Paper

Validity of a newly developed method to predict accumulated oxygen deficit

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Rowers transfer power output (PO) to the handle with the movement of their body toward back and forth which is defined as internal work, however, accumulated oxygen deficit (AOD) is estimated from only PO. This study aimed to investigate the validity of a newly proposed method, which estimates oxygen demand from PO and internal work, by comparing the relationships between AOD and blood lactate related parameters. Eight male university rowers performed a 2-minute supramaximal test, and blood lactate samples were obtained for 90-min of the following recovery period to evaluate blood lactate concentration at the end of the exercise ($[La]_{b(0)}$), its peak value ($[La]_{b\ peak}$), and a quantity of lactate accumulation (Q_{LaA}). Higher AOD was observed in the new method relative to the conventional method (change of mean, 13.8 %; 90 % confidence limits (CL), ± 5.1 %). Furthermore, the AODs estimated by the both conventional and the new methods were likely to correlate with Q_{LaA} (r = 0.65; 90 % CL, ± 0.43 and r = 0.59; 90 % CL, ± 0.47, respectively), however, unclear to correlate with $[La]_{b(0)}$ (r = 0.12; 90 % CL, ± 0.62 and r = -0.03; 90 % CL, ± 0.63, respectively) and $[La]_{b\ peak}$ (r = 0.13; 90 % CL, ± 0.62 and r = 0.10; 90 % CL, ± 0.62, respectively). In conclusion, our results suggest that the both of estimation methods provide AOD being likely to show a moderate correlation with Q_{LaA}, however, the value is higher in the new method.

Key words : Accumulated oxygen deficit; Stroke rate; Acceleration sensor; Quantitative lactate production

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1. Introduction

Rowing is a whole-body exercise modality generating a propulsion force defined as external work, with the rower's body moving back and forth defined as internal work (Hofmijster et al., 2009). The external work is utilized to calculate power output (PO) on ergometer, meanwhile, the internal work is not included into the PO calculation, and only number of strokes per minute (stroke rate: SR) is provided. Moreover, SR increases gradually and linearly with PO in submaximal intensity domain, however, it reaches significantly high rate in supramaximal intensity (**Fig. 1** (A)). This rowing's exercise specificity makes it difficult to assess the contribution of anaerobic energy in supramaximal exercise (Shirai and Nabekura, 2016). Anaerobic energy contribution is assessed by the maximal accumulated oxygen deficit (MAOD) method proposed by Medbø et al. (1988). In this method, anaerobic energy production is quantified by subtracting a measured oxygen uptake ($\dot{V}O_2$) from a predicted $\dot{V}O_2$ demand during a supramaximal exercise bout, and is termed as accumulated oxygen deficit (AOD) (Medbo, 1996; Medbø et al., 1988; Medbø and Tabata, 1993). In the case of rowing, the PO has been generally used to predict a $\dot{V}O_2$ demand (Russell et al., 1998; Pripstein et al., 1999). However, as mentioned above, PO is calculated from the external work, and the internal work is ignored in $\dot{V}O_2$ demand estimation. In addition, the internal work and the energy spent for this kind of work are reported to be higher at higher SR (Di Prampero et al., 1971; Hofmijster et al., 2009), suggesting that the conventional MAOD method underestimates $\dot{V}O_2$ demand during supramaximal rowing exercise bout which accompanies higher SR. To address this issue, we had designed and proposed a new

MAOD method that predicts $\dot{V}O_2$ demand from a PO and a tri-accelerometer output to assess both external and internal work, respectively (Shirai et al., 2016; Shirai et al., 2014; Shirai and Nabekura, 2016). The $\dot{V}O_2$ demand estimated by the new method was similar to that predicted by a SR-modified MAOD method, which minimized the effect of increased internal work in supramaximal intensity by increasing SR linearly with PO through submaximal to supramaximal intensities (Shirai and Nabekura, 2016). However, it has not been investigated how the AOD estimated by the new method relates with anaerobic metabolism related parameters.

The anaerobic energy production in an exercise bout lasting ~2-min predominantly depends on glycolysis (Bangsbo et al., 1990; Bangsbo, 1998), which results in lactate production, and several authors proposed end-exercise blood lactate concentration ($[La]_{b\ (0)}$) and/or its highest value obtained during the recovery period ($[La]_{b\ peak}$) as an index of nonoxidative glycolytic energy contribution during exercise (Saeki et al., 1999a, b; Craig et al., 1995; Saeki et al., 1998). While, most studies have not found significant correlations between $[La]_{b\ (0)}$ or $[La]_{b\ peak}$ and AOD (Medbø et al., 1988; Scott et al., 1991; Medbø, 1993; Pripstein et al., 1999; Bishop et al., 2002). These results were to be expected because $[La]_{b\ (0)}$ or $[La]_{b\ peak}$ reflect not only muscle lactate production but also lactate exchange between muscle cells and other tissues (van Hall et al., 2010).

Several studies reported that a mathematical model to evaluate $[La]_b$ kinetics during recovery period after an intense exercise bout was available to quantify the amount of lactate produced more precisely compared to blood lactate concentration, and this method has been used widely in various exercise modalities (Zouloumian and Freund, 1981b, a; Freund and Zouloumian, 1981b, a; Freund et al., 1986; Bret et al., 2003; Bret et al., 2013; Messonnier et al., 2006; Messonnier et al., 1997). More recently, Maciejewski et al. (2013) demonstrated that the quantity of lactate accumulation (Q_{LaA}) calculated with this model showed a correlation with AOD, even though the AOD was estimated by the conventional MAOD method in rowing (r = 0.85).

The purpose of this study was to investigate the validity of the new method estimating AOD in rowing by comparing the relationships between AOD and blood lactate related parameters ($[La]_{b(0)}$, $[La]_{b\ peak}$ and Q_{LaA}). Our hypothesis was that the AOD estimated by the new method would correlate with Q_{LaA}, which would show a stronger relationship with Q_{LaA} relative to the conventional method, but not with $[La]_{b\ (0)}$ and $[La]_{b\ peak}$. This study would be expected to contribute for further studies which investigate the anaerobic metabolism in 2000 m simulation test, because SR, or internal work in other words, during the test is high and not constant (Steinacker, 1993), therefore, a valid method estimating $\dot{V}O_2$ demand including internal work is necessary.

2. Methods

2. 1. Subjects

According to the previous study investigating the relationship between AOD and Q_{LaA} in rowing (Maciejewski et al., 2013), correlation coefficient (r) of 0.85 (n = 9) was reported, corresponding very large in effect size (ES, 0.7 to <0.9). In this study, to investigate the relationship with the same level of ES, a sample size of 7 subjects was recommended by a spread sheet estimating sample size for magnitude-based inference (Hopkins, 2006). We recruited eight male university rowers (mean height: 1.75 ± 0.05 m, mean weight: 70.5 ± 10.1 kg, and mean age: 19.8 ± 0.9 years), including 7 prize-winners of national university regatta, since the risk of drop out was considered. The personal record of 2000 m time trial on rowing ergometer was 410 ± 13 sec (range: 390 ~ 426 sec), and the performance level of the 6 subjects reached the standard criteria (420 sec) which was established by Japan amateur rowing association to give a qualification to compete at the national level regattas. Subjects were not allowed to perform strenuous exercises or ingest alcoholic and caffeine-containing beverages from 24 h before trials, and the last meal on the day of the trial was finished at least 2 h before the start of trials. The general rowing ergometer (Rowing Ergometer Type D, Concept II) was used, and subjects were instructed to adjust intensities (PO and SR) with confirming the display mounted on the ergometer when these parameters were designated. The experiment was conducted in accordance with the concepts of the Declaration of Helsinki regarding the participation of human subjects, and was approved by the Human Research Ethics Committee of the University of Tsukuba (体 26 - 9). Before giving their written consent,

subjects were informed of the objectives, possible discomfort, and potential benefits and risks of the experiment.

2. 2. Experimental design

Fig. 1 illustrated the process estimating oxygen demand in the conventional method (A) and the newly proposed method (B). To estimate AOD by the both methods, subjects performed 1) PO-incremental test assessing the individual linear relationship between PO and $\dot{V}O_2$, 2) SR-incremental test to assess the individual linear relationship between tri-accelerometer output and $\dot{V}O_2$ when increasing SR while keeping PO constant, and 3) 2-min supramaximal test. These tests were conducted at least 3 days apart. The $\dot{V}O_2$ demand in the 2-min supramaximal test was estimated by the conventional

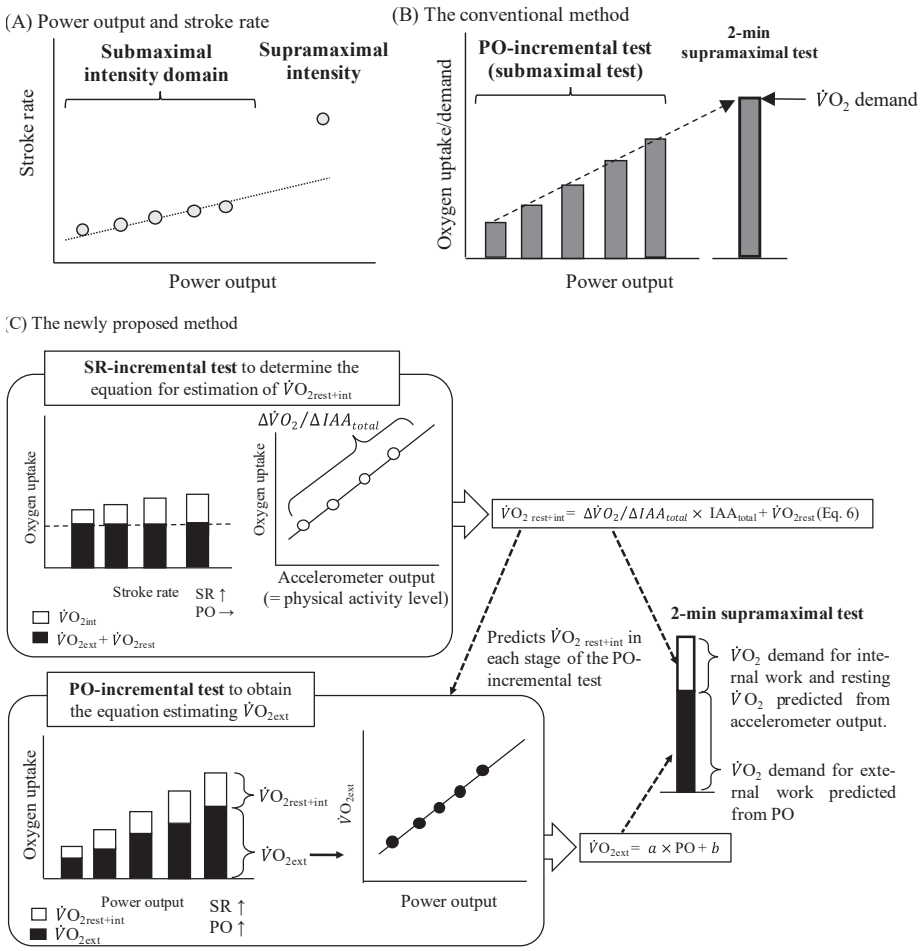

Fig 1. The schematic of the relationship between power output (PO) and stroke rate (SR) in each exercise intensity domain (A), oxygen demand ($\dot{V}O_2$ demand) estimation in the conventional method (B), and the newly proposed method (C). SR increases with PO gradually and linearly in submaximal intensity domain, however, SR in supramaximal intensity is significantly higher, meaning that the internal work, which do not be measured as PO, also increases drastically in supramaximal intensity (A). In the conventional method, $\dot{V}O_2$ demand was estimated by a linear equation obtained from the relationship between PO and oxygen uptake ($\dot{V}O_2$) in each stage of PO-incremental test (B). In the new method (C), $\dot{V}O_2$ demand was theoretically divided into $\dot{V}O_2$ demand for internal work (white bar in right panel) and for external work (black bar in right panel). To estimate $\dot{V}O_2$ demand for internal work, a linear equation (Eq.6) was calculated from a relationship between accelerometer output and $\dot{V}O_2$ obtained in SRincremental test (upper left panel of C) which increased only internal work (white bars) by increasing SR while keeping PO constant (corresponding to external work, black bars). Eq. 6 was also used to predict $\dot{V}O_2$ for internal work in each stage of PO-incremental test by assigning accelerometer output measured in each stage (white bars in lower left panel of C), and the rest of measured $\dot{V}O_2$ in each stage was defined as $\dot{V}O_2$ for external work (black bars in lower left panel of C). An estimation equation to predict $\dot{V}O_2$ demand for external work is calculated from the relationship between PO and $\dot{V}O_2$ for external work. These two equations were used to estimate $\dot{V}O_2$ demand from accelerometer output and PO in the supramaximal test, respectively

method, which used the liner relationship obtained from the PO-incremental test, and the new methods, which used the both linear relationships obtained from the PO-incremental test and SR-incremental test (see also further descriptions).

2. 2. 1. PO-incremental test session

PO-incremental test was constructed by a submaximal and a maximal test. Participants reported to the laboratory 45 min before starting a normalized warm-up comprising 10 min of light stretching exercise, and 10 min rowing exercise warm-up divided into 5-min of freely chosen intensity and 5-min of designated intensity equal to the intensity of the initial stage in the submaximal test. After the warm-up, a 15-min passive rest followed on the rowing ergometer. $\dot{V}O_2$ and tri-accelerometer output in resting status was recorded, then the submaximal test was commenced. Four-min exercise bouts, separated by 2-min rest to collect blood samples from finger tips, were repeated 7 times beginning from 40 % of average PO at the recent 2000 m time trial (PO_{2000}) and increased by 5 % PO_{2000} in each stage. Maximal test was started following 10-min of rest after submaximal test from 70 % PO_{2000}, and increased in increments of 5 % PO_{2000} per minute until exhaustion. PO, $\dot{V}O_2$ and tri-accelerometer output were measured in 1-min intervals at the last minute of submaximal stages and consecutive 1-min in the maximal test. The relationship between PO and blood lactate concentration ($[La]_b$) was analyzed by the log-log transformed method (Beaver et al., 1985) to determine PO corresponding to blood lactate threshold (PO_{LT}).

2. 2. 2. SR-incremental test

SR-incremental test, followed by the normalized warm-up, was conducted on another experimental day. Subjects were instructed to increase SR (24, 28, 32 and 36 strokes/min) with maintaining PO as the PO_{LT} determined in the PO-incremental test. The duration of each stage was 4-min, separated by 2-min rest, and $\dot{V}O_2$ and tri-accelerometer outputs were measured during the last minute of each stage. Subjects were asked to control PO and SR with monitoring the display mounted on the ergometer.

2. 2. 3. Supramaximal test

Supramaximal test, followed by the normalized warm-up, was conducted on another experimental day. Subjects kept 5 min of resting status on ergometer, then started the trial with all-out effort. PO, tri-accelerometer output and $\dot{V}O_2$ were recorded during the exercise bout. Blood samples were collected before the trial ($[La]_{b\ pre}$), at the end of the trial ($[La]_{b\ (0)}$), and thereafter at 0.5-, 1-, 1.5-, 2-, 2.5-, 3-, 3.5-, 4-, 4.5-, 5-, 6-, 8-, 10-, 12-, 15-, 20-, 25-, 30-, 40-, 50-, 60-, 70-, 80-, and 90-min of recovery.

2. 3. Measurements

Expired air was collected into a Douglas bag at 1-min intervals, and the fractions of O_2 and CO_2 were analyzed by an auto gas analyzer (Aeromonitor AE310-s, Minato medical, Japan) which was pre-calibrated by a gas mixture of known concentration. The volume of expired gas was measured by a dry gas test meter (Dry gas test meter type DC, Shinagawa, Japan). Heart rate was recorded by a heart rate monitor (S610i, Polar, Finland).

Accelerometer outputs were measured by a tri-accelerometer sensor (9 axes-wireless motion sensor, Logical product, Japan) which has ±16 *G* (gravitational acceleration) for detection range, and 57 mV/G for detection sensitivity. The measured data was stored in a flash memory built in the sensor and transferred to a laptop after exercise to conduct high-pass filtering procedure (0.3-25 Hz) by a software developed by the supplier of the sensor (LP-WSAPP01). **Figure 2** illustrates the mounted position of the sensor and the corresponding directions of its axes. The sensor was mounted on the upper part of subjects' back to detect rowing's specific motion. The measuring axes, x, y and z, corresponded to crosswise direction, head-to-low back direction and vertical direction against the contact surface of the sector, respectively, thus the data was measured in a local co-ordinate system rather than global co-ordinate system. The transferred data was integrated in 1-min interval to indicate physical activity level in each axis (integral absolute value of accelerometer output: IAA).

$$IAA_X = \int_{t=0}^{T} |a_x| dt \quad \text{(Eq. 1)}$$

$$IAA_y = \int_{t=0}^{T} |a_y| dt \quad \text{(Eq. 2)}$$

$$IAA_z = \int_{t=0}^{T} |a_z| dt \quad \text{(Eq. 3)}$$

Where a_x, a_y and a_z indicate accelerometer output obtained in each axis of the sensor. The sum of IAAs was calculated as an indicator of total physical activity level

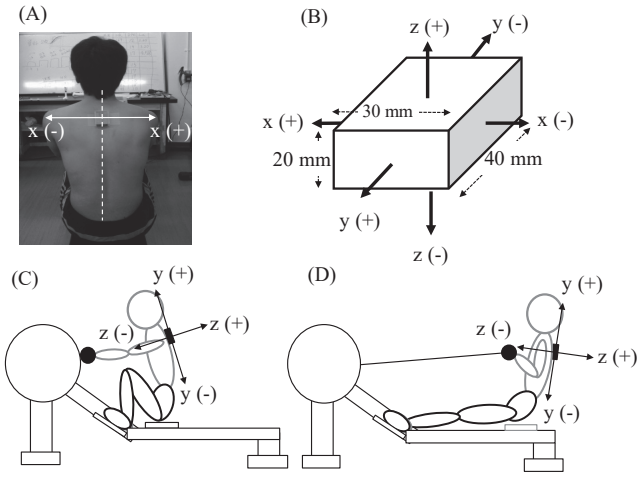

Fig 2. The mounting position of a tri-accelerometer sensor (A), the appearance of the sensor and measuring axes (B), and the relationship between posture and corresponding directions of measuring axes (C and D).

(total integral absolute value of accelerometer output: IAA_{total}).

$IAA_{total} = IAA_x + IAA_y + IAA_z$ (Eq. 4)

The IAA_{total} was expressed by using arbitrary unit (a.u.) because no defined criteria were provided in the previous studies.

2. 4. Calculations

2. 4. 1. $\dot{V}O_2$ demand and AOD

For the conventional MAOD method, $\dot{V}O_2$ demand was predicted by extrapolating an individual linear equation between PO and $\dot{V}O_2$ in the submaximal test until it intersects with the PO measured in supramaximal test (**Fig.1** (B)). AOD was calculated by subtracting measured $\dot{V}O_2$ during supramaximal test from the predicted $\dot{V}O_2$ demand (Hill and Vingren, 2011).

For the new MAOD method, the prediction of $\dot{V}O_2$ demand was conducted with the following assumptions suggested by Winter (1979): $\dot{V}O_2$ during exercise was constituted by resting $\dot{V}O_2$ and $\dot{V}O_2$ for exercise load ($\dot{V}O_{2exer}$), and $\dot{V}O_{2exer}$ can be divided into $\dot{V}O_2$ for external and internal work ($\dot{V}O_{2external}$ and $\dot{V}O_{2internal}$, respectively).

$\dot{V}O_2 = \dot{V}O_{2rest} + \dot{V}O_{2exer}$

$= \dot{V}O_{2rest} + \dot{V}O_{2int} + \dot{V}O_{2ext}$ (Eq 5)

$\dot{V}O_2$ during exercise moving rower's body toward back and forth without PO (zero external work) can be assumed as that a resting $\dot{V}O_2$ ($\dot{V}O_{2rest}$) corresponds to a baseline and $\dot{V}O_2$ is determined by the amount of the internal work performed by moving rower's body toward back and forth ($\dot{V}O_{2intenrnal}$, Secher et al., 1983). In previous study, IAA_{total} was used to assess $\dot{V}O_2$ during locomotion, including displacements of the center of gravity, such as running and walking (Iwashita et al., 2003). Moreover, we have previously demonstrated that IAA_{total} and $\dot{V}O_2$ increased with stroke rate, and that they showed a linear relationship (Shirai et al., 2014; Shirai and Nabekura, 2016; Shirai et al., 2014). From these evidences, the equation predicting $\dot{V}O_{2internal}$ from IAA_{total} with $\dot{V}O_{2rest}$ as a baseline was adopted in this study (**Fig. 1** (C), Eq. 6).

$$\dot{V}O_{2\,rest+int}(L\cdot min^{-1}) = \frac{\Delta \dot{V}O_2}{\Delta IAA_{total}} \cdot IAA_{total} + \dot{V}O_{2\,rest} \quad \text{(Eq. 6)}$$

where $\Delta\dot{V}O_2$ / ΔIAA_{total} is the slope of the linear relationship between IAA_{total} and $\dot{V}O_2$ measured in SR-incremental test in accordance with Shirai and Nabekura (2016). $\dot{V}O_{2rest+int}$ in each stage of submaximal test was predicted with equation 6, then $\dot{V}O_2$ for the external work ($\dot{V}O_{2external}$) in each stage was calculated by subtracting $\dot{V}O_{2rest+int}$ from measured $\dot{V}O_2$. The $\dot{V}O_{2external}$ and PO were used to determine an equation to predict the $\dot{V}O_{2external}$ from PO (**Fig. 1** (C)). These equations that predict $\dot{V}O_{2rest+int}$ and $\dot{V}O_{2external}$ were used to estimate $\dot{V}O_2$ demand in the supramaximal exercise from PO and IAA_{total}, then AOD was calculated by subtracting measured $\dot{V}O_2$ from the predicted $\dot{V}O_2$ demand.

2. 4. 2. Q_{LaA}

Individual blood lactate recovery curves after the supramaximal test were fitted by the biexponential time function.

$[La]_{b(t)} = [La]_{b(0)} + A_1(1-e^{-\gamma 1 t}) + A_2(1-e^{-\gamma 2 t})$ (Eq. 7)

where $[La]_{b(0)}$ and $[La]_{b(t)}$ ($mmol \cdot L^{-1}$) are lactate concentrations in arterialized capillary blood measured at the onset of recovery and at a given recovery time, respectively; concentration parameters A_1 and A_2 (mmol/L) are the amplitudes of the exponential functions; and γ_1 and γ_2 (per minute) are the velocity constants that describe lactate exchange and removal capacity, respectively (Freund and Zouloumian, 1981a; Freund et al., 1986). The blood lactate recovery curves were fitted to equation 7 by nonlinear regression on the Matlab software to determine the values of A_1, A_2, γ_1 and γ_2, with $[La]_{b(0)}$

being an experimental measurement.

$[La]_{b\,peak}$ occurs almost concomitantly with equilibrium between muscle and blood lactate concentrations during recovery (Freund and Zouloumian, 1981b; Freund et al., 1986). Consequently, the Q_{LaA} (in mmol) in the total lactate distribution space (V_{TLS}) at $[La]_{b\,peak}$ can be estimated according to the following equation:

$$Q_{LaA}\ at\ [La]_{b\,peak}=[La]_{b\,peak} \cdot V_{TLS} \text{ (Eq. 8)}$$

where V_{TLS} is the total lactate distribution space (muscles previously involved in exercise, blood, inactive muscles and kidney), which was calculated as 0.6 L per kg of body weight (Maciejewski et al., 2013; Freund and Zouloumian, 1981b; Zouloumian and Freund, 1981a). However, the quantity of lactate predicted by equation 8 is lower than that reached at the end of exercise, because $[La]_{b\,peak}$ occurs after several minutes of recovery, during which lactate removal is elevated. This quantity of lactate removed (Q_{LaR}, in mmol) from the end of exercise ($[La]_{b\,(0)}$) to $[La]_{b\,peak}$ can be estimated as follows (Maciejewski et al., 2013)

$$Q_{LaR}=\{([La]_{b\,peak}+[La]_{b\,(0)})/2\} \cdot \gamma_2 \cdot t[La]_{b\,peak} \cdot V_{TLS} \text{ (Eq. 9)}$$

where $t[La]_{b\,peak}$ is the time to reach the maximal lactate concentration during recovery. Therefore, Q_{LaA} at the end of exercise can be expressed as follows:

$$Q_{LaA}=Q_{LaA}\ at\ [La]_{b\,peak}+Q_{LaR} \text{ (Eq.10)}$$

2. 5. Statistical analysis

Values were presented as means ± standard deviations (SD), unless otherwise stated. The effects were estimated in percent unit via log-transformation, and uncertainty in the estimate was expressed as 90 % confidence limits (Batterham and Hopkins, 2006). The effect size (ES), which represents the magnitude of the difference between the two methods in term of SD, was calculated from log-transformed data by dividing the change in the mean by the average SD of the two methods. Thresholds for interpreting the ES were as follows: <0.2, trivial; 0.2 to <0.6, small; 0.6 to 1.2, moderate; >1.2, large (Hopkins et al., 2009). The outcome for $\dot{V}O_2$ demand and AOD were evaluated with the nonclinical version of magnitude-based inference: the effect was deemed unclear if the chance of positive and negative effect was sufficiently high (> 5 %), respectively; while the effect was deemed as clear and reported the qualitative probability that the true magnitude was at least as large as the observed magnitude. The quantitative probability was calculated and expressed as follows: <0.5%, mostly unlikely; 0.5% to <5%, very unlikely; 5% to <25%, unlikely; 25% to <75%, possibly;

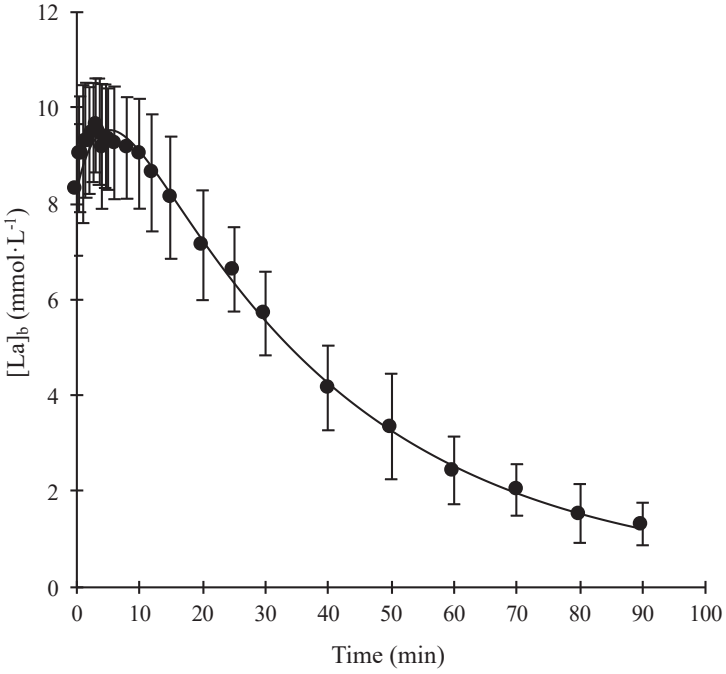

Fig 3. Means (± standard deviation) of blood lactate concentration ($[La]_b$) obtained during recovery period after 2-min all-out exercise. The curve described was obtained by fitting the equation 7. to the mean values.

75% to <95%, likely; 95% to <99.5%, very likely; >99.5%, most likely (Hopkins, 2007). The linearity between PO and $\dot{V}O_2$, and IAA_{total} and $\dot{V}O_2$ was evaluated by linear regression analysis, and its linearity was interpreted by Pearson's correlation coefficient (*r*). The relationships between variables were interpreted with *r* that was converted into 90% confidence limits using a spreadsheet (Hopkins, 2007). The relationship between variables deemed unclear if the chances that the true value of *r* is positive and negative were sufficient (> 5 %), respectively, while the relationship was deemed as clear and report the quantitative probability in accordance with the criterion described above. ES for correlation analysis was evaluated with following scale: <0.1, trivial; 0.1 to <0.3, small; 0.3 to <0.5; moderate; 0.5 to <0.7, large; 0.7 to <0.9, very large; >0.9, nearly perfect, when the relationship was deemed as clear (Hopkins et al., 2009).

3. Results

$\dot{V}O_{2rest}$ and $\dot{V}O_{2max}$ were 301.3 ± 45.0 and 4072.6 ± 465.8 $mL \cdot min^{-1}$, respectively. Strong correlations were found between PO and $\dot{V}O_2$ in the submaximal ($\dot{V}O_2$ = 15.4 ± 2.5 $mL \cdot min^{-1} \cdot W^{-1}$ × PO + 110.4 ± 495.9 $mL \cdot min^{-1}$, *r* = 0.96~1.00).

Table 1 shows PO, SR, and IAA_{total} obtained in the SR-incremental test. PO showed almost the same values in every SR condition, meanwhile, IAA_{total} and $\dot{V}O_2$ increased with SR and showed strong linear relationships ($\dot{V}O_2$ = 20.1 ± 7.3 $mL \cdot min^{-1} \cdot a.u.^{-1}$ ×IAA_{total}+ 1789.0 ± 376.4 $mL \cdot min^{-1}$, *r* = 0.91~1.00), and the slope of the line was defined as $\Delta\dot{V}O_2/\Delta IAA_{total}$. PO and $\dot{V}O_{2external}$, which indicates the difference between measured $\dot{V}O_2$ and predicted $\dot{V}O_{2internal}$ in the submaximal test, showed strong linear relationships ($\dot{V}O_{2internal}$ = 13.2 ± 3.1 $mL \cdot min^{-1} \cdot W^{-1}$ × PO + 717.1 ± 515.1 $mL \cdot min^{-1}$, *r* = 0.95〜1.00).

PO, SR and IAA_{total} during supramaximal test were 405.4 ± 38.5 watt, 40.4 ± 0.9 strokes/min and 69.4 ± 26.5 a.u., respectively. **Table 2** illustrates the variables obtained from the supramaximal test. $\dot{V}O_2$ demand predicted by the new method was very likely higher compared to that predicted by the conventional method (% of change in mean = 6.4 %; ES = 0.33; 90 % confidence limits, ± 0.10). Likewise, AOD estimated by the new method was very likely higher compared to that estimated by the conventional method (13.8 %, ES = 0.40; 90 % confidence limits, ± 0.14). The most likely and nearly perfect linear relationship was found between AODs estimated by each method (r = 0.98; 90 % confidence limits, ± 0.05).

Fig. 3 illustrates $[La]_b$ curve during recovery period, which showed rapid increase from the end of exercise ($[La]_{b\,(0)}$, 8.29 ± 1.37 $mmol \cdot L^{-1}$) and reached peak value ($[La]_{b\,peak}$, 9.75 ± 1.8 $mmol \cdot L^{-1}$) at 3.1 ± 1.5 min, thereafter it decreased gradually to reach almost the same value as that obtained before exercise ($[La]_{b\,rest}$, 1.28 ± 0.27 $mmol \cdot L^{-1}$) at 80- and 90-min (1.54 ± 0.61 and 1.32 ± 0.44 $mmol \cdot L^{-1}$, respectively). The mathematical model fitted well to the individually measured $[La]_b$ (R^2 = 0.969〜0.996), and the parameters obtained by the model were also reported in **Table 2**.

Fig. 4 illustrates the relationship between the AODs and blood lactate related parameters. The relationship between AOD and $[La]_{b\,(0)}$ was unclear in each method (*r* = 0.12, *p* = 0.77, 90 % confidence limits ± 0.62 and *r* = -0.03, *p* = 0.995, confidence limits ± 0.63 in the conventional and new methods, respectively). Likewise, the relationship between AOD and $[La]_{b\,peak}$ was unclear in each method (*r* = 0.13, *p* = 0.768, confidence limits ± 0.62 and *r* = 0.10, *p* = 0.81, confidence limits ± 0.62). However, the AOD and Q_{LaA} showed a moderate relationship in both methods (*r* = 0.65, *p* = 0.08, confidence limits ± 0.43 and *r* = 0.59, *p* = 0.12, confidence limits ± 0.47, respectively).

Table 1. Power output, stroke rate, IAA_{total}, and $\dot{V}O2$ in different stroke rate conditions (n = 8).

	24 strokes·min^{-1}	28 strokes·min^{-1}	32 strokes·min^{-1}	36 strokes·min^{-1}
Power output (W)	183.6 ± 25.2	183.2 ± 26.2	183.7 ± 25.3	184.1 ± 26.9
Stroke rate (strokes·min^{-1}	23.9 ± 0.3	27.7 ± 0.2	32.1 ± 1.1	34.8 ± 1.4
IAA_{total} (count·min^{-1})	40.1 ± 2.7	46.3 ± 3.7	54.7 ± 3.8	60.9 ± 2.6
$\dot{V}O_2$ (mL·min^{-1})	2697.9 ± 357.7	2727.5 ± 325.9	2856.2 ± 349.9	3047.0 ± 362.6

Table 2. MAerobic and anaerobic energy expenditure, blood lactate response and its related parameters in the supramaximal exercise (n = 8).

	Conventional method	New method	Change of mean (%)	ES (± 90%CL)	Qualitative inference[a]
Energy expenditure measures					
$\dot{V}O_2$ demand (LO_2eq)	12.4 ± 2.0	13.2 ± 1.9	6.4 *	0.33 (± 0.10)	Moderate +ive [b]
Accumulated $\dot{V}O_2$ (L)	6.3 ± 0.7				
AOD (LO_2eq)	6.1 ± 1.5	6.8 ± 1.5	13.8 *	0.40 (± 0.14)	Moderate +ive [c]
Blood lactate related measures					
$[La]_{b\ (0)}$	7.03 ± 1.81				
$[La]_{b\ peak}$	9.63 ± 1.11				
$t[La]_{b\ peak}$	3.13 ± 1.48				
A_1 ($mmol \cdot L^{-1}$)	5.15 ± 2.04				
γ_1 ($1 \cdot min^{-1}$)	0.42 ± 0.24				
A_2 ($mmol \cdot L^{-1}$)	-12.74 ± 2.31				
γ_2 ($1 \cdot min^{-1}$)	0.03 ± 0.01				
Q_{LaA} at $[La]_{b\ peak}$ (mmol)	409.7 ± 28.0				
Q_{LaR} (mmol)	28.9 ± 11.0				
Q_{LaA} (mmol)	435.5 ± 35.4				

AOD: accumulated oxygen deficit, $[La]_{b\ (0)}$: end-exercise blood lactate concentration, $[La]_{b\ peak}$: the highest value of blood lactate concentration during recovery period, $t[La]_{b\ peak}$: time $[La]_{b\ peak}$ obtained, A_1 and A_2: amplitude of exponential equation, γ_1 and γ_2: time constant of exponential equation, QLaA at $[La]_{b\ peak}$: lactate accumulation in its distribution space at $[La]_{b\ peak}$, Q_{LaR}: quantity of lactate removed from the end of exercise to $[La]_{b\ peak}$, and Q_{LaA}: total lactate accumulation at the end of exercise.
90%CL indicates 90% confidence limits. [a] Inference about the magnitude of effect on $\dot{V}O_2$ demand and AOD estimated by the conventional and new methods. [b] Very likely, 95-99.5 %. [c] Most likely, 99.5- %. * indicates that *p* value was smaller than 0.001.

4. Discussion

The purpose of this study was to investigate the validity of the new method estimating AOD in rowing by comparing the relationships between AOD and blood lactate related parameters ($[La]_{b\ (0)}$, $[La]_{b\ peak}$ and Q_{LaA}). We hypothesized that AOD estimated by the new method would be higher than that estimated by the conventional method, and would show a stronger relationship with Q_{LaA}, relative to the conventional method, but not with $[La]_{b(0)}$, $[La]_{b\ peak}$ in both the methods. The AOD estimated by the new method was very likely higher compared to that estimated by the conventional method (13.8 %). In addition, the relationship between AOD and blood lactate concentration parameters ($[La]_{b\ (0)}$ and $[La]_{b\ peak}$) were unclear in both the methods. Meanwhile, the AOD values estimated by each method likely showed moderate relationships with Q_{LaA}; however, a marked difference of correlation coefficient was not observed between both the methods.

Blood lactate concentration after supramaximal exercise ($[La]_{b\ (0)}$) or peak blood lactate concentration during recovery period following a supramaximal exercise ($[La]_{b\ peak}$) were considered as indicators of anaerobic capacity (Saeki et al., 1999a, b; Craig et al., 1995; Saeki et al., 1998). On the other hand, several studies have reported that $[La]_{b\ (0)}$ or $[La]_{b\ peak}$ did not show a significant correlation with AOD (Medbø et al., 1988; Scott et al., 1991; Medbo, 1993; Pripstein et al., 1999; Bishop et al., 2002). Our results were in line with these studies, and appear to be supported by the evidence that blood lactate concentration is not determined by lactate production in exercising muscle, and that it is influenced by both lactate exchange and removal abilities between muscle and other tissues involved in lactate metabolism (van Hall et al., 2009). Therefore, we fitted the mathematical model to the blood lactate concentration curve obtained during recovery period to assess the total amount of lactate accumulated (Q_{LaA}). This model has

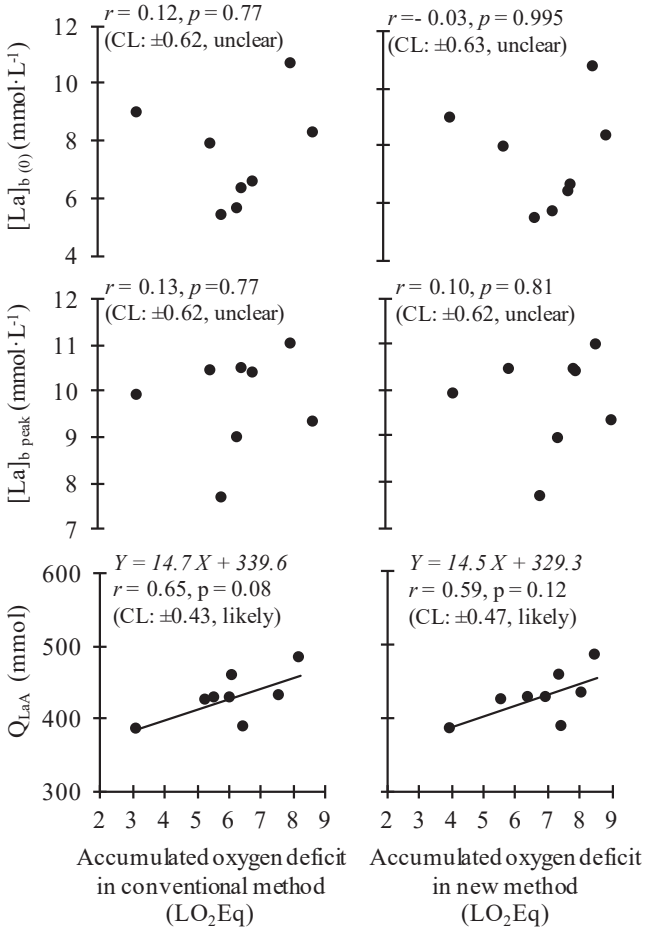

Fig 4. The relationships between blood lactate related parameters, which are end-exercise blood lactate concentration ($[La]_b$ (0)), the highest value of blood lactate concentration during recovery period ($[La]_{b\ peak}$) and quantity of lactate accumulation (Q_{LaA}), and accumulated oxygen deficit (AOD) in the conventional (left) and new (right) estimation methods. Correlation coefficients, *p* values, confidence limits (CL), and interpretations according to the distribution of CL are described (also see method section for details).

been applied to running (Aguiar et al., 2015a; Aguiar et al., 2015b; Bret et al., 2003; Bret et al., 2013; Chatel et al., 2016), cycling (Beneke et al., 2007; Beneke et al., 2010; Messonnier et al., 2001; Messonnier et al., 2006) and rowing (Maciejewski et al., 2013; Messonnier et al., 1997) in previous studies, and the coefficient of determination in this study was as high as previous studies (Beneke et al., 2007; Maciejewski et al., 2013; Bret et al., 2013). In addition, Maciejewski et al. (2013) have reported that AOD estimated by the conventional method demonstrated a linear relationship with Q_{LaA} in rowing, and the reported correlation coefficient was higher than this study (*r* = 0.85; 90 % confidence limits ± 0.22, corresponding to "*most likely*"). The weak relationship in this study might have been caused by either the duration of supramaximal test or the anthropometric characteristics of our subjects. At first, the test duration in our study was shorter than that in the previous study (2- vs 3-min) which might decrease the contribution of glycolysis to the total anaerobic energy provision and consequently increase the contribution of ATP-PCr, while AOD includes energy production except that from oxidative pathways (ATP-PCr and glycolysis). Therefore, AOD and Q_{LaA}, which do not include energy production by breaking down of PCr might show a lower correlation coefficient. Second, the variance of body weight in our subjects was larger than that in the previous study (72.1 ± 3.0 kg and 70.5 ± 10.5 kg, corresponding to 14.8 % and 4.2 % in coefficient of variance, respectively), however, such a large variance was not observed in height, which was 1.75 ± 0.05 m and 1.83 ± 0.03 m corresponding to 2.9 % and 1.6 % in coefficient of variance, respectively. This suggested that the body composition of our subjects might be more inhomogeneous relative to the previous study, which might reduce the precision to estimate the total lactate distribution space (V_{TLS}) by using the fixed value and body weight (0.6 L per kg of body weight). Therefore, the result of this study suggested that AOD estimated by the conventional method demonstrated a moderate linear relationship with Q_{LaA} even though the subjects might have a wide variance of body composition. Further study is required to investigate the effect of the variance of body composition on V_{TLS} estimation.

AOD estimated by the new and conventional methods likely showed a moderate linear relationship with Q_{LaA}, respectively. However, contrary to our hypothesis, the correlation coefficients did not differ between the methods. Although our results could not provide clear explanation for this point, the Q_{LaA} might be affected by the shorter exercise duration or the wide variance of subjects' body composition or both factors, and consequently the weak relationship between Q_{LaA} and AOD in the new method might be induced.

The remarkable result was that AOD estimated by the conventional and new methods most likely showed a nearly perfect correlation relationship, which suggests that both the methods can be interchangeable to evaluate the highness of anaerobic capacity in the group. However, the AOD of the new method showed a 13.8 % higher value compared to that of the conventional method. In addition,

we have reported that $\dot{V}O_2$ demand predicted by the new method, which uses IAA_{total} and PO to take into account the increase of SR, was higher than that predicted by the conventional method, and almost equal to that predicted by a SR-adjusted method, which gradually increased SR in submaximal test to minimize the effect of increased SR in supramaximal test (Shirai and Nabekura, 2016). Taking these previous results together, the higher value of $\dot{V}O_2$ demand in the new method might indicate that the new method could predict $\dot{V}O_2$ demand including the increased internal work induced by higher SR in supramaximal test. Moreover, the difference in AOD between both methods varied in each subject (4.2~26.5 %), suggesting that the new and conventional methods is not interchangeable to evaluate anaerobic capacity in the case of subjects who have small variance of AOD.

In 2000 m simulation test, SR is significantly high in the start period, and then sifts toward lower SR in the middle of race, and increases again in the final spurt period (Steinecker, 1993). To predict $\dot{V}O_2$ demand accurately, the change of internal work induced by the unstable SR should be considered when predicting $\dot{V}O_2$ demand. In the case of the SR-modified method, which gradually increases SR in submaximal test to minimize the effect of significant increase of SR in supramaximal test, rowers should repeat several submaximal tests to obtain proper linear equations for each period of 2000 m simulation test, meanwhile, the new method, which takes the internal work into account by using a tri-accelerometer, requires only one submaximal test and SR-incremental test to predict $\dot{V}O_2$ demand. Therefore, the new method would be less burden for rowers and researchers to investigate the metabolism during 2000 m simulation test.

The process of $\dot{V}O_2$ demand estimation in the new method includes the SR-incremental test, which requires subjects to increase SR with maintaining PO, suggesting that in the case of subjects whose technical level is not enough to control SR and PO simultaneously, the linearity between IAA_{total} and $\dot{V}O_2$ can be weaken due to the instability of SR and PO. The other limitations of this study were the shorter duration of the supramaximal test and heterogeneity of anthropometric characteristic. In the case that a longer duration of supramaximal exercise is applied, the contribution of glycolysis metabolism and lactate production increase, and consequently Q_{LaA} and AOD may show a closer relationship. Moreover, recruiting more homogenous subjects in anthropometric characteristic may improve the accuracy of V_{TLS} estimation and the relationship between Q_{LaA} and AOD.

5. Conclusion

This study demonstrated that 1) AOD estimated by either the conventional or new methods did not show linear relationship with $[La]_{b\ (0)}$ and $[La]_{b\ peak}$, but rather likely showed moderate relationships with Q_{LaA}, 2) AOD estimated by the conventional and new methods showed a nearly linear relationship to each other, however, 3) AOD estimated by the new method was 13.8 % higher than that of the conventional method, which consisted with the previous study (Shirai and Nabekura, 2016) , and 4) the difference in AOD between the methods involved large variance (4.2~26.5 %). These results suggest that the new method can estimate $\dot{V}O_2$ demand including the increased internal work, however, AOD estimated by both the methods have the same level of relevance with the amount of lactate accumulation. Furthermore, both the methods can be interchangeable in the case of subjects who have large variance of AOD, however, in the case of subjects who have smaller variance of AOD, the new method should be chosen to estimate $\dot{V}O_2$ demand including the increased internal work.

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