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COMPETITIVE STRESS ASSESSMENT USING SALIVARY BIOMARKERS IN RHYTHMIC GYMNASTS

OCENA TEKMOVALNEGA STRESA Z UPORABO BIOMARKERJEV SLINE PRI TEKMOVALCIH V RITMIČNI GIMNASTIKI

ABSTRACT

Stress and adaptation are inevitable components of the gymnastics training in competitive gymnasts. Salivary biomarkers, such as alpha-amylase activity (sAAA), concentration of protein (sP) and potassium (sK+) are useful non-invasive stress indicators. The aim of this study was to assess the stress levels before training and competitions in rhythmic gymnasts by using non-invasive biochemical salivary stress markers and anxiety questionnaires. The study included 10 national level rhythmic gymnasts (mean age: 14.7±1.57 years). Saliva was collected by salivates at three time points: nine days pre-competition at home (baseline), five days pre-competition before a training session, and just before the competition. The sAAA and the sP and sK+ were measured. Trait and state anxiety were evaluated by the State-Trait Anxiety Inventory. At baseline, the mean sAAA was 5.89±0.75 ln(U/mL) and increased significantly to 6.56±0.58 ln(U/mL) just before the training session ($p<0.05$) and increased further to 6.90±0.70 ln(U/mL) just before the competition ($p<0.05$ vs baseline). The mean sP increased progressively insignificantly 1.84±0.70 g/L vs 2.28±0.97 g/L vs 2.91±1.44 g/L, respectively. The mean sK+ was significantly higher before the competition vs the baseline value (35.73±8.3 mmol/L vs 23.94±4.83 mmol/L, $p<0.01$). The mean state anxiety score was significantly higher before the training session in contrast to the baseline (36.90±11.03 vs 30.80±10.26, $p<0.05$) and to that before the competition (40.10±9.57 vs 30.80±10.26, $p<0.05$). In conclusion, the sAAA and sK+ were both in agreement with the anxiety scores, and they can be applied as useful and objective non-invasive markers of stress.

Keywords: saliva analysis, salivary potassium, anxiety markers, performance anxiety

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IZVLEČEK

Stres in prilagoditev sta neizogibna sestavina treninga gimnastike pri tekmovalnih telovadcih. Biokemični markerji v slini, kot so aktivnost alfa-amilaze (sAAA), koncentracija beljakovin (sP) in kalija (sK+), so uporabni nenasilni kazalci stresa. Namen te študije je bil oceniti stopnje stresa pred treningi in tekmovanji pri ritmičnih telovadcih z uporabo neinvazivnih biokemijskih markerjev stresa v slini in vprašalnikov tesnobe. Študija je vključevala 10 tekmovadk, ki tekmujejo na nacionalni ravni (povprečna starost: 14,7±1,57 let). Slina je bila zbrana ob treh časovnih točkah: devet dni pred tekmovanjem doma (osnovna vrednost), pet dni pred tekmovanjem pred treningom in tik pred tekmovanjem. Meritve so zajemale sAAA, sP in sK+. Lastnosti in stanje tesnobe so bile ocenjene z Inventarjem tesnobe (STAI). Ob osnovni vrednosti je bila povprečna vrednost sAAA 5,89±0,75 ln(U/mL) in se je znatno povečala na 6,56±0,58 ln(U/mL) tik pred treningom ($p<0,05$), nato pa še na 6,90±0,70 ln(U/mL) tik pred tekmovanjem ($p<0,05$ v primerjavi z osnovno vrednostjo). Povprečna vrednost sP se je progresivno, a neznačilno, povečala z 1,84±0,70 g/L na 2,28±0,97 g/L in 2,91±1,44 g/L. Povprečna vrednost sK+ je bila znatno višja pred tekmovanjem v primerjavi z osnovno vrednostjo (35,73±8,3 mmol/L proti 23,94±4,83 mmol/L, $p<0,01$). Povprečni rezultat za stanje tesnobe je bil znatno višji pred treningom v primerjavi z osnovno vrednostjo (36,90±11,03 proti 30,80±10,26, $p<0,05$) in pred tekmovanjem (40,10±9,57 proti 30,80±10,26, $p<0,05$). Zaključek: sAAA in sK+ sta bila v skladu z rezultati tesnobe in ju je mogoče uporabiti kot koristne in objektivne neinvazivne markerje stresa.

Ključne besede: Analiza sline, kalij v slini, markerji tesnobe, tekmovalna tesnoba

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INTRODUCTION

According to the stress theory of Hans Selye, stress is not simply a nervous tension or a result of a damage, but it is the nonspecific response of the body to any demand made upon it (Selye, 1976). Physiological reactions related to stress can occur by direct effects on the human body, as well as by the effects from psychological factors, such as the loss of a relative, conflicts in the family or at work, and others. Lazarus defined stress as a dynamic, two-way continuously changing interaction between the person and the environment (R.S. Lazarus, 1966; R. S. Lazarus & Folkman, 1984). According to this definition, stress is actually a reflection of the individual's behavior towards the environment when the person assesses it as difficult, exhausting and threatening (R. S. Lazarus & Folkman, 1984). When a certain situation is assessed by the individual as dangerous, and the available resources are insufficient to deal with it, a physiological and mental reaction develops, which is referred to as mental stress. The physiological components of mental stress are identical to those described by Hans Selye for biological stress, however, the mental components are associated with different emotions depending on the type of stress (R.S. Lazarus, 1991; R. S. Lazarus, 1991).

A situation is perceived as a threat when the individual anticipates possible harm or loss, which can refer to both physical damage and interference with the individual's self-esteem or social status. According to A. Beck's theory, anxiety is perceived as physical or psychological threat to one's personal domain (Beck & Clark, 1988; Beck & Clark, 1991). In the literature, anxiety is often divided into state and trait anxiety and levels of anxiety have been assessed by the State Trait Anxiety Inventory, which is a questionnaire of 40 self-report items on a 4-point scale developed by Charles Spielberger (C. D. Spielberger, 1977). State anxiety refers to the feeling of a person at the moment (it is a temporary feeling), and it is defined as fear, nervousness, or discomfort induced by different situations that are perceived as dangerous (C. Spielberger & Sydeman, 1994). Trait anxiety is defined as feelings of stress, and discomfort that can be experienced on a daily basis (Heeren, Bernstein, & McNally, 2018).

Stress and adaptation are primary components of sport because of its competitive nature, which requires athletes to demonstrate their maximum physical potential under great psychological pressure. Anxiety can have a negative effect on simple motor tasks (Noteboom, Fleshner, & Enoka, 2001), and can lead to more interfering thoughts with greater disruptions in concentration in youth sport (McCarthy, Allen, & Jones, 2013). During competitions, athletes experience anxiety and the accompanying physiological stress reactions due to the uncertainty

of the results and the threat of loss of prestige and lowering of their self-esteem or evaluation by coaches, relatives, and admirers.

The possibilities for an objective assessment of pre-competition stress and the methods for influencing it are being considered with growing interest in many sports. Along with the physiological indicators, such as heart rate, arterial pressure and others, a number of biochemical parameters are also used (Nesheva, 2015, 2017a, 2017b; Petrov, Alexandrova, & Lefterov, 2017; Petrov, Bozhilov, Alexandrova, Mugandani, & Djarova, 2013; Petrov, Penov, Kolimechkov, & Alexandrova, 2018). While blood cortisol is a commonly used stress biomarker, obtaining venous blood samples is an unpleasant procedure for many athletes, posing a particular challenge for field testing and monitoring. In recent years, there has been a growing interest in using biochemical parameters for research through non-invasive methods (Groschl, 2008). A suitable non-invasive alternative of blood samples is the use of saliva, which has the following advantages: it is collected by a non-invasive procedure that does not require special expertise, it does not pose high risks of infection, and it is easier to store and process (Kaufman & Lamster, 2002). Some prospective parameters in saliva are alpha-amylase activity, protein, potassium, and sodium concentrations. Methods and devices for the application of these parameters have already been described in the scientific literature (Fuentes et al., 2011; Robles et al., 2011; Shetty, Zigler, Robles, Elashoff, & Yamaguchi, 2011; Yamaguchi et al., 2006; Yamaguchi et al., 2004; Yamaguchi & Sakakima, 2007).

Studies demonstrated the possibility of using salivary alpha-amylase activity (sAAA) as a parameter of physical (Li & Gleeson, 2004) and psychological stress (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Nater & Rohleder, 2009), as sAAA correlates with serum levels of adrenaline and cortisol (Chatterton et al., 1996). It has been documented that sAAA increases in response to stressors such as exercise, heat, cold, and behavioral tests (Chatterton et al., 1996; Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007).

It should be noted that the maximum sAAA is reached between the 15th and 20th minute before the increase of salivary cortisol concentration. This is due to the shorter response time of the sympathetic nervous system (SNS) compared to the response of the hypothalamic–pituitary–adrenal axis (HPA axis). While the sAAA is synthesized and secreted directly by the salivary glands in the oral cavity, cortisol is released from the adrenal gland to circulate in the blood, and then it diffuses passively to reach the saliva. This may be one of the reasons why some

studies assessing stress showed no correlation between sAAA and salivary cortisol (Granger et al., 2007).

The autonomic nervous system regulates the concentration of protein in saliva in the following way: the parasympathetic nerves release acetylcholine and stimulate fluid secretion, while sympathetic nerves release noradrenalin to induce flow at rest and protein production (M. Edgar, Dawes, & O'Mullane, 2012; W. M. Edgar, 1992). In this way, the increase in the concentration of protein in the saliva also indicates the increase in the activity of the sympathetic autonomic nervous system, which is seen in stressful situations.

Some studies noted changes in the saliva concentrations of Na+ (sNa+) and K+ (sK+) in under psychological stress. Most researchers noted an increase of sK+ under stress, while sNa+ either did not change or showed a decrease. Their ratio (sK+/sNa+) was used as an indicator reflecting this reciprocal behaviour of the concentration of the two ions (Hinton et al., 1992; Minasian, Gevorkian, Daian, Grigorian, & Grigorian, 2004; Richter, Hinton, Meissner, & Scheller, 1995). These changes may be due to stimulation of the sympathetic nervous system, which causes changes in salivary flow, reabsorption and secretion of electrolytes in secretory cells (Chicharro, Lucia, Perez, Vaquero, & Urena, 1998).

The main factor causing the described changes in the sK+ and sNa+ is probably the increased level of aldosterone under stress. Indeed, the level of mineralocorticoids, compared to the level of glucocorticoids, is less affected by the adrenocorticotropic hormone (ACTH), but under stress, the renin-angiotensin-aldosterone system (RAS) is also activated due to the influence of the SNS as shown in Figure 1 (Antonov, Markel, & Yakobson, 2011; Brown, Thou, & Whitworth, 1995; Kubzansky & Adler, 2010). Aldosterone increases K+ excretion and decreases Na+ excretion in both the kidney and salivary glands (Riad, Lefaivre, Tournaire, & Barlet, 1986). This mechanism, in our opinion, explains the already mentioned changes in the electrolyte composition of saliva under stress.

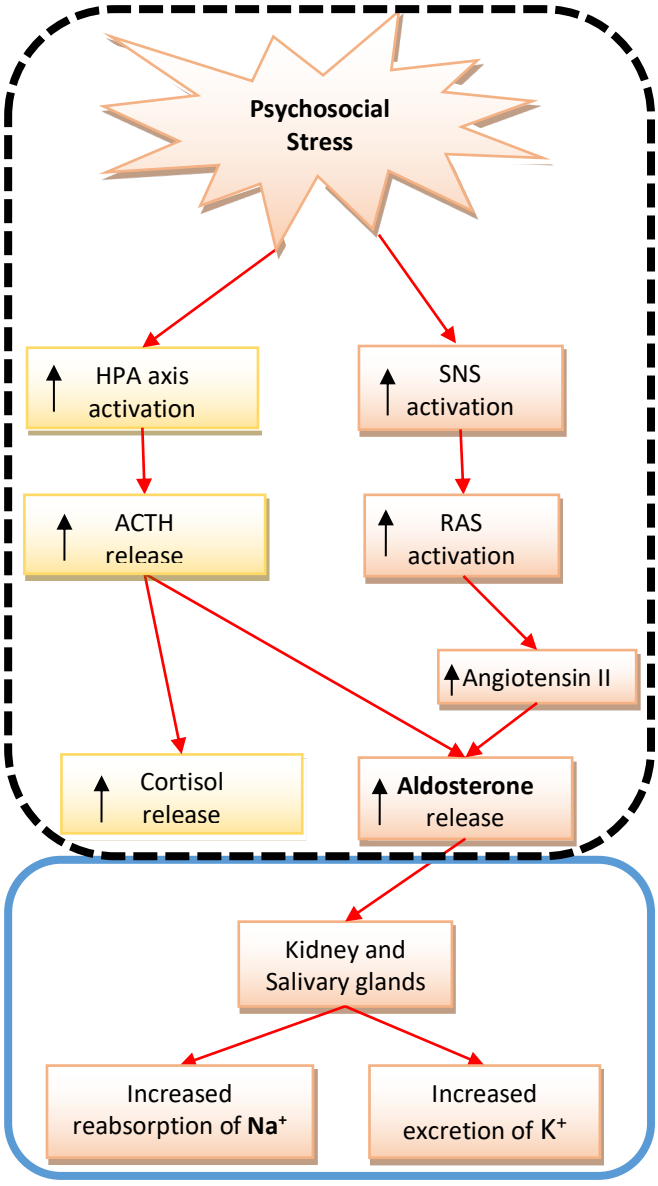


Figure 1. Mechanism of an increase in aldosterone level, and hence an increased sK+ and decreased sNa+ under stress. Legend: - - - - - - - according to (Kubzansky & Adler, 2010), ——— with our additions in blue.

Rhythmic gymnastics is an aesthetic sport characterized by unique physical and psychological demands, often involving young athletes. Special consideration is given to the body image with particular adherence to their diet and body composition (Miteva et al., 2020). Competitive rhythmic gymnasts spend many hours of intensive sessions per week, practice lots of repetitions and perform high level technical elements. Rhythmic gymnasts involved in competitions are associated with high levels of physical and psychological stress (Bobo-Arce & Méndez-Rial, 2013; Nassib, Mkaouer, Riahi, Wali, & Nassib, 2017), including the pressure to maintain a specific body image and adhere to strict diets, intensive training schedules with long hours of

repetitive practice, and the need to execute complex technical elements with precision (Miteva et al., 2020). Elite rhythmic gymnasts who travelled long distances to compete showed high risk for short sleep duration, abnormal daytime sleepiness and reduced sleep quality (Silva & Paiva, 2019). High stress levels in rhythmic gymnastics can lead to worse results and increased sports injuries (Kim & Park, 2020). Understanding of the personality traits and coping strategies to manage stress levels can help gymnastics coaches to develop effective interventions and assist their gymnasts to attain optimal performance in competitions (Kaplanova, 2019). The competitive environment further amplifies these stressors, with gymnasts experiencing anxiety related to performance outcomes, evaluation by coaches and judges, and the potential impact on self-esteem. This complex interplay of factors creates a high-stress environment that can affect athletes' well-being and performance (Kovacs, Keringer, Racz, Gyomber, & Nemeth, 2022; Tsopani, Dallas, & Skordilis, 2011). Therefore, a deeper understanding of the physiological and psychological stress responses in rhythmic gymnasts is crucial for developing effective support strategies.

The small number of studies focused on stress in rhythmic gymnastics used mostly psychological tests/questionnaires as research methods (Codonhato et al., 2018; Kim & Park, 2020; Nassib et al., 2017; Silva & Paiva, 2019; Tsopani et al., 2011). The complex and multi-dimensional nature of stress in rhythmic gymnasts was reported by using interviews in another study (Penna, Filho, Bentes, Ferreira, & Pires, 2023). A case study involving 3 men and 3 women artistic gymnasts analysed anxiety by questionnaires but also included salivary cortisol, heart rate and skin conductance, and the authors reported that the visualization of competitive situation causes stress and anxiety (Pineda-Espejel, Trejo, Terán, Cutti, & Galarraga, 2020). Researchers targeting a comprehensive assessment of stress in competitive sport should also involve objective biochemical or/and physiological parameters. For instance, saliva collection can be a suitable non-invasive biochemical method, which does not have a high risk of infection or require special skills, to assess stress levels in elite athletes, such as rhythmic gymnasts.

The aim of this study was to assess the pre-competition stress levels in rhythmic gymnasts by using non-invasive biochemical methods in addition to an anxiety questionnaire.

METHODS

This study was performed in accordance with the Declaration of Helsinki for Human Research (WMA, 2013), and an institutional ethics approval was granted by the Ethics Committee of the

Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 with the US Department of Health and Human Services). Informed consents were obtained from the parents of all participants prior to the study.

Participants

The study included 10 youth rhythmic gymnasts (mean age: 14.7 ± 1.57 years), who were group competitors at the Bulgarian Rhythmic Gymnastics Championships. Five of them were competing as a team at the group all-around event in the women's category (cases #1W to #5W) with a mean age of 16.0 ± 1.00 years (ranging from 15 to 17 years). The other five female gymnasts were competing as a team at the group all-around event in the adolescent's category (cases #1A to #5A) with a mean age of 13.4 ± 0.55 years (ranging from 13 to 14 years). The women's team was ranked 10th in the women's group all-around, and the adolescent's team won the gold medals in adolescent's group all-around out of 14 teams at the Bulgarian Rhythmic Gymnastics Championships.

Study design

Saliva was collected at three time points: nine days before the competition at home (HOME), five days before the competition just before a training session (TRAINING), just before the competition (COMPETITION). The first saliva collection was taken in the home of the participants by the gymnasts themselves on Monday at 10:00 o'clock, nine days before the Bulgarian Rhythmic Gymnastics Championships to obtain the baseline values of the parameters tested. In addition, the gymnasts also filled in two tests to measure trait and state anxiety. The second saliva collection was taken five days before the competition at the gymnastics hall at a similar time in the morning (around 30 minutes before the training session). The third collection was taken at the gymnastics centre on the day of the competition in the morning around one hour before the beginning of the gymnastics routines. After the second and third saliva collection, the gymnasts filled in only the state anxiety questionnaire.

To minimize the influence of external factors on salivary biomarkers, several control measures were implemented. Participants were instructed to refrain from consuming alcohol, smoking, or drinking coffee for 24 hours before each saliva collection. To standardize dietary intake, they were asked to avoid heavy meals and maintain their regular diet on the day before and the day of saliva collection. Furthermore, participants were asked to avoid eating or drinking anything other than water for at least 1 hour before providing a saliva sample.

The saliva was taken by cotton swab salivates without salivary stimulant (Sarstedt AG & Co, Nümbrecht, Germany). Prior to the saliva collection, the gymnasts rinsed their mouth with distilled water and placed the swab under their tongue for two minutes, and then put the swab back into the salivate. The salivates were marked with code numbers, time, and date, and were transported to the laboratory by using a portable cool bag. The salivates were centrifuged at 1000 g in the laboratory, and the extracted saliva was then stored at -20°C until the biochemical analysis, which determined the salivary alpha-amylase activity (sAAA), salivary protein concentration (sP), and salivary potassium concentration (sK+).

Salivary alpha-amylase activity (1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.1)

The Alpha-amylase Colorimetric test kit, (REF E12 218A, EMAPOL, Gdańsk, Poland) was used to determine the alpha-amylase activity. The samples were first diluted 1:100, and then 10 µl of the diluted saliva was added to 1000 µl of the reagent. This mixture was stirred vigorously and incubated for 1 min at 25°C. The samples' optical density (OD) at 410 nm (A410) were read immediately and after 1, 2 and 3 minutes. The average enzyme kinetics (ΔA410/min) was determined, and the salivary alpha-amylase activity (sAAA) was calculated by using the formula: sAAA = (ΔA sample/min) x 24820 [U/L], and presented as natural logarithm, ln(sAAA) in accordance with Kobayashi et al. 2012 (Kobayashi, Park, & Miyazaki, 2012).

Salivary protein concentration (sP)

The Total Protein test kit, (REF 10570, HUMAN Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) was used to determine the salivary protein concentration. Twenty µl of the saliva, as well as 20 µl standard protein solution (80 g/L) were separately added to 1000 µl biuret reagent. The mixtures were stirred vigorously and incubated for 10 min at 25°C. After that the OD at 540 nm (A540) of the samples and the standard were read, and the salivary protein concentration (sP) was calculated by using the formula: sP = 80 x (sample A540/standard A540) [g/L].

Salivary Potassium concentration (sK+)

The Potassium test kit, (REF 10118, HUMAN Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany) was used to determine sK+. A working reagent was prepared from the two reagents 1 and 2, which were mixed in a 1:1 ratio and stood for 15 - 30 min before being used. 500 µl precipitating solution was added to 50 µl of the saliva. The mixtures were stirred for 10 min with 10 000 rpm. 100 µl standard or 100 µl supernatant was added to 1000 µl

of the prepared reagent. The OD at 578 nm (A578) of the standard and the sample were measured against the reagent after 5 min. The sK+ was calculated by using the formula: sK+ = 5 x (ΔA578 sample/ΔA578 standard) [mmol/L].

State and Trait Anxiety

The State-Trait Anxiety Inventory (STAI) comprises two separate 20-item self-report questionnaires that measure state anxiety (S-Anxiety) and trait anxiety (T-Anxiety). Each item is rated on a 4-point Likert scale, ranging from 1 ("not at all") to 4 ("very much so"). For both S-Anxiety and T-Anxiety, scores are calculated by summing the item scores, with some items reverse-scored as indicated in the STAI manual. This summation yields a raw score that ranges from 20 to 80 for each scale. Higher scores indicate greater anxiety levels. While there are no universally defined cut-off scores, scores can be interpreted relative to normative data provided in the STAI manual (Spielberger, 1977) to categorize anxiety levels (e.g., low, moderate, high). In this study, we used the raw scores to compare anxiety levels across the three time points and to examine correlations with salivary biomarkers.

Statistical Analyses

The statistical analyses were conducted with GraphPad Prism 7.04 software, using descriptive statistics and the Shapiro-Wilk test of normality. All variables had a normal distribution, and the statistical significance of the differences between the mean values was evaluated by one-way repeated measures ANOVA with Tukey's post-hoc multiple comparisons test. The data in the text and the tables are presented as mean ± SD.

RESULTS

The individual results of the sAAA for each gymnast are presented in Figure 2. The sAAA was between 4.99 and 7.13 ln(U/mL) measured at time point HOME, between 5.58 and 7.58 ln(U/mL) at time point TRAINING, and between 6.24 and 7.29 ln(U/mL) at time point COMPETITION. The sAAA was higher before the training session and the highest just before the competition in comparison with the samples taken at home in 6 of the gymnasts (#1W, #2W, #5W, #1A, #2A and #5A). Three of the gymnasts (#3W, #4W, #3A) had sAAA higher before the training session in comparison with the samples taken just before the competition. There were no clear differences in sAAA from the three samples in one of the gymnasts (#4A).

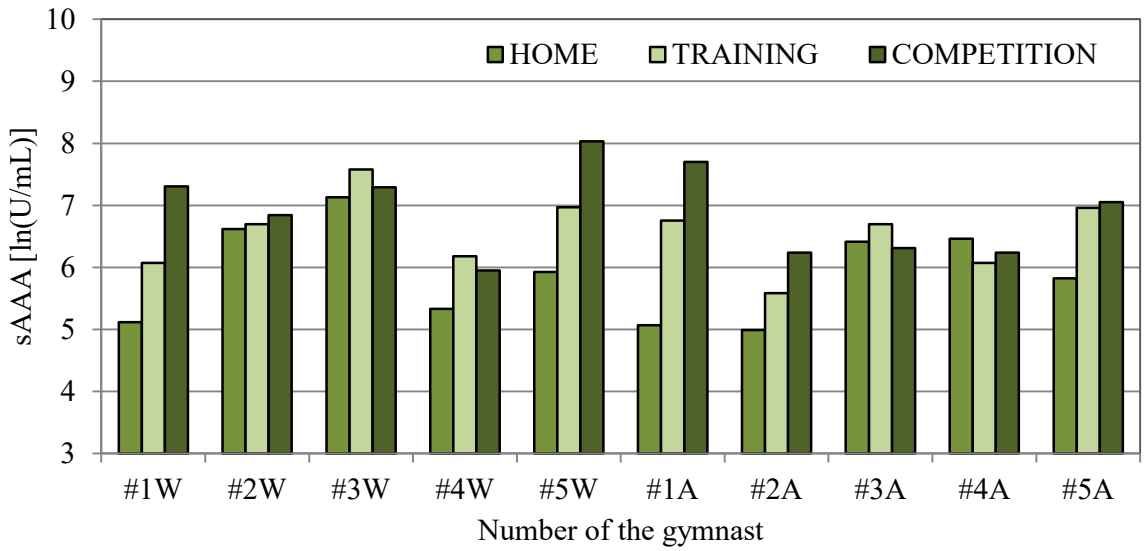


Figure 2. Salivary alpha-amylase activity of each gymnast on the 9th day before the competition (HOME), just before the training session (TRAINING), and before the competition (COMPETITION)

The individual results of the protein concentration for each gymnast are shown in Figure 3. The sP was between 0.86 and 2.66 g/L at HOME, between 0.50 and 3.88 g/L at TRAINING and between 0.76 – 5.16 g/L at COMPETITION. Overall, the lowest values of the gymnasts were registered in the samples taken at HOME. The values of sP in gymnasts #4W and #4A had a different pattern.

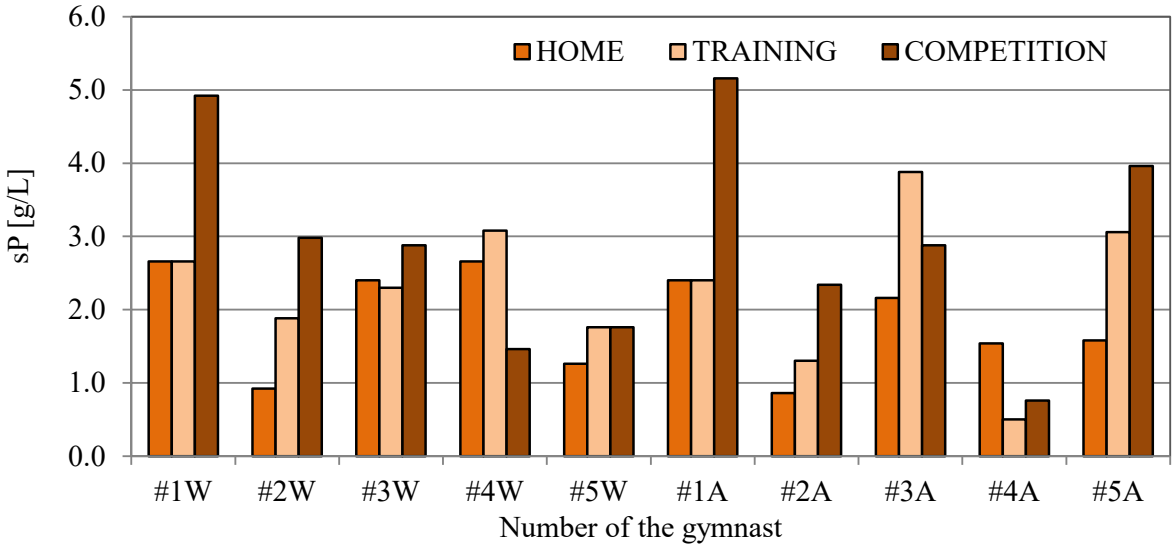


Figure 3. Salivary protein concentration of each gymnast on the 9th day before the competition (HOME), just before the training session (TRAINING), and before the competition (COMPETITION)

The individual values of the sK+ for each gymnast are shown in Figure 4. The sK+ was between 18.8 and 33.5 mmol/L at HOME, between 19.6 and 40.2 mmol/L at TRAINING, and between 20.8 and 48.3 mmol/L at COMPETITION. The lowest values (except gymnast #1A) were registered in the samples taken at HOME as it was the case in the sAAA and sP samples. In most of the cases, except for gymnasts #3W and #5A, the highest sK+ was registered at COMPETITION.

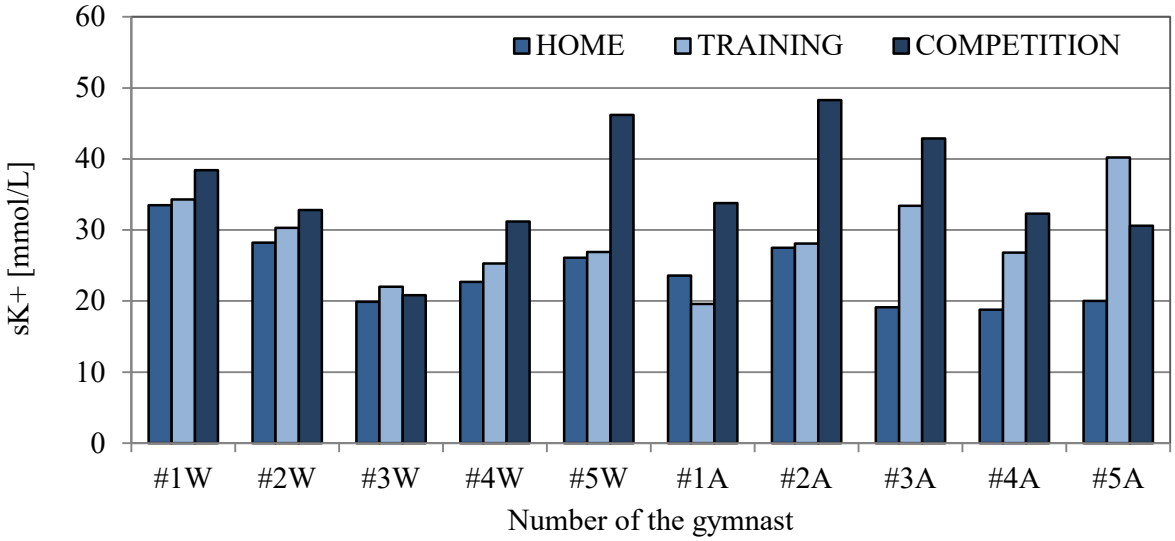


Figure 4. Salivary K+ concentration of each gymnast on the 9th day before the competition (HOME), just before the training session (TRAINING), and before the competition (COMPETITION).

The trait and state anxiety of the gymnasts are presented in Figure 5. The trait anxiety values were between 32 and 59. The state anxiety values were between 21 and 54 at HOME, 27 and 57 at TRAINING, and 24 and 52 at COMPETITION. In most cases, the values of the state anxiety levels were the lowest at HOME, and the highest at COMPETITION.

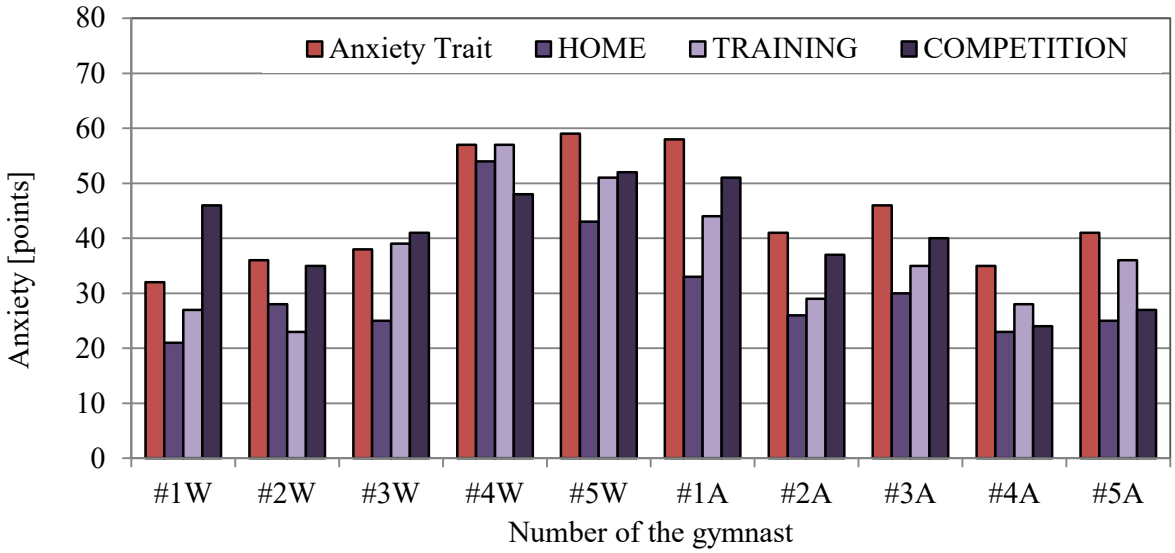


Figure 5. Trait anxiety at home and state anxiety of each gymnast on the 9th day before the competition (HOME), just before the training session (TRAINING), and before the competition (COMPETITION).

The mean results of the sAAA, sP, and sK+, and the trait and state anxiety results in all gymnasts from this study are presented in Table 1.

Table 1. Salivary alpha-amylase activity, salivary protein and K+ concentrations of all gymnasts (n=10) on the 9th day before the competition (HOME), just before the training session (TRAINING), and before the competition (COMPETITION).

	HOME	TRAINING	COMPETITION
sAAA [ln(U/mL)]	**5.89** ± 0.75 ^{bc}	**6.56** ± 0.58	**6.90** ± 0.70
sP [g/L]	**1.84** ± 0.70	**2.28** ± 0.97	**2.91** ± 1.44
sK^+ [mmol/L]	**23.94** ± 4.83 c	**28.69** ± 6.10	**35.73** ± 8.30
State Anxiety [points]	**30.80** ± 10.26 ^{bc}	**36.90** ± 11.03	**40.10** ± 9.57
Trait Anxiety [points]	**44.30** ± 10.20	NA	NA

Notes. ^{b} – $p < 0.05$ vs TRAINING; ^{c} – $p < 0.05$ vs COMPETITION; c – $p < 0.01$ vs COMPETITION

The mean values of each parameter progressively increased from the baseline results (HOME) to their highest values before the competition. The mean sAAA measured at HOME was 5.89 ± 0.75 ln(U/mL), which increased to 6.56 ± 0.58 ln(U/mL) at TRAINING, and then increased further to 6.90 ± 0.70 ln(U/mL) at COMPETITION. The gymnasts had significantly higher mean value of sAAA at TRAINING vs HOME ($p < 0.05$), and at COMPETITION vs HOME ($p < 0.05$), Table 1. The mean salivary protein concentration increased progressively but the differences were not significant. The mean sK+ was significantly higher at COMPETITION vs

HOME (35.73 ± 8.3 mmol/L vs 23.94 ± 4.83 mmol/L; $p < 0.01$). The registered mean values of state anxiety levels were significantly higher at TRAINING (36.90 ± 11.03 v.s. 30.80 ± 10.26; $p < 0.05$) and at COMPETITION (40.10 ± 9.57 v.s. 30.80 ± 10.26; $p < 0.05$) in comparison with that registered at HOME.

Corelations between parameters are presented in Table 2. Statistically significant correlation coefficients were observed between the sAAA in the samples obtained at HOME and the sAAA at TRAINING ($r=0.627$; $p < 0.05$).

Table 2. Correlation matrix of the biochemical parameters.

n=10		sAAA HOME	Anxiety HOME	Anxiety TRAINING	Anxiety COMPETITION
sAAA TRAINING	correlation	0.627			
	p	0.049			
Anxiety TRAINING	correlation	-0.206	0.876		
	p	0.568	0.001		
Anxiety COMPETITION	correlation	-0.398	0.588	0.629	
	p	0.255	0.074	0.049	
Trait Anxiety	correlation	-0.340	0.839	0.881	0.673
	p	0.336	0.002	0.001	0.033

Notes. The significant correlation coefficients are coloured in grey, and only the rows and columns with significant correlation are presented.

Correlations between state and trait anxiety, recorded at the three time points (Anxiety HOME, Anxiety TRAINING, Anxiety COMPETITION), are also significant and very high. High corelation was also registered between state anxiety levels before the training (Anxiety TRAINING) and before competition (Anxiety COMPETITION).

DISCUSSION

Many researchers reported that sAAA is a good non-invasive indicator of stress (Chatterton et al., 1996; Granger et al., 2007; Li & Gleeson, 2004; Nater & Rohleder, 2009). Therefore, the fact that sAAA was the lowest at baseline in eight out of the ten gymnasts from our study, the highest before competition in six out of ten, and the highest before training in only three of them (Fig. 2), indicated that most of the gymnasts (n=6) experienced the highest stress levels just before the competition, but in three of them, stress was at the highest level before training. This was also confirmed by the significantly higher mean sAAA before training and just before competition vs baseline mean value, with the ones before competition having the highest

average value (Table 1). These results coincide with the well-expected assumption that competitions are the events which stress gymnasts the most, but it should also be noted that some exceptions in individual gymnasts are possible. It should also be noted that sAAA showed large individual differences both in our studies and according to other published data (Kobayashi et al. 2012), which necessitates to present sAAA values as natural logarithm. This issue, as well as the need for specialized laboratory equipment for the measurement of sAAA, casts doubt on its wide applicability in sports practice, especially in the field.

Salivary protein concentration has been suggested as a potential non-invasive indicator of stress (M. Edgar et al., 2012; W. M. Edgar, 1992), and in our study, the changes in salivary protein levels across the different conditions (at home, before training, and before competition) showed a trend similar to that observed for sAAA. However, it is important to note that these changes in protein concentration did not reach statistical significance, and we did not find a significant correlation with other stress markers. Therefore, while salivary protein may contribute to a broader understanding of stress responses, our findings suggest that it may not be a reliable sole indicator of stress in this context. Further research with larger sample sizes and more frequent measurements may be needed to fully elucidate the role of salivary protein as a stress biomarker in athletes.

In our opinion, there is a link between changes in sK+ and stress levels, possibly mediated by increased aldosterone (Antonov et al., 2011; Brown et al., 1995; Kubzansky & Adler, 2010; Riad et al., 1986). We observed that individual sK+ values were highest just before competition in a majority of participants (n=8), and the mean sK+ values were significantly elevated compared to baseline. Furthermore, the sK+ measurements were within the sensitivity range of ion-selective electrodes (ISE), which allows for rapid and reagent-free measurement in field settings. These results indicate that sK+ warrants further investigation as a non-invasive marker of stress in sports practice, particularly in field conditions, but should be interpreted in the context of its statistical significance and alongside other stress indicators.

Collecting saliva by using cotton swab salivates is a very convenient method which can be repeated several times if necessary. That allows taking samples from the athletes themselves both at home and before training or competition. The coaches and gymnasts willingly participated in this study and did not note any disadvantages in their work or any issues that could affect their results. The use of non-invasive salivary biomarkers, particularly sAAA and sK+, offers a valuable tool for monitoring stress levels in athletes. Regular monitoring of these

biomarkers can help coaches and sports psychologists identify athletes who may be experiencing excessive stress, allowing for timely interventions to prevent potential negative consequences on performance and well-being. For instance, if an athlete consistently shows elevated sAAA and sK+ levels during training, coaches can adjust the intensity or volume of training, or incorporate stress management techniques such as mindfulness, visualization, or relaxation exercises. Furthermore, the pre-competition increase in stress highlighted by our results underscores the importance of psychological preparation for competitions. Implementing strategies to manage anxiety and enhance coping skills, such as cognitive-behavioral interventions or performance routines, may help athletes to optimize their performance under pressure. By integrating physiological and psychological assessments, a more holistic approach to athlete management can be achieved, promoting both performance enhancement and athlete welfare.

State anxiety levels were the highest before the competition in seven out of the ten gymnasts (Fig. 5) and its mean values were significantly greater vs the baseline, and vs the time before the training session (Table 1). The high positive correlation between trait anxiety and state anxiety in different stress conditions shows that trait anxiety is a good indicator for those competitors who demonstrate greater state anxiety before competition.

In our study, a significant correlation was obtained only in the values of sAAA measured at baseline and just before training. These findings align with previous research demonstrating that sAAA is a sensitive marker of stress in anticipation of demanding events. For example, studies on athletes in other sports have also reported significant elevations in sAAA in response to competitive stress (Chatterton et al., 1996; Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007). However, the magnitude of sAAA increase observed in our study is similar to previous findings in other sports (Petrov et al., 2017; Petrov et al., 2013), suggesting that the stress response may be consistent across different athletic populations. In contrast, the lack of correlation with sAAA before competition, and the absence of significant correlations between the other biochemical parameters and between them and the results of the anxiety questionnaire in our study may be due to the different mechanisms in which the stress influences the individual parameters and the different stress levels in individual athletes at baseline, before training and just before competition. For instance, as noted earlier, sAAA is primarily influenced by the sympathetic nervous system activation at the level of adrenaline, while the sK+ ions concentration in saliva is primarily affected by the increased aldosterone levels. Furthermore, a high level of physiological stress probably does not affect the level of anxiety

equally in different athletes, as some of them perceive the conditions with the increased level of adrenaline accompanying training and competitions positively, and others perceive them negatively. These distinct physiological mechanisms underlying the changes in sAAA and sK+ highlight the multi-faceted nature of the stress response.

Limitations of the study

The sample size in this study (n = 10) is a limitation that should be considered when interpreting the results. While this sample size allowed us to observe significant changes in salivary biomarkers and anxiety levels within this specific group of elite rhythmic gymnasts, it limits the generalizability of our findings to the broader population of rhythmic gymnasts. The small sample size might not fully capture the variability in stress responses that exist across different individuals, training regimens, or competitive levels. However, this sample size is consistent with previous studies in this field and allowed us to establish a foundation for future research. Future studies should aim to replicate these findings with larger and more diverse samples to enhance the generalizability and robustness of the conclusions.

CONCLUSION

In conclusion, this study demonstrates the utility of salivary alpha-amylase activity and salivary potassium concentration as non-invasive biomarkers for assessing stress in rhythmic gymnasts. The practical application of these findings lies in their potential to inform training and competition strategies. Regular monitoring of these biomarkers can provide valuable insights into athletes' stress levels, enabling coaches and sports psychologists to implement targeted interventions to optimize performance and support athlete well-being. By integrating biomarker assessment into routine practice, it may be possible to create a more supportive and effective training environment for rhythmic gymnasts.

Conflicts of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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