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Sequencing patterns of ventilatory indices in less trained adults

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Submaximal ventilatory indices, i.e., point of optimal ventilatory efficiency (POE) and anaerobic threshold (AT), are valuable indicators to assess the metabolic and ventilatory response during cardiopulmonary exercise testing (CPET). The order in which the ventilatory indices occur (ventilatory indices sequencing pattern, VISP), may yield additional information for the interpretation of CPET results and for exercise intensity prescription. Therefore, we determined whether different VISP groups concerning POE and AT exist. Additionally, we analysed fat metabolism *via* the exercise intensity eliciting the highest fat oxidation rate (Fat_{max}) as a possible explanation for differences between VISP groups. 761 less trained adults (41–68 years) completed an incremental exercise test on a cycle ergometer until volitional exhaustion. The ventilatory indices were determined using automatic and visual detection methods, and Fat_{max} was determined using indirect calorimetry. Our study identified two VISP groups with a lower work rate at POE compared to AT in $VISP_{POE < AT}$ but not in group $VISP_{POE = AT}$. Therefore, training prescription based on POE rather than AT would result in different exercise intensity recommendations in 66% of the study participants and consequently in unintended physiological adaptations. $VISP_{POE < AT}$ participants were not different to $VISP_{POE = AT}$ participants concerning VO_{2peak} and Fat_{max} . However, participants exhibiting a difference in work rate ($VISP_{POE < AT}$) were characterized by a higher aerobic capacity at submaximal work rate compared to $VISP_{POE = AT}$. Thus, analysing VISP may help to gain new insights into the complex ventilatory and metabolic response to exercise. But a methodological framework still must be established.

KEYWORDS

exercise testing (CPET), exercise physiology, aerobic capacity, anaerobic threshold (AT), point of optimal ventilatory efficiency (POE), fat oxidation, indirect calorimetry

1. Introduction

Cardiorespiratory fitness is an indicator of physical performance, and cardiovascular and metabolic health. Accordingly, the improvement of the cardiorespiratory fitness is recommended in prevention and therapy to lower the risk of morbidity and mortality (1–3). In cardiopulmonary exercise testing (CPET) two approaches developed over the decades to detect changes in cardiorespiratory fitness: the testing of the aerobic power, i.e., maximal oxygen uptake: VO_{2max} , and the aerobic capacity, i.e., percent of VO_{2max} expressed as AT (3, 4). VO_{2max} is the most common applied marker to monitor changes of the exercise performance level and to prescribe a health-enhancing exercise intensity in prevention and therapy. However, submaximal ventilatory indices have been shown to be an alternative basis for exercise intensity prescription and a better predictor of physical performance, morbidity and mortality (1, 2, 5). Different submaximal ventilatory indices (e.g., POE, AT, respiratory compensation point (RCP)) have been established within the last decades in CPET and a time-dependent sequence of these indices associated to the three-phase model of energy supply was reported (2, 3). Although these submaximal ventilatory indices have been investigated extensively, only few studies discussed the time-dependent sequencing patterns of these various ventilatory indices in adults (6–8). It is, therefore, important to analyse differences in VISP to improve the interpretation of CPET data regarding health-enhancing physical exercise prescription.

The determination of ventilatory indices from CPET data has a long tradition (3, 9) and these indices detected from ventilatory variables and measured by indirect calorimetry can be used to non-invasively detect exercise-induced changes of the metabolism (2, 10). Traditionally a three-phase model with two submaximal ventilatory indices, in particular the AT and the RCP, is used to discriminate three phases of energy supply during an incremental CPET (2, 11–13). The first increase in blood lactate concentration during an incremental exercise test leads to a disproportionate increase in carbon dioxide output (VCO_2) in relation to oxygen uptake (VO_2) due to the “excess CO_2 ” from the bicarbonate buffering of H^+ resulting from the dissociation of lactic acid. This gas exchange response characteristic is used for the determination of the AT using the v-slope method (3, 14) and marks the transition from a predominantly aerobic (mainly fatty acid oxidation and aerobic glycolysis; phase one) to a partially anaerobic energy metabolism (mainly aerobic and anaerobic glycolysis; phase two). As a consequence to the slightly increased end-tidal carbon dioxide partial pressure ($PETCO_2$), it is stated that there is a compensatory stimulus for ventilation mediated *via* the carotid bodies to regulate the lactic acid-induced acidosis (2). Theoretically, this leads to a simultaneous increase in minute ventilation (VE) at the AT

(3). Therefore, it is common practice to determine the AT *via* the POE-detection method, which is defined as finding the first disproportional increase of VE related to VO_2 (6, 11, 12, 15). The POE represents the best integration between the respiratory and the cardiovascular system, because it corresponds to the moment during an incremental CPET at which there is the lowest ventilation to take up one litre of oxygen (9, 15).

But some authors have reported differences in the time-dependent sequence of AT and POE in less trained adults (6, 8, 16–18). While in some individuals POE and AT are found at the same work rate, for a similar number of individuals POE occurs at a lower work rate than AT indicating an earlier increase in VE in relation to VO_2 (6). Therefore, it seems reasonable to distinguish between these two indices. Thus, the v-slope method for AT detection is the most direct approach to find the changes of the energy metabolism between phase one and phase two of the energy supply during incremental CPET. On the other hand, the POE reflects the ventilatory response of an individual to cope with the exercise strain during this initial phase of the CPET (2, 6). Therefore, training prescription based on POE could lead to training at other exercise intensities and, as a consequence, to different and unintended adaptations than training prescription based on AT (16).

In recent years the “Fatmax concept”, based on the crossover concept of fat and carbohydrate utilisation during exercise (19), has provided further ventilatory indices to describe changes concerning the fat metabolism due to exercise. Absolute fat oxidation rates increase during low to moderate exercise intensities until Fat_{max} (i.e., the exercise intensity, at which the maximal fat oxidation rate (MFO) occurs) and declines with a further increase in exercise intensity until it gets negligible, i.e., a minimum fat oxidation rate is reached (Fat_{min}). Concomitantly, the carbohydrate (CHO) oxidation increases with the exercise intensity and becomes the dominant energy source at Fat_{min} and above (10, 20).

The fat metabolism is of great relevance in performance and health settings. Extensive use of fat oxidation during exercise reduces the requirement for endogenous carbohydrate oxidation and therefore muscle glycogen depletion, which is linked to fatigue. The capacity for fat oxidation during exercise has been associated with insulin sensitivity, weight gain, metabolic flexibility, and lower metabolic risk factors (20, 21). It has been shown that Fat_{max} occurs at $48\%VO_{2peak}$ (22) or lower at $39\%VO_{2peak}$ (23) in untrained individuals equalling exercise intensities below AT (22, 24). Additionally, Fat_{max} was reported to increase with training and enhancement of habitual physical activity (21, 23, 25, 26). On the other hand, Fat_{max} and AT were reported to occur at almost the same relative exercise intensity (45 vs. $46\%VO_{2peak}$) in moderately trained men (27), indicating the interrelationship between Fat_{max} and AT. However, very large inter-individual variations and large

discrepancies in the exercise intensity at Fat_{max} between specific population groups have been reported by various studies (10, 22, 28), which may be related to differences in the experimental design (different CPET-protocols; single- vs. multiple exercise tests) and the ventilatory indices detection methods (10, 21, 24). Therefore, the determination of Fat_{max} during a single incremental CPET and the analysis of the association to other ventilatory indices may be a valuable addition. Especially in clinical settings as an indicator of metabolic health and in performance settings where the capacity to utilize fat as a metabolic substrate is of concern (24).

While the influence of different determinants on single indices has already been studied extensively (2, 10), the sequencing patterns and the inter-relationship between the different ventilatory indices Fat_{max} , POE and AT during a single CPET remain to be studied in order to understand differences between individuals (21, 24, 27).

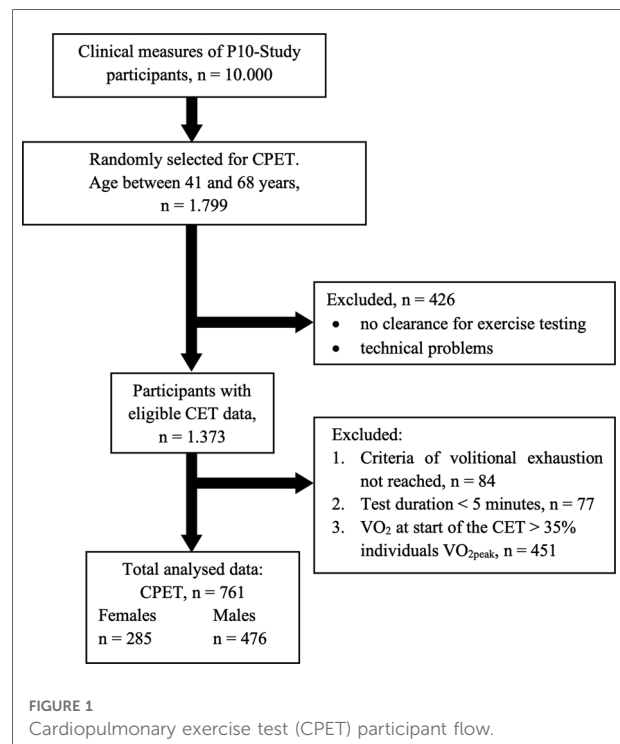
In short, the time-dependent sequence of Fat_{max} , POE and AT yield important insight into the metabolic and ventilatory response of individuals during low and moderate exercise intensities. Especially, the POE/AT-sequencing pattern may provide further information on different strategies to cope with the ventilatory and metabolic strain to exercise. It is, therefore, crucial to investigate the influence of determinants on the occurrence and the sequencing of these indices to further improve the assessment of the functional capacity of people to subsequently refine exercise-training prescription in less trained individuals.

Therefore, (1) the first aim of this study was to determine, whether different VISP groups concerning POE and AT exist in less trained adults. If distinct VISP groups were found, differences in participant characteristics, gas-exchange values and CPET results were presented. (2) The second aim was to investigate the association of the ventilatory index Fat_{max} with POE and AT.

2. Methods

2.1. Participants

This is a cross-sectional study with 761 data-sets (285 females and 476 males) drawn from a sub-sample of 1.799 participants of the Paracelsus 10.000 Study (P10-Study) who were randomly assigned for CPET (Figure 1 and Table 2). The P10-Study was conducted between 2013 and 2020, and 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 (29). 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 (415-E/1521/3–2012). All participants gave written informed consent.



2.2. Data collection

The measurements were supervised by the same investigators and were performed after overnight fasting at the Salzburg University Hospital, Austria between 7:00 a.m. and 3:00 p.m. The CPET measurements were performed between 1:00 and 3:00 p.m. Participants were instructed not to drink coffee or smoke on the test-day and were provided with standardized food. It consisted of a bread with cheese and water, consumed between 11:00 a.m. and noon.

2.2.1. Participant characteristics and medical examinations

The medical examinations performed by physicians included a detailed medical history and physical examinations, anthropometric and standard gas-exchange measurements, laboratory evaluations (including blood chemistry, haematology and urine analysis), and an electrocardiogram. Body fat mass (FM) and fat free mass (FFM) were estimated by multi-frequency bio-impedance analysis (B.I.A Nutriguard-M, Data Input, Darmstadt, Germany). Therefore, electrodes (Bianostic AT, Data Input, Darmstadt, Germany) were attached on the frontal site of the left wrist and ankle of the participant lying in supine position and the measurements were made according to the manufacture's guidelines (Data Input, Darmstadt, Germany).

2.2.2. CPET and gas exchange measurements

After the medical examination, participants were assigned for incremental 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, United States) 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 semipermeable 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, end-tidal partial pressure of oxygen and carbon dioxide (PETO₂, PETCO₂), ventilatory equivalents of O₂ and CO₂ (EQO₂, EQCO₂). 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% CO₂, 16% O₂, 79% N₂, Rießner Gase GmbH, Lichtenfels, Germany).

2.2.3. CPET and exercise protocol

Each participant underwent an incremental exercise test until volitional exhaustion. The exercise protocols were designed to reach volitional exhaustion after 8 to 12 min of test duration (Table 1) using individual starting workloads and increments regarding sex and body mass-range as reported elsewhere (1, 30). The exercise test was performed on a cycle ergometer (ergo select 200P, 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, an incremental 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 (3): (1) a plateau in VO_2 (changes of less than $2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ 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 (31); (5) pedalling rate $< 50 \text{ rpm}$ due to leg fatigue or shortness of breath. Exercise testing was terminated if any complications and contraindications occurred (1). During the incremental exercise test electrocardiogram was continuously recorded and blood pressure was determined every two minutes.

2.3. Data processing

Data from the stationary cycling test (CPET) regarding warm-up and recovery phase were excluded from further analyses and the recorded breath-by-breath data were averaged over 10 s epochs. The mean of the three consecutive highest 10 s VO_2 values at cessation was then taken as the peak value. Peak work rate (WR_{peak}) was determined as the mean work rate during the last minute of the exercise test (32, 33).

POE and Fat_{max} are found at low to moderate exercise intensities and were reported at 44%–57% VO_{2peak} (8, 17) and 38%–64% VO_{2peak} (10, 21), respectively. 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 POE and Fat_{max} . Furthermore, participants who could not complete at least five minutes of the CPET were excluded from further analysis.

The VO_2 – work rate relationship (VO_2/W slope) was assessed by linear regression of VO_2 vs. work rate considering all exercise values up until VO_{2peak} (3).

2.3.1. Determination of ventilatory indices

The ventilatory indices POE, AT and RCP were determined semiautomatic by combining automatic and visual detection methods (34). First, the indices were determined automatically using polynomial regression. Then, the time points of POE, AT and RCP during the exercise test were visually determined by finding the first disproportional increase in a VE (y-axis) vs. VO_2 (x-axis) plot (9, 15), in a VCO_2 vs. VO_2 plot (14), and in a VE vs. VCO_2 plot (3), respectively. In addition, EQCO₂, PETCO₂, EQO₂, and PETO₂ time plots were drawn as an additional guidance for the VI determination. The automatically detected indices were shown as a guide in these plots. Finally, the selected time points were then used to determine the work rate at POE, AT and RCP using the test protocol. The VO_2 and VCO_2 10 s averages were used to calculate fat oxidation rates according to the non-protein respiratory exchange ratio (RER) technique with the assumption that the urinary nitrogen excretion rate was negligible (10). For each participant the calculated values for fat oxidation were depicted graphically as a function of exercise intensity (% VO_{2peak}) and a 3rd degree polynomial function with intersection in (0,0) was constructed to

TABLE 1 Stationary cycling protocols of the P10-study for CPET.

	Females			Males			Females and Males
	50–69	50–69	70–94	50–69	70–94	70–94	
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, $W \cdot \text{min}^{-1}$	10	10, 15 ^a	10, 15 ^a	15	15, 20 ^a	20	20, 25 ^a

^aincrement rise after 6th minute to ensure a test duration of about 10 to 12 min.

determine the relative intensity that elicited the highest rate of fat oxidation (Fat_{max}) (10, 35). If less than six calculated fat oxidation values were available to construct the 3rd degree polynomial, the subject was excluded from further analysis (36).

2.3.2. Ventilatory indices sequencing pattern (VISP) groups

To determine differences between the VISP-groups, the work rates at POE and at AT were determined and the investigated participants were categorized into the following VISP groups: (1) $VISP_{POE < AT}$, participants with a lower work rate at POE compared to AT. (2) $VISP_{POE = AT}$, participants without a difference in work rate between POE and AT.

2.4. Statistical analysis

Data are given as means \pm standard deviation. Due to sex differences in energy metabolism and exercise performance, analysis were conducted separately for female and male participants. The Shapiro-Wilk test and visual inspection of histograms and quantile-quantile plots were used to verify the normal distribution of the data ($n < 50$). Differences in sex and in VISP groups for participant characteristics and CPET variables were tested using unpaired t-tests and Mann-Whitney non-parametric tests when appropriate. Mean biases [95% confidence intervals (CI)] \pm 95% limits of agreement according to Bland and Altman were calculated to evaluate the level of absolute agreement between the ventilatory indices Fat_{max} , POE and AT (37). The explained variance of the work rate differences between POE and AT and the work rate differences between Fat_{max} and AT on the aerobic capacity (measured as AT) were tested by linear regression analysis. Additionally, the relations between the relative oxygen uptake at Fat_{max} and the two ventilatory indices POE and AT were described by linear regression analysis using Pearson correlations.

A mixed-design ANOVA was completed to investigate differences between the ventilatory indices Fat_{max} , POE and AT (within-subjects factor, VI) and between VISP groups (between-subjects factor, G) in relative oxygen uptake, relative work rates, relative heart rates and RER. Bonferroni *post-hoc* comparisons were applied when ANOVA indicated significant interaction effects. 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, United States).

3. Results

3.1. Participant characteristics

Participant characteristics of the 285 females and 476 males who successfully completed the CPET and met the inclusion criteria for this study are listed in **Tables 2, 3**.

TABLE 2 Characteristics, comorbidity and main results of resting spirometry and cardiopulmonary exercise test (CPET) in females and males.

	Females		Males		df	t	p
	n						
	M	SD	M	SD			
Characteristics							
Age, yrs	54	3	55	4	759	4.41	<.001
Body mass, kg	67	11	83	12	759	19.18	<.001
FM, kg	21	8	19	6	696	-3.66	<.001
Body fat, %	30	7	22	5	696	-17.18	<.001
FFM, kg	46	5	65	8	696	34.67	<.001
Height, m	1.7	0.1	1.8	0.1	720	27.57	<.001
BMI, $kg \cdot m^{-2}$	24.2	3.8	26.1	3.2	720	7.46	<.001
Waist circumference, cm	85	10	96	10	711	14.80	<.001
Comorbidity							
Hypertension, N (%)	22 (8)		75 (16)				
Pulmonary disease, N (%)	20 (7)		43 (9)				
Diabetes mellitus, N (%)	2 (1)		14 (4)				
Cardiovascular disease, N (%)	12 (4)		34 (8)				
Resting Spirometry							
FVC, L	3.6	0.6	4.9	0.7	488	19.98	<.001
FEV ₁ , L	2.7	0.4	3.6	0.6	488	18.85	<.001
CPET							
Test duration, min	10.5	2.0	10.0	2.0	759	-3.53	<.001
%VO _{2peak} at start, %	30	4	28	4	759	-5.77	<.001
%VO _{2peak} at Fat_{max} , %	41	8	43	7	728	3.64	<.001
%VO _{2peak} at POE, %	46	9	46	8	758	-0.57	.567
%VO _{2peak} at AT, %	53	9	54	9	759	0.35	.726
%VO _{2peak} at RCP, %	83	10	84	10	725	1.29	.197
VO _{2peak} , $ml \cdot kg^{-1} \cdot min^{-1}$	26.0	5.4	31.0	6.3	759	11.28	<.001
WR at start, $W \cdot kg^{-1}$	0.7	0.1	0.9	0.1	757	16.93	<.001
WR _{peak} , $W \cdot kg^{-1}$	2.3	0.5	2.8	0.6	759	13.03	<.001
RER _{peak}	1.19	0.07	1.20	0.08	759	2.22	.027
HR _{peak} , min^{-1}	164	12	163	13	741	-0.75	.451
VO ₂ /WR slope, $ml O_2 \cdot W^{-1}$	10.4	1.3	10.2	1.2	759	-1.85	.065

Data are presented as mean (M) \pm standard deviation (SD) and frequencies (%); FM: fat mass; FFM: fat-free mass; BMI: body mass index; FVC: forced vital capacity; FEV₁: forced expiratory volume over 1 s; WR: work rate; VO₂: oxygen uptake; RER: respiratory exchange ratio; HR: heart rate; VO₂/WR slope: VO₂ – work rate relationship; p = significance level between males and females (unpaired t-tests).

TABLE 3 Characteristics, comorbidity and main results of resting spirometry and cardiopulmonary exercise test (CPET) in VISP_{POE < AT} and VISP_{POE = AT} participants, separately for females and males.

	VISP _{POE < AT}		VISP _{POE = AT}		df	t	p
	M	SD	M	SD			
Females, n							
		197	88				
Characteristics							
Age, yrs	54	3	54	3	283	1.32	.189
Body mass, kg	67	11	67	12	283	-0.47	.637
FM, kg	20	8	21	8	256	-0.55	.580
Body fat, %	30	6	30	7	256	-0.34	.736
FFM, kg	46	5	47	5	256	-0.26	.793
Height, m	1.7	0.1	1.7	0.1	269	0.27	.790
BMI, kg · m ⁻²	24.1	3.7	24.4	4.0	269	-0.65	.515
Waist circumference, cm	84	10	87	11	266	-1.85	.065
Comorbidity							
Hypertension, N (%)	12 (6)		10 (12)				
Pulmonary disease, N (%)	17 (9)		3 (4)				
Diabetes mellitus, N (%)	1 (1)		1 (2)				
Cardiovascular disease, N (%)	6 (3)		6 (7)				
Resting Spirometry							
FVC, L	3.6	0.6	3.7	0.5	170	-0.70	.484
FEV ₁ , L	2.7	0.4	2.8	0.5	170	-0.89	.372
CPET							
Test duration, min	10.7	2.0	10.0	2.1	283	2.84	.005
%VO _{2peak} at start, %	30	4	29	4	283	1.38	.169
%VO _{2peak} at RCP, %	84	10	81	11	274	1.83	.068
VO _{2peak} , ml · kg ⁻¹ · min ⁻¹	26.3	5.2	25.5	5.7	283	1.08	.281
WR at start, W · kg ⁻¹	0.7	0.1	0.8	0.1	282	-1.14	.255
WR at RCP, W · kg ⁻¹	1.9	0.4	1.8	0.5	274	0.96	.338
WR _{peak} , W · kg ⁻¹	2.3	0.4	2.2	0.5	283	1.64	.102
%HR _{peak} at RCP, %	90	6	90	6	269	-0.11	.913
HR _{peak} , min ⁻¹	165	12	162	12	277	1.42	.158
RER at RCP	1.08	0.06	1.07	0.07	274	1.31	.191
RER _{peak}	1.19	0.06	1.18	0.08	283	0.83	.410
VO ₂ /WR slope, ml O ₂ · W ⁻¹	10.2	1.3	10.7	1.4	283	-2.67	.008

(continued)

Compared to reference values published by Rapp et al. (2018), the female and male participants of this study are characterized by an average cardiorespiratory fitness

TABLE 3 Continued

	VISP _{POE < AT}		VISP _{POE = AT}		df	t	p
	M	SD	M	SD			
Males, n							
		307	169				
Characteristics							
Age, yrs	55	4	55	4	474	-0.76	.450
Body mass, kg	84	12	83	11	474	0.23	.820
FM, kg	18	7	19	6	438	-1.11	.269
Body fat, %	22	6	23	5	438	-1.85	.065
FFM, kg	65	8	64	8	438	1.50	.135
Height, m	1.8	0.1	1.8	0.1	449	0.83	.405
BMI, kg · m ⁻²	26.0	3.2	26.2	3.1	449	-0.56	.576
Waist circumference, cm	95	10	97	9	443	-1.38	.167
Comorbidity							
Hypertension, N (%)	42 (14)		33 (20)				
Pulmonary disease, N (%)	32 (11)		11 (7)				
Diabetes mellitus, N (%)	6 (3)		8 (7)				
Cardiovascular disease, N (%)	24 (8)		10 (6)				
Resting Spirometry							
FVC, L	4.8	0.7	4.9	0.7	316	-0.31	.755
FEV ₁ , L	3.6	0.6	3.6	0.5	316	-0.38	.705
CPET							
Test duration, min	10.1	2.0	9.7	1.9	474	1.99	.047
%VO _{2peak} at start, %	28	4	28	4	474	-0.55	.584
%VO _{2peak} at RCP, %	85	10	82	11	449	2.11	.036
VO _{2peak} , ml · kg ⁻¹ · min ⁻¹	31.4	6.6	30.3	5.6	474	1.85	.066
WR at start, W · kg ⁻¹	0.9	0.1	0.9	0.1	473	0.50	.619
WR at RCP, W · kg ⁻¹	2.3	0.6	2.2	0.6	449	2.15	.032
WR _{peak} , W · kg ⁻¹	2.9	0.6	2.8	0.6	474	1.77	.077
%HR _{peak} at RCP, %	88	7	88	7	438	0.78	.435
HR _{peak} , min ⁻¹	163	13	163	13	462	0.17	.863
RER at RCP	1.09	0.07	1.08	0.07	449	1.92	.055
RER _{peak}	1.21	0.08	1.19	0.07	474	2.15	.032
VO ₂ /WR slope, ml O ₂ · W ⁻¹	10.2	1.3	10.3	1.2	474	-0.92	.358

Data are presented as mean (M) ± standard deviation (SD) and frequencies (%); VISP: ventilatory indices sequencing pattern; FM: fat mass; FFM: fat-free mass; BMI: body mass index; FVC: forced vital capacity; FEV₁: forced expiratory volume over 1 s; VO₂: oxygen uptake; RCP: respiratory compensation point; WR: work rate; HR: heart rate; RER: respiratory exchange ratio; VO₂/WR slope: VO₂ – work rate relationship; p = significance level between the VISP groups (unpaired t-tests).

corresponding approximately to the 50th and 40th percentile (in terms of $\text{VO}_{2\text{peak}}$: 26.0 ± 5.4 vs. $31.0 \pm 6.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), respectively. $\text{VO}_{2\text{peak}}$ ranged from 13.3 to $44.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in females and 16.2 to $55.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in males. The females and males displayed normal pulmonary function at rest with an average FVC of $3.6 \pm 0.6 \text{ l}$ and $4.9 \pm 0.7 \text{ l}$, and an average FEV_1 of $2.7 \pm 0.4 \text{ l}$ and $3.6 \pm 0.6 \text{ l}$, respectively (38). The female participants in this study were marginally younger, were on average 16 kg lighter and had 11 cm lower waist circumference than the males. Additionally, females possessed a higher FM in kg as well as a higher percentage of body fat than males but a lower FVC and FEV_1 .

Furthermore, there was a sex difference in $\text{VO}_{2\text{peak}}$, WR_{peak} and $\% \text{VO}_{2\text{peak}}$ at Fat_{max} being significantly higher in males. But no significant sex differences were found at the remaining ventilatory indices POE, AT, and RCP (in terms of $\% \text{VO}_{2\text{peak}}$). Regarding sex differences in the test protocol, we found a significant higher starting work rate in males, and a longer test duration and higher $\% \text{VO}_{2\text{peak}}$ at test start in females.

3.2. Differences in work rates between POE and AT

Based on a difference in work rates between POE and AT, participants were divided into two VISP groups (Tables 3, 4). Thus, 197 females and 307 males who showed a difference in work rate between POE and AT were assigned to $\text{VISP}_{\text{POE} < \text{AT}}$. This corresponds to 66% of all study participants. The remaining 88 females and 169 males with no difference were assigned to $\text{VISP}_{\text{POE} = \text{AT}}$ (Tables 3, 4). The intra-group distribution ($\text{VISP}_{\text{POE} < \text{AT}}$ vs. $\text{VISP}_{\text{POE} = \text{AT}}$) was similar between females (69% vs. 31%) and males (64% vs. 36%). The female and male characteristics, gas-exchange values and CPET results in $\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$ participants differed in test duration (females: 10.7 ± 2.0 vs. $10.0 \pm 2.1 \text{ min}$; males: 10.7 ± 2.0 vs. $10.0 \pm 2.1 \text{ min}$), relative oxygen uptake and relative work rate at RCP (males: 85 ± 10 vs. $82 \pm 11\% \text{VO}_{2\text{peak}}$; 2.3 ± 0.6 vs. $2.2 \pm 0.6 \text{ W} \cdot \text{kg}^{-1}$), VO_2 – work rate relationship (females: 10.2 ± 1.3 vs. $10.7 \pm 1.4 \text{ ml O}_2 \cdot \text{W}^{-1}$) and RER_{peak} (males: 1.21 ± 0.08 vs. 1.19 ± 0.07) (Table 3).

3.3. Associations between the ventilatory indices Fat_{max} , POE and AT

The mean (\pm standard deviation) work rate difference between POE and AT of $\text{VISP}_{\text{POE} < \text{AT}}$ participants was significantly higher in males than in females ($28 \pm 21 \text{ W}$ vs. $17 \pm 12 \text{ W}$). There was a significant correlation between the aerobic capacity (in terms of VO_2 at AT) and the magnitude of the work rate differences between POE and AT, with $r = .47$ (females: $p < .001$) and $r = .55$ (males: $p < .001$) (39) and

with coefficient of determination (R_{adj}^2) explaining 22% and 31% of the variance in work rate difference of females and males, respectively (Figure 2A). Additionally, there was a significant correlation between the aerobic capacity and the magnitude of work rate difference between Fat_{max} and AT, with $r = .59$ (females: $p < .001$) and $r = .63$ (males: $p < .001$) (39) and with coefficient of determination (R_{adj}^2) explaining 35% and 40% of the variance in work rate difference of females and males, respectively (Figure 2B).

There was a significant interaction between the ventilatory indices (Fat_{max} , POE and AT) and VISP groups ($\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$) for the relative oxygen uptake (in terms of $\% \text{VO}_{2\text{peak}}$), the relative work rate (in terms of $\text{W} \cdot \text{kg}^{-1}$), the relative heart rate (in terms of $\% \text{HR}_{\text{peak}}$) and the respiratory exchange ratio at the different ventilatory indices in females as well as in males (Table 4). *Post-hoc* pairwise comparisons indicated significant differences between $\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$ participants (Figure 3). The relative oxygen uptake and the relative work rate at POE were significantly lower in $\text{VISP}_{\text{POE} < \text{AT}}$ compared to $\text{VISP}_{\text{POE} = \text{AT}}$ participants, while both were significantly higher at the AT in $\text{VISP}_{\text{POE} < \text{AT}}$ compared to $\text{VISP}_{\text{POE} = \text{AT}}$ participants. Additionally, AT was significantly higher compared to Fat_{max} in both groups.

We used a Bland-Altman limit of agreement analysis to investigate the absolute agreement between the ventilatory indices Fat_{max} , POE and AT. In $\text{VISP}_{\text{POE} < \text{AT}}$ participants, Fat_{max} was best associated with POE (mean bias [95% CI] ranging from -3 [-4 ; -2] to 0 [-1 ; 1] $\% \text{VO}_{2\text{peak}}$). The agreement with AT was lower (mean bias [95% CI] ranging from -13 [-14 ; -12] to -11 [-12 , -10] $\% \text{VO}_{2\text{peak}}$). In $\text{VISP}_{\text{POE} = \text{AT}}$ participants, there was a similar agreement of Fat_{max} with both, the POE and AT. As expected, there was a high agreement between POE and AT in $\text{VISP}_{\text{POE} = \text{AT}}$ participants (mean bias [95% CI] of -1 [-2 ; 0] and -1 [-2 ; -1] $\% \text{VO}_{2\text{peak}}$) and a low agreement in $\text{VISP}_{\text{POE} < \text{AT}}$ participants (mean bias [95% CI] of -10 [-11 ; -9] and -12 [-13 ; -11] $\% \text{VO}_{2\text{peak}}$). Results were similar in females and males (Table 5).

There was a strong correlation between the relative oxygen uptake at Fat_{max} and the two ventilatory indices POE and AT, with r ranging from .68 to .76 ($p < .001$) (39) and with coefficient of determination (R_{adj}^2) explaining 47% to 58% of the variance in VO_2 at Fat_{max} . No VISP group differences could be discerned (Figure 4).

4. Discussion

Ventilatory indices are valuable indicators to assess the metabolic and ventilatory response during exercise in individuals. Combining different indices into a single CPET may yield great potential to further improve the interpretation of CPET results. Therefore, we determined whether different

TABLE 4 Relative oxygen uptake, absolute and relative work rate, relative heart rate and respiratory exchange ratio at the ventilatory indices Fat_{max}, POE and AT in VISP_{POE < AT} and VISP_{POE = AT} participants.

Females, <i>n</i>	VISP _{POE < AT}		VISP _{POE = AT}		ANOVA			
	197		88					
	M	SD	M	SD	Effect	<i>F</i> ratio	df	η_p^2
Relative oxygen uptake, %VO_{2peak}								
Fat _{max}	42	8	41	8	G	0.35	1	.00
POE	45	8	49	9	VI	239.60***	2	.47
AT	55	9	50	8	G × VI	38.80***	2	.13
Absolute work rate, W								
Fat _{max}	55.6	12.0	56.5	12.2	G	0.24	1	.00
POE	59.3	14.7	68.2	14.3	VI	156.70***	2	.37
AT	75.3	18.7	68.0	14.1	G × VI	42.90***	2	.14
Relative work rate, W · kg⁻¹								
Fat _{max}	0.9	0.2	0.8	0.2	G	0.01	1	.00
POE	0.9	0.2	1.0	0.2	VI	153.60***	2	.36
AT	1.2	0.3	1.0	0.2	G × VI	41.50***	2	.13
Relative heart rate, %HR_{peak}								
Fat _{max}	64	6	65	6	G	2.05	1	.01
POE	66	7	70	7	VI	189.80***	2	.42
AT	72	8	70	6	G × VI	30.7***	2	.10
Respiratory Exchange Ratio								
Fat _{max}	0.85	0.05	0.84	0.07	G	2.40	1	.01
POE	0.86	0.06	0.87	0.08	VI	192.00***	2	.41
AT	0.92	0.07	0.88	0.08	G × VI	38.80***	2	.13
Males, <i>n</i>	VISP _{POE < AT}		VISP _{POE = AT}		ANOVA			
	307		169					
	M	SD	M	SD	Effect	<i>F</i> ratio	df	η_p^2
Relative oxygen uptake, %VO_{2peak}								
Fat _{max}	44	7	43	7	G	0.54	1	.00
POE	43	7	50	8	VI	346.80***	2	.43
AT	55	9	51	8	G × VI	99.70***	2	.18
Absolute work rate, W								
Fat _{max}	89.1	17.6	87.1	19.2	G	0.06	1	.00
POE	86.5	17.3	102.0	26.8	VI	234.00***	2	.34
AT	115.0	27.1	102.0	26.3	G × VI	111.00***	2	.20
Relative work rate, W · kg⁻¹								
Fat _{max}	1.1	0.2	1.1	0.3	G	0.04	1	.00
POE	1.1	0.2	1.2	0.4	VI	219.00***	2	.33
AT	1.4	0.4	1.2	0.3	G × VI	103.00***	2	.19

(continued)

TABLE 4 Continued

Males, <i>n</i>	VISP _{POE < AT}		VISP _{POE = AT}		ANOVA			
	307		169					
	M	SD	M	SD	Effect	<i>F</i> ratio	df	η_p^2
Relative heart rate, %HR_{peak}								
Fat _{max}	64	6	64	6	G	1.75	1	.00
POE	64	7	67	7	VI	249.50***	2	.36
AT	70	7	68	7	G × VI	72.50***	2	.14
Respiratory Exchange Ratio								
Fat _{max}	0.85	0.05	0.85	0.05	G	0.62	1	.00
POE	0.86	0.06	0.89	0.07	VI	366.00***	2	.45
AT	0.92	0.07	0.89	0.07	G × VI	100.00***	2	.18

Data are presented as mean (M) ± standard deviation (SD); ANOVA = analysis of variance; G = group (VISP_{POE < AT}, VISP_{POE = AT}); VI = ventilatory indices (Fat_{max}, POE and AT). ****p* < .001.

groups of sequencing patterns in ventilatory indices (VISP) concerning POE and AT in less trained females and males exist. Further, we analysed the fat metabolism (Fat_{max}) during CPET to support the hypothesis, that differences between VISP groups are to some extent explained by inter-individual differences in the ability to oxidize fat during exercise.

4.1. Participant characteristics and sex differences

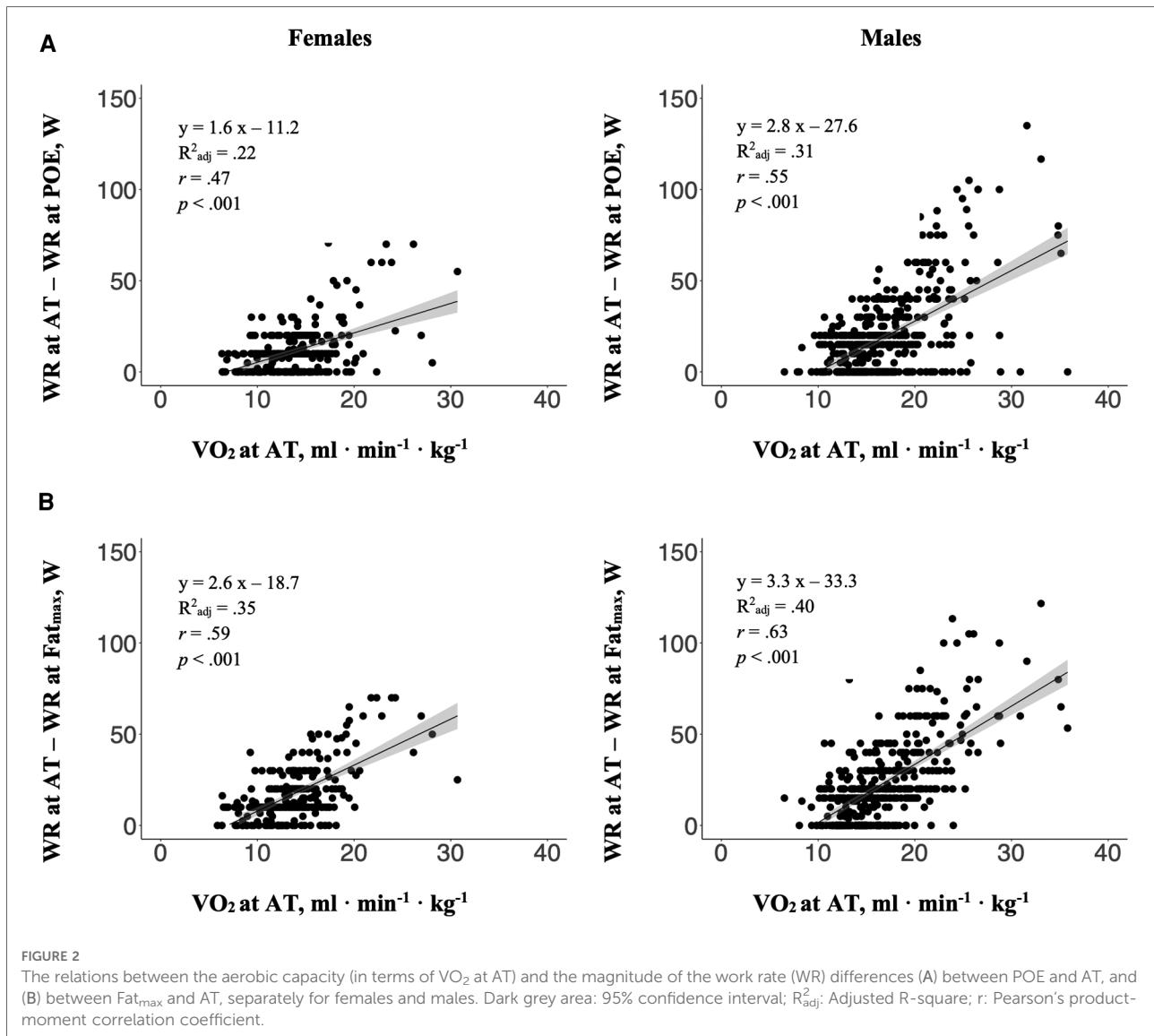
As expected, the females in this study presented with a lower cardiorespiratory fitness in terms of VO_{2peak} and a lower pulmonary function (FEV₁ and FVC) than the males (10, 38, 40). Additionally, the females possessed significant lower absolute work rates and oxygen uptake levels at the ventilatory indices Fat_{max}, POE and AT. This is in line with data reported by others and is related to differences in body mass and composition, lung size and a greater reliance on fat metabolism during exercise in females (21, 38). Interestingly, when work rate and oxygen uptake were expressed in relation to the peak values, no sex differences could be found. Only a marginal lower Fat_{max} (41 ± 8 vs. 43 ± 7%VO_{2peak}) was found in females compared to males (Table 2). This is in contrast to others reporting a minor tendency towards a greater Fat_{max} in females compared to males (56 ± 14 vs. 51 ± 14%VO_{2peak}) (21). It has been shown that there is a large inter-individual variation in fat oxidation with physical activity, VO_{2peak} and sex only explaining 12% of the inter-individual variation in MFO. Hence, genetic predisposition, diet or menopausal mechanisms may further contribute to variations in fat oxidation (22, 41). Nevertheless, it seems reasonable to analyse females and males separately when differences in fat metabolism during exercise are of interest.

4.2. Differences in work rates between POE and AT

Based on the work rate differences between POE and AT, we identified two VISP groups: 66% of the total sample presented with a significant lower work rate at POE compared to AT (VISP_{POE < AT}), while the remaining participants showed no POE - AT work rate difference (VISP_{POE = AT}). An early occurrence of POE compared to AT has also been reported by others (8, 17, 18). We calculated a mean work rate difference between POE and AT of 28 ± 21 W in females and 17 ± 12 W in males of the VISP_{POE < AT} group, corresponding to 12% and 11% of WR_{peak}, respectively. These differences cannot be neglected when CPET is used to evaluate and prescribe exercise training intensity or to assess the functional capacity of individuals.

Traditionally, POE and AT are considered the same ventilatory indices and are used interchangeable to detect the transition between phase one and phase two of the three-phase model of energy supply (2, 11, 12). AT is determined by identifying the first disproportional increase in VCO₂ related to VO₂, resulting from the “excess CO₂” due to the additional allocation of ATP by anaerobic glycolysis as the exercise intensity increases (14). This “excess CO₂” must be eliminated *via* enhanced ventilation and consequently, there will be a disproportional increase in VE related to VO₂, which corresponds to the POE (3, 12). But some factors seem to disrupt this close link between VE and VCO₂ in VISP_{POE < AT} participants of this study, as indicated by the early increase in VE (at POE) followed by the subsequent increase in VCO₂ at a higher work rate (at AT).

In a recent study (6) we tried to identify these factors and therefore, analysed breathing pattern differences by breathing frequency and tidal volume. But we could not find any



interrelationships to explain the early increase in VE independent of the later “excess CO_2 ”. Accordingly, we concluded in agreement with others that the VE vs. VO_2 relation (POE) is not suitable for determining the AT (5). Because of that observation, the POE should be seen as a distinct ventilatory index, describing the ventilatory adaptations and strategies of an individual in response to increasing exercise intensity (6, 8).

The participants of $\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$ are comparable in regard to age, anthropometric characteristics, resting spirometric values and peak CPET results. Additionally, $\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$ participants started the exercise test at comparable work rates and oxygen uptake levels (Table 3). Therefore, an influence of the test protocol can be excluded.

Further, no differences between $\text{VISP}_{\text{POE} < \text{AT}}$ and $\text{VISP}_{\text{POE} = \text{AT}}$ participants were found at Fat_{max} . Interestingly, while there

was no difference in the aerobic power ($\text{VO}_{2\text{peak}}$) we found a significant higher aerobic capacity (in terms of $\% \text{VO}_{2\text{peak}}$ at AT) and a significant higher relative work rate at AT (in terms of $\text{W} \cdot \text{kg}^{-1}$) in $\text{VISP}_{\text{POE} < \text{AT}}$ compared to $\text{VISP}_{\text{POE} = \text{AT}}$ participants. Additionally, the first disproportional increase in VE at POE occurred at a significant lower relative oxygen uptake and a significant lower relative work rate in $\text{VISP}_{\text{POE} < \text{AT}}$ compared to $\text{VISP}_{\text{POE} = \text{AT}}$ participants (Figure 3). Therefore, an early increase in ventilation, indicated by the POE at a lower work rate compared to AT, may lead to a delay in the appearance of the AT and can be seen as a ventilatory strategy adopted by some individuals ($\text{VISP}_{\text{POE} < \text{AT}}$).

There was a significantly lower VO_2 – work rate relationship in $\text{VISP}_{\text{POE} < \text{AT}}$ compared to $\text{VISP}_{\text{POE} = \text{AT}}$ females (10.2 ± 1.3 vs. $10.7 \pm 1.4 \text{ ml O}_2 \cdot \text{W}^{-1}$) but only a tendency towards a lower VO_2 – work rate relationship in $\text{VISP}_{\text{POE} < \text{AT}}$ males

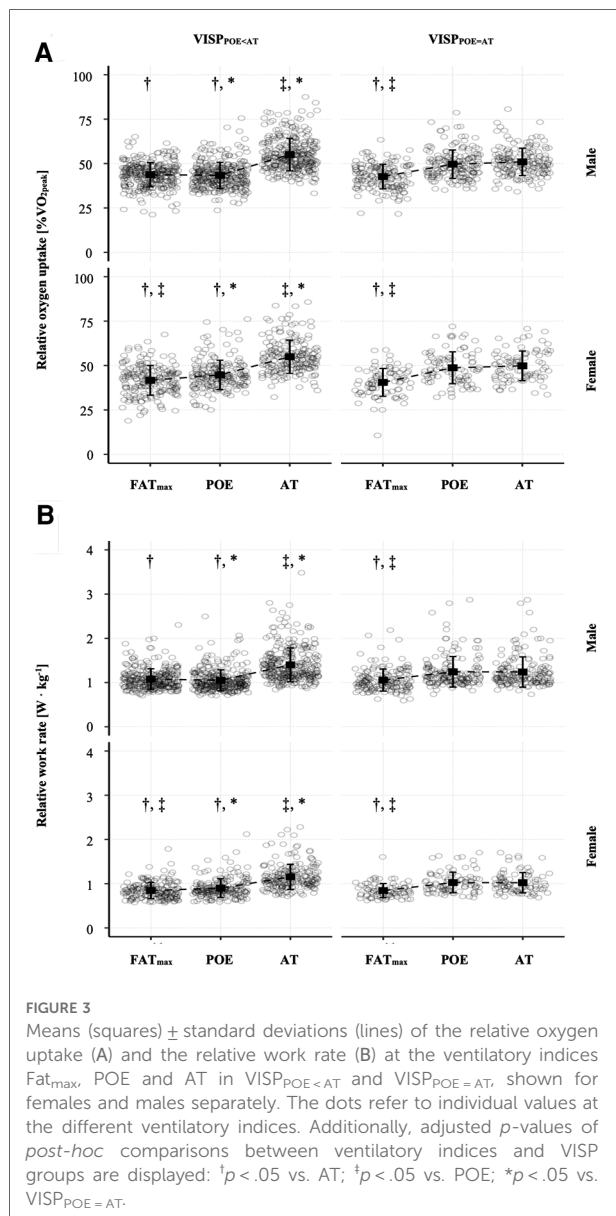


FIGURE 3 Means (squares) ± standard deviations (lines) of the relative oxygen uptake (A) and the relative work rate (B) at the ventilatory indices Fat_{max}, POE and AT in VISP_{POE < AT} and VISP_{POE = AT}, shown for females and males separately. The dots refer to individual values at the different ventilatory indices. Additionally, adjusted *p*-values of *post-hoc* comparisons between ventilatory indices and VISP groups are displayed: †*p* < .05 vs. AT; ‡*p* < .05 vs. POE; **p* < .05 vs. VISP_{POE = AT}.

(10.2 ± 1.3 vs. 10.3 ± 1.2 ml O₂ · W⁻¹). A flattening of the VO₂ – work rate relationship has been related to decreased work of breathing (3) and to improvements in the locomotor-respiratory coupling, associated with a decrease of the

metabolic requirements during exercise by reducing respiratory muscle fatigue (42). Therefore, an early increase in ventilation (POE at a lower work rate compared to AT) may therefore be another ventilatory adaption to regular exercise and decrease the work of breathing.

4.3. Associations between the ventilatory indices Fat_{max}, POE and AT

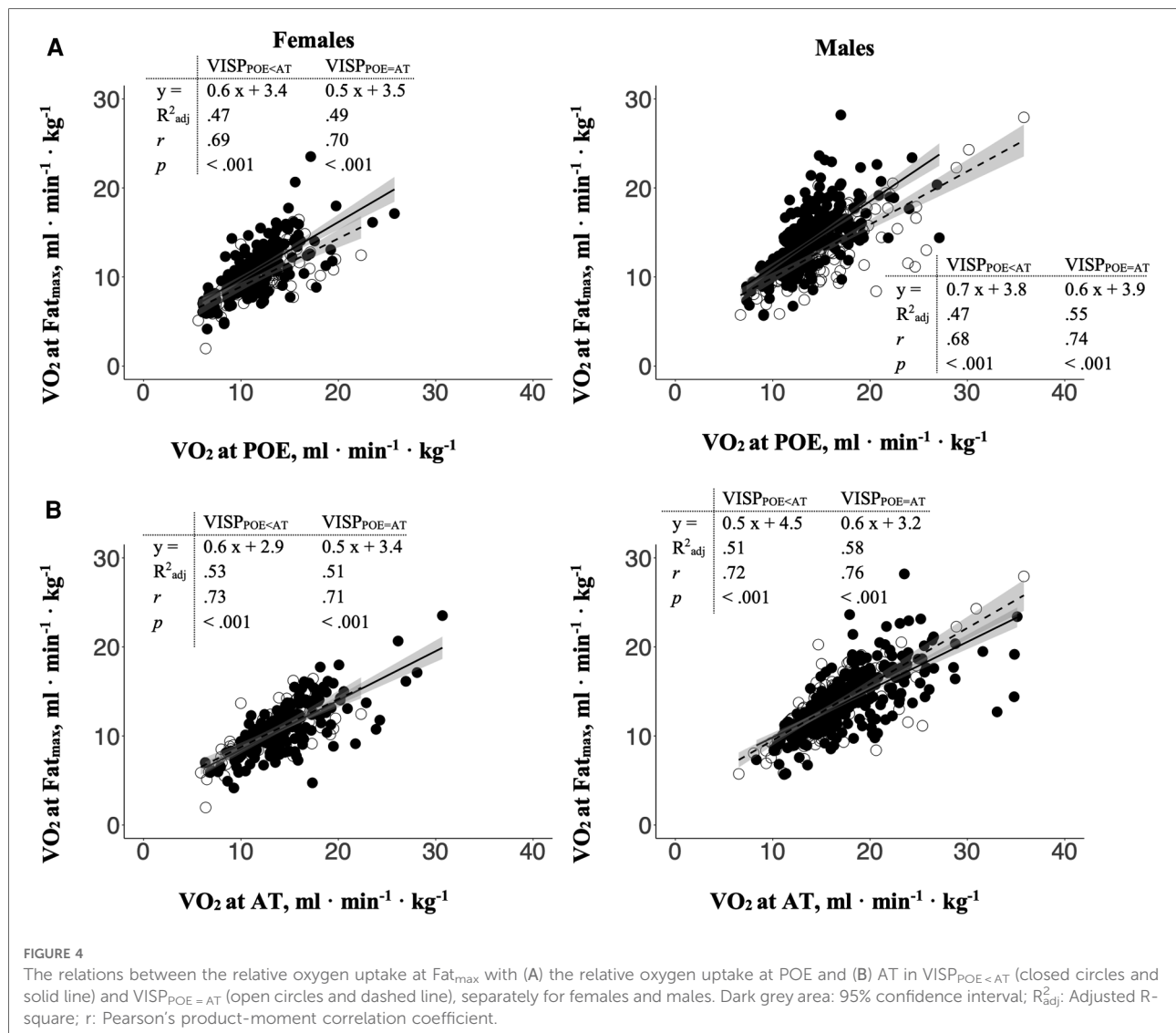
POE and AT were found at 46 ± 8 and 54 ± 9%VO_{2peak} (1.1 ± 0.3 and 1.3 ± 0.4 W · kg⁻¹) in the total sample, which is in agreement with other studies (2, 8, 17). The POE reflects the best integration of ventilation and the cardiorespiratory system with the most efficient ventilation in terms of O₂ extraction. It means that this is the point during an exercise test, where the least ventilation is required for the uptake of one litre of oxygen (8, 9). In a recent study by Ramos et al. (2012), POE was reported to occur at 44%VO_{2peak} (range: 30%–50% VO_{2peak}) in 2,237 untrained adults, always present at lower exercise intensities compared to AT. In our study, this result is in line with participants of the VISP_{POE < AT} but not the VISP_{POE = AT} group, shown by the Bland-Altman analysis (Table 5). Slightly different methods of POE determination (Ramos et al. (8) detected POE by determining the lowest value of the ratio between VE and VO₂) may explain these differences.

The ventilatory index Fat_{max} was found at a significant lower exercise intensity (in terms of %VO_{2peak} and W · kg⁻¹) compared to AT in both groups (Figure 3). This is in line with other studies, reporting Fat_{max} at lower exercise intensities compared to AT in untrained adults (22, 24). In contrast, similar values for Fat_{max} and AT (45 ± 8 vs. 47 ± 10%VO_{2peak}) were found in trained male cyclists (27). An increase in Fat_{max} was reported after endurance training, but only in previously sedentary individuals (26). In previously physically active or trained individuals, only a small tendency towards a Fat_{max} increase was reported (21). On the other hand, the submaximal fat oxidation rates (e.g., MFO) can largely increase with training in sedentary as well as physically active and trained individuals (10, 21, 26). In conclusion, it seems that the difference between Fat_{max} and AT decrease with endurance training because of

TABLE 5 Bland-Altman analysis. Comparison of the relative oxygen uptake (in terms of %VO_{2peak}) between the ventilatory indices Fat_{max}, POE and AT in VISP_{POE < AT} and VISP_{POE = AT} participants, separately for females and males.

	Females		Males	
	VISP _{POE < AT}	VISP _{POE = AT}	VISP _{POE < AT}	VISP _{POE = AT}
Fat _{max} vs. POE	-3 [-4; -2] ± 17	-8 [-10; -7] ± 17	0 [-1; 1] ± 15	-7 [-8; -6] ± 16
Fat _{max} vs. AT	-13 [-14; -12] ± 18	-9 [-11; -8] ± 16	-11 [-12; -10] ± 18	-8 [-10; -7] ± 15
POE vs. AT	-10 [-11; -9] ± 14	-1 [-2; 0] ± 06	-12 [-13; -11] ± 15	-1 [-2; -1] ± 05

Data are presented as mean bias [95% confidence interval] ± 95% limits of agreement.



increased full-body fat oxidation caused by skeletal muscle adaptations (e.g., mitochondrial biogenesis, increased tricarboxylic acid cycle enzyme and electron transport chain protein content, and increased fatty acid transporter and enzyme content (21)) leading to an increased Fat_{max} and MFO.

In our study, there was a strong positive linear relationship in $VISP_{POE < AT}$ and $VISP_{POE = AT}$ females and males (ranging from $r = .71$ to $r = .76$) between Fat_{max} and the aerobic capacity (in terms of $\%VO_{2peak}$ at AT) confirming the association of Fat_{max} and training status. Additionally, we found a strong positive linear relationship ($r = .59$ in females and $r = .63$ in males) between the aerobic capacity and the work rate difference between AT and Fat_{max} , but with the coefficient of determination (R^2_{adj}) only explaining 35% and 40% (in females and males, respectively) of the variance in the aerobic capacity (Figure 2B). This contradicts the

hypothesis stated above suggesting that the difference between Fat_{max} and AT decreases with an increase in aerobic capacity (e.g., as a consequence of endurance training). However, this could be related to the large inter-individual variations in this study, which diminish this effect. Specific experimental studies are needed to analyse this phenomenon.

No differences in Fat_{max} were found between $VISP_{POE < AT}$ and $VISP_{POE = AT}$ participants, and Fat_{max} was significantly lower than AT (in terms of $\%VO_{2peak}$ and $W \cdot kg^{-1}$) in both VISP groups. Interestingly, there was a strong agreement between Fat_{max} and POE in $VISP_{POE < AT}$ (mean bias [95% CI] ranging from -3 [$-4; -2$] to 0 [$-1; 1$] $\%VO_{2peak}$), but not in $VISP_{POE = AT}$ participants. This can be explained by the significant lower POE (in terms of $\%VO_{2peak}$ and relative work rate) in $VISP_{POE < AT}$ compared to $VISP_{POE = AT}$ participants. Consequently, differences in Fat_{max} between

VISP_{POE < AT} and VISP_{POE = AT} participants do not seem to explain the work rate difference between POE and AT.

There is a strong inverse relationship between fat oxidation and blood lactate, and there is a major effect of lactatemia in limiting fat oxidation in individuals with widely ranging exercise capacities. Lactatemia significantly affects and downregulates fat metabolism with increasing exercise intensity. It has been shown, that physically active and trained individuals have a higher capacity to oxidize fat compared to sedentary individuals or individuals with mitochondrial dysfunction as a result of type-2-diabetes-mellitus or the metabolic syndrome (20). In this study we confirmed these findings by showing a strong correlation between Fat_{max} and the aerobic capacity (Figure 4). However, great inter-individual variation in Fat_{max} must be kept in mind. Beside sex, comorbidities (e.g., hypertension and diabetes mellitus) and training status, the menopause status and diet are reported to influence Fat_{max}. Although, participants were instructed to realize overnight fasting and were provided with standardized food on the test day, the chronic nutritional status and the menstrual cycle of the female participants were not controlled. Hence, these factors should be taken into account in future studies when Fat_{max} is analysed and should be considered when interpreting the results of this study (22, 41).

Furthermore, the optimal test stage duration to determine submaximal and peak ventilatory indices in one single CPET does not exist. It has been shown, that MFO is slightly overestimated using a one minute incremental protocol, but Fat_{max} intensity (in terms of %VO_{2peak}) is not affected by step duration (36). There is a delay in the increase of VO₂ in response to increasing exercise intensity, which is known as the mean response time and which increases with exercise intensity and is dependent on the work rate increase per minute (43). Additionally, dependent on the fitness level of the participants, suitable stage durations may vary between individuals. But it has been shown that short time test protocols can be used to estimate different ventilatory indices in one single CPET (27, 36). Hence, we used different 1-minute stage-exercise protocols and increased the increment in some of our test protocols after the 6th minute of the test (Table 1) to realise, (1) a slow increase of the work rate during the early stages of the exercise test in order to minimise the VO₂ response time, and (2) to be able to achieve reliable VO_{2peak} values by realising the recommended test duration of 8 to 12 min (30). The mean VO₂ – work rate relationship in this study was found to be normal ($10.3 \pm 1.3 \text{ ml O}_2 \cdot \text{W}^{-1}$) (1) but marked inter-individual variations were found (range: 5.5–17.5 ml O₂ · W⁻¹). These differences must be considered when interpreting the results of this study and may explain the great inter-individual variations in Fat_{max}. Furthermore, the differences between the ventilatory indices POE and AT can be explained, at least partly, by the measurement error in determining the individual ventilatory indices. Consequently, further studies are needed to

establish reliable test protocols and reference values for different study populations in order to analyse VISP using one single CPET (27).

4.4. Conclusion

In summary, there are differences in the time-dependent sequence of the ventilatory indices POE and AT in less trained adults. In some individuals the POE occurs at a lower work rate compared to AT (VISP_{POE < AT}), while in the remaining individuals POE equals AT (VISP_{POE = AT}). Therefore, POE should not be used to determine AT. Training prescription based on POE may result in different exercise intensity recommendations compared to AT and therefore, lead to different and unintended physiological adaptations.

Differences in Fat_{max} have not been found to influence the POE – AT work rate differences. Although participants with a POE – AT work rate difference were characterized by a higher aerobic capacity (in terms of %VO_{2peak} and W · kg⁻¹ at AT), compared to participants with a work rate at POE equalling AT.

Therefore, it seems reasonable that the POE – AT work rate difference is mainly influenced by differences in the ventilatory response to the exercise strain at the early stages of the exercise test rather than by metabolic differences. And consequently, this ventilatory response leads to an increased aerobic capacity (in terms of %VO_{2peak}) in those individuals.

Finally, we consider the determination of different ventilatory indices in a single CPET useful for the evaluation and interpretation of the ventilatory and metabolic response to exercise to provide a more comprehensive picture of the performance capacity of the tested individual. Additionally, the inclusion of further indices like the crossover point (19, 24) or the mitochondrial threshold (44) may add further value to our understanding of cardiorespiratory fitness measured by CPET. Yet, because of the complexity of the CPET results (e.g., sequencing patterns), advanced methods like neural networks (45) are underway to support a more comprehensive interpretation of the exercise testing from the very sedentary to the highly performative individual.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Regional Ethics Committee of the federal

state of Salzburg (415-E/1521/3-2012). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: MP, SRD; Data curation: MP, VF; Formal analysis: MP; Investigation: MP; Methodology: MP, SRD; Project administration: MP, VF; Resources: BI, ET, BP; Supervision: SRD; Validation: SRD, BI, ET, BP; Visualisation: MP; Writing – original draft: MP; Writing – review; editing: MP, SRD, BI, VF, ET, BP. All authors contributed to the article and approved the submitted version.

References

- Ross RM. ATS/ACCP statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med.* (2003) 167:1451–1451. doi: 10.1164/ajrccm.167.10.950
- Meyer T, Lucia A, Earnest CP, Kindermann W. A conceptual framework for performance diagnosis and training prescription from submaximal gas exchange parameters—theory and application. *Int J Sports Med.* (2005) 26(Suppl 1): S38–48. doi: 10.1055/s-2004-830514
- Wasserman K, Hansen JE, Sue DY, Stringer WW, Sietsema KE, Sun X-G, et al. *Principles of exercise testing and interpretation.* 5th ed Baltimore: Lippincott Williams & Wilkins (2011).
- Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc.* (2000) 32:70–84. doi: 10.1097/00005768-200001000-00012
- Poole DC, Rössler HB, Brooks GA, Gladden LB. The anaerobic threshold: 50 + years of controversy. *J Physiol.* (2020) 0:1–31. doi: 10.1113/jp279963
- Pühringer M, Ring-Dimitriou S, Stöggel T, Iglseder B, Paulweber B. Differences in the point of optimal ventilatory efficiency and the anaerobic threshold in untrained adults aged 50 to 60 years. *Respir Physiol Neurobiol.* (2020) 282:103516. doi: 10.1016/j.resp.2020.103516
- Steding K, Buhre T, Arheden H, Wohlfart B. Respiratory indices by gas analysis and fat metabolism by indirect calorimetry in Normal subjects and triathletes. *Clin Physiol Funct Imaging.* (2010) 30:146–51. doi: 10.1111/j.1475-097X.2009.00918.x
- Ramos PS, Ricardo DR, Araújo CGS. Cardiorespiratory optimal point: a submaximal variable of the cardiopulmonary exercise testing. *Arq Bras Cardiol.* (2012) 99:988–96. doi: 10.1590/S0066-782X2012005000091
- Hollmann W. The relationship between pH, lactic acid, potassium in the arterial and venous blood, the ventilation, PoW and puls frequency during increasing spirometric work in endurance trained and untrained persons. *3rd Pan-American Congress for Sports Medicine.* Chicago (1959).
- Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med.* (2005) 26: S28–37. doi: 10.1055/s-2004-830512
- Binder RK, Wonisch M, Corra U, Cohen-Solal A, Vanhees L, Saner H, et al. Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *Eur J Cardiovasc Prev Rehabil.* (2008) 15:726–34. doi: 10.1097/HJR.0b013e328304fed4
- Westhoff M, Rühle KH, Greiwing A, Schomaker R, Eschenbacher H, Siepmann M, et al. [Positional paper of the German working group “cardiopulmonary exercise testing” to ventilatory and metabolic (lactate) thresholds]. *Dtsch Medizinische Wochenschrift.* (2013) 138:275–80. doi: 10.1055/s-0032-1332843
- Galán-Rioja MÁ, González-Mohino F, Poole DC, González-Ravé JM. Relative proximity of critical power and metabolic/ventilatory thresholds: systematic review and meta-analysis. *Sport Med.* (2020) 50:1771–83. doi: 10.1007/s40279-020-01314-8
- Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol.* (1986) 60:2020–7. doi: 10.1152/jappl.1986.60.6.2020
- Hollmann W. 42 Years ago - development of the concepts of ventilatory and lactate threshold. *Sports Med.* (2001) 31:315–20. doi: 10.2165/00007256-200131050-00002
- Gaskell SE, Walker AJ, Serfass RA, Bouchard C, Gagnon J, Rao DC, et al. Changes in ventilatory threshold with exercise training in a sedentary population: the heritage family study. *Int J Sports Med.* (2001) 22:586–92. doi: 10.1055/s-2001-18522
- Ring-Dimitriou S, Kedenko L, Kedenko I, Feichtinger RG, Steinbacher P, Stoiber W, et al. Does genetic variation in PPARGC1A affect exercise-induced changes in ventilatory thresholds and metabolic syndrome? *JEPonline.* (2014) 17:1–18. doi: 10.1519/JSC.0b013e3181874564
- Santos EL, Giannella-Neto A. Comparison of computerized methods for detecting the ventilatory thresholds. *Eur J Appl Physiol.* (2004) 93:315–24. doi: 10.1007/s00421-004-1166-6
- Brooks GA, Mercier J. Balance of carbohydrate and lipid utilization during exercise: the “crossover” concept. *J Appl Physiol.* (1994) 76:2253–61. doi: 10.1152/jappl.1994.76.6.2253
- San-Millán I, Brooks GA. Assessment of metabolic flexibility by means of measuring blood lactate, fat, and carbohydrate oxidation responses to exercise in professional endurance athletes and less-fit individuals. *Sport Med.* (2018) 48:467–79. doi: 10.1007/s40279-017-0751-x
- Maunder E, Plews DJ, Kilding AE. Contextualising maximal fat oxidation during exercise: determinants and normative values. *Front Physiol.* (2018) 9:1–13. doi: 10.3389/fphys.2018.00599
- Venables MC, Achten J, Jeukendrup AE. Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *J Appl Physiol.* (2005) 98:160–7. doi: 10.1152/jappphysiol.00662.2003
- Chrzanowski-Smith OJ, Edinburgh RM, Thomas MP, Haralabidis N, Williams S, Betts JA, et al. The day-to-day reliability of peak fat oxidation and FATMAX. *Eur J Appl Physiol.* (2020) 120:1745–59. doi: 10.1007/s00421-020-04397-3
- Michallet AS, Tonini J, Regnier J, Guinot M, Favre-Juvin A, Bricut V, et al. Methodological aspects of crossover and maximum fat-oxidation rate point determination. *Diabetes Metab.* (2008) 34:514–23. doi: 10.1016/j.diabet.2008.04.004
- Achten J, Jeukendrup AE. Maximal fat oxidation during exercise in trained men. *Int J Sports Med.* (2003) 24:603–8. doi: 10.1055/s-2003-43265
- Scharhag-Rosenberger F, Meyer T, Walitzek S, Kindermann W. Effects of one year aerobic endurance training on resting metabolic rate and exercise fat oxidation in previously untrained men and women. *Int J Sports Med.* (2010) 31:498–504. doi: 10.1055/s-0030-1249621
- Nikolovski Z, Barbaresi S, Cable T, Peric R. Evaluating the influence of differences in methodological approach on metabolic thresholds and fat oxidation points relationship. *Eur J Sport Sci.* (2020) 0:1–8. doi: 10.1080/17461391.2020.1717640

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28. Bircher S, Knechtle B. Relationship between fat oxidation and lactate threshold in athletes and obese women and men. *J Sport Sci Med.* (2004) 3:174–81.
29. Frey VN, Langthaler P, Raphaelis E, Ring-Dimitriou S, Kedenko L, Aigner E, et al. Paracelsus 10,000: an observational cohort study about the health status of the population of Salzburg, Austria. Rationale, objectives and study design. *Paracelsus Proc Exp Med.* (2023) 1:1–17. doi: 10.33594/000000600
30. American College of Sports Medicine. *Guidelines for exercise testing and prescription.* Philadelphia, PA: Lippincott Williams & Wilkins (2013). xv, 314 p. p.
31. Tanaka H, Monhah K, Seals D. Age-predicted maximum heart rate revisited. *J Am Coll Cardiol.* (2001) 37:153–6. doi: 10.1016/s0735-1097(00)01054-8
32. Robergs RA, Burnett AF. Methods used to process data from indirect calorimetry and their application to VO₂max. *J Exerc Physiol Online.* (2003) 6:44–57.
33. Merry KL, Glaister M, Howatson G, van Someren K. The exercise intensity at maximal oxygen uptake (iVO₂max): methodological issues and repeatability. *Eur J Sport Sci.* (2016) 1391:1–7. doi: 10.1080/17461391.2016.1183715
34. Pühringer M, Ring-Dimitriou S, Stöggel T, Iglseider B, Paulweber B. Comparison of visual, automatic and semiautomatic methods to determine ventilatory indices in 50 to 60 years old adults. *J Sports Sci.* (2020) 38:692–702. doi: 10.1080/02640414.2020.1725993
35. Stisen AB, Stougaard O, Langfort J, Helge JW, Sahlin K, Madsen K. Maximal fat oxidation rates in endurance trained and untrained women. *Eur J Appl Physiol.* (2006) 98:497–506. doi: 10.1007/s00421-006-0290-x
36. Takagi S, Sakamoto S, Midorikawa T, Konishi M, Katsumura T. Determination of the exercise intensity that elicits maximal fat oxidation in short-time testing. *J Sports Sci.* (2014) 32:175–82. doi: 10.1080/02640414.2013.815360
37. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Int J Nurs Stud.* (2010) 47:931–6. doi: 10.1016/j.ijnurstu.2009.10.001
38. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J.* (2012) 40:1324–43. doi: 10.1183/09031936.00080312
39. Cohen J. *Statistical power analysis for the behavioural sciences.* 2nd ed. Hillsdale, N.J.: L. Erlbaum Associates (1988). 567 p.
40. Rapp D, Scharhag J, Wagenpfeil S, Scholl J. Reference values for peak oxygen uptake: cross-sectional analysis of cycle ergometry-based cardiopulmonary exercise tests of 10 090 adult German volunteers from the prevention first registry. *BMJ Open.* (2018) 8:1–11. doi: 10.1136/bmjopen-2017-018697
41. Abildgaard J, Pedersen AT, Green CJ, Harder-Lauridsen NM, Solomon TP, Thomsen C, et al. Menopause is associated with decreased whole body fat oxidation during exercise. *Am J Physiol - Endocrinol Metab.* (2013) 304:1227–36. doi: 10.1152/ajpendo.00492.2012
42. Stickford ASL, Stickford JL. Ventilation and locomotion in humans: mechanisms, implications, and perturbations to the coupling of these two rhythms. *Springer Sci Rev.* (2014) 2:95–118. doi: 10.1007/s40362-014-0020-4
43. Iannetta D, De Almeida Azevedo R, Keir DA, Murias JM. Establishing the VO₂ versus constant-work-rate relationship from rampincremental exercise: simple strategies for an unsolved problem. *J Appl Physiol.* (2019) 127:1519–27. doi: 10.1152/jappphysiol.00508.2019
44. Baumbach P, Schmidt-Winter C, Hoefler J, Derlien S, Best N, Herbsleb M, et al. A pilot study on the association of mitochondrial oxygen metabolism and gas exchange during cardiopulmonary exercise testing: is there a mitochondrial threshold? *Front Med.* (2020) 7:1–15. doi: 10.3389/fmed.2020.585462
45. Zignoli A, Fornasiero A, Rota P, Muollo V, Peyré-Tartaruga LA, Low DA, et al. Oxynet: a collective intelligence that detects ventilatory thresholds in cardiopulmonary exercise tests. *Eur J Sport Sci.* (2021) 22:425–35. doi: 10.1080/17461391.2020.1866081