AGE-RELATED PROLONGATION OF PHASE I OF \( \dot{V}O_2 \) ON-KINETICS IN HEALTHY HUMANS

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Abstract

Data are lacking regarding age-related modifications of phase I (PhI) of pulmonary \( \dot{V}O_2 \) on-kinetics during moderate-intensity exercise. We studied 3 groups (aged 20-30, 40-50, and 60-70 years) of 10 normal subjects, who underwent one incremental and 4 below-gas exchange threshold constant-power cardiopulmonary exercise tests. Data from constant-power tests were time-aligned and averaged, and the PhI-phase II transition (PhI-IItr) determined when a sharp decrease from baseline of respiratory exchange ratio occurred. The \( \dot{V}O_2 \) phase II time constant (\( \tau \)) was obtained by an exponential fitting starting i) from PhI-IItr (‘experimental’ fitting strategy) and ii) after 20 s from exercise onset (‘fixed-duration’ fitting strategy). Assuming estimated arterial-venous \( O_2 \) concentration difference not to change with respect to resting value, cardiac output (CO) values at rest and PhI-IItr were obtained according to Fick’s principle. Average pulmonary flow acceleration (AFA) during PhI was calculated as the ratio between CO increase during PhI and PhI duration.

PhI duration was related to age (\( r = 0.74, p < 0.0001 \)), increasing from 21±3 s to 27±3 s to 32±4 s in the 20-30, 40-50, and 60-70 age-groups, respectively, and to AFA (\( r = -0.60, p < 0.001 \)), but not to CO increase during PhI. With respect to the experimental fitting strategy, the fixed-duration strategy overestimated \( \dot{V}O_2 \) phase II \( \tau \) the more the higher the subject’s age, with a lower goodness of fit in the 60-70 group (SE 0.035 vs. 0.056, \( p < 0.01 \)).

In conclusion, PhI duration is related to age in healthy male humans, and linked to CO acceleration - rather than to increase - during PhI. A significant overestimation of phase II \( \tau \) thus may occur in healthy elderly subjects and patients with a pathologically-
induced longer PhI duration when fitting data where the PhI-PhIItr was not experimentally determined but assumed to be a set value (i.e. 20 s).

**Key words:** ageing, oxygen consumption, on-response, moderate-intensity exercise.
Introduction

Phase II of pulmonary oxygen uptake (\(\dot{\text{VO}_2}\)) on-kinetics during moderate-intensity constant-power exercise has been found to slow down with advancing age, presumably due to an impaired capacity of both the circulatory system to deliver \(\text{O}_2\) and skeletal muscle to utilize it (19,20,49). On the other hand, phase I of \(\dot{\text{VO}_2}\) on-kinetics, during which the \(\dot{\text{VO}_2}\) increase relies mostly on pulmonary blood flow (i.e. cardiac output) increment in the presence of minor or null changes of resting arterial-mixed venous \(\text{O}_2\) concentration difference (Rest \(C_{(a-v)\text{O}_2}\)) (5,6,21,22,34,35,39,61-63), has never been specifically evaluated in the elderly. Available data in subjects up to 40-45 years of age suggest an age-related increase in phase I duration (24,41,43,61), but studies specifically addressing this point in older subjects are lacking. Given its distinct \(\text{O}_2\) delivery dependence, phase I has been reported to be longer in patients suffering from pathological conditions affecting cardiac output increase during exercise, such as chronic heart failure, congenital heart disease, pulmonary vascular disease, and heart transplantation (12,41,43,48,50,52), as a direct consequence of the prolonged blood transit time from exercising muscles to pulmonary capillaries. As cardiac output increase during exercise is known to be impaired as a result of the ageing process also in healthy subjects (11,42,54), we reasoned that a progressive lengthening of phase I with increasing age would be expected.

If this were the case, a lengthening of phase I could influence the phase II fitting accuracy. Indeed, in studies aimed at evaluating the \(\dot{\text{VO}_2}\) phase II time constant, the exponential fitting is often started after excluding a predetermined, fixed initial 20 s interval - i.e. an estimated phase I duration - from the on-transient data (10,15,16,37,45,52,63,64). As long as such an indirectly determined phase I duration is
close to the real one, this would not distort the goodness of fit of the phase II kinetics; vice versa, the broader the difference between such a fixed interval and the real phase I duration, the more phase II unrelated data would be included in the fitting window, thereby probably reducing the model goodness of fit during the transition phase.

The aim of this study was thus to evaluate in three groups of healthy subjects of different age (young, middle-aged, and elderly) the duration and amplitude of phase I of \( \dot{\text{VO}}_2 \) on-kinetics and to correlate them with cardiac output increase during phase I, in order to elucidate possible relationships between cardiac output and phase I descriptors changes over age. A secondary aim of this study was to verify the influence of possible age-dependent changes in phase I duration on the accuracy of the \( \dot{\text{VO}}_2 \) phase II fitting; to this end, we sought possible differences in both \( \dot{\text{VO}}_2 \) phase II time constant and \( \dot{\text{VO}}_2 \) phase II goodness of fit between a fitting strategy starting after a fixed, predetermined 20 s phase I and that starting from the experimentally determined phase I - phase II transition.
Methods

Ethical approval
This study conforms to the standards set by the latest revision of the Declaration of Helsinki. The protocol was approved by the Central Ethics Committee of the S. Maugeri Foundation and informed written consent was obtained from all participants.

Study population
Thirty healthy male subjects agreed to participate in the study. Recruitment criteria were as follows: 1) no history of cardiac or noncardiac disease; 2) normal resting ECG; 3) cardiopulmonary exercise test stopped for fatigue and/or dyspnea with peak respiratory exchange ratio of ≥1.10; 4) absence of any formal training in addition to habitual daily activities; 5) age within one of the following ranges: 20 to 30 years (20-30 group), 40 to 50 years (40-50 group), and 60 to 70 years (60-70 group). Each of the three age-groups was composed of 10 subjects.

Anthropometric evaluation
Three skinfold thicknesses were measured according to standard procedures (23) by means of a Lafayette caliper. The skinfold sites used were chest, abdomen, and thigh. Body density was calculated using the Jackson-Pollock prediction equations for white male adults (46) and percent body fat was determined by the Siri formula (46).

Habitual physical activity level evaluation
An activity score defining the intensity of habitual physical activity was calculated as previously described (44). Briefly, the activity scoring system is interview-based and
takes into consideration leisure-time and occupational activities during the preceding 6 months and recent deconditioning events (i.e. hospitalizations). Estimated energy cost of activities is classified as low ($\leq 2$ METs), moderate ($> 2 \leq 4$ METs), heavy ($> 4 \leq 6$ METs), or very heavy ($> 6$ METs) according to the Compendium of Physical Activities by Ainsworth et al. (1). To be classified at the moderate, heavy, or very heavy level, subjects have to have performed one or more of the listed activities corresponding to that level for at least 4 h/wk. Different combinations of leisure-time and occupational activity estimated energy costs can result in a final score ranging from 1 (lowest level) to 5 (highest level).

**Ergometric evaluation**

Each participant in the study underwent: a) one ramp cardiopulmonary exercise test for peak $\dot{V}O_2$ and gas exchange threshold (GET) assessment; b) 4 repetitions of a moderate-intensity constant-power exercise test for $\dot{V}O_2$ and cardiac output on-responses evaluation. All tests were performed on an electromagnetic bicycle ergometer (Ergo-metrics 800S; Sensormedics; Yorba Linda, CA, USA).

**Ramp incremental exercise test**

After a 1-min unloaded-cycling warm-up period, a ramp protocol of 15 or 20 W/min at a pedaling rate of 60 revs/min was started and participants were encouraged to exercise until exhaustion. Respiratory gas exchange measurements were obtained breath-by-breath using a computerized metabolic cart (Vmax29; Sensormedics; Yorba Linda, CA, USA). Peak $\dot{V}O_2$ was the mean $\dot{V}O_2$ value observed during the last 30 seconds of the exercise period. Predicted $\dot{V}O_2_{max}$ was determined using a sex-, age-, and protocol-
specific formula defined by Wasserman et al. (59), and GET was estimated by the V-slope and/or respiratory equivalents methods (59).

Moderate-intensity constant-power exercise tests

During a period of one week following ramp cardiopulmonary exercise testing, all subjects performed 4 moderate-intensity constant-power exercise tests with respiratory gas exchange measurement, at a power equal to 80% of the GET power. Following a 2-min resting baseline period, subjects started pedaling at the established power, which was maintained for 8 minutes at a pedaling rate of 60 revs/min. Tests were performed on 2 separate days at least 48 hours apart; the 2 tests performed on the same day were separated by a 30 min resting period to allow cardiovascular and metabolic measures to return back to pre-exercise, baseline levels.

Data analysis and modeling

For modeling of \( \dot{V}O_2 \) on-response, breath-by-breath data were derived from the Sensormedics Vmax29 metabolic system and analyzed using a software specifically developed by the Bioengineering Service of our Institute.

Breath-by-breath \( \dot{V}O_2 \) signals of each of the 4 constant-power tests were resampled with a 1-s sampling rate; the transitions were then time-aligned and ensemble averaged to provide a single response for each subject. In so doing, according to Lamarra et al. (36), the 95% confidence limit for kinetics parameters estimation was reduced to one half of that associated with the analysis of a single transition. As the ventilatory response can affect the accuracy of phase I description, both the number of breaths and the increase of ventilation with respect to the resting value during the first 30 s of exercise were evaluated in the 3 study groups.
The VO₂ basal value (Rest VO₂) was obtained by averaging data obtained during the last minute of the resting period and expressed in ml/min. The phase I - phase II transition (Phi-IItr) was visually determined as the point where a sharp decrease of both respiratory exchange ratio and end-tidal O₂ partial pressure from baseline values occurred, as previously proposed (61). As phase I cannot be reliably described by an exponential term due to the lack of evidence supporting its exponential nature, the limited number of data points, and uncertainty about its true asymptotic value (28,63), the relevant features of phase I were considered to be its duration (PhiI duration), measured in seconds from the start of the exercise period up to the time of Phi-IItr, and amplitude (PhiI amplitude), measured in ml/min as the difference between VO₂ at Phi-IItr (Phi-IItr VO₂) and Rest VO₂ (Fig. 1).

Phase II of VO₂ on-kinetics was calculated as a single exponential function (28,63) of the kind:

\[ \Delta\dot{\text{VO}}_2(t) = \Delta\dot{\text{VO}}_2(\text{ss}) \times (1 - e^{-\left(t-TD\right)/\tau}) \]

where \( \Delta\dot{\text{VO}}_2(\text{ss}) \) is the change of VO₂ from baseline to steady-state, and TD and \( \tau \) are the time delay and time constant of the response, respectively. An iterative exponential association model (CurveExpert 1.3., Daniel Hyams, 1997) was fitted on the VO₂ data of each subject using 2 different fitting strategies: 1) fitting window starting from the directly determined Phi-IItr (‘experimental’ fitting strategy); 2) fitting window starting after a fixed 20 s interval from exercise onset (‘fixed-duration’ fitting strategy) (Fig. 1). For goodness of fit comparison, the standard error of the fit was calculated in both models over a period equal to 4 time constants (i.e. 98% of the response), as differences
in residuals variance between the two fitting strategies were assumed to occur mostly during the transient phase rather than over the steady-state (see residuals in Fig. 1).

**Cardiac output assessment**

During phase I, an unvarying $C_{(a-v)O_2}$ with respect to the resting value has been hypothesized by several authors (5,6,21,22,28,39,61,62) on the grounds that a delay must logically exist between muscle and pulmonary $\dot{V}O_2$ (the longer the leg-to-lung blood transit time is, the longer the delay will be). As a consequence, according to the Fick principle, during phase I cardiac output changes should be strictly linked by a causal relationship to those of $\dot{V}O_2$ through a constant given by the Rest $C_{(a-v)O_2}$ value, as formerly hypothesized by Wasserman (58). Even though the concept of an unvarying $C_{(a-v)O_2}$ during phase I has been challenged in a previous paper (13), Lador et al gave recently provided a strong experimental corroboration of the hypothesis that PhI amplitude is almost completely accounted for by the pulmonary blood flow increase during phase I, showing similar $\dot{V}O_2$ and cardiac output phase I time constants for exercise performed at absolute powers comparable to those of our study (34,35). Accordingly, once Rest $\dot{V}O_2$, PhI-IItr $\dot{V}O_2$, and Rest $C_{(a-v)O_2}$ values are known, cardiac output values at rest (Rest CO) and PhI-IItr (PhI-IItr CO) can be reasonably estimated as:

$$\text{Rest CO (l/min)} = \frac{\text{Rest } \dot{V}O_2}{\text{Rest } C_{(a-v)O_2} \times 10}$$

and

$$\text{PhI-IItr CO (l/min)} = \frac{\text{PhI-IItr } \dot{V}O_2}{\text{Rest } C_{(a-v)O_2} \times 10}$$

respectively, where Rest $C_{(a-v)O_2}$ is expressed in ml/dl.
To measure the resting arterial O2 content (Rest CaO2) value, 5 ml of blood were drawn from an antecubital vein in the supine position in all study participants, and subsequently analyzed (XT-2000; Sysmex; Japan) for haemoglobin content. Arterial O2 saturation (SaO2) was measured at a fingertip by pulse oximetry (NPB-40; Nellcor Puritan Bennett Inc.; Pleasanton, CA) while subjects sat still on the ergometer. Rest CaO2 was then calculated as (31):

\[
\text{Rest CaO2 (ml/dl)} = [\text{Hb}] \times 1.34 \times \text{SaO2}
\]

where [Hb] is haemoglobin concentration in g/dl and 1.34 is the Hb O2 binding capacity in ml O2/g Hb; given its negligible amount, oxygen dissolved in plasma was not considered in the CaO2 calculation. As resting O2 extraction in healthy subjects is quite stable and ranges between 25 and 30% (25,55,57,60), a value of 27% was assumed in the study population and used to estimate the resting mixed venous O2 content (Rest CvO2) value in ml/dl and successively Rest C(a-v)O2 and, according to the Fick principle, Rest CO and PhI-IItr CO. Rest CO and PhI-IItr CO estimated assuming a resting O2 extraction of 25% and 30% resulted in values on average 8% higher and 10% lower, respectively, than those obtained as explained above.

The estimated CO change during phase I (PhI ΔCO) was expressed as the difference between Rest CO and PhI-IItr CO. In addition, the incremental ratio between changes in CO and time during phase I, which represents dimensionally the average pulmonary blood flow (i.e. CO) acceleration during PhI (PhI AFA), was also calculated as:

\[
\text{PhI AFA (ml/s}^2) = \text{PhI ΔCO/PhI Δt}
\]
where PhI $\Delta CO$ is expressed in ml/s and PhI $\Delta t$ is, by definition, PhI duration.

**Statistics**

Unpaired and paired t-tests and one-way analysis of variance with Fisher’s PLSD *post hoc* tests were used to compare the means of quantitative variables, and F test to compare goodness of fit (standard error) of phase II fitting strategies. Regression and Pearson product moment coefficients were used to determine the correlation between measured variables. The level of statistical significance was set at a 2-tailed p value of $\leq 0.05$.

On the basis of available data (24,41,43,61), for one-way analysis of variance evaluating differences in mean PhI duration among the 3 study groups, the smallest difference physiologically worth detecting was considered to be 15%; accordingly, for such an effect size and a two-tailed $\alpha$ value $= 0.05$, a sample size of at least 6 subjects for each of the 3 study groups was required to yield a statistical power of $\geq 80\%$. The StatView® 5.0.1. (SAS Institute, Inc.; Cary, NC, USA) and Power And Precision™ 2 (Biostat, Inc.; Englewood, NJ, USA) software packages were used for statistical calculations.
Results

Demographic and ergometric characteristics

The study groups had by design significantly different mean ages and were well matched as to weight and body mass index, whereas percent lean body mass mean values were significantly lower in the 60-70 compared to 20-30 group (Table 1). The level of habitual physical activity, expressed as Activity Score mean value, was similar in all groups (Table 1).

Peak \( \dot{V}O_2 \) mean values decreased significantly over age, showing a trend towards higher values in the 60-70 than in the 20-30 group when expressed as a percentage of age- and sex-predicted maximum; similarly, significantly decreasing values of GET \( \dot{V}O_2 \) with increasing age were observed, but GET \( \dot{V}O_2 \) tended to be higher in the 60-70 than in the 20-30 group when expressed as a percentage of peak \( \dot{V}O_2 \) (Table 2). GET and peak power mean values were significantly higher in the 20-30 group than in the 40-50 and 60-70 groups; the 60-70 group reached a significantly lower peak HR value when compared to younger subjects (Table 2).

Moderate-intensity constant-power exercise tests workload was significantly higher in the 20-30 than in the 40-50 and 60-70 groups (87±15 W vs. 71±10 W and 65±15 W, respectively, p < 0.01), but did not differ between the 40-50 and 60-70 groups; accordingly, also steady-state \( \dot{V}O_2 \) values were significantly higher in younger than in middle-aged and elderly subjects (1293±378 ml/min vs. 1054±118 ml/min, and 966±139 ml/min, respectively, p < 0.01).
Relationship between age and PhI duration

The number of breaths during the first 30 s of the exercise phase during constant-power exercise tests did not differ among the 3 study groups (10.0± 1 breaths vs. 9.8± 2 breaths vs. 10.3± 2 breaths in the 20-30, 40-50, and 60-70 age-groups, respectively), nor did ventilation increase at phase I-phase II transition with respect to resting value (9.3±1.4 l/min vs. 8.6±2.3 l/min vs. 9.1±2.0 l/min in the 20-30, 40-50, and 60-70 age-groups, respectively). Shortly after exercise onset, a sharp decrease of both respiratory exchange ratio and end-tidal O₂ partial pressure from stable or slightly increasing values with respect to baseline was evident in all subjects, allowing an accurate identification of PhI-IItr.

Phase I duration was found to be strongly related to age (r = 0.74, p < 0.001 - Fig. 2), and mean PhI duration vs. age values reported by others up to an age of about 45 years (24,41,43,61) fitted well with the regression line of our study population (Fig. 2). PhI duration increased significantly by 29% from the 20-30 to the 40-50 group, and by a further 19% from the 40-50 to the 60-70 group (21±3 s vs. 27±3 s vs. 32±4 s, respectively, all p <0.01). Moreover, a significant inverse correlation was observed between PhI duration and peak \( \dot{V}O_2 \) (r = -0.61, p <0.003).

Relationship between age and increase of \( \dot{V}O_2 \) and CO during phase I

No differences in Hb, SaO₂, \( C_aO_2 \), estimated \( C_vO_2 \), and estimated Rest \( C_{(a-v)}O_2 \) values were detected among the three study groups (Table 3). On the contrary, a trend towards significantly lowering Rest \( \dot{V}O_2 \) values over age was observed; as a consequence, also estimated Rest CO values tended to be the lower the higher the subjects’ age (Table 3).
PhI amplitude, estimated PhI ΔCO, and estimated PhI-IItr CO, i.e. parameters describing the amount of VO$_2$ and CO increase during phase I, did not differ among the 20-30, 40-50, and 60-70 groups (Table 3), nor were they related to age, PhI duration, or peak VO$_2$.

On the contrary, AFA, i.e. a parameter describing the rate of CO increase during PhI, correlated inversely with PhI duration (Fig. 3) and directly with peak VO$_2$ ($r = 0.68$, $p <0.0001$), showing progressively decreasing values with increasing age (Table 3).

**Relationship between PhI duration and phase II fitting strategy**

VO$_2$ phase II $\tau$ values obtained by the fixed-duration and the experimental fitting strategy were significantly correlated (Fig. 4). However, both the slope and the y-intercept of the regression line were different from those of the line of identity (0.42 vs. 1 and 15.9 vs. 0, respectively, both $p<0.001$), with all points but one lying below the latter. This finding indicates that the fixed-duration fitting strategy systematically overestimated VO$_2$ phase II $\tau$ with respect to the experimental strategy, as shown in Table 4. Checking agreement between phase II $\tau$ evaluations by a Bland and Altman plot (Fig. 5) confirmed this concept, showing both a VO$_2$ phase II $\tau$ value on average 10 s higher when assessed by the fixed-duration vs. the experimental fitting strategy and a clear trend towards larger differences with increasing age. As a consequence, given the age-related increase of PhI duration, a significant direct correlation was observed between the difference of $\tau$ values obtained by the two fitting strategies and PhI duration ($r = 0.51$, $p < 0.005$). Finally, a significantly higher phase II goodness of fit for the experimental fitting strategy with respect to the fixed-duration one was observed in the 60-70 group (Table 4), which implies inclusion in the fitting window of more and
more phase II-unrelated data with increasing PhI duration (and thus with increasing age) when using the fixed-duration strategy.

**Discussion**

The main findings of this study were that: 1) phase I of pulmonary \( \dot{V}O_2 \) on-kinetics during constant-power moderate-intensity exercise, when directly estimating PhI-IItr using gas exchange indices, shows a progressive lengthening over age in healthy male humans; 2) such a lengthening is associated to a progressive decrease of the average pulmonary blood flow acceleration during phase I with increasing age, but is independent of cardiac output increase during phase I. PhI duration seems thus to be linked to the rate -and not the amount- of cardiac output increase at exercise onset; 3) given the age-related phase I prolongation, using a \( \dot{V}O_2 \) phase II fitting strategy assuming a fixed 20 s PhI duration instead of fitting phase II starting from the experimentally determined PhI-IItr can result in a significant overestimation of phase II \( \tau \), especially in elderly subjects and/or patients with a pathology-induced phase I prolongation.

**PhI duration changes over age**

Phase I of pulmonary \( \dot{V}O_2 \) on-kinetics during moderate-intensity exercise has been evaluated so far mostly in young and adult healthy subjects over a relatively narrow age range (24,27,35,41,43,51,61). Available evidence suggests an increase in the PhI duration up to about 45 years of age, whereas no data have been reported for older
subjects. VO2 increment during phase I is considered to closely mirror the immediate pulmonary blood flow (i.e. cardiac output) increase at the start of exercise, in the presence of an unchanging C\((a-v)O_2\) due to the exercising muscle-to-lung blood transit delay (5,6,21,22,34,35,39,61-63); as a consequence, a phase I prolongation is probably due to a slower increase of cardiac output at exercise onset. Even if no direct evidence has been provided as yet to demonstrate slower cardiac output on-kinetics in the elderly, some indirect data, such as the finding of slowing heart rate on-kinetics over age (4,15,19,49), suggest that this could be the case.

Our data fit well with this picture, showing a strong direct relationship between age and PhI duration (Fig. 2). The latter increased progressively with age, namely, from a 21 s mean value in the 20-30 group to 27 s and 32 s in the 40-50 and 60-70 groups, respectively. Of note, mean PhI duration vs. age values previously reported by others (24,41,43,61) for subjects up to an age of about 45 years lie very close to the regression line found in this study (Fig. 2). The existence of a link between PhI duration and the exercising muscle-to-lung blood transit time is supported by the finding of a significant inverse relationship between the average pulmonary flow acceleration (i.e. the rate of cardiac output increase) during phase I and PhI duration (Fig. 3); in other words, a faster accelerating cardiac output response at exercise onset would determine a shortening of the exercising muscle-to-lung delay and hence of PhI duration. These data are in agreement with the modeling study by Barstow et al (6), who proposed that PhI duration would depend on ‘the rate of adjustment of blood flow’ at exercise onset and the size of venous volume. Moreover, our finding of an age-induced reduction of the average pulmonary flow acceleration during phase I is in line with available data describing a decrease of resting pulmonary artery flow acceleration over age (56) in normal subjects, which finds its biological plausibility in the acknowledged reduction of right ventricular
systolic and diastolic function (33), increase of pulmonary arterial pressure and pulmonary vascular resistances (17,30), and augmentation of pulmonary artery stiffness (40) with increasing age. Also, the 60-70 group showed a lean body mass significantly lower than that of the 20-30 group; as a greater muscle mass has been shown to enhance venous emptying of the healthy subject’s leg (31), a higher skeletal muscle pump-induced venous return to the pulmonary flow acceleration during phase I may have played a contributing role in the youngest groups of our study population. Of note, however, the observed relationship between average pulmonary flow acceleration and phase I duration explained only 36% of PhI duration variance, which underlines the presence of other factors potentially influencing PhI duration in normal subjects; among these, the previously proposed role of venous volume (6) is still to be elucidated.

In the present work, both Rest $\dot{V}O_2$ and Rest CO values fit well with those found in normal subjects of comparable age (47,54,60), showing a trend towards a significant reduction over age which is in agreement with previously reported data (32,47,54). On the contrary, PhI amplitude did not vary with increasing age, and was very similar to values previously reported in other papers using similar protocols, i.e. constant-power exercise starting from a resting baseline (27,34,35,51,61,65); consequently, also PhI $\Delta$CO did not differ among the study groups, nor did PhI-IItr CO values. The finding of age-independent PhI amplitude, PhI $\Delta$CO, and PhI-IItr CO values may be due to the relatively small differences in absolute workloads between the 3 study groups and/or to the random occurrence of a $\dot{V}O_2$ overshoot during phase I observed in this and other studies (51), but it may also suggest that the entity of $\dot{V}O_2$ and cardiac output increase during phase I mainly depends on the relative workload with respect to peak $\dot{V}O_2$. It should also be kept in mind that the rapid increase of blood flow during phase I could be
due more to skeletal muscle pump and vagal withdrawal than to selective vasodilation in active muscles, so representing a quite unspecific response poorly matched to the actual O₂ demand at the skeletal muscle level (22,26,35,51).

Finally, PhI-IItr CO values in the three age-groups were very similar to those reported at comparable times from exercise onset and relative intensities (18,35,38,65); this suggests the reasonableness of our estimates, supporting the reliability of the indirect method of cardiac output measurement utilized in this study.

**PhI duration and phase II fitting strategy**

The exclusion of a fixed initial 20 s-period of the on-response from the fitting window is a commonly used procedure in studies dealing with VO₂ on-kinetics phase II τ evaluation (10,15,16,37,45,52,63,64), on the basis of direct measurements originally carried out in young subjects (39). This fitting strategy has also been used in papers evaluating the relationship between age and VO₂ phase II τ (10,15,16), and even in studies on populations with diseases potentially affecting PhI duration (37,52).

In the present study, notwithstanding the existence of a significant direct correlation between VO₂ on-kinetics phase II τ values obtained with the fixed-duration and the experimental fitting strategy, the regression line of such relationship differed significantly from the line of identity, both as to slope and y-intercept. Of note, as evident in Figure 4, a systematic overestimation of phase II τ values occurred with the fixed-duration fitting strategy as compared to the experimental one. These findings are better evidenced by analyzing the agreement concerning VO₂ phase II τ evaluation between the two fitting strategies using a Bland and Altman plot (Fig. 5), which shows:

1) a large mean positive difference between VO₂ phase II τ evaluated by the fixed
duration vs. the experimental fitting strategy, thus confirming the existence of a
systematic and physiologically significant overestimation of phase II $\tau$ when using the
former; 2) a clear trend towards an increase of differences between fitting strategies
with increasing evaluations mean value (i.e. with increasing divergence between the
actual regression line and the identity line in Fig. 4) and with increasing age. Indeed,
when evaluated by the fixed-duration fitting strategy, $\dot{V}O_2$ phase II $\tau$ values
significantly overestimated those obtained by the experimental fitting strategy by 6 s
(19%), 8 s (22%), and 13 s (38%) in the 20-30, 40-50, and 60-70 groups, respectively
(Table 4). In fact, the more the actual PhI duration exceeds a 20 s duration, the greater
the amount of phase II-unrelated data that is included in the first part of the fitting
window when using the fixed-duration fitting strategy, as also evidenced by the plot of
residuals in Figure 1. Our results were further supported by: 1) the finding of a
significant direct correlation between the difference of phase II $\tau$ values obtained by the
two fitting strategies and PhI duration ($r = 0.51$, $p < 0.005$); 2) a significantly higher
phase II goodness of fit for the experimental fitting strategy with respect to the fixed-
duration one in the 60-70 group. These data stress the concept that, when evaluating
$\dot{V}O_2$ on-kinetics phase II in ageing subjects and/or patients with a possible pathology-
induced phase I lengthening (i.e. patients with chronic heart failure or pulmonary
vascular disease) it is necessary to directly measure PhI duration and have the phase II
fitting window start from the real phase I - phase II transition.

$\dot{V}O_2$ phase II $\tau$ mean values obtained by the fixed-duration fitting strategy showed a
clear trend to increase with increasing age, whereas those obtained by the experimental
fitting strategy did not (Table 4). The latter finding seems to contradict data previously
reported by others, who found phase II $\tau$ values, evaluated with an experimental fitting
strategy analogous to ours, to increase with increasing age (19,49). This result could be at least partly explained on the grounds that the energy expenditure due to habitual activities did not differ among our study groups (Table 1), which suggests that older subjects performed daily activities at a higher relative percentage of peak $\dot{VO}_2$ compared to younger ones. Doing so, they could have been exposed to a more intense ‘training stimulus’ than their younger counterparts, as suggested by a clear trend towards higher mean values of both %pred. $\dot{VO}_2$ and GET $VO_2\%$ in the 60-70 than in the 20-30 group. Indeed, not only $\dot{VO}_2$ on-kinetics, but also peak power and peak $\dot{VO}_2$ have all been found to be related to the level of habitual physical activities in the elderly (2,29,44).

On the other hand, according to the elegant modeling study of Barstow et al. (5), in the presence of normal muscular $\dot{VO}_2$ kinetics the phase II $\tau$ of pulmonary $\dot{VO}_2$ on-kinetics should indeed be shortened when phase I is prolonged, due to a slower cardiovascular adjustment. In fact, for a given rate of muscle $O_2$ utilization increase, a slower cardiovascular on-response (i.e. an increased blood transit time from active muscles to the lungs) would require a greater peripheral $O_2$ extraction and contribute to both a prolonged PhI duration and a faster increase in phase II of pulmonary $\dot{VO}_2$ (i.e. faster pulmonary $\dot{VO}_2$ kinetics relative to muscle $O_2$ utilization kinetics). This notion has already been discussed in previous studies evaluating $\dot{VO}_2$ kinetics in heart transplant recipients (12,43), who typically present a sluggish cardiovascular adjustment to exercise. In these studies, phase I of pulmonary $\dot{VO}_2$ on-kinetics was found to be longer in patients than in controls, whereas phase II $\tau$ (evaluated starting from the experimentally determined phase I - phase II transition) did not differ between the two
groups, leading these authors to conclude that skeletal muscle \( \dot{\text{VO}}_2 \) kinetics were anyhow presumably slower in patients. Thus, in the presence of a lengthened phase I, the observation of a phase II \( \tau \) in the elderly not significantly different from that of young subjects may indirectly indicate a slower adjustment of skeletal muscle oxidative metabolism in the former group (see review 8), as previously suggested (11,20). Finally, the reduction over age of the rate of cardiac output increase at exercise onset observed in this study could also impact \( \text{O}_2 \) delivery to exercising skeletal muscle, supporting recent experimental data in a rat model (9) which suggest blunted dynamics of \( \text{O}_2 \) delivery to skeletal muscle (as compared to those of \( \text{O}_2 \) uptake) during rest-to-exercise transition in aged animals.

**Study limitations**

Several factors could affect phase I as evaluated by breath-by-breath \( \dot{\text{VO}}_2 \) data measured at the mouth (62); among these, most relevant could be the influence of changes in pulmonary \( \text{O}_2 \) stores (7,14,28). Although quite stable over the long term in steady-state conditions, pulmonary \( \text{O}_2 \) stores may vary during the transient \( \dot{\text{VO}}_2 \) on-response (7,14,28), which could result in differences between the alveolar \( \dot{\text{VO}}_2 \) on-kinetics and those observed at the mouth. Even though several algorithms have been proposed to resolve this problem (14), the issue does not seem settled yet. In any case, several authors who evaluated phase I in normal subjects did not use such algorithms (24,27,43,52,61,65), reporting values similar to those found by others who did correct their breath-by-breath \( \dot{\text{VO}}_2 \) data for changes in pulmonary \( \text{O}_2 \) stores between consecutive breaths (34,35,51). Moreover, a recent study by Aliverti et al (3) showed no difference between the phase I time constant as fitted on \( \dot{\text{VO}}_2 \) breath-by-breath values
measured at the mouth and that obtained by fitting $\dot{\text{VO}}_2$ data accurately corrected for changes in pulmonary $\text{O}_2$ stores using opto-electronic plethysmography. Finally, no information is currently available on the possible influence of ageing on pulmonary $\text{O}_2$ stores changes during exercise transitions.

**Perspectives and significance**

This study demonstrates that, when the phase I - phase II transition is estimated directly using gas exchange indices, an age-related prolongation of the phase I of pulmonary $\dot{\text{VO}}_2$ on-kinetics during moderate-intensity exercise is evident in healthy male humans. Such a prolongation is associated to a reduction with increasing age of the average pulmonary flow acceleration during phase I, but is independent of cardiac output increase during phase I, being thus linked to the *rate* - and not the *amount* - of cardiac output increase at exercise onset. As a consequence of such age-related phase I prolongation, an overestimation of the $\dot{\text{VO}}_2$ phase II $\tau$ can occur in elderly subjects when fitting the $\dot{\text{VO}}_2$ data after excluding a fixed, *a priori* determined 20 s phase I from the on-response, i.e. through the inclusion in the fitting window of more phase II-unrelated data. These findings highlight the need for an experimental determination of the phase I-phase II transition in studies dealing with pulmonary VO$_2$ on-kinetics, especially when considering older subjects and/or patients with pathologies potentially prolonging phase I duration (i.e. chronic heart failure). Finally, the results of the present study lend support to the hypothesis that an impairment of $\text{O}_2$ delivery to skeletal muscle likely contribute to generate the reduced aerobic performance typical of ageing, providing, at the same time, indirect evidence of a slower adjustment of skeletal muscle oxidative metabolism in the elderly.
To confirm the findings of the present study, prospective experimental protocols should be designed aimed at evaluating changes over age of pulmonary VO\textsubscript{2} on-kinetics phase I and phase II by both experimentally determining the phase I-phase II transition and directly measuring the cardiac output on-response.

**Acknowledgements**

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**Figure legends**

Figure 1. Averaged \( \dot{VO}_2 \) and respiratory exchange ratio response as a function of time in a 70-year-old subject.

The phase I - phase II transition is identified as the point where a sharp decrease of respiratory exchange ratio from baseline values occurs; phase I duration is 38 s. The phase II experimental (i.e. starting from the phase I-phase II transition - black line) and fixed-duration (i.e. starting after a fixed 20 s interval from exercise onset - gray line) fitting strategies are shown. A marked overestimation of the phase II time constant (57 s vs. 32 s, +78%) is observed with the latter model. See text for further details.

\[ \tau \times 4 = \text{end of the 4 time constant-period considered for comparison of goodness of fit between the two fitting strategies; } \]

\[ \text{PhI} = \text{phase I; } \text{PhI-IItr} = \text{phase I-phase II transition; } \]

\[ \text{Rest } \dot{VO}_2 = \text{resting } \dot{VO}_2; \text{ RER = respiratory exchange ratio.} \]

Figure 2. Phase I duration as a function of age.

Full circles are individual points from this study. Open circle represents mean values ± SE from reference 61; open diamonds are mean values ± SD from reference 24; open square represents mean values ± SD from reference 41; and open triangle mean values ± SD from reference 43. Regression line and statistics are from fitting of this study’s data only.

\[ \text{PhI = phase I.} \]
Figure 3. Phase I duration as a function of average pulmonary blood flow acceleration during phase I.

AFA = average pulmonary flow acceleration.

Figure 4. Correlation between $\dot{\text{VO}}_2$ phase II time constant as evaluated by the experimental fitting strategy vs. the fixed-duration fitting strategy.

Dotted line is the line of identity. Gray circles = 20-30 age-group; black circles = 40-50 age-group; white circles = 60-70 age-group.

EXP = experimental fitting strategy; FD = fixed duration fitting strategy; $\tau$ = time constant.

Figure 5. Bland and Altman analysis of the agreement between different fitting strategies for $\dot{\text{VO}}_2$ on-kinetics phase II $\tau$ evaluation.

The plot reports the bias and the limits of reproducibility as 95% confidence limits (dashed lines) between $\dot{\text{VO}}_2$ on-kinetics phase II $\tau$ as measured by two different fitting strategies (i.e. fixed-duration vs. experimental). The y-axis shows the difference between measurements and the x-axis shows their mean value. A systematic overestimation of phase II $\tau$ values by the fixed-duration with respect to the experimental fitting strategy is evident, with a clear trend towards an increase with increase of both age and mean values. See text for further details.

Gray circles = 20-30 age-group; black circles = 40-50 age-group; white circles = 60-70 age-group.
Table 1
Baseline characteristics

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>20-30</th>
<th>40-50</th>
<th>60-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74±12</td>
<td>77±8</td>
<td>76±11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1±3.3</td>
<td>25.7±2.7</td>
<td>24.9±2.6</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>63±5</td>
<td>62±4</td>
<td>58±4 *</td>
</tr>
<tr>
<td>AS</td>
<td>2.1±0.4</td>
<td>2.0±0</td>
<td>2.3±0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD or number. * = p < 0.01 vs. 20-30 group; † = p < 0.01 vs. 20-30 and 40-50 groups.
20-30 = 20-30 years age-group; 40-50 = 40-50 years age-group; 60-70 = 60-70 years age-group; n. = number; BMI = body mass index; AS = activity score.
Table 2

Incremental exercise testing

<table>
<thead>
<tr>
<th></th>
<th>20-30</th>
<th>40-50</th>
<th>60-70</th>
</tr>
</thead>
</table>
| Peak $\dot{V}O_2$ (ml/min) | 3005±469  | 2704±352  | 2181±238  *
| Peak $\dot{V}O_2$ (ml/kg/min) | 41.6±7.9  | 35.4±4.8 †| 28.7±3.9  *|
| % pred. $\dot{V}O_2$ max | 100±10    | 103±15    | 109±17**  |
| Peak power (W) | 239±39‡   | 202±21    | 183±20    |
| Peak HR (bpm)  | 182±15    | 167±16    | 159±14†   |
| GET $\dot{V}O_2$ (ml/kg/min) | 19.8±4.5  | 17.0±3.7  | 14.9±1.8† |
| GET $\dot{V}O_2$ % | 47±4       | 48±8      | 52±7**    |
| GET power (W) | 109±19‡   | 89±12     | 81±17     |
| Peak RER       | 1.12±0.01 | 1.12±0.01 | 1.11±0.01 |

Values are mean ± SD. * = p < 0.01 vs. 20-30 and 40-50 groups; ** = p < 0.1 vs. 20-30; † = p < 0.01 vs. 20-30 group; ‡ = p < 0.01 vs. 40-50 and 60-70 groups.
20-30 = 20-30 years age-group; 40-50 = 40-50 years age-group; 60-70 = 60-70 years age-group;
% pred. $\dot{V}O_2$ max = percentage of predicted $\dot{V}O_2$ max; HR = heart rate; GET = gas exchange
threshold; GET $\dot{V}O_2$ % = $\dot{V}O_2$ at gas exchange threshold as percentage of peak $\dot{V}O_2$; RER =
respiratory exchange ratio.
Table 3

Phase I cardiac output-related parameters

<table>
<thead>
<tr>
<th></th>
<th>20-30</th>
<th>40-50</th>
<th>60-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (mg/dl)</td>
<td>15.5±0.8</td>
<td>15.9±1.0</td>
<td>15.6±0.7</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>98±0.9</td>
<td>97±0.7</td>
<td>98±1.0</td>
</tr>
<tr>
<td>C₅O₂ (mg/dl)</td>
<td>20.3±1.1</td>
<td>20.8±1.3</td>
<td>20.5±0.8</td>
</tr>
<tr>
<td>CᵥO₂ (mg/dl)</td>
<td>14.8±0.8</td>
<td>15.2±0.9</td>
<td>15.0±0.6</td>
</tr>
<tr>
<td>Rest Cₐ₋ᵥO₂ (mg/dl)</td>
<td>5.5±0.3</td>
<td>5.6±0.4</td>
<td>5.5±0.2</td>
</tr>
<tr>
<td>Rest VO₂ (ml/min)</td>
<td>324±28</td>
<td>304±44</td>
<td>284±37   *</td>
</tr>
<tr>
<td>Rest CO (l/min)</td>
<td>5.9±0.6</td>
<td>5.4±1.0</td>
<td>5.1±0.6  **</td>
</tr>
<tr>
<td>PhI amplitude (ml/min)</td>
<td>399±48</td>
<td>405±85</td>
<td>379±76</td>
</tr>
<tr>
<td>PhI ΔCO (l/min)</td>
<td>7.3±1.6</td>
<td>7.3±1.4</td>
<td>6.9±2.0</td>
</tr>
<tr>
<td>PhI-IItr VO₂ (ml/min)</td>
<td>723±90</td>
<td>709±106</td>
<td>663±139</td>
</tr>
<tr>
<td>PhI-IItr CO (l/min)</td>
<td>13.2±1.2</td>
<td>12.7±2.0</td>
<td>12.0±2.4</td>
</tr>
<tr>
<td>PhI AFA (ml/s²)</td>
<td>5.63±1.0 #</td>
<td>4.51±1.0 †</td>
<td>3.58±0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * = ANOVA’s F = 2.81, p < 0.07; ** = ANOVA’s F = 2.65, p < 0.08; # = p <0.01 vs. 40-50 and 60-70; † = p <0.01 vs. 60-70.

20-30 = 20-30 years age-group; 40-50 = 40-50 years age-group; 60-70 = 60-70 years age-group; Hb = haemoglobin; SaO₂ = arterial O₂ saturation; C₅O₂ = arterial O₂ content; CᵥO₂ = estimated venous O₂ content; estimated Cₐ₋ᵥO₂ = arterial-venous O₂ concentration difference; Rest = resting; estimated CO = cardiac output; PhI = phase I; PhI ΔCO = estimated cardiac output increase from baseline to phase I - phase II transition; PhI-IItr = phase I - phase II transition; PhI AFA = average pulmonary flow acceleration during phase I.
<table>
<thead>
<tr>
<th></th>
<th>20-30</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXP τ (s)</strong></td>
<td>32± 5</td>
<td>37± 9</td>
<td>34± 10</td>
</tr>
<tr>
<td><strong>FD τ (s)</strong></td>
<td>38± 9 *</td>
<td>45± 13 *</td>
<td>47± 20 *</td>
</tr>
<tr>
<td><strong>EXP SE</strong></td>
<td>0.047± 0.011</td>
<td>0.047± 0.024</td>
<td>0.035± 0.008</td>
</tr>
<tr>
<td><strong>FD SE</strong></td>
<td>0.049± 0.012</td>
<td>0.051± 0.025</td>
<td>0.056± 0.019 **</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * = p <0.05 vs. EXP τ; ** = p <0.01 vs. EXP SE.
20-30 = 20-30 years age-group; 40-50 = 40-50 years age-group; 60-70 = 60-70 years age-group; EXP = experimental fitting strategy; FD = fixed duration fitting strategy; τ = VO₂ phase II time constant; SE = standard error of the estimate.
$\dot{V}O_2$ (ml/min)

Exercise

Time (s)

Rest $\dot{V}O_2$

$\tau \times 4$

PhI-PhIItr

PhI duration

RER

0.9

0.75

20 s

240

320

400

480

Residual
PhI duration (s)

Age (years)

y = 16 + 0.25 \cdot x
r = 0.74
r^2 = 0.55
p < 0.0001
y = 38.4 - 2.5 \cdot x
r = -0.60
r^2 = 0.36
p < 0.001
\[ y = 14.4 + 0.46 \cdot x \]

\[ r = 0.77 \]

\[ r^2 = 0.60 \]

\[ p < 0.0001 \]