Examination of gas exchange and blood lactate thresholds in Paralympic athletes during upper-body poling

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Abstract

Objectives

The primary aim was to compare physiological and perceptual outcome parameters identified at common gas exchange and blood lactate (BLa) thresholds in Paralympic athletes while upper-body poling. The secondary aim was to compare the fit of the breakpoint models used to identify thresholds in the gas exchange thresholds data versus continuous linear and curvilinear (no-breakpoint) models.

Methods

Fifteen elite Para ice hockey players performed seven to eight 5-min stages at increasing workload until exhaustion during upper-body poling. Two regression lines were fitted to the oxygen uptake (VO₂)-carbon dioxide (VCO₂) and minute ventilation (VE)/VO₂ data to determine the ventilatory threshold (VT), and to the VCO₂-VE and VE/VCO₂ data to determine the respiratory compensation threshold (RCT). The first lactate threshold (LT1) was determined by the first rise in BLa (+0.4mmol L⁻¹ and +1.0mmol L⁻¹) and a breakpoint in the log-log transformed VO₂-BLa data, and the second lactate threshold (LT2) by a fixed rise in BLa above 4mmol·L⁻¹ and by employing the modified Dmax method. Paired-samples t-tests were used to compare the outcome parameters within and between the different threshold methods. The fit of the two regression lines (breakpoint model) used to identify thresholds in the gas exchange data was compared to that of a single regression line, an exponential and a 3rd order polynomial curve (no-breakpoint models) by Akaike weights.

Results

All outcome parameters identified with the VT (i.e., breakpoints in the VO₂-VCO₂ or VE/VO₂ data) were significantly higher than the ones identified with a fixed rise in BLa (+0.4 or +1.0mmol·L⁻¹) at the LT1 (e.g. BLa: 5.1±2.2 or 4.9±1.8 vs 1.9±0.6 or 2.3±0.5mmol·L⁻¹, p<0.001), but were not significantly different from the log-log transformed VO₂-BLa data.
The outcome parameters identified with breakpoints in the VCO₂-VE data to determine the RCT (e.g. BLa: 5.5±1.4mmol L⁻¹) were not different from the ones identified with the modified Dmax method at the LT2 (5.5±1.1mmol L⁻¹) (all p>0.06), but were higher compared to parameters identified with VE/VCO₂ method (4.9±1.5mmol L⁻¹) and a fixed BLa value of 4mmol L⁻¹ (all p<0.03). Although we were able to determine the VT and RCT via different gas exchange threshold methods with good fit in all 15 participants (mean R²>0.931), the continuous no-breakpoint models had the highest probability (>68%) of being the best models for the VO₂₋VCO₂ and the VCO₂₋VE data.

**Conclusions**

In Paralympic athletes who exercise in the upper-body poling mode, the outcome parameters identified at the VT and the ones identified with fixed methods at the LT1 showed large differences, demonstrating that these cannot be used interchangeably to estimate the aerobic threshold. In addition, the close location of the VT, RCT and LT2 does not allow us to distinguish the aerobic and anaerobic threshold, indicating the presence of only one threshold in athletes with a disability exercising in an upper-body mode. Furthermore, the better fit of continuous no-breakpoint models indicates no presence of clear breakpoints in the gas exchange data for most participants. This makes us question if breakpoints in the gas exchange data really exist in an upper-body exercise mode in athletes with disabilities.

**Introduction**

In able-bodied endurance athletes performing lower-body or whole-body exercise, gas exchange and blood lactate (BLa) threshold concepts are well-established in the diagnosis of endurance performance as well as in the prescription of systematic training with different exercise intensity zones [1]. Two thresholds are commonly described in the literature: 1) The aerobic threshold (AT)—determined by the ventilatory threshold (VT) or the first lactate threshold (LT1)—separates low- from moderate-intensity exercise [2, 3]. 2) The anaerobic threshold (ANT)—determined by the respiratory compensation threshold (RCT) or the second lactate threshold (LT2)—separates moderate- from high-intensity exercise [2, 3]. However, to what extent the outcome parameters identified at the VT and LT1 as well as the RCT and LT2 coincide in Paralympic sitting sport athletes who exercise in an upper-body mode remains to be investigated.

Various methods have been employed to determine the VT and the RCT, as well as the LT1 and the LT2 [3–6]. The VT is based on a disproportionate increase (i.e. a breakpoint) in carbon dioxide production (VCO₂) and minute ventilation (VE) in relation to oxygen uptake (VO₂) [3, 7], and the LT1 on an onset in BLa concentration above resting levels that marks the beginning of exercise [5] or on a breakpoint in the log-log transformed VO₂-BLa data [4]. Even though these physiological changes occur above the VT and LT1, the body is still able to maintain equilibrium at intensities up to the ANT, and aerobic metabolism (indicated by measurements of oxygen uptake and the corresponding energy equivalent) reflects overall energy expenditure [2]. The ANT marks the point beyond which any attempt of the body to maintain metabolic equilibrium at a constant rate of work fails [6]. The RCT is based on a disproportionate increase (i.e. a breakpoint) of VE in relation to VCO₂ [3], a mechanism that has been suggested to correspond with the point where BLa starts to accumulate with constant workload.
In contrast, it has been argued that the changes in gas exchange with increasing work rate are continuous transitions where fatigue gradually accumulates rather than clear breakpoints [8].

The assumption that the VT corresponds with the LT1, and the RCT with the LT2, are based on the initial studies by Beaver et al. [3] and Wassermann et al. [6, 9, 10] from the 1980’s. However, there has been a continuous debate around the existence of and the physiological link between these different thresholds [2, 11–14]. Although physiological parameters identified at the VT and LT1, and at the RCT and LT2 have shown high correlations in able-bodied participants during cycling and running in some studies [15, 16], others find low correlations [17]. In wheelchair basketball and wheelchair rugby athletes with a spinal cord injury, the % of VO$_{peak}$ was lower at the LT1 compared to the VT, whereas it did not significantly differ at the LT2 and RCT [18]. In contrast, in able-bodied swimmers, there were no significant differences in physiological outcome parameters at the LT1 and the VT [19].

Whereas a range of studies have investigated the VT during upper-body exercise in able-bodied participants and participants with a disability [20–29], knowledge is limited on whether gas exchange and BLa threshold concepts can be used interchangeably in athletes with disabilities who exercise in an upper-body mode, or whether breakpoints exist in the gas exchange data of these athletes. Therefore, the primary aim of this study was to compare physiological and perceptual outcome parameters at the gas exchange and BLa thresholds in the data obtained from Paralympic athletes while upper-body poling. The secondary aim was to compare the fit of breakpoint models used to identify gas exchange thresholds with continuous linear or curvilinear (no-breakpoint) models.

Methods

Participants

Fourteen male and one female endurance-trained Norwegian Para ice hockey players participated in this study. Anthropometrics and training hours per month of the participants are depicted in Table 1. All participants were healthy and free of injuries at the time of testing. The study was approved by the Norwegian Data Protection Authority and conducted in accordance with the Declaration of Helsinki. All participants signed an informed consent form prior to voluntarily take part in the study, and were made aware that they could withdraw from the study at any point without providing an explanation.

Experimental design

The testing consisted of two consecutive test days at similar test times, during which participants performed an incremental test to exhaustion on day one, followed by seven to eight 5-min stages at gradually increasing effort for each stage until exhaustion on day two. All tests were performed in upper-body poling on a Concept2 ski ergometer 1 (Concept2, Inc., Morrisville, USA, http://www.concept2.com/service/skierg/skierg-1), while sitting in an ice sledge hockey seat.

Test set-up

After being equipped with an oro-nasal mask (Hans Rudolph Inc, Kansas City, MO, USA) and a heart rate monitor (Polar Electro Inc., Port Washington, NY, USA), the participants were tightly strapped around the thighs and hips into an ice sledge hockey seat that was mounted on a wooden platform (Fig 1). The distance of the seat to the Concept2 ski ergometer and the position of the feet depended on personal preference but was the same for test day one and
two. The ski ergometer uses wind resistance, which is generated by the spinning flywheel. The ski ergometer has a spiral damper with settings from one to ten, which works like a gearing system. We had this damper set at “eight” for all participants. Power output was measured with the ergometer’s software, which was previously validated with force and velocity measurements using a force cell (Noraxon USA inc., Scottsdal, AZ, USA) and the Oqus cameras of the Qualisys motion capture system (Qualisys AB, Gothenburg, Sweden) as described by Hegge et al. [30]. The Metamax II ergospirometer (CORTEX Biophysik GmbH, Leipzig, Germany) was calibrated against a known mixture of gases (16% O₂ and 4% CO₂) and ambient air prior to the testing procedure of every second participant. Before each athlete was tested, the flow transducer was calibrated with a 3 L syringe and then connected to the oro-nasal mask, which allowed for the measurement of breath-by-breath respiratory parameters.

Test protocol

The participants were instructed to refrain from heavy training and alcohol consumption 24 hours before, caffeine intake the day of, and food intake two hours before testing. Additionally, the participants were instructed to void their bladder directly before arriving at the laboratory. A questionnaire was filled out on each of the two test days to monitor if the participants followed these instructions, as well as to exclude any prior illness or injury that might have interfered with the testing.

Test day one. A standardized warm-up of five 5-min submaximal stages with a 2- to 3-min break between stages was performed in the upper-body poling mode at an overall rating of perceived exertion (RPE) of 7 (very light), 9 (very light), 11 (light), 13 (somewhat hard) and 15 (hard). Next to serving as a warm-up, the submaximal stages were used to familiarize the participants with the use of the Borg scale [31] to indicate RPE after the incremental test and

Table 1. Sex, age, anthropometric and disability characteristics as well as monthly training hours of the 15 Norwegian national team Para ice hockey players participating in this study.

|   | Sex  | Age  (years) | Body mass (kg) | Height (cm) | Disability (level of injury) | Training hrs/month |
|---|------|-------------|----------------|-------------|-----------------------------|-------------------|
| 1 | Male | 53         | 83.3           | 186         | Paraplegia (Th12-L1)        | 25                |
| 2 | Male | 18         | 75.7           | 160         | Spina bifida (L5)           | 49                |
| 3 | Male | 27         | 61.0           | 160         | Athrogryposis multiplex congenita | 63                |
| 4 | Male | 31         | 69.4           | 184         | Hereditary spastic paraplegia | 45                |
| 5 | Male | 28         | 90.0           | 173         | Paraplegia (Th10)           | 26                |
| 6 | Male | 21         | 70.4           | 164         | Spina bifida (ns)           | 59                |
| 7 | Male | 33         | 70.5           | 160         | Spina bifida (Th12)         | 67                |
| 8 | Male | 34         | 75.3           | 173         | Paraplegia (Th11-12)        | 48                |
| 9 | Female | 22       | 70.0           | 167         | Spina bifida (L3-S1)        | 33                |
| 10 | Male | 22        | 63.4           | 164         | Paraplegia (Th11-12)        | 28                |
| 11 | Male | 18        | 64.2           | 154         | Spina bifida (ns)           | 54                |
| 12 | Male | 20        | 68.0           | 186         | Paraplegia (Th12)           | 40                |
| 13 | Male | 20        | 77.0           | 163         | Cerebral Palsy (motor only) | 23                |
| 14 | Male | 28        | 66.5           | 173         | Amputation (single leg above the knee) | 80                |
| 15 | Male | 32        | 63.2           | 165         | Paraplegia (ns)             | 56                |
| Mean ± SD | 27.1±8.9 | 71.2±8.0 | 170±10 | - | 47±18 |

* Players are from the Norwegian national B-team
All other players are from the Norwegian national A-team.
Thoracic (Th), lumbar (L), sacral (S), not specified (ns)

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Fig 1. Test set-up. The participants were strapped in around the hips and thighs in an ice sledge hockey seat mounted on a platform in front of the Concept2 ski-ergometer.

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each of the 5-min stages on day two. After a 5-min break, the incremental test started at the individual power output of the third submaximal stage (rounded to the nearest 10-point value), and participants were instructed to continuously increase power output by 10 W every 30 s. The test was terminated when the participant, despite strong verbal encouragement, could no longer maintain the required power output of the 30-s stage and the VO$_2$ values either plateaued or decreased (a drop of more than 2 mL·kg$^{-1}$·min$^{-1}$). After the incremental test, participants recovered passively for five min and actively for three min (at the power output of the first submaximal stage). They then performed a verification stage at a 10% higher power output than the peak power output of the incremental test (rounded to the nearest 10-point value) to verify the attainment of a true VO$_{2peak}$ [32]. The verification stage was terminated when the participant dropped more than 10% of target power output for more than five s.

Test day 2. Seven to eight 5-min stages were performed with a 2- to 3-min break between stages and in the same upper-body poling mode. The first stage started at 20% of the individual peak power output obtained during the incremental test on day one, with increases of 10% (of the individual peak power output) for each consecutive stage. The last stage was terminated when the participant, despite strong verbal encouragement, could no longer maintain the power output of that stage and dropped more than 10% in the target power output for longer than five s. The intermittent exercise protocol was chosen to take a BLa sample from the fingertip in between stages. The duration of five min per stage was chosen, since in an upper-body mode two to three min are needed to achieve steady-state of physiological outcome parameters [33].

Outcome measurements

Heart rate was measured every second with a Polar heart rate monitor, and respiratory parameters (i.e., VO$_2$, VCO$_2$, VE, and respiratory exchange ratio (RER)) were measured breath-by-breath and averaged over 10 s by the in-built software of a Metamax II. A blood sample was taken from the fingertip and BLa analysed with a Lactate Pro device (Arkray Inc., Japan) at rest and directly after each of the submaximal stages on day one and day two, and one and three min after the incremental test and the verification stage on day one as well as the last stage of day two. Overall RPE was recorded after each of the submaximal stages on day one and two, as well as after the incremental test on day one and the last stage on day two. Power output was displayed per stroke and saved as 20-s averages during the submaximal stages on day one and day two by the in-built Concept2 software (Concept2, Morrisville, VT, USA). Peak power output during the incremental test and during the verification stage was registered as the highest 30-s average.

Data analysis

Data processing. Peak power output and gas exchange outcome parameters were calculated as the highest 30-s moving average and peak heart rate (HR$_{peak}$) as the highest 3-s moving average of the incremental test performed on test day one. The gas exchange, heart rate and power output data of the last two min (12 x 10-s averages) of each complete 5-min stage conducted on test day two was included for data analysis in MATLAB (R2016a; Mathworks Inc., Natick, MA). The analyses in the following were based on the concatenated 2-min gas exchange data for the VT and RCT and on the BLa values after each 5-min stages for the LT1 and LT2.

Different methods were used to determine both the VT and the RCT, as well as the LT1 and the LT2. For the determination of the VT, VO$_2$ was plotted against VCO$_2$ (V-slope method)
[3] as well as time against VE/VO₂ and VE/VCO₂ (ventilatory equivalent method) [7] and two regression lines fit to the data. For a valid detection of the VT with the ventilatory equivalent method, the VE/VO₂ had to increase before an increase in VE/VCO₂ [15, 34]. For the detection of the RCT, VCO₂ was plotted against VE [3] and two regression lines fit to the data. The LT1 was determined in two different ways: the first fixed rise in BLa concentration by 0.4 and 1 mmol·L⁻¹ above the lowest individual BLa value [5, 35]. Additionally, the LT1 was determined by breakpoints in the log-log transformed VO₂-BLa relationship [4]. The LT2 was determined by a fixed BLa concentration of 4 mmol·L⁻¹ [36]. Additionally, the LT2 was determined by the modified Dmax method, which identifies the point on the 3rd order polynomial curve fitted to the BLa values that yields the maximal perpendicular distance to the straight line formed by the first stage with an increase of 0.4 mmol·L⁻¹ and the BLa measured after the last stage [5]. Outcome parameters (% of peak power output, % of VO₂peak, % of HRpeak, as well as BLa and RPE) were interpolated at the thresholds identified with each of the above described methods used to determine the VT, LT1, RCT and LT2.

Statistical analyses. Paired-samples t-tests were used to compare the physiological and perceptual outcome parameters within the VT, LT1, RCT and LT2, and between all four different thresholds. Pearson’s r was used to investigate relationships between the outcome parameters identified with the different methods used to determine VT, LT1, RCT and LT2. Ranges of 0.26–0.49, 0.50–0.69, 0.70–0.89 and 0.90–1.0 were used to indicate low, moderate, high and very high correlations according to Munro’s criteria [37]. An α level of 0.05 was used to indicate statistical significance.

To compare the fit of breakpoint models versus continuous linear or curvilinear (no-breakpoint) models to the gas exchange data, two regression lines (Eq 1) versus a single linear regression line (Eq 2), an exponential curve (Eq 3), and a 3rd order polynomial curve (Eq 4) were fitted to the VO₂-VCO₂, VE/VO₂, VE/VCO₂, and VCO₂-VE data by linear least squares fitting.

\[
y = \begin{cases} 
  a_1 + b_1 x , & t < k \\
  a_2 + b_2 x , & t \geq k 
\end{cases} \quad (1)
\]

\[
y = a + bx \quad (2)
\]

\[
y = a + c \cdot \exp\left(\frac{t - g}{d}\right) \quad (3)
\]

\[
y = a + b_1 x + b_2 x^2 + b_3 x^3 \quad (4)
\]

y is the variable of interest, a the y-axis offset, b the slope coefficients, c and d spreading coefficients, g the x-axis offset and k the point where the first and the second regression line of the piecewise function cross. To compare the fit of the four models, the Akaike information criterion (AIC) (Eq 5) [38] and the Akaike weights (wi) (Eq 7) for each model i relative to the set of R candidate models were calculated based on the delta AIC (Δi) (Eq 6) [39, 40].

\[
AIC = n \cdot \log\left(\frac{SS}{n}\right) + 2 \cdot K \quad (5)
\]

\[
\Delta_i = AIC_i - AIC_{2og} \quad (6)
\]
AIC weight = \( w_i = \frac{\exp\left(-\frac{D_i}{2}\right)}{\sum_{j=1}^{n} \exp\left(-\frac{D_j}{2}\right)} \)  

\( n \) is the number of data points, \( \text{SS}_e \) the error sums of squares, and \( K \) the number of parameters +1 of each model. Our rationale was that a better fit of the two regression lines (breakpoint model) as compared to the linear/curvilinear models (continuous no-breakpoint models), would suggest the presence of a breakpoint.

**Results**

The outcome parameters identified with different methods used to determine the VT, LT1, RCT and LT2 (Fig 2) are presented as percentage of the respective peak power output, VO\(_{2\text{peak}}\) and peak HR obtained during the incremental test (Table 2).

All outcome parameters identified at VT with either the V-slope or the ventilatory equivalent method were significantly higher than the ones at both the LT1 (+0.4) and LT1 (+1.0) (all \( p < 0.001 \)), but not significantly different from the ones identified with the log-log transformed VO\(_2\)-BLa method (all \( p > 0.06 \)) (Fig 2). Additionally, most of the outcome parameters identified at the VT did not significantly correlate with the corresponding ones at LT1 (+0.4).
or LT1 (+1.0) (exception: power output and BLa at LT1 (+0.4): r > 0.55, p < 0.04; all other outcome parameters: r < 0.38, p > 0.16) (S1 File, sheet “correlations”). All outcome parameters at LT1 (+0.4) and LT1 (+1.0) were highly or very highly correlated (all r > 0.83, p < 0.001). In addition, some of the outcome parameters identified with breakpoints in the log-log transformed VO\(_2\)-BLa moderately correlated with the outcome parameters identified by the V-slope method (HR: r = 0.64, p = 0.01; BLa: r = 0.54, p = 0.04) and the breakpoints in the VE/VO\(_2\) data of the ventilatory equivalent method (HR: r = 0.54, p = 0.04).

The outcome parameters identified with breakpoints in the VCO\(_2\)-VE data at the RCT (e.g. BLa: 5.5 ± 1.4 mmol·L\(^{-1}\)) were not significantly different from the ones identified with the modified D\(_{\text{max}}\) method at the LT2 (5.5 ± 1.1 mmol·L\(^{-1}\)) (all p > 0.53), but were higher compared to parameters identified with VE/VCO\(_2\) method (4.9 ± 1.5 mmol·L\(^{-1}\)) and a fixed BLa value of 4 mmol·L\(^{-1}\) (all p < 0.03). Furthermore, there was no significant difference between the outcome parameters identified with V-slope method used to determine the VT and the ones identified with breakpoints in the VE/VCO\(_2\) and VCO\(_2\)-VE data used to determine the RCT (p > 0.22). However, most outcome parameters identified at the breakpoints in the VE/VO\(_2\) and VE/ VCO\(_2\) data (ventilatory equivalent method) were highly or very highly correlated with those identified at the breakpoints in the VCO\(_2\)-VE data (RCT) (exception: % of VO\(_2\)peak r = 0.67, p = 0.006; all other outcome parameters: r > 0.73, p < 0.01) (Fig 2). In addition, most outcome parameters identified at the thresholds in the VE/VCO\(_2\) data were moderately to highly correlated with the outcomes parameters identified with the modified D\(_{\text{max}}\) method (exception: % of peak power output: r = 0.43, p = 0.11; all other outcome parameters: r > 0.57, p < 0.03). Furthermore, there was no significant difference between the outcome parameters identified with breakpoints in the VE/VCO\(_2\)-VE and VCO\(_2\)-VE data used to determine the RCT and at a fixed BLa concentration of 4 mmol·L\(^{-1}\) (all p > 0.43).

For the gas exchange data, all fitting procedures for the VO\(_2\)-VCO\(_2\) and the VCO\(_2\)-VE plots, including the single linear regression line, showed very good fit on the data for all 15 participants (mean \(r^2 > 0.97\) (Table 3). However, the fit of the breakpoint model compared to the continuous no-breakpoint models on the VO\(_2\)-VCO\(_2\) and the VCO\(_2\)-VE data was only better among five participants. Accordingly, the continuous no-breakpoint models had 71% and 68% probability of being the best models for the VO\(_2\)-VCO\(_2\) and the VCO\(_2\)-VE data, respectively (Table 4). Exemplary VO\(_2\)-VCO\(_2\) and VCO\(_2\)-VE plots are illustrated in Figs 3 and 4, respectively.

In the gas exchange data displayed in the VE/VO\(_2\) plots and the VE/VCO\(_2\) plots, the breakpoint model fitted better than the continuous no-breakpoint models in six and seven of the athletes, respectively (Fig 5). Accordingly, it is unclear if in general the breakpoint (41 and
increases in workload every 5 min while upper-body poling.

Table 3. The coefficient of determination (mean $r^2 \pm SD$ (range) for the two regression lines (breakpoint model) and the single regression line, exponential and 3rd order polynomial curve (continuous no-breakpoint models) fitted to the gas exchange data of 15 elite Para ice hockey players following a protocol with stepwise increases in workload every 5 min while upper-body poling.

|                      | Two regression lines | Single regression line | Exponential curve | 3rd order polynomial curve |
|----------------------|----------------------|------------------------|-------------------|-----------------------------|
| VO$_2$-VCO$_2$ plots | 0.995 ± 0.005 (0.993–0.998) | 0.994 ± 0.005 (0.991–0.996) | 0.993 ± 0.005 (0.991–0.996) | 0.996 ± 0.005 (0.993–0.998) |
| VE/VO$_2$ plots      | 0.931 ± 0.069 (0.896–0.966) | 0.764 ± 0.094 (0.716–0.811) | 0.919 ± 0.064 (0.886–0.951) | 0.932 ± 0.064 (0.900–0.964) |
| VE/VCO$_2$ plots     | 0.940 ± 0.044 (0.918–0.962) | 0.700 ± 0.142 (0.628–0.772) | 0.920 ± 0.044 (0.898–0.942) | 0.940 ± 0.041 (0.919–0.961) |
| VCO$_2$-VE plots     | 0.995 ± 0.003 (0.994–0.997) | 0.968 ± 0.015 (0.960–0.976) | 0.992 ± 0.006 (0.989–0.994) | 0.996 ± 0.003 (0.994–0.998) |

Oxygen uptake (VO$_2$), carbon dioxide production (VCO$_2$), minute ventilation (VE)

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Table 4. Akaike weights ($w_i$) representing a measure of strength of evidence for probability of best fit of the two regression lines (breakpoint model) and the single regression line, exponential and 3rd order polynomial curve (continuous no-breakpoint models) (mean $w_i \pm SD$ (95% CI) [# of participants with better fit of the respective model compared to the two regression lines]) fitted to the gas exchange data of 15 elite Para ice hockey players following a protocol with stepwise increases in workload every 5 min while upper-body poling.

|                      | Two regression lines | Single regression line | Exponential curve | 3rd order polynomial curve |
|----------------------|----------------------|------------------------|-------------------|-----------------------------|
| VO$_2$-VCO$_2$ plots | 0.29 ± 0.35 (0.11–0.46) | 0.07 ± 0.18 (-0.03–0.16) [#0] | 0.03 ± 0.10 (-0.01–0.08) [#0] | 0.61 ± 0.37 (0.42–0.79) [#10] |
| VE/VO$_2$ plots      | 0.41 ± 0.45 (0.18–0.64) | 0.00 ± 0.00 (0.00–0.00) [#0] | 0.13 ± 0.24 (0.01–0.26) [#2] | 0.46 ± 0.40 (0.25–0.66) [#7] |
| VE/VCO$_2$ plots     | 0.47 ± 0.49 (0.22–0.72) | 0.00 ± 0.00 (0.00–0.00) [#0] | 0.09 ± 0.19 (-0.01–0.19) [#2] | 0.44 ± 0.43 (0.22–0.66) [#6] |
| VCO$_2$-VE plots     | 0.31 ± 0.44 (0.09–0.54) | 0.00 ± 0.00 (0.00–0.00) [#0] | 0.00 ± 0.01 (0.00–0.01) [#0] | 0.68 ± 0.44 (0.46–0.91) [#10] |

Oxygen uptake (VO$_2$), carbon dioxide production (VCO$_2$), minute ventilation (VE)

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47%, respectively) or continuous no-breakpoint (59 and 53%, respectively) models fit the VE/VO$_2$ and the VE/VCO$_2$ data best (Table 4). The rise in VE/VO$_2$ occurred earlier than the VE/VCO$_2$ only in four athletes (S1 Fig). The VT detection by the VE/VO$_2$ relationship was, therefore, only valid in these four athletes. In none of these four athletes, did the breakpoint model fit the VE/VO$_2$ data better than the continuous no-breakpoint models.

Discussion

The main aim of this study was to compare physiological and perceptual outcome parameters identified with common gas exchange and Bla thresholds methods used to determine the VT, LT1, RCT and LT2 in Paralympic athletes while upper-body poling. Furthermore, we compared the fit of breakpoint models used to determine gas exchange thresholds to the fit of continuous linear or curvilinear (i.e., no-breakpoint) models. The LT1 occurred at much lower exercise intensity than the VT although both are used as indicators of AT, whereas there were no or minor differences between the methods used to identify the RCT and LT2 that determine the ANT. Furthermore, the RCT and LT2 did not differ from the VT. In addition, the outcome parameters corresponding to the LT1 and LT2 using the log-log transformed VO$_2$-Bla data and the modified $D_{max}$ method, respectively, were significantly higher than ones identified with fixed Bla values at the LT1 and LT2 (i.e., rise in Bla of +0.4/1.0 at LT1 or Bla concentration of 4 mmol·L$^{-1}$ at LT2). We were able to determine breakpoints at the VT and RCT with different gas exchange methods with good fit in all 15 participants, although continuous no-breakpoint models showed even better fit for the majority of participants.

The physiological and perceptual outcome parameters identified with a fixed rise in Bla at the LT1 were significantly lower than the ones at the VT, and the outcome parameters using these methods only low or moderately correlated with each other. Overall, this indicates that these two thresholds cannot be used interchangeably to determine the AT. In addition,
Fig 3. Exemplary VO\textsubscript{2}-VCO\textsubscript{2} plots. The VO\textsubscript{2}-VCO\textsubscript{2} data was fitted with a single regression line, a bilinear regression line, an exponential curve, and a 3\textsuperscript{rd} order polynomial curve for an athlete without breakpoint (the four plots to the left) and with suggested breakpoint presence (the four plots to the right). (Note that the plots of the five athletes with a suggested breakpoint also show a rather linear increase in the VO\textsubscript{2}-VCO\textsubscript{2} relationship). Oxygen uptake (VO\textsubscript{2}), carbon dioxide production (VCO\textsubscript{2}).

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Fig 4. Exemplary $\text{VCO}_2$-VE plots. The $\text{VCO}_2$-VE data was fitted with a single regression line, a bilinear regression line, an exponential curve, and a third order polynomial curve for an athlete without breakpoint (the four plots to the left) and with suggested breakpoint presence (the four plots to the right). (Note that the plots of the five athletes with a suggested breakpoint show a rather curvilinear increase in the $\text{VCO}_2$-VE relationship).

Carbon dioxide production ($\text{VCO}_2$), minute ventilation (VE).

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Fig 5. Exemplary VE/VO₂ and VE/VCO₂ plots. Exemplary VE/VO₂ data fitted with a bilinear regression line and a 3rd order polynomial curve for an athlete without breakpoint (upper two plots to the left) and with suggested breakpoint presence (upper two plots to the right). Exemplary VE/VCO₂ data fitted with a bilinear regression line and a 3rd order polynomial curve for an athlete without breakpoint (lower two plots to the left) and with suggested breakpoint presence (upper two plots to the right). Oxygen uptake (VO₂), carbon dioxide production (VCO₂), minute ventilation (VE).

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thresholds identified by a fixed BLa increase at the LT1 were significantly lower compared with the breakpoints identified in the log-log transformed VO₂-BLa data, showing that individually adjustable BLa methods did not correspond with fixed methods in determining the LT1. The early occurrence of a rise in BLa in upper-body exercise is in accordance with Beneke et al. [41], who found BLa to be higher at a given workload in activities involving smaller muscle mass, where power output per kg of active muscle mass and, thus, local metabolic stress is increased compared to lower body exercise. In addition, BLa accumulation after cessation of exercise was shown to be faster in individuals with a spinal cord injury as compared to able-bodied individuals [42]. However, although outcome parameters identified with breakpoints in the log-log transformed VO₂-BLa data are not significantly lower than the ones identified at the VT, outcome parameters identified with methods using fixed BLa values to identify the LT1 are much lower than the VT.

As estimates of the ANT, the outcome parameters identified with the Dmax method to determine LT2 did not significantly differ from the ones identified with breakpoints in the VCO₂-VE data at the RCT, whereas most of the outcome parameters identified with breakpoints in the VE/VCO₂ data were significantly lower than these. However, the outcome parameters identified by the latter method differ only marginally from the two other ANT methods (VCO₂-VE, Dmax), indicating that the exercise intensity where a disproportionate increase in BLa and in VE occurs is relatively similar. Note that we decided to not correct for multiple comparisons and rather present the uncorrected p-values from paired samples t-tests instead. Although we are aware of the subsequent increased chances of making a type 1 errors, the decreased chances of making a type 2 errors were regarded more important, which is in accordance with Rothman [43]. However, if Bonferroni corrections would have been used in this specific case, there would have been no significant differences between the outcome measures identified at with these three methods.

Furthermore, most of the outcome parameters identified with the different methods at the LT2 and RCT are low to moderately correlated, coinciding with high individual variation in the outcome parameters within each of the methods used to identify the LT2 and RCT. This indicates that an individual with a high LT2 does not necessarily display a high RCT. The high individual variation may be explained by disability-related differences in the cardio-respiratory system that might affect physiological responses to upper-body exercise. For example, athletes with a spinal cord injury exercising in an upper-body mode were shown to vary considerably in their VO₂peak depending on their level of injury [44], which might also reflect differences in the % of VO₂peak that can be sustained during exercise. In addition, the inclusion of one participant that was much older than the rest and one female participant may have contributed to the high variation. Furthermore, individual variation in physiological responses may be higher in upper-body exercise compared to lower-body exercise. Altogether, it is questionable whether the similar outcome parameters identified at the LT2 and the RCT on a group basis, result in similar outcome parameters at the LT2 and RCT for the individual sitting athlete when training in an upper-body mode.

The thresholds identified by the breakpoints in the VE/VO₂ at the VT and in VE/VCO₂ at the RCT did not significantly differ and were highly correlated. This, together with the rather linear increase in the VO₂-VCO₂ relationship suggests that it is solely the disproportionate rise in VE that leads to a rather rapid increase in the data of the VE/VO₂ and the VE/VCO₂ plots, and to discernible breakpoints in approximately half of the participants. Together with the close location of the breakpoints identified in the VCO₂-VO₂ data at the VT and the VCO₂-VE data at the RCT, this indicates that a two-phase (low-high) rather than a three-phase (low-moderate-high) intensity zone model could be applicable in athletes with a disability who exercise in an upper-body mode. This is in contrast to significant differences between the VT and
the RCT in Dekerle et al. [45], who test able-bodied participants in the arm crank ergometry mode, and Leicht et al. [18], who tested wheelchair athletes in the wheelchair treadmill mode. However, our findings are in line with a study of Pires et al. [46], who also found one rather than two thresholds in the gas exchange data in upper-body trained able-bodied participants during exercise in the arm crank ergometry mode. Whether the discrepancies between studies are related to employment of e.g. different populations, protocols or exercise modes needs to be examined further in other experimental designs.

All gas exchange threshold methods have in common that there is an a priori assumption of the presence of a breakpoint, defined as “a place where an interruption or change occurs” [47]. However, the presence or absence of breakpoints in the gas exchange data is a debated topic [8, 12]. Thus, in addition to the breakpoint models used to identify the VT and the RCT in the present study, we fitted continuous no-breakpoint models to the data to investigate if there are clear breakpoints in our data. Here, we found good fit for the breakpoint model used to identify the gas exchange thresholds, but better fit for the curvilinear no-breakpoint models in most cases. We, hence, question if clear breakpoints really exist in the gas exchange data of athletes with disabilities in an upper-body exercise mode.

Conclusion

In Paralympic athletes who exercise in upper-body poling, the physiological and perceptual outcome parameters identified at the VT and the LT1 showed large differences, which demonstrates that these cannot be used interchangeably to identify the AT. In addition, the close location of the VT, RCT and LT2 does not allow us to distinguish the AT and ANT, indicating that there might only be one threshold in athletes with a disability exercising in an upper-body mode. Furthermore, continuous no-breakpoint models fit the gas exchange data better than breakpoint models in most participants. We, hence, question if clear breakpoints in the gas exchange data really exist in an upper-body exercise mode in athletes with disabilities.

Supporting information

S1 File. Data. Data and analyses conducted in this study of gas exchange and blood lactate thresholds in Paralympic sitting athletes. (XLSX)

S1 Fig. VE/VO\textsubscript{2} and VE/VCO\textsubscript{2} plots fitted with two regression lines. The data is of the six or seven completed stages of each of the 15 athletes. Breakpoint presence is indicated above each individual plot. Furthermore, it is indicated in the second row above the figures whether the two thresholds occur at the same time, or the VE/VO\textsubscript{2} occurs before or after the VE/VCO\textsubscript{2} threshold. Oxygen uptake (VO\textsubscript{2}), carbon dioxide production (VCO\textsubscript{2}), minute ventilation (VE). (TIF)

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References
1. Seiler S, Toennessen E. Intervals, Thresholds, and Long Slow Distance: the Role of Intensity and Duration in Endurance Training. Sports science. 2009; 13:32–53.
2. Binder RK, Wonisch M, Corra U, Cohen-Solal A, Vanhees L, Saner H, et al. Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. Eur J Cardiovasc Prev Rehabil. 2008; 15(6):726–34. https://doi.org/10.1097/HJR.0b013e328304fed4 PMID: 19050438
3. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. J Appl Physiol (1985). 1986; 60(6):2020–7.
4. Beaver WL, Wasserman K, Whipp BJ. Improved detection of lactate threshold during exercise using a log-log transformation. J Appl Physiol (1985). 1985; 59(6):1936–40.
5. Bishop D, Jenkins DG, Mackinnon LT. The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. Med Sci Sports Exerc. 1998; 30(8):1270–5. PMID: 9710868
6. Wasserman K. The anaerobic threshold: definition, physiological significance and identification. Adv Cardiol. 1986; 35:1–23.
7. Reinhard U, Muller PH, Schmulling RM. Determination of anaerobic threshold by the ventilation equivalent in normal individuals. Respiration. 1979; 38(1):36–42. https://doi.org/10.1159/000194056 PMID: 493728
8. Myers J, Ashley E. Dangerous curves. A perspective on exercise, lactate, and the anaerobic threshold. Chest. 1997; 111(3):787–95. PMID: 9118720
9. Wasserman K. The anaerobic threshold measurement to evaluate exercise performance. Am Rev Respir Dis. 1984; 129(2 Pt 2):S35–40. https://doi.org/10.1164/arrd.1984.129.2P2.S35 PMID: 6421216
10. Wasserman K. Determinants and detection of anaerobic threshold and consequences of exercise above it. Circulation. 1987; 76(6 Pt 2):VI29–39. PMID: 3315297
11. Faude O, Kindermann W, Meyer T. Lactate threshold concepts: how valid are they? Sports Med. 2009; 39(6):469–90. https://doi.org/10.2165/00007256-200939060-00003 PMID: 19453206
12. Hopker JG, Jobson SA, Pandit J. Controversies in the physiological basis of the ‘anaerobic threshold’ and their implications for clinical cardiopulmonary exercise testing. Anaesthesia. 2011; 66(2):111–23. https://doi.org/10.1111/j.1365-2044.2010.06604.x PMID: 21254986
13. Peronnet F, Aguilaniu B. Lactic acid buffering, nonmetabolic CO2 and exercise hyperventilation: a critical reappraisal. Respir Physiol Neurobiol. 2006; 150(1):4–18. https://doi.org/10.1016/j.resp.2005.04.005 PMID: 15890562

14. Brooks GA. Anaerobic threshold: review of the concept and directions for future research. Med Sci Sports Exerc. 1985; 17(1):22–34. PMID: 3884959

15. 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(11):1841–8. PMID: 11689733

16. Maffulli N, Testa V, Capasso G. Anaerobic threshold determination in master endurance runners. J Sports Med Phys Fitness. 1994; 34(3):242–9. PMID: 7830387

17. Chichorro JL, Perez M, Vaquero AF, Lucia A, Legido JC. Lactic threshold vs ventilatory threshold during a ramp test on a cycle ergometer. J Sports Med Phys Fitness. 1997; 37(2):117–21. PMID: 9239989

18. Leicht CA, Griggs KE, Lavin J, Tolfrey K, Goosey-Tolfrey VL. Blood lactate and ventilatory thresholds in wheelchair athletes with tetraplegia and paraplegia. Eur J Appl Physiol. 2014; 114(8):1635–43. https://doi.org/10.1007/s00421-014-2886-x PMID: 24751928

19. Ribeiro J, Figueiredo P, Sousa M, De Jesus K, keskien K, Vilas-Boas JP, et al. Metabolic and ventilatory thresholds assessment in front crawl swimming. J Sports Med Phys Fitness. 2015; 55(7–8):701–7. PMID: 25069963

20. Bernardi M, Guerra E, Di Giacinto B, Di Cesare A, Castellano V, Bambhani Y. Field evaluation of paralympic athletes in selected sports: implications for training. Med Sci Sports Exerc. 2010; 42(6):1200–8. PMID: 19997027

21. Bambhani YN, Holland LJ, Steadward RD. Anaerobic threshold in wheelchair athletes with cerebral palsy: validity and reliability. Arch Phys Med Rehabil. 1993; 74(3):305–11. PMID: 8439260

22. Coutts KD, McKenzie DC. Ventilatory thresholds during wheelchair exercise in individuals with spinal cord injuries. Paraplegia. 1995; 33(7):419–22. PMID: 7478733

23. Davis JA, Vodak P, Wilmore JH, Vodak J, Kurtz P. Anaerobic threshold and maximal aerobic power for three modes of exercise. J Appl Physiol. 1976; 41(4):544–50. https://doi.org/10.1152/jappl.1976.41.4.544 PMID: 985399

24. Keyser RE, Mor D, Andres FF. Cardiovascular responses and anaerobic threshold for bicycle and arm ergometer exercise. Arch Phys Med Rehabil. 1989; 70(9):687–91. PMID: 2774887

25. Lin KH, Lai JS, Kao MJ, Lien IN. Anaerobic threshold and maximal oxygen consumption during arm cranking exercise in paraplegia. Arch Phys Med Rehabil. 1993; 74(3):515–20. PMID: 849362

26. Orr JL, Williamson P, Anderson W, Ross R, McCafferty S, Fettes P. Cardiopulmonary exercise testing: arm crank vs cycle ergometry. Anesthesia. 2013; 68(5):497–501. https://doi.org/10.1111/anae.12195 PMID: 23573945

27. Schneider DA, Sedlock DA, Gass E, Gass G. VO2peak and the gas-exchange anaerobic threshold during incremental arm cranking in able-bodied and paraplegic men. Eur J Appl Physiol Occup Physiol. 1999; 80(4):292–7. PMID: 10483798

28. Vinet A, Le Gaillass D, Bernard PL, Poulain M, Varray A, Mercyier J, et al. Aerobic metabolism and cardioventilatory responses in paraplegic athletes during an incremental wheelchair exercise. Eur J Appl Physiol Occup Physiol. 1997; 76(5):455–61. https://doi.org/10.1007/s004210050275 PMID: 9367286

29. Yasuda N, Gaskill SE, Ruby BC. No gender-specific differences in mechanical efficiency during arm or leg exercise relative to ventilatory threshold. Scand J Med Sci Sports. 2008; 18(2):205–12. https://doi.org/10.1111/j.1600-0838.2007.00637.x PMID: 17490463

30. Hegge AM, Bucher E, Ettema G, Faude O, Holmberg HC, Sandbakk O. Gender differences in power production, energetic capacity and efficiency of elite cross-country skiers during whole-body, upper-body, and arm poling. Eur J Appl Physiol. 2015.

31. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982; 14(5):377–81. PMID: 715463

32. Leicht CA, Tolfrey K, Lenton JP, Bishop NC, Goosey-Tolfrey VL. The verification phase and reliability of physiological parameters in peak testing of elite wheelchair athletes. Eur J Appl Physiol. 2013; 113(2):337–45. https://doi.org/10.1007/s00421-012-2441-6 PMID: 22718268

33. Inbar O, Faina M, Demarie S, Whipp BJ. VO2 Kinetics during Moderate Effort in Muscles of Different Masses and Training Level. ISRN Physiology. 2012; 2013.

34. Powers SK, Dodd S, Garner R. Precision of ventilatory and gas exchange alterations as a predictor of the anaerobic threshold. Eur J Appl Physiol Occup Physiol. 1984; 52(2):173–7. PMID: 6598852
35. Buckley JD, Bourdon PC, Woolford SM. Effect of measuring blood lactate concentrations using different automated lactate analysers on blood lactate transition thresholds. J Sci Med Sport. 2003; 6(4):408–21. PMID: 14723391

36. Stegmann H, Kindermann W. Comparison of prolonged exercise tests at the individual anaerobic threshold and the fixed anaerobic threshold of 4 mmol.l⁻¹ lactate. Int J Sports Med. 1982; 3(2):105–10. https://doi.org/10.1055/s-2008-1026072 PMID: 7107102

37. Plichta SB, Kelvin EA, Munro BH. Munro’s statistical methods for health care research: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013.

38. Bozdogan H. Model selection and Akaike’s information criterion (AIC): The general theory and its analytical extensions. Psychometrika. 1987; 52(3):345–70.

39. Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach: Springer Science & Business Media; 2003.

40. Wagenmakers E-J, Farrell S. AIC model selection using Akaike weights. Psychonomic bulletin & review. 2004; 11(1):192–6.

41. Beneke R, Leithäuser R, Hüttler M. Dependence of the maximal lactate steady state on the motor pattern of exercise. Br J Sports Med. 2001; 35(3):192–6. https://doi.org/10.1136/bjsm.35.3.192 PMID: 11375880

42. Leicht C, Perret C. Comparison of blood lactate elimination in individuals with paraplegia and able-bodied individuals during active recovery from exhaustive exercise. J Spinal Cord Med. 2008; 31(1):60–4. PMID: 18533413

43. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990; 1(1):43–6. PMID: 2081237

44. Bhambhani Y. Physiology of wheelchair racing in athletes with spinal cord injury. Sports Med. 2002; 32(1):23–51. PMID: 11772160

45. Dekker J, Dupont L, Caby I, Marais G, Vanvelcenaher J, Lavoie JM, et al. Ventilatory thresholds in arm and leg exercises with spontaneously chosen crank and pedal rates. Percept Mot Skills. 2002; 95(3 Pt 2):1035–46. https://doi.org/10.2466/pms.2002.95.3f.1035 PMID: 12578244

46. Pires FO, Hammonds J, Lima-Silva AE, Bertuzzi RC, Kiss MA. Ventilation behavior during upper-body incremental exercise. J Strength Cond Res. 2011; 25(1):225–30. https://doi.org/10.1519/JSC.0b013e3181b2b999 PMID: 20993972

47. Definition of a break point in English [Internet]. Oxford University Press;2017 [cited 2017 May 19]. https://en.oxforddictionaries.com/definition/break_point.