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ORIGINAL ARTICLE

A new interpolation-free procedure for breath-by-breath analysis of oxygen uptake in exercise transients

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Abstract
Introduction Interpolation methods circumvent poor time resolution of breath-by-breath oxygen uptake ($\dot{V}O_2$) kinetics at exercise onset. We report an interpolation-free approach to the improvement of poor time resolution in the analysis of $\dot{V}O_2$ kinetics.
Methods Noiseless and noisy (10 % Gaussian noise) synthetic data were generated by Monte Carlo method from pre-selected parameters (Exact Parameters). Each data set comprised 10 ($\dot{V}O_2$)-on transitions with noisy breath distribution within a physiological range. Transitions were superposed (no interpolation, None), then analysed by bi-exponential model. Fitted model parameters were compared with those from interpolation methods (average transition after Linear or Step 1-s interpolations), applied on the same data. Experimental data during cycling were also analysed. The 95 % confidence interval around a line of parameters' equality was computed to analyse agreement between exact parameters and corresponding parameters of fitted functions.
Results The line of parameters' equality stayed within confidence intervals for noiseless synthetic parameters with None, unlike Step and Linear, indicating that None reproduced Exact Parameters. Noise addition reduced differences among pre-treatment procedures. Experimental data provided lower phase I time constants with None than with Step.
Conclusion In conclusion, None revealed better precision and accuracy than Step and Linear, especially when phenomena characterized by time constants of <30 s are to be analysed. Therefore, we endorse the utilization of None to improve the quality of breath-by-breath $\dot{V}O_2$ data during exercise transients, especially when a double exponential model is applied and phase I is accounted for.

Keywords Gas exchange · Humans · Kinetics · Procedure

Abbreviations

a_1	Amplitude of response of first exponential
a_2	Amplitude of response of second exponential
b	Baseline resting $\dot{V}O_2$ value
d_1	Time delay of first exponential
d_2	Time delay of second exponential
T	Time
s	Second
$\dot{V}O_2$	Oxygen uptake
θ	Heaviside function
τ_1	Time constant of first exponential
τ_2	Time constant of second exponential
$\in$	Mathematical symbol meaning "is an element of"

Introduction

Technical developments in the study of the pulmonary gas exchange dynamics at the onset of exercise were characterized by the attempts at getting rid of two problems, namely:

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(1) the low time resolution and, (2) the poor signal-to-noise ratio. The most significant advancements in the latter were achieved through the progressive improvement of computational algorithms for the determination of alveolar gas transfer on a breath-by-breath basis.

The first breath-by-breath algorithm was proposed by Auchincloss et al. (1966), who used fixed pre-defined values of functional residual capacity as end-expiratory lung volumes in their estimate of the changes in lung gas stores over each breath. Subsequently, several authors introduced variants to the Auchincloss algorithm (Busso and Robbins 1997; Swanson and Sherrill 1983; Wessel et al. 1979), to better assess end-expiratory lung volumes. However, di Prampero and Lafortuna (1989) demonstrated that, as long as end-expiratory lung volume cannot be measured for each single breath, it is not possible to establish the extent to which the alveolar gas exchange variability depends on physiological phenomena using Auchincloss-like algorithms. Other tracks were to be taken. On one side, Cala et al. (1996) tried to use external devices. On the other side, Capelli et al. (2001) resumed an alternative algorithm (Grønlund 1984), which got rid of the need of estimating end-expiratory lung volume. With this algorithm, they doubled the signal-to-noise ratio in breath-by-breath determination of alveolar gas transfer as compared with Auchincloss-like algorithms.

Algorithm improvements, however, did not act on the time resolution, although this is a fundamental aspect in the study of gas exchange dynamics. In fact, an abrupt increase in ventilation and oxygen uptake ($\dot{V}O_2$) at the mouth was frequently observed at exercise start. Some authors attributed this increase to a sudden increase in pulmonary blood flow (Cummin et al. 1986; Wasserman et al. 1974; Weissman et al. 1982). On these bases, Barstow and Molé (1987) formalized the dynamics of $\dot{V}O_2$ at exercise start with a double exponential model. However, the time constant (τ_1) of the first exponential (phase I) turned out to be so low (so fast), that it was systematically undersampled because it was resolved within one, at most two breaths, from exercise start. If a double exponential model of the kinetics of $\dot{V}O_2$ upon exercise onset is accepted, as is usually the case nowadays, then a correct and precise determination of τ_1 and of the amplitude of phase I (a_1) becomes crucial. To this aim, an improvement of the time resolution in gas exchange analysis is a need. Previous authors tried to increase the time resolution by means of interpolation methods on the 1-s basis (Beaver et al. 1981; Hughson et al. 1993; Lamarra et al. 1987), yet introducing distortion of the physiological signal. For this reason, Lamarra et al. (1987) suggested that their procedure was to be applied only for analysing phenomena with a time constant of at least 30 s.

The aim of this study was to present, evaluate and test a different, new approach to the problem of poor time resolution in the analysis of breath-by-breath $\dot{V}O_2$ kinetics at exercise onset. With the proposed procedure, possible signal distortion was prevented using superposition of raw data obtained during several identical $\dot{V}O_2$-on transients instead of interpolation, on the assumption that each repetition on a given subject is representative of the same physiological situation. Murgatroyd et al. (2011) already used superposition of several repetitions together, without interpolation, yet they then averaged the data by n breaths, where n is the total number of repetitions performed, without improving time resolution.

Synthetic data generated by Monte Carlo simulation procedures were used without and with added noise. Experimental data collected during moderate intensity exercise on a cycle ergometer were also obtained. The results were compared with those provided by previous methods of interpolation, applied on the same data sets.

Methods

Synthetic $\dot{V}O_2$ data generation

A set of synthetic data was generated using the following bi-exponential model (Barstow and Molé 1987; Cautero et al. 2002; Hughson et al. 1993; Lador et al. 2006):

$$\dot{V}O_2 = b + a_1\theta(t-d_1)\left(1-e^{-\frac{t-d_1}{\tau_1}}\right) + a_2\theta(t-d_2)\left(1-e^{-\frac{t-d_2}{\tau_2}}\right) \quad (1)$$

where t is time. The parameter b (in L min^{-1}) represents the oxygen consumption at rest. The parameters a_1 and a_2 (both in L min^{-1}) are the amplitudes of the first and second exponential, respectively, and the parameters τ_1 and τ_2 (both in s) the corresponding time constants. The times d_1 and d_2 are the time delays for the two exponentials. The function θ is the Heaviside function ($\theta(t) = 0$ if $t < 0$ and $\theta(t) = 1$ if $t \geq 0$).

By means of Eq. (1), 1,000 noiseless sets of $\dot{V}O_2$ data had to be generated by Monte Carlo method, to encompass the non-uniform Gaussian distributions on the following intervals: $b \in [0.1, 0.6]$ L min^{-1}, $a_1 \in [0.2, 0.8]$ L min^{-1}, $a_2 \in [0.3, 1]$ L min^{-1}, $\tau_1 \in [0.1, 5]$ s, $\tau_2 \in [5, 45]$ s, $d_2 \in [8, 35]$ s and with $d_1 = 0$ s. The amplitudes, time constants and time delays of the 1,000 data sets were defined as Exact Parameters, which were used as reference to define the precision of simulation and experimental results.

Experimental $\dot{V}O_2$ data generation

Eight healthy non-smoking male subjects (age 30.1 ± 5.5 years, height 181 ± 7 cm, body mass

73.9 ± 11.4 kg) took part in the experiments. Only subjects with maximal aerobic power equal or higher than 225 W were included. This ensured that the intensity of 80 W that was selected for further experimental tests was in the moderate, fully aerobic exercise domain, so that a metabolic steady state was always attained and the τ_2 was neither affected by "early" lactate accumulation (di Prampero and Ferretti 1999) nor influenced by the appearance of the slow component of the $\dot{V}O_2$ kinetics (Whipp and Wasserman 1972). All subjects were preliminarily informed of all procedures and risks associated with the experimental testing. Informed consent was obtained from each volunteer, who was aware of his right of withdrawing from the study at any time without jeopardy. The study was conducted in accordance with the Declaration of Helsinki and was approved by local ethical committee.

The experimental protocol consisted of 10 repetitions of 5-min cycling at 80 W on an electrically braked cycle ergometer (Ergo-metrics 800S, Ergo-line, Blitz, Germany). Successive trials were separated by 5 min of recovery at rest. The first repetition of 5-min exercise was preceded by 2 min of rest. The pedalling frequency was kept in the 60–90 rpm range. Each subject, aided by visual feedback, was asked to keep his own pedalling frequency as invariant as possible within each trial and across the 10 trials.

Respiratory gas traces and ventilation were continuously measured at the mouth, using a metabolic portable unit (K4b^2, Cosmed, Rome, Italy), consisting of a zirconium oxygen analyser, an infrared CO_2 metre and a turbine flowmeter. The gas analysers were calibrated with ambient air and with a gas mixture of known composition (O_2 16 %, CO_2 5 %, N_2 as balance), and the turbine by means of a 3-L syringe. Breath-by-breath $\dot{V}O_2$ and carbon dioxide output were then computed off-line by means of a modified version of the Grønlund's algorithm (Capelli et al. 2001; Grønlund 1984). A software purposely written under the Labview® developing environment (Labview® 5.0, National Instruments, Austin, TX, USA) was used.

The None, Linear and Step procedures were applied also to the treatment of the 10 repetitions of the experimental data sets. However, since the use of 10 repetitions is unlikely in actual experimental conditions, where 3 repetitions are performed in most cases, the three pre-treatment procedures were applied to experimental data also using the first 3 repetitions only instead of the entire set of 10 repetitions. So, for each pre-treatment procedure, we had 9 curves of experimental data (one per subject) resulting from 10 repetitions, and 9 curves resulting from the use of the first 3 of these 10 repetitions.

Accounting for irregular time distribution in synthetic data

Once the Exact Parameters had been combined to define the synthetic data set, we accounted for the fact that, in a real experiment, t is not uniformly sampled. In fact t identifies the instant of the actual occurrence of each breath, whose time distribution is irregular and varies with exercise time (the breathing frequency increases) and among experiments. To account for this phenomenon, for each of the 1,000 data sets, 10 repetitions were generated. These had the same Exact Parameters, and differed among them only for the time at which each breath occurred. Breath duration in the Monte Carlo simulations was let to vary randomly within minima and maxima that were calculated from experimental data. These minima and maxima were defined for each 20-s time window, beginning from exercise start. This implied longer breath duration at exercise start, and progressively shorter breath duration towards the steady state, since minima and maxima tended to decrease moving from one time window to the successive. The 10 repetitions for each data set were generated on an exercise time basis of 5 min, as in actual experimental data acquisition. To give an idea of the degree of breathing frequency variation, we note that breathing frequency of experimental data increased from 16.1 ± 3.9 breaths per minute at rest to 19.4 ± 4.7 breaths per minute at exercise steady state.

These 10 repetitions are noiseless repetitions, in which only t sampling was let to vary. These were subsequently used to calculate noiseless estimated parameters by "Fitting procedure" (see below). Actual breathing, however, is affected by noise. To account for the effects of noise, we reanalysed the 10 repetitions, with the same t sampling, after having added also Gaussian noise to the synthetic data. The noise added in the present study had a constant standard deviation of 10 %. This value was derived from the statistical analysis of noise performed by Lamarra et al. (1987), showing that noise (1) was independent of the work rate, (2) had a typical amplitude for a single transition of ≈10 %, and (3) this amplitude remained unchanged during the entire exercise transient. This second set of data, after addition of noise, was then used to calculate noisy estimated parameters by "Fitting procedure" (see below).

Data pre-treatment

Three pre-treatment procedures were applied on the 10 repetitions of the 1,000 data sets for the analysis of the $\dot{V}O_2$ kinetics. The first procedure, called None, which is the new procedure proposed in this study, requires that the data be used as they came out from the simulation or the actual experiment. Thus, no real pre-treatment was performed, apart from pooling all the breaths from the 10 repetitions together on the same plot for further fitting. As a consequence, None should merely reconstruct the data curve characterized by the Exact Parameters.

The second procedure, called Linear, consisted of a linear interpolation performed between two consecutive

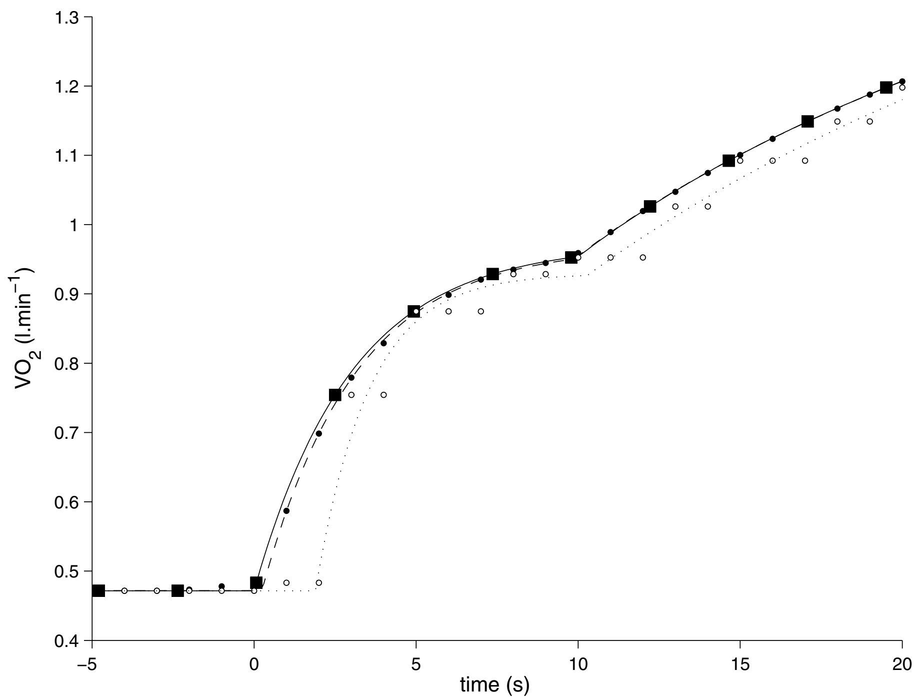

Fig. 1 Schematic representation of the two interpolation methods utilized for data pre-treatment. For the sake of clarity the last 5 s of rest, the entire phase I and only the initial seconds of phase II are represented. The *filled squares* represent the raw points of one repetition of synthetic data. The *filled dots* refer to the 1-s linear interpolation between consecutive breaths (Linear). The *open dots* refer to the 1-s flat interpolation between consecutive breaths with sudden step increase at the next breath (Step). The *lines* represent the fitted bi-exponential model: *continuous line* for None, *dashed line* for Linear and *dotted line* for Step. The exact parameters were: $b = 0.472$ L min^{-1}; $a_1 = 0.5$ L min^{-1}, $a_2 = 0.6$ L min^{-1}, $\tau_1 = 3$ s, $\tau_2 = 23$ s, $d_2 = 10$ s and $d_1 = 0$ s. The estimated parameters with None were $b = 0.472$ L min^{-1}; $a_1 = 0.500$ L min^{-1}, $a_2 = 0.600$ L min^{-1}, $\tau_1 = 3.000$ s, $\tau_2 = 23.000$ s, $d_2 = 10.000$ s and $d_1 = 0.000$ s. The estimated parameters with Linear were $b = 0.472$ L min^{-1}; $a_1 = 0.496$ L min^{-1}, $a_2 = 0.604$ L min^{-1}, $\tau_1 = 2.870$ s, $\tau_2 = 22.897$ s, $d_2 = 9.875$ s and $d_1 = 0.242$ s. The estimated parameters with Step were $b = 0.472$ L min^{-1}; $a_1 = 0.458$ L min^{-1}, $a_2 = 0.642$ L min^{-1}, $\tau_1 = 1.645$ s, $\tau_2 = 21.257$ s, $d_2 = 10.277$ s and $d_1 = 1.893$ s

breaths on the data of each of the 10 repetitions. Mean $\dot{V}O_2$ values were re-computed at 1-s intervals along the interpolation line (Barstow and Molé 1991; Hughson et al. 1993), which reconstructed an apparent segmental linearized slope of the $\dot{V}O_2$ kinetics.

The third procedure, called Step, consisted of a flat interpolation performed on the data of each of the 10 repetitions, with a 1-s sampling, until the next breath started, at which time a step increase in $\dot{V}O_2$ was admitted (Lamarra et al. 1987; Whipp et al. 1982). This procedure is based on the assumption that the mass flow rates of CO_2 and O_2 are relatively constant across each breath (Cumming 1981; Lamarra et al. 1987).

For Linear and Step, within each trial, the interpolated $\dot{V}O_2$ value obtained at the end of each second was retained. Then, the 10 pre-treated trials were averaged and the mean $\dot{V}O_2$ at each second was computed, thus obtaining the average file on which the fitting procedure was applied. Figure 1 is a schematic representation of the interpolation procedures applied with Linear and Step.

The None, Linear and Step procedures were applied to the treatment of both noiseless (no noise, but with irregular t sampling distribution) and noisy (presence of both noise and irregular t sampling distribution) synthetic data sets.

Fitting procedure

The curves obtained by superposing data from 10 repetitions (None) and the average curves generated after application of Linear and Step, provided 1,000 $\dot{V}O_2$ versus t series of noiseless or noisy synthetic data, and 8 $\dot{V}O_2$ versus t series of experimental data, for each pre-treatment procedure. Moreover, we had 8 $\dot{V}O_2$ versus t series of experimental data with 3 repetitions instead of 10. The t at which each breath occurred was defined as the time when an inspiration began. This was identified as the time when the flow attains zero between an exhalation and the subsequent inspiration. These series were fitted using a non-linear least squares procedure (Levenberg–Marquardt algorithm, see Lavenberg 1944; Marquardt 1963, and trust-region-reflective algorithm, see Coleman and Li 1996) with constraints. Equation (1) was used as the fitting model. In other words, the estimated model parameters b, a_1, a_2, τ_1, τ_2, d_1 and d_2 were obtained by minimizing the squared difference between the model function and the synthetic or experimental $\dot{V}O_2$ data. Constraints were set as follows: (1) parameters b, a_1, a_2, τ_1, τ_2, d_1 and d_2 could not be negative; (2) τ_2 and d_2 were to be larger than τ_1 and d_1, respectively. The instant at which exercise started was defined as time 0.

The non-linear least square procedure was performed using a function available in the Matlab toolbox (version 7.13.0.564, MathWorks, Natick, MA, USA). For synthetic data, the starting values were 0.2, 1 L min^{-1}, 5, 30, 0 and 20 s, respectively, for a_1, a_2, τ_1, τ_2, d_1 and d_2. Starting value for b was the individual mean calculated over the last minute of rest before exercise start. For experimental data, the starting values were individually selected based on visual inspection. This carried along the need of testing the robustness of the applied fitting procedure. This was done on synthetic data, by setting different starting values from the Exact Parameters and checking the ensuing output parameters, which turned out independent of the applied starting values.

This fitting procedure was applied to both noiseless and noisy synthetic data sets, to obtain, respectively, noiseless estimated parameters and noisy estimated parameters, as defined above. If pre-treatment procedures were not introducing errors, noiseless estimated parameters would result equal to the Exact Parameters. In this case, if we plot the former as a function of the latter parameters, all points should lie on the line of parameters' equality. Because of noise, noisy estimated parameters should not lie on the line of parameters' equality; however, the precision and accuracy of their distribution around the line of parameters' equality will be best, the highest is the coincidence between noiseless estimated parameters and Exact Parameters. The parameters estimated on the experimental data were defined as experimental parameters.

Statistical analysis

For the experimental data, the effects of the pre-treatment procedure and of the number of repetitions on the experimental parameters were analysed by two-way analysis of variance for repeated measures. When applicable, a Tukey post hoc test was used to locate significant differences. These analyses were performed using commercial statistical software (Statistica version 11, Stat Soft. Inc., Tulsa, OK, USA). The results were considered significant if $p < 0.05$. For each parameter, individual confidence intervals were calculated based on the Jacobian provided by the fitting function, using a function available in the Matlab toolbox (version 7.13.0.564, MathWorks, Natick, MA, USA).

For the synthetic data with all pre-treatment methods, the 95 % confidence intervals were estimated for a_1, a_2, d_2, τ_1 and τ_2 by bootstrap, using the bias corrected and accelerated percentile method using a function available in the Matlab toolbox (version 7.13.0.564, MathWorks, Natick, MA, USA), and encompassing the whole range of Exact Parameters used in the Monte Carlo simulation (see "Synthetic $\dot{V}O_2$ data generation"). The 95 % bootstrap confidence interval was computed on 5,000 bootstrap samples (random sampling from the original data set with replacement), on which a linear regression was calculated, yielding a total of 5,000 slopes. The input of the function included either the 1,000 noiseless or the 1,000 noisy synthetic data. The output was a vector containing the lower and upper bounds of the confidence interval, calculated for the whole range of the Exact Parameters.

The influence of d_2 value on the other parameters of the noiseless synthetic data was estimated by calculating, for bins of exact d_2 values, the percentage of estimated parameters that are closer than a certain error margin from the corresponding Exact Parameters. The bins of exact d_2 were 8–12, 12–16, 16–20, 20–24, 24–30, 30–35 s, covering all the intervals of d_2 values selected for the Monte Carlo method, by which the synthetic $\dot{V}O_2$ data were generated. The error margins selected were equivalent to once the width of the confidence intervals for None, as calculated by bootstrap, at the median of the range of exact values for each parameter. At these medians, the retained error margins resulted 0.0717, 0.1208 s, 0.0055 and 0.0054 L min^{-1}, for τ_1, τ_2, a_1 and a_2, respectively, for noiseless estimated parameters. The corresponding retained error margins for noisy estimated parameters were 0.1387, 0.3215 s, 0.0064 and 0.0061 L min^{-1}, respectively, for τ_1, τ_2, a_1 and a_2.

Results

The differences between noiseless estimated d_2 values and the corresponding exact d_2 values are shown in the upper panels of Fig. 2 as a function of exact d_2. Whereas None and Linear provided good estimates of d_2, Step overestimated d_2, as demonstrated by the line of parameters' equality lying below the lower confidence interval curve. The differences between noisy estimated d_2 values and the corresponding exact d_2 values are shown in the lower panels of Fig. 2 as a function of exact d_2. Although noisy data were more scattered than noiseless data for all three methods, None and Linear provided good noisy estimated d_2 values, whereas Step again overestimated d_2.

The same type of analyses reported in Fig. 2 for d_2 was performed for estimated τ_1, τ_2, a_1 and a_2. The difference between these estimated parameters and the corresponding Exact Parameters were plotted as a function of the

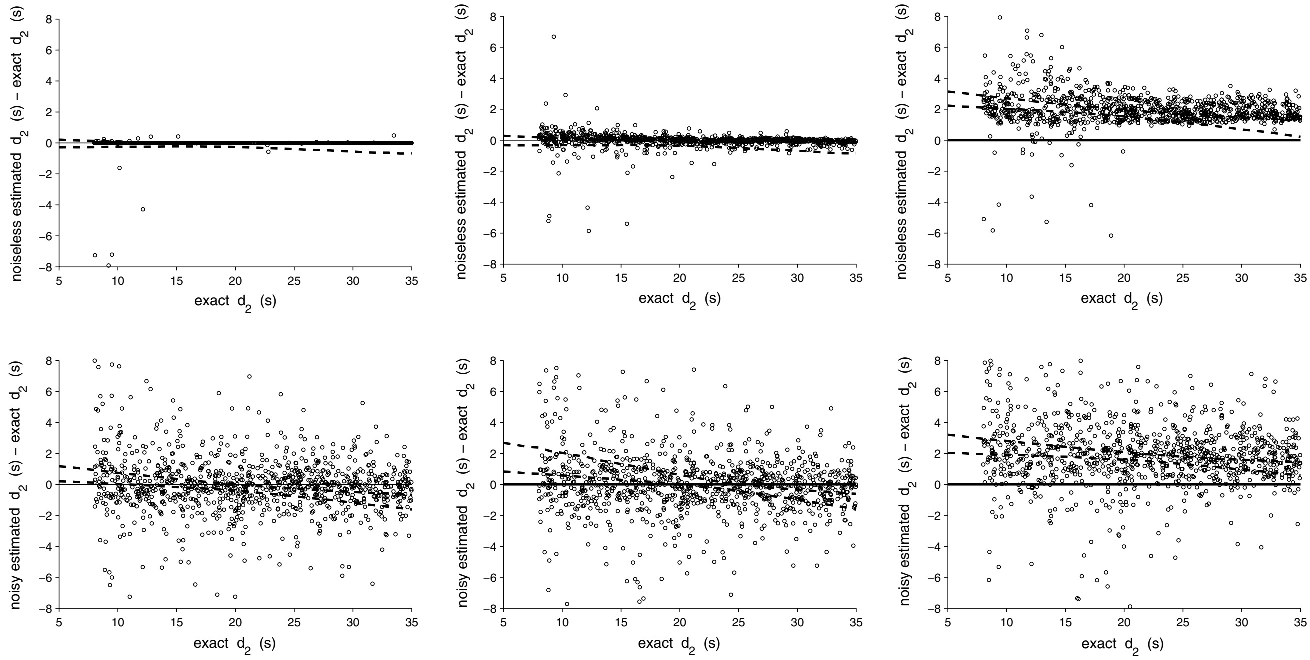

Fig. 2 *Top* The difference between noiseless estimated d_2 and exact d_2 is plotted as a function of exact d_2. For None (*left panel*), Linear (*middle panel*) and Step (*right panel*). The *horizontal line* is the line of parameter's equality ($y = 0$); the *dotted lines* represent the confidence intervals calculated by bootstrap (5,000 resampling). *Bottom* The difference between noisy estimated d_2 and exact d_2 is plotted as a function of exact d_2, for None (*left panel*), Linear (*middle panel*) and Step (*right panel*). The *horizontal line* is the line of parameter's equality ($y = 0$); the *dotted lines* represent the confidence intervals calculated by bootstrap (5,000 resampling). $N = 1{,}000$

corresponding Exact Parameters. The plots concerning τ_1 are shown in Fig. 3, the plots concerning, τ_2, a_1 and a_2 are presented as Supplementary material. Noiseless estimated parameters appear in the upper panels, noisy estimated parameters in the lower panels. As far as None is concerned, it is noteworthy that several data still lay outside the confidence intervals. Obviously enough, the fraction of data outside the confidence intervals was greater for noisy than for noiseless estimated parameters. With None the line of parameters' equality stayed within the confidence intervals for all noiseless estimated parameters. These tendencies were less evident in the case of noisy estimated parameters (lower panels of Figs. 2, 3, and of figures provided as Supplementary material), due to wider data dispersion. In this case, the line of parameters' equality was close to the upper or the lower confidence interval for d_2, τ_1, τ_2, a_1, $a_{2.}$

When also Linear and Step were accounted for, in both cases, it appeared that the number of noiseless estimated parameters lying outside the line of parameters' equality was more numerous than for None. Symmetrically, for all fitting parameters, the fraction of synthetic values lying exactly on the line of parameters' equality was larger with None than with Linear and Step. Concerning Step and Linear, it is noteworthy that both procedures provided line of parameters' equality below the lower confidence interval curve for τ_1, indicating that they overestimated τ_1 (Fig. 3, upper panels). Moreover, as can be seen in the Figures that we uploaded as Supplementary material, both procedures overestimated τ_2, and tended to overestimate a_1 and to underestimate a_2. Yet concerning noiseless τ_2, we remark a tendency to approach the line of parameter's equality at high exact τ_2 values. All pre-treatment procedures provided a good estimate of the total $\dot{V}O_2$ change between rest and steady state, which is equal to the sum of a_1 plus a_2.

The experimental data are shown in Table 1, where the experimental parameters obtained with the three pre-treatment procedures are reported. With 10 repetitions, τ_1 was significantly shorter with None than with the two other procedures. No differences among pre-treatment procedures were found for phase II parameters. No significant differences between 10 and 3 repetitions were found. Nevertheless, with three repetitions, τ_1 with None was significantly shorter than that with Step, but not than that with Linear.

The fraction of estimated data that deviated from the Exact Parameters by less than the width of the confidence intervals was reported in Table 2 for each considered range of exact d_2 values, for None, Linear and Step. For τ_1, the fraction of noiseless estimated data deviating from the Exact Parameters by less than once the width of the confidence intervals with Linear and Step remained always below 5 %, even with the longest d_2 values. For all parameters, this fraction for None was above 94 % for all the d_2

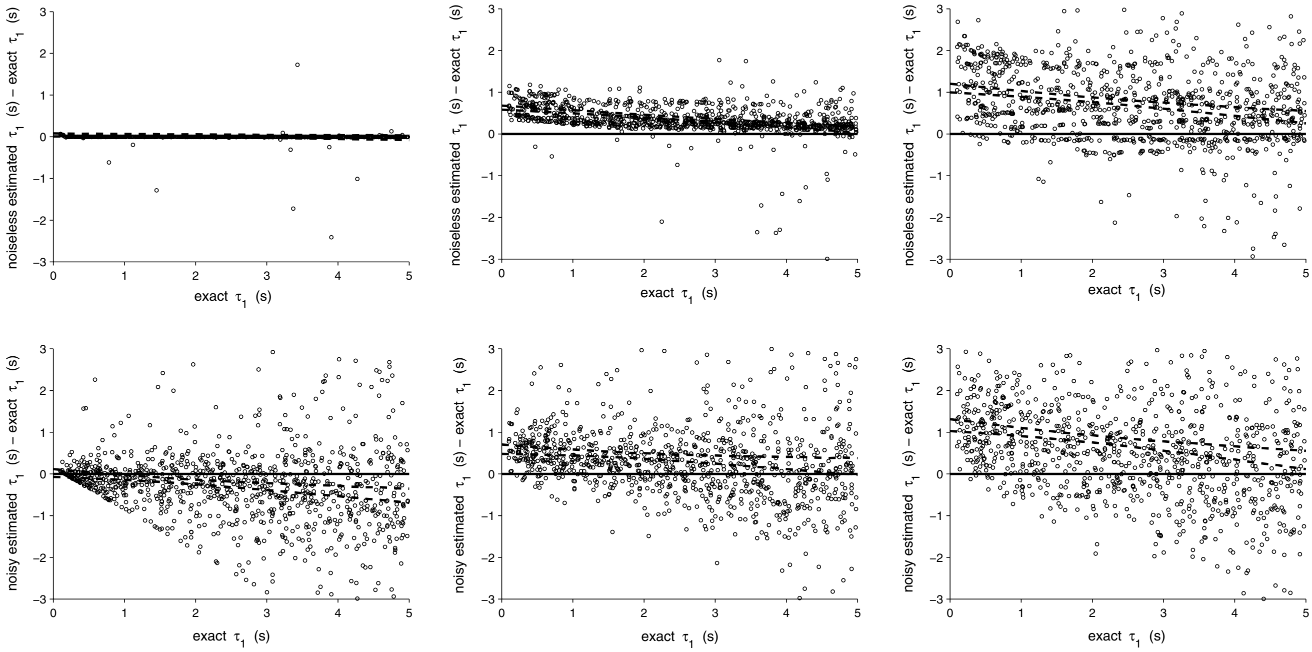

Fig. 3 *Top* The difference between noiseless estimated time constant of the first exponential (noiseless estimated τ_1), obtained after application of None (*top left panel*), Linear (*top middle panel*) and Step (*top right panel*), and the corresponding exact values (exact τ_1), is reported as a function of the corresponding exact τ_1. Each point ($N = 1{,}000$) was obtained using the combined data of the 10 repetitions. The *horizontal line* is the line of parameter's equality ($y = 0$); the *dotted lines* represent the confidence intervals calculated by bootstrap (5,000 resampling). *Bottom* The difference between noisy estimated τ_1 and exact τ_1 is plotted as a function of exact τ_1, for None (*left panel*), Linear (*middle panel*) and Step (*right panel*). The *horizontal line* is the line of parameter's equality ($y = 0$); the *dotted lines* represent the confidence intervals calculated by bootstrap (5,000 resampling). $N = 1{,}000$

ranges. By contrast, the corresponding fractions of noisy estimated τ_1 and τ_2 data deviating from the Exact Parameters by less than once the width of the confidence intervals was never higher than 24, 19 and 16 %, respectively, for None, Linear and Step. A better precision was attained in the estimate of amplitudes than in that of time constants, for all pre-treatment methods.

Discussion

In this study, we presented a new procedure for breath-by-breath analysis of $\dot{V}O_2$-on kinetics without intra-breath interpolation treatment (None). The results obtained with None were compared with those obtained by applying on the same data two commonly used pre-treatment procedures, both characterized by application of 1-s intra-breath interpolation methods with subsequent computation of the mean $\dot{V}O_2$ value at each second over several repetitions of the same test (here defined as Step and Linear). This was done on (1) high-quality noise-free synthetic data obtained by means of Monte Carlo simulations, (2) the same sets of synthetic data after addition of 10 % Gaussian noise, and (3) physiological data obtained on exercising humans (experimental data). In the first case, we stressed the distortion effect of the interpolation procedures with respect to the None method—which on this basis may theoretically be considered a more correct approach—without other confounding factors; in the second, we added a dumping effect of signal noise, common to all tested conditions, tending to minimize differences among methods; this dumping effect was even enlarged in the third case, where other sources of random noise, actually present in real experimental conditions, were further introduced. As a consequence, noiseless synthetic data highlighted the effect of pre-treatment alone, in absence of other confounding factors, thus providing a direct demonstration of the impact of pre-treatment on model parameters. The addition of Gaussian noise to synthetic data, plus that of random noise coming from experimental data, allowed then an evaluation of the tendencies revealed on noiseless data in conditions progressively approaching the "physiological" one.

The three procedures only differed by the pre-treatment, whereas the $\dot{V}O_2$ computation algorithm and the fitting procedure were the same. The results of the noiseless synthetic data demonstrated that None was the best approach to the estimate of τ_1. In fact, Fig. 3 showed that None provided the least data shift with respect to the exact data, represented by the line of parameters' equality. This conclusion can be confirmed after analysis of noisy estimated data and,

Table 1 Experimental Parameters as calculated by the model from the experimental data: comparison of results from the three pre-treatment procedures, for the whole set of 10 repetitions (top) and for the first 3 repetitions only (bottom)

	None			Linear			Step		
	Mean ± SD	CI−	CI+	Mean ± SD	CI−	CI+	Mean ± SD	CI−	CI+
10 repetitions									
d_2 (s)	27.91 ± 5.05	20.59 ± 6.50	33.22 ± 5.89	27.31 ± 5.61	20.28 ± 15.02	34.34 ± 11.42	29.27 ± 5.69	26.20 ± 5.20	32.34 ± 6.27
τ_1 (s)	1.17 ± 1.87	−2.08 ± 2.44	4.42 ± 4.77	3.01 ± 2.24	−0.93 ± 6.41	6.94 ± 4.31	4.09 ± 3.44*	1.29 ± 2.34	6.89 ± 4.72
τ_2 (s)	13.43 ± 3.23	6.87 ± 3.70	19.99 ± 4.68	13.56 ± 4.49	8.57 ± 6.08	18.56 ± 6.87	13.45 ± 4.11	9.08 ± 5.00	17.81 ± 3.50
a_1 (L min^{-1})	0.368 ± 0.084	0.311 ± 0.085	0.425 ± 0.087	0.390 ± 0.085	0.360 ± 0.080	0.420 ± 0.089	0.387 ± 0.090	0.345 ± 0.081	0.429 ± 0.100
a_2 (L min^{-1})	0.464 ± 0.068	0.410 ± 0.070	0.519 ± 0.068	0.450 ± 0.052	0.421 ± 0.054	0.480 ± 0.050	0.449 ± 0.066	0.408 ± 0.071	0.490 ± 0.064
3 repetitions									
d_2 (s)	24.35 ± 9.61	12.89 ± 9.46	35.81 ± 12.31	28.22 ± 7.72	24.84 ± 7.85	31.60 ± 7.71	30.74 ± 7.52*	25.82 ± 7.52	35.67 ± 7.77
τ_1 (s)	1.60 ± 1.99	−5.13 ± 4.59	8.33 ± 5.54	2.55 ± 1.62	0.30 ± 1.57	4.80 ± 1.86	4.53 ± 3.73*	0.11 ± 2.42	8.96 ± 5.87
τ_2 (s)	15.60 ± 7.27	3.28 ± 9.73	27.92 ± 6.80	12.62 ± 4.56	8.14 ± 4.80	17.09 ± 4.69	13.57 ± 5.83	6.26 ± 6.78	20.89 ± 5.55
a_1 (L min^{-1})	0.374 ± 0.076	0.262 ± 0.097	0.486 ± 0.075	0.401 ± 0.093	0.356 ± 0.100	0.447 ± 0.089	0.414 ± 0.113	0.341 ± 0.113	0.487 ± 0.124
a_2 (L min^{-1})	0.474 ± 0.094	0.365 ± 0.098	0.582 ± 0.108	0.451 ± 0.102	0.406 ± 0.095	0.495 ± 0.111	0.427 ± 0.087	0.356 ± 0.082	0.499 ± 0.103

Data are given as mean and standard deviation (SD), with the average of the lower (CI−) and upper (CI+) individual 95 % confidence intervals bounds

d_2 time delay of the second exponential, τ_1 time constant of the first exponential, τ_2 time constant of the second exponential, a_1 amplitude of the first exponential, a_2 amplitude of the second exponential

* Significantly different from None. $N = 8$

Table 2 Percentage of model's parameters from synthetic data with estimated errors (difference between noiseless estimated parameters and the corresponding exact parameters and between noisy estimated parameters and the corresponding exact parameters) that are smaller than the confidence intervals: comparison of results from the three pre-treatment procedures, as a function of exact d_2

Exact d_2 (s)	τ_1						τ_2					
	Noiseless			Noisy			Noiseless			Noisy		
	None	Linear	Step	None	Linear	Step	None	Linear	Step	None	Linear	Step
8–12	94.94	4.43	0.00	17.09	10.76	5.70	94.30	24.68	5.70	10.13	8.86	12.03
12–16	97.16	2.84	4.26	15.60	12.06	7.09	97.16	27.66	11.35	10.64	13.48	13.48
16–20	99.32	2.74	0.68	17.12	13.01	8.22	100.00	34.93	19.86	17.81	12.33	6.85
20–24	99.32	1.37	5.48	23.97	17.12	10.96	98.63	44.52	20.55	10.96	17.81	11.64
24–30	99.57	2.56	4.27	23.50	13.68	8.97	99.57	35.04	15.38	11.97	14.10	12.82
30–35	98.86	2.86	4.57	20.00	18.86	6.86	98.29	41.71	5.71	17.71	12.57	16.00
Exact d_2 (s)	a_1						a_2					
	Noiseless			Noisy			Noiseless			Noisy		
	None	Linear	Step	None	Linear	Step	None	Linear	Step	None	Linear	Step
8–12	96.20	36.08	18.99	13.92	11.39	9.49	96.20	33.54	18.35	12.03	12.03	8.23
12–16	97.87	56.74	27.66	19.15	14.18	15.60	98.58	52.48	27.66	19.86	14.18	15.60
16–20	100.00	77.40	47.95	21.23	19.18	15.75	100.00	73.29	47.95	22.60	19.18	13.01
20–24	100.00	91.78	71.92	27.40	28.77	23.29	100.00	86.99	69.18	26.71	24.66	19.86
24–30	99.57	89.32	79.49	32.05	29.91	30.34	99.57	94.02	78.63	30.34	32.91	29.49
30–35	98.86	93.71	90.29	37.71	33.71	33.71	98.86	95.43	88.00	33.71	36.57	30.86

Data are given as a percentage of the total noiseless estimated and noisy estimated parameters for bins of exact d_2, for each pre-treatment procedure

d_2 time delay of the second exponential, τ_1 time constant of the first exponential, τ_2 time constant of the second exponential, a_1 amplitude of the first exponential, a_2 amplitude of the second exponential

although at a lesser extent, after analysis of experimental data.

Intuitively, the apparent best performance of None can be explained by the fact that Linear and Step distort the recorded data and thus indirectly influence the constants describing the model's function. In the case of Linear and Step, this effect is not completely predictable because we are in presence of non-uniform breath sampling—that is random-like—in the time domain. This means that the influence of the interpolation procedures on two identical $\dot{V}O_2$ kinetics repetitions, and thus on the model's constants, is not the same in different trials, because the sampling times change at each repetition of the experimental protocol. In other words, the distortive effect depends on the sampling's characteristics.

Fitting procedures are such that any fitting model, which moves smoothly through the data, is tantamount to a precise filter for the data. Assuming that all repetitions carried out by one given subject, who performs a given exercise with identical protocol, are indeed representative of the same experimental condition, superposing data points from different repetitions of the same well-controlled event, as done with None, provides the necessary amount of data points allowing for a highly accurate computation of model parameters by the fitting procedure. This is a general concept, which holds for any type of experimental condition and model fitting procedure, wherein repeated discrete measurements of the same event in the same condition are made. This being the case, there is no need of introducing data interpolation procedures in the analysis of $\dot{V}O_2$-on kinetics, as done with Step and Linear.

The model analysis of the $\dot{V}O_2$-on kinetics in this study was carried out using the double exponential model that Barstow and Molé (1987) developed after an original idea of Wasserman et al. (1974). The model was widely used ever since, and was thoroughly criticized by Lador et al. (2006), who first applied the same concept to the analysis of beat-by-beat kinetics of cardiac output. We are nevertheless aware of the incompleteness of this model, which does not account for pulsatile phenomena affecting pulmonary blood flow, as a consequence of the rhythmic activity of the heart and of the lungs and of the heterogeneous recruitment of lung capillaries (Arieli 1983; Marconi et al. 1988; Meyer et al. 1989; West et al. 1964). Further advances in modelling should account also for these phenomena.

In the context of the double exponential model of Barstow and Molé (1987), a comparison of the three pre-treatment methods, carried out on noiseless synthetic data, showed that None was more precise than Linear and Step in describing the $\dot{V}O_2$-on kinetics, as demonstrated by the distribution of points on the line of parameters' equality. Among the parameters estimated by the bi-exponential model, the largest differences between the three pre-treatment procedures were observed for τ_1 (Fig. 3), as expected. None showed less point scattering (imprecision) than Linear, and particularly than Step. It provided the best estimate of τ_1 with respect to the exact values, whereas Linear and Step clearly and systematically overestimated τ_1. In other terms, τ_1 resulted smaller (faster) with None than with any of the two other methods. This is in line with the expectations associated with the introduction of interpolation procedures with Linear and Step. To sum up, due to the absence of added data interpolation, None can be considered as the best of the three tested procedures when fast temporal parameters, like τ_1, are to be estimated.

All three procedures provided more precise results in estimating τ_2 than τ_1, because τ_2 was computed on a greater number of data points, due to the longer duration of phase II of the $\dot{V}O_2$ kinetics and to the concomitant increase in breathing frequency. In spite of this, the analysis of both noiseless and noisy synthetic data shows that Linear and Step still overestimated τ_2, especially when it was low, but this tendency was progressively reduced as long as τ_2 was increased, to disappear for τ_2 values higher than 35 s. This is coherent with the concept that the distortive effect of Step and Linear on calculated parameters was inevitably less, the higher was τ_2. This effect was not such as to affect experimental τ_2 values, which were similar, and did not differ significantly among the three pre-treatment procedures. The low experimental τ_2 values are therefore not a consequence of None with respect to Step and Linear. They may rather be an intrinsic consequence of the Grønlund algorithm, as demonstrated by Cautero et al. (2003), combined with the lightness of the selected power, well below the ventilatory threshold in all subjects. At comparable powers, similar results to the present ones (τ_2 of 14.1 ± 6.6 s, Aliverti et al. 2009) were obtained using a direct estimate of end-expiratory lung volumes with an opto-electronic plethysmography technique (Cala et al. 1996).

Concerning amplitudes, tendencies to overestimate a_1 by Linear and Step were counteracted by opposite tendencies to underestimate a_2. As a consequence, the total amplitude of the $\dot{V}O_2$ response ($a_1 + a_2$) was fairly well estimated by all pre-treatment procedures, in agreement with the notion that they all are sufficiently accurate when used in steady state conditions, because the "flatness" of the breath time series underscores the distortive effect of the interpolation method.

The presence of noise reduced in all cases the method's precision, as shown by the increase in data dispersion around the line of parameters' equality and by the smaller fraction of data within one confidence interval for noisy synthetic data than for noiseless synthetic data (Table 2). This, however, did not affect accuracy, as long as in Fig. 3 and in Supplementary material, the line of parameters' equality remained outside the confidence intervals for Linear and Step, contrary to None. To sum up, when noiseless synthetic data are accounted for, None resulted both very precise and accurate, because there was no noise and no data distortion, whereas Linear and Step were less precise than None, despite the absence of noise, and inaccurate, due to data distortion. When noisy synthetic data are accounted for instead, there was in all cases a loss of precision due to the introduction of noise, but since noise did not affect accuracy, None remained accurate and Linear and Step remained inaccurate.

Lamarra et al. (1987), who first proposed and discussed Step, were aware of the fact that Step introduces a filter whose time constant is the mean breath duration, which removes only high-frequency fluctuations. Coherently, we demonstrated on noiseless synthetic data that the accuracy of Step tended to increase with increasing τ_2. Although Step provided a correct estimate of τ_2 when this was higher than 35 s, it must not be used when time constants lower than 30 s are expected.

Linear (Barstow and Molé 1991; Hughson et al. 1993) tried to circumvent the limits of Step through an apparently less distortive interpolation. The noisy synthetic data reported in Table 2 indicated that it succeeded at least partly, since for a large number of d_2 ranges Linear provided better performance than Step, especially as far as the computation of τ_1 was concerned. The physiological concept behind Linear is that the rate of alveolar gas exchange varies within a single breath, so that we may assume a continuous $\dot{V}O_2$ increase from one breath to the next during the $\dot{V}O_2$-on kinetics. This $\dot{V}O_2$ increase was considered to be linear. Yet the distortive effect was still maintained, especially in the determination of τ_1, whose values may be within the duration of a single breath. In fact Linear still remained an inaccurate method, as demonstrated by its overestimate of τ_1, and by no means had it attained the accuracy and precision of None (Fig. 3).

Coherently with the synthetic data, experimental data showed the same amplitudes for the three pre-treatment methods. Significant differences were observed only for τ_1, as None provided, as expected, faster (lower) values than Step. As discussed above, this may be due to the filter-like effect implicit in the latter procedures. The 2-s difference for d_2 with Step, although non-significant, corresponds to the shift with respect to the line of parameters' equality observed for Step on noisy data (Fig. 2, lower right panel).

When 3 repetitions were considered instead of 10, the lack of significant differences with respect to the latter suggested that 3 repetitions may be enough indeed for a satisfactory analysis of the $\dot{V}O_2$-on kinetics. A closer inspection of the data, however, revealed some ambiguities. With three repetitions in fact (1) d_2 turned out significantly lower with None than with Step; (2) τ_2 tended to be slower with None. Despite the suggestions of this study, a more specific study, aimed at identifying more accurately the minimum number of repetitions for the establishment of a correct $\dot{V}O_2$-on kinetics should be envisaged.

In this study, we did not consider d_2 values lower than 8 s, because d_2 represents the blood transit time from contracting muscles to lungs at exercise start, which is between 7 and 9 s at the present steady state metabolic rate (Barstow and Molé 1987; Lindholm et al. 2006; Whipp et al. 1982). In fact Table 2 shows that for None there was no effect of d_2 on the fraction of synthetic noiseless data that deviated from the Exact Parameters by less than the width of the confidence intervals. By contrast, this was not so with Linear and Step, for which the lower were the *exact* d_2 values, the less precise were the corresponding estimated τ_2, a_1 and a_2.

Conclusions

In conclusion, None revealed better precision and accuracy than Step and Linear, especially when phenomena characterized by time constants of <30 s are to be analysed. The addition of noise, however, reduced the differences among pre-treatment procedures, so that None maintained an added value with respect to Linear only for the computation of time constants, but not for the computation of amplitudes. Therefore, we endorse the utilization of None as a tool to improve the quality of breath-by-breath pulmonary gas exchange analysis during exercise transients, especially when a double exponential model (Barstow and Molé 1987) is applied and phase I is accounted for. We are nevertheless aware that, although these results represent a significant step forward in the treatment of experimental breath-by-breath data under unsteady state conditions, further computational developments, accounting also for the effects of concomitant oscillatory mechanisms, are still necessary to attain a fully satisfactory comprehension of the breath-by-breath $\dot{V}O_2$ data.

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