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SALIVARY ALPHA-AMYLASE AND CORTISOL CHANGES IN RESPONSE TO BASKETBALL TRAINING IN MEN AND WOMEN

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Abstract

Introduction. This study aimed to determine changes in the level of alpha-amylase and cortisol in the saliva of male and female basketball players after one session of basketball training. Additionally, the study examined whether differences existed between females and males in the levels of these biomarkers in response to physical exertion. **Material and Methods.** The study involved 30 athletes from academic leagues (17 females and 13 males). The training session consisted of warm-up, conditioning, and tactical drills and lasted for 90 minutes. A sample of saliva was obtained before and after the training session. Salivary flow, alpha-amylase, and cortisol were measured. Wilcoxon Signed-Rank and U-Mann were used for statistical analysis at a significance level of 0.05. **Results.** After the training session, a significant increase in salivary cortisol levels was observed in females ($p < .05$), whereas in males, a significant increase ($p < .05$) was noted in salivary alpha-amylase levels. There were no significant differences in alpha-amylase and cortisol levels between females and males before and after exercise. **Conclusions.** These results suggest that the response to physical exertion may vary depending on gender, which highlights a need for further research targeting the relationship between exercise intensity, hormonal response, and body adaptation.

Keywords: alpha-amylase, basketball, cortisol, saliva, training

Introduction

Saliva is the biological fluid that plays a key role in maintaining oral health and supporting digestive processes. Due to its non-invasive collection method, saliva is a convenient and easily accessible material for research purposes [1, 2]. Saliva secretion is regulated by the autonomic nervous system, involving both sympathetic and parasympathetic systems. Stimulation of α - or β -adrenergic receptors can influence the quantity, viscosity, and content of proteins and ions. The average daily salivary secretion volume ranges from 0.5 to 2 litres and is influenced by the amount of sleep, the meals, as well as emotional stimuli [3].

Under normal conditions, without external stimulation, the salivary flow rate varies between 0.25 mL/min and 0.35 mL/min. In response to gustatory stimulation, it can increase to 1.50-2.30 mL/min [4]. During sleep, when the salivary secretion process slows down, the flow rate decreases to approximately 0.05 mL/min [5]. The primary function of saliva is to maintain proper homeostasis within the oral cavity. In addition, it plays a crucial role in regulating the pH of the oral environment due to its buffering capacity (acid neutralisation). The continuous flow of saliva facilitates the removal of food debris and microorganisms from the oral cavity. Saliva supports remineralisation of hard dental tissues through its content of calcium (Ca), phosphate, and fluoride ions [6, 7]. Furthermore, saliva plays a role in initial digestion, protection (anti-viral, anti-fungal, anti-oxidative), buffering, conduction, and facilitation of syllable pronunciation.

Physical exercise induces changes in the activity of the autonomic nervous system, leading to modifications in both sali-

vary secretion and its composition. Consequently, the intensity, type, course, duration, and time of day of the exercise play a crucial role in modifying salivary parameters [8].

Recently, saliva has become a matter of concern as a biomarker for physiological and psychophysiological stress [9]. Objective markers of psychophysiological stress, commonly used across various sports disciplines, include activation of the hypothalamic-pituitary-adrenal (HPA) axis, reflected by cortisol secretion, and activation of the sympathetic nervous system, reflected by alpha-amylase secretion [10, 11].

Some studies have shown that salivary alpha-amylase levels increase more rapidly than salivary cortisol, suggesting that alpha-amylase may serve as a more immediate indicator of stress than cortisol [12]. The assessment of selected salivary components can be used to monitor physical exertion during participation in various sports disciplines [13]. The correlation between the increase in cortisol and alpha-amylase levels in saliva was shown in a study conducted during a handball match. It revealed the close cooperation between the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, which becomes even more intense during physical and psychological stress [14]. Comprehensive analysis of salivary constituents provides critical data for understanding pathophysiology and helps assess its diagnostic potential [15, 16]. Salivary alpha-amylase is the main enzyme of saliva, representing about 20% of the total salivary proteins. Its functions include not only the breakdown of glycogen and starch but also the suppression of bacterial adhesion and growth by forming an acquired pellicle on the tooth surface, which inhibits the binding of bacterial lipopolysaccharides [17].

Measuring salivary alpha-amylase level is a non-invasive method of examining the activity of the sympathetic adreno-medullary system, which controls the salivary glands in highly stressful situations such as physical activity [18, 9]. During exercise, the level of alpha-amylase in saliva increases. This is caused by the stimulation of β -adrenergic neurons and increased release of catecholamines, such as norepinephrine and epinephrine [19]. The binding of neurotransmitters to neurons causes activation of adenylcyclase and [20] phosphorylation of proteins, leading to exocytosis of amylase enclosed in zymogen granules. The secretion of this peptide depends on the type, intensity and duration of exercise [9]. The incremental exercise test revealed an initial decrease in alpha-amylase levels, which began to rise rapidly with increasing exercise intensity, especially when reaching the salivary threshold (the first moment of continuous growth of the enzyme in the saliva) and the anaerobic threshold [19, 20]. Both points correlate with the plasma catecholamine threshold, allowing the assessment of the adrenal glands during physical stress [19]. Physical activity based on rapid body movements and high intensity of physical exercise causes increased digestive activity, preparing the body for a state of increased energy demand. In addition, Wunsch et al. [21] proved that only very high-intensity exertion stimulates the activation of the autonomic nervous system (ANS), causing an increase in salivary protein levels. In other studies [22], it was proven that the exertion of a marathon runner caused a seven-fold increase in amylase at the end of the run and its return to basic values just one hour later.

After short-term, intensive exercise, such as taekwondo, the alpha-amylase level returns to the initial level after about 30 minutes [23]. Similar results were obtained in the studies performed on swimmers [24], cyclists [25], rugby [26] and football players [27]. Hill et al. proposed the so-called dose-response profile, which allows for the presentation and scaling of the body's response, including hormonal changes, to a stress factor. This model allows for the demonstration of the relationship between physical activity and changes in alpha-amylase levels, while also enabling the prediction of the body's response to a given stimulus [28].

Recently, the analysis of salivary cortisol concentration has become increasingly important for determining physiological and psychophysiological stress. It is a steroid hormone produced in the zona fasciculata of the adrenal glands [29]. During exercise, this hormone increases the availability of energy substrates in skeletal muscles and decreases the activity of the immune system, which maintains homeostasis [30]. Its release is controlled by the hypothalamic-pituitary-adrenal axis [29]. The response of the adrenal cortex is controlled by the secretion of CRH (corticotropin-releasing hormone) by the hypothalamus and ACTH by the anterior pituitary gland [31]. The cortisol secretion is regulated by two mechanisms: tonic and phasic. The phasic pathway, unlike the tonic mechanism, is active during intense stress [32]. Cortisol is used as a biomarker of HPA axis activity, enabling the examination of cardiovascular diseases and sleep disorders. Its release may also be associated with depression and physiological stress caused by, for example, physical activity [29]. Cortisol levels during exercise depend on the type of physical activity, which varies in intensity, duration, and nature of the exercise [31]. High cortisol activity occurs in response to acute, intense, long-term stress [30]. A single bout of endurance exercise causes a single spike in cortisol, while regular exercise increases the basal level of this hormone. Single endurance exercises stimulate the HPA axis, causing the secretion of proopiomelanocortin and its derivatives β -endorphin

and β -lipotropin. The minimum exercise intensity that causes an increase in cortisol occurs after reaching 60% of maximum oxygen uptake. Above this level, there is a linear correlation between exercise intensity and the concentration of this hormone. If VO_{2max} is less than 60%, longer exercise time is required to reach the threshold [33]. A prolonged return of the hormone level to the baseline has been demonstrated, allowing the body to prepare for the next series of exercises [30]. It has also been noted that a single high-intensity workout increases cortisol levels, but regular exercise lowers them [33].

Alpha-amylase and cortisol levels can be influenced by various factors, such as age, gender and diet, as well as tooth brushing. Studies between women and men have shown that they have clearly different responses to stress, reflected in changes in saliva parameters. In women, alpha-amylase activity and the rate of its secretion increased significantly during exercise, whereas in men, they remained relatively unchanged. It was also noted that the relationship between alpha-amylase activity and cortisol levels showed opposite trends in women and men. It is believed that the phase of the menstrual cycle may influence these differences. This suggests a noticeable gender difference in the SMA and HPA response to stress [34].

Previous studies have rarely analysed changes in alpha-amylase and cortisol levels in team games, including basketball, during a single training session. This sport is characterised by a very high variability of situations, which causes players to perform physical exertion with different intensities and in different emotional and stressful situations [16, 35]. An important feature of basketball players is economy and efficiency, which allows them to plan their strength for four quarters of a match or a training session [36]. Prolonged, exhausting basketball training or participation in a match increases maximum oxygen uptake, improving the metabolism, endurance, and cardiovascular capacity of players. Physical activity increases the level of stress, both physiological and psychophysiological. However, changes in these parameters may be lower due to adaptation to this type of physical exertion [37]. A study of free throws in basketball players showed a decline in performance and accuracy under the influence of environmental pressure [38]. High environmental pressure, fear and anxiety related to making a successful shot at the basket affect the mental load, which is directly related to changes in the concentration of stress hormones. It has also been proven that higher inhibitory control ability reduces mental workload and thus improves basketball players' performance [39].

Material and Methods

The aim of the study was to focus on determining changes in the levels of two stress biomarkers – alpha-amylase and cortisol – in the saliva of male and female basketball players after one session of basketball training. Both biomarkers are commonly used to assess the body's response to stress, including physical stress associated with intense exercise. The objective of the study was to determine how the levels of alpha-amylase and cortisol change during training. Additionally, the study examined whether there are significant differences in the levels of these biomarkers between women and men in response to physical exertion.

Participants

A priori power analysis was conducted using G*Power 3.1 to estimate the minimum required sample size. The analysis assumed a medium effect size ($f = 0.50$), an alpha level of 0.05,

and statistical power ($1-\beta$) of 0.75. Based on these parameters, the estimated sample size was 28 participants. The minimum required total sample size was estimated to be 30 participants for repeated measures. Thirty adult basketball players from the academic league who had had significant achievements in the National University Basketball Championships were involved in this study. The sample size was determined by the availability of participants among AWF students. The group consisted of 17 females (age 20.8 years \pm 1.36; body height: 169.50 cm, \pm 6.14 cm; body mass: 62.47 kg, \pm 8.56 kg) and 13 males (age 21.7 years \pm 1.75; body height: 188.46 cm, \pm 8.04 cm; body mass: 85.69 kg, \pm 12.72 kg) who were the members of the University Sports Club. Both groups trained separately, but the training conditions for both groups were the same. The participants had taken part in regular training sessions twice a week before the experiment in the same sports hall. The exclusion criteria were injuries of the musculoskeletal system, chronic pain syndromes, or other medical conditions which would have got worse if the players had taken part in this trial. The study protocol was approved by the Bioethics Committee (KB-327/2009) of Wrocław Medical University and was conducted according to the ethical regulations concerning clinical research included in the World Medical Association Declaration of Helsinki. The study meets the "Ethical Standards in Sports and Physical Exercise Science Research" [40]. Every participant was required to provide a written informed consent, which was given prior to the study. In this consent, the purpose and characteristics of the study were explained. The menstrual cycle of the participants was not monitored.

Experimental procedure

Every training session took place on the same day between 7.00 p.m. and 10.00 p.m. in the same sports hall and lasted for 90 minutes. Those training sessions were prepared by the coaching staff and focused on various technical, tactical and fitness aspects. Firstly, the players were required to do a warm-up. Then, the main part of the session consisted of basic conditioning, specific conditioning and tactical conditioning exercises. At the end, the team had the time to cool down. Each training unit was conducted during the competition season. The basketball players consumed water during their training session. Before and directly after the session (9.00 p.m.), a sample of unstimulated mixed saliva was obtained. In the research sample, the saliva was drawn twice – before and after the training session. To ensure consistency and to account for the diurnal fluctuation of salivary parameters, all salivary samples of mixed saliva were taken, without external stimulation, in the evening. In the study group, salivary samples were collected twice – before and after the workout; a total of 60 samples were obtained. Samples of saliva were collected after thoroughly rinsing the mouth with distilled water. The aim of collecting samples after oral rinsing and using clear supernatants for the assays was to minimise the contribution of other sources of the tested salivary parameters, such as oral bacteria, epithelial cells, leukocytes, and dietary residues. The subjects were sitting with their heads bent down and their mouths open. Approximately 5 ml of saliva was taken with the use of a plastic pipette, and the sample was put into a graded test tube stored on crushed ice. The time and volume of saliva collected were measured separately for each participant. Based on the volume and time of saliva collection, the salivary flow rate was calculated. Mean salivary flow time was not calculated because we were only interested in salivary flow rate, which for all participants was: V ml/min salivary flow rate mL/min before training: 0.39 ± 0.19 , and after training: 0.36 ± 0.27 . The difference was statistically significant ($p \leq 0.001$). Sampling

took place in the gymnasium. The velocity of the salivary flow was calculated [ml/min (V)] based on the volume of the sample and the time of collection. Before performing the biochemistry tests, the samples were centrifuged for 10 minutes (3500 revolutions per minute). The evaluated parameters included: alpha-amylase (Caraway's colorimetric method – Alpha Diagnostic Kit) and cortisol (ELISA kit by R&D Systems).

Data analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 21 (SPSS Inc., Chicago, Ill., United States). Normality was tested with the Shapiro-Wilk test. As the distributions were not adjusted to normal, non-parametric tests were used. The median, interquartile range, and percentiles 25 and 75 were calculated for each variable using traditional statistical techniques. A Wilcoxon signed-rank test for intragroup comparisons was conducted to analyse differences between pre- and post-test measurements. The Mann-Whitney U test (Z) was used to determine the differences between female and male basketball players. The significance level was set at $p < 0.05$. All data calculations were performed using Statistica 13.0 software (Stat Soft Poland, Krakow, Poland).

Results

According to the Wilcoxon signed-rank test, there were significant differences in the level of cortisol in females. Before the training session, the value was 1.44, and after it increased to 2.27 ($p < 0.05$). On the contrary, in male basketball players there was a significant increase in alpha-amylase, rising from 122.45 before to 200 ($p < 0.05$) after the exertion. However, no statistically significant changes were noted in alpha-amylase in women and in cortisol in men.

Table 1. The level of amylase and cortisol before (PRE) and after (POST) the training session.

Variable	alpha-amylase PRE [IU/L]	alpha-amylase POST	Δ alpha-amylase	cortisol PRE [μ g/ml]	cortisol POST	Δ cortisol
male basketball players						
Median	122.45	200.00	Z = 1.99 p = 0.046	1.29	1.67	Z = 1.57 p = 0.116
IR	75.62	188.80		0.62	1.90	
P25	90.91	88.89		1.08	1.34	
P75	166.53	277.69		1.70	3.24	
female basketball players						
Median	62.00	82.59	Z = 1.44 p = 0.149	1.44	2.27	Z = 1.96 p = 0.049
IR	115.57	240.63		1.38	3.82	
P25	23.33	57.17		0.92	1.40	
P75	138.90	297.80		2.30	5.22	
Δ K - M	Z = 1.46 p = 0.143	Z = 1.13 p = 0.258		Z = 0.38 p = 0.706	Z = 0.88 p = 0.379	

Note: Δ alpha-amylase – difference in alpha-amylase before and after the session, Δ cortisol – difference in cortisol before and after the session, Δ K-M – difference between females and males, IR – interquartile range, P25 – percentile 25, P75 – percentile 75.

In addition, the Mann-Whitney U test was performed to determine if there were differences in levels of alpha-amylase before the exercise between male and female players. This test proved that the difference was insignificant ($p > 0.05$). The same result was obtained when it comes to the comparison of cortisol levels in sportsmen and sportswomen after the exertion ($p > 0.05$).

Discussion

The main purpose of this study was to determine changes in salivary alpha-amylase and cortisol levels in male and female basketball players before and after exercise. The Wilcoxon signed-rank test was performed and a significant difference ($p < 0.05$) in the levels of cortisol in females was observed. Moreover, only basketball players showed a statistically significant increase ($p < 0.05$) in alpha-amylase in response to physical activity.

In women, median cortisol increased significantly after exercise. This finding may be supported by a study conducted at the University of Memphis. The purpose of that study was to compare the cortisol response from a regular-season game and a typical practice session. Salivary cortisol levels and anxiety about competitive sports were monitored. The results clearly demonstrate the psychological and physiological differences between competitive soccer training and college women's training. Both physiological and psychological variables appear to contribute to a large stress hormone response to actual competitive play [41]. These hypotheses put forward by the researchers are confirmed in another study, conducted by scientists from the University of São Paulo. The data they obtained indicate that the state of mental fatigue is a key factor regulating the technical performance of athletes (in the case of this particular study, basketball players) and influences the responses of the endocrine and autonomic systems [42].

In men, median cortisol increased after exercise, but these changes were not significant. These results differ from the study by Sansone et al. [43], which showed a significant increase in salivary cortisol after training based on a small-sided game in basketball. This variability may be a result of different intensities of physical exercise, the diversity of movements, and dynamic changes occurring on the court. Games may be more stressful than traditional exercise-based training. Another study [44] worth mentioning shows pre-training and post-training salivary cortisol in male basketball players in comparison to non-athletes. In sportsmen, it was observed that the salivary cortisol rose from pre- to post-training. On the other hand, the group of untrained individuals manifested no significant differences [44]. In our study, the participants were neither professional players nor non-athletes; however, the study described above suggests that one's fitness could possibly impact the changes in cortisol levels. Foretić et al. also emphasise a possible correlation between body height and cortisol levels, since in handball some playing positions tend to involve more contact, which has an impact on catabolism and, therefore, on cortisol response. The athletes who play those positions are usually taller [14].

What is more, research by Arruda et al. [45] indicates a significant rise in salivary cortisol in basketball players post-match when compared to pre-match. Additionally, it was proven that the stage of competition (quarter of the match) did not have a significant impact on the levels of salivary cortisol [45]. Other factors that may have an impact on the level of cortisol in saliva are age and experience in training. For example, in a study involving boys aged 14-18 years who participated in morning basketball training, cortisol levels were much lower post-exercise

than pre-exercise [13]. A similar result to our findings was noted in rugby players who performed a morning preconditioning session. The salivary cortisol level did not change after this session [46].

When discussing the changes of cortisol in sports, many factors could possibly have an impact on the hormonal response to exercise. The element of competition plays a great role in team sports, and it may have an impact on the endocrine response. It was proved by a 2023 study performed on male handball players, since the salivary cortisol had higher values after playing a match than after participating in training. In conclusion, the environment of a match tends to be far more stressful, thus it triggered a much stronger response in salivary biomarkers [47]. The impact of environmental stress on basketball players is, without a doubt, complex. Environmental pressure can increase anxiety and mental workload, negatively affecting performance. It also decreases both the accuracy and the rapidity of response. Higher inhibitory control allows players to maintain satisfying results [39], whereas individuals with higher salivary cortisol present lower overall task performance [48]. In addition, increased salivary cortisol was linked to intensified perceived stress [49], noise annoyance [50], high work demands and lack of social support [51]. The ubiquitous presence of stressors is undeniable; therefore, it is crucial to comprehend the dose-response interactions of stress factors and motor performance. This was presented using the example of crew rowing, where the coordination of crew members was dependent on common patterns of hormesis. It means that large variations in stressors can interfere with the intended result. By changing the intensity of the stress stimulus, the ideal dose can be adjusted to each individual. It can be helpful in the process of creating training regimes tailored to personal needs. In this way, triggering undesirable behaviour can be avoided [28]. Another approach to this topic was presented in a systematic review of the behaviour of players in free-throw shooting under pressure by Goldschmied et al. Due to the controlled nature of free-throw shooting, it has become a useful tool for research on the topic of stress. The authors highlight the phenomenon of choking, which can be defined as the inability to achieve satisfying results in stressful conditions due to anxiety. Evidence for the existence of this occurrence in free-throw shooting is shown in a study by Cao et al., who showed that players' shooting performance was 5-10% worse than average in the final seconds of close games [52].

When it comes to psychical factors, the data suggest that mental fatigue potentially plays a role in modulating both endocrine and autonomic response and regulates technical performance of basketball players [42]. Yet another key factor needs to be taken into consideration when discussing the endocrine response in team sports – it is the contact nature of the discipline. It is crucial not only in basketball but also in other sports, such as handball, as mentioned previously. In a study performed on male handball players, a group that performed contact playing positions (CCP) had a significantly higher response in cortisol when compared to a group with non-contact playing positions (NCCP). It highlights the fact that contact actions are a vital factor that needs to be considered when it comes to calculating training loads or injury prevention [46].

In our study, women responded to a lesser extent with changes in alpha-amylase levels after the training session; this increase was not statistically significant ($p > 0.05$). When it comes to alpha-amylase level in sportswomen, the median before the workout was 62 and after it increased to 82.59. The Wilcoxon signed-rank test showed that the rise in alpha-amylase was not significant ($p > 0.05$). According to the literature, alpha-amylase

may be a direct indicator of stress, since it increases much faster than salivary cortisol during exercise [53]. The activation of the hypothalamic-pituitary-adrenal (HPA) axis tends to be slower than the response of the sympathetic nervous system (SNS) to a stressor. It is because the release of cortisol into the bloodstream requires a cascade of hormones. Therefore, the peak of cortisol level can be observed 15-30 minutes after the triggering factor. In addition, the HPA axis may be active chronically or before the stressor occurs. The SNS activates rapidly in response to stress-inducing factors (the peak activation can be noticed about 14 minutes before cortisol), and it returns to the baseline as soon as the stressor is resolved [54]. However, the results of the conducted experiment are not consistent with this. Unfortunately, very few studies have been found that investigated the changes of salivary biomarkers in female basketball players in response to matches or training sessions. One study measured the effects of training on alpha-amylase in women who played basketball. It is worth mentioning that only four females were among the participants of this experiment, and the conclusions were not drawn in relation to genders separately due to a small number of examined women. It was estimated that the difference in alpha-amylase pre- and post-exercise was not statistically significant [55]. On the contrary, another study assessed salivary parameters in response to a free-throw shooting training session in collegiate female basketball players. Consequently, in this experiment, no statistically significant difference concerning alpha-amylase was reported. Nevertheless, there are noteworthy differences between the following research and our study. The free-throw competition lasted 20 minutes and the participants were expected to perform shots only, whereas the training session in our experiment was 90 minutes long, and it included various exercises. Additionally, it was highlighted that in the free-throw tournament, certain measures were taken to induce psychological stress in athletes. They were told that their individual scores would be given to their head coach. In conclusion, despite those differences, the results were consistent with our findings [56]. The impact of pressure on the performance of players in free-throw shooting was highlighted by Goldschmied et al. The authors emphasised that a stressful environment can significantly change the athletes' results; however, some players are perceived to perform better in stressful conditions [38].

In our study, median salivary alpha-amylase levels increased significantly in men after a training session. This is the first study of adult college basketball players to measure salivary alpha-amylase levels after a single training session. However, the impact of exercise on the level of this biomarker had been assessed before. Similar results were obtained in a 2020 study by Foretić et al., where the male handball team was examined before and after the match. It may be associated with both physical stress and the metabolism of carbohydrates. The authors suggested that the supplementation of carbohydrates during halftime may be beneficial for the players. When it comes to salivary cortisol, its level rose in the second half of the match. The association between the rise in these two biomarkers was attributed not only to the simultaneous effect of psychological stress but also to a close correlation between the sympathetic system and the hypothalamic-pituitary-adrenal axis [14]. What is more, in the study by Azarbayjani et al. [57], the amylase response was dependent on exercise intensity, which was consistent with previous findings. Further research should be carried out because there is a dose-dependent impact of stress factors on motor performance [28], which would be valuable to study in the context of salivary alpha amylase levels. The planned studies should analyse the reactions of these parameters to various types of phys-

ical exercise. As mentioned, salivary cortisol levels increased significantly in male basketball players, while no significant changes in this parameter were observed in women. There are results in the literature suggesting that the response to exercise differs depending on gender. In the study by Kim et al. [58], it was found that gender has an impact on the response to stress in golf among elite junior players. It was also shown that cortisol increased more in men during competitions than during training, while no such differences were found in women. Other studies suggest [59] that after high-intensity interval training, both men and women experienced an increase in salivary cortisol levels in endurance athletes. In the study by Rutherford-Markwick et al. [34], salivary cortisol levels remained unchanged during 60-minute ergometer training at 70% of maximum power. Our study was performed to determine if there were differences in levels of alpha amylase before exercise between male and female players. Similarly, the comparison of results after exercise showed no significant differences between athletes of both sexes. Other studies indicate differences in salivary parameters in response to physical activity. A study conducted by Yasuda et al. [60] in adolescents of both sexes with similar cardiorespiratory fitness found an increase in alpha-amylase activity immediately after endurance exercise (one-hour cycling training at 65% of maximal oxygen uptake). However, when comparing boys and girls, no differences were found in this salivary parameter. These results suggest that adolescents of both sexes show a similar salivary response after intense exercise. Another study by Shiraiwa et al. [61] involving college students performing exercises of the same intensity showed that in men, the level of salivary alpha-amylase after exercise did not differ significantly from the baseline, while in women, a significant increase was observed.

These results indicate a difference in the effects of exercise on alpha-amylase levels between men and women.

Limitations

To obtain reliable results, it should be emphasised that this study has some limitations. Firstly, the sample size was relatively small with only 30 participants (17 women and 13 men). With a small group, certain irregularities, such as sampling error, are more likely to occur. In the future, it would be worthwhile to expand the research population to include a larger number of players. The study did not include detailed information about the athletes' training experience or workload, which may limit the interpretation of the findings. Additionally, the absence of psychological measures, such as perceived stress or mental workload, is a methodological limitation that may have influenced the results.

Furthermore, in the case of female athletes, no data were collected on their menstrual phase, sex hormone concentrations (e.g., progesterone, estrogen) or potentially used hormonal contraception. This uncontrolled hormonal variability may therefore act as a confounder, potentially impacting the study outcomes. Another limitation is the lack of control over the endogenous circadian modulation of cortisol secretion. Unmonitored and unstandardized circadian variability may have contributed to the bias in the study results. Furthermore, only one measurement was conducted before and after the training session. For this reason, there was no possibility of assessing the repeatability of the results. A larger database would have allowed for a more reliable analysis of potential variability. Collecting such data could allow for expanding knowledge about changes in performance and response to stress related to the above-mentioned factors [62].

Conclusions

The study demonstrated that a single training session in basketball caused significant changes in cortisol in women and in alpha-amylase in men. The present study draws attention to the need to continue research, with the aim to determine the reactions of male and female basketball players to a wide variety of physical exercises, which will take into consideration their intensity, duration and the characteristics of movements. Collecting saliva to measure the levels of alpha-amylase and cortisol is an easy method of assessing the stress response, which can help understand the changes in physiological condition during basketball training. It may also be used to monitor players' responses not only during training but also during matches. The study emphasises the potential of salivary biomarkers as non-invasive, practical indicators for individualised monitoring of training intensity and stress responses in team sports and potentially many other areas of life. More studies need to be done, which will compare the levels of alpha-amylase and cortisol in a bigger group of players. Assessing the activation of the HPA axis in regard to sex, age, training experience, or physical activity may be a promising direction of further research.

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