Abstract

Purpose

The aim of the study was to compare the kinetics responses of heart rate (HR), pulmonary ($\text{V'O}_2\text{pulm}$) and predicted muscular ($\text{V'O}_2\text{musc}$) oxygen uptake between two different pseudo-random binary sequence (PRBS) work rate (WR) amplitudes both below anaerobic threshold.

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Results

HR and $\text{V'O}_2\text{musc}$ kinetics seem to be independent of WR intensity ($p > 0.05$). $\text{V'O}_2\text{pulm}$ kinetics show prominent differences in the lag of the CCF maximum ($39 \pm 9s; 31 \pm 4s; p < 0.05$).

Conclusions

A mean difference of 14 W between the PRBS WR amplitudes impacts venous return significantly, while HR and $\text{V'O}_2\text{musc}$ kinetics remain unchanged.
Analysis of Heart Rate and Oxygen Uptake Kinetics Studied by Two Different Pseudo-Random Binary Sequence Work Rate Amplitudes

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Keywords

Kinetics analysis, oxygen uptake, venous return, physiological modelling, moderate exercise

intensity
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<tr>
<td>AT</td>
<td>Anaerobic threshold</td>
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<td>Cross-correlation function</td>
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<td>HR</td>
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<td>PRBS</td>
<td>Pseudo-random binary sequence</td>
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<td>Q'</td>
<td>Cardiac output</td>
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<td>Q'_rem</td>
<td>Perfusion of non-exercising tissues</td>
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<td>SDS</td>
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1. Introduction

The measurement of oxygen uptake (\(V'O_2\)) kinetics implies valuable indications of the aerobic metabolism (Wassermann 1984, Bassett & Howley 2000), which is commonly used as a general proxy for exercise-induced muscle contractions (Wasserman et al. 1973, Di Prampero 1981). \(V'O_2\) kinetics are positively correlated with maximal \(V'O_2\) capacity (Chilibeck et al. 1996) and are improved by or following aerobic exercise training (Cerretelli et al. 1979; Fukuoka et al. 2002; Murias et al. 2010, Koschate et al. 2016a).

\(V'O_2\) kinetics are relevant for a performance evaluation in all kinds of endurance sports (e.g. running (Millet et al. 2002), swimming (Rodriguez et al. 2003), skating (Hettinga et al. 2009)), in daily life activities (e.g. stair climbing (Koufaki et al. 2002)), in some kinds of profession (e.g. fire fighters (Harvey et al. 2008)), in rehabilitation processes (e.g. in chronic obstructive pulmonary disease (Casaburi et al. 1997)) as well as for older adults (e.g. quality of life (Alexander et al. 2003)).

The mentioned examples comprise different exercise intensities and accordingly specific metabolic demands in each case. It is therefore essential to account for these differences in exercise intensity to evaluate the specific aerobic exercise performance for an assessment of the muscular and circulatory health status and functionality of the involved physiological systems.

A common approach for the analysis of \(V'O_2\) kinetics is the realization of repeated step changes in work rate (WR). This procedure utilizes the application of double exponential mathematical equations to differentiate the pulmonary \(V'O_2\) (\(V'O_2\)pulm) response into a cardio dynamic (phase I) and a fundamental (phase II) component (Barstow & Molè 1987; Barstow & Molè 1991). Phase I of \(V'O_2\)pulm implies the fast cardiovascular responses to exercise during the transients (Whipp et al. 1982; Barstow & Molè 1987; Barstow et al. 1990; Grassi et al. 1996; Koga et al. 1996; Lador et al. 2006; Lador et al. 2008). The beginning of Phase II of \(V'O_2\)pulm denotes the arrival of the deoxygenated blood draining through the
venous volume from the exercising muscles to the lungs. Thus, the kinetics responses of
\( V'O_2 \) in phase II reflect closely muscle \( V'O_2 \) (Grassi et al. 1996) and phosphocreatine
kinetics (Binzoni et al. 1992; Binzoni et al. 1997; Rossiter et al. 2002).

It could be shown, that the time constants of phase II \( V'O_2 \) are independent of power
output if the baseline is in the low WR range (Whipp & Ward 1990; Barstow et al. 1993;
Whipp 1996; Xu & Rhodes 1999; Hughson 2009; Robergs 2014). With elevated baseline WR
phase II \( V'O_2 \) becomes sluggish and time constants are slowed (Brittain et al. 2001;
MacPhee et al. 2005; Bowen et al. 2001; Spencer et al. 2011; Williams et al. 2013; Keir et al.
2014). Keir et al. (2016) demonstrated that below anaerobic threshold (AT) time constants of
phase II \( V'O_2 \) are slowed with increasing baseline WR. In addition, Keir et al. (2016)
could show, that below AT time constants of phase II \( V'O_2 \) are not altered with
progressively increasing WR amplitudes from a constant baseline WR. In contrast, DiMenna
et al. (2010) illustrated that for high exercise intensities (261 ± 34W) the baseline WR is not
influencing phase II \( V'O_2 \) time constants. As can be seen, this issue is still controversial
and it remains to be reconciled in prospective studies (DiMenna & Jones 2009).

However, the double exponential procedure cannot be applied for chirp (e. g. Roman et al.
2012; O’Connor et al. 2016), sinusoidal (e. g. Casaburi et al. 1977; Fukuoka et al. 2017) and
fast repetitive transitions in WR (pseudo-random binary sequence protocols (PRBS)). In this
context, the practical approaches of Hughson et al. (1991), Edwards et al. (2003) and
Hoffmann et al. (2013) enable the kinetics analysis of cardiovascular and pulmonary
parameters with rapid WR changes, by application of time-series analysis (e.g. cross-
correlation functions (CCF)) and PRBS WR protocols. The novel computational approach of
Hoffmann et al. (2013) comprises a circulatory transfer model with a pulmonary and an active
muscle site accounting for non-linear venous return conjunctures between exercising
musculature and gas exchange at the lungs. The model of Hoffmann et al. (2013) allows a
differentiation between predicted muscle (as backward calculation) and measured pulmonary
\( V'O_2 \) kinetics by considering venous return derived from cardiac output (Q’) estimates.
For this reason, we aimed to check whether the novel computational approach of Hoffmann et al. (2013) provides comparable results as from previous studies with the traditional double exponential model. In particular, investigating the impact of two different PRBS WR amplitudes – below AT – with the same baseline WR on cardiovascular and pulmonary parameters. Therefore, we tested the following hypothesis: The kinetics responses of heart rate (HR), V'O₂pulm and muscle oxygen uptake (V'O₂musc) are independent of WR amplitudes with equal baseline WR.

2. Methods

2.1 Subjects

Eight healthy and physically active subjects [seven men, one woman, age: 26 ± 4 years; height: 177 ± 7 cm; weight: 77 ± 11 kg; body mass index: 24.5 ± 2.8 kg/m²; V'O₂peak: 4.2 ± 0.9 L · min⁻¹; relative V'O₂peak: 54.2 ± 8.9 mL · min⁻¹ · kg⁻¹; AT (Beaver et al. 1986): 2.6 ± 0.6 L · min⁻¹ (mean ± SD)] participated in this study. All participants were informed about the study aims and the measurements and gave their written informed consent before participating. The study was approved by the Ethics Committee of the German Sport University Cologne which was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.2 Exercise protocols

Each subject performed three exercise tests in a seated position (seat back at 45°, leg exercise device at 42° relative to ground level) on a recumbent cycle ergometer (Cardiac Stress Table, Lode B. V., Netherlands). Two of the tests comprise PRBS WR changes between two different intensities: a) between 30 and 80 W and b) between 30 and 110 W. In each of the two protocols we applied two PRBS phases in series, in which each PRBS phase last 300s. Before and after the PRBS phases low (30 W in each case) and high (80 W, 110 W) constant WR phases were conducted in both cases (see Fig. 1). Followed by two
incremental WR steps each with 30 W · 2 min⁻¹, which are not in focus of the kinetics analysis and will therefore not be considered subsequently.

<< Fig. 1 >>

The third exercise test implied three WR steps of 50 W, 100 W and 150 W each for 3 minutes, followed by incremental WR steps of 25 W · min⁻¹ until voluntary exhaustion. This test was always performed one day after the moderate exercise test with the low PRBS WR amplitude (30W – 80W). Three of the high PRBS WR amplitude tests (30W – 110W) were conducted 10 ± 9 days before and five tests 15 ± 21 days after the low PRBS WR amplitude test. Three of the high PRBS WR amplitude tests were carried out 11 ± 9 days before and five tests 14 ± 21 days after the exercise test until voluntary exhaustion. All exercise tests were performed within a time span of 14 ± 17 days. All subjects were requested to maintain their daily life activities to avoid remarkable strain conditions (e.g. endurance or resistance exercise training procedures) while participating in the study.

2.3 Cardio pulmonary exercise testing (CPET)

VO₂pulm was measured breath-by-breath (ZAN 680, ZAN Messgeräte GmbH, Oberthulba, Germany) during the exercise tests. For corrections of alveolar gas exchange the algorithms of Beaver et al. (1981) were applied. HR and stroke volume were estimated beat-to-beat continuously during the exercise tests by R-R intervals from electrocardiography (ECG) and by impedance cardiography (ICG), respectively (TaskForce®Monitor, CNSystems Medizintechnik AG, Graz, Austria; see Fortin et al. 2006). All measurement devices were calibrated in accordance to the manufacturer’s instructions before testing.

2.4 Data processing

For the subsequent analysis all data were time-aligned and computed into equidistant (1 s) time intervals applying a linear interpolation procedure. To account for influences of the interpolation interval (1 s) on the calculations of the kinetics responses we estimated the
deviations from the 1s-intervals (1 Hz) compared to different measurement frequencies (0.25 Hz, 0.5 Hz, 2 Hz, 3 Hz; see Fig. 4 and section Results for further explanation) as approximation of beat-to-beat and breath-by-breath data.

For the calculation of HR and V'O₂pulm slopes as a function of WR we averaged these parameters at 4 points in time for both exercise protocols (Fig. 1). Then, we calculated the mean values separately during the whole PRBS time periods (300 s each) for both PRBS amplitudes (low, high). In addition, we estimated mean values for HR and V'O₂pulm at rest within 30 s prior to exercise, during the last 30 s at the end of low (30 W) and high (80 W and 110 W, respectively) constant WR phases for both exercise protocols.

2.5 Kinetics analysis

For the kinetics analysis of the parameters of interest we applied the procedure presented by Hoffmann et al. (2013). The application of CCF enables a direct and feasible alternative to the commonly used multi-phase exponential model (Barstow & Molé 1991, Hughson 2009, Ma et al. 2010). The CCF approach constitutes procedures that do not need data fitting efforts by definition which is a benefit for the analysis. Time-series analysis is a useful method for complex dynamics and time shifts in distorted and sluggish responses (Lamarra et al. 1987). Additionally, time-series analysis improves the signal-to-noise ratio, and is therefore well suited for a meaningful analysis of dynamics responses (Hoffmann et al. 2013). The kinetics analysis provides different kinetics information: a) the maximum (CCFₘₐₓ) and the lag (CCFₗ₉₉) of the CCF course, and b) the CCF time course itself as extensive kinetics quality information. Higher CCFₘₐₓ-values denote faster system responses (independent of system properties) of the parameter analyzed, and vice versa. Longer CCFₗ₉₉-values imply time-delayed system responses (independent of system properties) like a rightward shift of the CCFₘₐₓ, and also vice versa. In this regard, the CCFₘₐₓ and the CCFₗ₉₉-values have to be seen as more rough estimations, because they comprise compressed and reduced information about the kinetics responses. In contrast, the entire CCF course implies the more complex and overall kinetics responses.
As introduced by Hoffmann et al. (2013) the CCF of 1\textsuperscript{st} order exponential systems illustrate a distinct CCF profile as response to the ACF of WR as a virtual WR protocol (see Fig. 2b; Hoffmann et al. 2013). In this regard, deviations from or deformations of this specific CCF profile may suppose contradictions to 1\textsuperscript{st} order model properties. These contradictions could be related to asymmetries in on- and/or off-kinetics or distortions of Q’ in V’O\textsubscript{2}pulm kinetics for instance. If we assume a 1\textsuperscript{st} order exponential system and we calculate the kinetics responses in the correlation domain as CCF course, then the maximum of the CCF (CCF\textsubscript{max}) can be used as a proxy for describing the dynamics of the system within 100% accuracy and without further information. However, if the CCF profile is distorted and the CCF\textsubscript{max} is not affected, then it would not be appropriate to use the CCF\textsubscript{max} value as a reliable representative of the entire CCF course, because the system response is in contradiction to 1\textsuperscript{st} order system properties. From there, inferences about the entire 1\textsuperscript{st} order system dynamics using only CCF\textsubscript{max} values have to be taken with caution. As illustrated by Koschate et al. (2016; Fig. 3c) the CCF profile of V’O\textsubscript{2}pulm during treadmill walking shows a biphasic course with one absolute and one local maximum. This is a practical example showing that CCF\textsubscript{max} values may not represent the entire CCF course of the dynamic 1\textsuperscript{st} order system responses in principal. Therefore, the entire CCF course is of major importance for an integrated evaluation of the kinetics responses.

The CCF\textsubscript{max} value of V’O\textsubscript{2}pulm and also the entire CCF profile comprises the integrated response of V’O\textsubscript{2} kinetics without any data exclusions. That means that the information ‘stored’ in CCF\textsubscript{max} can be described as pooling of phase I (cardiodynamic) and phase II (primary) of multi-exponential models.

In the successive kinetics analysis we will focus on both the comprehensive CCF\textsubscript{max}- and CCF\textsubscript{lag}-values as well as the entire CCF course.

The circulatory distortions in V’O\textsubscript{2}pulm comprise the variable (non-linear) muscle-to-lung transit times of desaturated blood and the flow-weighted mixing of muscle oxygen content with venous blood of the remainder of the body in the transient phases (Hoffmann et al.}
Taken this entry, $V'O_2\text{musc}$ was calculated as described by Hoffmann et al. (2013) applying a circulatory model allowing for venous return ($V_v$ – venous blood volume) between exercising leg muscles and the lungs, as well as oxygen consumption ($V'O_2\text{rem}$) and perfusion ($Q'_\text{rem}$) of the non-exercising body compartments. This approach was applied to account for the distortive and time-delaying effects of venous return and $Q'$ between $V'O_2\text{musc}$ and $V'O_2\text{pulm}$. By means of this approach it is possible to equalize the $V'O_2\text{pulm}$ response from the cardiovascular distortions and 'extract' the predicted $V'O_2\text{musc}$ kinetics responses.

In dependence on Hoffmann et al. (2013) assuming 1st order system properties, $CCF_{\text{max}}$- and $CCF_{\text{lag}}$-values can be transferred into time constants ($\tau$) and time delays (TD), which allows a comparison with multi-phase exponential models. TDs were calculated as the difference between the measured $CCF_{\text{lag}}$ of $V'O_2\text{pulm}$ with WR and from the anticipated $CCF_{\text{lag}}$ calculated from the real-estimated $CCF_{\text{max}}$-value of $V'O_2\text{pulm}$.

### 2.6 Statistical analysis

The slopes of HR and $V'O_2\text{pulm}$ as functions of WR, the $Q'-V'O_2\text{pulm}$ relationships, $CCF_{\text{max}}$, $CCF_{\text{lag}}$, $\tau$ and TD were analyzed for differences between the PRBS amplitudes with Wilcoxon signed ranks tests.

For analysis of static linearity comparisons in the time domain (HR, $V'O_2\text{pulm}$) a one-way repeated measures analysis of variance (ANOVA) were applied to account for Work rate effects at low (30 W), high (80 W & 110 W), and during the dynamic PRBS phases (53.3 W & 67.3 W). Pairwise Post-hoc comparisons were performed via Bonferroni tests.

To account for comparisons of kinetics responses between CCF courses at specific lag times (10s-intervals) and dimension of the PRBS WR amplitudes a two-way ANOVA with factors $Lag$ and $Amplitude$ was performed for HR, $V'O_2\text{pulm}$, and $V'O_2\text{musc}$ kinetics, respectively. In case of significant factors or interactions Post-hoc pairwise comparisons by Bonferroni were conducted.
Spearman's rank correlation coefficient was calculated for the analysis of relationships between parameters. Statistical significance was set to 0.05 for the alpha level (software package: IBM SPSS statistics 23).

3. Results

3.1 Heart rate and pulmonary V'O₂ responses in the time domain

To account for WR influences on HR and V'O₂pulm we applied a one-way ANOVA with repeated measures with factor Work rate in one analysis design with eight mean values (2 x 4) implying both PRBS WR tests (see Tab. 1). For both, HR and V'O₂pulm a significant Work rate effect was observed (p < 0.001).

<< Tab. 1 >>

In contrast, no significant differences in the slopes of HR and V'O₂pulm, respectively, were revealed between the two different RPBS WR amplitudes.

3.2 The Q' – V'O₂pulm relationship

We calculated the slopes of Q' as function of V'O₂pulm for the low (5.6 ± 1.6) and the high (5.5 ± 1.2) PRBS WR amplitudes. Comparing both slopes against each other showed no significant difference between the conditions (p > 0.05).

<< Fig. 2 >>

Fig. 2 displays the means (± SE) of Q' as function of V'O₂pulm, for both PRBS WR amplitude tests. In each case, both PRBS WR amplitudes show a high linear relationship with a coefficient of determination > 0.97.

3.3 Respiratory exchange ratio (RER)
The respiratory exchange ratios (RER, Fig. 3) imply that during the dynamic leg exercise all subjects are well below a value of 1.0 for both, the low and the high PRBS WR amplitude.

3.4 Impact of interpolation procedure on kinetics analysis

For the assessment of different measure frequencies (e.g. beat-to-beat HR, breath-by-breath gas exchange) on the 1s-interpolation values we calculated HR and breathing frequencies (BF) during the time span (time: 200s to 800s) in which the kinetics analysis was performed. During the low PRBS WR amplitude we calculated HR and BF as 96.9 ± 3.6 min⁻¹ and 19.3 ± 1.1 min⁻¹, and for the high PRBS WR amplitude as 106.1 ± 5.5 min⁻¹ and 19.0 ± 0.8, respectively. In accordance, the physiological triggered beat-to-beat and breath-by-breath frequencies are estimated for HR and BF for the low PRBS WR amplitude as 1.61 Hz & 0.32 Hz and as 1.77 Hz & 0.32 Hz for the high PRBS WR amplitude, respectively.

For the calculation of the temporal profiles for VO₂ we applied a mono-exponential equation with time constants of 10s, 20s, 30s, 40s, and 50s without TD for 0.25 Hz, 0.5 Hz, 2 Hz and 3 Hz, respectively. As amplitude between the low (30 W: 0.983 L·min⁻¹) and high (80 W: 1.374 L·min⁻¹) WR constant phase we calculated the averages from the low PRBS WR amplitude exemplarily. Thereafter we applied the kinetics analysis as described in the methods section kinetics analysis.

Fig. 4a illustrates the per cent changes in the CCFₘₐₓ values derived from the relative difference between the CCFₘₐₓ of either 0.25 Hz, 0.5 Hz, 2 Hz or 3 Hz and the CCFₘₐₓ values of the 1Hz triggered data (as basis) as function of actual time constants.

Negative values in Fig. 4a comprise an underestimation (slowed dynamics) and positive values an overestimation (speeded dynamics) in the kinetics information; these data are shown in consideration of the original-approximated physiological triggered data (0.25 Hz,
0.5 Hz, 2 Hz, 3 Hz) and the applied 1s-interval (1 Hz) interpolation method as basis of comparison. For time constants of 10s the kinetics responses will be slowed dependent on the frequencies of the physiological data (0.25 Hz: -0.54%; 0.5 Hz: -0.19%; 2 Hz: -0.05% 3 Hz: -0.06%). For time constants of 20s, frequencies of 0.25 Hz (-0.24%) and 0.5 Hz (-0.04%) will be estimated as slowed and frequencies of 2 Hz (+0.06%) and 3 Hz (+0.12%) will be speeded. For time constants of 30s frequencies of 0.25 Hz (-0.10%) will be estimated as slowed and frequencies of 0.5 Hz (+0.07%), 2 Hz (+0.15%) and 3 Hz (+0.23%) will be speeded. For time constants of 40s frequencies of 0.25 Hz (-0.04%) will be estimated as slowed and frequencies of 0.5 Hz (+0.12%), 2 Hz (+0.21%) and 3 Hz (+0.28%) will be speeded. For time constants of 50s frequencies of 0.25 Hz (-0.01%) will be estimated as slowed and frequencies of 0.5 Hz (+0.14%), 2 Hz (+0.22%) and 3 Hz (+0.31%) will be speeded.

Fig. 4b displays the per cent changes in time constants derived from the relative difference between the time constants derived from the CCF$_{max}$ of 0.25 Hz, 0.5 Hz, 1 Hz, 2 Hz or 3 Hz and the actual time constants as function of actual time constants. In addition, Fig 4c illustrates the absolute changes in time constants derived from the relative difference between the time constants derived from the CCF$_{max}$ of 0.25 Hz, 0.5 Hz, 1 Hz, 2 Hz or 3 Hz and the actual time constants as function of actual time constants as well. Considering the mentioned findings above, we suppose, that the 1s-interpolation method has small but negligible influence on the applied kinetics analysis.

It can be recognized, that fast time constants from 10s to 30s seem not to be impacted by the kinetics analysis using the approach of Hoffmann et al. (2013). However, slower kinetics responses with time constants of 40s and 50s are slightly dampened, which is in the range of −0.50% (−0.20s) and −1.82% (−0.91s), respectively.
3.5 Kinetics analysis

Fig. 5 illustrates the kinetics responses of HR (5a), V'O₂pulm (5b) and V'O₂musc (5c). For a deeper matter kinetics analysis we applied a two-way ANOVA with factors Lag and Amplitude at specific lag times; we calculated relevant mean values from lag '-20 s' to '80 s' with 10-s-intervals for the parameters in Fig. 5, which can be observed in the upper right graphs.

For HR, V'O₂pulm and V'O₂musc the factor Lag was significant (p < 0.05) implying that within the lag times of each parameter significant differences between at least two mean values exist. We did not perform post-hoc comparisons between the different lag times for all three parameters because these significant differences display the typical and specific responses as a result of the triangular WR pattern of each of the parameter analyzed and need no further investigation. This is so, because the ACF of WR illustrates a virtual WR pattern, and its responses (e.g. HR, V'O₂pulm, V'O₂musc) will respond as a function of this WR pattern with its particular system properties. For instance, HR and V'O₂pulm show a positive (linear) relationship as a function of WR. In the correlation domain (ACF, CCF) these system properties are still existing and the same as in the time domain. Therefore, the triangular WR pattern (ACF) can be seen as an increasing followed by a decreasing ramp. Both parameters (HR, V'O₂pulm) will respond to the increasing part of the ACF with increasing values, and related to the decreasing part of the ACF with declining values. For this reason, the factor Lag illustrates (if significant) the typical responses of the parameters of interest as a function of the WR pattern.

For factor Amplitude and interactions no significance (p > 0.05) was observed.

<< Fig. 5 >>

Fig. 6 shows the grouped mean data of the kinetics responses of HR, V'O₂pulm and V'O₂musc for the different PRBS WR amplitudes by means of CCF<sub>max</sub> as a function of CCF<sub>lag</sub>.

<< Fig. 6 >>
The CCF\textsubscript{max} values of HR, V'O\textsubscript{2}pulm and V'O\textsubscript{2}musc showed no significant difference between the two PRBS WR protocols (p>0.05 each), implying that the kinetics responses (assuming a 1\textsuperscript{st} order system) of these parameters are identical among the two different PRBS WR amplitudes (Tab. 2). For the CCF\textsubscript{lag} values of HR and V'O\textsubscript{2}musc no significant differences could be identified (p>0.05 each), but for CCF\textsubscript{lag} of V'O\textsubscript{2}pulm a significant difference across the PRBS WR amplitudes was observed (p<0.05). It seems that higher perfusion rates for instance during 110W (13.0 ± 1.3 L·min\textsuperscript{-1}) at the higher PRBS WR amplitude in contrast to lower perfusion rates during 80W (12.3 ± 1.4 L·min\textsuperscript{-1}) at the smaller PRBS WR amplitude move CCF\textsubscript{lag} to the left side, implying shorter venous transit times; lower perfusion is moving it to the right side, implying longer venous transit times.

The parameter V'O\textsubscript{2}pulm illustrates the typical right-shifted orientation of the CCF peak values (abscissa: CCF\textsubscript{lag}, ordinate: CCF\textsubscript{max}) compared to HR and V'O\textsubscript{2}musc as described earlier (Hoffmann et al. 2013; Drescher et al. 2015; Drescher et al. 2016; Koschate et al. 2016a; Koschate et al. 2016b; Koschate et al. 2016c).

The model parameters V\textsubscript{v}, Q\textsuperscript{rem} and V'O\textsubscript{2}rem of the remainder of the body revealed no significant differences between the two applied PRBS WR amplitudes (p > 0.05).

In addition to the CCF\textsubscript{max} and CCF\textsubscript{lag} values, we calculated the time constants and TD for HR, V'O\textsubscript{2}pulm and V'O\textsubscript{2}musc as a basis of comparison with the double exponential approach (see Tab. 2).

Fig. 7 comprises the comparisons of measured and simulated CCF courses for HR (7a, 7b), V'O\textsubscript{2}pulm (7c, 7d) and V'O\textsubscript{2}musc (7e, 7f), respectively, for both PRBS WR amplitudes. For simulation of the 1\textsuperscript{st} order system responses we utilized the CCF\textsubscript{max} values from each parameter and each PRBS WR condition and derived the corresponding \(\tau\). For estimation of each TD we placed the simulated CCF curve with its maximum exactly on the same lag such as the measured averaged CCF, again for each parameter and for both PRBS WR.
amplitudes. Additionally, we estimated the residuals (bottom in each graph) as difference between the simulated (expected) and the measured CCF courses. Furthermore, the sum of deviation (equal to residuals) squares (SDS) were calculated and displayed in the upper right corner in each main graph. SDS demonstrates contradiction of 1st order mono-exponential system properties with increasing values; smaller SDS-values indicate closer 1st order mono-exponential system characteristics.

<< Fig. 7 >>

Fig. 8 summarizes the SDS-values of HR, V'O₂pulm and V'O₂musc as measure of contradictions of 1st order system responses. HR shows the smallest deviations from 1st order system characteristics (30W–80W: 0.11; 30W–110W: 0.17) in contrast to V'O₂pulm which displays the greatest deviation during the 30W – 80W PRBS WR amplitude (0.48). But the 30W – 110W condition illustrated that SDS for V'O₂pulm is clearly reduced (0.15). Equally, V'O₂musc shows deviations with SDS-values of 0.15 (30W–80W) and 0.14 (30W–110W), respectively. It has to be noticed that in both PRBS WR amplitude conditions the mean CCF courses of V'O₂musc an early undershoot has been observed, which is a non-linear, anticipatory component and therefore contradictory to 1st order system characteristics. This early undershoot seems to be responsible for the most part for deviations from 1st order system properties in V'O₂musc kinetics responses.

<< Fig. 8 >>

Between CCF_{max} of V'O₂pulm and CCF_{max} of V'O₂musc we found a significant correlation for both PRBS WR conditions (30W – 80W: r = 0.905, p < 0.01; 30W – 110W: r = 0.762, p < 0.05). For CCF_{max} of V'O₂pulm significant relationships could be identified with V'O₂peak (r = 0.738, p < 0.05), relative V'O₂peak (r = 0.810, p < 0.05) and WR_{max} (r = 0.723, p < 0.05) for the 30W – 80W, but not for the 30W – 110W PRBS WR amplitude (p > 0.05). In addition, a significant relationship between CCF_{max} of HR and CCF_{max} of V'O₂musc for the 30W – 110W PRBS WR
amplitude could be detected \( r = 0.714, p < 0.05 \) but not for the 30W – 110W PRBS WR amplitude \( p > 0.05 \).

Analyzing the CCF\(_{\text{lag}}\) values we found significant correlations between HR with \( V'O_2\text{peak} \) \( r = -0.786, p < 0.05 \) as well as HR with relative \( V'O_2\text{peak} \) \( r = -0.810, p < 0.05 \) in the 30W – 80W PRBS WR condition. Additionally, a significant correlation in CCF\(_{\text{lag}}\) of HR between the two PRBS WR conditions could be identified \( r = 0.732, p < 0.05 \).

4. Discussion

The aim of the present study was to compare two different PRBS WR protocols in terms of the kinetics responses of HR, \( V'O_2\text{pulm} \) and \( V'O_2\text{musc} \) kinetics in the moderate WR domain below AT. The significant findings are the following:

a) In each case, HR and \( V'O_2\text{musc} \) kinetics show no significant differences in CCF\(_{\text{max}}\) and in CCF\(_{\text{lag}}\) between the two PRBS conditions.

b) A significant difference in CCF\(_{\text{lag}}\) of \( V'O_2\text{pulm} \) kinetics between the PRBS WR protocols was observed, while CCF\(_{\text{max}}\) of \( V'O_2\text{pulm} \) remains unchanged.

4.1 Static linearity

Static linearity is a prerequisite for the dynamic analysis (e.g. time-series analysis) for \( V'O_2 \) kinetics for instance. If there are marked deviations from static linearity, kinetics analysis of transients would be inadequate. In the present study HR and \( V'O_2\text{pulm} \) show highly linear characteristics for both PRBS WR amplitudes. For both parameters a significant Work rate effect could be identified applying a one-way repeated measures ANOVA with increasing WR. The static responses of both parameters reflect the fundamentals in exercise physiology, even the relationship between increased demands and the subsequent response – namely an increasing response – of the physiological parameters.

For the slopes of HR and \( V'O_2\text{pulm} \) no significant differences between the two PRBS WR conditions could be observed. Therefore, we assume that HR as well as \( V'O_2\text{pulm} \)
regulations may be unaltered in their linear characteristics as a function of WR in the exercise intensities analyzed here – below AT.

Interestingly, in this study the slopes of $V'O_2pulm$ seem to be somewhat smaller compared to other studies which should be in the range of $10 \text{ ml} \cdot W^{-1} \cdot \text{min}^{-1}$ (Whipp 1996). Calculating the 95%-confidence intervals for the $V'O_2pulm$ slopes for both PRBS WR conditions ($30W – 80W: 6.9 – 8.7 \text{ ml} \cdot W^{-1} \cdot \text{min}^{-1}$; $30W – 110W: 5.8 – 8.8 \text{ ml} \cdot W^{-1} \cdot \text{min}^{-1}$), these values are comparable to the extreme values of the slopes of pulmonary ($8.2 – 11.6 \text{ ml} \cdot W^{-1} \cdot \text{min}^{-1}$) and muscular $V'O_2$ ($5.4 – 13.8 \text{ ml} \cdot W^{-1} \cdot \text{min}^{-1}$) described by Poole et al. (1992). However, the direct comparison between the two PRBS WR conditions reveals no differences on the basis of the examined participants in the current study.

Regarding the slopes of Q’ as a function of $V'O_2pulm$, we found no significant differences between both PRBS WR conditions ($30W – 80W: 5.6 \pm 1.6; 30W – 100W: 5.5 \pm 1.2; p > 0.05$). This result is coherent with the computations of Lador et al. (2006, 2008) concerning phase I of $V'O_2pulm$ and reinforces the concept of a tight link between Q’ and $V'O_2pulm$ in exercise transients. Comparing our data with the literature (Rowell 1993), we are in line with an expected increase in Q’ of $6 \text{ L} \cdot \text{min}^{-1}$ per $1 \text{ L} \cdot \text{min}^{-1} V'O_2pulm$ increase.

4.2 Linear 1s-interpolation procedure

For impacts of the 1s-interpolation of the beat-to-beat and breath-by-breath data of HR and pulmonary gas exchange on the kinetics analysis by means of the $CFF_{max}$-values, we calculated relative changes as difference between the $CFF_{max}$ with various frequencies and $CCF$ of the standardized applied 1s-interpolation data. The results reveal, that minimal impacts result on the derived kinetics responses using the $CFF_{max}$-values. For instance, breath-by-breath responses with a $\tau$ of 10s and a frequency of 0.25 Hz will be slowed by about 0.54%, which results in a $\tau$ difference of about −0.156s. For example, beat-to-beat responses with a $\tau$ of 50s and a frequency of 3 Hz, will be speeded by about 0.31%, which is
a τ difference of about +0.210s. This is in line with Lamarra et al. (1987) who indicated that the filtering technique applying 1s-interpolations remove high-frequency fluctuations in the breath-by-breath responses which have insignificant influence on the estimated data. Summarizing the impact of the linear 1s-interpolation procedure on the beat-to-beat and breath-by-breath data applied in the present study, seems to be negligible, and should have therefore no practical implication on the entire kinetics analysis.

4.3 Kinetics analysis

The comparisons of the kinetics responses of HR, V'O₂pulm and V'O₂musc across the entire CCF courses by applying a two-way ANOVA with factors Lag and Amplitude revealed no significant differences between the two PRBS WR amplitudes.

Focusing on the CCF_{max} and CCF_{lag}-values of the mentioned parameters, only for the CCF_{lag} of V'O₂pulm a significant difference could be observed. This finding denotes that with increased WR the transit time of deoxygenated blood traveling from the exercising leg musculature to the lungs is shorter due to a higher Q' during the 30W – 110W PRBS WR condition. The mean difference in WR between the two PRBS amplitudes is 14 W (= 67.3 W – 53.3 W), which provokes a significant time-delaying change in venous return that is represented by the reduction in the CCF_{lag}-value of V'O₂pulm kinetics, resulting in a difference in the venous transit time of 8 s (= 39 s – 31 s). Due to these findings, it seems that such minimal WR changes, of only 14 W increase in mean exercise intensity have an obvious impact on venous return (dynamic transit time) that can be clearly specified on the leftward shift of the CCF_{lag}-value of V'O₂pulm – from increased perfusion.

Because the CCF_{max}-value and also the entire CCF course – aside from the CCF_{lag}-value – is not different between the two PRBS WR amplitudes, which fits in the current picture of V'O₂pulm kinetics control, implying an invariant and independent relationship between time constant of phase II V'O₂pulm (τV'O₂pulm) and WR intensity below AT (Mahler & Whipp

It has to be noted, that τ $V'O_2^{\text{pulm}}$ is not one-to-one comparable to CCF max of $V'O_2^{\text{pulm}}$. This is due to the fact, that τ $V'O_2^{\text{pulm}}$ is corrected for cardio dynamic influences during the early transients by discarding of phase I $V'O_2^{\text{pulm}}$. The CCF max-value of $V'O_2^{\text{pulm}}$ is not corrected for those cardio dynamic impacts, because the methodological approach does not provide such a feasibility. From there, it is expected that derived τ from CCF max-values of $V'O_2^{\text{pulm}}$ may differ from phase II τ $V'O_2^{\text{pulm}}$. That is why the CCF max of predicted $V'O_2^{\text{musc}}$ and basically the entire CCF course of $V'O_2^{\text{musc}}$ is estimated, which allows a comparison between phase II τ $V'O_2^{\text{pulm}}$ and τ derived from CCF max of $V'O_2^{\text{musc}}$, because both values are corrected for cardio dynamic influences.

In this regard, we could show that CCF max of $V'O_2^{\text{musc}}$ (or τ $V'O_2^{\text{musc}}$) are not significantly different in the moderate exercise domain by comparing the two PRBS WR amplitudes with the same baseline WR – this is in line with the current understanding of $V'O_2$ kinetics (Whipp & Ward 1990; Barstow et al. 1993; Whipp 1996; Xu & Rhodes 1999; Hughson 2009; Robergs 2014).

For the analysis of contradictions to 1st order systems responses the following aspects are relevant: a) the location of the CCF lag-value (abscissa) in relation to its CCF max-value (ordinate) b) the entire CCF course of the response, c) CCF increases at the earliest at lag –20s, and d) no CCF maximum before lag of 0s. If we assume a 1st order mono-exponential system without dead time, then the CCF max-value has to be located on or very close to the descending part of the ACF of WR. The entire, temporal CCF profile has to fit to mono-exponential characteristics, implying only one CCF maximum and monotonic increases (as response to the increasing part of the ACF of WR) or decreases (as response to the declining part of the ACF of WR) without superimposed oscillations. Additionally, increases of the CCF response earlier than at lag-values of –20s indicate clear discrepancies to 1st order
system responses, because such a response implies an increase in $V'O_2$ before the WR was increased.

If a dead time or a TD exist and can be determined in kinetics responses, e.g. in $V'O_2$pulm, then the difference between the measured $CCF_{lag}$ of $V'O_2$pulm and its predicted $CCF_{lag}$ derived from the measured $CCF_{max}$ can be applied for the evaluation of 1st order system responses. The first step would be, to test whether the calculated TD are not different from zero, which suggests conformity with 1st order system responses. If the TD is different from zero, then it has to be checked (2nd step) whether a left-shift of the entire CCF profile around the calculated TD would result in an early increase in the CCF response before lag-values of $-20s$ and a CCF maximum before lag $0s$. If so, contradictions to 1st order system responses are given. If not, the 3rd step would imply to check whether the entire CCF profile illustrate prominent disagreements to a mono-exponential profile derived by the sum of deviation squares (SDS) between measured and predicted CCF courses.

For the CCF course of $V'O_2$pulm during the 30W – 80W PRBS WR amplitude, there was the greatest deviation from 1st order system responses as a measure of residuals and summarized deviation squares (SDS: 0.48). For the other PRBS WR amplitude condition (30W – 110W) these deviations were markedly reduced for $V'O_2$pulm (0.15). This is in contrast to other publications (Mahler & Whipp 1980; Barstow & Molè 1991; Özyener et al. 2001; Scheuermann & Barstow 2003; Spencer et al. 2013), suggesting $V'O_2$pulm as a 1st order kinetics model.

For HR both CCF courses show similar deviations from 1st order system responses in the range between 0.11 and 0.17, respectively.

For $V'O_2$musc the deviations from 1st order system properties are nearly equal comparing the two PRBS WR amplitudes (0.14; 0.15). It has to be noticed that in both PRBS WR amplitude conditions the mean CCF courses of $V'O_2$musc, an early undershoot has been observed, which is a non-linear, anticipatory component and therefore contradictory to 1st order system
characteristics. This early undershoot seems to be responsible for the most part for deviations from 1st order system properties in V'O₂musc kinetics responses. In this regard, Grassi et al. (1996) demonstrated a time delay in V'O₂ in humans during transient leg exercise. This is contrasting to Behnke et al. (2002) who illustrated that V'O₂ at muscular site increases directly without an observable time delay. Also, Grassi et al. (1998a; 1998b) described a sluggish V'O₂ increase in isolated muscle which does not show mono-exponential responses during the transients. However, the comparison between the current literature and our results does not show any comparability in regard to the undershoot phenomenon. Therefore, the deviations from 1st order system properties for V'O₂musc – obviously based on the undershoot – have to be related to other origins in our study. We assume that the undershoot in the CCF courses of V'O₂musc may be related to collaterally modified Q' dynamics and possibly varying venous blood volumes during the rapid transients, which may limit the applicability of the circulatory model (Hoffmann et al. 2013).

4.4 Model parameters

The model parameters (Vᵥ, Q'rem, V'O₂rem) for the assessment of V'O₂musc kinetics indicate no significant differences between the two PRBS WR amplitudes. We therefore assume that equal conditions regarding the individual physiological condition of each participant was given (e.g. hydration status). The values of Vᵥ, Q'rem and V'O₂rem of the non-exercising body compartment are in the range comparable to other publications applying the same approach (e.g. Hoffmann et al. 2013, Drescher et al. 2015, Drescher et al. 2016).

4.5 Conclusion

In the present study we analyzed the kinetics responses of HR, V'O₂pulm and V'O₂musc between two PRBS WR amplitudes (30W – 80W and 30W – 110W), each well below AT and with the same baseline WR. To account for cardio dynamic differences between exercising muscles and the lungs, we separated V'O₂ into a pulmonary (V'O₂pulm) and a muscular parameter (V'O₂musc).
Summing up, the results reveal that (a) HR and $V'O_2$ musc kinetics seem to be independent of WR intensity applying different PRBS WR amplitudes below AT with equal baseline WR, and (b) $V'O_2$ pulm kinetics show prominent differences in $CCF_{lag}$, representing altered venous return between the two PRBS WR amplitudes, whereat $CCF_{max}$ of $V'O_2$ pulm remains unaltered.

The current findings describe important differences between two physiological points of interest in $V'O_2$ kinetics: a) exercising tissue (muscles) and b) measurement at the mouth (lung site). Therefore, it is a requirement to account for the cardio dynamic differences between exercising musculature and the lungs to describe and analyze $V'O_2$ kinetics adequately.

A mean increase of 14 W in WR impacts venous return significantly. Hence, the applied method in the current study may be of interest for the assessment of the kinetics responses during low and moderate WR intensities for patients and older populations (e.g. Koschate et al. 2016b) to evaluate their cardio-respiratory and circulatory health status.

**Acknowledgement**

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5. References


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**Figure and Table legends**

**Fig. 1** Work rate (WR) protocol for exercise testing of the two different pseudo-random binary sequence (PRBS) amplitudes. Grey horizontal bars indicate time periods for mean value calculations during rest (30 s), at low (30 W) and high (80 W & 110 W) constant phases (30 s), and during the PRBS phases (300 s) for both PRBS WR amplitudes.

**Fig. 2** Means (± SE) of cardiac output (Q’) as function of pulmonary oxygen uptake (V’O₂pulm; means ± SE) estimated at low (30 W) and high (80 W & 110 W) constant phases (30 s), and during the PRBS phases (300 s) for both PRBS WR amplitudes.

**Fig. 3** Means (± SE) of respiratory exchange ratio (RER) estimated at low (30 W) and high (80 W & 110 W) constant phases (30 s), and during the PRBS phases (300 s) for both (low & high) PRBS WR amplitudes.

**Fig. 4a** Relative changes in cross-correlation maximum (CCFₘₐₓ) of 1ˢᵗ order system responses as function of actual time constants (in silico). The basis for comparisons of the different sample rates (0.25 Hz, 0.5 Hz, 2 Hz, 3 Hz) is the 1s-interpolation sample which is equal to 1 Hz. The lower small graph displays averages (±SE) derived from the main diagram as function of actual time constants.

**Fig. 4b** Relative changes (per cent) in estimated time constants (τ) of 1ˢᵗ order system responses as function of actual time constants (in silico) for different sample rates (0.25 Hz, 0.5 Hz, 1 Hz, 2 Hz, 3 Hz). The lower small graph displays averaged per cent changes (±SE) derived from the main diagram as function of actual time constants.

**Fig. 4c** Absolute changes (s) in estimated time constants (τ) of 1ˢᵗ order system responses as function of actual time constants (in silico) for different sample rates (0.25 Hz, 0.5 Hz, 1 Hz, 2 Hz, 3 Hz). The lower small graph displays averaged per cent changes (±SE) derived from the main diagram as function of actual time constants.

**Fig. 5** Means (± SE) of the cross-correlation functions (CCF) of work rate (WR) with heart rate (HR, 3a), pulmonary (V’O₂pulm, 3b) and muscular (V’O₂musc, 3c) oxygen uptake, respectively, for the different PRBS amplitudes. The grey triangle displays the auto-correlation function (ACF) of WR. The upper right enhanced graphs display the mean values (± SE) at specific lag times (-20 s, -10 s, 0 s, 10 s, 20 s, 30 s, 40 s, 50 s, 60 s, 70 s, 80 s) for a comparison applying a two-way ANOVA with factors Lag & Amplitude. See text for further explanation.

**Fig. 6** Mean (± SE) of the cross-correlation functions (CCF) [maxima (CCFₘₐₓ) as a function of lags (CCFₗₐ₉)] of work rate (WR) with heart rate (HR), pulmonary (V’O₂pulm) and muscular (V’O₂musc) oxygen uptake, respectively, for the two different PRBS WR amplitudes. The grey line illustrates the auto-correlation function of WR (in part). Data points are close to or on this line represent therefore possible assumptions of a linear 1ˢᵗ order response of the physiological system or parameter behind the computational rational.
**Fig. 7** Cross-correlation functions (mean ± SE) of work rate (WR) with pulmonary oxygen uptake \( (V'O_2\text{pulm}; 7a, 7b) \), heart rate (HR; 7c, 7d), and muscle oxygen uptake \( (V'O_2\text{musc}; 7e, 7f) \) of the low \( (30W-80W) \) and high \( (30W-110W) \) pseudo-random binary sequence WR amplitude. The grey triangles illustrate the auto-correlation function of WR. The black dotted lines illustrate the simulated CCF course of a 1\textsuperscript{st} order system with time delays (TD) and time constants (\( \tau \)), derived from the CCF\textsubscript{max} of each of the displayed parameter. The lower panels show the residuals between the measured and the simulated CCF course as indicator for deviations from 1\textsuperscript{st} order system responses. Each of the upper panels represents the cumulative residuals as sum of deviation squares (SDS) from each main panel from lag -50 s to 200 s in each case.

**Fig. 8** Sum of deviation squares (SDS) for heart rate (HR), muscular \( (V'O_2\text{musc}) \) as well as for pulmonary oxygen uptake kinetics \( (V'O_2\text{pulm}) \) displayed for both the low and the high PRBS WR amplitude. The deviance is calculated between simulated CCF courses derived from the corresponding CCF\textsubscript{max} value and the measured parameter (from lag -50 s to 200 s). Smaller values indicate better agreements with 1\textsuperscript{st} order system properties.

**Tab. 1** Averages (± SD) and slopes (± SD) of heart rate (HR) and pulmonary oxygen uptake \( (V'O_2\text{pulm}) \) during low constant WR (30 W), dynamic WR changes (PRBS 1 & 2: 53.3 W, 67.3 W) and high constant WR (80 W, 110 W) for the low \( (30W-80W) \) and high \( (30W-110W) \) PRBS WR amplitudes.

**Tab. 2** Means (± SD) of model parameter estimates and time series analysis data (CCF\textsubscript{max}, CCF\textsubscript{lag}) for heart rate (HR), muscular \( (V'O_2\text{musc}) \) and pulmonary \( (V'O_2\text{pulm}) \) oxygen uptake for the two different PRBS WR amplitudes. Time constants (\( \tau \)) were derived from CCF\textsubscript{max}-values and time delays (TD) from the CCF\textsubscript{lag}-values assuming 1\textsuperscript{st} order system properties.
Figures

Fig. 1

Fig. 2
Fig. 3

![Graph showing RER vs. Work rate [W].]

- 30W – 80W
- 30W – 110W
- RER = 1.0

Fig. 4a

![Graph showing ΔCF_max [%] vs. Actual time constant [s].]

- 3 Hz
- 2 Hz
- 1 Hz
- 0.5 Hz
- 0.25 Hz
Fig. 4b

Fig. 4c
Fig. 5a

Fig. 5b
**Fig. 5c**

![Cross-correlation function](image1)

**Fig. 6**

![CCF graph](image2)
Fig. 7a, 7b

Fig. 7c, 7d
Fig. 7e, 7f

Fig. 8
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<td>30 W</td>
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<td>110 W</td>
<td>123.0 ± 15.1</td>
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<td>1.612 ± 0.189</td>
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* Work rate effect: p < 0.001, one-way repeated measures ANOVA

Slope data were derived from the WR-HR and the WR-VO₂pulm relationship, respectively.
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<td>V_v</td>
<td>Q_rem</td>
<td>VO2_rem</td>
<td>HR</td>
<td>VO2_pulm</td>
<td>VO2_musc</td>
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</table>

V_v: venous blood volume between exercising muscles and lungs
Q_rem and VO2_rem: perfusion and oxygen uptake for the remainder of the body
HR: heart rate
VO2_pulm: oxygen uptake in the pulmonary vessels
VO2_musc: oxygen uptake in the musculature
CCFmax: maximum of cross-correlation function
CCF_lag: lag (abscissa) of CCFmax
τ, TD: kinetics characteristics of the system time constant and time delay

*: significantly different between 30W─80W and 30W─110W, P < 0.05
n. s.: not significantly different between 30W─80W and 30W─110W, P > 0.05
#: significantly different from zero, P < 0.05
Highlights

- Two different pseudo-random binary work rate amplitudes are applied below anaerobic threshold to analyze heart rate and oxygen uptake kinetics
- Heart rate and muscular oxygen uptake kinetics seem to be independent of work rate
- Pulmonary oxygen uptake kinetics showed altered responses due to changes in venous return during the transients