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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Muscle blood flow as an indicator of anaerobic threshold in young athletes – A near infrared spectroscopy study

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abstract

Background In this study we evaluated the effect of exercise with increasing intensity until exhaustion on muscle tissue oxygenation in children. Furthermore, we tested the hypothesis that a decrease in muscle indirect blood flow (O_2Hb) predicts the anaerobic threshold determined by analysis of ventilation parameters.

Material/Methods Five, young, school-aged tennis players were engaged in this study (age 16.2 ±1.63 years, VO_{2max} 56.7 ±2.00 ml · kg^{-1} · min^{-1}). Changes in oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (HHB), blood volume (tHb = HbO_2 + HHB) and muscle tissue oxygenation (TOI%) in the right vastus lateralis muscle, using a near-infrared spectrometer were recorded.

Results The hemodynamic values (HHB, tHb) increased as expected depending on the exercise intensity. Therefore, NIRS data precedes the occurrence of AT determined by the analysis of ventilation parameters, which in the case of the V-slope method was achieved at 75% VO_{2max}.

Conclusions A correlation between the parameters of muscle tissue oxygenation and ventilation parameters shows that the anaerobic threshold occurs as a result of deoxygenation of a muscle tissue.

Key words anaerobic threshold, incremental exercise, children, athletes

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INTRODUCTION

The anaerobic threshold (AT), a measure that has been introduced into the diagnostics of the amount of work in sports training is an additional indicator of the level of aerobic effort abilities. It is undoubtedly a significant source of information about the aerobic performance of athletes and non-athletes. The anaerobic threshold is defined as the intensity of work load or oxygen consumption in which anaerobic metabolism is accelerated [1, 2]. Defining AT raises a lot of controversy, primarily from the method of measurement [3].

Despite a very advanced state of knowledge, terminology of AT is still under discussion, and possible reasons are:

- during incremental exercise there are two types of metabolism functions; however, only one of them dominates. A situation when only one of them functions is impossible;
- kinetics of lactate acid (LA), ventilation and gas exchange response are dependent on the exercises protocol (i.e. dynamics of load increase) [4], the type of exercises (e.g. running, swimming, rowing or cycling) [5], and the source of blood samples (vein capillaries, arteries) [6];
- the “cause and effect” relationship between selected invasive (e.g. LA^-, pyruvate, pH, HCO_3^-, adrenaline) and non-invasive (e.g. heart rate, ventilation, gas exchange) parameters, which was recorded during cardiopulmonary exercise testing (CPET) seems to be strong, but it does not have to be a source of physiological information [3].

During an exercise with an increasing intensity until exhaustion, the contribution of aerobic and anaerobic processes in energy supply of working muscle is determined by the intensity of exercise.

The increase in an exercise load generates an increase in the concentration of lactic acid in the muscle and lactate (La^-) and hydrogen ions (H^+) in the blood. There are two defined ventilation thresholds and two lactate thresholds. It means that during exercise to exhaustion the increase in ventilation (linear and non-linear) and LA^- compared to a linear increase in the workload. To visualize this situation, a three-phase model of the relationship was developed between the LA^- concentration and the intensity of physical activity (Fig. 1).

Therefore, the aim of this study was to compare the non-invasive methods of calculating the anaerobic threshold based on the muscle oxygenation parameters and ventilation parameters. We hypothesized that the kinetics of muscle tissue oxygenation coincides with the kinetics of ventilation parameters. This was based on an increase in the exercise intensity and a pronounced reduction in muscle tissue oxygenation as a predictor of the anaerobic threshold.

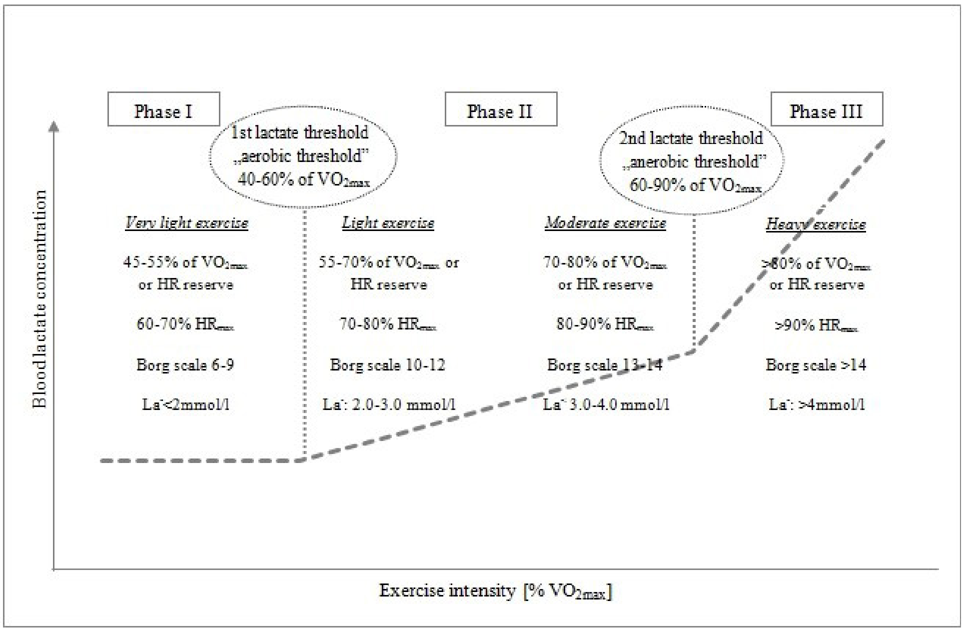

Fig. 1. The three-phase model of the relationship between the concentration of blood lactate (BL) and the intensity of physical activity [3, 7] (HR – heart rate, VO_2 – oxygen consumption volume, BL – blood lactate)

MATERIAL AND METHODS

SUBJECTS

Five, right-handed, school-aged, table tennis players (boys – 16.2 ±1.3 yr) participated in the study. They were representatives of the Junior Polish National Team. The study group had 7.9 ±1.1 years of training practicing. Among the participants there were no diseases relating to the cardiovascular, respiratory, endocrine, or nervous systems. During the experiment, players were not taking any medications. Respondents were asked not to participate in any training two days before CPET. Detailed anthropometric characteristics of the respondents are presented in Table 1. The study was approved by the Local Ethics Committee and all subjects gave their informed consent before the start of the study.

EXPERIMENTAL PROTOCOL

A week before the experimental procedure, the participants visited the exercise laboratory. That time was used to familiarize each child with the research apparatus, the laboratory environment and the people conducting the experiment. This educational/informational stage was also attended by parents and coaches. To evaluate selected parameters and anthropometric measures, the anaerobic threshold, oxygenation of the muscle tissue, and gas exchange parameters, the following methods were used.

ANTHROPOMETRIC MEASUREMENTS

The measurements of selected parameters and anthropometric measures were performed before the CPET. We measured the total body weight [kg], the percentage of body fat – FAT [%] using bioelectrical impedance (Body Composition Analyzer TBF – 300 A Body Fat Monitor/Scale Tanita Japan), and body height [cm] using anthropometer (GPM-skinfold caliper).

ANAEROBIC THRESHOLD DETERMINING

Participants exercised on a bicycle ergometer VIAsprint TM 150P Ergoline (Germany) with an increasing load until exhaustion (CPET). The protocol used in the test is a modification of the protocol proposed by Wasserman in 1987 [8]. The first phase of the test was preceded by a five-minute period in which the resting measurement indicators characterizing the activity of the cardiovascular and respiratory system were determined. Then subjects performed the test until exhaustion. The continuous progressive intensity protocol started at 1.5W · kg^{-1} for 5 min (v = 55 rpm) and increased 25W every 1 minute until exhaustion. The anaerobic threshold (AT) was established with a noninvasive method on the basis of the load measure (%VO_{2max}) with the respiratory exchange ratio (RER = 1) in the test effort as well as the maximal oxygen uptake (VO_{2max}). After the test, the participants recovered for 5 minutes sitting on a cycle ergometer.

At rest, during the exercise and during the 5-minute recovery we used exhaled gas analyzer Oxycon Pro Jaeger (Viasys, Germany) and Breath by Breath module in the JLab 5.31 software. The following parameters characterize the cardio-respiratory activity: oxygen pressure in the terminal exhaustion air – $PETO_2$ [kPa] and the pressure of carbon dioxide in the terminal exhaustion air – $PETCO_2$ [kPa], oxygen uptake – VO_2 and carbon dioxide production – VCO_2 in relative [mL · kg^{-1} · min^{-1}] and absolute values [L · min^{-1}]. The tidal volume – VT [L], the respiratory rate – BF [1 · min^{-1}], minute ventilation – VE [L · min^{-1}], and the heart rate – HR [b · min^{-1}] also were recorded. Based on the measured parameters we calculated: the respiratory exchange ratio (RER), oxygen consumption to ventilation (VE · VO_2^{-1}) and ventilation to carbon dioxide production (VE · VCO_2^{-1}) equivalent. CPET allowed assessing the indicators determining cardiovascular fitness: maximal oxygen uptake (VO_{2max} – a direct method) and the anaerobic threshold (AT) – non-invasive methods described below, based on graphs of exhaled breath and oxygenation of muscle tissue parameters analysis.

ANAEROBIC THRESHOLD DETERMINATION – THE V-SLOPE METHOD

Determination of AT with the V-slope method relied on graph analysis of VO_2 and VCO_2. AT was determined when the first straight lines according to VO_2 and VCO_2 became steeper in connection with the disproportionate increase in VCO_2 production in relation to oxygen consumption without signs of hyperventilation [9, 10].

ANAEROBIC THRESHOLD DETERMINATION – THE EQUIVALENTS METHOD

The determination of AT using the equivalents method relied on graph analysis of the ventilation equivalent for oxygen (VE · VO_2^{-1}), which is the volume of minute ventilation from which at this stage of effort 1 liter of oxygen is captured, and the ventilation equivalent for carbon dioxide (VE · VCO_2^{-1}), characterized in minute ventilation volume that is required to remove 1 liter of carbon dioxide. AT was determined, when a systematic increase in VE · VO_2^{-1} started, without a corresponding increase in VE · VCO_2^{-1} [11].

Anaerobic threshold determination – the relationship between $PETO_2$ and $PETCO_2$

Using the course of the relationship between the pressure of O_2 and CO_2 in the terminal exhaustion air ($PETO_2$ and $PETCO_2$), AT was determined when $PETO_2$ reached its minimum and began to grow steadily at the same time unchanging the course of $PETCO_2$ [12].

ANAEROBIC THRESHOLD DETERMINING – RER AND EXERCISE LOAD

Using the course of the relationship between the gas exchange ratio (RER) and the load during the test, the AT was determined when plotted on a course initially flat or slowly ascending into a steeper one, and the value of the RER was approaching, but remained less than 1.0 [9, 11].

ANAEROBIC THRESHOLD DETERMINATION – NIRS

Muscle tissue oxygenation measurement was carried out using a dual-channel near-infrared spectrometer NIRO 200 (Hamamatsu – Japan). The light source and detector of the second channel NIRS were placed on the skin in the area of the vastus lateralis muscle in the central line along the vertical axis, and in a third of the lateral epicondyle of the greater trochanter of the femur [13]. The distance between sensors was 4.0 cm. Each sensor was attached to the skin with a black patch with a dense structure in order to eliminate the access of sunlight. The hemoglobin concentration was expressed in conventional units (a.u.) as the delta value in relation to the baseline recorded in the calibration phase (at rest). Wavelength NIRS was set at 775 to 850 nm. Differential path length factor (DPF) was set at 4.0. The data passed in real time with the frequency of 1 Hz. After measurement, the data were averaged over the 20-second time intervals, then a graph of oxygenated hemoglobin (O_2Hb) and load stress was created. Using the process of this dependency, we determined the AT when the chart of the course initially flat or slowly descending changed the course to a steeper one [14]. In addition to O_2Hb, deoxygenated hemoglobin (HHB) and muscle tissue oxygenation index (TOI,%) were recorded.

STATISTICAL ANALYSIS

All data were collected in order to create a single data sheet for statistical analysis. Statistical analysis was performed using the tools of STATISTICA 10 StatSoft Poland [15]. Variables have been revised in terms of the nature of the distribution and homogeneity of variance. Following the calculation of basic statistics, the differences between the averages were subjected to an evaluation in order to determine their significance. For this purpose we used a one-way analysis of variance. The Newman-Keuls test was used to determine when the differences were found. Relationships between variables were examined using Pearson's correlation. The alpha level was set at $p < 0.05$, and data are presented as a mean and standard deviation (±SD) and the minimum and maximum.

RESULTS

The values of the parameters characterizing the aerobic capacity are shown in Table 2. Obtaining VO_{2max} in the study group was accompanied by a decrease in oxygenation (TOI) of muscle tissue from 70% to 40%.

The anaerobic threshold (AT), determined on the muscle tissue oxygenation (NIRS) graph parameters analysis, was achieved at 61% of the maximum aerobic power (MP) and 65% of oxygen uptake (VO_{2max}).

The anaerobic threshold, determined on the exhaled breath parameters analysis, has been achieved in the subsequent intensities of exercise, in the following order, according to the methods:

- AT determined based on the relationship between the pressure of oxygen and carbon dioxide in the terminal exhaustion air: 63% MP and 69% VO_{2max}
- AT designated by the equivalents method: MP 66% and 73% VO_{2max}
- AT designated by the V-slope: 69% MP and 75% VO_{2max}
- AT appointed based on the relationship between RER and the exercise load: 75% MP and 78% VO_{2max} – (Fig. 2 and 3).

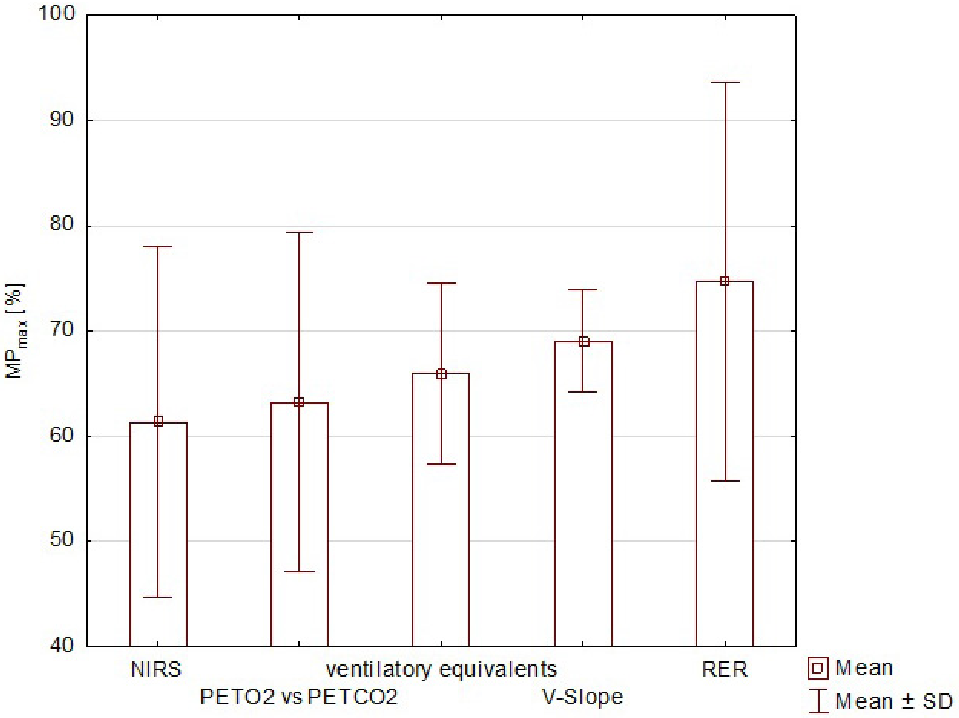

Fig. 2. The intensity of physical activity as a percentage of maximum aerobic power (MP) for the anaerobic threshold determined based on the graphs parameters of muscle tissue oxygenation (NIRS) and the analysis of exhaled breath parameters. ($PETO_2$ vs $PETCO_2$ – the relationship between the pressure of the oxygen in the terminal exhaustion air and the pressure of carbon dioxide in the terminal exhaustion air, Equivalents – the relationship between the ventilated oxygen equivalent and ventilated carbon dioxide equivalent; V-Slope – the relationship between VO_2 and CO_2 and RER – the relationship between the coefficient gas exchange and the exercise load)

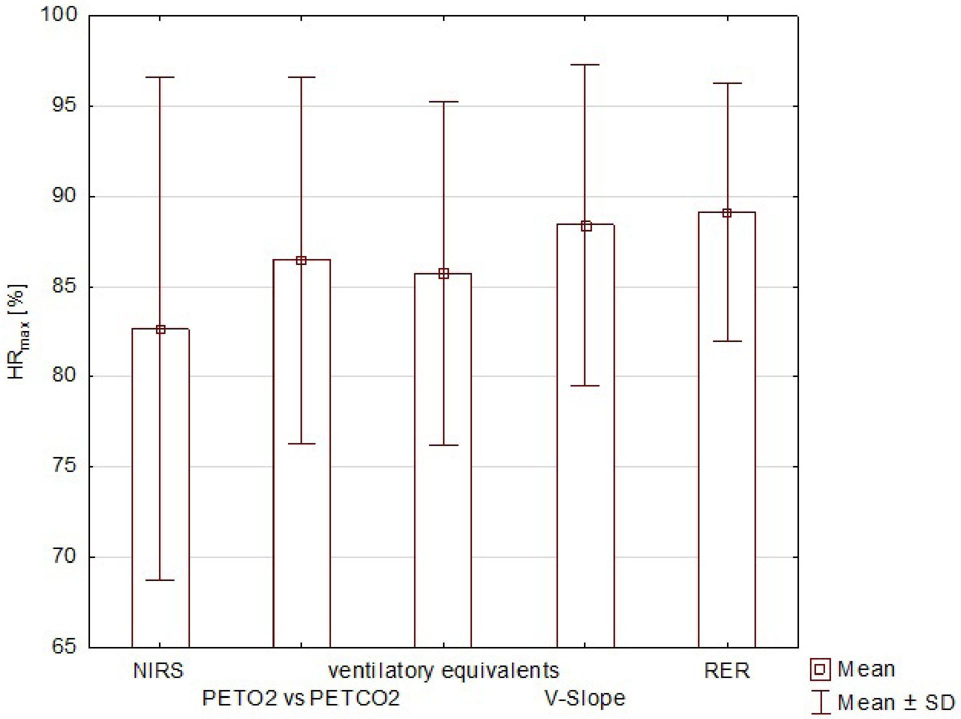

Fig. 3. The intensity of physical activity as a percentage of maximum heart rate (maximum heart rate [%]) for the anaerobic threshold determined based on the graphs of muscle tissue oxygenation parameters (NIRS) and the analysis of exhaled breath parameters. (PETO$_2$ vs PETCO$_2$ - the relationship between the pressure of oxygen through the terminal exhaustion air and carbon dioxide pressure through the terminal exhaustion air; Equivalents – the relationship between the ventilated oxygen equivalent and ventilated carbon dioxide equivalent; V-Slope – the relationship between VO$_2$ and CO$_2$; RER - the relationship between the coefficient gas exchange and the exercise load)

AT estimated as a percentage of the maximum heart rate has slightly changed in the case of ventilation methods. In this case AT determined by NIRS was achieved at 83% of the maximum heart rate, while AT was designated based on:

- the relationship between pressure of oxygen and carbon dioxide in the terminal exhaustion air – 87% of the maximum heart rate,
- equivalents was at 86% of the maximum heart rate,
- V-slope at 88% of the maximum heart rate.
- the relationship between the ratio of gas exchange and the exercise load taking place at 89% of the maximum heart rate (Fig. 4).

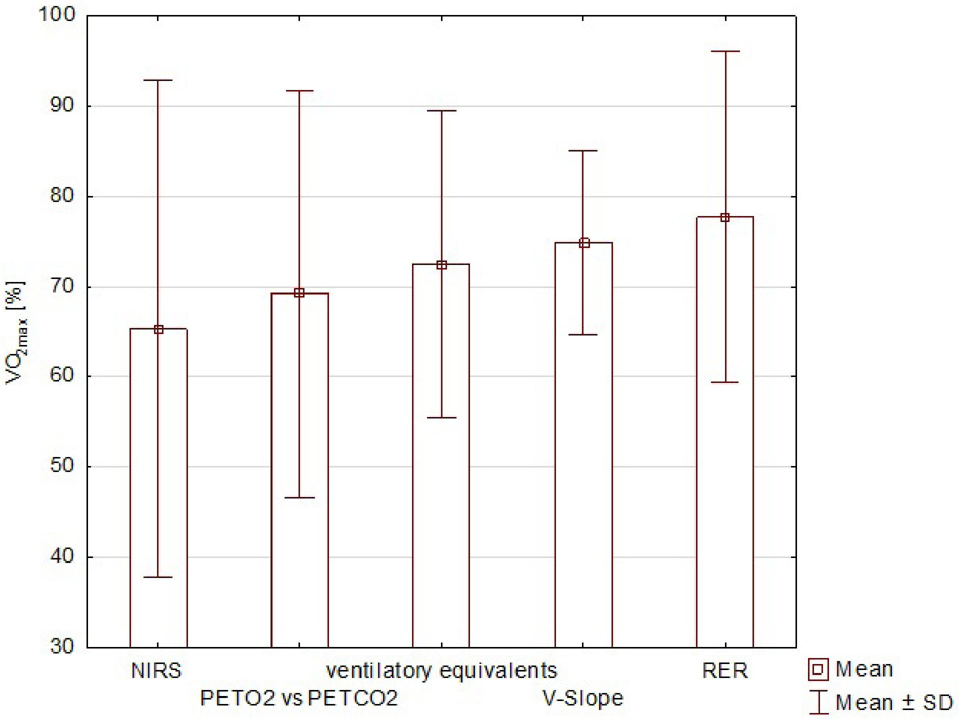

Fig. 4. The intensity of physical activity expressed as a percentage of the maximum volume of oxygen consumption (VO_{2max} [%]) for the anaerobic threshold determined based on the graphs of muscle tissue oxygenation parameters (NIRS) and the analysis of exhaled breath parameters ($PETO_2$ vs $PETCO_2$ – the relationship between the pressure of the oxygen through the terminal exhaustion air and carbon dioxide pressure through the terminal exhaustion air; Equivalents – the relationship between the ventilated oxygen equivalent and ventilated carbon dioxide equivalent; V-Slope – the relationship between VO_2 and CO_2; RER – the relationship between the coefficient gas exchange and the exercise load)

Positive and statistically significant relationships between the ventilation parameters and the work load was found. The strength of these relationships fluctuated around the level r = 0.98 for VO_2, 0.90 for VE to 0.94 for the RER (Fig. 5(d) to (f)).

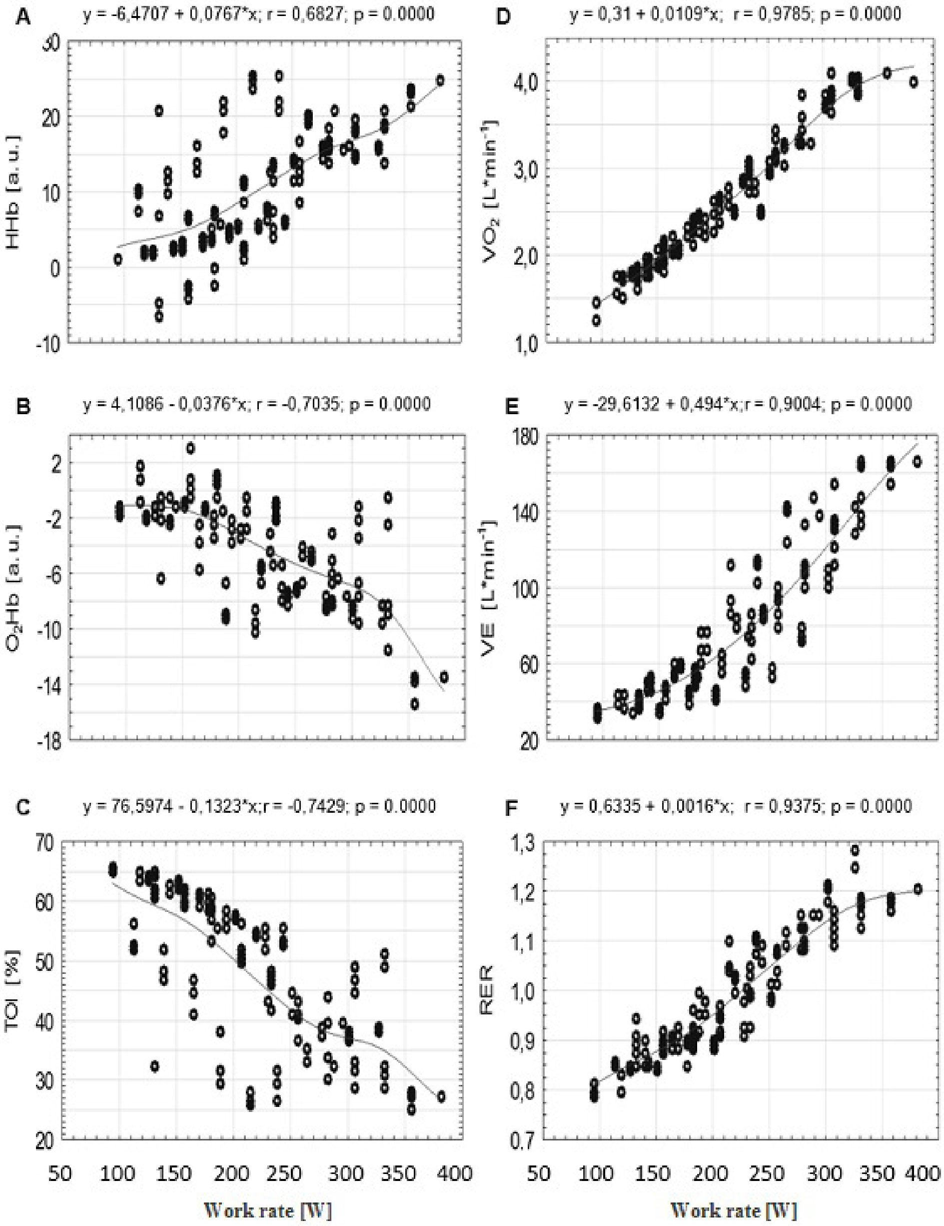

Fig. 5. The relationship between the parameters of muscle tissue oxygenation (a) to (c) and the parameters of ventilation (d) to (f) and the work load [W] (HHB – deoxyhemoglobin, O_2Hb – oxyhemoglobin, TOI – index of muscle tissue oxygenation)

Between the parameters of muscle tissue oxygenation and the work load there was a positive, statistically significant correlation in the range of deoxygenated hemoglobin (HHB) r = 0.68. The analysis of the relationship between the oxygenated hemoglobin (O_2Hb) and the muscle tissue oxygenation index (TOI) showed a negative correlation with the work load at r = -0.70 for O_2Hb and -0.74 for TOI (Fig. 5 (a) to (c)).

DISCUSSION

The aim of our study was to compare the anaerobic threshold method of determination. In our research we assumed that the kinetics of muscle tissue oxygenation coincides with the kinetics of the ventilation parameters during an exercise of an increasing intensity and that a significant reduction in oxygenation of the muscle tissue is necessary to establish the anaerobic threshold.

The study group consisted of young table tennis players, who are characterized by a possibility of making short-term efforts of high power requiring high efficiency anaerobic–phosphogenate energy processes and the ability to carry out the efforts that require an average efficiency of glycolytic processes [16]. Due to the subjects’ participation in competition, high aerobic fitness is required. The creation of the above exercise conditions runs due to a thorough planning and implementation of training procedures.

It is known that the onset of anaerobic metabolism during an exercise of an increasing intensity can be a result of an imbalance between supply and demand of oxygen in a muscle tissue, which can be determined by analyzing the parameters of the exhausted air as the anaerobic threshold (AT).

The obtained results indicate that the ventilation parameters are followed by the muscle tissue oxygenation parameters. In addition, the curves that characterize these parameters have different slope in successive phases of increasing load effort. It was observed that deoxygenated hemoglobin (HHB) increases with work load, which may indicate an increased oxygen utilization by the working muscles. The direction of the curve changes in the oxygenated hemoglobin (O_2Hb), and the muscle tissue oxygenation index (TOI) is reversed to the ventilation parameters. In O_2Hb an initially mild decrease in the curve accelerates rapidly at 65% VO_{2max}. Considering the fact that O_2Hb indirectly reflects blood flow [17] by decreasing the ratio of oxygen supply and demand, it can be suggested that the above mentioned intensity is followed by a reduced blood flow in muscles. Reduced blood supply leads to a restriction in the effect of oxygen on working muscles which use another source of energy, i.e. glycogen. This could also evoke blood buffering capability [18].

In this situation, even a low concentration of lactic acid results in an increase in free protons in the muscle cell. The consequence of this are disturbances in the transmission of stimulation for neuromuscular synapses and metabolism. Possibly the tested subjects, characterized by reduced blood buffer capacity, were not able to increase intensity or even continue the exercise [18].

In the CPET, it is possible to monitor continuously a group of parameters, such as aerobic and anaerobic metabolism. Additionally, the use of near infrared spectroscopy (NIRS) may increase the number of measured parameters of muscle tissue oxygenation. Such a method can also allow the AT determination maintaining the non-invasiveness, which is very important especially among children athletes.

Ventilation parameters allow for monitoring the changes of exercises metabolism. The results indicate that the anaerobic threshold determined by NIRS preceded the AT determined by an analysis of exhaust air parameters.

This result corresponds with published Timinkul data, which showed that deoxygenation of the brain tissue during exercise with increasing intensity reduces the activity of motor neurons and switch off individual motor units, resulting in a decrease in generating power by the muscles and work interruption [19].

A correlation between the parameters of muscle tissue oxygenation and work load was found (r = 0.70); however, a relationship between ventilation parameters and the load shows the strongest correlation coefficient (r = 0.93). This suggests a more consistent AT determination based on the analysis of exhaust gas parameters which we wanted to clarify due to our hypothesis.

Our data shows that the decrease in muscle indirect blood flow (O_2Hb) by decreasing the ratio of oxygen supply and demand predicts the anaerobic threshold determined by other ventilation parameters graph analysis methods. We also showed that the anaerobic threshold occurs as a result of the muscle tissue deoxygenation. It could be a good way to obtain AT while maintaining non-invasiveness especially when we do not want to add additional stress to children by giving them masks with a gas analyzer. It could also be an alternative way to examine those children who are afraid to do the CPET in a traditional way.

LIMITATION

The muscle tissue oxygenation measurement is limited to a number of external factors (temperature, sunshine, thickness of the fat tissue) [20]. This makes it difficult to interpret the results and can be a source of error in determination of the anaerobic threshold.

On the other hand, numerous papers [21, 22, 23] have confirmed that NIRS applied to measure the local muscle oxygenation can be useful in monitoring oxidative muscle metabolism, detecting adaptations of skeletal muscles to a training as well as examining the differences in the physical fitness level [24, 25, 26, 27]. In the future the examined groups should be expanded; however, this report may be used as a reference in further study.

CONCLUSIONS

Due to a practical application of this study, we would like to sum up the results of our research, which are:

a. muscle tissue oxygenation kinetics in the range of HHB coincides with the kinetics of selected ventilation parameters in response to exercise with increasing load;
b. kinetics of muscle tissue oxygenation in the area O_2Hb and TOI has a reverse dependence in relation to the work load;
c. correlations between the pressure of oxygen and carbon dioxide in the terminal exhaustion air at the anaerobic threshold determined by NIRS analysis and exhaust air parameters indicate that the anaerobic threshold occurs as a result of deoxygenation of the muscle tissue, which confirms NIRS as a method for determining AT;
d. the anaerobic threshold determined by NIRS precedes the anaerobic threshold determined based on the analysis of exhaust air parameters,

which can be helpful in obtaining AT among patients who cannot strike their maximal amount of work load due to various factors (poor disposition, a low level of VO_{2max}, obesity, etc.) [28];

e. we confirmed the use of NIRS as a non-invasive method for determining the anaerobic threshold in children, which used alone can be less stressful than a normal CPET test, especially for children who are afraid of the whole equipment.

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