



## Association between different Non-Invasively Derived Thresholds with Lactate Threshold during graded incremental exercise

CONOR RALEIGH\*, BERNARD DONNE‡, and NEIL FLEMING‡

Human Performance Laboratory, Departments of Anatomy and Physiology, School of Medicine, Trinity College Dublin, IRELAND

\*Denotes undergraduate student author, ‡Denotes professional author

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### ABSTRACT

*International Journal of Exercise Science 11(4): 391-403, 2018.* We compared lactate threshold ( $T_{Lac}$ ) with non-invasive markers of an aerobic-anaerobic transition; namely, ventilatory (VT) and tissue saturation index ( $TSI_T$ ) thresholds. While identification of a breakpoint in blood lactate concentration ([BLa]) is common for determination of an aerobic-anaerobic transition, non-invasive measures, VT and NIRS, have also received attention as a means of determining this critical exercise intensity. We hypothesised that one or other of these non-invasive measures would have a strong association with  $T_{Lac}$ . Thirty-one ( $n=31$ ) competitive male athletes (mean  $\pm$  SD, age  $29\pm 9$  yr, height  $1.81\pm 0.1$  m, body mass  $77.7\pm 10.0$  kg) performed graded incremental cycling to volitional exhaustion. Heart rate, TSI and gas exchange data were measured throughout and [BLa] was determined at fixed intervals. Threshold detection involved a segmented linear regression analysis minimising the squared sum of the residuals to determine  $T_{Lac}$ ,  $TSI_T$  and VT. Workload and HR at  $T_{Lac}$ , VT and  $TSI_T$  were analysed using repeated measures ANOVA and correlation assessed using Pearson's and interclass correlation coefficients. Thresholds at  $TSI_T$  and  $T_{Lac}$  were not significantly different ( $255\pm 35$  vs.  $249\pm 30$  W,  $P>0.05$ ), suggesting that limitations in  $O_2$  delivery could be closely linked to an aerobic-anaerobic transition. However, poor correlation ( $r=0.55$ , ICC=0.54 and 95%LoA of +67 and -54 W) suggested other factors may exert an influence. Mean VT occurred at a significantly higher workload than  $T_{Lac}$  ( $271\pm 35$  vs  $249\pm 30$  W,  $P<0.001$ ). Consequently, VT proved less useful, giving an indication of when an aerobic-anaerobic transition had already occurred. In conclusion, non-invasive markers of the aerobic transition are not concurrent with  $T_{Lac}$ .

KEY WORDS: Aerobic, anaerobic, muscle oxygenation, NIRS, ventilation

### INTRODUCTION

The term "aerobic-anaerobic transition" was first used to describe an exercise intensity above which there is an exponential increase in blood lactate (BLa) concentration and associated changes in gas exchange variables (20, 21). Determination of the transition in athletes has become critical as a performance indicator, and for heart rate controlled training prescription. To date, physiological mechanisms underlying the transition are highly debated amongst researchers resulting in several definitions and methods of determination. While the identification of a breakpoint in [BLa] remains the most common method of determining the transition, reflecting the balance between lactate production and clearance, usage of non-

invasive measures, such as gas exchange variables, and more recently near-infrared spectroscopy (NIRS), have received attention due to their potential for determining this critical exercise intensity.

Tissue saturation index (TSI) is the ratio of oxy-haemoglobin ( $\text{HbO}_2$ ) to total haemoglobin (tHb) and is commonly measured using NIRS. This ratio gives an indication of the balance between  $\text{O}_2$  delivery and consumption at a tissue specific level (3). While NIRS has been widely used in clinical scenarios, particularly in brain surgery to monitor cerebral oxygenation (3), the recent development and commercial availability of portable, user-friendly devices have led to growing applications in the field of sport performance (3). Athletes and coaches can now measure muscle specific TSI during exercise with relative ease. Exercise intensity increases the demand for  $\text{O}_2$  by the exercising musculature, and during a graded exercise test (GxT) the musculature becomes progressively deoxygenated as  $\text{O}_2$  delivery lags uptake. An exercise intensity is reached where the delivery of  $\text{O}_2$  by the circulation reaches a maximum and the tissue must therefore rely increasingly on anaerobic metabolism to sustain the effort. A TSI threshold ( $\text{TSI}_T$ ), detected using NIRS, could therefore potentially be used as a marker of a transition; however, there is still debate as to its efficacy.

As lactic acid accumulates in the blood it rapidly dissociates into lactate and  $\text{H}^+$  ions. The  $\text{H}^+$  ions are buffered by bicarbonate ( $\text{HCO}_3^-$ ) forming carbonic acid ( $\text{H}_2\text{CO}_3$ ) which is then converted to  $\text{H}_2\text{O}$  and  $\text{CO}_2$  catalysed by carbonic anhydrase. This  $\text{CO}_2$  is termed non-metabolic  $\text{CO}_2$  and along with metabolic  $\text{CO}_2$  contributes to an increase in minute ventilation (VE) as detected by ventilatory chemoreceptors (14). It is possible to detect this increase in VE relative to  $\text{VO}_2$ , and, therefore, infer the transition as an inflection point measured through gas exchange variables (1) known as a gas exchange or ventilatory threshold (VT). Some previous studies identified two ventilatory thresholds.  $\text{VT}_1$  can be identified as an inflection in  $\text{VE}/\text{VO}_2$  vs workload while  $\text{VT}_2$  can be identified using a plot of  $\text{VE}/\text{VCO}_2$  vs workload. Racinicas *et al.* (14) identified  $\text{VT}_1$  and  $\text{VT}_2$  and reported that muscle oxygenation in the *Vastus Lateralis* (VL) displayed a threshold at, or after,  $\text{VT}_2$ . In the current study  $\text{VT}_1$  was the only threshold identified using gas exchange variables as it is more commonly used in published literature (6, 15, 18, 19, 22).

Several studies have previously investigated the relationship between lactate, ventilatory and NIRS derived markers of an aerobic-anaerobic transition in different combinations. Grassi *et al.* (8) reported significant correlation between onset of muscle deoxygenation and the onset of blood lactate accumulation (OBLA, a nominal  $[\text{BLa}]$  of  $4\text{mmol}\cdot\text{L}^{-1}$ ). Another study (6) reported a significant correlation between workload at  $\text{TSI}_T$  and  $\text{VT}_1$ ; but reported no significant correlation between workload at  $\text{TSI}_T$  and  $T_{\text{Lac}}$ ; however, the technique used to determine  $T_{\text{Lac}}$  was not reported. Rao *et al.* (15) assessed the association between  $\text{VT}_1$  and NIRS derived thresholds in visceral organs rather than in exercising muscle. A moderate to strong correlations in  $\text{O}_2$  saturation in the kidney and brain with  $\text{VT}_1$  was reported, but wide limits of agreement were also identified. These authors also commented that the NIRS measured threshold slightly underestimated  $\text{VT}_1$ . In recent years, several portable NIRS measurement devices have become available facilitating application of this non-invasive technique to athletic

populations (4, 5, 19, 23). However, little published research is available to assess the efficacy of such devices as the majority of published studies have used laboratory based non-portable units.

Consequently, there are mixed opinions, and an ongoing debate, as to whether non-invasive markers of the aerobic-anaerobic transition are concurrent with more established blood derived measures. Certain ambiguity exists because of differing opinions on specific methods of transition detection and different variables associated with each measurement technique. The aim of the current study was to investigate if any association existed between  $T_{Lac}$  and non-invasive physiological markers of the aerobic-anaerobic transition, such as VT and NIRS assessed  $TSI_T$ . The current study hypothesised that one or more of these non-invasive measures would have a strong association with  $T_{Lac}$  and thus occur at a non-significantly different exercise intensity.

## **METHODS**

### *Participants*

Thirty-one (n=31) competitive male cyclists and triathletes between the age of 18 and 46 participated in the current study (mean  $\pm$  SD: age  $29 \pm 9$  yr, height  $1.81 \pm 0.1$  m, body mass  $77.7 \pm 10.0$  kg). All participants were actively involved in their chosen sport and trained 5 to 7 times per week. Before agreeing to participate in the current study all participants were informed of the associated risks and requirements, both verbally and in writing. A 7-day period was allowed for participants to fully familiarise themselves with the risks and requirements associated with participation and make any queries known to investigators. Having agreed to participate, enlisted participants provided written informed consent. The Faculty of Health Sciences Research Ethics Committee in Trinity College Dublin granted ethical approval.

Participants were required to attend the laboratory on one occasion in a rested state, carbohydrate loaded, well hydrated and clean shaven. They were also required to abstain from both alcohol and caffeine in the 24 h prior to testing. All participants underwent a medical examination by a qualified medical doctor prior to testing. Any person presenting with cardiac abnormalities, respiratory difficulties, symptoms of cold or influenza, musculoskeletal injury that could impair performance, diabetes, hypertension, metabolic disorders or any other contra-indicatory symptoms were excluded. In addition, participants completed a medical questionnaire detailing previous personal and family health abnormalities, recent illness or injury, as well as details of recent travel and vaccinations.

### *Protocol*

A GxT to volitional exhaustion was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Groningen, The Netherlands). Participants' initially identified a cycling position in which they were most comfortable by adjusting saddle height, saddle to handlebar distance and handlebar height. Participant's feet were secured to the ergometer using their own cycling shoes with cleats and accompanying pedals. The protocol commenced with a 15-min warm-up at a workload of 120 Watt (W). The GxT began with a 3-

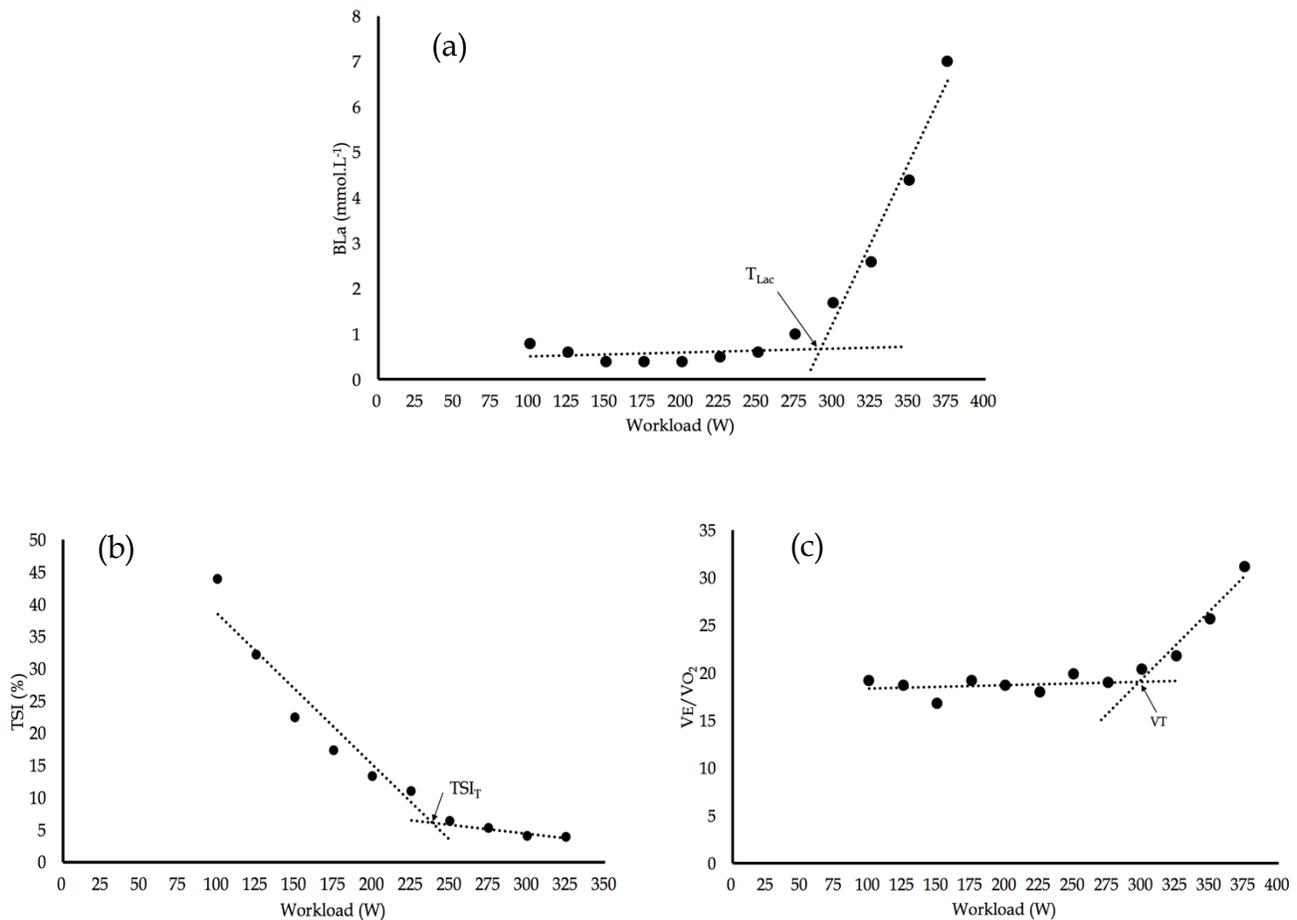
min stationary phase followed by an active phase commencing at a workload of 100 W and subsequently increasing by 25 W every 3-min. During assessment participants maintained a constant self-selected cadence (permitted range was 5 rev.min<sup>-1</sup>) and the test was terminated when a participant was no longer able to maintain a constant cadence.

Heart rate (HR) data was recorded continuously by radio-telemetry using a Cosmed HR monitor (Cosmed, Rome, Italy). During the test, blood samples were collected from the middle finger of the right hand at the end of the second minute of each 3-min interval. The fingertip was cleaned to remove any sweat or blood and lanced using a long point sterile lancet (Braun, Melsungen, Germany). The blood sample was collected into a heparinised capillary tube (Brand, Wertheim, Germany) by holding the tube horizontal to the droplet and allowing transfer by capillary action. Subsequently, a 25µL aliquot of blood was drawn from the capillary tube using a YSI syringepet (YSI, OH, USA) and added into the chamber of a YSI 1500 Sport lactate analyser (YSI, OH, USA) for determination of [BLa] in mmol.L<sup>-1</sup>. The lactate analyser was calibrated to the manufacturer's requirements ( $\pm 0.05$  mmol.L<sup>-1</sup>) before each test using a standard solution (YSI, OH, USA) of known concentration (5 mmol.L<sup>-1</sup>).

Gas exchange variables including VE, VO<sub>2</sub> and carbon dioxide production (VCO<sub>2</sub>), were measured on a breath by breath basis throughout the test, using a cardiopulmonary exercise testing unit (CPET) and an associated software package (Cosmed, Rome, Italy). Participants wore a face mask (Hans Rudolf, KA, USA) which was connected to the CPET unit. The metabolic unit was calibrated each day using ambient air and an  $\alpha$  certified gas mixture containing 16% O<sub>2</sub>, 5% CO<sub>2</sub> and 79% N<sub>2</sub> (Cosmed, Rome, Italy). Volume calibration was performed using a 3L gas calibration syringe (Cosmed, Rome, Italy) Pressure calibration was performed by recording barometric pressure using a laboratory grade barometer. TSI data was measured continuously from the VL musculature of the left leg using a portable near infrared spectroscopy unit (MOXY, MN, USA) and associated software (Peripedal, IN, USA). TSI data assessed oxy-haemoglobin as a percentage of total haemoglobin within the tissue. Both oxy- and deoxy-haemoglobin are chromophores, groups of atoms within a molecule that absorb light differently at different wavelengths (9). Light emitted into the tissue at one location is detected at another and the amount of light absorbed is computed. Consequently, it is possible to make quantitative measurements of the concentrations of chromophores using the Beer-Lambert law via the following equation:

$$\text{Log } I_0/I_1 = \epsilon \cdot \text{Pl} \cdot c$$

where I<sub>0</sub> is the starting intensity, I<sub>1</sub> is the intensity exiting the tissue,  $\epsilon$  is the molar absorptivity, a property of the chromophore available in published literature, Pl is the path length and c is the concentration. NIRS technology uses LEDs as light emitters that fire in rapid sequence, the exiting light intensity is recorded simultaneously at a detector located a fixed distance from the emitter. In the current study an opaque black support strap was placed around the leg and over the apparatus to prevent intrusion of any unwanted ambient light during data recording.



**Figure 1.** (a) BLa vs workload displaying  $T_{Lac}$  (294 W), (b) TSI vs workload displaying  $TSI_T$  (239 W) and (c)  $VE/VO_2$  vs workload displaying VT (316 W).

#### Statistical Analysis

[BLa] was plotted as a function of workload. Mean ventilatory equivalent for oxygen ( $VE/VO_2$ ) and TSI data were determined during the final minute of each 3-min phase and subsequently plotted as a function of workload. Several methods have been devised to determine thresholds during the aerobic-anaerobic transition. In the current study, the method of threshold detection involved a segmented regression analysis. Two linear segments were plotted that minimised the squared sum of the residuals between the plotted points and best fit lines. The intersection of these the two linear segments was defined as the relevant breakpoint or threshold. This method was recently used by Driller *et al.* (5) to detect  $T_{Lac}$ , and proposed by Beaver *et al.* (1) to detect VT. This analysis procedure was used to identify  $T_{Lac}$ , VT and  $TSI_T$  using commercially available software (SigmaPlot 13.0, Systat, IL, USA), see Figure 1. A linear best fit line was applied to each individual's HR data set and HR at each computed threshold determined by extrapolation.

The workload and HR at  $T_{Lac}$ , VT and  $TSI_T$  were analysed using a one-way repeated measure analysis of variance (ANOVA). Tukey *post-hoc* multiple comparison tests quantified detected significant differences. Pearson's product moment correlation coefficients ( $r$ ) were computed

between threshold variables to determine association; coefficients of determination ( $r^2$ ) were also computed. Bland-Altman analysis, with calculation of upper and lower 95%LoA, was performed to establish agreement between measures. For all statistical analysis a value of  $P < 0.05$  inferred significance. All statistical analysis and graphical presentation of data were performed using Prism 6 (GraphPad, CA, USA).

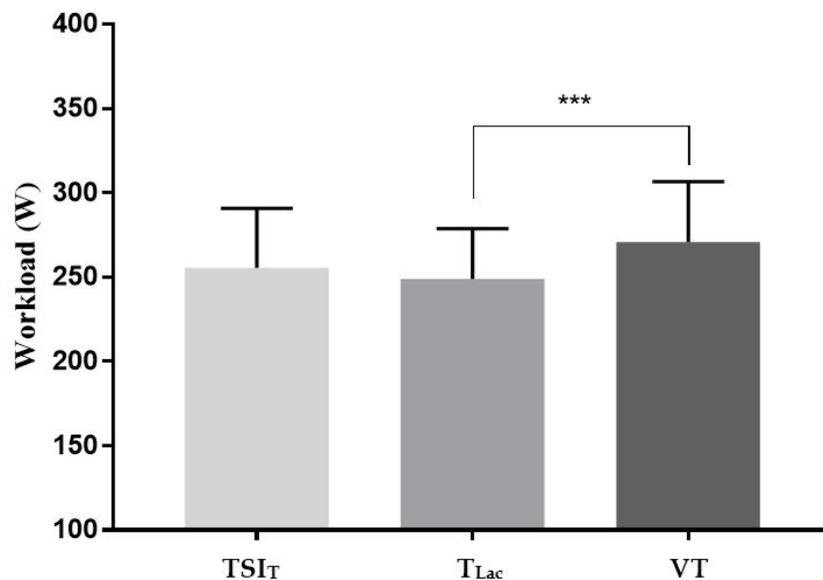
## RESULTS

Thirty-one male individuals ( $n = 31$ ) participated in the current study. Mean and standard deviation of GxT performance indicators are displayed in Table 1. Mean data for workload at computed thresholds are depicted in Figure 2. A repeated measures ANOVA revealed that mean workloads at computed thresholds were significantly different ( $F = 4.73$ ,  $P \leq 0.001$ ). Tukey *post-hoc* tests quantified that load at VT was significantly higher than at  $T_{Lac}$  ( $P \leq 0.001$ ).

**Table 1.** Mean GxT performance indicators,  $n=31$ .

	BLa at $T_{Lac}$ (mmol.L <sup>-1</sup> )	Workload at OBLA (W)	Pmax (W)	VO <sub>2</sub> max (mL.kg <sup>-1</sup> .min <sup>-1</sup> )	HRmax (beats.min <sup>-1</sup> )
Mean	2.4	308	349	62.5	183
SD	1.0	33	31	8.1	11

The coefficient of determination ( $r^2$ ) in the comparison of VT with  $T_{Lac}$  was 0.55, revealing that only a moderate proportion of the variance in  $T_{Lac}$  was attributable to VT. In addition, only moderate reproducibility was evident in the comparison of VT and  $T_{Lac}$  (ICC = 0.56). The wide limits of agreement in the comparison of VT vs  $T_{Lac}$  (+70 to -26 W) indicated moderate threshold concurrency, see Table 2 and Figure 3.

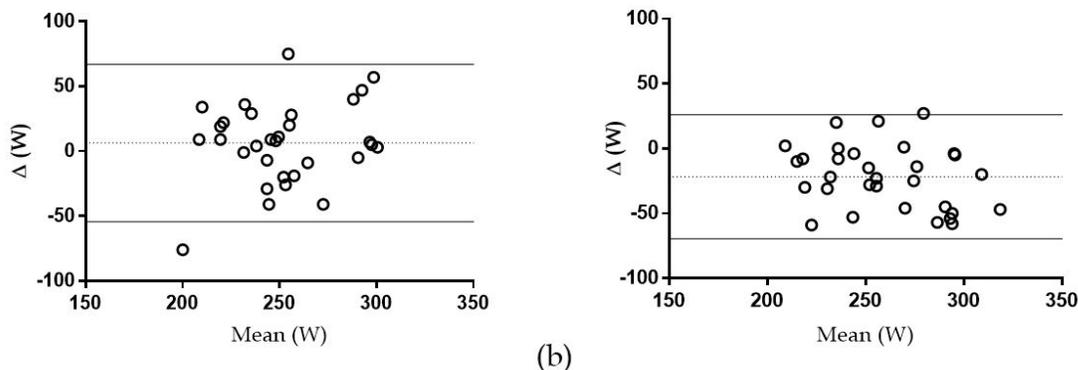


**Figure 2.** Mean workload at each computed threshold. \*\*\* denotes significant difference of  $P \leq 0.001$ . Bars denote SD,  $n=31$ .

Although workload at  $TSI_T$  was not significantly different from any other computed threshold, the coefficient of determination ( $r^2$ ) in the comparison of  $TSI_T$  with  $T_{Lac}$  was 0.31, indicating that only small to moderate proportion of the variance in  $T_{Lac}$  was attributable to  $TSI_T$ . The computed ICC of  $TSI_T$  with  $T_{Lac}$  was 0.54. The wide limits of agreement in the comparison of  $TSI_T$  vs  $T_{Lac}$  (+67 to -54 W) also indicated moderate threshold concurrency, see Table 2 and Figure 3.

**Table 2.** Correlation and association analysis of computed thresholds, n=31.

	r	ICC	Mean bias (W)	95% LoA (W)
$TSI_T$ vs $T_{Lac}$	0.56	0.54	6.387	+67 to -54
$TSI_T$ vs VT	0.41	0.36	-15.35	+60 to -91
VT vs $T_{Lac}$	0.74	0.56	-21.74	+70 to -26



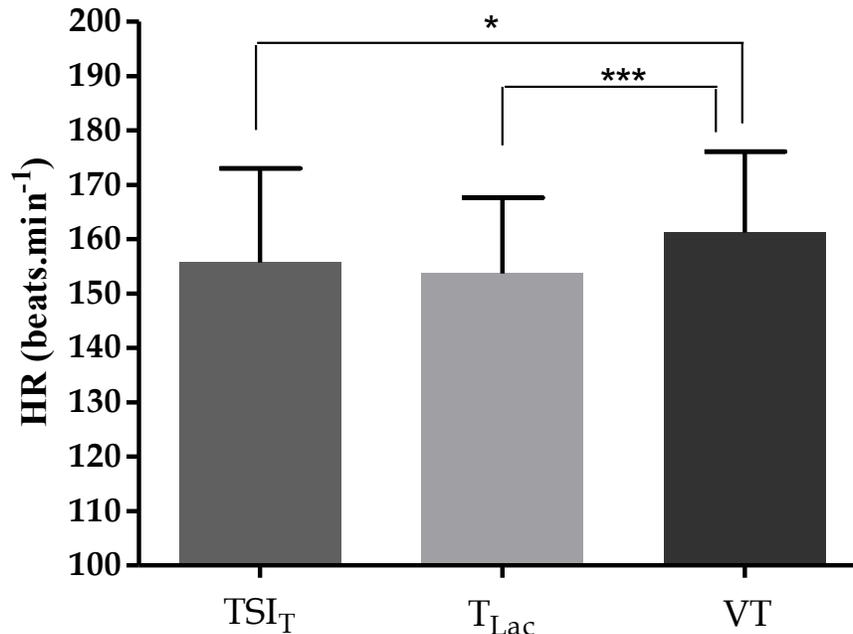
**Figure 3.** (a) Bland-Altman plot displaying mean bias with upper and lower 95% LoA for (a)  $TSI_T$  vs  $T_{Lac}$ ; (b) VT vs  $T_{Lac}$ ; n=31.

Mean HR at each computed threshold displayed a similar pattern of results to workload at each threshold, see Figure 4. Analysis revealed that HR data at computed thresholds were significantly different ( $F = 8.06$ ,  $P \leq 0.001$ ). Tukey *post-hoc* analysis revealed that HR at VT was significantly higher than both  $TSI_T$  ( $P < 0.05$ ) and  $T_{Lac}$  ( $P < 0.001$ ). Upper and lower 95% LoA and mean bias for HR data at computed threshold comparing  $TSI_T$  and  $T_{Lac}$  were 18 to -22 and +2 beats.min<sup>-1</sup>, respectively, and comparing VT and  $T_{Lac}$  were 24 to -8 and +8 beats.min<sup>-1</sup>, respectively.

## DISCUSSION

The aerobic-anaerobic transition is a concept used by physiologists, coaches and athletes to attain training benefits through specifically designed training programmes and for monitoring an athlete's performance during training and racing. Ever since it was first described there has been an ongoing debate as to the specific mechanisms underlying it, and the most appropriate and accurate way to pinpoint it. It is now widely accepted that measuring [BLa] during incremental exercise is the 'gold standard' method, however, this is an invasive measure. Accordingly, non-invasive measures, such as VT and NIRS derived TSI, have been developed.

The aim of the current study was to establish if correlation and association existed between invasive and non-invasive measures. Several key findings were made:  $TSI_T$  occurred at a non-significantly different ( $P > 0.05$ ) mean workload to  $T_{Lac}$ . However, VT occurred at a significantly higher workload than both  $T_{Lac}$  and  $TSI_T$ . There was a small to moderate correlation in the comparisons of  $TSI_T$  with  $T_{Lac}$ , and a slightly stronger correlation in comparisons of VT with  $T_{Lac}$ .



**Figure 4.** Mean HR at each assessed threshold. \* denotes significantly different to VT, \* infers  $P < 0.05$  and \*\*\* infers  $P < 0.001$ . Bars denote SD,  $n=31$ .

Studies in the past have compared different methods of pinpointing the aerobic-anaerobic transition with many contrasting outcomes. The methodology and specific variables compared differ across studies making it difficult to draw comparisons and conclusions. Soller *et al.* (18) described NIRS as a method of estimating  $[H^+]$  and concluded that  $T_{Lac}$  was significantly higher than  $VT_1$  and NIRS  $[H^+]$  threshold. Zhang *et al.* (23) identified two inflexions in muscle oxygenation during incremental exercise. A significant correlation was identified between the first inflexion and  $VT_1$ . Consequently, they concluded that NIRS could be used to detect  $T_{Lac}$ ; however, this was potentially an errant conclusion as they did not show any comparisons to  $[BLa]$  or  $T_{Lac}$ . van der Zwaard *et al.* (19) concluded that during incremental exercise the oxygenation threshold was reproducible and coincided with  $VT_1$ . However, they also documented that  $VT_1$  recorded higher reproducibility than NIRS derived oxygenation threshold and therefore favoured it in the context of determining an aerobic-anaerobic transition. In their opinion, the unknown influence of adipose tissue thickness on absorbance may have led to this conclusion. The finding reported by Grassi *et al.* (8) that the onset of muscle deoxygenation was correlated with OBLA was not supported by the current study. The workload at OBLA ( $308 \pm 33$  W) was significantly higher ( $P < 0.001$ ) than all other computed thresholds in the current study, poorly correlated with  $TSI_T$  ( $r=0.5$ ) and VT ( $r=0.54$ ) and

moderately correlated with  $T_{Lac}$  ( $r=0.82$ ). The current study used the same method, namely a segmented linear regression model to detect  $TSI_T$ ,  $T_{Lac}$  and  $VT$ , and although several methods of threshold detection currently exist, it was decided that using the same mathematical procedure for threshold detection would eliminate potential sources of bias.

A recent study by Driller *et al.* (5) used a commercially available 'wearable lactate threshold sensor' and reported good agreement between it and other traditional markers of  $T_{Lac}$ . However, the study reported by Driller *et al.* (5) focused on the *gastrocnemius* musculature which would not be considered a primary force generating muscle group during cycling. The VL musculature was used in the current study as it has been shown, through EMG analysis, to be a primary force generating muscle during the crank cycle (11). *Vastii* musculature are primarily active during the pedal down-stroke with peak activity recorded around  $55^\circ$ , in contrast, the *gastrocnemius* musculature is only recruited for a short period after  $90^\circ$ , peaking around  $107^\circ$  (17).

In the current study, during the GxT the only variable which participants had control over was cycling cadence and it was a study requirement to maintain cadence within a  $5 \text{ rev}\cdot\text{min}^{-1}$  range between  $75$  and  $95 \text{ rev}\cdot\text{min}^{-1}$ . Gotshall *et al.* (7) investigated the effect of 3 different pedal cadences during  $200 \text{ W}$  cycling on haemodynamic responses. Their data suggested that increasing the cadence resulted in a greater metabolic cost with the haemodynamic response favouring higher cadence. It was proposed that the action of the skeletal muscle pump improved across cadence resulting in increased muscle blood flow and cardiac output. Accordingly, an analysis was performed (data not shown) to determine if variation within this range ( $75$  to  $95 \text{ rev}\cdot\text{min}^{-1}$ ) had any effect on the workload and  $TSI$  at which each threshold occurred. No obvious pattern was uncovered, consequently, we concluded that the narrow cadence range used in the current study minimised any significant effect of cadence on the haemodynamic response and thus had little impact on transition detection.

The pattern of results obtained in the current study can be explained by a simple model assessing supply, demand and consumption of oxygen in the exercising musculature from a cellular and tissue level. Exercising at low intensities, such as, during the early stages of a GxT, relies primarily on the recruitment of slow oxidative aerobic fibers. During this phase there is no increase in  $[BLa]$  as slow oxidative fibers do not produce excessive amounts of lactate, are consumers of lactate and, consequently, the rate of lactate production equals the rate of lactate clearance. Under such conditions the supply of  $O_2$  equals demand through appropriate increases in cardiac output and local vasodilation. As exercise intensity progressively increases,  $O_2$  consumption increases and this can be seen through a steady decline in  $TSI$  as the musculature becomes deoxygenated. This decline in  $TSI$  eventually reaches a breakpoint and plateaus, indicative of  $O_2$  delivery reaching a maximum. As a result of this deoxygenation and an imbalance of  $O_2$  delivery relative to uptake and consumption, additional recruitment of anaerobic fibers occurs. Firstly, fast oxidative glycolytic fibers and eventually fast fatigable fibers which are potent producers of lactic acid. There is a resultant imbalance in the rates of lactate production and clearance, and, consequently  $[BLa]$  increases giving rise to  $T_{Lac}$ . The fact that  $TSI_T$  does not occur at a significantly different workload to  $T_{Lac}$  could potentially indicate

that the limiting of O<sub>2</sub> delivery occurs in unison with, or is closely related to, the increase in [BLa]. However, the poor correlation and wide 95% LoA identified in the current study between TSI<sub>T</sub> and T<sub>Lac</sub> indicates that other factors may also influence this process. Unlike T<sub>Lac</sub> and VT, which are reflective of whole body status, TSI<sub>T</sub> is not a whole-body measure and uses shallow light penetration to sample a very small volume of tissue under investigation. In addition, while VL is a primary force producing muscle during cycling, other involved muscle groups may influence production of lactate and therefore non-metabolic CO<sub>2</sub> and thus have an influence on the comparison of NIRS derived threshold with whole body measures.

The increase in [BLa] has a knock-on effect on ventilation and gas exchange variables. Lactic acid has a pKa of 3.87 and rapidly dissociates in body fluids (22). The resulting H<sup>+</sup> ions are buffered by bicarbonate to form carbonic acid which dissociates into CO<sub>2</sub> and H<sub>2</sub>O. Therefore, in addition to metabolically produced CO<sub>2</sub>, this non-metabolic production of CO<sub>2</sub> is detected by ventilatory chemoreceptors. Changes in VE as a function of VO<sub>2</sub> remain steady and linear during the aerobic phase as there is no non-metabolic production of CO<sub>2</sub>. During the anaerobic phase production of non-metabolic CO<sub>2</sub> increases as H<sup>+</sup> ions dissociated from lactic acid are buffered. This increase in CO<sub>2</sub> production is manifested as an increase in VE but not in VO<sub>2</sub>. VE and VO<sub>2</sub> become desynchronised giving rise to a detectable threshold in VE/VO<sub>2</sub> data; namely, VT. In the current study, the workload at which VT occurred was significantly higher than the workload at T<sub>Lac</sub>. This can be explained in the context of the underlying physiological mechanisms involved. Increases in lactic acid occur at the level of the exercising muscle. There is a time-lag before the resultant increase in non-metabolic CO<sub>2</sub> can be detected by central chemoreceptors. The blood must travel through the circulatory system, diffuse across the blood brain barrier and be detected by central chemoreceptors before a change in ventilation can take place. Although VT was easily detectable, VT occurs after an appreciable increase the production of lactate. In endurance based activities, the athlete aims to limit the effects of fatigue and remain in an aerobic zone of energy production. Therefore, in this context, VT may not be as useful an indicator of the aerobic-anaerobic transition, since it appears significantly after the non-linear increase in blood lactate occurs.

Endurance based activities require athletes to sustain a relatively high exercise intensity for extended period of times. Athletes training for endurance events frequently maintain exercise HR in designated individualised zones based on laboratory based GxT to maximise their adaptive responses. Results of the current study imply that usage of [BLa] correlated HR zones remain the most effective method of training personalisation. The current wide limits of agreement depicted for TSI<sub>T</sub> vs T<sub>Lac</sub> and VT vs T<sub>Lac</sub> (Figure 3) suggest that non-invasive measures could potentially lead to inaccuracies for threshold identification on an individual basis. The wide limits of agreement observed following analysis of HR data at each detected threshold confirm this concern. Therefore, we would caution that usage of non-invasive threshold assessment could potentially inaccurately determine critical exercise intensities to effectively attain the desired training benefits.

The use of NIRS technology to detect critical exercise intensities has several limitations which have been highlighted in the past. Firstly, as indicated by van der Zwaard *et al.* (19) variations

in adipose tissue thickness between participants can have an effect on the amplitude of NIRS signals. Adipose tissue thickness in the path of light penetration and detection was not measured and therefore its influence on the amplitude of NIRS signals could not be quantified. There are mixed consensus regarding the specific placement of NIRS devices over the musculature. Mizuno *et al.* (12) and Koga *et al.* (10) stated that differences exist in the NIRS signal between the proximal and distal ends of the VL during static knee extension and cycling, respectively. However, Kime *et al.* (9) and Boone *et al.* (2) reported no difference in NIRS signal between the proximal and distal portions of the VL during cycling ramp exercise. The MOXY NIRS unit used in the current study is relatively new to the consumer market and a small body of research exists on its use in laboratory based environments. Little is known about the exact specifications and algorithms which MOXY uses to measure TSI/ SmO<sub>2</sub>. Previous studies using NIRS as a means of measuring haemodynamic responses to exercise have used either a laboratory based (Hammatsu NIRO-200NX) or wireless portable unit (Artinis Portomon system) units. One problem encountered during the current study was the fact that MOXY reports data as 'SmO<sub>2</sub>' whereas studies using other systems report data as TSI, HHb, O<sub>2</sub>Hb or variations of the same. Following a review of the literature it was revealed that TSI and SmO<sub>2</sub> are in fact the same and give an indication of the ratio of HbO<sub>2</sub> to total Hb mass (tHb) in the musculature of interest expressed as a percentage. Cornachione *et al.* (4) stated that 'SmO<sub>2</sub>, which has also been referred to the tissue oxygenation index and tissue saturation index, is considered a measure of the O<sub>2</sub> saturation within the capillaries of subdermal skeletal muscle. As such, changes in SmO<sub>2</sub> during exercise are a direct reflection of the balance between the availability of oxygenated blood and the use of O<sub>2</sub> by the active muscles'. Therefore, TSI was used in the current study as it is a more universal measure, and in the future, it would be easier to compare apparatus and studies if the majority report data as TSI. A useful piece of research would be to compare the current NIRS unit to a laboratory standard NIRS unit, or to other portable NIRS units that have been used extensively in the scientific research to date. It would also prove useful to carry out a test retest study to assess the reliability of these various devices and methods of threshold detection.

In conclusion, the current study evaluated association of VT and TSI<sub>T</sub> with T<sub>Lac</sub> during graded incremental exercise. No significant differences were observed for either workload or heart rate data at TSI<sub>T</sub> compared to T<sub>Lac</sub>, possibly suggesting that limitations in O<sub>2</sub> delivery may be closely linked to the aerobic-anaerobic transition. However, weak correlation between the two thresholds suggests that other factors may also have an influence on this process. VT occurred at a significantly higher workload and HR to T<sub>Lac</sub> which would prove less useful to an athletic population as it gives an indication of when an aerobic-anaerobic transition has occurred rather than when it is occurring. Therefore, it would appear that neither TSI<sub>T</sub> nor VT are suitable non-invasive determinant of the aerobic-anaerobic transition when both are simultaneously compared to the invasive measure of T<sub>Lac</sub>.

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