Evidence of a double anaerobic threshold in healthy subjects

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Aims The anaerobic threshold (AT) is an important cardiopulmonary exercise test (CPET) parameter both in healthy and in patients. It is normally determined with three approaches: V-slope method, ventilatory equivalent method, and end-tidal method. The finding of different AT values with these methods is only anecdotic. We defined the presence of a double threshold (DT) when a ΔVO_2 > 15 mL/min was observed between the V-slope method (met AT) and the other two methods (vent AT). The aim was to identify whether there is a DT in healthy subjects.

Methods and results We retrospectively analysed 476 healthy subjects who performed CPET in our laboratory between 2009 and 2018. We identified 51 subjects with a DT (11% of cases). Cardiopulmonary exercise test data at rest and during the exercise were not different in subjects with DT compared to those without. Met AT always preceded vent AT. Compared to subjects without DT, those with DT showed at met AT lower carbon dioxide output (VCO_2), end-tidal carbon dioxide tension ($PetCO_2$) and respiratory exchange ratio (RER), and higher ventilatory equivalent for carbon dioxide (VE/VCO_2). Compared to met AT, vent AT showed a higher oxygen uptake (VO_2), VCO_2, ventilation, respiratory rate, RER, work rate, and $PetCO_2$ but a lower VE/VCO_2 and end-tidal oxygen tension. Finally, subjects with DT showed a higher VO_2 increase during the isocapnic buffering period.

Conclusion Double threshold was present in healthy subjects. The presence of DT does not influence peak exercise performance, but it is associated with a delayed before acidosis-induced hyperventilation.

Keywords Anaerobic threshold • Cardiopulmonary exercise test • Healthy subjects • Double threshold

Introduction

The so-called anaerobic threshold (AT), also known as the lactate threshold, first ventilatory threshold, first threshold, gas exchange threshold, or lactic acidosis threshold, is considered the intensity, during an incremental work rate exercise, where an increasing contribution of anaerobic metabolism to overall metabolism begins and consequently AT is where, in a progressive increasing workload exercise, VCO_2/work rate relationship shows an increase.[1–5] Literally the term AT implies dysoxia, but dysoxia is not needed for lactic acid production during exercise so the classical term AT may be physiologically deceptive.[6] The AT is an important cardiopulmonary exercise test (CPET) parameter both in healthy individuals and in patients. Indeed, the AT has a definite role in assessing preoperatively surgical risk and post-surgery need for intensive care, in the prognosis of chronic heart failure or respiratory patients, and in cardiac rehabilitation, both for exercise prescription and for training programs.[7–19] Furthermore, the simple presence/absence of AT in heart failure patients who reached anaerobiosis has a relevant prognostic role, as regards cardiovascular death, i.e., in those in which at peak exercise VCO_2/VO_2 is >1.05.[16,18,20,21] The AT can be detected invasively through arterial blood samples to identify blood lactate level

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increase.[3,4] However, since the historical paper of Beaver *et al.*, AT detection is conventionally done noninvasively using three approaches: V-slope method, a ventilatory equivalent method, or end-tidal method, as original described by Beaver *et al.*[5,22] Usually, the V-slope method is the first considered in the AT analysis, and the other two methods are thereafter applied as confirmatory measurements.[4,23–26] Of note, in the V-slope method, the role of ventilation in AT definition disappears, since it is present in both oxygen uptake (VO_2) and carbon dioxide output (VCO_2). Differently, in both the ventilatory equivalent and in end-tidal methods, ventilation changes are among the drivers for AT definition. The finding of different AT values between the V-slope and the other two methods that, for convenience, we will call ventilation-derived methods (the ventilatory equivalent and the end-tidal methods) has been reported anecdotally.[6,27,28] To the best of our knowledge, the presence of concordance or discrepancy between the various methods to determine AT has not been systematically analysed in a population of normal subjects, and the physiological reasons behind the presence of a double anaerobic threshold (DT) are unknown. The aim of our study was, therefore, to identify AT both with the V-slope method and with ventilation-derived methods in healthy individuals who performed a CPET in our laboratories for any reason.

Study design and methods

We retrospectively analysed the data of healthy individuals who performed CPET in our exercise laboratories between 2009 and 2018, either as healthy volunteers for previous studies or as self-presented individuals willing to know their exercise performance.[29,30]

Study inclusion criteria were age greater than or equal to 18 years, ability to perform a CPET, no recent or previous major diseases, and no chronic treatment except for hormonal replacement therapy or oral contraceptive medications. Exclusion criteria were the presence of any ongoing treatment or diagnosis of any physical condition potentially affecting exercise performance. Cardiopulmonary exercise tests interrupted for any reason before a self-reported maximal effort was reached were also excluded. Similarly, CPET during which respiratory manoeuvres were done at mid-exercise either for flow/volume analysis or for cardiac output determination by rebreathing techniques were done, were also excluded from the present analysis.[30,31] Similarly, athletes, i.e., people who exercise regularly, at least three times a week, elderly patients (over 85 years old), and obese subjects (BMI >30) were excluded. At enrolment, we recorded patients' clinical history, and we performed a physical examination, a resting ECG, and CPET.

The present retrospective study has been approved by the Centro Cardiologico Monzino IRCCS scientific committee, and it has been registered by the Centro Cardiologico Monzino IRCCS ethics committee as CCM 06-21.

Cardiopulmonary exercise test

In every case, a personalized ramp exercise protocol, preceded by at least 1 min of unloaded pedalling and a resting time needed to adapt subjects with mask-breaths, was performed. We used an electronically braked cycle ergometer (Erg 800S, SensorMedics, or Lode Corival), to achieve peak exercise in ~10 min.[32] As standard, before each testing we performed CPET calibration procedures. We collected and analysed ventilation and respiratory data breath by breath, and we reported AT and peak VO_2 data as an average over 10 s (229D Spectra metabolic cart, SensorMedics, or Innocor rebreathing system, Innovision A/S, Odense, Denmark). Cardiopulmonary exercise tests were re-analysed for the present study by two experts (S.R. and P.G.) using a standard technique.[25] Predicted values of VO_2 were calculated according to Hansen *et al.*'s formula.[33] Anaerobic threshold was identified by three methods: the V-slope, the ventilatory equivalent, and the end-tidal methods. Specifically, AT was identified with the V-slope method when the relationship between VO_2 and VCO_2, plotted on an equal axis scale squared graph, showed an increased slope of the linear previously relationship between VO_2 and VCO_2. The AT assessment by the ventilatory equivalent method involved the simultaneous analysis vs. time (or work rate) of the ventilation equivalent for carbon dioxide (VE/VCO_2) and of the ventilation equivalent for oxygen (VE/VO_2). The AT corresponds to the VO_2 at which VE/VO_2, after reaching the lowest point, starts to increase consistently, while VE/VCO_2 remains unchanged. The ventilatory equivalent method is matched by the end-tidal method, which identifies AT when end-tidal oxygen tension ($PetO_2$) begins to increase while end-tidal carbon dioxide tension ($PetCO_2$) is stable.[3,4] A DT was identified when there was a VO_2 discrepancy between the V-slope and ventilation-derived methods (>15 mL/min). In these cases, we named the AT detected by V-slope method as metabolic AT (met AT) and the AT identified by ventilation-derived methods as ventilation AT (vent AT). In *Figure 1*, the data of two subjects are described, one with a single AT (left) and one with a DT (right). In the left panel, the AT has the same value regardless of the method used for its detection; in the right panel, met AT precedes vent AT. The respiratory compensation point (RCP) was identified by an increase of VE/VCO_2 and a reduction of $PetCO_2$. The isocapnic buffering period was delimited by met AT and the respiratory compensation point.[4]

Statistical analysis

Continuous variables are presented as mean ± standard deviation or as median and interquartile range in case of non-normally distributed variables. We reported categorical variables as absolute values and percentages. We compared subjects with DT with those with a single AT by ANOVA for normally distributed variables and Kruskal–Wallis test for non-normally distributed variables. Considering only the subjects with DT, we compared the values at met AT and vent AT using paired *t*-test for normally distributed variables and non-parametric Wilcoxon–Mann–Whitney test for non-normally distributed variables. Assuming a sample size of 400 subjects and an α of 0.05, we will be able to identify 80% of subjects with DT with an accuracy of ~6% and a statistical power of 80%. A *P*-value <0.05 was used to define statistical significance.

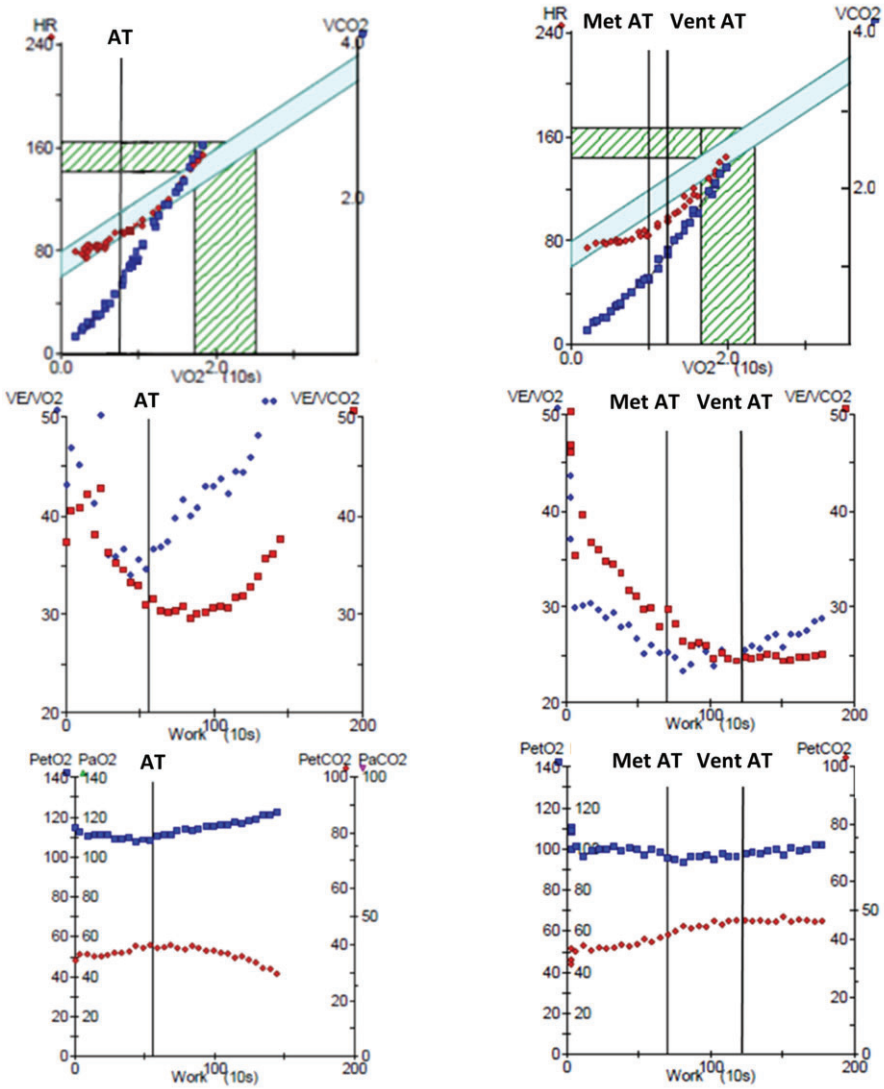

Figure 1 On the left the response of a healthy subject to an incremental work rate test and the three methods to calculate the anaerobic threshold (AT). The anaerobic threshold is clearly detectable, and it has the same value with all methods, from top to bottom: V-slope method, ventilatory equivalent method, and the end-tidal oxygen tension ($PetO_2$) and end-tidal carbon dioxide tension ($PetCO_2$) method. On the right the response of another healthy subject to an incremental work rate test. In this case, the AT value, detected with the three different methods, has given two different measures. Specifically, the anaerobic threshold value detected with the V-slope method (*met AT*) (upper graph) is different from the anaerobic threshold value calculated using the ventilatory equivalent methods and the $PetO_2$ and $PetCO_2$ methods (*vent AT*). HR, heart rate; VE/VCO_2, ventilatory equivalent for carbon dioxide; VE/VO_2, ventilatory equivalent for oxygen.

We collected all data in an Excel database, and we performed analyses using IBM SPSS Statistics 25.

Results

In the present analysis, out of 476 subjects who fulfilled the study inclusion/exclusion criteria, 51 showed a DT (11%, 20 females and 31 males), while in 425 cases met AT and vent AT were superimposable so that a single AT was observed. Demographic and CPET data at peak exercise are reported in *Table 1*. No differences were found between subjects with DT and those without. At rest, variables such as RER between 0.7 and 1 and constant ventilation, VO_2 and VCO_2 for at least 2 min, confirmed the stability of resting cardiorespiratory data and absence of voluntary hyperventilation. No differences among the usual CPET variables between DT and non-DT cases were found at rest and at the beginning of exercise (*Table 2*).

Compared with subjects without a DT, subjects with DT, considering met AT, showed lower VCO_2, $PetCO_2$, and RER, and higher VE/VCO_2 ratio at AT (*Table 3*). In the DT subjects, met AT always preceded vent AT which showed higher VO_2, VCO_2, ventilation, respiratory rate, RER, work rate, and $PetCO_2$ but lower $PetO_2$ and VE/

Table 1 **Cardiopulmonary exercise test data at rest and at the first minute of exercise**

	All ($n = 476$)	DT no ($n = 425$)	DT yes ($n = 51$)	*P*-value
Age (years)	46 ± 14	45 ± 14	49 ± 15	0.098
Sex male (%)	278 (58%)	244 (57%)	31 (61%)	0.765
Height (cm)	172 (165; 179)	172 (165; 179)	172 (168; 177)	0.708
Weight (kg)	69.5 (59; 79)	69 (59; 79)	71 (60; 77)	0.980
BMI (kg/m^2)	23.2 (21.1; 25.4)	23.2 (21.1; 25.4)	23 (21.5; 24.8)	0.832
Peak work rate predicted (W)	161 (116; 209)	161 (116; 209)	164 (123; 209)	0.995
Peak work rate measured (W)	147 (112; 194)	147 (110; 194)	153 (124; 209)	0.358
Peak VO_2 (mL/min)	2012 (1543; 2612)	2013 (1539; 2600)	2001 (1665; 2631)	0.796
Peak VO_2 (%)	97 ± 20	97 ± 19	99 ± 23	0.408
Peak VCO_2 (mL/min)	2244 (1761; 2951)	2267 (1746; 2954)	2219 (1910; 2948)	0.998
Peak RER	1.12 (1.04; 1.20)	1.12 (1.05; 1.20)	1.11 (1.03; 1.21)	0.503
Peak VE (L/min)	65.9 (54.1; 82.3)	65.8 (53.9; 82.3)	66 (55.4; 82.6)	0.734
Peak RR (breath/min)	30 ± 7	30 ± 7	31 ± 6	0.296
Peak $PetO_2$ (mmHg)	118 (113; 122)	118 (113; 122)	117 (111; 120)	0.246
Peak $PetCO_2$ (mmHg)	42 (39; 46)	43 (39; 47)	42 (38; 44)	0.239
VE/VCO_2 slope	24.6 (22.3; 27.8)	24.7 (22.3; 27.8)	24.2 (22.1; 28.3)	0.871
VO_2/Work rate slope (mL/min/W)	11 (10.1; 12)	11 (10.1; 12)	10.9 (10.2; 11.8)	0.642

Data are presented as mean ± standard deviation and as median and interquartile range.
DT, double threshold; $PetCO_2$, end-tidal carbon dioxide tension; $PetO_2$, end-tidal oxygen tension; RER, respiratory exchange ratio; RR, respiratory rate; VCO_2, carbon dioxide output; VE, ventilation; VE/VCO_2 slope, slope of ventilation to carbon dioxide output; VO_2%, oxygen uptake as percentage of predicted value; VO_2, oxygen uptake.

Table 2 **Demographic and cardiopulmonary exercise test at peak exercise data of healthy individuals**

	All ($n = 476$)	DT no ($n = 425$)	DT yes ($n = 51$)	*P*-value
REST				
VO_2 (mL/min)	338 (282; 407)	337 (282; 407)	340 (283; 420)	0.668
VCO_2 (mL/min)	299 (238; 374)	299 (238; 373)	293 (239; 399)	0.912
VE (L/min)	14.3 (11.9; 17.5)	14.1 (11.9; 17.2)	14.9 (11.5; 19)	0.381
RR (breath/min)	15 (13; 18)	15 (13; 17)	17 (13; 18)	0.101
RER	0.89 ± 0.14	0.89 ± 0.13	0.89 ± 0.16	0.970
1 min of loaded exercise				
VO_2 (mL/min)	561 (463; 661)	563 (463; 661)	535 (456; 657)	0.599
VCO_2 (mL/min)	460 (375; 556)	463 (380; 557)	425 (334; 555)	0.211
VE (L/min)	17.8 (14.9; 20.9)	17.8 (15.2; 20.9)	17.8 (14; 20.5)	0.608
RR (breath/min)	17 ± 5	17 ± 5	18 ± 5	0.298
RER	0.81 (0.74; 0.91)	0.81 (0.74; 0.91)	0.79 (0.73; 0.86)	0.305

Data are presented as mean ± standard deviation and as median and interquartile range.
DT, double threshold; RER, respiratory exchange ratio; RR, respiratory rate; VCO_2, carbon dioxide output; VE, ventilation; VO_2, oxygen uptake.

VCO_2. VE/VO_2 was unchanged between met AT and vent AT (*Table 4* and *Figure 2*). Average ΔVO_2 between the two ATs was 186 (88; 317) mL/min and 2.8 (1.5; 5.2) mL/kg/min.

The respiratory compensation point was identified in 94% (449 out of 476) of healthy individuals (95% of subjects without a DT and 92% of subjects with a DT) (*Table 5*). Subjects with a DT showed a higher VO_2 increase during the isocapnic buffering period, 451 (287; 781) mL/min vs. 392 (238; 683) mL/min (*P*-value <0.04).

Discussion

The major finding of the present study is that a DT was identified in 11% of healthy subjects and, in these cases, met AT always preceded vent AT.

Our population is made up of middle-aged, healthy subjects with normal BMI and, on an average, an exercise performance within normal limits. Of note, athletes, elderly and obese subjects were excluded from the analysis, as were subjects who did not complete

Table 3 Cardiopulmonary exercise test data at the anaerobic threshold (AT) detected with the V-slope method (met AT) in healthy subjects

	DT no ($n = 425$)	DT yes ($n = 51$)	*P*-value
Met AT VO_2 (mL/min)	1279 (1025; 1604)	1197 (956; 1512)	0.193
Met AT VCO_2 (mL/min)	1124 (859; 1441)	1016.2 (813; 1244)	0.019
Met AT VO_2 (mL/kg/min)	19 (16; 23)	18 (14; 22)	0.086
Met AT VE/VO_2	26.1 (23.1; 30.0)	26.0 (23.1; 28.6)	0.695
Met AT VE/VCO_2	29.8 (26.3; 34.0)	31.7 (28.2; 34.5)	0.043
Met AT $PetCO_2$ (mmHg)	45 ± 5	43 ± 4	0.007
Met AT $PetO_2$ (mmHg)	109 (103; 114)	109 (100; 112)	0.062
Met AT VE (L/min)	33.5 (27.5; 40.7)	32.2 (25.8; 38.5)	0.135
Met AT RR (breath/min)	21 ± 5	20 ± 6	0.720
Met AT workload (watt)	89 (66; 114)	77 (62; 104)	0.220
Met AT RER	0.88 ± 0.09	0.83 ± 0.07	0.0001

Data are presented as mean ± standard deviation and as median and interquartile range.
DT, double threshold; $PeTCO_2$, end-tidal carbon dioxide tension; $PeTO_2$, end-tidal oxygen tension; RER, respiratory exchange ratio; RR, respiratory rate; VCO_2, carbon dioxide output; VE, ventilation; VE/VCO_2, ventilatory equivalent for carbon dioxide; VE/VO_2, ventilatory equivalent for oxygen; VO_2, oxygen uptake; W, watt.

Table 4 Cardiopulmonary exercise test data at the anaerobic threshold (AT) detected with the V-slope method (met AT) and with the ventilatory equivalent method (vent AT) in the subjects with double AT ($n = 51$)

	Met AT	Vent AT	*P*-value
VO_2 (mL/min)	1197 (956; 1512)	1440 (1145; 1816)	0.0001
VCO_2 (mL/min)	1016 (813; 1244)	1302 (1032; 1636)	0.0001
VO_2 (mL/min/kg)	18 (14; 22)	21 (16; 27)	0.0001
VE/VO_2	26.0 (23.1; 28.6)	25.6 (23.6; 30.1)	0.708
VE/VCO_2	31.7 (28.2; 34.5)	29.5 (25.9; 33.2)	0.0001
$PetCO_2$ (mmHg)	43 ± 4	44 ± 4	0.0001
$PetO_2$ (mmHg)	109 (100; 112)	108 (102; 113)	0.012
VE (L/min)	32.2 (25.8; 38.5)	38.4 (31.2; 45.2)	0.0001
RR (breath/min)	20 ± 6	22 ± 6	0.003
Workload (watt)	77 (62; 104)	101 (75; 130)	0.0001
RER	0.83 ± 0.07	0.90 ± 0.09	0.0001

Data are presented as mean ± standard deviation and as median and interquartile range.
DT, double threshold; $PetCO_2$, end-tidal carbon dioxide tension; $PetO_2$, end-tidal oxygen tension; RER, respiratory exchange ratio; RR, respiratory rate; VCO_2, carbon dioxide output; VE, ventilation; VE/VCO_2, ventilatory equivalent for carbon dioxide; VE/VO_2, ventilatory equivalent for oxygen; VO_2, oxygen uptake.

what they considered a maximal exercise test. In this setting, a DT was identified in 11% of cases, extending our anecdotal case report to a relevant minority of cases.[27] None of our demographic or exercise variables differentiated these subjects.

The AT identified by the V-slope method, which we named met AT, indicates the beginning of metabolic acidosis buffered by the bicarbonate system. From a physiological point of view, according to Koike *et al.*,[34] met AT separates the VO_2 increase independent from cardiac output increase (below AT) from that dependent from cardiac output increase (above AT). Anaerobic threshold determination by V-slope analysis is identified as the point where the slope of the relationship between VCO_2 and VO_2 starts to increase systematically. This increase is due to the buffering of the anaerobic metabolism that causes an extra production of CO_2 by the muscles and a consequent increase of ventilation to eliminate it. Consequently, this ventilation increase is out of proportion to VO_2 increase but in proportion to the VCO_2 rise, paralleled by a constant $PetCO_2$ and an increasing $PetO_2$. The isocapnic buffering period starts above AT and it ends at the RCP, which is characterized by the beginning of compensatory hyperventilation as represented by VE/VCO_2 ratio increase and $PetCO_2$ decrease. The VO_2 during the isocapnic buffering period is proportional to exercise performance, so that the longer the isocapnic buffering period, the better the exercise performance.[35]

In the present study, we describe, in a sizable minority of normal subjects (11%), a peculiar respiratory behaviour after met AT, i.e., a delay in onset of respiratory compensation previously described only anecdotally.[27] As a matter of fact, several authors identified before the AT defined by the V-slope method an increase of the VE/VO_2

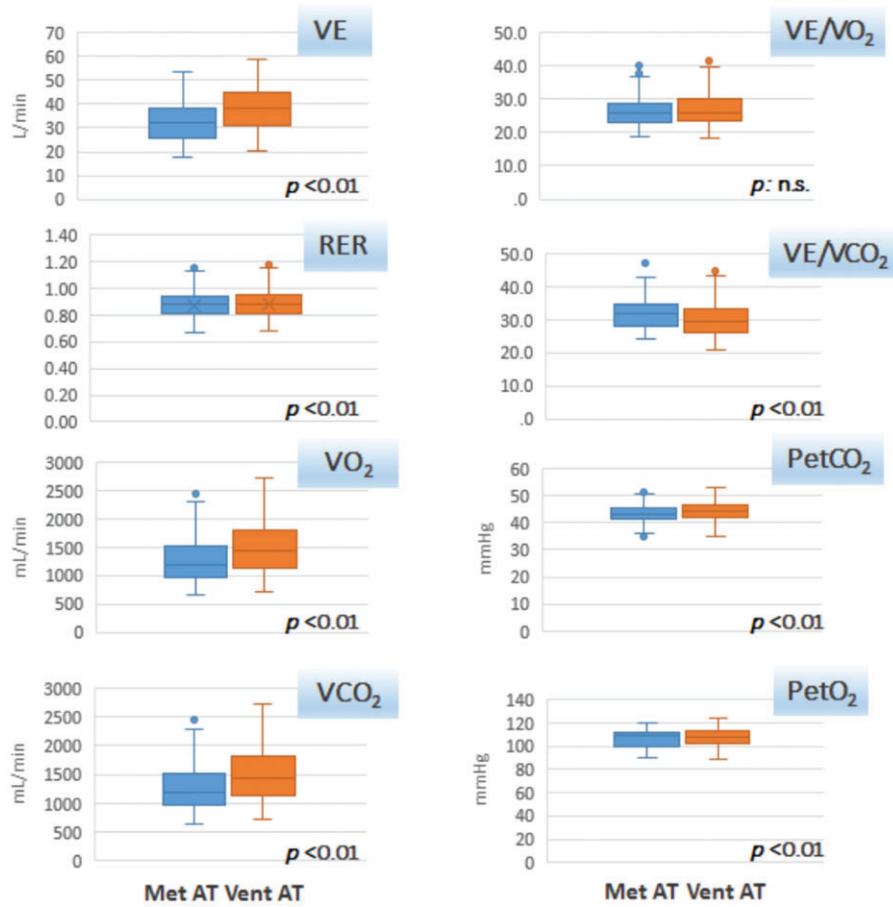

Figure 2 Representation of the changes of some cardiopulmonary test values between the anaerobic threshold calculated with the V-slope method (met AT) and the anaerobic threshold detected with the ventilatory equivalent method (vent AT) in healthy subjects with double threshold using box and whiskers plot. Outliers are plotted as individual points. $PetCO_2$, end-tidal carbon dioxide tension; $PetO_2$, end-tidal oxygen tension; RER, respiratory exchange ratio; VCO_2, carbon dioxide output; VE, ventilation; VE/VCO_2, ventilatory equivalent for carbon dioxide; VE/VO_2, ventilatory equivalent for oxygen; VO_2, oxygen uptake.

Table 5 **Cardiopulmonary exercise test data at the respiratory compensation point**

	Respiratory compensation point			
	All (*n* = 449)	**DT no (*n* = 402)**	**DT yes (*n* = 47)**	***P*-value (DT no vs. DT yes)**
VO_2 (mL/min)	1704 (1348; 2193)	1685 (1336; 2202)	1772 (1394; 2107)	0.777
VCO_2 (mL/min)	1836 ± 671	1837 ± 669	1830 ± 692	0.949
VE (L/min)	50.5 (42.1; 61.1)	50.4 (42.1; 60.9)	51.6 (43.7; 62.3)	0.676
Workload (W)	129 (98; 170)	129 (96; 170)	131 (44; 62)	0.741
RER	1.01 (0.94; 1.06)	1.01 (0.94; 1.06)	0.99 (0.93; 1.05)	0.437

DT, double threshold; RER, respiratory exchange ratio; VCO_2, carbon dioxide output; VE, ventilation; VO_2, oxygen uptake.
Data are presented as mean ± standard deviation and as median and interquartile range.

ratio, which was named optimal ventilatory efficiency point (POE).[1,17,36–38] Several reasons have been postulated for this anticipated ventilatory response observed in a few normal subjects during exercise, such as psychogenic stress, interindividual differences in alveolar gas tension for CO_2, differences in chemoreceptor sensitivity, dead space to tidal volume ratio changes, and differences

in the locomotor-respiratory coupling. Optimal ventilatory efficiency point has been recently identified in ~80% of normal cases by Puhringer *et al.*[36] Our findings are however unrelated to the presence/absence of POE.

The discrepancy between met AT and vent AT is relevant in terms of VO_2 [ΔVO_2 = 3 (2; 5) mL/kg/min and ΔVO_2 = 186 (88; 317) mL/min] and of ventilation [6.0 (3.3; 8.8) L/min]. Moreover, the increase of VO_2 from 1197 to 1440 mL/min, of VCO_2 from 1016 to 1302 mL/min and of RER from 0.84 to 0.91 is considerable, and all these data clearly speak in favour of an increased aerobic but also anaerobic metabolism. The period between met AT and vent AT is characterized by the lack of the ventilatory response normally induced by extra VCO_2 as shown by the constant VE/VO_2 and the reduced VE/VCO_2, paralleled by a significant reduction of $PetO_2$ and an increase of $PetCO_2$. The reason behind this unusual behaviour seems to be, in a minor part, associated with an anticipated anaerobic metabolism occurring on met AT in DT cases (lower VCO_2 compared to subjects without a DT) and, for a major part, to a delayed ventilation response to acidosis. In any case, the interval between the two ATs is likely due to either a delayed central chemoreceptor response to CO_2 changes or a delayed arrival of CO_2 changes at the central chemoreceptors site. The presence or absence of DT does not influence peak exercise performance, which is similar between DT and non-DT cases. The greater ΔVO_2 during the isocapnic buffering period found in subjects with DT suggests that they can tolerate more exercise before acidosis-induced hyperventilation, although it is unknown if this is due to a greater aerobic fitness level as suggested by Algul *et al.* and Carriere *et al.*, and/or if they are more keen to some physical activities, such as endurance efforts.[35,39–43] The present study was performed in healthy subjects but it is possible that DT is present also in patients with cardiovascular diseases such as heart failure or pulmonary hypertension. Indeed, in these patients alteration of the respiratory behaviour during exercise is frequently observed and they have a relevant prognostic impact.[44,45] At present, the presence, incidence, and prognostic meaning of DT in cardiovascular patients is unknown.

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. Indeed, this is a retrospective study and blood lactate was not routinely collected and, moreover, very frequent blood samples would be needed to be obtained for a precise definition of lactate threshold, which is not a standard procedure during CPET. Second, we used an average over 10 s to calculate the mean value of each variable. This is an admittedly short interval, but it allows the detection of small differences. Third, the minimum VO_2 difference to assess a discrepancy between ATs is 15 mL/min. This value is both small and totally arbitrary. However, the average ΔVO_2 between the two ATs is much larger: 186 (88; 317) mL/min. Fourth, albeit AT was measured by CPET recognized expert, intra-observed variability was not assessed. Fifth, differences between ventilator equivalent and end-tidal method were not observed, albeit not specifically assessed. Sixth, we do not know whether the ergometer used, cycle or treadmill, influence our findings, being used only the cycle ergometer. Finally, these data were collected in 'normal' healthy individuals, so that we do not know whether DT exists in athletes of different sport activities or in patients. Similarly, the effects of training and drugs on DT are unknown.

Interpretation

We showed the existence of DT in a sizable minority of healthy subjects, likely due to a delayed central chemoreceptor response. Double threshold does not influence peak exercise performance, but it is associated with a greater exercise capacity before the beginning of acidosis-induced compensatory hyperventilation.

Conflict of interest: Authors declare that they have no conflict of interest.
Funding: None.

References

1. Westhoff M, Ruhle 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 Med Wochenschr* 2013;**138**:275–280.
2. Forman DE, Myers J, Lavie CJ, Guazzi M, Celli B, Arena R. Cardiopulmonary exercise testing: relevant but underused. *Postgrad Med* 2010;**122**:68–86.
3. American Thoracic Society, American College of Chest Physicians. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2003; **167**:211–277.
4. Sietsema KE SD, Stringer WW, Ward SA (eds). *Wasserman & Whipp's Principles of exercise testing and interpretation*. 6th edn. Philadelphia: Wolters-Kluwer, 2020. p 70–74.
5. Binder RK, Wonisch M, Corra U, Cohen-Solal A, Vanhees L, Saner H, Schmid J-P. Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *Eur J Cardiovasc Prev Rehabil* 2008;**15**: 726–734.
6. Poole DC, Rossiter HB, Brooks GA, Gladden LB. The anaerobic threshold: 50+ years of controversy. *J Physiol* 2021;**599**:737–767.
7. Vanhees L, De Sutter J, GeladaS N, Doyle F, Prescott E, Cornelissen V, Kouidi E, Dugmore D, Vanuzzo D, Börjesson M, Doherty P; EACPR. Importance of characteristics and modalities of physical activity and exercise in defining the benefits to cardiovascular health within the general population: recommendations from the EACPR (Part I). *Eur J Prev Cardiol* 2012;**19**:670–686.
8. Pelliccia A, Sharma S, Gati S, Back M, Borjesson M, Caselli S, *et al.* 2020 ESC Guidelines on sports cardiology and exercise in patients with cardiovascular disease. *Eur Heart J* 2020;**42**:17–96.
9. Mezzani A, Hamm LF, Jones AM, McBride PE, Moholdt T, Stone JA, Urhausen A, Williams MA. Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: a joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitation and the Canadian Association of Cardiac Rehabilitation. *Eur J Prev Cardiol* 2013;**20**:442–467.
10. Tamburus NY, Kunz VC, Salviati MR, Castello Simoes V, Catai AM, Da, Silva E. Interval training based on ventilatory anaerobic threshold improves aerobic functional capacity and metabolic profile: a randomized controlled trial in coronary artery disease patients. *Eur J Phys Rehabil Med* 2016;**52**:1–11.
11. Zheng H, Luo M, Shen Y, Ma Y, Kang W. Effects of 6 months exercise training on ventricular remodelling and autonomic tone in patients with acute myocardial infarction and percutaneous coronary intervention. *J Rehabil Med* 2008;**40**: 776–779.
12. Maté-Muñoz JL, Domínguez R, Lougedo JH, Garnacho-Castaño MV. The lactate and ventilatory thresholds in resistance training. *Clin Physiol Funct Imaging* 2017; **37**:518–524.
13. Older P. Anaerobic threshold, is it a magic number to determine fitness for surgery? *Perioper Med (Lond)* 2013;**2**:2.
14. Older PO, Levett DZH. Cardiopulmonary exercise testing and surgery. *Ann Am Thorac Soc* 2017;**14**:S74–S83.
15. Gitt AK, Wasserman K, Kilkowski C, Kleemann T, Kilkowski A, Bangert M, Schneider S, Schwarz A, Senges J. Exercise anaerobic threshold and ventilatory efficiency identify heart failure patients for high risk of early death. *Circulation* 2002;**106**:3079–3084.

16. Carriere C, Corrà U, Piepoli M, Bonomi A, Merlo M, Barbieri S, Salvioni E, Binno S, Mapelli M, Righini F, Sciomer S, Vignati C, Moscucci F, Veglia F, Sinagra G, Agostoni P. Anaerobic threshold and respiratory compensation point identification during cardiopulmonary exercise tests in chronic heart failure. *Chest* 2019; **156**:338–347.
17. 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.
18. Paolillo S, Agostoni P. Prognostic role of cardiopulmonary exercise testing in clinical practice. *Ann Am Thorac Soc* 2017;**14**:S53–S8.
19. Palermo P, Corra U. Exercise prescriptions for training and rehabilitation in patients with heart and lung disease. *Ann Am Thorac Soc* 2017;**14**:S59–S66.
20. Agostoni P, Corrà U, Cattadori G, Veglia F, Battaia E, La Gioia R, Scardovi AB, Emdin M, Metra M, Sinagra G, Limongelli G, Raimondo R, Re F, Guazzi M, Belardinelli R, Parati G, Magrì D, Fiorentini C, Cicoira M, Salvioni E, Giovannardi M, Mezzani A, Scrutinio D, Di Lenarda A, Mantegazza V, Ricci R, Apostolo A, Iorio A, Paolillo S, Palermo P, Contini M, Vassanelli C, Passino C, Piepoli MF. Prognostic value of indeterminable anaerobic threshold in heart failure. *Circ Heart Fail* 2013;**6**:977–987.
21. Radtke T, Crook S, Kaltsakas G, Louvaris Z, Berton D, Urquhart DS, Kampouras A, Rabinovich RA, Verges S, Kontopidis D, Boyd J, Tonia T, Langer D, De Brandt J, Goërtz YMJ, Burtin C, Spruit MA, Braeken DCW, Dacha S, Franssen FME, Laveneziana P, Eber E, Troosters T, Neder JA, Puhan MA, Casaburi R, Vogiatzis I, Hebestreit H. ERS statement on standardisation of cardiopulmonary exercise testing in chronic lung diseases. *Eur Respir Rev* 2019;**28**:180101.
22. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol (1985)* 1986;**60**:2020–2027.
23. Hopker J, Coleman D, Passfield L, Wiles J. The effect of training volume and intensity on competitive cyclists' efficiency. *Appl Physiol Nutr Metab* 2010;**35**:17–22.
24. Myers J, Ashley E. Dangerous curves. A perspective on exercise, lactate, and the anaerobic threshold. *Chest* 1997;**111**:787–795.
25. Agostoni P, Dumitrescu D. How to perform and report a cardiopulmonary exercise test in patients with chronic heart failure. *International Journal of Cardiology* 2019;**288**:107–113.
26. Reinhard U, Muller PH, Schmulling RM. Determination of anaerobic threshold by the ventilation equivalent in normal individuals. *Respiration* 1979;**38**:36–42.
27. Whipp BJ, Agostoni P. Noninvasive estimation of the lactate threshold in a subject with dissociated ventilatory and pulmonary gas exchange indices: a case report. *Chest* 2007;**132**:1994–1997.
28. Vainshelboim B, Rao S, Chan K, Lima RM, Ashley EA, Myers J. A comparison of methods for determining the ventilatory threshold: implications for surgical risk stratification. *Can J Anaesth* 2017;**64**:634–642.
29. Salvioni E, Corra U, Piepoli M, Rovai S, Correale M, Paolillo S. Gender and age normalization and ventilation efficiency during exercise in heart failure with reduced ejection fraction. *ESC Heart Fail* 2020;**7**:371–380.
30. Agostoni P, Vignati C, Gentile P, Boiti C, Farina S, Salvioni E, Mapelli M, Magrì D, Paolillo S, Corrieri N, Sinagra G, Cattadori G. Reference values for peak exercise cardiac output in healthy individuals. *Chest* 2017;**151**:1329–1337.
31. Bussotti M, Agostoni P, Durigato A, Santoriello C, Farina S, Brusasco V, Pellegrino R. Do maximum flow-volume loops collected during maximum exercise test alter the main cardiopulmonary parameters? *Chest* 2009;**135**:425–433.
32. Agostoni P, Bianchi M, Moraschi A, Palermo P, Cattadori G, La Gioia R, Bussotti M, Wasserman K. Work-rate affects cardiopulmonary exercise test results in heart failure. *Eur J Heart Fail* 2005;**7**:498–504.
33. Hansen JE, Sue DY, Wasserman K. Predicted values for clinical exercise testing. *Am Rev Respir Dis* 1984;**129**:S49–55.
34. Koike A, Wasserman K, Beaver WL, Weiler-Ravell D, McKenzie DK, Zanconato S. Evidence supporting the existence of an exercise anaerobic threshold. *Adv Exp Med Biol* 1990;**277**:835–846.
35. Carriere C, Corrà U, Piepoli M, Bonomi A, Salvioni E, Binno S, Magini A, Sciomer S, Pezzuto B, Gentile P, Schina M, Sinagra G, Agostoni P. Isocapnic buffering period: from physiology to clinics. *Eur J Prev Cardiol* 2019;**26**:1107–1114.
36. Puhringer M, Ring-Dimitriou S, Stoggl 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.
37. Hollmann W. Historical remarks on the development of the aerobic-anaerobic threshold up to 1966. *Int J Sports Med* 1985;**6**:109–116.
38. Gaskill SE, Ruby BC, Walker AJ, Sanchez OA, Serfass RC, Leon AS. Validity and reliability of combining three methods to determine ventilatory threshold. *Med Sci Sports Exerc* 2001;**33**:1841–1848.
39. Algul S, Ozcelik O, Yilmaz B. Evaluation of relationship between aerobic fitness level and range of isocapnic buffering periods during incremental exercise test. *Cell Mol Biol (Noisy-le-Grand)* 2017;**63**:78–82.
40. Bentley DJ, Vleck VE, Millet GP. The isocapnic buffering phase and mechanical efficiency: relationship to cycle time trial performance of short and long duration. *Can J Appl Physiol* 2005;**30**:46–60.
41. Chicharro JL, Hoyos J, Lucia A. Effects of endurance training on the isocapnic buffering and hypocapnic hyperventilation phases in professional cyclists. *Br J Sports Med* 2000;**34**:450–455.
42. Oshima Y, Miyamoto T, Tanaka S, Wadazumi T, Kurihara N, Fujimoto S. Relationship between isocapnic buffering and maximal aerobic capacity in athletes. *Eur J Appl Physiol Occup Physiol* 1997;**76**:409–414.
43. Lucia A, Pardo J, Durantez A, Hoyos J, Chicharro JL. Physiological differences between professional and elite road cyclists. *Int J Sports Med* 1998;**19**:342–348.
44. Agostoni P, Emdin M, De Martino F, Apostolo A, Masè M, Contini M, Carriere C, Vignati C, Sinagra G. Roles of periodic breathing and isocapnic buffering period during exercise in heart failure. *Eur J Prev Cardiol* 2020;**27**:19–26.
45. Salvioni E, Bonomi A, Re F, Mapelli M, Mattavelli I, Vitale G, Sarullo FM, Palermo P, Veglia F, Agostoni P. The MECKI score initiative: development and state of the art. *Eur J Prev Cardiol* 2020;**27**:5–11.