  $t = 10.3$ ) work rate intensity.

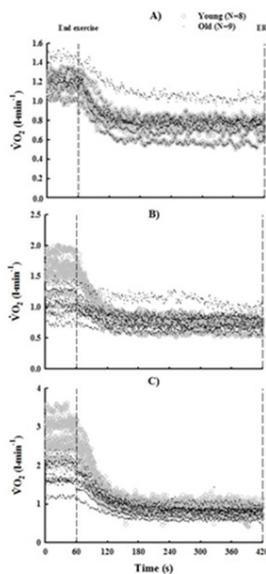
### [Off]-estimated temporal parameters baseline 1min ( $A_0$ , $\text{ml} \cdot \text{min}^{-1}$ )

Analyses showed that  $A_0$  for groups times intensity exercise AbsMod (YG= $1188.8 \pm 33.6$ , OG= $1180.0 \pm 50.4$ ) ModRel (YG= $1630.0 \pm 81.5$ , OG= $1049.0 \pm 65.5$ ) and HvyRel (YG= $2783.1 \pm 162.1$ ,

OG=1773.0±109.3) resulted in a statistically significant interaction for relative work rate exercise only; in other words, ModRel  $A_0$  in the OG resulted in 581  $\text{mlAmin}^{-1}$  low compared YG ( $t=4$ ,  $P<0.001$ ), and HvyRel  $A_0$  in the OG resulted in 1010  $\text{mlAmin}^{-1}$  low compared YG ( $t=8$ ,  $P<0.001$ ). The end-exercise  $\text{VO}_2$  ( $\text{ml}\cdot\text{min}^{-1}$ ) in YG (1182±87) resulted similar compared OG (1180±145) ( $t=3$ ,  $P\leq 0.05$ ).

**Age-related low fundamental gain ( $G_{A_2}$ ,  $\text{ml}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ ), age-related slow time delayed ( $\delta_2$ , s) and age-related slow time constant ( $\tau_2$ , s) did not depend on the level of exercise intensity**

Analyses showed between groups significant ( $t=5$ ,  $P<0.001$ ) low  $G_{A_2}$  age-related (YG-OG=2.1). Analysis showed significant ( $t=4.5$ ,  $P<0.001$ ) slow  $\delta_2$  age-related (OG-YG=7) and, significant slow  $\tau_2$  age-related (OG-YG=19.8,  $t=7.3$ ,  $P<0.001$ ) (Figure 3) but all of them were not dependent on the level of exercise intensity. In other words, gain, time delayed, and time constant from phase two [off] resulted numerically similar between ModRel and HvyRel exercise intensity but the mentioned parameters were significant low in the OG compared YG.

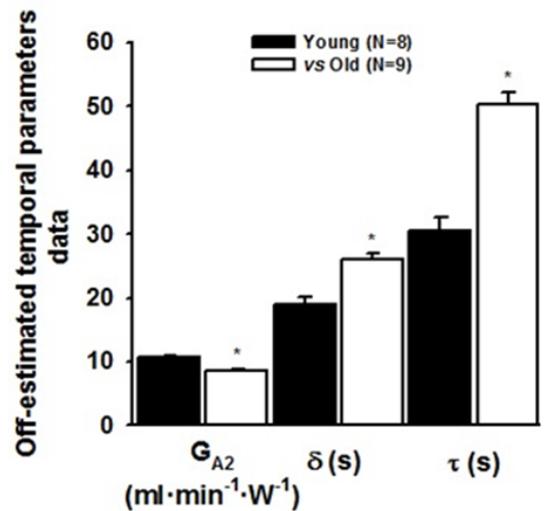


**Figure 2** [Off]-transient pulmonary oxygen uptake ( $\dot{V}\text{O}_2$ ) response profiles of young and old groups to

(A) absolute moderate (50W).

(B) Relative moderate (80% $\dot{V}\text{eT}$ ).

(C) Relative heavy (120% $\dot{V}\text{eT}$ ) square wave exercise. The time course (min) corresponded to each intensity exercise were consisted in 6 min loadless pedalling followed by 6 min work rate and finally, 6 min loadless pedalling for absolute-, relative moderate-, and relative heavy- square wave exercise. End-exercise (offset). Data points (symbols) were the breath-by-breath interpolated to second-by-second pulmonary  $\text{VO}_2$  (experimental data) from one min baseline (quasi 120% $\dot{V}\text{eT}$  baseline) submaximal exercise to the entire off-transient response (six min, from offset to the end recovery exercise (ERE)). The eight young and nine old subjects submaximal exercise at each intensity ( $N=8$ ,  $N=9$ ) were displayed.  $\dot{V}\text{eT}$ , Ventilatory threshold.



**Figure 3** Groups [off]-estimated fundamental temporal parameters data from submaximal exercise modelled with twocomponent (phase two) and threecomponent exponential (phase two) mathematical models.  $G_{A_2}$  refers to the decrease in oxygen uptake during phase two in response to a simultaneous decrease in work rate. Phase 2 referred to the period following the offset of exercise when the mixed venous blood gas concentrations decrease to change because of changes in the effluent from the exercising muscles. Phase two [off] reflects the "kinetic phase" of the gas exchange that begins at the end of phase one [off] and continues until a recovery steady state is obtained. [Off], refers to end recovery exercise transient response;  $\delta_2$ , refers to the latency when phase two [off] first become apparent.  $\tau_2$  refers to the time required for phase two [off] to reach its 63% of the response. \*.Significant differences ( $P<0.0001$ ) between young and old subjects for gain ( $G_{A_2}$ ), time delayed ( $\delta_2$ ) and time constant ( $\tau_2$ ).

## Discussion

This study sought to experimentally estimate the duration of phase [off]-transient  $\dot{V}\text{O}_2\tau$  using the best fitting exponential mathematical models, previously published<sup>12,13</sup> in old subjects compared young individuals in the study of oxygen uptake kinetics, looking for an insightful understanding of the age-related mechanisms regulating the rate at which oxidative phosphorylation adapts to loadless step changes, in exercise intensities and energy requirement by assessing  $\dot{V}\text{O}_2$  kinetics parameters from the end submaximal exercise recovery (50W, 80% $\dot{V}\text{eT}$ , 120% $\dot{V}\text{eT}$ )  $\text{VO}_2$  transient response.

## Physical characteristics and ramp exercise test

In spite of the different age between the OG and YG, we observed both lacks of significant differences in physical characteristics for height, total body mass, and body mass index and also a significantly high  $\dot{V}\text{eT}$  in the OG compared the YG. We explain these observations as indicators that the old subjects were in good physical fitness in terms of their general anthropometry and estimated ventilatory threshold, specially because the response to ramp test exercise is an essential component of the physiological evaluation of subjects across the entire spectrum of fitness and physical activity; from elite athletes to patients with a variety of disease states.<sup>8,20</sup>

## Submaximal exercise test

Since it has been observed aged low-on-transient  $\text{VO}_2$  response profiles to submaximal exercise previously<sup>9,10,21</sup> and aging is associated

with progressive declines in resting and energy expenditure and total energy expenditure<sup>22</sup> it was not a surprise, that the young and old subjects [off]-transient  $\text{VO}_2$  response profiles to ModRel recovery from 80% eT, and HvyRel recovery from 120% eT were significant aged-low work rate intensity,<sup>10,23</sup> resulting differences from both the ModRel OG-YG=-47.6W and the HvyRel OG-YG=-70.3W. However, this aged-low submaximal exercise was multifactorial in origin.<sup>1,9,21,22</sup> Evermore, for moderate exercise condition, the  $\text{O}_2$  deficit represented the energy equivalent to the depletion of high-energy phosphate (Creatine phosphate and ATP) and  $\text{O}_2$  stored in the body at the start of the exercise.<sup>7,23</sup> For heavy exercise condition, the  $\text{O}_2$  deficit included the energy equivalent of the anaerobic.<sup>7,23</sup> Therefore, the estimation of the  $\text{O}_2$  deficit during heavy exercise transitions could also be considered the slow component of  $\text{VO}_2$  as an additional deficit component with delayed start.<sup>23</sup> Nevertheless, we considered that it did not affect the differences in  $\text{O}_2$  deficit previously observed between YG and OG for heavy exercise condition.<sup>21</sup> The high  $\text{O}_2$  age-related deficit observed<sup>21</sup> for the moderate absolute- intensity exercise was mainly because ageing was associated with poor muscle function<sup>24</sup> that yielded slow  $\text{VO}_2$  kinetics and a large  $\text{O}_2$  deficit but the causes of lactate threshold production are a matter of debate.

### [Off]-estimated temporal parameters

The [off]-transient (post-exercise  $\text{VO}_2$  recovery) responses to the exercise tests for 50 W, absolute moderate exercise; relative moderate exercise and; for relative heavy exercise (submaximal exercise) constant [off]-loadless ([off]-transient) cycling were analysed with best statistically and/or physiologically exponential mathematical fitting models<sup>12,13</sup> that characterised the [off]-  $\Phi_2\text{VO}_2$  kinetics ([off]- $\tau\Phi_2\text{VO}_2$ ) for this submaximal exercise in young healthy adult and old healthy men. Nevertheless, the aged-low submaximal exercise observations are multifactorial in origin.<sup>1,24</sup>

### Baseline I min ( $\text{A}_0$ , ml min<sup>-1</sup>)

The  $\text{VO}_2\text{A}_0$  values from the YG and OG resulted similar to each other, probably because these AbsMod work were performed without a lactic acidosis by our subjects. In this condition, the  $\text{O}_2$  flow through the muscles is adequate to supply all of the  $\text{O}_2$  needed for the aerobic regeneration of ATP in the steady state, and the patterns of  $\text{VO}_2$  and  $\text{VCO}_2$  increase reaching the steady-state exercise baseline without lactic acidosis.<sup>25</sup> In contrast, our analyses showed that  $\text{VO}_2\text{A}_0$  values from young and old groups times intensity exercise (ModRel and HvyRel) resulted in a statistically significant interaction for relative work rate exercise only, and probably this is due to the fact that by applying two different relative exercise intensities, 80% eT and 120% eT, our subjects performed these tests at different energy level energy.<sup>14,25</sup> In consequence, the  $\text{VO}_2\text{A}_0$  values (ml·min<sup>-1</sup>) in the YG were 581 and 1010 high compared OG for ModRel and HvyRel intensity exercise, respectively.

### Age-related low [off]- $\Phi_2\text{VO}_2$ functional gain ( $\text{GA}_2$ , ml min<sup>-1</sup>·W<sup>-1</sup>)

Analyses showed between groups significant low [off]-  $\text{VO}_2\text{G}_{\text{A}_2}$  age-related; in the OG the [off]-  $\text{VO}_2\text{G}_{\text{A}_2}$  was 2.1 ml·min<sup>-1</sup>·W<sup>-1</sup> low compared YG and thus, the decrease in pulmonary oxygen uptake in response to a simultaneous decrease in work rate resulted diminished in the OG probably due to less efficiency for muscular work.<sup>8</sup>

### Age-related slow [off]- $\Phi_2\text{VO}_2$ time delay ( $\delta$ )

The  $\delta\Phi_2\text{VO}_2$  [off]- transient response in the OG was 6.95 s longer than that in the YG from submaximal exercise [off]-response. Slow [off]-transient  $\Phi_2\text{VO}_2$  time delay ( $\delta_2$ ) age-related, can be explained by an inertia of both the feedforward of ventilation and the time needed for down blood to flow from working muscles to lungs related with temporal physiological considerations modulating muscle efficiency<sup>26</sup> indicating that it is necessary to take account of this transit delay “from muscle to mouth” if pulmonary  $\text{OVVO}_2$  kinetics are to be used to estimate the end recovery exercise kinetics of muscle  $\text{VO}_2$  consumption also.<sup>26</sup>

### Age-related slow [off]- $\Phi_2\text{VO}_2$ kinetics ( $\tau_2$ )

Our finding of long fundamental [off]-time constant age-related from submaximal exercise, not dependent on the level of exercise intensity, is in partial agreement with the previous observation that during high-intensity leg exercise in humans where exercise mode had no discernible effect on the kinetics of  $\text{VO}_2$  in a subsequent recovery phase.<sup>27</sup> In this study the [off]-  $\Phi_2\text{VO}_2$  kinetics resulted in 19.8 s prolonged in the OG compared the YG and this observation in older adults, probably means that the [off]-  $\Phi_2\text{VO}_2$  kinetics may be limited by a slow adaptation of muscle blood flow and  $\text{O}_2$  delivery, due to the fact that in various studies have been observed increased total peripheral resistance,<sup>28</sup> reduced capillary density,<sup>29</sup> endothelial dysfunction,<sup>30</sup> sarcopenia<sup>21</sup> and altered capillary hemodynamics,<sup>31</sup> which suggest that the convective delivery of  $\text{O}_2$  to working muscle during exercise may be reduced in OG compared with YG, postulating that muscle  $\text{O}_2$  delivery may limit  $\text{VO}_2$  kinetics in older adults.<sup>1,32,33</sup> Therefore, potential differences in the physical properties of the muscle vascular system could account, at least in part, for the age-related slow  $\text{VO}_2$  [off] kinetics.<sup>6,7</sup> In brief, in this work we observed a significant low fundamental gain, long fundamental both time delayed and time constant age-related in a subsequent recovery phase that was not dependent (except the fundamental gain) on the level of exercise intensity.

### Conclusion

There was a slow kinetics ( $\tau_2$  of prolonged duration) related to age during  $\text{VO}_2$  in phase two of age-related recovery of submaximal exercise, markedly influenced by the dynamics of  $\text{VO}_2$  during submaximal muscle exercise in adult men.

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### Conflict of interest

Author declares that there is no conflict of interest.

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