

# Anaerobic Threshold and Salivary $\alpha$ -amylase during Incremental Exercise

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**Abstract.** [Purpose] The purpose of this study was to clarify the validity of salivary  $\alpha$ -amylase as a method of quickly estimating anaerobic threshold and to establish the relationship between salivary  $\alpha$ -amylase and double-product breakpoint in order to create a way to adjust exercise intensity to a safe and effective range. [Subjects and Methods] Eleven healthy young adults performed an incremental exercise test using a cycle ergometer. During the incremental exercise test, oxygen consumption, carbon dioxide production, and ventilatory equivalent were measured using a breath-by-breath gas analyzer. Systolic blood pressure and heart rate were measured to calculate the double product, from which double-product breakpoint was determined. Salivary  $\alpha$ -amylase was measured to calculate the salivary threshold. [Results] One-way ANOVA revealed no significant differences among workloads at the anaerobic threshold, double-product breakpoint, and salivary threshold. Significant correlations were found between anaerobic threshold and salivary threshold and between anaerobic threshold and double-product breakpoint. [Conclusion] As a method for estimating anaerobic threshold, salivary threshold was as good as or better than determination of double-product breakpoint because the correlation between anaerobic threshold and salivary threshold was higher than the correlation between anaerobic threshold and double-product breakpoint. Therefore, salivary threshold is a useful index of anaerobic threshold during an incremental workload.

**Key words:** Salivary  $\alpha$ -amylase, Salivary threshold, Anaerobic threshold

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## INTRODUCTION

With the rapid increase in the proportion of elderly in Japan, the proportion of adult diseases such as cancer, ischemic heart disease, cerebrovascular disease, and diabetes mellitus has also increased, now accounting for about 60% of the causes of mortality<sup>1, 2)</sup>. Exercise plays a crucial role in the prevention of adult disease and is recommended to maintain or improve health<sup>3, 4)</sup>.

It has been reported that a certain intensity of exercise is required for improvements in health and physical strength<sup>5–7)</sup>. At least a moderate exercise intensity is required to improve the health of adults<sup>6)</sup>, but comorbidities in the elderly may restrict their ability to exercise. Therefore, exercise should be prescribed at a suitable intensity for the elderly, i.e., the intensity at which the benefits of exercise can be gained but risks of exercise avoided. The balance between benefit and safety varies with the physical

condition of the individual but is considered to occur at a lower intensity in elderly people with existing disease than in healthy young adults. Therefore, exercise intensity in the elderly must be carefully adjusted.

Anaerobic threshold (AT) has been proposed as an objective criterion for precise adjustment of exercise intensity<sup>8)</sup>. AT is the exercise intensity just after which energy production by anaerobic metabolism is added to aerobic metabolic energy production during incremental exercise. It is estimated by the lactate threshold (LT), which is the workload at which the blood lactate concentration abruptly increases, and by the ventilatory threshold (VT), which is the workload at which the rate of pulmonary minute ventilation abruptly increases nonlinearly during a progressive exercise test<sup>9, 10)</sup>. Wasserman et al. contend that the ventilatory threshold is the same as the lactate threshold<sup>9)</sup>. It has been reported that both thresholds are safe and effective criteria<sup>11, 12)</sup>. However, both thresholds have disadvantages; specifically, the gas analyzer used to measure expired gas for determination of VT is expensive and is limited to use in a laboratory setting, and blood collection for determination of LT is invasive.

Therefore, some alternatives for estimating AT simply have recently been developed. One is the double-product breakpoint (DPBP)<sup>13)</sup>, or the point at which double product

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(DP), the product of heart rate and systolic blood pressure, abruptly increases during a progressive exercise test. The DPBP can be calculated from the intersection of two regression lines, and its validity has already been established based on the high correlation between DPBP and AT ( $r = 0.87, p < 0.001$ )<sup>14–16</sup>. Another method for estimating AT is heart rate threshold (HRT), which uses only heart rate<sup>17, 18</sup>. HRT is obtained from the relationship between running speed and heart rate. The running speed–heart rate relationship is linear from low to submaximal speeds and curvilinear from submaximal to maximal speeds. The speed of transition from the linear to the curvilinear phase coincides with the beginning of a sharp accumulation of blood lactate<sup>18</sup>. However, it has been reported that the DPBP method is inferior to expired gas analysis in detecting AT<sup>14</sup> and that the HRT occurs at a higher workload than AT<sup>19</sup>. For this reason, it is necessary to develop a more sensitive indicator or to create a method combining a number of indicators to improve the ability to detect AT and thus prescribe suitable exercise to subjects with reduced exercise tolerance.

Both systolic blood pressure and heart rate, the components of DP, are controlled by the sympathetic nervous system. The plasma catecholamine concentration, which reflects sympathetic nervous system activity during incremental exercise, has been shown to start to rise abruptly at a workload similar to the LT. In addition, it has been reported that the plasma catecholamine concentration correlates with the blood lactate concentration<sup>20, 21</sup>. This suggests that DP may reflect changes in sympathetic nervous system activity and blood lactate concentration through the plasma catecholamine concentration. Therefore, it is possible to detect the AT more precisely if an index is used that is a more sensitive reflection of sympathetic nervous system activity. The proposed index of sympathetic nervous system activity is salivary  $\alpha$ -amylase, an enzyme in saliva that has been reported to be controlled by the sympathetic-adrenal-medullary system<sup>22, 23</sup> and to be a sensitive indicator of the activity of the sympathetic nervous system<sup>24</sup>. Therefore, it is possible to detect changes in blood lactate concentration indirectly by measuring salivary  $\alpha$ -amylase, a measurement that can be performed quickly under test conditions. Calvo et al. investigated the relationship between the salivary threshold (Tsa), the workload at which salivary  $\alpha$ -amylase abruptly increases, and the LT by measuring salivary  $\alpha$ -amylase and blood lactate concentrations during a multistage incremental exercise<sup>25</sup>. The authors concluded that Tsa had a high correlation with the LT ( $r = 0.95, p < 0.001$ ), and the detection rate was 80%. The results suggested that the high correlation between the Tsa and LT, as well as the ease of measuring salivary  $\alpha$ -amylase, makes Tsa a valid and useful estimate of AT. However, there are few reports on the relationship between AT and Tsa. Moreover, no report has investigated the relationship between Tsa and other methods of estimating AT. Determination of whether the detection rate is improved by combining Tsa and DPBP would provide meaningful information for exercise prescription, particularly for elderly people with preexisting disease.

The purpose of this study was to assess the validity of

salivary  $\alpha$ -amylase as a method for estimating AT quickly and to clarify the relationship between salivary  $\alpha$ -amylase and DPBP in order to establish a way to adjust exercise intensity to a safe and effective range.

## SUBJECTS AND METHODS

The subjects of this study were 11 healthy young adults ( $23.8 \pm 1.8$  years, height  $173 \pm 5.0$  cm, body weight  $64.6 \pm 6.3$  kg, body mass index  $21.7 \pm 1.3$  kg/m<sup>2</sup>). None of the subjects reported any neurological or vestibular disorders, orthopedic conditions, or oral cavity diseases before participating in this study. All subjects provided written informed consent before commencing the experiment. This study was conducted in accordance with the Declaration of Helsinki.

We used an electrically braked cycle ergometer (AERO-BIKE 75XL, Combi Co. Ltd., Tokyo, Japan) for the exercise test. The timing of the experiment (6:00 p.m. to 8:00 p.m.) was chosen to minimize the influence of circadian variation in salivary  $\alpha$ -amylase<sup>26</sup>. Room temperature was set to 24°C. Since the activity of salivary  $\alpha$ -amylase can be influenced by alcohol, medications, food, and caffeine, all subjects were instructed not to drink any alcohol the day before measurement and not to consume food or caffeine 2 hours before measurement.

After a rest period of 3 min on the ergometer, subjects were instructed to try to maintain a cadence of 50 rpm while the workload level was increased by 20 watts per 3 min beginning with 10 watts. The exercise test was continued until subjects could no longer maintain the prescribed cadence due to fatigue or until completion of 2 stages following that at which AT was observed.

Oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>), and ventilatory equivalent (VE) were measured using a breath-by-breath gas analyzer (AE-300, Minato Medical Science, Osaka, Japan). The V-slope method was used to determine AT<sup>27</sup>.

To calculate DP, systolic blood pressure and heart rate were measured during the last 30 seconds of each stage of the incremental exercise test. The same examiner measured systolic blood pressure using a stethoscope and mercury sphygmomanometer. Heart rate was measured using an electrocardiogram monitor (BSM-2401, Nihon Kohden, Tokyo, Japan). The DPBP was determined using a computer algorithm as follows: The linear regression lines of DP as a function of workload were calculated for all possible divisions of the data. DPBP was determined by choosing the intersection, or breakpoint, of the 2 lines representing the minimum residual sum of squares from among various intersections of the 2 lines.

Salivary  $\alpha$ -amylase was measured using a portable salivary amylase analyzer (Salivary amylase monitor, NIPRO, Osaka, Japan). It was verified that within the analyzer's linear range (10–230 kU/L), this handheld monitor's accuracy ( $R^2 = 0.989$ ), precision (coefficient of variation < 9%), and measurement repeatability (range –3.1% to +3.1%) approached those of a more elaborate laboratory-based automated clinical chemistry analyzer (Olympus America Inc., Center Valley, PA, USA). Salivary  $\alpha$ -amylase was measured

from saliva samples collected during the last 30 seconds of each stage of an incremental exercise test by inserting the reagent test strip directly into the subject's oral cavity. The mask for expired gas analysis was briefly removed to obtain the saliva sample and then replaced after saliva collection. Tsa was determined in the same manner as DPBP.

IBM SPSS for Windows Version 20.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analysis. Workload at the AT, Tsa, and DPBP were compared using one-way ANOVA to investigate the difference in workload between each index. Pearson's correlation coefficients were used to assess the relationship between workload at the AT and Tsa and between the AT and DPBP. In addition, with Tsa-1 (or DPBP-1) representing 1 workload prior to that corresponding to Tsa (or DPBP), Tsa-2 representing 2 workloads prior, Tsa-3 representing 3 workloads prior, and so on, Tsa, Tsa-1, Tsa-2, and Tsa-3 and workloads for DPBP, DPBP-1, DPBP-2, and DPBP-3 were compared by one-way ANOVA with repeated measures to identify the dynamics of salivary  $\alpha$ -amylase and DPBP associated with an incrementally increasing workload. We chose an alpha level of 0.05 to indicate significant effects.

## RESULTS

Subject characteristics are shown in Table 1. In the present study, AT could be determined in all subjects (100%). However, Tsa and DPBP could not be detected in 2 of the subjects (detection rate of 82% for both methods) because the increase was not abrupt enough to identify 2 regression lines clearly. In the following analysis, therefore, data for the subjects whose Tsa and DPBP could not be detected were excluded. The results of one-way ANOVA revealed that there was no significant difference among workloads at the AT, DPBP, and Tsa (Table 2).

There were significant correlations between AT and Tsa ( $r = 0.951$ ,  $p < 0.01$ ) and between AT and DPBP ( $r = 0.940$ ,  $p < 0.01$ ). The results of one-way ANOVA with repeated measures identified the significant main effects of salivary  $\alpha$ -amylase ( $F_{1,58, 12.6} = 24.0$ ,  $p < 0.01$ , partial  $\eta^2 = 0.75$ ) and DP ( $F_{1,34, 10.3} = 44.7$ ,  $p < 0.01$ , partial  $\eta^2 = 0.85$ ), and post hoc analysis using Bonferroni's test was executed. The results showed that there were significant differences between Tsa and Tsa-1 ( $p < 0.05$ ), between Tsa and Tsa-2 ( $p < 0.01$ ), and between Tsa and Tsa-3 ( $p < 0.01$ ). However, there were no significant differences between Tsa-1 and Tsa-2, between Tsa-1 and Tsa-3, or between Tsa-2 and Tsa-3. On the other hand, DP showed significant differences between all corresponding workloads.

## DISCUSSION

The purpose of this study was to assess the validity of Tsa as a workload index by clarifying the relationship between AT, which was measured by analysis of expired gas, and Tsa, which represented the workload at which salivary  $\alpha$ -amylase abruptly increased. We also investigated the relationship between DPBP, which has been established as a method for quickly estimating AT, and Tsa in order to assess the usability of Tsa as another method for quickly

**Table 1.** Subject characteristics

Age (yr)	23.8 $\pm$ 1.8
Height (m)	1.73 $\pm$ 0.05
Weight (kg)	64.6 $\pm$ 6.3
BMI (kg/m <sup>2</sup> )	21.7 $\pm$ 1.3
HR at rest (beats/min)	70.0 $\pm$ 11.4
HR at AT (beats/min)	116.2 $\pm$ 16.4
VO <sub>2</sub> at AT (mL/min)	1,170.2 $\pm$ 189.3
VO <sub>2</sub> /W at AT (mL/min·kg)	18.1 $\pm$ 2.3
Salivary $\alpha$ -amylase at rest (kU/L)	22.2 $\pm$ 27.5
Salivary $\alpha$ -amylase at Tsa (kU/L)	40.0 $\pm$ 18.1
DP at rest (mmHg · beats/min)	8,740.4 $\pm$ 1,994.7
DP at DPBP (mmHg · beats/min)	14,868.2 $\pm$ 3,153.1

Data presented as mean  $\pm$  SD. BMI, body mass index; AT, anaerobic threshold; DP, double product; DPBP, double-product breakpoint; HR, heart rate; Tsa, salivary threshold; W, Watt

**Table 2.** Power output at AT, Tsa, and DPBP

AT (W)	70.0 $\pm$ 28.3
Tsa (W)	61.1 $\pm$ 28.5
DPBP (W)	65.6 $\pm$ 21.9

AT, anaerobic threshold; DPBP, double-product breakpoint; Tsa, salivary threshold; W, watt

estimating AT.

The results of this study indicate significant correlations between AT and Tsa ( $r = 0.951$ ,  $p < 0.01$ ). Furthermore, a significant difference was not observed in the workload at the AT and Tsa. This result suggests that Tsa has validity as a method for estimating AT quickly, which confirms results reported by Calvo et al.<sup>25</sup>. Yamamoto et al. investigated the activity of the parasympathetic and sympathetic nervous systems during exercise using heart rate variability. The authors revealed that the indicator of parasympathetic nervous system activity decreased progressively from rest to a workload equivalent to 60% VT and that the indicator of sympathetic nervous system activity increased only when exercise intensity exceeded VT<sup>28</sup>). These results suggest that the activity of the sympathetic nervous system changed at the VT, which was equivalent to the AT, and that AT might be estimated by monitoring the activity of the sympathetic nervous system. Moreover, it has been reported that the salivary  $\alpha$ -amylase concentration increases with increased physical activity, such as treadmill exercise<sup>29</sup>), running<sup>30, 31</sup>), and cycle exercise<sup>24, 32</sup>). However, physical activity per se does not necessarily increase salivary  $\alpha$ -amylase. Chatterton et al. investigated salivary  $\alpha$ -amylase during walking, jogging, and running and reported that jogging and running increased salivary  $\alpha$ -amylase but that walking did not affect salivary  $\alpha$ -amylase<sup>24</sup>). This result confirmed that the increase in salivary  $\alpha$ -amylase was caused by physical activity exceeding a certain workload (i.e., the AT). Chatterton et al. also investigated the correlation between salivary  $\alpha$ -amylase and plasma catecholamines and showed significant correlations between them ( $r = 0.64$  for

norepinephrine and  $r = 0.49$  for epinephrine)<sup>24</sup>). This result directly indicates that salivary  $\alpha$ -amylase reflects the activity of sympathetic nervous system. Since salivary  $\alpha$ -amylase is an index that sensitively reflects the activity of the sympathetic nervous system and the activity of the sympathetic nervous system is caused by workloads above the AT, it is suggested that Tsa, which is the point of inflection of salivary  $\alpha$ -amylase, is a good index by which to estimate AT. We noted significant strong correlation between AT and Tsa in the present study.

The results of the present study also indicated that Tsa was at least as good as DPBP as a method for estimating AT quickly because the correlation between the AT and Tsa was 0.951 ( $p < 0.001$ ), whereas the correlation between the AT and DPBP was 0.940 ( $p < 0.001$ ). This result suggests that salivary  $\alpha$ -amylase reflects the activity of the sympathetic nervous system more sensitively than DP, the components of which are controlled by the sympathetic nervous system. Spence et al. compared the systolic and diastolic blood pressure responses to exercise before the AT with those after the AT by using a cycle ergometry<sup>33</sup>). The authors concluded that the rate of increase of systolic blood pressure significantly increased after the AT. Tanaka et al. also demonstrated that the slope of a regression line of systolic blood pressure and heart rate increased after the AT by comparing the slope of a regression line of systolic blood pressure and heart rate before and after the AT<sup>13</sup>). However, other researchers reported that the slope of a regression line of systolic blood pressure and heart rate may not increase after the AT. Riley et al. investigated the relationship between DPBP and AT, as determined from analysis of expiration gas, by measuring systolic blood pressure and heart rate during an exercise test<sup>14</sup>). Their results indicated that, although DPBP and AT are strongly correlated, the rates of increase of systolic blood pressure and heart rate do not always increase after the AT. In the study of Riley et al., the rate of increase of systolic blood pressure increased after the AT for some subjects but that for heart rate did not; other subjects indicated the opposite. Furthermore, Conconi et al. researched the relationship between HRT and LT by obtaining the HRT, which was calculated from running speed and heart rate<sup>18</sup>). The results indicated that there was a strong correlation between HRT and LT and that increased running speed involved an increase in heart rate. However, the rate of increase of heart rate after the HRT decreased, which is in contrast to the results of Tanaka<sup>13</sup>). Both Riley et al.<sup>14</sup>) and Conconi et al.<sup>18</sup>) demonstrated that changes in systolic blood pressure and heart rate before and after the AT differed among individuals. DP is highly correlated with AT because it consists of 2 variables, so that even if only 1 variable shows unique changes, the other variable is able to change in a complementary manner. However, using the product of variables that vary among individuals may lead inaccurate estimation of AT. Salivary  $\alpha$ -amylase is therefore superior to DP in the degree of correlation to AT because salivary  $\alpha$ -amylase can measure the activity of the sympathetic nervous system using only 1 variable.

There are other ways in which Tsa is superior to DPBP for estimation of AT. To calculate DP, blood pressure and heart rate must be measured, but blood pressure measure-

ment temporarily restricts subjects' physical activity. Additionally, measurement of heart rate requires equipment such as an electrocardiograph monitor, thus restricting the measurement environment. In contrast, measurement of salivary  $\alpha$ -amylase requires only insertion of the reagent test strip directly into the subject's oral cavity to collect saliva samples without the need to interrupt activity, and it can be performed in various environments. Thus, salivary  $\alpha$ -amylase estimates AT simply and has the advantages of easier measurement and more sensitive reflection of sympathetic nervous system activity than can be achieved with DP.

Interestingly, the results of the present study suggest that a more correct estimation of AT can be achieved by combining Tsa and DPBP. In this research, although AT was identified in all subjects using expired-gas analysis, Tsa and DPBP were unable to be determined in 2 subjects. However, because data allowing determination of DPBP were always collected, combining Tsa and DPBP allowed identification of AT in all subjects. This finding suggests that the variables salivary  $\alpha$ -amylase, systolic blood pressure, and heart rate are controlled by the sympathetic nervous system but that the expression of these variables in vivo differs among individuals. Therefore, inclusion of all the variables in the estimation of AT may allow a more precise estimation. AT estimation using salivary  $\alpha$ -amylase is useful for subjects in whom Tsa can be identified even if this is the only method used because Tsa and AT are strongly correlated. However, the combination of DPBP and Tsa might be desirable for some subjects, since Tsa was unable to be identified in about 18% of our study subjects. While it is better to measure salivary  $\alpha$ -amylase to estimate AT because of its superiority to DPBP in the degree of correlation with AT, if Tsa is not able to be determined, DP can be calculated to complement the detection of AT. As a result, AT can be determined with a detection rate as high as that for expired-gas analysis. This result is in line with the study of Calvo et al.<sup>25</sup>), who mentioned that differences among subjects in their hydration state before reporting to the laboratory or in the contribution of each salivary gland to the production of total saliva explained the inability to detect Tsa in 20% of their subjects. In this study, although we encouraged subjects to drink water before measurement and allocated a point under the tongue for saliva collection in order to minimize these influences, the detection rate was not improved. Further studies are needed to improve the precision of measurement of salivary  $\alpha$ -amylase itself.

The results of this study clarified that Tsa is a useful index for estimating AT during an incrementally increasing workload. Tsa was not able to be detected in 2 subjects, but in such cases, the rate of determination of AT can be improved by combining Tsa with DPBP. Determination of AT using salivary  $\alpha$ -amylase is superior to other methods because the measurement device is easily transportable, making measurement possible in various environments, and because of the noninvasiveness of the technique. We believe that the ability to adjust exercise to the optimal intensity in various environments using salivary  $\alpha$ -amylase can make exercise safer and more effective.

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