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Differences in the point of optimal ventilatory efficiency and the anaerobic threshold in untrained adults aged 50 to 60 years

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ABSTRACT

Background: The point of optimal ventilatory efficiency (POE) and the anaerobic threshold (AT) are traditionally considered the same ventilatory indices, but recently differences between them have been reported. Therefore, the aim of this study was to identify different response patterns regarding POE and AT, and to analyse differences in breathing patterns as a possible explanation.

Methods: 118 females and 199 males aged 50 to 60 years performed an exercise test with gas analysis. POE and AT were determined, and the breathing patterns concerning ventilation, breathing frequency and tidal volume were assessed.

Results and Conclusion: Our study identified two different response patterns concerning the ventilatory indices POE and AT. Participants with a work rate difference between POE and AT (82% of all participants) were not different regarding breathing patterns of breathing frequency and tidal volume. However, the difference in work rate was explained by an early increase in ventilation and a higher aerobic capacity.

1. Introduction

The anaerobic threshold (AT), derived from gas exchange measures during cardiopulmonary exercise testing (CPET), is widely used to assess the functional capacity and to prescribe exercise intensity in patients, healthy people as well as athletes (Meyer et al., 2005; Ross, 2003). A precise identification of the AT is important in order to assess the actual aerobic capacity (%VO_{2peak}) and consequently prescribe reliable exercise training (work rate) intensities. Especially in patients with cardiac or pulmonary disease, an incorrectly selected exercise intensity could lead to undesirable complications. Different methods for AT detection exist, with the v-slope method currently being the gold standard. Nevertheless, it is common practice to determine the AT via the point of optimal ventilatory efficiency (POE), which is defined as the first disproportional increase of ventilation (VE) related to oxygen uptake (VO_2) (Binder et al., 2008; Hollmann, 2001; Meyer et al., 2005; Westhoff et al., 2013). But some authors have reported differences in the work rates between AT and POE (Gaskill et al., 2001; Ring-Dimitriou et al., 2014; Santos and Giannella-Neto, 2004; Tschentscher and Ring-Dimitriou, 2010). Therefore, it is important to point out the difference between AT and POE to avoid incorrect exercise intensity prescription.

Traditionally a three-phase model with two submaximal ventilatory indices, in particular the anaerobic threshold (AT) and the respiratory compensation point (RCP), is used to discriminate three phases of energy supply during an incremental CPET (Binder et al., 2008; Meyer et al., 2005; Westhoff et al., 2013). The AT characterises an individual's performance level, where a transition from a predominantly aerobic to a partially anaerobic energy metabolism occurs, and the RCP indicates the transition from a partly anaerobic to a predominantly anaerobic energy metabolism. A detailed description of these concepts is given elsewhere (Meyer et al., 2005; Wasserman et al., 2011; Westhoff et al., 2013).

A reason for the POE-AT differences may be found in the detection methods. Thereby, POE is determined by identifying the first disproportional increase of VE related to VO_2 (Hollmann, 2001, 1959), while the AT is determined by the first disproportional rise of expired carbon dioxide (VCO_2) related to VO_2 (v-slope method; Beaver et al., 1986; Wasserman et al., 2011).

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Although the increase in VE is closely linked to a rise in CO_2 production especially during the transition from moderate to heavy exercise intensity (Haouzi, 2006; Wasserman et al., 2011), other factors may explain differences between work rates at POE compared to AT. Reasons for that variation in the ventilatory response to incremental testing are factors like psychogenic stress, inter-individual differences in the alveolar gas pressure of carbon dioxide ($PaCO_2$), the sensitivity of the chemoreceptors to $PaCO_2$, or the dead space to tidal volume ratio (Meyer et al., 2005; Sun et al., 2002; Wasserman et al., 2011). Another possible factor influencing ventilatory response may be locomotor-respiratory coupling. In some individuals, breathing becomes integrated to exercise rhythms to minimize the work of breathing and therefore, this may be a potential adaption to regular exercise (Fulton et al., 2018; O'Halloran et al., 2012; Stickford and Stickford, 2014; Tipton et al., 2017). These factors may account for the large inter-individual differences found in the ventilatory response during incremental exercise between individuals and may explain the differences between POE and AT (Cross et al., 2012; Gravier et al., 2013).

VE is the product of tidal volume (V_T) and breathing frequency (f_B). Normally, at low work rates the ventilatory response is primarily achieved by an increase in V_T until it reaches a plateau at 50% to 60% of the forced vital capacity (FVC). During these low work rates, f_B remains relatively stable or increases only slightly until the V_T-plateau is reached. With increasing work rate beyond the V_T plateau, the rise in VE is predominantly achieved by increasing f_B, resulting finally in a "tachypnoeic shift" at RCP. However, a high inter-individual variability of this breathing pattern has been reported (Duffin et al., 2000; Scheuermann and Kowalchuk, 1999).

Alterations in the breathing patterns, like abnormal rapid and shallow breathing at any given VE, were found in chronic heart disease patients (Myers, 2000; Taguchi et al., 2015) and obese individuals (Chlif et al., 2009). After an improvement in aerobic power (VO_{2peak}) due to exercise training, a normalisation of the breathing patterns via a decrease of f_B during the moderate exercise intensity could be demonstrated. Moreover, in recreationally active individuals (Cross et al., 2012) and in athletes (Carey et al., 2008), different breathing patterns compared to untrained healthy individuals have been observed. Although, the plateauing in V_T is more commonly observed at RCP, some individuals adopt a V_T-plateau already at AT, or increase V_T till exhaustion without showing a V_T-plateau (Cross et al., 2012), or reduce V_T beyond AT (Gravier et al., 2013). Consequently, the response pattern of f_B is affected as well. Some individuals show higher f_B rates below RCP compared to others, who only show an increase in f_B at work rates after RCP. The most common observed exercise induced breathing pattern is that V_T and f_B change at separate work rates, while some individuals show an increase in V_T and f_B at the same time (Cross et al., 2012; Duffin et al., 2000; Gravier et al., 2013). It is speculated, that f_B and V_T are under separate control and that f_B can also be regulated by non-metabolic factors, like psychogenic stress or locomotor-respiratory coupling, as well (Ohashi et al., 2013; Tipton et al., 2017). Additionally, the central nervous respiratory control system exhibits considerable the magnitude of its modulation and plasticity due to the exercise stimuli, which may explain differences in breathing patterns between individuals as a result to training, the amount and intensity of daily activities or a sedentary lifestyle (Babb et al., 2010).

Therefore, we hypothesize that inter-individual differences in the breathing patterns at POE and AT in response to incremental exercise exist in untrained adults. And as a consequence different work rates at POE and AT will be detected (Binder et al., 2008; Gaskill et al., 2001; Ring-Dimitriou et al., 2014; Santos and Giannella-Neto, 2004; Westhoff et al., 2013). In order to prescribe reliable exercise intensities based on CPET, it is therefore important to confirm the difference between the POE and the AT and to analyse the possible underlying ventilatory mechanisms.

Thus, (1) the first aim of this study was to support the hypothesis, that differences in work rates between the ventilatory indices POE and AT exist. (2) The second aim was to analyse differences in breathing patterns between participants with different and equal work rates at POE and AT.

2. Methods

2.1. Participants

317 data-sets were drawn from a sub-sample of 916 participants of the Paracelsus 10.000 Study (P10-Study) who were randomly assigned for CPET. The P10-Study is a population based, observational study with the aim to investigate the state of health in 10.000 randomly selected 40 to 70 years old inhabitants of Salzburg, Austria (Salk, 2016). Hence, our sample consisted of 118 females and 199 males (Fig. 1 and Table 2). The P10-Study conformed to the principles outlined in the Declaration of Helsinki and was approved by the regional ethics committee of the federal state of Salzburg (E1521). All participants gave written informed consent.

2.2. Data collection

The measurements were supervised by the same investigators and were performed at the Pulmonary Diseases Outpatient Clinic of the Salzburg University Hospital, Austria between 13:00 and 15:00. Participants were instructed not to drink coffee or smoke on the test-day and were provided with standardized food.

2.2.1. Resting measurements

Each participant underwent standard spirometry measurements to determine FVC and forced expiratory volume over 1 s (FEV_1) using a portable spirometer (Easy One™ Spirometer, ndd Medical Technologies, Zurich, Switzerland). The tests were performed in sitting position and in triplicate with values being accepted when consecutive maneuvers yielded values within 10% of each other (Miller et al., 2005).

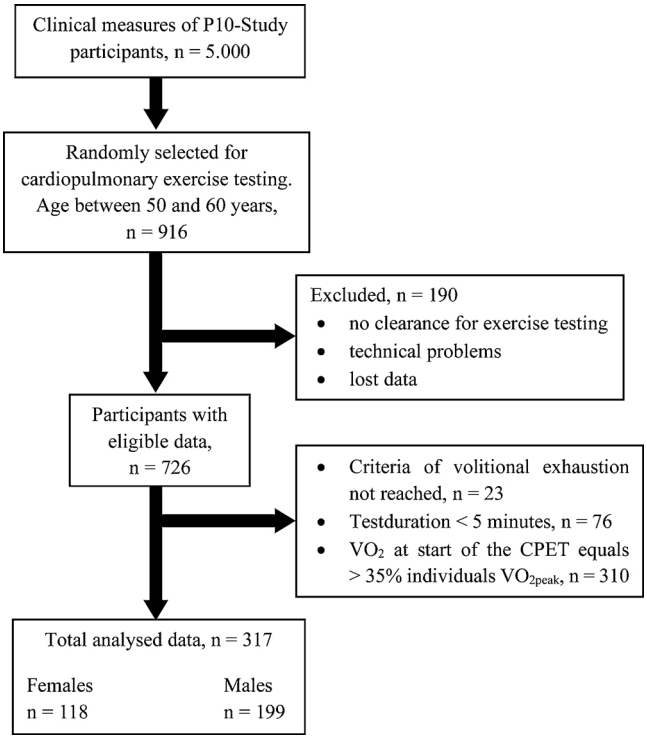

Fig. 1. Participant Flow.

2.2.2. Exercise measurements

After a medical examination performed by physicians including a detailed medical history and physical examinations, anthropometric measurements, laboratory evaluations (including blood chemistry, haematology and urine analysis) and an electrocardiogram, participants were assigned for graded exercise testing. Exclusion criteria for CPET were anaemia, cardiovascular disease, paralysis, abnormality of extremities, or other subjective limitations like pain or musculoskeletal disorders. During exercise, continuous respiratory gas analysis and volume measurements were performed using a facemask (Hans Rudolph, Kansas, USA) to ensure an airtight seal over the participant's nose and mouth with an attached volume sensor (Triple-V®) and a gas analyser (Master Screen CPX), which was connected using a semi-permeable sampling tube (Twin Tube, all products are manufactured by Jaeger, Höchberg, Germany). The following parameters were recorded breath-by-breath throughout the exercise and registered as raw data: VO_2, VCO_2, VE, V_T, f_B, end-tidal partial pressure of oxygen and carbon dioxide ($PETO_2$, $PETCO_2$), ventilatory equivalents of O_2 and CO_2 (EQO_2, $EQCO_2$). Calibration of the equipment was performed every day by medical technicians according to the instruction manual using the inbuilt calibration tools and a reference gas (mixture of 5% CO_2, 16% O_2, 79% N_2, Rießner Gase GmbH, Lichtenfels, Germany).

2.2.3. Exercise protocol

Each participant underwent a graded exercise test until volitional exhaustion. The exercise protocols were designed by the co-author (SRD) to reach volitional exhaustion after 8-12 min of test duration (Table 1) using different starting workloads and increments regarding sex and body mass-range as reported elsewhere (American College of Sports Medicine, 2013; Ross, 2003). The exercise test was performed on a cycle ergometer (ergo select 200 P, ergo line GmbH, Bitz, Germany), and the height of the seat and the position of the handlebar was adjusted individually. After a 2-min stationary phase with no pedalling to allow the participants to become accustomed to breathing through the mask and a 2-min warm-up period at 10 W, a graded exercise test with increasing workload every minute was performed until volitional exhaustion at a pedalling rate of 60 rpm. A 5-min recovery phase at 10 W was performed after exhaustion. Attainment of volitional exhaustion (and therefore VO_{2peak}) was confirmed by at least two of the following criteria (Ekkekakis et al., 2008; Ring-Dimitriou et al., 2014; Wasserman et al., 2011): (1) a plateau in VO_2 (changes of less than 2 ml · kg^{-1} · min^{-1} following an increase in workload); (2) $EQO_2 > 30$; (3) respiratory exchange ratio (RER) > 1.1; (4) achieving 90% of age predicted maximum heart rate (Tanaka et al., 2001); (5) pedalling rate < 50 rpm due to leg fatigue or shortness of breath. Exercise testing was terminated if any complications and contraindications occurred (Ross, 2003). During the graded exercise test electrocardiogram was continuously recorded and blood pressure was determined every two minutes.

2.3. Data processing

Spirometric values (FVC and FEV_1) were taken as the mean of the triplicate maneuvers performed prior to the graded exercise test. Breath-by-breath data were recorded and averaged over 10 s epochs through the graded testing procedure.

Table 1
Stationary cycling protocols of the P10-Study for CPET.

	Females			Males			Females and Males
Body mass range, kg	50-69	50-69	70-94	50-69	70-94	70-94	95-119
Initial workload, W	40	50	60	50	70	70	90
Increment, W · min^{-1}	10	10, 15[a]	10, 15[a]	15	15, 20[a]	20	20, 25[a]

Note: [a] increment rise after 6th minute of exercise test duration.

Data from the stationary cycling test (CPET) regarding warm-up and recovery phase were excluded from further analyses. The mean of the three consecutive highest 10 s VO_2, VE, V_T and f_B values at cessation was then taken as the respective peak value. Peak work rate (WR_{peak}) was determined as the mean work rate during the last minute of the exercise test (Merry et al., 2016; Robergs and Burnett, 2003). Participants who could not complete at least five minutes of the CPET were excluded from further analysis. It has been shown that POE occurs between 44% (Ramos et al., 2012) and 57% of VO_{2peak} (Ring-Dimitriou et al., 2014). Therefore, participants with a VO_2 of more than 35% of the individual VO_{2peak} at the onset of stationary cycling were excluded from further analysis because the initial applied work rate might have been too high in these individuals to determine the POE (Fig. 1).

2.3.1. Determination of ventilatory indices and group assignment

The ventilatory indices (POE, AT, RCP) were determined semi-automatic by combining automatic detection methods using polynomial regression and visual detection methods (Pühringer et al., 2020). POE, AT und RCP were determined by finding the first disproportional increase in a VE (y-axis) vs. VO_2 (x-axis) plot (Hollmann, 2001, 1959), in a VCO_2 vs. VO_2 plot (Beaver et al., 1986) and in a VE vs. VCO_2 plot (Wasserman et al., 2011), respectively.

To determine differences between the ventilatory indices POE and AT, work rate at the POE and at the AT were determined, and the investigated participants were categorized into the following sub-groups: (1) group 1, participants with a difference in work rate between POE and AT. (2) group 2, participants without a difference in work rate between POE and AT. Thus, 95 females and 164 males were assigned to group 1, and 23 females and 35 males were assigned to group 2.

2.3.2. Determination of the different breathing patterns

To determine differences in breathing patterns, absolute values of VE, V_T and f_B at the ventilatory indices POE and AT were computed and analysed. Additionally, the different components of the breathing patterns (VE, V_T and f_B) were plotted against the relative oxygen uptake (VO_{2rel}, expressed in terms of $\%VO_{2peak}$) of each participant. If an RCP occurred, data above the RCP were removed. First, these plots were divided into two segments separated by AT, and the slopes of the fitted linear least square regressions of the data before the AT (pre-AT) and after the AT (post-AT) were calculated. Furthermore, the Adjusted R-squares (R^2_{adj}) of these fitted linear least square regressions were calculated to evaluate the goodness of the fit. Second, the same procedure was applied to the data separated by the POE and the slopes (pre-POE, post-POE) as well as the corresponding R^2_{adj} were calculated. Third, the R^2_{adj} of the fitted linear least square regressions of f_B vs. VO_{2rel} post-AT and post-POE were compared. If the R^2_{adj} post-POE was higher than post-AT, we assumed that the f_B started to change at POE and therefore, the slopes of the fitted linear least square regressions pre-POE and post-POE were used as pre- and post-VI for further analysis. Otherwise, the pre-AT and post-AT slopes were used as pre- and post-VI. Finally, the investigated participants were categorized into the following sub-groups concerning their breathing patterns: (1) Breathing pattern 1, participants with a constant or decreasing increase rate of f_B till RCP, while V_T increases disproportionately at POE or AT. (2) Breathing pattern 2, participants with a disproportionate increase in f_B beginning at AT. (3) Breathing pattern 3, participants with a disproportionate increase in f_B beginning at POE (see Fig. 3). Thus, 22, 25 and 75 females and 22, 61 and 116 males were assigned to breathing pattern 1, 2 and 3, respectively.

2.3.3. Determination of the ventilatory efficiency and the oxygen uptake-work rate relationship

Ventilatory efficiency was assessed by the slope of the linear regression of VE vs. VCO_2 for all exercise values up until the RCP (VE/VCO_2 slope) and by finding the lowest VE/VCO_2 output ratio during

exercise (VE/VCO_2 min) (Sun et al., 2002). The VO_2 – work rate relationship (VO_2/WR slope) was assessed by linear regression of the VO_2 vs. work rate slope for all exercise values up until VO_{2peak} (Wasserman et al., 2011).

2.4. Statistical analysis

Data are given as means ± standard deviation. Due to sex differences in energy metabolism and exercise performance, analysis were conducted separately for male and female participants (Guenette et al., 2009). The Shapiro-Wilk test and visual inspection of histograms and quantile-quantile plots were used to verify the normal distribution of the data ($n < 50$). Participant characteristics and CPET variables at rest, at POE, AT and at the end of exercise were compared between groups using unpaired *t*-tests (Table 2 and Table 3). Differences in work rate, heart rate (HR), VO_2, RER, VE, V_T and f_B between POE and AT were assessed by performing paired t-tests (Table 3). To test for statistical differences concerning the changes in the slopes of the regression lines from pre- to post-VI (within-subjects) and between groups and breathing patterns (between-subjects), mixed-design analysis of variances (ANOVA) were used for VE, V_T and f_B separately. Bonferroni post hoc tests were applied when ANOVA indicated significant interaction effects (Table 4). The relations between the aerobic capacity (in terms of VO_2 at the AT) and the magnitude of the work rate differences between POE and AT were described by linear regression analysis using Pearson correlations. A Chi-square test was executed to test the differences in the frequency (%) of breathing patterns (1, 2 or 3) between group 1 and group 2. The level of significance was set at $\alpha \leq .05$. The statistical analyses were performed using RStudio version 1.2.5001 (RStudio Inc., Boston, Massachusetts, USA).

3. Results

3.1. Participant characteristics

No significant differences were observed between group 1 (different work rates at POE and AT) and group 2 (equal work rates at POE and AT) participants in age, anthropometric characteristics and results of resting spirometry. The female and male participants of this study displayed normal pulmonary function at rest with an average FVC of 3.6 ± 0.5 L and 4.9 ± 0.7 L, and an average FEV_1 of 2.7 ± 0.4 L and 3.6 ± 0.6 L, respectively (Quanjer et al., 2012). The participants are characterized by an average cardiorespiratory fitness (in terms of VO_{2peak}) of 25.9 ± 4.7 $ml \cdot kg^{-1} \cdot min^{-1}$ (females) and 31.1 ± 6.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (males) (Rapp et al., 2018).

In females as well as in males $\%VO_{2peak}$ at the POE was significantly higher in group 2, while $\%VO_{2peak}$ at the AT was significantly lower in group 2 compared to group 1. Significant lower RER at POE compared to AT in group 1 females and males, but not in group 2 participants were observed. RER at the POE differed significantly between group 1 and group 2 in females as well as in males. Further, the ventilatory efficiency and the VO_2 – work rate relationship differed significantly between group 1 and group 2 participants (Table 2 and 3).

3.2. Differences in work rates between POE and AT

Participants were divided into two groups: 259 participants (95 females and 164 males) showed a difference between work rates at POE

Table 2
Mean (± standard deviation) age, anthropometric characteristics and main results of resting spirometry and CPET for participants with (group1) and without (group 2) a difference in the work rate between the point of optimal ventilatory efficiency (POE) and the anaerobic threshold (AT), reported for females and males separately.

	Females			Males		
	Group 1	Group 2	*p*-value	Group 1	Group 2	*p*-value
N	95	23		164	35	
Characteristics						
Age, yrs	55 ± 3	53 ± 2	n.s.	55 ± 3	54 ± 3	n.s.
Body mass, kg	66 ± 11	70 ± 13	n.s.	83 ± 11	81 ± 10	n.s.
Height, m	1.7 ± 0.1	1.7 ± 0.1	n.s.	1.8 ± 0.1	1.8 ± 0.1	n.s.
BMI, $kg \cdot m^{-2}$	23.8 ± 3.6	24.7 ± 4.4	n.s.	25.9 ± 3.2	25.7 ± 3.0	n.s.
Comorbidity						
Hypertension, N (%)	7 (7)	4 (17)	-	23 (14)	7 (20)	-
Pulmonary disease, N (%)	6 (6)	0 (0)	-	20 (12)	4 (11)	-
Diabetes mellitus, N (%)	1 (1)	0 (0)	-	5 (3)	1 (3)	-
Cardiovascular disease, N (%)	2 (2)	0 (0)	-	12 (7)	1 (3)	-
Resting Spirometry						
FVC, L	3.6 ± 0.5	3.6 ± 0.6	n.s.	4.8 ± 0.7	5.0 ± 0.6	n.s.
FEV_1, L	2.7 ± 0.4	2.7 ± 0.5	n.s.	3.6 ± 0.6	3.8 ± 0.6	n.s.
FEV_1 / FVC, %	75 ± 6	76 ± 6	n.s.	75 ± 7	75 ± 7	n.s.
[b] MVV, $L \cdot min^{-1}$	108.5 ± 15.2	108.7 ± 19.1	n.s.	144.4 ± 23.5	151.0 ± 23.0	n.s.
CPET						
WR_{peak}, $W \cdot kg^{-1}$	2.3 ± 0.4	2.2 ± 0.4	n.s.	2.8 ± 0.6	2.6 ± 0.5	.022[#]
$\%VO_{2peak}$ at RCP, %	84 ± 10	78 ± 14	.032*	84 ± 10	82 ± 11	n.s.
VO_{2peak}, $ml \cdot kg^{-1} \cdot min^{-1}$	26.2 ± 4.8	25.1 ± 4.4	n.s.	31.5 ± 6.3	29.3 ± 4.8	n.s.
RER_{peak}	1.21 ± 0.06	1.22 ± 0.11	n.s.	1.23 ± 0.08	1.23 ± 0.08	n.s.
VE_{peak}, $L \cdot min^{-1}$	64.0 ± 13.4	61.1 ± 12.3	n.s.	95.0 ± 21.1	91.1 ± 15.6	n.s.
VE_{peak} / MVV, %	61 ± 15	54 ± 9	n.s.	67 ± 15	64 ± 14	n.s.
V_{Tpeak}, L	1.9 ± 0.3	1.9 ± 0.4	n.s.	2.9 ± 0.5	2.8 ± 0.5	n.s.
V_{Tpeak} / FVC, %	52 ± 11	53 ± 7	n.s.	60 ± 9	59 ± 6	n.s.
f_{Bpeak}, min^{-1}	36 ± 6	33 ± 6	n.s.	35 ± 7	33 ± 7	n.s.
HR_{peak}, min^{-1}	163 ± 12	162 ± 11	n.s.	157 ± 31	161 ± 13	n.s.
VO_2/WR slope, ml $O_2 \cdot W^{-1}$	10.5 ± 1.4	11.4 ± 1.7	.003*	10.3 ± 1.2	10.8 ± 1.4	.032[#]
VE/VCO_2 slope	25.8 ± 3.4	23.8 ± 2.5	.010*	25.0 ± 3.0	26.3 ± 3.9	.040[#]
VE/VCO_2 min	26.0 ± 2.4	24.6 ± 1.6	.007*	25.5 ± 2.2	26.2 ± 2.2	n.s.

Note: BMI: body mass index; FVC: forced vital capacity; FEV_1: forced expiratory volume over 1 s; [b] MVV = FEV_1 x 40 (Wasserman et al., 2011): maximal voluntary ventilation; WR: work rate; VO_2: oxygen uptake; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; VE: ventilation; V_T: tidal volume; f_B: breathing frequency; HR: heart rate; VO_2/WR slope: VO_2 – work rate relationship; VE/VCO_2 slope and VE/VCO_2 min: ventilatory efficiency; n.s.: not significant; *significant difference ($p \leq .05$) between group 1 and group 2 females; [#]significant difference ($p \leq .05$) between group 1 and group 2 males.

Table 3
Mean (± standard deviation) work rate (WR), heart rate (HR), oxygen uptake (VO_2), respiratory exchange ratio (RER), ventilation (VE), tidal volume (V_T) and breathing frequency (f_B) at the point of optimal ventilatory efficiency (POE) and the anaerobic threshold (AT) in group 1 and group 2 participants, reported for females and males separately.

	Females			Males		
	Group 1	Group 2	*p*-value	Group 1	Group 2	*p*-value
N	95	23		164	35	
WR at POE, W · kg^{-1}	0.91 ± 0.23	1.09 ± 0.26	.001*	1.06 ± 0.22	1.20 ± 0.27	< .001$^{\#}$
WR at AT, W · kg^{-1}	1.19 ± 0.29	1.08 ± 0.25	n.s.	1.46 ± 0.39	1.20 ± 0.27	< .001$^{\#}$
***p*-value**	<.001†	n.s.		<.001$^{\$}$	n.s.	
%WR_{peak} at POE, %	40 ± 8	51 ± 11	< .001*	38 ± 8	47 ± 7	< .001$^{\#}$
%WR_{peak} at AT, %	52 ± 10	51 ± 11	n.s.	52 ± 10	47 ± 7	.002$^{\#}$
***p*-value**	<.001†	n.s.		<.001$^{\$}$	n.s.	
HR at POE, min^{-1}	110 ± 14	116 ± 12	.040*	104 ± 12	109 ± 11	.023$^{\#}$
HR at AT, min^{-1}	120 ± 15	118 ± 11	n.s.	115 ± 14	111 ± 11	n.s.
***p*-value**	<.001†	.019†		<.001$^{\$}$	<.001$^{\$}$	
%HR_{peak} at POE, %	68 ± 7	72 ± 8	.009*	65 ± 8	68 ± 5	.034$^{\#}$
%HR_{peak} at AT, %	74 ± 7	73 ± 7	n.s.	72 ± 8	69 ± 5	.043$^{\#}$
***p*-value**	<.001†	n.s.		<.001$^{\$}$	<.001$^{\$}$	
%VO_{2peak} at POE, %	46 ± 9	51 ± 11	.044*	44 ± 7	49 ± 7	< .001$^{\#}$
%VO_{2peak} at AT, %	57 ± 10	52 ± 10	.016*	58 ± 9	52 ± 7	< .001$^{\#}$
***p*-value**	<.001†	n.s.		<.001$^{\$}$	<.001$^{\$}$	
VO_2 at POE, ml · min^{-1} · kg^{-1}	12.0 ± 3.2	12.6 ± 3.3	n.s.	13.7 ± 2.9	14.5 ± 3.5	n.s.
VO_2 at AT, ml · min^{-1} · kg^{-1}	15.0 ± 3.8	13.0 ± 3.3	.020*	18.2 ± 4.6	15.4 ± 3.5	< .001$^{\#}$
***p*-value**	<.001†	.038†		<.001$^{\$}$	<.001$^{\$}$	
RER at POE	0.88 ± 0.06	0.91 ± 0.10	.023*	0.86 ± 0.07	0.90 ± 0.06	.001$^{\#}$
RER at AT	0.94 ± 0.06	0.93 ± 0.09	n.s.	0.94 ± 0.07	0.92 ± 0.06	n.s.
***p*-value**	<.001†	.020†		<.001$^{\$}$	<.001$^{\$}$	
VE at POE, L · min^{-1}	20.3 ± 5.6	21.4 ± 5.6	n.s.	27.8 ± 5.3	30.5 ± 6.1	.009$^{\#}$
VE at AT, L · min^{-1}	26.1 ± 7.1	22.2 ± 5.7	.016*	38.0 ± 8.6	32.6 ± 6.1	< .001$^{\#}$
***p*-value**	<.001†	.038†		<.001$^{\$}$	<.001$^{\$}$	
V_T at POE, L	1.0 ± 0.3	1.1 ± 0.3	n.s.	1.6 ± 0.4	1.7 ± 0.4	n.s.
V_T at AT, L	1.3 ± 0.4	1.2 ± 0.3	n.s.	2.0 ± 0.5	1.8 ± 0.4	.008$^{\#}$
***p*-value**	<.001†	.037†		<.001$^{\$}$	<.001$^{\$}$	
f_B at POE, min^{-1}	20 ± 4	19 ± 3	n.s.	18 ± 4	19 ± 3	n.s.
f_B at AT, min^{-1}	21 ± 4	19 ± 3	.007*	20 ± 4	19 ± 4	n.s.
***p*-value**	<.001†	n.s.		<.001$^{\$}$	.016$^{\$}$	

Note: n.s.: not significant; *significant difference ($p \leq .05$) between group 1 and group 2 females; †significant difference ($p \leq .05$) between POE and AT in females in group 1 or group 2, respectively; $^{\#}$significant difference ($p \leq .05$) between group 1 and group 2 males; $^{\$}$significant difference ($p \leq .05$) between POE and AT in males in group 1 or group 2, respectively

and AT and were included in group 1. The 58 participants (23 females and 35 males) with no difference were included in group 2. The mean (± standard deviation) difference in work rates of the group 1 participants was significantly higher in males than in females (32 ± 25 W vs. 18 ± 10 W; Table 2). Interestingly, there were significant correlations between the aerobic capacity (in terms of VO_2 at the AT) and the magnitude of the work rate differences between POE and AT (Fig. 2).

There was a significant higher work rate at POE (W · kg^{-1}) in group 2 than in group 1 females and males. A significant difference between work rate at the AT (W · kg^{-1}) and the POE (W · kg^{-1}) in group 1, but not in group 2 participants was observed. VO_2 (ml · min^{-1} · kg^{-1}) was found to be significantly higher at AT compared to POE in group 1 and group 2 (Table 3).

3.3. *Differences in breathing patterns between group 1 and group 2 participants*

In both groups and for both sexes, VE, V_T and f_B were significantly higher at the AT than at the POE, with the exception of the f_B in group 2 females. Between group 1 and group 2, we found significant differences mainly at the AT. Group 1 participants reached a significant higher VE at the AT than participants of group 2. In females the f_B at the AT and in males the V_T at the AT were significantly higher in group1 than in group 2 participants, respectively (Table 3).

The mean values of the slopes of VE, V_T and f_B vs. VO_{2rel} regression lines pre- and post-VI are shown in Table 4. The mixed-design ANOVA revealed significant main effects between the slopes of pre- and post-VI for VE (females: $F(1, 112) = 92.9$, $p < .001$; males: $F(1, 193) = 287.7$, $p < .001$) and f_B (females: $F(1, 112) = 18.0$, $p < .001$; males: $F(1, 193) = 47.0$, $p < .001$). No significant main effects between group 1 and group 2 participants could be found. Concerning the slopes of the fitted linear least square regressions of f_B vs. VO_{2rel}, we identified 3 different breathing patterns. Fig. 3 illustrates the evolution of VE, V_T and f_B changes during the exercise test of three participants with different breathing patterns, all of them presented with the three ventilatory indices (POE, AT and RCP).

Significant interaction between pre- and post-VI and breathing patterns could be found for VE (females: $F(2, 112) = 3.8$, $p = .026$; males: $F(2, 193) = 3.0$, $p = .050$), V_T (females: : $F(2, 112) = 13.4$, $p < .001$, males: $F(2, 193) = 15.1$, $p < .001$) and f_B (females: $F(2, 112) = 28.9$, $p < .001$; males: $F(2, 193) = 30.0$, $p < .001$). *Post-hoc* pairwise comparisons indicated significant differences between the three breathing patterns. In all three breathing patterns, the slope of the fitted linear least square regression of VE vs. VO_{2rel} increased significantly after the ventilatory indices (POE or AT), with the increase in breathing pattern 2 and 3 tending to be higher than in breathing pattern 1. Participants with breathing patterns 2 and 3 achieved this increase in ventilation by significantly increasing f_B at POE or AT, respectively. Participants with breathing pattern 1 showed a decrease in the slope of f_B vs. VO_{2rel} (significantly in females but only with a tendency to decrease f_B in males) and realised the increase in ventilation mainly by a significant increase in V_T (Table 4).

The percentage of the three different breathing patterns (1 vs. 2 vs. 3) did not differ between group 1 (females: 19% vs. 20% vs. 61%; males: 17% vs. 26% vs. 57%) and group 2 (females: 17% vs. 26% vs. 57%; males: 14% vs. 29% vs. 57%) participants (females: χ^2 (2, $N = 118$) = 0.41, $p = .8$; males: χ^2 (2, $N = 199$) = 0.47, $p = .8$).

Table 4
Changes in slopes of the fitted linear least square regression of ventilatory variables below (pre-VI) and above (post-VI) the ventilatory indices, reported for females and males separately.

			Females				Males			
		Breathing pattern	Group 1	Group 2	Total	p-value	Group 1	Group 2	Total	p-value
N			95	23	118		164	35	199	
		1	0.386 ± 0.079	0.354 ± 0.039	0.334 ± 0.099		0.499 ± 0.205	0.462 ± 0.110	0.490 ± 0.186	
	pre-VI	2	0.359 ± 0.077	0.326 ± 0.065	0.351 ± 0.075		0.529 ± 0.134	0.474 ± 0.157	0.520 ± 0.138	
		3	0.383 ± 0.107	0.351 ± 0.090	0.327 ± 0.101		0.429 ± 0.166	0.498 ± 0.110	0.441 ± 0.160	
Slope VE vs. VO_{2rel}		1	0.520 ± 0.120	0.443 ± 0.119	0.537 ± 0.129	.010*	0.790 ± 0.189	0.792 ± 0.240	0.791 ± 0.196	< .001#
	post-VI	2	0.506 ± 0.241	0.554 ± 0.128	0.517 ± 0.217	< .001*	0.922 ± 0.177	0.848 ± 0.214	0.910 ± 0.183	< .001#
		3	0.596 ± 0.162	0.604 ± 0.209	0.608 ± 0.161	< .001*	0.888 ± 0.198	0.925 ± 0.147	0.895 ± 0.190	< .001#
		1	0.008 ± 0.013	0.013 ± 0.004	0.009 ± 0.012		0.013 ± 0.015	0.024 ± 0.008	0.016 ± 0.014	
	pre-VI	2	0.018 ± 0.006	0.015 ± 0.006	0.017 ± 0.006		0.030 ± 0.009	0.028 ± 0.008	0.029 ± 0.009	
		3	0.020 ± 0.012	0.024 ± 0.008	0.021 ± 0.011		0.031 ± 0.023	0.030 ± 0.009	0.031 ± 0.021	
Slope V_T vs. VO_{2rel}		1	0.024 ± 0.008	0.024 ± 0.006	0.024 ± 0.008	.005*	0.030 ± 0.012	0.046 ± 0.028	0.034 ± 0.018	< .001#
	post-VI	2	0.008 ± 0.015	0.012 ± 0.006	0.009 ± 0.013	n.s.	0.019 ± 0.008	0.021 ± 0.006	0.019 ± 0.008	n.s.
		3	0.014 ± 0.006	0.015 ± 0.006	0.014 ± 0.006	.007*	0.024 ± 0.009	0.024 ± 0.006	0.024 ± 0.009	0.047#
		1	0.193 ± 0.221	0.166 ± 0.142	0.188 ± 0.206		0.209 ± 0.200	0.025 ± 0.093	0.167 ± 0.196	
	pre-VI	2	−0.004 ± 0.078	0.039 ± 0.074	0.007 ± 0.078		−0.026 ± 0.105	−0.055 ± 0.110	−0.031 ± 0.105	
		3	−0.068 ± 0.178	−0.108 ± 0.149	−0.076 ± 0.172		−0.104 ± 0.191	−0.025 ± 0.134	−0.090 ± 0.184	
Slope f_B vs. $VO2_{rel}$		1	0.037 ± 0.110	−0.022 ± 0.130	0.026 ± 0.113	.024*	0.067 ± 0.153	−0.050 ± 0.166	0.040 ± 0.160	n.s.
	post-VI	2	0.214 ± 0.113	0.229 ± 0.120	0.217 ± 0.112	< .001*	0.221 ± 0.065	0.184 ± 0.091	0.215 ± 0.070	< .001#
		3	0.193 ± 0.075	0.211 ± 0.127	0.196 ± 0.086	< .001*	0.168 ± 0.091	0.185 ± 0.072	0.171 ± 0.088	< .001#

Note: VO_{2rel}: relative oxygen uptake (in terms of $\%VO_{2peak}$); VE: ventilation; V_T: tidal volume; f_B: breathing frequency; n.s.: not significant; *significant difference ($p \leq .05$) between pre-VI and post-VI in females (total); #significant difference ($p \leq .05$) between pre-VI and post-VI in males (total).

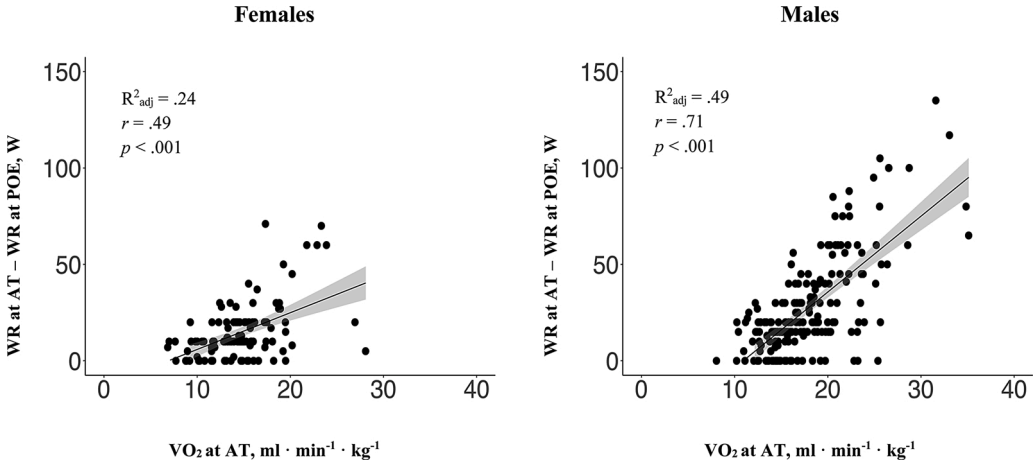

Fig. 2. The relations between the aerobic capacity (in terms of VO_2 at AT) and the magnitude of the work rate (WR) differences between the point of optimal ventilatory efficiency (POE) and the anaerobic threshold (AT), separately for females and males. R^2_{adj}: Adjusted R-square; r: Pearson's product-moment correlation coefficient.

4. Discussion

In this study we investigated, whether differences in work rates between the two ventilatory indices POE and AT exist in untrained adults. Further, we wanted to support the hypothesis that these work rate differences can be explained by inter-individual differences in breathing patterns at the POE and the AT.

4.1. Participant characteristics and differences in work rates between POE and AT

Our study strongly supports the existence of two different response patterns concerning the two ventilatory indices POE and AT during an incremental exercise test. While only 19% of the females and 18% of the males in this study had their POE and AT at the same work rate (group 2), the remaining participants had their POE at a significantly lower work rate than their AT (group 1). The mean difference between POE and AT in group 1 was 18 ± 10 W in females and 32 ± 25 W in males. This is in line with other studies, who found the POE at a lower work rate than the AT (Ramos et al., 2012; Ring-Dimitriou et al., 2014).

Aside from the POE - AT work rate difference, the two groups were comparable concerning age, anthropometric characteristics, resting spirometric values and peak CPET results. The POE and AT were found at 46 ± 8 and 57 ± 9 $\%VO_{2peak}$ in the total sample, which is in agreement with other studies (Meyer et al., 2005; Ramos et al., 2012; Ring--Dimitriou et al., 2014).

In group 2, the disproportionate increase in VE coincides with the disproportionate increase in VCO_2, while group 1 participants increase VE and VCO_2 independently. In group 1, the VE is increased first (at POE) while the disproportionate increase in VCO_2 occurs at a higher work rate (at AT) during the incremental exercise test. This result is in contrast to the traditional three-phase model with two ventilatory indices, for which POE and AT are considered the same ventilatory indices explained by the close link between VE and VCO_2 (Binder et al., 2008; Meyer et al., 2005; Westhoff et al., 2013). But it seems that some factors interrupt this close link in more than 80% of our participants and

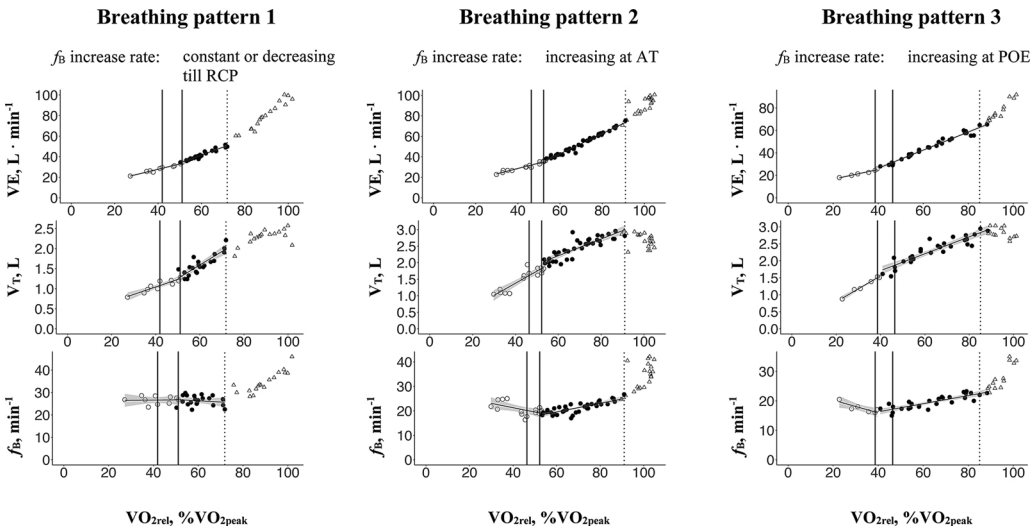

Fig. 3. Exemplary data of three participants with different breathing patterns based on the occurrence of the changes in the slopes of breathing frequency (f_B) vs. relative oxygen uptake (VO_{2rel}). The data-points (open and closed symbols) represent the 10-second time based averaged breath-by-breath results of the CPET. The solid lines along the data-points represent the linear regressions with their 95% confidence intervals (dark grey area) = slopes pre- and post-VI. The left and right vertical solid lines represent the point of optimal ventilatory efficiency (POE) or the anaerobic threshold (AT), respectively. The vertical dotted lines represent the respiratory compensation point (RCP). VE: ventilation; V_T: tidal volume.

therefore POE and AT cannot be considered the same ventilatory index.

While there was no difference in the aerobic power (VO_{2peak}) between group 1 and group 2 participants, we found a significant higher aerobic capacity (in %VO_{2peak} at the AT) in group 1 compared to group 2 participants and a significant greater work rate at AT in group 1 males but only a tendency in group 1 females. In addition, the disproportional increase in VE at POE occurred at a significant lower relative VO_2 (in % VO_{2peak} at the POE) in group 1 compared to group 2 participants (Table 3). These differences may be explained by a different strategy of group 1 participants to cope with the metabolic acidosis caused by the increasing workload during the exercise test. By increasing ventilation earlier to improve VO_2, the acid-base homeostasis may be maintained longer, which leads to a delay in the appearance of the AT. Although, the AT in group 1 participants was found at a higher relative VO_2 compared to the AT in group 2, there was no significant difference in the RER at the AT between the two groups. The RER is closely linked to the respiratory quotient and therefore indicates the kind of substrates being oxidized by the energy metabolism to supply the body with energy (Jeukendrup and Wallis, 2005). The same RER-value in both groups points toward an equal amount of fat and carbohydrates as a fuel source at the AT (Table 3). Consequently, in group 1 the fat metabolism contributes a higher proportion of the energy needed compared to group 2 participants at comparable relative VO_2 levels till the AT. Additionally, RER at the POE was found to be lower than at the AT in group 1 participants ($p < .001$). Consequently, group 1 participants increase ventilation at a lower RER where more fat is used as a fuel for energy metabolism compared to the fuel mix at POE in group 2 participants (Jeukendrup and Wallis, 2005).

The AT reflects, at least partly, the degree of adaption of humans to endurance exercise and their fitness level (Meyer et al., 2005). Therefore, it is an important index that describes the highest work rate that can be sustained aerobically and can be endured for a prolonged period of time (Wasserman et al., 2011). In endurance-trained individuals the AT is found at relatively higher aerobic capacity compared to healthy sedentary individuals, i.e. at 65-75 vs. 50-58 %VO_{2peak} (Meyer et al., 2005). The adaption of ventilatory strategies by regular exercise (VE increase at the POE before the occurrence of the AT) may therefore be responsible for the higher relative values of AT in addition to the known metabolic adaptions (Meyer et al., 2005; Wasserman et al., 2011). Ventilatory adaptions to regular physical activity (like locomotor-respiratory coupling) have already been shown to minimize the work of breathing (Fulton et al., 2018; O'Halloran et al., 2012; Stickford and Stickford, 2014; Tipton et al., 2017) and may therefore be the reason of the decreased metabolic requirement during exercise in group 1 participants indicated by the significantly lower VO_2 – work rate relationship. A flattening of the VO_2 - work rate relationship has been attributed to decreased work of breathing (Wasserman et al., 2011). Consequently, this may lead to the increased aerobic capacity (VO_2 at AT) found in this group. An early increase in ventilation, indicated by the detection of POE at a lower work rate compared to AT, may therefore be another ventilatory adaption to regular exercise.

4.2. Differences in breathing patterns between group 1 and group 2 participants

In all participants, the mean values of the slopes of VE increased significantly at the POE or the AT (Table 4) but marked inter-individual differences in breathing patterns concerning f_B and V_T have been found. Based on the changes of f_B, we identified three different breathing patterns (Fig. 3). Participants who presented with the breathing patterns 1 and 2 (14% and 27% of all participants) increased the rate of VE significantly at the AT. While participants with breathing pattern 2 realized the VE increase at the AT mainly by significantly increasing f_B, participants with breathing pattern 1 maintained or even decreased f_B until the RCP. In contrast, they increased V_T at the AT to achieve the increase in VE. These two breathing patterns have already been described by others (Carey et al., 2008; Cross et al., 2012; Duffin et al., 2000; Gravier et al., 2013; Lucía et al., 1999) and it has been stated, that the respiratory control by peripheral vagal afferents may prevail in those who increase mainly f_B and not V_T above the AT (Gravier et al., 2013). Additionally, rapid shallow breathing patterns with an increased f_B have also been reported in patients with chronic heart failure (Myers, 2000) and it has been demonstrated that the f_B is more susceptible than V_T to behavioural factors like psychogenic stress (Ohashi et al., 2013).

Besides the two well-known breathing patterns, we found a third breathing pattern, which is characterized by an early increase in f_B at the POE and is found in 59% of all participants. To our knowledge, this is the first study to describe three different breathing patterns. Adding up the number of participants who present with breathing pattern 2 and 3, 86% of the participants in this study had an early increase in f_B during the exercise test. This is in contrast to typical models who state that normally V_T is increased first till a plateau with a later increase in f_B as a result of the chemostimulation of the respiratory centres by an increasing blood acidosis (Carey et al., 2008; Duffin et al., 2000; Lucía et al., 1999). But in contrast to these models, 86% of the untrained adult participants in this study preferably increase f_B at the low and moderate exercise intensity. Therefore, further investigations concerning the origin of inter-individual breathing pattern differences are needed.

As the Chi-square analysis indicated no difference in the breathing patterns between group 1 and group 2 participants, breathing pattern

differences concerning f_B and V_T do not seem to be responsible for the work rate differences between POE and AT.

4.3. Limitations of the study

Because of the highly variable working capacity in the study population, different exercise protocols utilizing different starting workloads and increments for sex and body mass-ranges were used to yield an exercise duration of 8-12 min. This may have influenced the ventilatory indices determination. However, as previous studies did not find differences in ventilatory indices determined by gas analysis when using different exercise protocols (Wasserman et al., 2011), we believe that this is of minor influence to our results.

Although we paid close attention to maintain a constant pedalling rate of 60 rpm during the exercise test to eliminate the influence of the locomotor-respiratory coupling in the f_B changes, small fluctuations during the measurements could not be avoided and might have influenced the breathing patterns.

5. Conclusion

The point of optimal ventilatory efficiency (POE) and the anaerobic threshold (AT) are two different ventilatory indices in 82% of the participants in this study, while in the remaining participants, those two indices occur simultaneously during an incremental exercise test. Participants with a difference in work rates at POE and AT began to increase VE earlier (in terms of $\%VO_{2peak}$) and were characterized by a higher aerobic capacity (in terms of $\%VO_{2peak}$ at the AT) compared to participants without a POE – AT difference. Consequently, an early ventilatory increase may account for the higher aerobic capacity. Breathing pattern differences concerning f_B and V_T do not appear to be responsible for the work rate differences between POE and AT. Finally, the results of this study indicate that the AT cannot be detected by the POE in untrained adults aged 50 to 60 years. Furthermore, the POE should be seen as a distinct ventilatory index, and the theoretical and practical implication of a POE – AT difference for the evaluation and prescription of exercise training and the assessment of the functional capacity of individuals should be further investigated.

Author Contributions Statement

M.P, S.RD and S.T conceived and designed research. M.P conducted experiments, analysed data and drafted the manuscript. B.I and B.P intellectually contributed the content of the manuscript. All authors read and approved the final version of the manuscript.

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Declaration of Competing Interest

The authors report no conflict of interest.

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