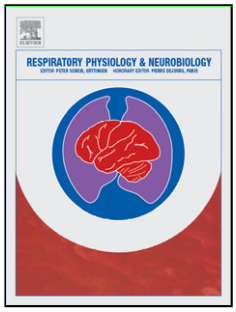

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Oxygen Uptake Kinetics Following Six Weeks of Interval and Continuous Endurance Exercise Training – An explorative pilot study

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Highlights

- Nine untrained individuals performed PRBS work rate changes (30 W – 80 W) before and after six weeks of endurance exercise training
- Significant increase in $V'O_2$peak, but no changes in cardiopulmonary or respiratory kinetics responses
- Negative relationship between muscle oxygen uptake kinetics and training work rate

Abstract

Purpose

The aim of the study was to compare the responses of pulmonary ($V'O_2$pulm) and muscle ($V'O_2$musc) oxygen uptake kinetics before (PRE) and after (POST) six weeks of endurance exercise training.

Methods

Nine untrained individuals performed pseudo-random binary sequences work rate changes between 30 W and 80 W at PRE and POST training intervention. Heart rate (HR) and $V'O_2$pulm were measured beat-to-beat and breath-by-breath, respectively. $V'O_2$musc was estimated applying the approach of Hoffmann et al. (Eur J Appl Physiol 113: 1745-1754, 2013).

Results

Maximal oxygen uptake showed significant increases from PRE (3.2 ± 0.3 $L \cdot min^{-1}$) to POST (3.7 ± 0.2 $L \cdot min^{-1}$; $p < 0.05$). For HR, $V'O_2$pulm and $V'O_2$musc kinetics no significant changes from PRE to POST training intervention were observed ($p > 0.05$).

Conclusions

Discrepancies in the adaptations of the involved exercise induced physiological systems seem to be responsible for the observed significant alterations in maximal $V'O_2$ after six weeks of the training intervention in contrast to no changes in the kinetics responses.

Keywords

Aerobic exercise, physiological modelling, pseudo-random binary sequence (PRBS), heart rate, cycling ergometry, gas exchange

List of specific abbreviations

CCF		cross-correlation function
CCF_{lag}	[s]	lag of CCF_{max}
CCF_{max}	[a.u.]	maximum (peak) of cross-correlation function
CON		continuous
HR	[min^{-1}]	heart rate
INT		interval
POST		after
PRE		before
PRBS		pseudo-random binary sequence
Q’	[$L \cdot min^{-1}$]	cardiac output
Q'_{rem}	[$mL \cdot min^{-1}$]	perfusion of non-exercising tissues
SV	[mL]	stroke volume
τ	[s]	time constant
TD	[s]	time delay
$V'O_2$	[$L \cdot min^{-1}$]	oxygen uptake
$V'O_2peak$	[$L \cdot min^{-1}$]	maximum $V'O_2$ attained during incremental exercise test to exhaustion
$V'O_2musc$	[$L \cdot min^{-1}$]	exercising muscle oxygen uptake
$V'O_2pulm$	[$L \cdot min^{-1}$]	pulmonary oxygen uptake
$V'O_2rem$	[$L \cdot min^{-1}$]	oxygen uptake in non-exercising tissues
V_v	[mL]	venous volume
WR	[W]	work rate

1. Introduction

Oxygen uptake ($V'O_2$) kinetics is a key parameter to evaluate the multi-systemic responses of the body to exercise stress (Wasserman 1984; Bassett and Howley 2000). For example, the efficiency of $V'O_2$ kinetics depend on the oxygen (O_2) supply and the O_2 extraction, and the O_2 delivery relies on the capillary density and the amount of muscle perfusion. The O_2 extraction is affected by muscle fiber distribution, enzyme activities, and by the number and size of mitochondria (e.g. Hughson, 2009).

To quantify $V'O_2$ kinetics, the time constant tau (τ) can be estimated. The τ is defined as the time to reach 63% of the difference amplitude between two work rate (WR) intensities or as transition from rest to moderate or WR intensities. In this regard, smaller τ of $V'O_2$ imply faster kinetics responses, which in turn reflect a high rate of adenosine triphosphate (ATP) delivery through the aerobic metabolism to the ATP demands during transient exercise. Therefore, faster $V'O_2$ kinetics are associated with increased exercise tolerance. In this regard, improvements in $V'O_2$ kinetics are also linked with greater aerobic capacities (Chilibeck, Paterson, Petrella and Sunningham, 1996) and are speeded as a result of endurance exercise training (e. g. Fukuoka et al., 2002; Murias, Kowalchuk and Paterson, 2010).

$V'O_2$ kinetics are of interest for all kinds of sports with focus on the endurance component of the complex sport specific performance (e.g. Millet et al., 2003; Rodriguez, Keskinen, Keskinen and Malvela, 2003; Hettinga, De Koning and Foster, 2009). Endurance exercise training is also a prevalent treatment for patients suffering from diabetes mellitus (Ivy, 1997), obesity (Boulé, Haddad, Kenny, Wells and Sigal, 2001), arterial hypertension (Guimaraes et al., 2010), and chronic obstructive pulmonary disease (Casaburi et al., 1997). For elderly, endurance exercise training and a speeding of V'O2 kinetics is of relevance for quality of life (Alexander, Dengel, Olson and Krajewski, 2003) as well. Nowadays, training performance and exercise testing is a practical and relevant application, for instance, for astronauts involved in long-term missions staying on the International Space Station (Moore et al., 2014; Hoffmann, Moore, Koschate, and Drescher, 2016).

The mentioned examples comprise different training procedures or methods (e.g. work durations, intensities, repetitions, series, work-to-rest ratios) with its particular objectives. A more fundamental discrimination is interval-type and continuous-type endurance exercise training. Available investigations with focus on only continuous (CON) exercise training reveal that $V'O_2$ kinetics are significantly improved after the training intervention (Hickson, Bomze and Hollozy, 1978; Fukuoka et al., 2002; Zoladz et al., 2014; Phillips, Green, MacDonald and Hughson, 1995). In contrast, investigations with focus on only interval-type

training show a speeding of $V'O_2$ kinetics as well (Christensen et al., 2016; Turnes et al., 2016a; Turnes et al., 2016b; Jacobs et al., 2013). Furthermore, studies comprising both, interval (INT) and CON exercise training, indicate that either INT (Daussin et al., 2008) or CON (Murias et al., 2010) improve $V'O_2$ kinetics, or both training procedures are equal in speeding of $V'O_2$ kinetics (Berger, Tolfre, Williams and Jones, 2006; McKay, Paterson and Kowalchuk, 2009; Da Boit, Bailey, Callow, DiMenna and Jones, 2014). However, there is no clear consensus, whether INT or CON may provoke superior improvements in $V'O_2$ kinetics.

Concerning this matter and regardless of INT or CON exercise training, the impacts of endurance exercise training can roughly be categorized into adaptations related to capacities and kinetics. Capacities are the maximal limits of the physiological systems involved during exercise, like maximal $V'O_2$, ($V'O_2$peak) maximal heart rate, and maximal cardiac output (Q'). In contrast, the kinetics reflect the speed of adaptation to exercise demands to relative changes, like the dynamic responses of $V'O_2$, heart rate (HR), or Q' in response to changing metabolic demands during transient exercise.

Both the capacities and the kinetics seem to be interconnected with each other and should not be subdivided into physiological-independent variables or parameters for an isolated evaluation of the endurance performance (Wüst, van der Laarse and Rossiter, 2013). Accordingly, the complex endurance performance depends on both the capacities and the kinetics responses of the cardiopulmonary and respiratory system.

In this context, different cardiopulmonary exercise testing procedures can be applied accounting for either testing at submaximal and maximal levels (e.g. $V'O_2$max testing) or in moderate exercise intensities below gas exchange threshold for kinetics testing. However, kinetics testing can also be applied above gas exchange threshold, which intensifies the complexity of the observed responses (Whipp and Rossiter, 2005).

From a practical point of view, the interest for evaluation of the aerobic metabolism is on the muscle site (Christensen et al., 2016), because adapted mitochondria and the micro-cellular alterations are the predominant explanations for an increased exercise tolerance. In this regard, during non-steady-state conditions, there could be prominent differences between the pulmonary measurement of $V'O_2$ at the mouth and the oxygen consumption at exercising musculature (Grassi et al., 1996; Benson, Grassi, and Rossiter; 2013; Hoffmann, Drescher, Benson, Rossiter, and Essfeld, 2013; Drescher, Koschate, Schiffer, and Hoffmann, 2016). This possible mismatch is based on the time-delaying and distortive effects of venous return which is based on the non-linear transit times of deoxygenated blood draining from exercising muscles to lungs and additionally the smearing effect of Q' on pulmonary $V'O_2$. Therefore, it is essential to account for these differences by appropriate approaches

(Barstow and Molè, 1987; Barstow and Molè, 1991; Eßfeld, Hoffmann, and Stegemann, 1991; Cochrane and Hughson 1992; Benson et al., 2013; Hoffmann, et al., 2013). The discrimination between pulmonary ($V'O_2$pulm) and muscle $V'O_2$ ($V'O_2$musc) kinetics becomes therefore of relevance by focusing on the systemic (e.g. changes in venous return and whole body perfusion) and peripheral (e.g. alterations in mitochondria cells, muscle perfusion) points of interest.

Therefore, we aimed to compare the kinetics responses of $V'O_2$pulm and $V'O_2$musc before and after CON and INT in untrained participants. The following hypothesis was tested: endurance exercise training speeds the responses of $V'O_2$pulm and $V'O_2$musc kinetics, respectively. In addition, we tried to illustrate possibly different impacts of CON and INT on $V'O_2$musc kinetics in form of a pilot trial.

2. Methods

2.1 Subjects

The study was approved by the Ethics Committee of the German Sport University Cologne according to the Helsinki Declaration (1964) and its later amendments or comparable ethical standards. Furthermore a medical check-up of the volunteers and their written informed consent was needed to participate in the study.

Nine healthy and physically non-active male volunteers participated in the endurance exercise training study with cardiopulmonary exercise testing before (PRE) and after (POST) the intervention (age: 44 ± 9 years; height: 179 ± 5 cm; body weight (PRE, POST): 89.9 ± 10.1 kg, 89.8 ± 8.4 kg [mean ± SD]).

2.2 Cardiopulmonary exercise testing

At PRE and POST intervention, all subjects performed an identical WR protocol for the cardiopulmonary exercise testing (see Fig. 1) on a cycle ergometer (Cardiac Stress Table, Lode B.V., Netherlands) in a semi-recumbent position (seat back at 45°, leg exercise device at 42° relative to ground level) to focus mostly on exercising leg musculature. The PRE exercise tests were performed 10 ± 5 days before and the POST tests 4 ± 2 days after the training intervention.

After a resting phase in which the subjects were instructed to sit calmly, the cardiopulmonary exercise testing started with a constant WR phase at 30 W for 200 s. The next phase contained two identical pseudo-random binary sequences (PRBS) which were switching between 30 W and 80 W, each sequence for 300 s. This section was followed by a high constant WR phase (80 W; 200 s) which then lead to the last section which included step increases (25 W · min^{-1}) until voluntary exhaustion. During the cardiopulmonary exercise testing the frequency of the pedals was set to 60 rpm during the moderate and PRBS phases (time: 0 s to 1000 s), while during the increasing WR steps (time > 1000 s) the pedal frequency was chosen between 60 rpm and 90 rpm individually.

<< Fig. 1 >>

During the cardiopulmonary exercise testing, $V'O_2$pulm was estimated breath-by-breath (ZAN 680, ZAN Messgeräte GmbH, Oberthulba, Germany) with applying the algorithms of Beaver et al. (1981) for the corrections of alveolar gas exchange, which was included in the metabolic cart software.

HR and stroke volume (SV) were determined beat-to-beat by electrocardiography (ECG; by R–R intervals) and impedance cardiography (Task Force Monitor, CNSystems Medizintechnik AG, Austria; see Fortin et al., 2006). All measurement devices were calibrated in accordance with the manufacturer's instructions before testing.

2.3 Intervention procedures

After the initial cardiopulmonary exercise test, the nine volunteers were divided into two equal groups matched by the absolute peak $V'O_2$.

The difference between resting oxygen consumption and peak $V'O_2$ was defined as 100% $V'O_2$ reserve ($V'O_2$Reserve; ACSM's guidelines for exercise testing and prescription, 2010). Four subjects were trained with a CON intervention method at 60% $V'O_2$Reserve (equal to 73% of peak power output) in the first week of intervention of PRE testing values, the other five subjects with an INT training method with upper limits of WR at 90% $V'O_2$Reserve (equal to 98% of peak power output) in the first week of intervention of PRE testing values and lower limits of WR at 50 W. Both training groups were trained three times per week for six weeks.

<< Fig. 2 >>

For both training groups each of the training sessions comprised a five minute warm-up phase at 50 W, followed by either a constant WR phase (CON) or by dynamic WR changes (INT), followed by a five minute cool-down phase at 50 W (see Fig. 2).

The training intervention was performed with Life Fitness Integrity Series Upright Lifecycle® Heimtrainer (CLSC) cycling ergometers. With these ergometers it was possible to program specific WR protocols for each participant individually as needed for the intervention. During each training session, HR was monitored (Sigma Sport RC 14.11, Sigma-Elektro GmbH, Neustadt, Germany) and the study participants were supervised by experienced trainers. While participating in the study, the participants were encouraged to keep their common nutrition and to avoid additional supplementations.

Both groups were matched to identical total work demands (CON: 4474 ± 308 kJ (n = 4); INT: 4423 ± 433 kJ (n = 5)) on a leg cycle ergometer with 70 rpm pedal frequency in the upright position during the six weeks of intervention.

One participant of the INT group suffered from arterial hypertension, who took 5mg of Amlodipin (calcium-channel-blocker) per day. Another participant of the INT group suffered from hemochromatosis, who took no medications. These two participants showed no contraindications at PRE and POST exercise testing and during the training intervention.

2.4 Data processing

The breath-by-breath as well as the beat-to-beat data were time-aligned and linearly interpolated in equidistant time intervals (1 s) for the subsequent time-series analysis. The 1s-interpolation procedure is a prerequisite for calculating the auto-correlation function (ACF) of WR and the cross-correlation functions (CCF) of WR with HR, $V'O_2$pulm and $V'O_2$musc, respectively. The calculations of ACF and CCF imply stepwise shiftings of 1 s of one data set against the same (resulting in ACF) or another data set (resulting in CCF). This shifting is defined as lag (see next section for detailed information). In addition, the estimation of $V'O_2$musc kinetics utilizes a circulatory model which requires equidistant data for calculating the venous volume transit times between exercising musculature and the lungs. Thus, the 1s-interpolation is twofold essential: 1st for the application of the time-series analysis procedures (e.g. ACF, CCF) and 2nd for the estimation of $V'O_2$musc kinetics by means of the circulatory model. For parameters of interest, averages were calculated during resting phase, both PRBS (53.3 W), and at the end of the low (30 W) and high (80 W) constant WR phases (see Fig. 1).

2.5 Muscle oxygen uptake prediction and kinetics analysis

Muscle oxygen uptake ($V'O_2$musc) was estimated 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 ($V'O_2$rem) and perfusion (Q'_{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 $V'O_2$musc and $V'O_2$pulm. By means of this approach, it is possible to discriminate between $V'O_2$pulm responses with its cardiovascular distortions and the estimated $V'O_2$musc kinetics responses. For further information see Hoffmann et al. (2013).

For the analysis of the kinetics responses of HR, $V'O_2$pulm, and $V'O_2$musc, the cross-correlation functions (CCF) of WR and the specific parameter (HR, $V'O_2$pulm, $V'O_2$musc) were estimated in consideration of the two PRBS phases. The maximum of the CCF (CCF_{max}) and its related lag (CCF_{lag}) indicate the kinetics responses of the parameter, whereas higher CCF_{max}-values indicate faster and greater CCF_{lag}-values denote delayed system responses, and vice versa.

Applying the approach of Hoffmann et al. (2013), which assumes 1st order system properties for $V'O_2$musc kinetics, CCF_{max}- and CCF_{lag}-values can be transferred into time τ and time delays (TD), which allows a comparison with multi-phase exponential models (Barstow & Molè 1987; Barstow & Molè 1991). TDs were calculated as the difference between the

measured CCF_{lag} of the specific parameter with WR and the anticipated CCF_{lag} calculated from the real-estimated CCF_{max}-value of the respective parameter. The CCF_{max}- and the CCF_{lag}-values are linked straightly (see Hoffmann et al. 2013, Fig. 2c), so it is possible to derive the CCF_{lag}-value by the CCF_{max}-value, and vice versa, and this is meant with 'anticipated' (see above). Assuming 1st order system properties no TD is expected in the system response and all CCF_{lag}–CCF_{max} data points are placed on the descending part of the ACF of WR by definition. The $V'O_2$pulm response does not show pure 1st order system properties due to the distorting effects of venous return and Q', so it is intended to calculate the TD for $V'O_2$pulm to demonstrate possible discrepancies to 1st order system characteristics. Accordingly, deviation from zero (positive as well as negative values) of TD-values denote, that the CCF_{max}-values of the measured parameter (e.g. CCF(WR/$V'O_2$pulm), CCF (WR/$V'O_2$musc)) are left- (negative TD values) or right-shifted (positive TD values) compared to the ACF of WR, which imply therefore contradictions to 1st order system properties as mentioned above. In this regard, negative TD-values are logical results from the computational rationale behind the approach using time-series analysis and are not one-to-one comparable to negative TD of the mono-exponential function fitting applying the repeated step transition approach.

2.6 Statistical analyses

We applied the non-parametric Wilcoxon signed-rank-test for single PRE to POST comparisons of variables of interest, because of the small sample size (n = 9). Whereas, we utilized two-way (repeated) measures analysis of variance (ANOVA) for the evaluation of interaction effects, which cannot be realized by the Wilcoxon signed-rank-test. Subsequently, we will present the applied statistical tests in detail as follows:

To compare the peak values and exercise-relevant parameters between PRE and POST training intervention we applied the non-parametric Wilcoxon signed-rank-test, irrespective of the training group; dependent variables were: peak power output, peak $V'O_2$, resting heart rate, peak heart rate, and exercise time (n = 9 each).

To test for static linear relationships between WR with HR and $V'O_2$pulm, respectively, we applied a two-way repeated measures analysis of variance (ANOVA) with factors *Work rate* (30 W, PRBS1 (53.3 W), PRBS2 (53.3 W), 80 W) and *Time* (PRE, POST), irrespective of training groups; dependent variables were: HR, $V'O_2$pulm (n = 9 each).

For the analysis of the slopes of HR and $V'O_2$pulm as functions of WR between PRE and POST intervention, the non-parametric Wilcoxon signed-rank-test was applied, irrespective of training group; dependent variables were: HR, $V'O_2$pulm (n = 9 each).

For the dynamic kinetics analysis of the CCF courses we applied a two-way repeated ANOVA with factors *Lag* (10 s-intervals from –20 s to 120 s) and *Time* (PRE, POST) accounting for possible differences in the entire CCF course and between PRE and POST changes, irrespective of training groups; dependent variables were: HR, $V'O_2$pulm, $V'O_2$musc (n = 9 each).

The non-parametric Wilcoxon signed-rank-test was used to test for differences in CCF_{max}-values between PRE and POST intervention, irrespective of training group; dependent variables were: HR, $V'O_2$pulm, $V'O_2$musc (n = 9 each).

On a trial basis, we applied a two-way ANOVA with factors *Group* (INT, CON) and *Time* (PRE, POST) trying to show possible interaction effects between the training groups and PRE and POST testing; dependent variables were: HR, $V'O_2$pulm, $V'O_2$musc (INT: n = 5 each; CON: n = 4 each).

For the analysis of the relationship between the relative changes in CCF_{max} (ΔCCF_{max}) of $V'O_2$musc kinetics and the average WR intensities during the six weeks of intervention, the Spearman's rank correlation coefficient was applied, irrespective of training group; dependent variable was ΔCCF_{max} (n = 9).

Statistical significance was set to 0.05 for the alpha level (software package: IBM SPSS statistics 24).

3. Results

Fig. 3 shows in each case an example of the measured breath-by-breath data of one participant of the INT (3a) and CON (3b) training group. In addition, the individual CCF courses for $V'O_2$pulm and $V'O_2$musc kinetics of PRE and POST intervention testing is displayed as embedded panels.

<< Fig. 3a, 3b >>

3.1 Intervention characteristics

Tab. 1 gives an overview about the average week-to-week recordings of the training realization for the mean training WR and its percentage peak power output related to PRE intervention testing, the training duration with its range (minimum, maximum) for both, the CON and INT group. In addition, the upper limits of WR for the INT training group are also recorded during the six weeks of intervention.

<< Tab. 1 >>

3.2 Peak responses

Tab. 2 summarizes the peak responses of power output, $V'O_2$, HR, as well as the resting values of HR and the exercise time PRE and POST the exercise training intervention as averages over all nine subjects. In addition, the same parameters were separated for the INT and CON training group. Significant differences between PRE and POST intervention have been observed for all parameters ($p < 0.05$ and $p < 0.01$, respectively, Wilcoxon signed-rank-test), with exception of peak HR ($p > 0.05$).

<< Tab. 2 >>

By comparing the two training groups, the INT group showed a smaller increase in peak $V'O_2$ from PRE to POST, in contrast to a greater increase in the CON group. However, this numerical smaller increase cannot be statistically analyzed because of the small sample sizes of the two training groups (CON = 4; INT = 5). The mentioned relation in peak $V'O_2$ can also be observed in peak power output. For resting HR this relationship is reversed comparing the two training groups.

3.3 Static kinetics analysis

The indication of a linear relationship (static linearity) between WR and both HR and $V'O_2$pulm is an essential requirement for the subsequent time-series analysis at moderate exercise intensities – in particular in the range of the PRBS WR phases (30 W to 80 W).

Tab. 3 illustrates the averages of PRE and POST HR and V'O_2pulm in the time domain for WRs at 30 W, 53.3 W and 80 W, respectively.

Applying a two-way repeated measures ANOVA with factors *Time* (PRE, POST) and *Work rate* (30 W, PRBS 1 & 2 (53.3 W), 80 W), both HR and V'O_2pulm showed significant effects of factor *Time* ($p < 0.05$) and *Work rate* ($p < 0.001$), respectively. For V'O_2pulm, but not for HR, significant interactions (*Time* x *Work rate*; $p < 0.05$) could be observed.

In addition, the slopes of HR and V'O_2pulm as functions of WR were estimated for static linearity analysis. The PRE to POST comparisons of HR and V'O_2pulm slopes – by Wilcoxon signed-rank-test – revealed no significant differences ($p > 0.05$) for both parameters. The coefficients of determination derived from the WR comprising 30 W, PRBS1 & 2 (53.3 W each) and 80 W were calculated as $R^2 = 0.9765 \pm 0.0204$ (PRE) and $R^2 = 0.9614 \pm 0.0262$ (POST) for HR and as $R^2 = 0.9828 \pm 0.0281$ (PRE) and $R^2 = 0.9944 \pm 0.0066$ (POST) for V'O_2pulm.

<< Tab. 3 >>

3.4 Dynamic kinetics analysis

Fig. 4 illustrates the PRE and POST kinetics responses for all study participants (n = 9) of HR (4a), V'O_2pulm (4b) and estimated V'O_2musc (4c) as CCF of the specific parameter with WR, regardless of the training group. The upper smaller panel in each graph demonstrates the intervention group-specific CCF maxima with its lags (CCF_{lag}, CCF_{max}; INT versus CON) for each parameter, also as PRE and POST averages and as individual data points.

<< Fig. 4a-c >>

The CCF_{max}-values, its lags (CCF_{lag}) and derived parameters (τ, TD) of the kinetics responses are commonly used as an approximation for analysis of the kinetics responses. Therefore, we will focus on these values for a first evaluation of the kinetics responses. For all illustrated parameters in Tab. 4 no significant differences ($p > 0.05$, $n = 9$, Wilcoxon signed-rank-test) could be observed between PRE and POST training intervention independent of groups. In addition, the TD values of HR, V'O_2pulm, and V'O_2musc kinetics were analyzed for differences to zero, at PRE and POST intervention, separately. The TDs of HR and V'O_2pulm kinetics showed marked differences to zero ($p < 0.05$, one-sample Wilcoxon signed-rank-test) at PRE and POST intervention, respectively. In contrast, the TD of estimated V'O_2musc kinetics showed no differences to zero ($p > 0.05$, one-sample Wilcoxon signed-rank-test) at PRE and POST intervention testing.

<< Tab. 4 >>

For a sophisticated analysis of the kinetics responses we performed a two-way repeated measures ANOVA with factors *Time* (PRE, POST) and *Lag* (10-s-intervals from -20 s to 120 s) for HR, V'O_2pulm and V'O_2musc kinetics, regardless of training group. In each case, we found no significant influences of factor *Time* and interactions (*Time* x *Lag*; $p > 0.05$), but each factor *Lag* was significant ($p < 0.001$).

Noteworthy, for HR, V'O_2pulm as well as for V'O_2musc in each case the CCF_{max}-values show negative trends from PRE to POST for the CON group and positive trends for the INT group. Due to these observations we performed a two-way ANOVA with factors *Group* (INT, CON) and *Time* (PRE, POST) on a trial basis for the CCF_{max}-values of HR, V'O_2pulm and V'O_2musc kinetics separately. For HR and V'O_2pulm kinetics no significant effects for factor *Group* and *Time* as well as for interactions (*Group* x *Time*) could be observed ($p > 0.05$ each). For V'O_2musc kinetics the factors *Group* and *Time* showed no significant effects ($p > 0.05$), but for the interaction (*Group* x *Time*) a trend ($p = 0.054$) could be identified.

<< Fig. 5 >>

Fig. 5 shows the relationship between the changes (Δ) in CCF_{max} of V'O_2musc kinetics from PRE to POST intervention as function of averaged training WR, with highlighting the INT (open rectangles) and CON (full rectangles) training groups. It can be observed that all participants of the INT group show increases in ΔCCF_{max} of V'O_2musc kinetics. In contrast, three of the four participants of the CON training group show declines in ΔCCF_{max} of V'O_2musc kinetics, with the exception of one participant, who illustrated increases in V'O_2musc kinetics. For analysis of a systematic relationship between ΔCCF_{max} of V'O_2musc kinetics and the averaged training WR we calculated the Spearman-rho. A negative relationship can be observed ($r_{SPEARMAN} = -0.633$, $p = 0.034$ (1-tailed), $n = 9$), independent of training group.

4. Discussion

The aim of the present study was to analyze the influence of endurance exercise training in terms of the responses of HR, $V'O_2$pulm and estimated $V'O_2$musc kinetics. The major findings are:

a) $V'O_2$pulm, $V'O_2$musc and HR kinetics showed no significant changes from PRE to POST intervention, regardless of training group.
b) Endurance exercise training showed significant increases in peak $V'O_2$ independent of training group.
c) A relationship between the PRE to POST alterations in the kinetics responses of $V'O_2$musc as function of averaged training WR could be observed ($r_{SPEARMAN}$ = -0.633; p = 0.034; 1-tailed; n = 9).
d) On a trial basis: For $V'O_2$musc kinetics an interaction effect (*Group* x *Time*) could be identified in tendency (p = 0.054), denoting that INT training may accelerate $V'O_2$musc kinetics at moderate WR intensities.

4.1 Peak responses

The overall alterations of peak responses between PRE and POST intervention represent the typical and expected results of endurance exercise training (Jacobs et al., 2013; Burgomaster, Heigenhauser, and Gibala, 2006; Lindsay et al., 1996; Little et al., 2011a; Zoladz et al., 2013, Murias et al., 2010, McKay et al., 2009, Dausin et al., 2008, Helgerud et al., 2007, Berger et al., 2006, Fukuoka et al., 2002, Hickson et al., 1978). Peak power output, peak $V'O_2$ and exercise time show significant increases POST intervention. In contrast, but also a typical adaptation onto endurance training, resting HR is significantly reduced after the training intervention.

Milanovic, Sporiš and Weston (2015) conclude in their review, that both endurance and high-intensity INT training can improve $V'O_2$max, which was also mentioned by Poole and Gaesser (1985). They also assume that less fit participants would profit more than trained individuals, regardless of the applied training procedure. Additionally, Milanovic and colleagues constitute, that the high-intensity INT training mode would produce greater gains in $V'O_2$max adaptations. Turnes et al., (2016a) performed four weeks of two different training procedures in the severe intensity domain, and conclude these intensities seem to be a promising application for endurance adaptations, whereas higher exercise intensities may improve sprint performance much more clearly. The mentioned studies above support the results in our study: higher training WR may provoke higher improvements in peak $V'O_2$. This

is also in line with Helgerud et al., (2007) who demonstrated that aerobic high-intensity intervals improve V'O_2peak more than moderate exercise intensities.

Furthermore, there is suggestion from high-intensity INT training, that positive adaptations in peak power output and peak V'O_2 is related to increases in skeletal muscle mitochondrial function (Jacobs et al., 2013). Because the focus of the present study was on the kinetics responses of V'O_2pulm and estimated V'O_2musc, we have no additional information about mitochondrial enzyme activity and protein expression. Therefore, it is not possible to reflect about fat oxidation, tricarboxylic acid cycle or the electron transport chain (Burgomaster, Hughes, Heigenhauser, Bradwell, and Gibala, 2005; Burgomaster et al., 2006; Perry et al., 2010; Talanian et al., 2010; Hood, Little, Tarnopolsky, Myslik, and Gibala, 2011; Little, Safdar, Bishop, Tarnopolsky, and Gibala, 2011b) in relation to the present study results.

4.2 Kinetics analysis

4.2.1 Static linearity

The slopes of HR and V'O_2pulm show no significant changes from PRE (0.0097 ± 0.0018 L·min^{-1}·W^{-1}) to POST (0.0112 ± 0.0014 L·min^{-1}·W^{-1}) intervention testing. The slopes of V'O_2pulm are in the expected range compared to other studies (Poole, Gaesser, Hogan, Knight, and Wagner, 1992; Whipp, 1996). As demonstrated above, there is a high linear relationship between V'O_2pulm responses as function of WR with coefficients of determination > 0.96, which is a prerequisite for the subsequent kinetics analysis.

4.2.2 Time-series analyses

For the analysis of HR, V'O_2pulm and V'O_2musc kinetics we performed a two-way repeated measures ANOVA with factors *Time* (PRE, POST) and *Lag* (10s-intervals) resulting in no significant effects for factor *Time* and interactions, but factor *Lag* was significant (Fig. 4a-c).

Because factor *Time* and interactions are not significant for HR, V'O_2pulm and V'O_2musc kinetics, it has to be assumed that the endurance exercise training – regardless of training procedure – has no effect on HR, V'O_2pulm or V'O_2musc kinetics.

Because of single CCF_{max}-values are located at different CCF_{lag}-values in comparison to the other participants, the fundamental information about the kinetics responses may be distorted when averaging the entire CCF course across conditions and participants. Therefore, a common approach to analyze kinetics responses of HR, V'O_2pum and V'O_2musc, are the comparisons of the CCF_{max}-values in each case. The CCF_{max}-values are based on the changes over time of the respective parameter and comprise therefore compressed kinetics information, which seem to be sensitive to changes in the dynamics of

the observed parameter (e.g. Drescher, Koschate, and Hoffmann, 2015). By analysis of these CCF_{max}-values, we found an interaction effect (*Group* x *Time*) by trend ($p = 0.054$) for $V'O_2$musc only. The potential interaction effect for $V'O_2$musc kinetics between the INT and CON training group from PRE to POST testing is encouraging to assume, that the two different training procedures may have complex impacts on the exercising musculature. To support this assumption we computed the post hoc achieved power for the interaction effect (G*Power 3.1.9.2) for the CCF_{max}-values of $V'O_2$musc kinetics with effect size $f = 0.874$ (partial $\eta^2 = 0.433$), revealing a power ($1-\beta$) of 0.941. If the number of trained subjects would be increased the difference between the training groups could either become statistically significant or, on the opposite, loose the observed tendency. However, this assumption is speculative, because the sample size is very low and limits therefore the predication.

The available literature reveal that INT (Christensen et al., 2016; Turnes et al., (2016b; Zoladz et al., 2013), CON (Hickson et al., 1978; Fukuoka et al., 2002; Phillips et al., 1995; Zoladz et al., 2014) as well as both INT and CON training procedures (Berger et al., 2006; McKay et al., 2009; Da Boit et al., 2014; Murias et al., 2010) have positive effects on the $V'O_2$ kinetics responses. The literature also shows, that there is no clear indication, whether INT training may have specific impacts on the kinetics responses of the cardiopulmonary and respiratory system measured at pulmonary level, and in particular the impact on $V'O_2$musc kinetics. Further, the inability to compare the training investigations (e.g. different work durations, intensities, repetitions, series, work-to-rest ratios) impede precise conclusion about the effects of its applied exercise training regime.

4.2.3 Context analysis

The observed relationship between the changes in $V'O_2$musc kinetics (ΔCCF_{max}) and the averaged training WR of each participant (see Fig. 5) illustrate, that all INT group participants show increases in the kinetics responses. In contrast, only one participant of the CON group shows an increase in CCF_{max} of $V'O_2$musc kinetics from PRE to POST testing. The other three participants of the CON group illustrate negative differences in CCF_{max} of $V'O_2$musc kinetics. These – for the most parts – consistent group-specific adaptations may be based on the two different training procedures, which was an additional aim on a trial basis of the present study. Namely, fast and repetitive changes in WR as INT training may provoke increases in the kinetics responses of the cardiopulmonary und respiratory system.

There is the possibility that smaller averaged training WR may be related to higher increases in CCF_{max}-values of $V'O_2$musc kinetics as can be seen in Fig. 5. This means, that exercise intensities which are too high would not provoke positive increases in $V'O_2$musc kinetics. Derived from Fig. 5, the limit for a positive or negative adaptation of $V'O_2$musc kinetics could

be determined at about 150 W to 160 W, which represents ~75% and ~68% of peak WR at PRE and POST testing, respectively.

4.2.4 Limitations

The small sample sizes for the CON (n=4) and the INT (n=5) training group is a clear limiting factor in the present study. Concerning this matter, the applied non-parametric statistical procedures (e.g., Wilcoxon signed-rank-test, Spearman's rank correlation coefficient) which were used regardless of the training groups, show no limitations with respect to the statistical significance and conclusions.

Both trainings groups started with five participants. During the training phase one participant of the CON group became ill and withdrew from the study. All participants were untrained at the beginning of the study and followed their normal life habits and occupations. Due to the study restrictions (e.g., three training sessions per week for six weeks at specific time slots) only few participants could be recruited for the study, which were compliant with the study conditions.

For the estimation of $V'O_2$musc kinetics Hoffmann et al. (2013) applied a circulatory model comprising the variables V_v (venous blood volume), Q'_{rem} (perfusion of the non-exercising tissue), and $V'O_2$rem which is a proxy for the O_2 consumption of the remaining body compartments (e.g., ventilation). However, this is just a simplification and has to be mentioned as limitations of the approach of Hoffmann et al. (2013).

4.3 Practical considerations

For sports with emphasis on the endurance exercise component it is of particular interest to improve both, the capacities and the kinetics fractions for an overall improvement of the specific sports related endurance performance. The kinetics responses are of relevance during exercise transients, e.g. from lower to higher exercise intensities and therefore during increasing exercise demands. The capacitive fractions seem to be more important for maximal performances of the cardiopulmonary and respiratory system. However, the kinetics responses may be a limiting factor for the capacitive fractions of the endurance sports performance, in fact, if the kinetics are very slow, so that the time duration to higher exercise demands cannot be sufficiently conducted within the aerobic metabolism. Therefore, the kinetics responses of the cardiopulmonary and respiratory systems are some kind of basis for the development and usability of the capacities. From there, all kinds of sports related to mostly endurance performance should consider the simultaneous and complex improvement of both, the capacities and regulative components of the involved physiological systems.

Another practical point in the view of exercise testing is, that without the differentiation between measured V'O_2pulm and estimated V'O_2musc the analysis of the different exercise training procedures would not be possible or hardly impeded. This is in particular of interest for those who are interested in a more discriminative evaluation of influences of different impacts on the cardiopulmonary und respiratory system. Often the muscle site is of greater interest than the pulmonary measurement at the mouth, and it is known that there could be differences between the measurement at the mouth and the oxygen consumption within the exercising muscles in transient WR phases (Barstow and Molè, 1987; Barstow and Molè, 1991; Eßfeld et al., 1991; Cochrane and Hughson 1992; Benson et al., 2013; Hoffmann et al. 2013). Therefore, the practical aspect is a differentiation between lungs and muscle site for a more sophisticated evaluation of the site of interest: exercising musculature.

4.4 Conclusion

In the present study we analyzed the kinetics responses of V'O_2 and HR before and after six weeks of an INT and CON endurance training. To account for cardio dynamic and venous return distortions between exercising musculature and the lungs, we separated V'O_2 into a pulmonary (V'O_2pulm) and an estimated muscular component (V'O_2musc).

In summary, the results reveal that **(a)** V'O_2pulm, V'O_2musc and HR kinetics showed no significant changes from PRE to POST training intervention **(b)** endurance exercise training caused in a significant increase in peak V'O_2, **(c)** a relationship between the changes in the kinetics responses of V'O_2musc (PRE to POST) as function of averaged training WR was observed, and **(d)** on a trial basis: for V'O_2musc kinetics an interaction effect (*Group* x *Time*) could be identified in tendency ($p = 0.054$), denoting that INT training may increase V'O_2musc kinetics.

Six weeks of endurance exercise training increases V'O_2peak, but it has no effect on the cardiopulmonary and respiratory kinetics responses. Discrepancies in the adaptations of the involved exercise induced physiological systems seem to be responsible for the observed significant alterations in V'O_2peak after six weeks of the training intervention in contrast to no changes in the kinetics responses. Possible alterations in the kinetics responses of the cardiopulmonary and respiratory systems could be assumed as delayed in time adaptations, if the training intervention would be extended.

Currently, there is no clear consensus, whether INT or CON endurance training may provoke an explicit speeding of V'O_2 kinetics. Nevertheless, the discrimination between V'O_2pulm and estimated V'O_2musc kinetics seem to be a promising approach to differentiate between different impacts of interval-like or continuous-like exercise endurance training on the site of interest: the exercising musculature, involved mitochondria and muscle perfusion.

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Conflict of interest

The authors declare that there is no conflict of interest.

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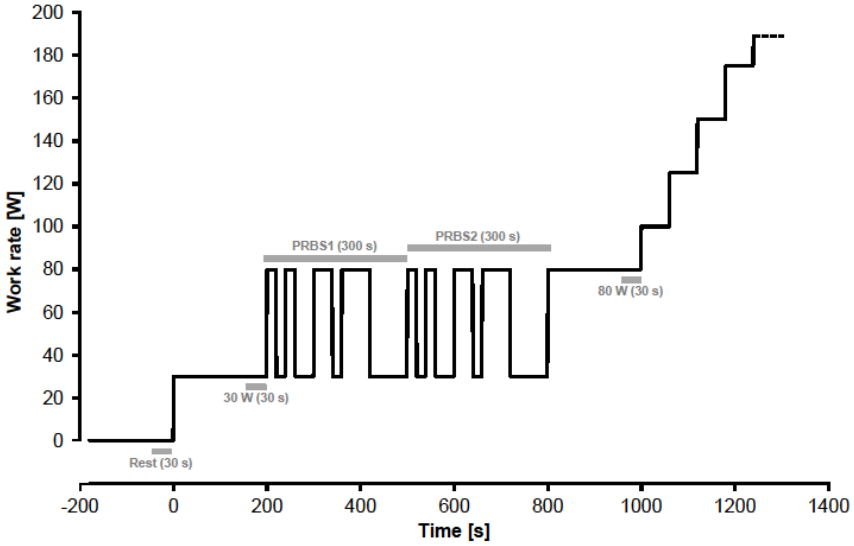
Legends



b

Fig. 1: Work rate (WR) protocol for cardiopulmonary exercise testing before and after the training intervention. Grey horizontal bars indicate time periods for mean value calculations during rest (30 s), at low and high constant phases (30 s), and during the two pseudo-random binary sequence (PRBS) WR phases (300 s each).

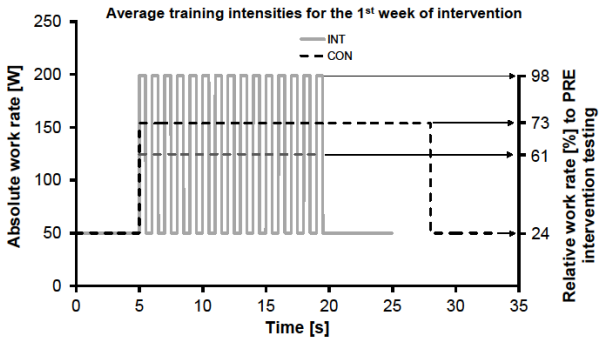


Fig. 2: One example of the training protocols during the first week of intervention for the continuous (CON: black dotted line; work rate is equal to 73% PRE peak power output: 154 W) as well as for the interval training group (INT: grey line; upper work rate limit is equal to 98% PRE peak power output: 199 W; lower work rate limit is equal to 24% PRE peak power output: 50 W; average work rate is displayed by the horizontal grey dashed line: 61% PRE peak power output (125 W)). Each training

session started with a warm-up phase (duration: 5 minutes at 50 W), followed by the interval or continuous procedures, and was finished with a cool-down phase (duration: 5 minutes at 50 W).

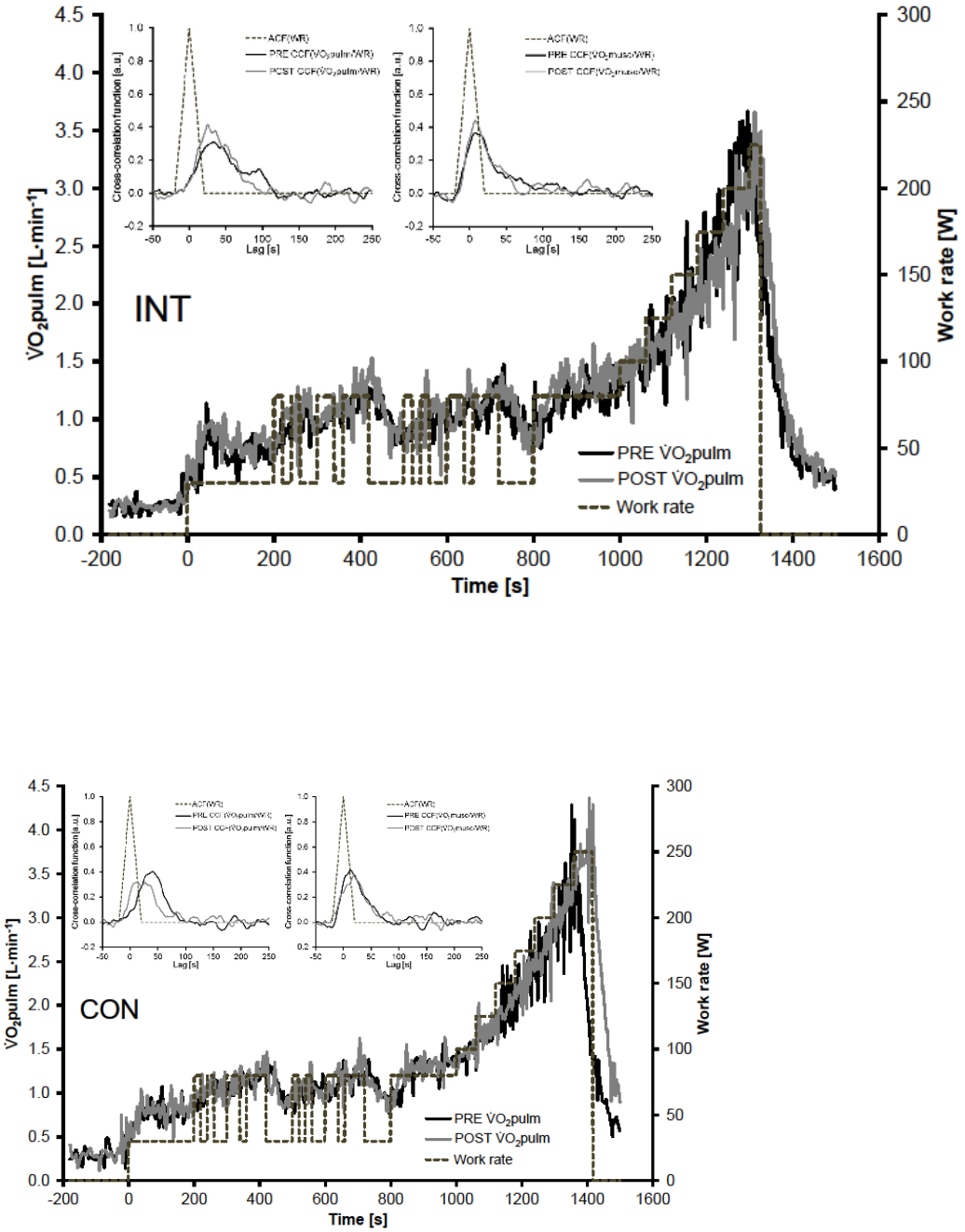
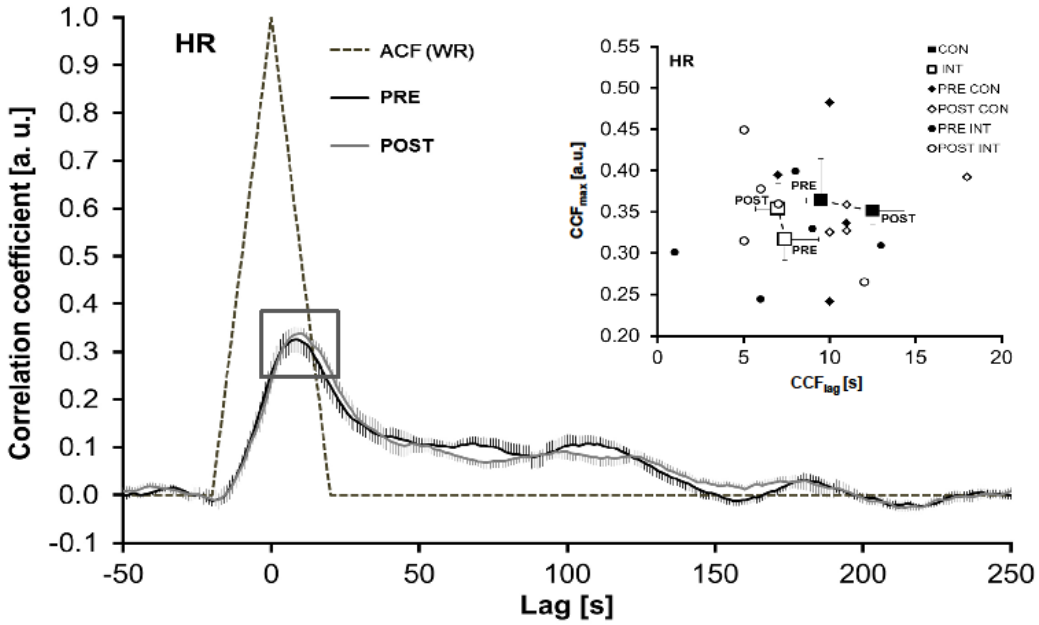
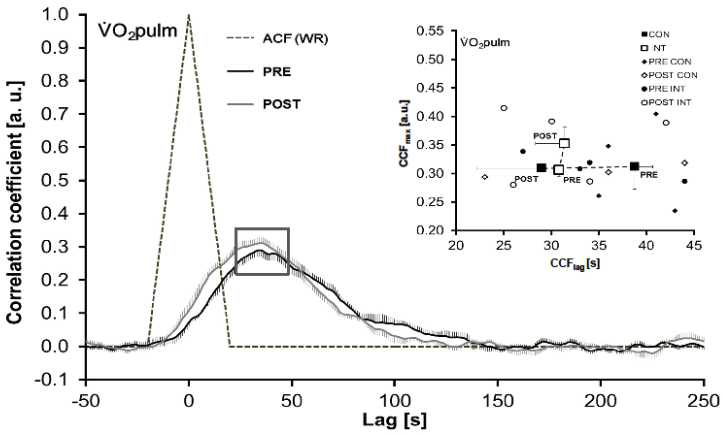


Fig. 3: Individual PRE and POST V'O_2pulm data of one participant of the INT (3a) and the CON (3b) training group. In each case, in the main graph the grey dotted line represents the work rate protocol, the black solid line show the PRE and the grey solid line the POST V'O_2pulm data (mean ± SE). The upper left panels show the auto-correlation function (ACF) of WR as grey dotted line, and the black

solid line the PRE and the grey solid line the POST cross-correlation function (CCF) of WR with $V'O_2$pulm. The upper right panels show the $V'O_2$musc responses equivalent to the left panel.





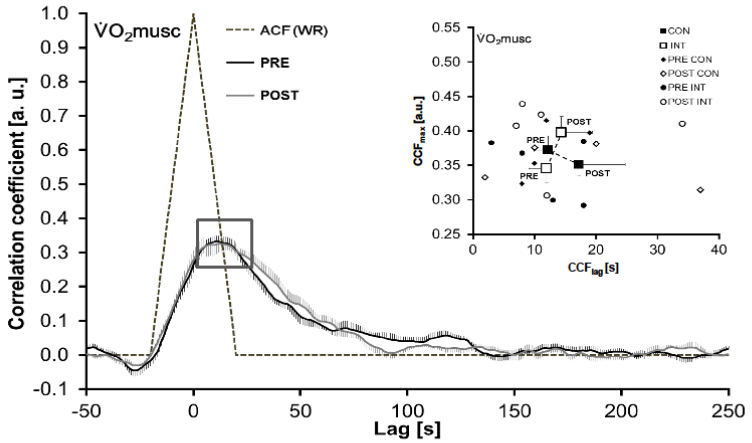


Fig. 4: Cross-correlation functions (means ± SE) of work rate (WR) with heart rate (HR; 4a), pulmonary ($V'O_2$pulm; 4b) and estimated muscle ($V'O_2$musc; 4c) oxygen uptake before (PRE) and after (POST) the endurance training intervention for all subjects (n=9), regardless of training group. The grey dotted triangles illustrate the auto-correlation function (ACF) of WR. In addition, the upper panels show the training group-specific (interval (n=5) versus continuous (n=4)) maxima of the cross-correlation functions (CCF_{max}; means ± SE) for the respective parameters from the main panel. For completeness, we added in the upper panels all data points of the INT and CON training group before (PRE) and after (POST) the training intervention.

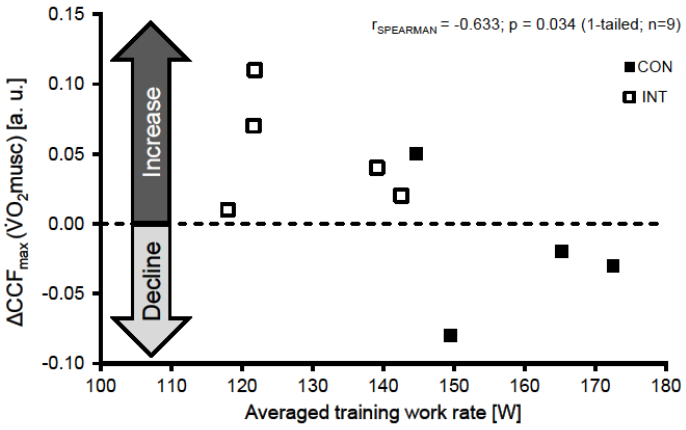


Fig. 5: Relationship (Spearman-rho) between the difference in the cross-correlation function maxima (ΔCCF_{max}: POST-PRE) between work rate (WR) and estimated muscle oxygen uptake ($V'O_2$musc) as function of averaged training WR during the intervention. Open rectangles display the individual responses of the interval (INT) and full rectangles of the continuous (CON) training group.

Tab. 1 Averaged (mean ± SD) absolute and relative training work rates, training durations (inclusively range [minimum, maximum]) for both, the continuous (CON) and the interval (INT) exercise training group. In addition, for the INT group the upper limits (UP) of the high intervals were presented as mean ± SD values and percentage of peak power output (PRE).

Group	Training description	Week					
		1	2	3	4	5	6
CON	Mean training work rate (W)	154 ± 5	155 ± 7	157 ± 13	161 ± 16	162 ± 19	158 ± 12
	Percentage of peak power output (PRE)	73	73	74	76	76	75
	Training duration (min)	23.0 ± 5.2	20.2 ± 0.3	20.7 ± 0.4	21.4 ± 0.4	22.3 ± 2.0	31.1 ± 0.4
	Range [minimum – maximum] (min)	20.0 ─ 29.0	20.0 ─ 20.5	20.5 ─ 21.0	21.0 ─ 21.8	20.5 ─ 24.5	26.5 ─ 33.8
INT	Mean training work rate (W)	125 ± 8	126 ± 8	131 ± 14	131 ± 14	130 ± 15	130 ± 14
	Percentage of peak power output (PRE)	61	62	64	64	64	64
	Mean training work rate UP limit (W)	199 ± 16	201 ± 16	210 ± 29	210 ± 28	210 ± 28	213 ± 27
	Percentage of peak power output (PRE)	98	99	103	103	103	105
	Training duration (min)	15.0 ± 0.0	19.7 ± 0.6	25.7 ± 1.2	30.3 ± 0.6	35.7 ± 0.6	39.8 ± 0.2
	Range [minimum – maximum] (min)	15.0 ─ 15.0	19.0 ─ 20.0	25.0 ─ 27.0	30.0 ─ 31.0	35.0 ─ 36.0	39.5 ─ 40.0

Tab. 2 Peak responses, exercise time and resting heart rate (means ± SD) before (PRE) and after (POST) exercise training intervention, and for the continuous (CON) and interval (INT) training group separately.

	Overall (n=9)			INT (n=5)		CON (n=4)	
	PRE	POST	Sig.	PRE	POST	PRE	POST
Peak power output (W)	208 ± 18	229 ± 23	a	203 ± 19	219 ± 10	214 ± 17	243 ± 28
Peak power output ($W \cdot kg^{-1}$)	2.3 ± 0.3	2.6 ± 0.3	a	2.3 ± 0.3	2.4 ± 0.1	2.5 ± 0.4	2.8 ± 0.4

Peak oxygen uptake ($L \cdot min^{-1}$)	3.2 ± 0.3	3.7 ± 0.2	b	3.3 ± 0.3	3.5 ± 0.2	3.3 ± 0.2	3.8 ± 0.3
Peak oxygen uptake ($mL \cdot min^{-1} \cdot kg^{-1}$)	37 ± 5	41 ± 4	b	36 ± 4	39 ± 1	38 ± 7	43 ± 6
Resting heart rate (min^{-1})	77 ± 14	66 ± 10	b	77 ± 15	70 ± 5	76 ± 15	61 ± 13
Peak heart rate (min^{-1})	164 ± 14	163 ± 11	n. s.	164 ± 17	163 ± 13	161 ± 8	163 ± 8
Exercise time (s)	1317 ± 40	1369 ± 54	a	1307 ± 45	1344 ± 35	1333 ± 41	1402 ± 68

a: significant difference between PRE and POST, P < 0.01 (2-tailed; Wilcoxon signed-rank-test)
b: significant difference between PRE and POST, P < 0.05 (2-tailed; Wilcoxon signed-rank-test)
n. s.: not significantly different between PRE and POST, P > 0.05 (Wilcoxon signed-rank-test)

Tab. 3 Averages (± SD) and slopes (± SD) before (PRE) and after (POST) training intervention of heart rate (HR) and pulmonary oxygen uptake ($V'O_2pulm$) during low constant WR (30 W), dynamic WR changes (PRBS 1 & 2: 53.3) and high constant WR (80 W) phases.

		HR		_O_2pulm	
Time	**Work rate**	Average [min^{-1}] a,b	Slope [$min^{-1} \cdot W^{-1}$] n.s.	Average [$L \cdot min^{-1}$] a,b,c	Slope [$L \cdot min^{-1} \cdot W^{-1}$] n.s.
PRE	30 W	93.9 ± 14.1	0.3831 ± 0.0989	0.897 ± 0.140	0.0097 ± 0.0018
	PRBS 1 (53.3 W)	102.0 ± 13.9		1.143 ± 0.116	
	PRBS 2 (53.3 W)	104.0 ± 14.8		1.157 ± 0.122	
	80 W	113.1 ± 14.9		1.386 ± 0.096	
POST	30 W	85.5 ± 12.0	0.4193 ± 0.1188	0.937 ± 0.121	0.0112 ± 0.0014
	PRBS 1 (67.3 W)	94.2 ± 12.2		1.202 ± 0.139	
	PRBS 2 (67.3 W)	97.0 ± 12.6		1.210 ± 0.132	

80 W	106.5 ± 14.1	1.496 ± 0.137

Slope data were derived from the WR-HR and the WR-$\dot{V}O_2$pulm relationship, respectively.
a: *Work rate* effect: $p < 0.001$ (two-way repeated measures ANOVA)
b: *Time* effect: $p < 0.05$ (two-way repeated measures ANOVA)
c: Interaction effect (*Time* x *Work rate*): $p < 0.05$ (two-way repeated measures ANOVA)
n.s.: not significantly different between PRE and POST $p > 0.05$ (Wilcoxon signed-rank-test)

Tab. 4 Means (± SD) of model parameter estimates and time series analysis data (CCF_{max}, CCF_{lag}) for heart rate (HR), pulmonary ($V'O_2$pulm) and muscle ($V'O_2$musc) oxygen uptake for both, the continuous (CON) and the interval (INT) exercise training group. Time constants (τ) were derived from CCF_{max}-values and time delays (TD) from the CCF_{lag}-values assuming 1st order system properties.

				V_v		**$\dot{Q}_{rem}$**		**$\dot{V}O_2$rem**		**HR**					**$\dot{V}O_2$pulm**					**$\dot{V}O_2$musc**			
				[mL]		[mL·min^{-1}]		[L·min^{-1}]		CCF_{max} [a.u.]	τ [s]	CCF_{lag} [s]	TD [s] *		CCF_{max} [a.u.]	τ [s]	CCF_{lag} [s]	TD [s] *		CCF_{max} [a.u.]	τ [s]	CCF_{lag} [s]	TD [s]
Group	**Time**		**Sig. (PRE-POST)**	n. s.		n. s.		n. s.		n. s.	n. s.	n. s.	n. s.		n. s.	n. s.	n. s.	n. s.		n. s.	n. s.	n. s.	n. s.
CON	PRE		Mean	2825		3535		0.303		0.364	39	10	-4.7		0.312	48	39	24.1		0.372	35	12	-1.9
			SD	435		995		0.041		0.101	18	2	1.7		0.078	17	4	4.0		0.042	6	5	5.0
	POST		Mean	2025		1675		0.269		0.351	38	13	-1.8		0.309	45	29	14.3		0.351	38	17	3.0
			SD	1372		222		0.040		0.031	5	4	4.0		0.013	3	14	13.8		0.033	5	15	15.0
INT	PRE		Mean	2500		2540		0.290		0.317	45	7	-7.2		0.306	46	31	16.1		0.345	39	12	-2.5
			SD	543		493		0.053		0.056	11	4	4.5		0.025	5	10	10.3		0.046	8	7	6.3
	POST		Mean	2340		2820		0.275		0.354	39	7	-7.3		0.352	39	31	17.1		0.398	32	14	0.5
			SD	1009		942		0.035		0.069	12	3	2.5		0.064	11	7	7.0		0.053	8	11	11.2

V_v: venous blood volume between exercising muscles and lungs Q_{rem} and $_O_2$rem: perfusion and oxygen uptake for the remainder of the body τ, TD: kinetics characteristics of the system time constant and time delay CCF_{max}: maximum of cross-correlation function CCF_{lag}: lag (abscissa) of CCF_{max}		n. s.: not significantly different between PRE and POST, $P > 0.05$ (n=9; Wilcoxon signed-rank-test) *: significantly different from zero (in PRE and in POST), $P < 0.05$ (n=9; one-sample Wilcoxon signed-rank-test)