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Link to publication

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Summary

The respiratory gas exchange and ventilation during an incremental cycle exercise test were analysed in a group of 19 healthy, moderately fit men. Different computer algorithms were used to estimate the $\dot{V}O_2$ values where: (i) the rate of $\dot{V}CO_2$ increase just exceeds the rate of $\dot{V}O_2$ increase (DX, derivative crossing), (ii) $\dot{V}CO_2/\dot{V}O_2 = 1{\cdot}00$ (PX, point of crossing) and (iii) ventilation ($\dot{V}E$) increases disproportionately in relation to $\dot{V}CO_2$ (PQ, point of $\dot{V}CO_2$ equivalent rise). The DX and PQ measurements were analysed using a new approach employing polynomial regression and the value of PX was determined following low-pass filtration of raw data. The repeatability of the measurements was evaluated with a 5–6 week interval between the tests. The correlations between tests were $0{\cdot}75$ at DX, $0{\cdot}85$ at PX and $0{\cdot}62$ at PQ. The mean differences between the repeated tests were not statistically significant. The repeatability of $\dot{V}O_2$, in absolute values expressed as ± 2 SD of the differences between the tests, had values of $5{\cdot}0$, $6{\cdot}1$ and $9{\cdot}5$ ml min^{-1} kg^{-1} for DX, PX and PQ, respectively. The mean value of $\dot{V}O_2$ for each measurement point expressed as a percentage of $\dot{V}O_{2max}$ was 54% at DX, 68% at PX and 70% at PQ. The most common sequence of the measured values was DX < PX < PQ, but the sequence DX < PQ < PX was also observed. It is concluded that the gas exchange responses to developing anaerobic metabolism during progressive exercise can be characterized by a series of thresholds. However, the considerable variation in absolute values in the two testing occasions requires further attention.

Introduction

It is of interest to estimate the development of anaerobic metabolism during an incremental exercise test, and a number of methods have been described for this purpose such as anaerobic thresholds, lactate thresholds and ventilatory thresholds (for review, see Bosquet *et al.*, 2002). The measures defined in such methods can be used to quantify the functional working capacity and working endurance of an individual, both in health and disease. Patients at high risk of early death from cardiac failure can be identified (Gitt *et al.*, 2002), as well as patients with obstructive pulmonary disease who may benefit from exercise (Sue *et al.*, 1988). Rehabilitation programmes including physical exercise can be planned based on the results of such measurements. The development of anaerobic metabolism during progressive exercise can be detected by increasing lactate

levels in the working muscle and in the circulating blood (see Tokmakidis, 1990). The transition can also be detected by using respiratory gas exchange analysis (for review, see Davis, 1985). The classical V-slope method (Beaver *et al.*, 1986) is based on the analysis of the CO_2 elimination ($\dot{V}CO_2$) in relation to O_2 uptake ($\dot{V}O_2$). By plotting $\dot{V}CO_2$ against $\dot{V}O_2$ the initial slope of $1{\cdot}0$ is followed by a steeper slope when lactic acid is buffered by bicarbonate and CO_2 is formed. The inflection point can be detected with the V-slope method. The point at which $\dot{V}CO_2 = \dot{V}O_2$ (the respiratory exchange ratio, RER = $1{\cdot}0$) can also be used to define the transition to significant anaerobic metabolism (Dickstein *et al.*, 1990a). Other methods employ ventilation ($\dot{V}E$) in relation to $\dot{V}O_2$, and the point at which $\dot{V}E/\dot{V}O_2$ and end-tidal oxygen pressure (PetO_2) increase while $\dot{V}E/\dot{V}CO_2$ and PetCO_2 remain constant (Wasserman *et al.*, 1973; Orr *et al.*, 1982). $\dot{V}E$ can also be related to $\dot{V}CO_2$ and a second

ventilatory threshold can be defined as the point at which $\dot{V}E/\dot{V}CO_2$ increases while $PetCO_2$ decreases (Beaver et al., 1986). This last point indicates hyperventilation or respiratory compensation for the metabolic acidosis that has developed during exercise. During a graded exercise test these different points do not describe the same threshold, but rather a transition between different physiological responses during an exercise test.

However, the measurements described above demand experienced observers since the analysis is often performed manually and when computerized analysis is used, the results must be carefully checked to avoid erroneous conclusions. To minimize the errors introduced by the observer it is of value to further develop the analysis techniques.

Studies of repeatability have mostly focused on the day-to-day variability and the results have been reported as correlation coefficients or as P-values (Simonton et al., 1988; Dickstein et al., 1990b) rather than to provide absolute values. Usually there are some weeks of exercise training between two physical tests. Thus, it is of special interest to study the repeatability with a prolonged period between the repeated tests.

Therefore, the aim of this study was to develop a refined computerized technique to obtain different indirect measures of the anaerobic metabolism during progressive exercise and to analyse the sequence of the measures. A further aim was to determine the repeatability of the measurements in a group of healthy men performing two exercise tests at a 5–6 week interval. Comparisons are made with earlier reported results regarding repeatability (Wergel-Kolmert et al., 2002) based on the same group of men.

Methods

Subjects

Nineteen healthy, non-smoking, male volunteers, aged 20–48 years, took part in this study (Table 1). The study group and test procedure were the same as described previously (for details see Wergel-Kolmert et al., 2002). These participants were recruited to participate in a medical study and later on randomly selected to a control group. The Ethics Committee of Lund University Hospital had approved the study. The subjects underwent a medical examination performed by a physician. The examination included medical history, a physical examina-

tion, laboratory evaluations (including blood chemistry, haematology, virology and urine analysis) and an electrocardiogram (ECG). Only non-smoking healthy individuals were included. In order to include non-exercising or moderately fit participants, the subjects first participated in a conventional symptom-limited ramp ergometer cycle 'inclusion pretest' with ECG and blood pressure (BP) measurements. The test followed the common clinical routine and started with a workload of 50 W and was gradually increased (5 W/20 s) until exhaustion. Subjects were included if 80–130% of the predicted maximal workload was reached without signs of any disease (Nordenfelt et al., 1985).

In accordance with clinical practice, prior the test, body weight was determined to the nearest kilogram on an ordinary physician's beam scale, with the participant dressed in light underwear and wearing no shoes. Height was measured to the nearest centimetre on a standardized wall-mounted height board and the body mass index (BMI) was calculated. Subject characteristics are given in Table 1.

Test procedure

Each subject underwent two graded exercise tests with continuous measurements of the respiratory gases. The two measurements were performed within the same hour of the day for each individual, 5–6 weeks apart. All participants were instructed not to take part in any sporting activities 48 h before the test, not to eat 3 h before the test and not to drink coffee 2 h before the test. The subjects were asked to maintain their ordinary food intake, but otherwise no specific diet instructions were given. They were also asked not to alter their general level of activity between the test sessions. Prior to the test it was ascertained whether the subject had followed the pretest instructions concerning physical activity, food and beverages. All measurements (tests 1 and 2) were supervised by the same investigators and were performed at the Department of Clinical Physiology at the Lund University Hospital, Lund, Sweden. The exercise test was performed on an ergometer cycle (Rodby 380, Siemens-Elema, Solna, Sweden). The height of the seat was adjusted individually (extended leg slightly bent at the knee) and the position of the handlebars was comfortably set. These settings were documented and applied on both test occasions. The participant was encouraged to maintain a pedalling rate of 60 r.p.m. during the test, with green and red lamps as aids. The test started with a workload of 50 W and was increased by 5 W/20 s until 40% of the individual maximum workload, determined in the inclusion pretest, had been attained. This level was maintained for 4 min, after which the intensity was gradually increased (5 W/20 s) until exhaustion. According to the clinical routine an ECG was continuously recorded (ECG Megachart, Siemens Elema, Solna, Sweden), BP and rating of the perceived exertion (RPE) on the 6–20 scale (Borg, 1982) were determined every 2 min.

Continuous respiratory gas analysis and volume measurements were performed breath-by-breath during the test. A facemask was

Table 1 Characteristics of the men (n = 19). BMI = body mass index (weight/height^2). The tests were performed with a 5–6 week interval.

Variable	Test 1		Test 2	
	Mean (SD)	Range	Mean (SD)	Range
Age (years)	27·4 (7·0)	20–48		
Height (cm)	179 (6)	167–189		
Weight (kg)	75·4 (7·4)	66–92	75·7 (7·5)	66–93
BMI	23·4 (2·2)	20–27	23·6 (2·2)	20–28

held in place by straps to ensure an airtight seal over the subject's nose and mouth. Ambient air inspired via the facemask and expired air passed over the flow/gas sensor. The facemask, was connected to a Triple-V® (venture vane volume) flow transducer and a Twin Tube® (which is a semipermiable sample tube) to a gas analyser (Oxycon Champion®, all products are manufactured by Jaeger, Höchberg, Germany). The vane flow transducer consisting of a low-mass helical impeller inserted in an electronic segment consisting of pairs of light-emitting diodes registered the revolution rate due to the inspiration and the expiration flow. Gas samples for O_2 and CO_2 concentration measurements were continuously taken directly at the Triple-V unit and transported in the Twin Tube, via a water trap and a dryer (which eliminated the water vapour), to a paramagnetic O_2 analyser and an infrared CO_2 analyser (sample rate 200 Hz). Time based (every 10 s) mean values of $\dot{V}O_2$, $\dot{V}CO_2$, HR and $\dot{V}E$ were obtained from the Oxycon gas analyser and registered as raw data.

Sitting on the cycle, gas collection started with a 2-min resting phase to allow the participant to become accustomed to breathing through the mask. These data were not included in the following analysis, but were collected in order to ensure that the start level of the exercise test was resting values (without hyperventilation). After the test, the participant was asked to remain sitting on the cycle for 3 min, with the facemask still in place.

Medical technicians supervised the calibration of the equipment, which was performed every day according to the instruction manual. Volume calibration was carried out using a manual 2 l syringe pump. The calibration was carried out under ATP conditions (current temperature, humidity and barometric pressure). Calibration of the gas analysers using the O_2 concentration in air and a reference gas (at a concentration of 5% CO_2 95% N_2, Air Liquide, Kungsängen, Sweden) and the transit time correction of gas from the mouthpiece through the tubes to the gas sensors were also made. The changes in volumes during the exercise test were automatically converted to BTPS (body temperature, current barometric pressure and water vapour saturated). Before each test a zero adjustment of the gas analysers were performed automatically.

Determination of DX

The essence of the classical V-slope method is to determine the point at which the rate of increase in $\dot{V}CO_2$ exceeds $\dot{V}O_2$. This point is determined by plotting $\dot{V}CO_2$ against $\dot{V}O_2$ and finding the point where the slope of the relation is just above 1·0. This can be done visually from graphs or mathematically by analysing the regression of $\dot{V}CO_2$ against $\dot{V}O_2$ (regression analysis of a biphasic response) (Beaver et al., 1986; Dickstein et al., 1990b) (Fig. 1b) in order to find a disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$. In this study, we also attempted to identify this point, during the progressive exercise test described, using a computerized method. To this end, polynomials were fitted to the relations of $\dot{V}CO_2$ versus time and $\dot{V}O_2$ versus time, since polynomials are easily handled and are

flexible functions often used to characterize a varying input signal. We have tested different polynomials and found that sixth degree polynomials followed the raw data well (Fig. 1a upper curves). The first derivative of the polynomials was determined in a region of interest (lower curves in Fig. 1a), excluding the early and late stages of the test. The last point at which $d\dot{V}CO_2/dt$ was just below $d\dot{V}O_2/dt$ was identified and this crossing point was designated DX (derivative crossing). By this technique the point where the increase in $\dot{V}CO_2$ just exceed the increase in $\dot{V}O_2$ could be determined.

Determination of PX

The point at which RER $= \dot{V}CO_2/\dot{V}O_2 = 1\cdot0$ has also been taken as a measure of the anaerobic threshold (Dickstein et al., 1990a). In this study, the very last point at which $\dot{V}CO_2$ was just below $\dot{V}O_2$ was taken as a measure of the crossing point PX (Fig. 1a, upper curves). The analysis of PX was performed using low-pass-filtered values (see section of analysis and statistics) of $\dot{V}CO_2$ and $\dot{V}O_2$.

Determination of PQ and PQo

The point of respiratory compensation has been defined as when $\dot{V}E$ increases curvilinearly in relation to $\dot{V}CO_2$. This point can be found by analysing the graph of $\dot{V}E$ versus $\dot{V}CO_2$ (see Fig. 1d) by means of linear regression (Beaver et al., 1986; Dickstein et al., 1990b). The point can also be found by analysing $\dot{V}E/\dot{V}CO_2 = EQCO_2$ versus time. In this study, a polynomial of sixth degree was fitted to the $EQCO_2$ data (Fig. 1c, upper curves) and $d(EQCO_2)/dt$ was calculated (Fig. 1c, lower curves). The point PQ was taken as the last point at which $d(EQCO_2)/dt$ was below zero, i.e. just before hyperventilation occurred in relation to $\dot{V}CO_2$.

The point at which $\dot{V}E$ increases non-linearly in relation to $\dot{V}O_2$ can be analysed by linear regression (Orr et al., 1982). This point (PQo in Fig. 1c) was identified from the $d(EQO_2)/dt$ curve using the same principle as for PQ.

Correction of the determinations

In some cases, a manual correction was necessary after visual inspection of the graphical presentations. Changes in the offset value from zero towards slightly greater values made DX, PX and PQ move in small steps to the right on the screen (in order to find the points described above). Different offset values were tested to obtain the smallest correction that provided satisfactory adjustment of the measurements, as judged by the two authors jointly. The corrections were made without knowledge about the actual values.

Analysis and statistics

Data were collected breath by breath by the gas analyser (Oxycon Champion®) and time-based averages (10 s) (whole breath
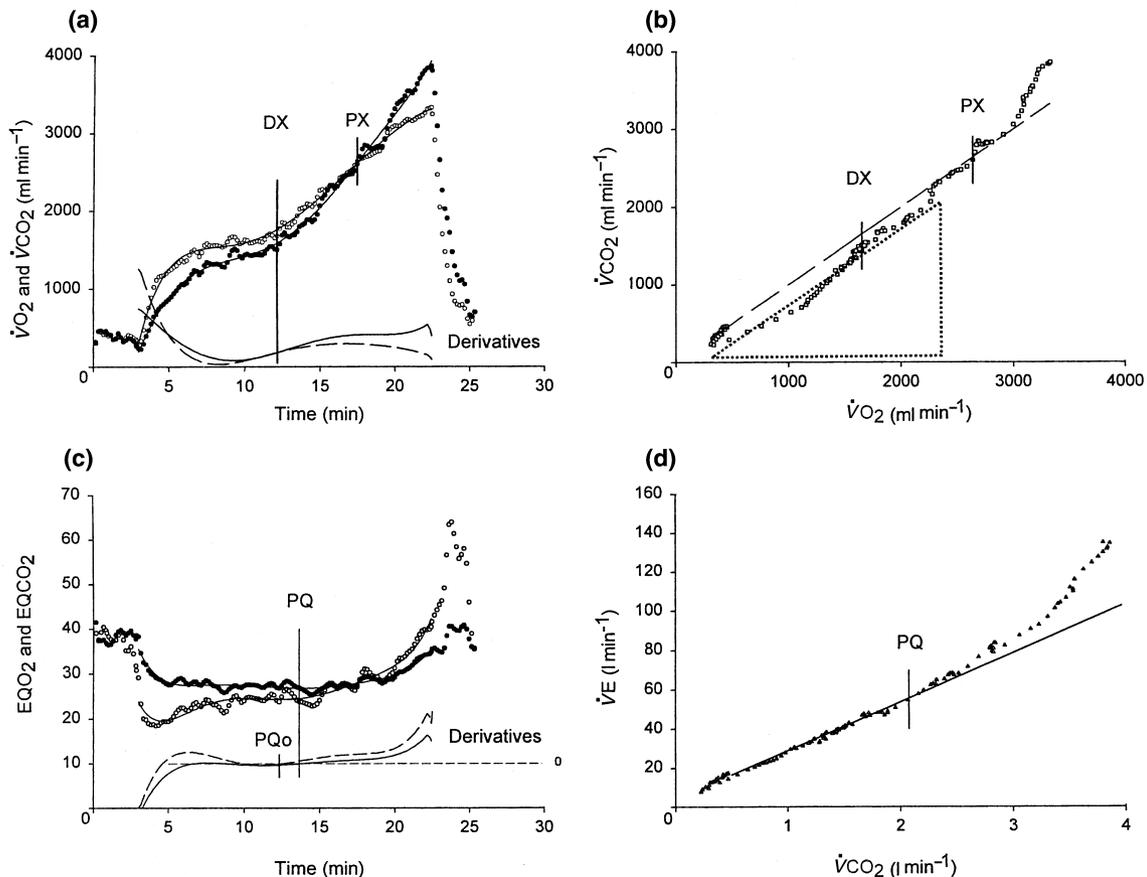
Figure 1 The method of determining anaerobic measurements during a cycle exercise test is illustrated in (a) and (c). Comparisons with more traditional methods are shown in (b) and (d). (a) shows the oxygen uptake ($\dot{V}O_2$, open circles) and carbon dioxide elimination ($\dot{V}CO_2$, dots) during a resting period followed by the ramp exercise, which included a 4-min steady-state period and a recovery period ($n = 1$). Polynomials (solid lines) were fitted to the original $\dot{V}O_2$ and $\dot{V}CO_2$ data. PX was determined as the last crossing point of $\dot{V}CO_2$ and $\dot{V}O_2$. Derivatives (lower curves) for $\dot{V}O_2$ (dashed line) and $\dot{V}CO_2$ (solid line) were calculated using the polynomials and rescaled to fit in the graph. DX was determined as the crossing point of the derivatives of $\dot{V}CO_2$ and $\dot{V}O_2$. (b) shows $\dot{V}CO_2$ versus $\dot{V}O_2$ during the exercise test ($n = 1$). The point at which the value of $\dot{V}CO_2$ increased more rapidly than the value of $\dot{V}O_2$ (the ordinary V-slope method) corresponds to DX. The point at which the value of $\dot{V}CO_2$ versus $\dot{V}O_2$ passes the line of identity (or RER = 1) corresponds to PX. (c) shows the ventilation $\dot{V}E/\dot{V}O_2$ (EQO$_2$, open circles) and $\dot{V}E/\dot{V}CO_2$ (EQCO$_2$, dots) during the same exercise test as in (a) ($n = 1$). Polynomials (solid lines) were fitted to the recorded data. Derivatives (lower curves) of EQO$_2$ (dashed line) and EQCO$_2$ (solid line) were calculated from the polynomials. PQo and PQ were determined as the points at which the corresponding derivatives exceeded zero. (d) shows $\dot{V}E$ versus $\dot{V}CO_2$ during the exercise test ($n = 1$). The point at which $\dot{V}CO_2$ increased disproportionately in relation to $\dot{V}CO_2$ (the ventilatory threshold) corresponds to PQ.

average) were obtained as raw data, which were further analysed on a personal computer. Signal processing, analysis of data, and generation of preliminary graphs were performed in Matlab® (The Math Works Inc., Natick, MA, USA). Heart rate (HR) was calculated, from the ECG, by the Oxycon Champion. The signals were low-pass-filtered using a filter algorithm provided in the Matlab program. The signal was processed by a linear phase, finite impulse response filter (15th order) having its 3 dB cut-off frequency at 0·01 Hz; the filter was designed by the window method using a Hamming window. In order to improve the attenuation of higher frequencies, the signal was filtered in both forward and reverse direction. Filtered values were generally used in the presentation in order to avoid the most extreme occasional fluctuations in the original signals. Filtered values were also used to determine the PX. In addition, the time-based original signals were also smoothened using the polynomials in order to find crossing

points DX, PQ and PQo, as described. The means, SDs and ranges of selected variables were calculated with Microsoft Excel®. Statistical comparisons between mean values (relating to DX, PX and PQ) were performed with Kruskal–Wallis ANOVA and pairwise comparisons by Tukey tests using Sigma stat® (SPSS inc. Chicago, IL, USA). The repeatability of the measurements obtained from each of the two exercise sessions was compared by plotting the differences between the individual measurements against their mean (Bland & Altman, 1986). The mean of the differences between the repeated measurements was calculated, and the hypothesis of zero bias was tested using Student's t-test. A significance level of P = 0·05 was chosen. The repeatability was quantified as the SD between the tests or as the coefficient of repeatability (COR) = 2 SD of the difference between two measurements according to the British Standards Institution (1979). Graphs were created in SigmaPlot® (SPSS inc. Chicago, IL, USA).

Results

$\dot{V}O_2$ and $\dot{V}CO_2$ followed in general the load protocol (Fig. 1a). After a resting period the cycling load gradually increased towards a sub-maximal plateau level, and thereafter towards a maximum. After exercise $\dot{V}O_2$ and $\dot{V}CO_2$ declined. Polynomials (solid curves in Fig. 1a) were fitted to the original data (see Methods) and their first derivatives were calculated.

The measure PX was taken from the filtered data as the last point at which $\dot{V}CO_2$ crossed $\dot{V}O_2$ and, in an analogous way, DX as the point at which the first derivative of $\dot{V}CO_2$ crossed the first derivative of $\dot{V}O_2$. In Fig. 1b, $\dot{V}CO_2$ is plotted against $\dot{V}O_2$ in the conventional manner to determine the 'anaerobic threshold' and our measures DX and PX are also indicated. As can be seen, DX refers to the point at which the slope passes 1·0 and PX the point at which the data cross the line of identity. PQ (Fig. 1c) was defined as the point at which respiratory compensation occurred and was determined as the point at which the first derivative of $EqCO_2$ was just above zero. Fig. 1d shows that PQ corresponds to the point at which ventilation increased disproportionately in relation to $\dot{V}CO_2$. PQo, hyperventilation in relation to $\dot{V}O_2$, (Fig. 1c) was determined using the same principle.

The mean $\dot{V}O_2$ at PQo (1691 ml min^{-1}) was similar to mean $\dot{V}O_2$ at DX (1591 ml min^{-1}) and therefore not further analysed in this study. Mean $\dot{V}O_2$ at DX, PX and PQ are given in Table 2. DX was significantly different from PX and PQ ($P<0·05$), but PX was not different from PQ.

The graphs were manually checked and in most of the tests the computerized determinations gave correct values. However, in 13 of 38 tests DX had to be adjusted by a manual correction procedure based on inspection of the graphs, as described. Corrections were made in 9 of 38 tests for PX and in 15 of 38 tests for PQ and PQo. Corrections were made by introducing the smallest possible offset value that gave an acceptable graph of the type shown in Fig. 1a,c.
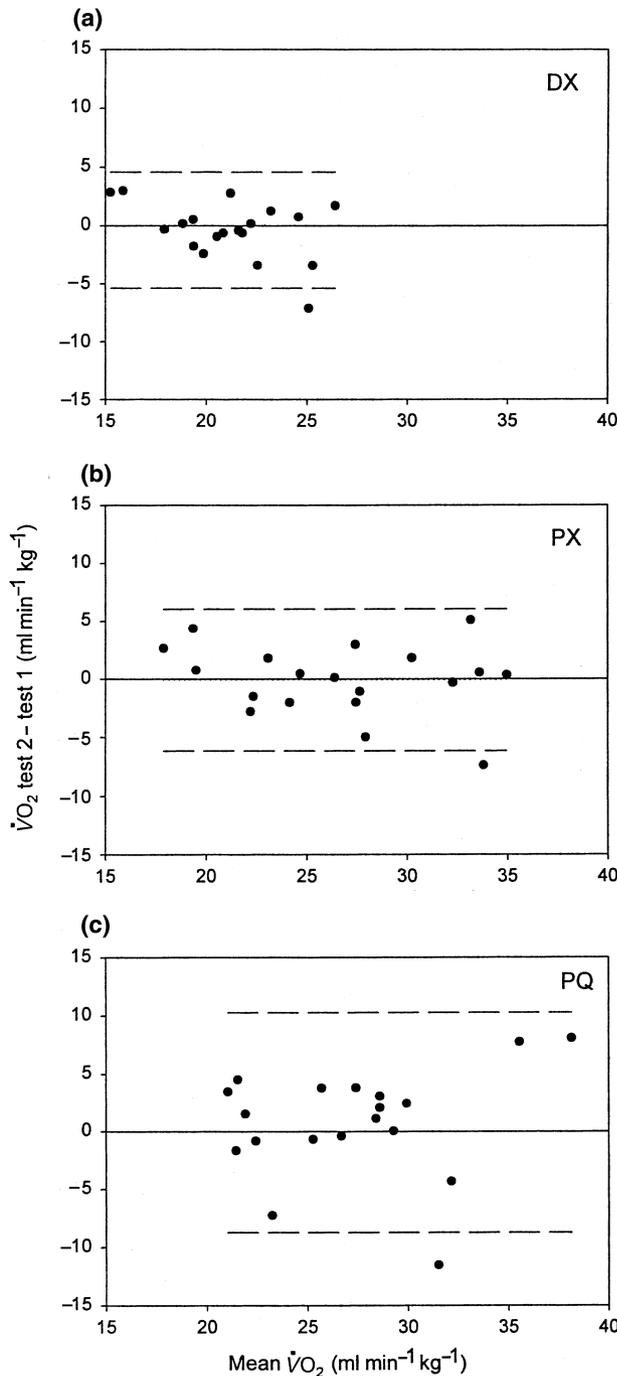
Repeatability of the measures

Each person performed a second exercise test within 5–6 weeks of the first test. The overall means of test retest at $\dot{V}O_2$, HR and load at DX, PX and PQ and maximum exercise test did not differ between the tests. The correlation coefficient for $\dot{V}O_2$ at DX, PX and PQ between the tests was between 0·65 and 0·86, which is somewhat less than for $\dot{V}O_{2max}$ (R = 0·92) (Table 2). To analyse the repeatability of the various measures, each individual difference between the tests were calculated. The repeatability was then quantified as the SD between the tests. Values are given in Table 2. The SD of $\dot{V}O_2$ at DX, PX and PQ was 194, 237 and 348 ml min^{-1}, respectively, and these values correspond to 12, 12 and 17% of the corresponding mean values. Thus the greatest variation was seen at PQ, while there were somewhat smaller variations at DX and PX. Greater variations at PQ than at DX and PX were also observed for HR and load (Table 2). A graphical presentation of the repeatability is given in Fig. 2 where the differences in $\dot{V}O_2$ (at DX, PX and PQ) are plotted against the mean values of the tests for each individual. Pairs of horizontal lines in the figure show the upper and lower limits of repeatability, defined as ±2 SD. Expressed in units of ml min^{-1} kg^{-1} the values of the COR for $\dot{V}O_2$ were 5·0, 6·1 and 9·5 at DX, PX and PQ, respectively.

The variation in $\dot{V}O_2$ and $\dot{V}CO_2$ between the tests could be due to technical and/or physiological factors. In an attempt to focus on the physiological variation RER was plotted against $\dot{V}O_2$ as loops representing the two tests. Since RER = $\dot{V}CO_2/\dot{V}O_2$, this variable is not as sensitive to differences in flow calibration or in breathing pattern as the variables $\dot{V}CO_2$ and $\dot{V}O_2$. Examples

Table 2 Oxygen uptake ($\dot{V}O_2$), heart rate (HR) and load at DX, PX, PQ and at the maximum exertion.

	DX	PX	PQ	Maximum
$\dot{V}O_2$ (ml min^{-1})				
Test 1/test 2	1603/1578	2011/2012	2028/2089	2910/2963
Mean (SD)	1591 (233)	2011 (399)	2058 (380)	2936 (410)
SD between the tests	194	237	348	166
Correlation coefficient	0·77	0·86	0·65	0·92
$\dot{V}O_2$ (ml min^{-1} kg^{-1})				
Mean (SD)	21·1 (3·0)	26·7 (5·3)	27·3 (4·9)	39·1 (5·7)
SD between the tests	2·5	3·0	4·9	2·2
$\dot{V}O_2$ (% VO$_{2\,max}$)	54	68	70	100
HR (bpm)				
Test 1/test 2	132/130	147/147	151/153	185/186
Mean (SD)	131 (12)	147 (13)	152 (17)	186 (15)
SD between the tests	11	12	15	5
Load (W)				
Test 1/test 2	124/119	162/160	167/171	249/254
Mean (SD)	122 (22)	161 (32)	169 (32)	251 (36)
SD between the tests	18	20	29	12

Mean values of tests 1 and 2 and mean values (SD) for the two tests are given, as well as the correlation between the two tests. SD of the individual differences between the tests is presented (n = 19).

Figure 2 The repeatability of $\dot{V}O_2$ (ml min^{-1} kg^{-1}) at DX, PX and PQ is shown in (a), (b) and (c), respectively ($n = 19$). The difference in $\dot{V}O_2$ between the two tests is plotted against the mean value of $\dot{V}O_2$ for each subject (dots). The means of the differences at DX, PX and PQ were not significantly different from zero. The upper and lower dashed lines show ± 2 SD in each panel.

of recordings are given in Fig. 3 where (a) and (b) clearly show different loops for the two tests, indicating a significant physiological variation. Fig. 3c,d show similar initial loops during the exercise test (where DX, PX and PQ were measured)

and therefore small physiological variations. However, the final parts of the loops (recovery from exercise) clearly differ.

The sequence of DX, PX and PQ

The sequence of DX, PX and PQ has a potential value in characterizing the anaerobic metabolism within an individual and was therefore analysed. The mean values of $\dot{V}O_2$ of the two test measurements expressed in per cent of $\dot{V}O_{2max}$ at DX, PX and PQ were 54, 68 and 70% (Table 2). The sequence DX < PX < PQ was observed in 12 of 19 individuals in the study group and the alternative sequence DX < PQ < PX in 7 of 19 individuals. Thus, in all the tests PX and PQ were greater than DX, whereas the individual order of PX and PQ could vary. Most individuals showed the same sequence at both tests but in 6 of 19 the order of PX and PQ changed mainly due to a greater variability in PQ. Figure 4 shows that DX, PX and PQ were positively correlated to $\dot{V}O_{2max}$. The correlation coefficients were 0·94, 0·89 and 0·79 for DX, PX and PQ, respectively. The slope of PX versus $\dot{V}O_{2max}$ was significantly greater than the slope of DX versus $\dot{V}O_{2max}$ ($P<0·05$). This means that the difference between DX and PX became wider for increasing values of $\dot{V}O_{2max}$. It is interesting to note that PX tended to be smaller than PQ for subjects exhibiting lower values of $\dot{V}O_{2max}$ and greater for those with higher values of $\dot{V}O_{2max}$. However, this difference was not statistically different in this group of healthy men.

Discussion

The aim of this study was to develop computerized methods to analyse and quantify the gradual increase in the supplementary anaerobic metabolism during an incremental exercise test, measured by gas analysis. To this end, different measures such as DX, PX and PQ were defined, and the sequence of these variables, as well as the repeatability, were analysed in a group of healthy men.

Physiological rational and determinations of DX, PX and PQ

The increase in $\dot{V}O_2$ and $\dot{V}CO_2$ during the incremental test reflects the enhanced muscle metabolism, although somewhat delayed. There is an additional increase in $\dot{V}CO_2$ due to (1) a gradual relative shift from lipid towards carbohydrate metabolism, (2) excess CO_2 production from bicarbonate as the H^+ from lactic acid is buffered, and (3) excess CO_2 elimination from the blood to counteract metabolic acidosis. The ventilation follows $\dot{V}CO_2$ during process (1) and (2), but is increased in relation to $\dot{V}CO_2$ during process (3). The gradual increase in anaerobic metabolism is a continuous process that includes several physiological mechanisms and may not be well described by one 'anaerobic threshold'. Different indices, or thresholds, during the transition can thus together be used to characterize the metabolism in a given
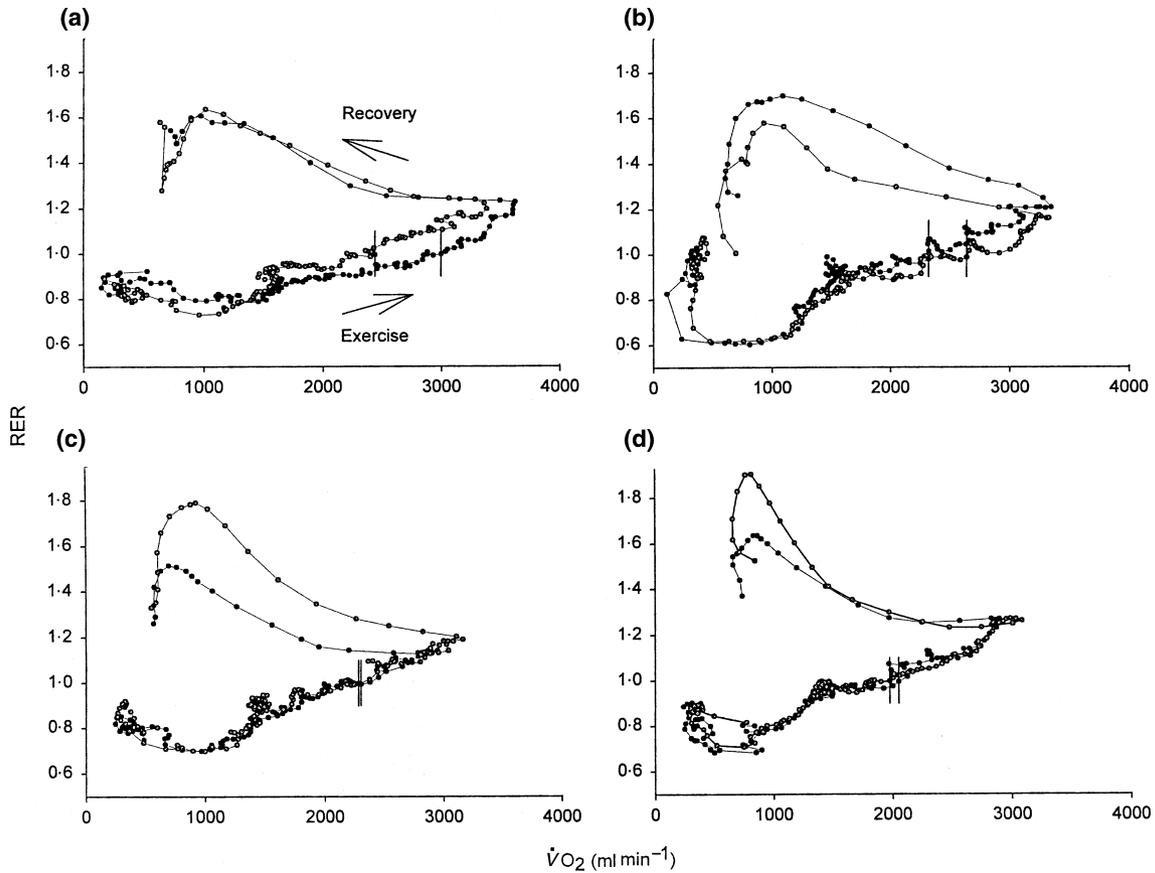
Figure 3 Similarities and dissimilarities in RER loops between the two tests. Examples from four subjects are shown. Recordings of RER ($\dot{V}CO_2/\dot{V}O_2$) versus $\dot{V}O_2$ (ml min^{-1}), during rest, exercise and recovery for an individual during the two tests are shown in each panel. Arrows (a) show the direction of the loops. It can be seen that physiological variation occurs in the production of CO_2 in relation to $\dot{V}O_2$ in panel (a) and (b), whereas the variation is less in panel (c) and (d).
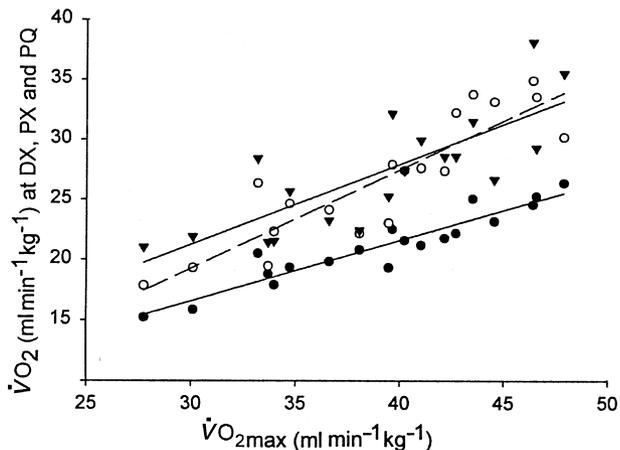
Figure 4 DX, PX and PQ in relation to $\dot{V}O_{2max}$. Mean $\dot{V}O_2$ at DX (dots), PX (open circles) and PQ (triangles) versus mean $\dot{V}O_{2max}$ for all subjects (n = 19). Trend lines for DX (solid lower line), PX (dashed line) and PQ (solid upper line) are shown. It can be seen that the distance between the trend lines increases at higher values of $\dot{V}O_{2max}$. The trend line of PX shows a steeper slope and passes the PQ trend line at a higher value of $\dot{V}O_{2max}$. Thus, both the sequences DX < PX < PQ and DX < PQ < PX can be seen.

individual. The measure DX defines the point at which the *increase* in $\dot{V}CO_2$ becomes greater than the *increase* in $\dot{V}O_2$ while PX is the point where $\dot{V}CO_2 = \dot{V}O_2$ (RER = 1·0). PQ indicates the onset of hyperventilation in relation to $\dot{V}CO_2$. The point PQo, i.e. hyperventilation in relation to $\dot{V}O_2$, is conceptually similar to DX. This point probably reflects the onset of lactate accumulation (Beaver *et al.*, 1986).

Polynomials were fitted to the original raw data. We tested different polynomials and found that sixth degree polynomials had to be used for $\dot{V}O_2$, $\dot{V}CO_2$, EQO_2 and $EQCO_2$ to adequately describe the original data. (The polynomials in itself do not describe the physiological process but is used as a flexible ruler in order to find the crossing points described above.) The polynomials were differentiated to obtain the *rates* of rise. Differentiation of the polynomials provides a less noisy signal than differentiation of original or filtered values and therefore more reliable values can be obtained. DX was defined as the crossing of the derivatives of $\dot{V}CO_2$ and $\dot{V}O_2$. In a similar way, PX was defined as the last crossing of $\dot{V}CO_2$ and $\dot{V}O_2$. PQ was calculated from the derivative of $EQCO_2$ and taken as the point at which the derivative became greater than zero. The measures

DX, PX and PQ correspond to more traditional thresholds described by others (Beaver et al., 1986; Dickstein et al., 1990a).

We found it important to visually inspect the original data and fitted curves before accepting the results, as has been recommended by others (Cooper & Storer, 2001). In some cases, we had to introduce offset values in the graphical analysis to obtain reasonable values of DX, PX and PQ. However, better algorithms may still be developed to obtain more stable results.

The exercise protocol

The exercise protocol, used in the pretest, with the inclination rate described is commonly used in Swedish clinical laboratories. In this study, we included a period with a fixed load into this protocol, with the purpose to reach steady state of the recorded variables. This protocol has advantages since not only maximal variables can be measured. During the fixed load various extra measurements like blood samples can be made. $\dot{V}O_2$ at the sub-maximal fixed load at test and re-test will be discussed below. In addition, the presence of a fixed load, provides the possibility to calculate other variables that describe the physical fitness such as time constant for $\dot{V}O_2$ ($\dot{V}CO_2$) to reach steady-state and the amount of $\dot{V}O_2$-cost per W external work (manuscript under preparation). Usually these two measurements are obtained by using several steady-state exercise tests, which certainly is more time consuming. However, it is not necessary to have a fixed load included in the ramp test in order to determine DX, PX and PQ, but this study shows the feasibility to perform these measurements. As pointed out by Tokmakidis (1990) there are indeed many different protocols and techniques in use for characterizing the anaerobic metabolism during exercise testing. The special protocol used in this study should be taken into consideration when comparisons with other studies are made. Since, exactly the same exercise protocol was used in test and re-test it is possible to examine the repeatability of the different variables.

The repeatability of DX, PX and PQ

We have previously reported on other aspects of this study in which the subjects and the exercise protocol were the same. The earlier reported variations (2 SD) of $\dot{V}O_{2max}$ and sub-maximal $\dot{V}O_2$ were 332 and 209 ml min^{-1}, respectively (Wergel-Kolmert et al., 2002). This latter value should be compared with the value for the variation of DX (2 SD) in this study (389 ml min^{-1}), which is almost twice as large. The absolute variation of DX (corresponding to the V-slope method) has, to our knowledge, not been reported before. The variations in absolute values of PX (2 SD, 474 ml min^{-1}) and PQ (696 ml min^{-1}) were even greater. However, these large absolute variations in DX, PX and PQ are to some degree, understandable since the measurements are based on two variables ($\dot{V}O_2$ and $\dot{V}CO_2$) and both these variables fluctuate during the exercise test. Moreover, DX, PX and PQ are measurements obtained under dynamic conditions, whereas

$\dot{V}O_2$ at sub-maximal load refers to a steady state (i.e. constant load). In addition, some subjects showed significant physiological variation between the tests. This is clear from studying the loops of RER versus $\dot{V}O_2$ (Fig. 3), which in some cases, differed considerably. However, there was no systematic difference between the two tests. The average values of $\dot{V}O_2$ between the tests were similar (Table 2) and the degree of correlation between the tests was high for DX, PX and PQ. Others have reported good repeatability merely based on a good correlation between two tests or a similar average in two tests (Simonton et al., 1988; Cohen-Solal et al., 1991; Weston & Gabbett, 2001). Tokmakidis (1990) made an extensive review of test–retest repeatability and it is striking that the correlation coefficients are used to describe the variability. Then again, it is of clinical importance to gain knowledge about the actual variations in absolute values. During 5–6 weeks between the tests certainly a lot of factors can influence the effect analysed, such as diseases, increased or decreased physical activity and weight. As described we have given rigid pretest restrictions and prior the test it was ensured that the recommendations had been followed. We also made great effort to eliminate differences in the test procedure and the test conditions through careful instructions, the assistance of experienced staff and by checking environmental measurements. Caution was also taken to avoid hyperventilation prior to the test since this might affect the results (Ozcelik et al., 1999). As discussed in our previous study (Wergel-Kolmert et al., 2002) technical differences in volume calibration between tests were small and would correspond to <40 ml min^{-1} in $\dot{V}O_2$ and $\dot{V}CO_2$. Another factor that is not usually taken into consideration is the that the variations of carbohydrate and fat in the meal just prior the test may alter the results, such as the arterial lactate concentrations during exercise has been shown to be smaller after a fat-rich diet giving a lower RER than after a carbohydrate rich diet (Jansson, 1980). We did not control the content of carbohydrates and fat in the meal prior the test and it is one of the speculative reasons for the physiological changes that seem to appear between some of the tests (Fig. 3).

The variations between the tests in heart rate at DX (22 bpm) and PX (25 bpm) were similar to the variation at a sub-maximal load (20 bpm), as described earlier (Wergel-Kolmert et al., 2002). The variations in the load at DX and PX were similar (about 40 W) but greater at PQ (about 60 W).

In exercise prescription the level of intensity is often determined from the HR, $\dot{V}O_2$ and load at the threshold and monitored with HR (Boulay et al., 1997). It is recommended that aerobic endurance training be performed at intensities lower than the threshold, i.e. the functional working capacity. It is, therefore, also of interest to have knowledge of the test–retest variations of these measurements in healthy, moderately fit men (see Table 2).

Sequence of DX, PX and PQ

It seems likely that different sequences of DX, PX and PQ reflect different strategies for an individual to cope with the evolution

of supplementary anaerobic metabolism, such as onset of lactate accumulation, isocapnic buffering and respiratory compensation. The mean value of the $\dot{V}O_2$ measurements occurred at 54, 68 and 70% for DX, PX and PQ, respectively. The sequence DX < PX < PQ was more frequently observed than the sequence DX < PQ < PX in this group of healthy men. We have also observed that the sequence DX < PQ < PX characterized a group of endurance-trained runners (n = 6) in comparison with a group of weight-lifters (n = 5) who showed the sequence DX < PX < PQ, although the two groups did not differ in their values of $\dot{V}O_{2max}$ (unpublished observations). This is interesting to note together with our findings that the trend-line of PX crossed the trend-line of PQ at the higher $\dot{V}O_{2max}$ (Fig. 4). The tendency to increasing distance between DX and the endpoint (PX or PQ) at higher $\dot{V}O_{2max}$ might indicate a greater buffering capacity. It remains to be investigated whether different patterns characterize subgroups of individuals with different activity levels, aerobic capacity and/or different types and severity of diseases.

Conclusions

Three different measures to describe the increasing anaerobic metabolism obtained from respiratory gas exchange during a progressive cycle exercise test were determined using either filtration (PX) or polynomial fittings to raw data and calculations of their derivatives (DX, PQ). In a group of 19 healthy young men the measures occurred as a percentage of VO_{2max} at 54% (DX), 68% (PX) and 70% (PQ). The sequence DX < PX < PQ was most frequently seen but the sequence DX < PQ < PX was also observed. It seems likely that various patterns characterize different individuals response to exercise, but this remains to be investigated. However, the considerable absolute variation between the duplicated tests, with 5–6 weeks between, requires further attention.

Acknowledgments

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