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Assessment of double anaerobic threshold in trained subjects

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ABSTRACT

Background: Regular training increases Anaerobic Threshold (AT) and improves ventilatory efficiency during exercise. A presence of a Double AT (DT), a delayed ventilatory response to CO_2 increase during exercise, was described in some healthy subjects, and may represent a favorable adaptation mechanism for trained subjects. The aim was to investigate the prevalence of DT in trained individuals and the relationship between training and ventilatory efficiency.
Methods: Maximal CardioPulmonary Exercise Test (CPET) of trained subjects were analysed for assessment of AT with the metabolic (V-slope analysis, MetAT) and the ventilatory methods (end-tidal and ventilatory equivalent methods, VentAT). Trained subjects were assigned to groups DT+ or DT- based on the presence/absence of DT, respectively. Trained subjects were also classified by type of sport practiced. Determinants of ventilatory efficiency were assessed at adjusted multivariate regression analysis.
Results: DT was identified in 52 cases out of 205 enrolled (25.4%). No differences by age, sex, body mass index and peak CPET parameters were observed between groups. DT+ subjects had a longer duration and greater ΔVO_2 values of isocapnic buffering period. Ventilatory efficiency was negatively associated (better values) with the presence of a DT ($p = 0.017$). Type of sport practiced did not influence the prevalence of DT nor the ventilatory efficiency response.
Conclusion: DT was found in 25.4% of trained people. The presence of DT is not influenced by the type of training and is not related to maximal aerobic capacity; however, subjects with a DT have a greater isocapnic buffering period and a better ventilatory efficiency.

1. Introduction

The anaerobic threshold (AT) is the point where, during an incremental work rate exercise, an increasing contribution of anaerobic metabolism to overall metabolism begins (Sietsema et al., 2020; Binder et al., 2008). The AT has an important role in several situation including the choice of exercise training modalities in cardiac rehabilitation, assessment of surgical risk, and prognosis of chronic heart failure or respiratory diseases (Mezzani et al., 2013; Meyer et al., 2005; Carriere et al., 2019a). The determination of AT is of importance also for both evaluation of physical performance of trained subjects, and in structuring exercise prescription programs (Pelliccia et al., 2021; Maffulli et al., 1991; Ghosh, 2004). Regular training, especially aerobic exercise, can increase AT VO_2 and workload (Henritze et al., 1985; Joyner and Coyle, 2008; Ready and Quinney, 1982; Staff et al., 2023). The non-invasive investigation of AT is usually performed during a cardiopulmonary exercise test (CPET) with an incremental ramp exercise. In this case, AT is measured using three approaches: V-slope method, the

Abbreviations: AT, anaerobic threshold; CPET, cardiopulmonary exercise test; DT, double threshold; Met AT, metabolic anaerobic threshold; RCP, respiratory compensation point; RER, respiratory exchange ratio; VE, ventilation; VE/VCO_2 slope, ventilation equivalent for carbon dioxide; VE/VO_2, ventilation equivalent for oxygen; Vent AT, ventilatory anaerobic threshold; VO_2peak, peak of oxygen uptake.

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ventilatory equivalent method and the end-tidal method (Sietsema et al., 2020; Beaver et al., 1986).

Usually, the V-slope method is the first considered in the AT analysis, and the other two methods are subsequently applied as confirmatory measurements (Sietsema et al., 2020; Beaver et al., 1986; Myers and Ashley, 1997). In the V-slope method, ventilation is not considered in AT definition being included in both axis; conversely in both the ventilatory equivalent and in end-tidal methods, also called ventilation-derived methods, ventilation changes are among the drivers for AT definition (Beaver et al., 1986). A discrepancy between the AT values found with the metabolic or ventilatory approach has been recently described and defined as Double Threshold (DT). This finding has been reported initially anecdotally (Poole et al., 2021; Vainshelboim et al., 2017; Whipp and Agostoni, 2007), and then reported with a prevalence of 11% in healthy individuals (Rovai et al., 2022a) and 42% in patients with heart failure (Rovai et al., 2022b). In these patients, the presence of DT was associated with a decreased ventilatory response during exercise. Indeed, a delay in the chemoreceptor response to excess exercise-induced metabolic acidosis has been suggested as DT leading causes (Rovai et al., 2022a). As a matter of facts, a delayed response of ventilation increase during exercise and a consequent increased ventilatory efficiency may represent a favorable adaptation mechanism for trained subjects including, on top of the delayed ventilatory response to acidosis, a prolonged isocapnic buffering period. Thus, the aim of our study was to investigate the prevalence of DT among a cohort of trained subjects, to identify factors associated to its presence and the relationship with ventilatory efficiency. Indeed, according to K. Wasserman the response of the healthy population to exercise induced acidosis can be classified as responder subject, those who increase ventilation and keep pH constant, and non-responder subjects, those who do not promptly respond to acidosis and show unbuffered acidosis, lower ventilation and higher $PaCO_2$ levels.

Thus, the aim of our study was to investigate the prevalence of DT among a cohort of trained subjects, to identify factors associated to its presence and the relationship with ventilatory efficiency.

2. Methods

2.1. Study cohort, procedures and ethics

We retrospectively (from 2007 to 2021) and prospectively (from 2022 to 2024) analysed data of healthy trained subjects who referred for a CPET in three laboratories (IRCCS Monzino, Milan; AOU Careggi, Florence; Ospedali Riuniti, Foggia). Study inclusion criteria were age ≥ 16 yearsand history of training for at least 4 h per week regardless of the type of sport (McKinney et al., 2019). All those who for any reason interrupted the test before maximal effort (respiratory exchange ratio, RER, < 1.05) were also excluded. Trained subjects were classified based on the type of sport practiced in endurance (cycling, athletics, swimming, triathlon, skiing) and non-endurance (all other sports). The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the IRCCS Centro Cardiologico Monzino ethics board, n. prot. R 1613/22 – CCM 1719, on 09/02/2022. Informed written consent was obtained from each participant.

2.2. Cardiopulmonary exercise test

A ramp exercise protocol was performed for each subject. Electronically braked cycle ergometers were used (Ergoline, SensorMedics), and the exercise was personalized with the aim of attaining the peak of the exercise in ~10 min. CO_2 exhaled and O_2 uptake were measured using the breath-by-breath method with a metabolic cart (COSMED Quark CPET, Vyntus CPX). All data were than averaged over 10 s. Predicted values of VO_2 were calculated according to the Hansen's formula (Hansen et al., 1984). AT was identified by the three methods: the V-slope, the ventilatory equivalent, and the end-tidal methods. AT identified with the V-slope method was defined as metabolic AT (Met AT). Met AT was graphically determined when, considering VO_2 and VCO_2 plotted on equal axis scale squared graph, their initially linear relationship showed an increased slope (Fig. 1). The ventilatory AT (Vent AT) assessment was performed with the other two methods. In particular, ventilatory equivalent method involved the simultaneous analysis vs. time of the ventilation equivalent for carbon dioxide (VE/VCO_2) and for oxygen (VE/VO_2). The point where VE/VO_2, after reaching the lowest point, starts to increase consistently while VE/VCO_2 remains unchanged is considered the Vent AT (Fig. 1). The end-tidal method identifies Vent AT when $PetO_2$ begins to increase while $PetCO_2$ is stable (Fig. 1). The presence of a DT was arbitrarily determined when a VO_2 discrepancy between Met AT and Vent AT > 15 ml/min and a time gap of at least 30 s were observed. The presence of a DT was arbitrarily determined when a VO_2 discrepancy between Met AT and Vent AT > 15 ml/min and a time gap of at least 30 s were observed. This criteria were chosen for the present analysis since the former was adopted in the healthy non athletes study and the latter to reduce the risk of random variaiblity of VO_2 (Rovai et al., 2022a). Cardiopulmonary exercise tests were reanalyzed by experienced operators; in doubtful cases DT was defined in agreement with a second operator. Trained subjects were assigned to groups DT+ or DT- based on the presence or absence of DT, respectively. All other CPET parameters were calculated and reported as standard. Specifically, peak VO_2 was reported as the highest recorded VO_2 values (average of 20 s), the oxygen pulse (O_2-pulse) as VO_2 over heart rate, and the VE/VCO_2 was determined as the slope of the linear relationship between ventilation and VCO_2 until the respiratory compensation point (RCP). Isocapnic buffering period defines the portion of the test between Met AT and RCP.

2.3. Statistical analysis

Continuous variables are presented as mean ± SD. Categorical variables are reported as absolute values and percentages. Differences between groups were assessed by ANOVA for normally distributed variables and chi square test for non-normally distributed variables. In subjects with DT, comparisons of values between Met AT and Vent AT were made using paired *t*-test. The association between VE/VCO_2 slope and the presence of a DT was investigated using multiple linear regression analysis. Assuming a sample size of 201 subjects, assuming a precision of 6% and a confidence level of 95%, we can estimate a prevalence of 25% with a statistical power of 80%. Statistical significance is a two-sided level of 0.05. Data were analyzed using the Statistical Package for the Social Sciences (SPSS Statistics v. 28, IBM, Armonk, NY).

3. Results

3.1. Prevalence of a double anaerobic threshold

Out of 228 trained subjects who performed a CPET, 205 fulfilled the study inclusion/exclusion criteria and were enrolled. 52 subjects showed a DT (25.4%, 10 females and 42 males), while in 153 cases Met AT and Vent AT were superimposable. No differences in DT prevalence were observed within the study centers.

Demographic and CPET data at Met AT, RCP and peak exercise are reported in Table 1. No differences were observed between DT+ and DT- subjects for age, sex, anthropometric measures or sport category. The two groups also showed comparable values of RCP and peak exercise parameters (Fig. 2).

Considering Met AT, VE/VCO_2 was higher in DT+ than DT- trained subjects. No other differences were observed between the two groups (Table 1).

In DT+ subjects, Met AT always preceded Vent AT. At Vent AT, VO_2, VO_2/kg, %VO_2peak, VCO_2, RER, VE/VCO_2, $PetCO_2$, ventilation, work rate, heart rate, oxygen pulse values were higher than at Met AT. As

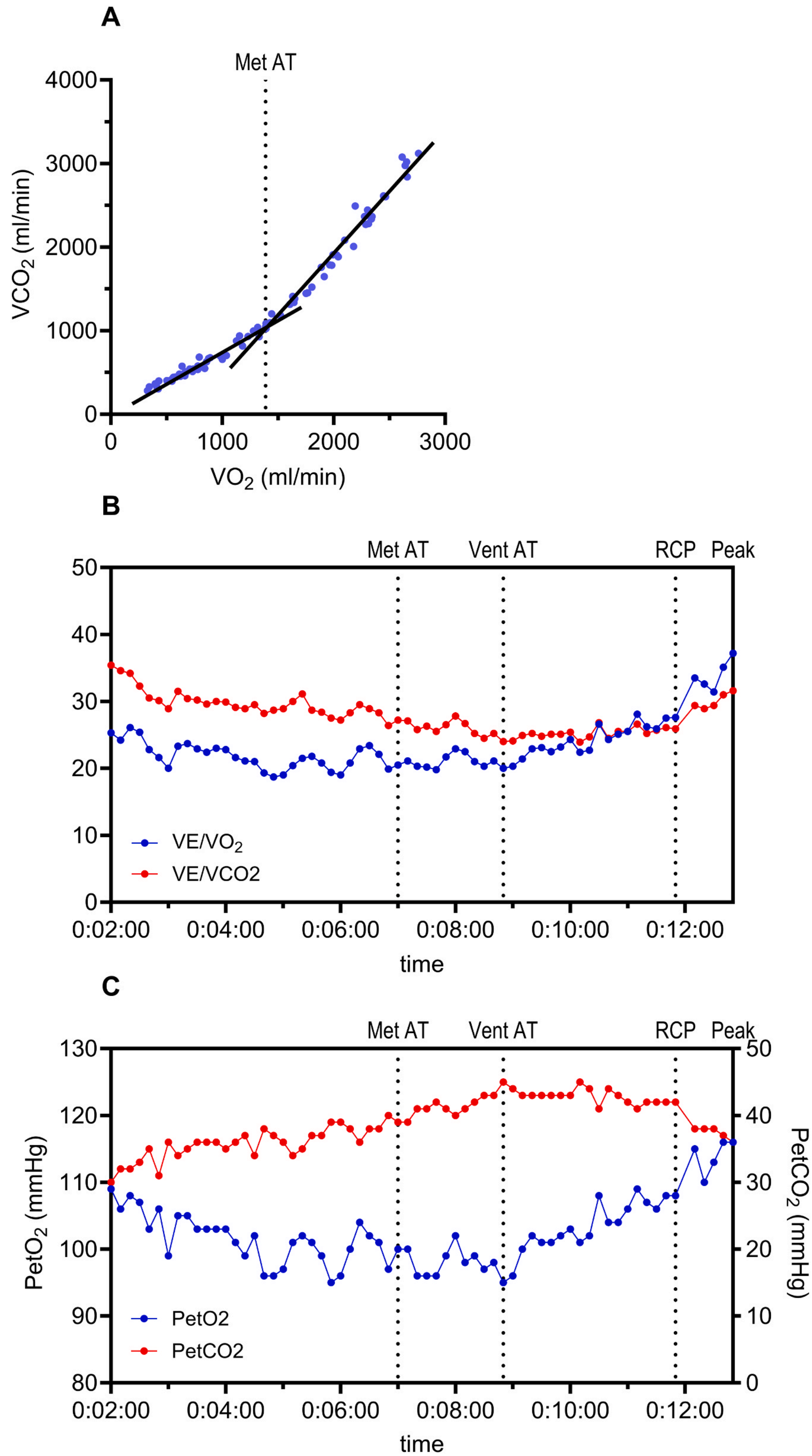

Fig. 1. Example case of a trained subject with double anaerobic threshold (DT). The determination of metabolic (Met AT) and ventilatory (Vent AT) thresholds is performed after CPET. Met AT is firstly assessed with the V-slope method (panel A). Vent AT is then determined with the ventilatory equivalent methods (panel B) and the $PetO_2$ and $PetCO_2$ methods (panel C). RCP, respiratory compensation point.

expected, no significant difference was found regarding VE/VO_2, $petO_2$ and respiratory rate (Table 2).

In DT+ subjects, the isocapnic buffering period had a longer duration and a greater difference in terms of VO_2 between the two thresholds (both in ml/min and %peak) than DT- subjects (Table 1, Fig. 2).

Comparing Vent AT parameters among the two groups, DT+ subjects showed higher VCO_2 (1727 ± 821 vs. 2056 ± 866, $p = 0.015$), RER (0.85 ± 0.08 vs 0.92 ± 0.07, $p < 0.001$) and $petCO_2$ (42.9 ± 4.4 vs. 44.2 ± 3.9, $p = 0.044$) values with a comparable respiratory rate (23 ± 7 vs. 24 ± 6, $p = 0.265$), confirming that ventilatory drive had not yet

Table 1
Demographic, anthropometric and cardiopulmonary exercise test data at anaerobic threshold detected with the V-slope method (met-AT), RCP, and peak exercise, in subjects with (DT+, n = 52) and without (DT-, n = 153) a double anaerobic threshold.

		All (n = 205)	DT- (n = 153)	DT+ (n = 52)	p
Demographic and anthropometric data					
Age (years)		33.8 ± 13.8	33.2 ± 13.3	35.8 ± 15	0.244
Sex					0.405
	female	32 (15.6)	22 (14.4)	10 (19.2)	
	male	173 (84.4)	131 (85.6)	42 (80.8)	
Sport category					0.671
	non endurance	72 (35.1)	55 (35.9)	17 (32.7)	
	endurance	133 (64.9)	98 (64.1)	35 (67.3)	
Weight (kg)		70.6 ± 10.6	70.6 ± 10.9	70.4 ± 9.5	0.888
Height (cm)		176 ± 7.5	176 ± 7.6	176.2 ± 7.5	0.818
BMI (kg/m^2)		22.7 ± 2.6	22.7 ± 2.7	22.6 ± 2.3	0.752
Met-AT					
VO_2 (ml/min)		1969 ± 899	2001 ± 894	1875 ± 912	0.386
VO_2 (ml/min/kg)		28.3 ± 13.4	28.9 ± 13.8	26.5 ± 12.2	0.269
VO_2 (% VO_2peak)		57.7 ± 11.8	58.7 ± 11.7	55.0 ± 11.7	0.052
VCO_2 (ml/min)		1689 ± 817	1718 ± 822	1603 ± 804	0.381
RER		0.85 ± 0.08	0.85 ± 0.08	0.85 ± 0.07	0.793
VE (l/min)		45.1 ± 19.8	45.6 ± 19.8	43.7 ± 19.7	0.541
RR (breath/min)		22.7 ± 6.3	22.7 ± 6.7	22.9 ± 4.8	0.757
VE/VO_2		23.2 ± 3.3	23.0 ± 3.4	23.7 ± 2.9	0.198
VE/VCO_2		27.0 ± 3.1	26.7 ± 3.0	27.9 ± 3.1	0.011
petO_2 (mmHg)		98.4 ± 5.8	98.2 ± 6.0	99.3 ± 5.1	0.226
petCO_2 (mmHg)		42.6 ± 4.2	42.8 ± 4.4	42.2 ± 3.5	0.395
Work (watt)		158.9 ± 82.8	161.7 ± 83.1	150.6 ± 82.1	0.404
HR (bpm)		125.9 ± 20.7	127.0 ± 21.1	122.7 ± 19.3	0.197
RCP					
VO_2 (ml/min)		2933 ± 1012	2918 ± 1004	2973 ± 1043	0.601
VO_2 (% VO_2peak)		87.2 ± 9.4	86.7 ± 10.0	88.6 ± 7.4	0.214
VCO_2 (ml/min)		3023 ± 1028	2989 ± 1014	3120 ± 1069	0.739
RER		0.97 ± 0.09	0.98 ± 0.10	0.95 ± 0.06	0.520
VE (l/min)		80.8 ± 26.2	80.1 ± 26.3	82.8 ± 26	0.525
Work (watt)		252.5 ± 92.3	250.4 ± 92	258.2 ± 93.6	0.431
Peak					
VO_2 (ml/min)		3322 ± 993	3322 ± 974	3322 ± 1058	0.998
VO_2 (ml/min/kg)		47.6 ± 14.7	47.7 ± 14.9	47.2 ± 14.3	0.815
VO_2 (% predicted)		122.1 ± 27.7	121.4 ± 28.1	124.1 ± 26.5	0.542
VCO_2 (ml/min)		3675 ± 1108	3657 ± 1086	3729 ± 1179	0.685
RER		1.15 ± 0.07	1.14 ± 0.07	1.16 ± 0.07	0.266
VE (l/min)		113.6 ± 33.1	113.6 ± 33.1	113.3 ± 33.6	0.955

Table 1 (*continued*)

	All (n = 205)	DT- (n = 153)	DT+ (n = 52)	p
RR (breath/min)	44.2 ± 10.3	44.2 ± 11.1	44.2 ± 7.8	0.987
Work (watt)	299.8 ± 95.8	300 ± 94.9	299.2 ± 99.2	0.959
Work (% predicted)	141.4 ± 34.9	139.9 ± 35.9	145.9 ± 31.5	0.278
Slopes				
VE/VCO_2 slope	25.4 ± 3.3	25.7 ± 3.3	24.7 ± 3.1	0.056
VO_2/Work slope (ml/min/watt)	10.3 ± 0.9	10.2 ± 0.9	10.3 ± 1.0	0.471
Isocapnic buffering period				
Δtime Met AT-RCP (sec)	205.5 ± 73.8	193.5 ± 73.3	237.8 ± 65.4	< 0.001
ΔVO_2 Met AT-RCP (ml/min)	974 ± 414	929 ± 420	1097 ± 371	0.012
ΔVO_2 Met AT-RCP (ml/min/kg)	14.0 ± 5.9	13.3 ± 6.0	15.6 ± 5.1	0.016
ΔVO_2 Met AT-RCP (% peak)	29.8 ± 11.2	28.5 ± 11.2	33.6 ± 10.4	0.004

Data are expressed as mean±standard deviation or as count (percentage). BMI = body mass index; Met AT = metabolic anaerobic threshold, identified by the V-slope method; Vent AT = ventilatory anaerobic threshold, identified by the ventilatory equivalent and/or end-tidal methods; RCP = respiratory compensation point; VO_2 = oxygen uptake; VCO_2 = carbon dioxide production; RER = respiratory exchange ratio (VCO_2/VO_2); VE = min ventilation; VE/VO_2 = ventilatory equivalent for oxygen; VE/VCO_2 = ventilatory equivalent for carbon dioxide; PetO_2 = end-tidal partial pressure of oxygen; PetCO_2 = end-tidal partial pressure of carbon dioxide; RR = respiratory rate; HR = heart rate; VO_2/W slope = slope of the relationship between oxygen uptake and work rate; VE/VCO_2 slope = slope of the linear relationship between ventilation and carbon dioxide production; Δ = difference between values; DT = double anaerobic threshold.

started in DT+ group.

3.2. Endurance vs. non endurance sports

Considering the difference between endurance trained subjects and non-endurance trained subjects, significant differences were found. Endurance trained subjects showed higher age, and CPET parameters as VO_2, VCO_2, VE, and work rate, at Met AT, RCP and peak exercise. Duration in isocapnic buffering period was similar between endurance and non-endurance groups. No difference was observed in Δtime between Met AT and Vent AT in the DT+ subgroup (Table 3).

3.3. Ventilatory efficiency

Ventilatory efficiency expressed as VE/VCO_2 slope was lower in subjects who had a DT, despite not reaching the significance criteria ($p = 0.056$) (Table 1, Fig. 2). However, at multivariate regression analysis (adjusted for sex, age, BMI and sport category), VE/VCO_2 slope was negatively associate with the presence of DT, suggesting an increased ventilatory efficiency in this subgroup (B= −1.225, 95%Cl [-2.227 to −0.223], $p = 0.017$). Sport category did not influence VE/VCO_2 slope.

4. Discussion

The main results of the present study were that: 1) a DT was identified in 25% of trained subjects; 2) the presence of a DT is not related to

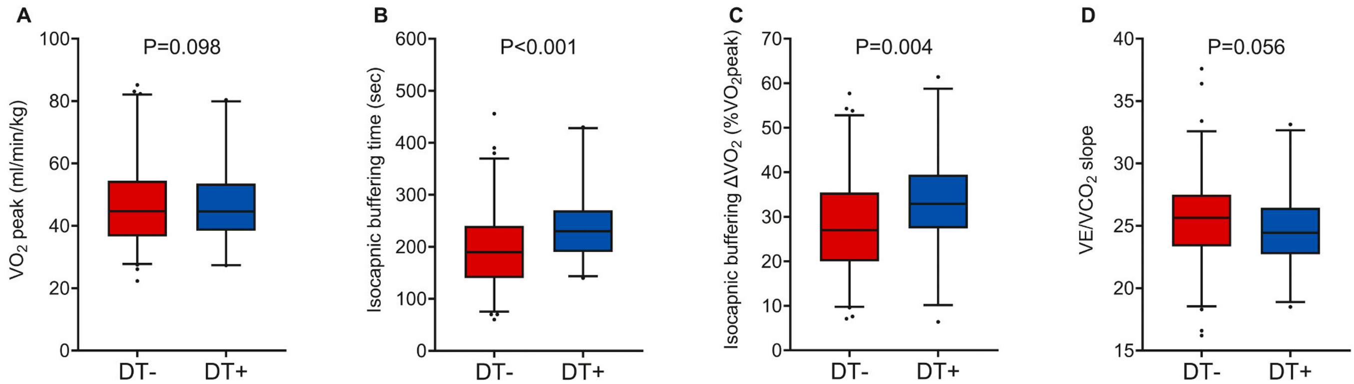

Fig. 2. Representation of main results characterizing subjects with (DT+, n = 52) and without a (DT-, n = 153) a double anaerobic threshold. Results are expressed for the two groups as box-plots (length of the box is the difference between the 75th and 25th percentiles, ends of the box are 2.5–97.5th percentile, outliers are plotted as individual points). No differences in VO_2peak were observed between DT+ and DT- subjects (panel A). Conversely, isocapnic buffering period of DT+ subjects had a longer duration (panel B) and a greater difference in terms of VO_2 between the two thresholds (panel C) than in DT- subjects. DT + subjects had lower values of VE/VCO_2 slope (panel D).

Table 2
Cardiopulmonary exercise test data at the anaerobic threshold (AT) detected with the V-slope method (met-AT) and with ventilatory equivalent method in subjects with a DT (n = 52).

	Met AT	Vent AT	p
VO_2 (ml/min)	1876 ± 912	2248 ± 962	< 0.001
VO_2 (ml/min/kg)	26.5 ± 12.2	31.8 ± 12.9	< 0.001
VO_2 (% VO_2peak)	55.0 ± 11.7	66.6 ± 11.6	< 0.001
VCO_2 (ml/min)	1603 ± 804	2056 ± 866	< 0.001
RER	0.85 ± 0.07	0.92 ± 0.07	< 0.001
VE/VO_2	23.7 ± 2.9	24.0 ± 2.8	0.167
VE/VCO_2	27.9 ± 3.1	26.2 ± 2.8	< 0.001
petO_2 (mmHg)	99.3 ± 5.1	100.0 ± 5.3	0.072
petCO_2 (mmHg)	42.2 ± 3.5	44.2 ± 3.9	< 0.001
VE (l/min)	43.7 ± 19.7	53.2 ± 21.1	< 0.001
RR (breath/min)	22.9 ± 4.8	23.9 ± 6.1	0.077
Work (watt)	150.6 ± 82.1	191.3 ± 85.7	< 0.001
HR (bpm)	122.6 ± 19.5	138.5 ± 19.2	< 0.001

Data are expressed as mean±standard deviation.
DT= double anaerobic threshold; Met AT = metabolic anaerobic threshold, identified by the V-slope method; Vent AT = ventilatory anaerobic threshold, identified by the ventilatory equivalent and/or end-tidal methods; VO_2 = oxygen uptake; VCO_2 = carbon dioxide production; RER = respiratory exchange ratio (VCO_2/VO_2); VE = min ventilation; VE/VO_2 = ventilatory equivalent for oxygen; VE/VCO_2 = ventilatory equivalent for carbon dioxide; PetO_2 = end-tidal partial pressure of oxygen; PetCO_2 = end-tidal partial pressure of carbon dioxide; RR = respiratory rate; HR = heart rate.

the type of training and does not translate in advantages in VO_2peak values; 3) conversely, DT is associated with an increased isocapnic buffering period and increased ventilatory efficiency.

The present study is the first to evaluate the presence of a delayed ventilatory response, i.e. a DT, in trained subjects, that was observable in the 25% of cases. In particular, we described a prevalence twice that of a healthy population (Rovai et al., 2022a) but lower than that of patients with heart failure (Rovai et al., 2022b). It was previously hypothesized that the delayed ventilatory response to exercise-induced acidosis could be linked to a reduced chemoreflex activity (Rovai et al., 2022c). Regular training, as associated with an increased vagal response and reduced sympathetic stimulation at rest (Hautala et al., 2009), may indeed act on chemoreceptor sensitivity in an inhibitory sense. However, this is not the only mechanism involved in the ventilatory response to exercise, as other reflexes such as metaboreflexes or lung stretch receptors may have a role in ventilatory response to exercise, and indeed three out of four trained subjects still do not exhibit this peculiar ventilatory response.

Within our population, we did not find factors associated with the presence of DT. In particular, none of our demographic or exercise variables differentiated these subjects. With regard to the type of sport, as expected, endurance trained subjects had higher VO_2peak values and an AT shifted forward with respect to their VO_2peak compared to non-endurance trained subjects. However, there is no difference in the prevalence of DT between these two groups.

The presence or absence of DT did not influence peak exercise performance, which was similar between DT+ and DT- cases. Differently, a greater isocapnic buffering period both in terms of duration and ΔVO_2 values was observed in subjects with DT, suggesting that they can tolerate more exercise before acidosis-induced hyperventilation. It can be speculated that this subgroup of trained subjects may have a greater tolerance to acidosis at intermediate intensities of exercise. In heart failure patients, the VO_2 during the isocapnic buffering period is positively related to exercise performance (Carriere et al., 2019b). A linear relationship between aerobic fitness level (VO_2peak values) and magnitude in VO_2/kg values of the isocapnic buffering period was also observed in trained subjects (Algul et al., 2017; Oshima et al., 1997). This relationship was observed in our cohort.

Lastly, trained subjects with a DT showed a slightly better ventilatory efficiency, while no influence of the type of sport was observed. This pattern was also observed in heart failure patients with a DT (Rovai et al., 2022b), and may represent a favorable adaptation mechanism for trained subjects and patients addressed to an exercise prescription program. Future studies may investigate the prognostic role of DT.

4.1. Limitations

Our study has some limitations that should be acknowledged. First of all, blood lactate was not measured, so that we cannot say which threshold, Met AT, or Vent AT, is associated with the blood sample measured lactate threshold. Second, the use of cycle ergometry for all subjects may lead to a marked underestimation of VO_2 max and consequently the position of metabolic and ventilatory thresholds during the incremental test in trained runners compared to trained subjects practicing other sports (Pannier et al., 1980).

5. Conclusions

For the first time, the prevalence of a delayed ventilatory response was assessed in a population of trained subjects. The presence of DT appears to be not influenced by the type of sport practiced and it is not related to maximal aerobic capacity; however, subjects with a DT have a greater isocapnic buffering period and a better ventilatory efficiency.

Table 3
Characteristics of non endurance (n = 72) and endurance (n = 133) subjects.

		Non endurance	Endurance	p
Demographics				
Age (years)		31.1 ± 13.2	35.3 ± 13.9	0.037
Sex				0.480
	female	13 (18.1)	19 (14.3)	
	male	59 (81.9)	114 (85.7)	
Weight (kg)		73.2 ± 11.2	69.2 ± 9.9	0.009
Height (cm)		177.5 ± 7.9	175.3 ± 7.2	0.045
BMI (kg/m2)		23.2 ± 2.7	22.5 ± 2.4	0.055
Met-AT (all)				
VO_2 (ml/min)		1467 ± 448	2241 ± 964	< 0.001
VO_2 (ml/min/kg)		20.0 ± 5.0	32.8 ± 14.4	< 0.001
VO_2 (% VO_2peak)		52.2 ± 9.1	60.7 ± 12.0	< 0.001
VCO_2 (ml/min)		1275 ± 461	1912 ± 880	< 0.001
RER		0.86 ± 0.09	0.85 ± 0.07	0.450
VE (l/min)		34.7 ± 12.0	50.8 ± 20.8	< 0.001
RR (breath/min)		20.1 ± 5.7	24.1 ± 6.1	< 0.001
VE/VO_2		23.5 ± 3.6	23.0 ± 3.1	0.251
VE/VCO_2		27.3 ± 3.0	26.8 ± 3.1	0.244
$petO_2$ (mmHg)		99.5 ± 5.7	97.9 ± 5.8	0.061
$petCO_2$ (mmHg)		42.2 ± 4.3	42.9 ± 4.1	0.251
Work (watt)		113.9 ± 43.0	183.3 ± 88.8	< 0.001
HR (bpm)		119.6 ± 17.7	129.2 ± 21.4	0.001
RCP				
VO_2 (ml/min)		2338 ± 644	3241 ± 1032	< 0.001
VO_2 (% VO_2peak)		83.6 ± 11.1	89.0 ± 7.9	< 0.001
VCO_2 (ml/min)		2447 ± 716	3322 ± 1040	< 0.001
RER		0.96 ± 0.10	0.98 ± 0.09	0.101
VE (l/min)		65.8 ± 19.0	88.6 ± 26.1	< 0.001
Work (watt)		198.6 ± 58.1	280.5 ± 94.4	< 0.001
HR (bpm)		155.2 ± 16.3	162.0 ± 16.1	0.007
Peak				
VO_2 (ml/min)		2806 ± 625	3601 ± 1044	< 0.001
VO_2 (ml/min/kg)		38.5 ± 7.4	52.5 ± 15.4	< 0.001
VO_2 (% predicted)		101.5 ± 15.8	133.2 ± 26.4	< 0.001
VCO_2 (ml/min)		3081 ± 806	3997 ± 1118	< 0.001
RER		1.13 ± 0.07	1.15 ± 0.07	0.074
VE (l/min)		93.6 ± 23.3	124.4 ± 32.7	< 0.001
RR (breath/min)		39.0 ± 7.6	47.0 ± 10.5	< 0.001
Work (watt)		244.8 ± 56.7	329.6 ± 99.6	< 0.001
Work (% predicted)		114.5 ± 25.5	155.9 ± 30.4	< 0.001
Slopes				
VE/VCO_2 slope		25.6 ± 3.5	25.3 ± 3.2	0.560
VO_2/W slope (ml/min/W)		10.2 ± 1.0	10.3 ± 0.9	0.236
Isocapnic buffering period				
Δtime Met AT-RCP (sec)		214.3 ± 67.8	200.9 ± 76.6	0.233
ΔVO_2 Met AT-RCP (ml/min)		908 ± 400	1008 ± 418	0.109
ΔVO_2 Met AT-RCP (ml/min/kg)		12.7 ± 5.7	14.6 ± 5.9	0.026
ΔVO_2 Met AT-RCP (% peak)		32.1 ± 10.6	28.6 ± 11.3	0.039
Only DT+ subjects (n = 52)		*n = 17*	*n = 35*	
Δtime Met AT-Vent AT (sec)		85.5 ± 35.3	94.8 ± 48.5	0.483

Data are expressed as mean±standard deviation or as number (percentage). BMI = body mass index; Met AT = metabolic anaerobic threshold, identified by the V-slope method; Vent AT = ventilatory anaerobic threshold, identified by the ventilatory equivalent and/or end-tidal methods; RCP = respiratory compensation point; VO_2 = oxygen uptake; VCO_2 = carbon dioxide production; RER = respiratory exchange ratio (VCO_2/VO_2); VE = min ventilation; VE/VO_2 = ventilatory equivalent for oxygen; VE/VCO_2 = ventilatory equivalent for carbon dioxide; $PetO_2$ = end-tidal partial pressure of oxygen; $PetCO_2$ = end-tidal partial pressure of carbon dioxide; RR = respiratory rate; HR = heart rate; VO_2/W slope = slope of the relationship between oxygen uptake and work rate; VE/VCO_2 slope = slope of the linear relationship between ventilation and carbon dioxide production; Δ = difference between values; DT = double anaerobic threshold; DT= double anaerobic threshold;.

CRediT authorship contribution statement

Edoardo Falconi: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Mauro Contini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Conceptualization. **Alessio Pellegrino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Pietro Amedeo Modesti:** Writing – review & editing, Visualization, Validation, Investigation. **Michele Correale:** Writing – review & editing, Visualization, Validation, Investigation. **Lucia Tricarico:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Data curation. **Alessandro Nava:** Writing – review & editing, Visualization, Validation, Investigation, Data curation. **Piergiuseppe Agostoni:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare no conflict of interests.

Data availability

Data will be made available on request.

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