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INVESTIGATION OF THE RELATIONSHIP BETWEEN MALE YOUNG ELITE WRESTLERS' BLOOD AND SALIVARY LACTATE DENSITIES THROUGH THE APPLICATION OF NON-INVASIVE LACTATE THRESHOLD ESTIMATION METHOD SUBSEQUENT TO THE EFFECTUATION OF STATIONARY PERIODIC WRESTLING-BASED EXERCISES

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ABSTRACT

The purpose of the present study was to scrutinize the relationship between male young and elite wrestlers' blood and salivary lactate densities and, heart rate through the non-invasive estimation of lactate threshold after the effectuation of a certain periodic wrestling-based exercise protocol. To this end, 20 male young elite wrestlers (Age 17 ± 2.5; Height: 178.73 \pm 4.4; Weight: 65.2 \pm 6.4; Fat percentage: 13.45 \pm 1.2) were incorporated into the study. The subjects effectuated the periodic wrestling-based exercise protocol in 5 minute phases so that their heart rates could be recorded using a Polar heart rate meter. The blood and salivary lactate sampling involved six phases including: resting mode, after warming up for 15 minutes and, after effectuation of four 5-minute exercise phases at intensities of 60%, 70%, 80% and, 90%. Blood and salivary lactate samples were taken during the passive 5-minute resting periods between the exercise phases. The data were analyzed through the application of the ANOVA test with repeated measurements and the Spearman correlation test at the significance level of 0.05. Considering the results, a statistically significant difference exists between the values of blood and salivary lactate between the aforementioned six phases. On the other hand, an acceptable correlation exists between the phase five's and the phase six's (at intensities of 80% and 90%) values of blood and salivary lactate (p<0.05, R5-69, p<0.05, R6-59) while there are is also a high correlation evident between heart rate and lactate levels in phases number five and six (p<0.05, R5-59, p<0.05, R6-63). Therefore the method of salivary lactate estimation could possibly be used as a non-invasive method in physiology studies for the objective determination of anaerobic threshold for such exercise protocols.

Key words: Male young elite wrestlers. Wrestling based stationary exercises. Salivary lactate. Blood lactate. Stationary periodic exercise.

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RESUMO

Investigação da relação entre o sangue de lutadores de elite de jovens do sexo masculino e as densidades de lactato salivar por meio da aplicação do método de estimativa do limiar de lactato não invasivo após a realização de exercícios periódicos de luta livre estacionários

O objetivo do presente estudo foi examinar a relação entre as densidades de lactato salivar e sangue de homens jovens e de elite e a frequência cardíaca através da estimativa não invasiva do limiar de lactato após a efetivação de um determinado protocolo de exercícios periódicos baseados em lutas. Para tanto, 20 jovens lutadores de elite do sexo masculino (idade de 17 ± 2,5; altura: $178,73 \pm 4,4$; peso: $65,2 \pm 6,4$; percentual de gordura: $13,45 \pm 1,2$) foram incorporados ao estudo. Os sujeitos efetuaram o protocolo periódico de exercício baseado em luta livre em fases de 5 minutos, para que suas frequências cardíacas pudessem ser registradas usando um medidor de frequência cardíaca Polar. A coleta de lactato sanguíneo e salivar envolveu seis fases, incluindo: modo repouso, após aquecimento por 15 minutos e, após a realização de quatro fases do exercício de 5 minutos, nas intensidades de 60%, 70%, 80% e 90%. Amostras de lactato sanguíneo e salivar foram coletadas durante os períodos de repouso passivo de 5 minutos entre as fases do exercício. Os dados foram analisados através da aplicação do teste ANOVA com medidas repetidas e teste de correlação de Spearman ao nível de significância de 0,05. Considerando os resultados, existe diferença estatisticamente significante entre os valores de sangue e lactato salivar entre as seis fases acima mencionadas. Por outro lado, existe uma correlação aceitável entre a fase cinco e a fase seis (em intensidades de 80% e 90%) dos valores de lactato sangüíneo e salivar (p<0,05, R5-69 e p<0,05, R6-59). enquanto há também uma alta correlação evidente entre a frequência cardíaca e os níveis de lactato nas fases cinco e seis (p<0,05, R5-59 e p<0,05, R6-63). Portanto, o método de estimativa de lactato salivar poderia possivelmente ser usado como um método não invasivo em estudos de fisiologia para a determinação objetiva do limiar anaeróbio para tais protocolos de exercícios.

Palavras-chave: Jovens lutadores de elite do sexo masculino. Exercícios estacionários baseados em wrestling. Lactato salivar. Lactato sanguíneo. Exercício periódico estacionário.

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INTRODUCTION

During the wrestling activity, human body develops certain special physical requirements including both anaerobic (strength, speed, lactate tolerance and anaerobic stamina) and aerobic features; hence wrestling is considered as an intense and heavy activity (Ion, 2002) while also being a sport based on anaerobic strength. This is why a major part of pre-seasonal exercises of wrestlers is occupied by lactate-Acid-Production-Based intense exercise (John and collaborators, 2015). Until today, various studies on elite wrestlers have shown that currently the wrestlers are under more pressure during exercises, matches and seasons compared to the past.

Therefore a need has been developed for more precise and professional exercises that would guarantee a suitable level of physical preparedness for wrestlers. In general, wrestling is an intense sport including periodic activities. Hence, anaerobic stamina and explosive speeds are highly important in this field (Rashid Lamar and collaborators, 2012).

Wrestling coaches give a high priority to the desirability and progressiveness of the athletic performance of their elite wrestlers. Through designing and implementing the most effective wrestling exercise protocols, these coaches could achieve their goal. In the field of wrestling, it is crucially important to highlight the anaerobic stamina and aerobic preparedness of wrestlers. By anaerobic stamina it is referred to the body's capacity for tolerance of the effects of participation in national and international tournaments without feeling weaknesses.

Nowadays, many trainers and scholars of sport sciences point to the importance of lactate threshold, initiation of blood lactate accumulation and, heart rate as indices of physiological pressure for the evaluation and determination of athletes' ability in endurance activities. In fact the basis of using the blood lactate's response to sport activities as a tool for the estimation of endurance performance and, the designing of specific exercise protocols has roots in several different studies. This shows that compared to the VO2max, the blood lactate's response to sport activities is a better index. The importance and the sensitivity of lactate-related studies, applied measurement methods and the effective factors on the former are excessively high. The most credible method of investigation of lactate and its changes is the blood sampling method which is considered as an invasive method. Due to the need for several samples at different phases of activity, it is rather difficult to use invasive methods and therefore, some studies have suggested a few non-invasive methods including the analysis of salivary lactate and electrolytes (Segora and collaborators, 1996).

In addition, some other studies have also used non-invasive methods in the contexts of aerobic endurance, anaerobic endurance and lactate threshold estimation (Plato and collaborators, 2008). Overall, noninvasive methods put the subjects under less stress while also compared to taking blood samples; it is many times easier to collect saliva samples (Navazesh and collaborators, 1982).

In this regard, Amir Sassan and collaborators (2011) conducted a study regarding the relationship between blood and salivary lactate densities subsequent to playing football on rather small fields. They stated that due to the similarity between the breakage point of blood and salivary lactate and heart rate, by taking a look at the heart rate curves, one can subjectively determine the anaerobic threshold.

On the other hand, Chicharo (1994); Segora and collaborators (1996) and Amir Sassan and collaborators (2011) have made efforts to estimate salivary lactate and electrolytes including Chloride Ions, Sodium, Potassium and salivary IgA subsequent to various exercise protocols. However, there has been no absolute agreement on the credibility of a specific method. In addition, none of the previously conducted similar studies have proposed a training protocol specialized for wrestling.

On this basis, aiming at investigation of the relationship between male young elite wrestlers' blood and salivary lactate densities and, heart rate, the present study makes an effort for non-invasive estimation of lactate threshold through the application of stationary wrestling-based periodic exercises while also pursuing the indirect goal of objective estimation of lactate.

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MATERIALS AND METHODS

The sample of the present study is comprised of male young elite wrestlers who exercised wrestling at least three times a week and at the time of conduction of study had at least 6 years of regular experience in addition the membership of a specific team. The entire subjects were controlled in terms of athletic records, healthcare records, disease records, medication records, age and maximum oxygen consumption.

One week prior to the execution of the research, the subjects were familiarized with the manner of effectuation of the proposed training protocol and received the necessary instructions. They subjects were banned from undertaking intense activities during the 20 hours prior to the administration of tests while their nutritional patterns were also homogenized using diet reminder forms (Larson, 2006).

Table 1 suggests subjects' anthropometric characteristics. Blood samples were taken from the fingertips of the subjects in six phases using the Germany made lactate analyzer device of Scout. The former six phases included: resting, after 15 minutes of warming up, after effectuation of four phases of stationary periodic exercises at intensities of 60, 70, 80 and 90 percent.

Nevertheless, the Cronin's formula of activity intensity control (5 minutes of working and 5 minutes of resting) was used. The salivary samplings during the mentioned 6 phases were carried out in an unstimulated fashion (Navazesh and Christenson, 1982) in 3 to 4 minutes during the resting period, after the fifteen minutes of warming-up and, during the rest times between the four phases. The subjects were seated and their saliva samples were stored on special plastic containers. Nonetheless, the entire samples have been collected between 4:30p.m and 6:30p.m.

For the purpose of measurement of heart rate, the Polar OreGon SCIENTIFIC 120 heart analyzers was used. To this end, subjects' heart rates were recorded during the resting mode, after the fifteen minutes of warming up and, during each 5-minute activity phase (once every 30 seconds with ten reps during each phase; 40 times during 4 phases) and overall, 42 readings were recorded.

The exercise protocol administered in the study was a researcher-made protocol

during which every technic is effectuated once and there is no resting time between stations and laps. In addition, there distance between stations is 10 meters. The subjects run the distances at maximum speed and get a 30 seconds resting at the end every of two consecutive executions. The subjects will have a 5 minute rest before the initiation of the next round of the stationary periodic exercise. The whole time length of execution of the administered stationary wrestling-based periodic exercise protocol is 6 minutes and includes: [(3sets X 2min Rest) + (30sec rest between each set X 2)]. After giving the necessary instructions to the subjects, they made presence in the predetermined testing site for participation in the research and effectuation of the exercise protocol (Rashid Lamar and collaborators, 2011).

The entire activities of the subjects were controlled during the 48hours prior to the execution of testing. The subjects were not allowed to undertake intense physical activity during the 24hours prior to the testing. Nevertheless, the nutrition of the subjects was also homogenized using a diet reminder.

During the resting and prior to warming up, every subject's heart rate was recorded using a wrist heart monitor gadget. The warming-up phase was 15 minutes long and was also undertaken at an intensity of 30-40 percent. Immediately after warming up, heart rates were recorded and blood and saliva samples were taken with a 5-minute gap in between. Afterwards, the third phase of the exercise protocol was executed at an intensity of 60% for 6 minutes. During this phase, heart rates were recorded ten times (once every thirty seconds). Afterwards, a five minute rest was administered for the collection of blood and saliva samples. The phases 4, 5 and 6 (intensities of 70, 80 and, 90 percent) were also executed in the same fashion (Dellal and collaborators, 2008; Fernandez and collaborators, 2012).

Salivary lactate measurement was undertaken through enzymatic methods in a laboratory using a Spectrophotometer. The collected saliva samples were kept in a fridge at 4 degrees (Celsius) from the time of taking until the time of analysis. The ANOVA method with repeated measurements and the Scheffe test have been used for the investigation of the changes in the indices of interest between the various phases of sampling. Afterwards, in

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order to investigate the relationships between the variables, the Spearman's correlation coefficient test was used. The collected data have been analyzed using the SPSS v.21.0 and Excel 2013 software at the significance level of $P \ge 0.05$.

RESULTS

Table 1 shows the average and standard deviation values of the physiological and anthropometric characteristics of the subjects. In addition, table 2 shows the changes in blood lactate, salivary lactate and, heart rate between the previously mentioned 6 phases.

Blood Lactate Density

According to the results of the ANOVA test with repeated measurements, a statistically significant difference exists between the values of blood lactate corresponding to each of the six sampling phases. In other words, it can be stated that one session of stationary wrestlingbased periodic exercise results in a significant change in the blood lactate density of male young elite wrestlers (P < 0.05, F: 33.82). considering the former significant difference between the values of blood lactate corresponding to various sampling phases, application of the Scheffe test indicated that the statistically significant differences observed between the phases 6, 1, 2, 3 and, 4of sampling shows a statistically significant increase in the density of blood lactate after one session of stationary wrestling-based periodic exercise ($P \ge 0.05$).

Table 1 - Physiological and Anthropometric characteristics of the subjects.

Measured indices	Average	Standard deviation
Age (year)	17	25
Height (CM)	178	2.4
Weight (Kg)	68.27	6.4
Fat Percentage	13.45	1.2
Resting heart rate	67.2	2.7

Phases	Prior to first activity (rest mode)	Warming-up	Immediately after activity at 60% intensity	Immediately after activity at 70% intensity	Immediately after activity at 80% intensity	Immediately after activity at 90% internist
Heart rate (per minute)	67.2 ± 2.7	128.0 ± 4.3	151.2 ± 3.2	167.0 ± 2.5	179.8 ± 4.2	194.0 ± 1.2
Blood lactate density	1.3 ± 1.5	1.6 ± 2.1	2.3 ± 1.4	3.9 ± 4.2	5.8 ± 5.0	6.5 ± 2.6
Salivary lactate density	0.2 ± 2.1	0.29 ± 1.2	0.4 ± 2.0	0.52 ± 0.1	0.7 ± 2.2	0.7 ± 1.4

Salivary Lactate Density

According to the results of the ANOVA repeated measurements. test with а statistically significant difference exists between the values of salivary lactate corresponding to each of the six sampling phases. In other words, it can be stated that one session of stationary wrestling-based periodic exercise results in a significant change in the salivary lactate density of male young elite wrestlers (P < 0.05, F: 12.17). Considering the former significant difference between the values of salivary lactate corresponding to various sampling phases, application of the Scheffe test indicated that the statistically significant differences observed between the phases 6, 1, 2, 3 of sampling shows a statistically significant increase in the density of salivary lactate after one session of stationary wrestling-based periodic exercise (P ≥ 0.05).

The difference between the scopes of changes of blood and salivary lactate densities was only statistically significant in the fifth and sixth phases; therefore considering the statistically significant and positive relationship between these two indices at the intensities of 80 and 90 percent, it can be stated that salivary lactate density increases as the density of blood lactate increases (table 3).

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The difference between the scopes of changes of salivary lactate density and heart rates was only statistically significant in the fifth and sixth phases; therefore considering the statistically significant and positive relationship between these two indices at the intensities of 80 and 90 percent, it can be stated that with the increase of heart rate, salivary lactate density increases as well. On this basis, in subjects who showed an increase in the scope of changes of the heart rate, salivary lactate was also changed similarly (table 4).

Table 3 - Estimated relationship between the scopes of changes of blood and salivary lactate densities at intensities of 80 and 90 percent.

Blood and salivary lactate	Correlation	0.85		
densities in the fifth phase	coefficient	0.05		
(80% intensity	Significance	0.05		
Blood and salivary lactate	Correlation	0.76		
densities in the sixth phase	coefficient	0.70		
(90% intensity	Significance	0.05		

Table 4 - Heart rate and salivary lactate density.

Heart rate and salivary lactate density in the fifth phase (80%	Correlation coefficient	0.67
intensity	Significance	0.045
Heart rate and salivary lactate density in the sixth phase (90%	Correlation coefficient	0.78
intensity	Significance	0.015

Considering the above content, it can be concluded that the statistical findings of the present study suggest that one session of stationary wrestling-based periodic exercise results in statistically significant increase of both blood and salivary lactate densities (P \geq 0.05). in addition, the result of investigation of the relationships between the variables showed that there exists a statistically significant relationship between the scopes of changes of both blood and salivary lactate densities and heart rate at highly intense (intensities of 80 and 90%) stationary wrestling-based periodic exercises.

DISCUSSION AND CONCLUSION

The purpose of the present study was to investigate the relationship between blood and salivary lactate densities and heart rate aiming to validate a non-invasive lactate threshold estimation method after the effectuation of a stationary wrestling-based periodic exercise protocol. A statistically significant difference was observed between the values of blood and salivary lactate in all phases.

On the other hand, an acceptable correlation was observed between the values of blood and salivary lactate density in the fifth and sixth phases of the prescribed exercise protocol. In addition, a high correlation between heart rate and salivary lactate density was also observed in the fifth and sixth phases. The controlling of the intensity of exercises was made possible through the ballshaped protocol of the present study. since the literature of the subject matter includes only a few studies in the context of salivary lactate and electrolytes, only a limited number of studies have been referred to.

The present study is consistent with the studies conducted by Amir Sassan and collaborators (2011), Chicharo and collaborators (1994), Dumke and collaborators (2006), Santos and collaborators (2006), Segora and collaborators (1996), Valenzano and collaborators (2008). It is also essential to point out that salivary lactate is probably formed through an inactive transfer from blood and salivary glands and therefore it increases in density when blood lactate's density increases. although it is still unknown that how much time it takes the salivary lactate to form after the entry of blood into the salivary glands, this issue has not influenced the results of the present study since the samples have been taken simultaneously. In this condition, the lack existence of a statistically significant of difference between the phase six and phases four and five may be because of the closeness of the times of sampling. This is mostly because in contrast to blood lactate, salivary lactate forms with a little bit of delay (Santos and collaborators, 2006).

On the other hand, lack of significant differences between the phases one, two and three is probably due to the low intensity of exercise and the balance between the production and elimination rates of lactate. Nevertheless, in the present study's exercise protocol, the slope of the curve corresponding to salivary lactate has adopted a sudden increasing trend at a specific intensity. This could be related to the crossing of the line between the aerobic and the anaerobic. In other words, the salivary lactate threshold has

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taken place at the point where slope changes (Valenzano and collaborators, 2008).

According to the results of analyses of the relationships between the variables, the difference between the scopes of changes of blood and salivary lactate densities is significant only at the intensities of 80 and 90 percent. On this basis, it can be stated that as the blood lactate density increases, salivary lactate density increases as well. In other words, salivary lactate density has increased in subjects who have experienced an increase in the scope of changes of their blood lactate density. This finding is consistent with the results obtained by Amir Sassan and collaborators (2011), Santos and collaborators (2006), Segora and collaborators (1996).

In the present study, the correlation between the densities of blood and salivary lactate was desirable at the phases five and six. This result is consistent with the results obtained by Amir Sassan and collaborators (2011) and Santos and collaborators (2006). Increase in the blood lactate density in the initial phases of the exercise and the increase in the density of salivary lactate after a while may be due to the delay in secretion of lactate from salivary glands compared to the speed of blood lactate formation. It has been turned out that secretion of saliva is usually the result of responding to the spontaneous stimulation of glands and catecholamine agents may be somehow involved in the controlling of salivary electrolytes. In addition, the natural secretion of saliva depends on the cooperation between sympathetic and Para-sympathetic nerves. It has been stated that Para-sympathetic stimulation results in excessive secretion of saliva with less densities of mineral and organic compounds.

On the other hand, it is known that Sympathetic stimulation reduces the volume of saliva. In addition, the factors of intensity and length of stimulation of glands can also be effective on the salivary compounds. While undertaking prolonged exercises at low to medium intensities (VO2max of less than 60%), it doesn't seem that if any statistically significant changes take place in the secretion of saliva.

However, at higher intensities, salivary secretion reduces (Dumke and collaborators, 2006). Among the factors related to intense activity it can be referred to dehydration and evaporation of saliva due to over-ventilation. Regarding salivary lactate it has been claimed that this Ion is probably formed through inactive transfer from blood and salivary glands (Folke, 2008). It is still unknown that how much time does it take the salivary lactate to form after the entry of blood into the salivary glands (Saguaro and collaborators, 1996; Santos and collaborators, 2006).

Nonetheless, the results of the study conducted by Asgari and collaborators (2004) are inconsistent with the results of the present study. Asgari and collaborators (2004) concluded that there is no relationship between the densities of blood and salivary lactate and therefore salivary lactate cannot be a replacement for blood lactate. It seems that since the samples from blood and saliva were taken without a proper time-gap, no significant relationships have been observed between the salivary and blood lactate densities. It should always be taken into account that if the length of the test is less than 3 minutes and if the time-gaps between the sampling trials are too small, some sort of imbalance between the blood and salivary lactate densities may be observed (Santos and collaborators, 2006).

Although there was no relationship between the blood and salivary lactate densities during the initial phases, the mere of statistically existence а significant relationship in fifth and sixth phases shows that salivary lactate testing can be considered as a non-invasive method for estimation of lactate threshold. Many of previous studies during the past few years have investigated the relationship between heart rate and blood lactate and all of these studies have verified the existence of a statistically significant relationship between blood lactate density and heart rate (Alvarez and Gastanga, 2007; Soler and collaborators, 2008).

There are no previous studies investigating the relationship between the heart rate and salivary lactate density in the form of the protocol administered in the present study. in the present study, a statistically significant relationship was recorded between the scope of changes of blood and salivary lactate densities during the fifth and sixth phases of the exercise protocol.

Considering the significance and the positivity of the relationship between the former and latter indices and heart rate at the intensities of 80 and 90 percent, it can be stated that as the heart beat increases in

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consistence with the increase in the intensity of exercise, salivary lactate density increases as well. In other words, subjects who have experienced an increase in their heart rate at the formerly mentioned 6th and 5th phases have also experienced an increase in their salivary and blood lactate densities as well (Candiotti and collaborators, 2008; Karapetian and collaborators, 2008).

Since with increased exercise intensity, the blood lactate level and heart rate increase as well and considering the formerly explained relationship between the blood and salivary lactate densities (Andersen and Mahoney, 2007), it can be claimed that with increased heart rate, salivary lactate density increases consequent to the increased density of blood lactate. The results of the present study have shown that the administered exercise protocol can be used for conduction of several various scientific studies on young elite wrestlers.

Quantification and evaluation of the physiological stresses imposed on young wrestlers during various exercise activities are two important factors that should be considered during every planning. Since heart rate is recorded through non-invasive methods. it would cause the least stress and therefore it can be stated that monitoring or recording the heart rate can be considered as a flexible key to the evaluation intensity during the games. As it is evident from the results of the present study, the pattern of changes in the blood and salivary lactate densities are similar to the pattern of changes in the heart rate. Another important result in terms of heart rate and physiological responses including lactate response is that heart rate can change at the lactate threshold according to the exercise type.

While using heart rate as the instrument of estimation of lactate threshold, one must be sufficiently cautious. Most of the previous studies regarding the relationship between lactate density and heart rate have been conducted using turntables and mills in which the roles of the torso and the lower body are distinct. In general it can be stated that wrestling-technics based exercises cause statistically significant changes in the levels of both blood and salivary lactate.

Nevertheless, a significant correlation was recorded between the blood and salivary lactate densities in the present study's exercise protocol's high intensity activities (80 and 90 percent). On this basis, making use of the noninvasive method of salivary sampling and measurement of salivary lactate density can probably be a good solution for determination of the changes in blood lactate. In addition, in the present study it is also possible to subjectively determine the breakage point of blood and salivary lactate curves and the lactate threshold as well.

Additionally, it has been indicated that the patterns of changes in blood and salivary lactate densities and heart rate are similar. The highly important point in here is that conducting performance related physiology studies outside of the laboratories and in field study fashions, reduces the required amounts of time and money, although it is accompanied by certain difficulties.

The present study has contributed to the formation of a new technic for field measurement of salivary lactate which is one of the most important applicable achievements of the present study.

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