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ORIGINAL ARTICLE

Effect of competition on stress salivary biomarkers in elite and amateur female adolescent inline skaters



Effet d'une compétition sur les biomarqueurs salivaires du stress chez des patineuses à roues adolescentes d'élite et amatrices

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Received 3 March 2017; accepted 30 April 2018

Available online 25 September 2018

KEYWORDS

Skate competition;
Anticipatory stress;
Adrenal activity;
Salivary α -amylase;
Cortisol

Summary

Objectives. – The purpose of the present study was to investigate the effect of competition on stress salivary biomarkers alpha-amylase (sAA), cortisol, and dehydroepiandrosterone (DHEA) in elite or amateur female adolescent inline skaters.

Material and methods. – Eighteen female adolescent inline skaters (9 elites and 9 amateurs) who have competed in official skate competitions, participated in the present study. Unstimulated whole saliva was collected during their rest day, 1 h and immediately before competition (pre-competition), as well as immediately and 1 h after the competition (post-competition). Free cortisol, sAA, DHEA, and total protein concentrations were assessed.

Results. – Cortisol concentrations of elite group significantly increased 1 h before and pre-competition compared to rest day ($P < 0.05$) whereas no significant changes observed in amateur group ($P > 0.05$). The sAA concentrations were significantly higher in the elite group at 1 h before

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<https://doi.org/10.1016/j.scispo.2018.04.011>

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MOTS CLÉS

Compétition de patinage ;
Stress anticipé ;
Activité surrénalienne ;
 α -amylase salivaire ;
Cortisol

and at pre-competition compared to the rest day ($P < 0.05$). sAA concentrations of amateur group were significantly higher at pre-competition, post-competition, and 1 h after competition compared to the rest day ($P < 0.05$). DHEA levels of both groups markedly decreased 1 h after competition compared to the rest day ($P < 0.05$). No significant differences in DHEA concentrations were observed between the two groups ($P > 0.05$). The ratio of DHEA to cortisol exhibited significant difference among the five measurement stages in the amateur group ($P < 0.05$), whereas no significant changes in this ratio were observed in the elite group ($P > 0.05$). The total protein concentrations of the elite group increased significantly during three stages, pre-competition, post-competition, and 1 h after competition compared to rest day ($P < 0.05$). Additionally, the total protein concentrations at pre-competition and 1 h after competition were significantly higher in elite group ($P < 0.05$).

Conclusion. – Our results confirmed that the optimum increase in adrenal activity occurs before a competition, which can improve athletic performance. However, a similar increase after a competition suggests the body's attempt to maintain homeostasis. Therefore, anticipatory stress indicates that athletes need to relax before a competition.

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Résumé

Objectif. – Le but de la présente étude était d'étudier l'effet d'une compétition sur les biomarqueurs salivaires du stress alpha-amylase (sAA), cortisol et dehydroépiandrosterone (DHEA) chez les patineuses à roues adolescentes d'élite et amatrices.

Matériels et méthodes. – Dix-huit patineuses adolescentes (9 d'élites et 9 amatrices) qui ont participé à des compétitions de patinage officielles, ont participé à la présente étude. La salive entière non stimulée a été prélevée pendant leur journée de repos, 1 h et immédiatement avant la compétition ainsi qu'immédiatement et 1 h après la compétition. Les concentrations du cortisol libre, sAA, DHEA et des protéines totales ont été évalués.

Résultats. – Les concentrations de cortisol du groupe d'élite ont augmenté de manière significative 1 h avant et avant la compétition par rapport au jour de repos ($p < 0,05$) alors qu'aucun changement significatif n'a été observé dans le groupe amateur ($p > 0,05$). Les concentrations de sAA étaient significativement plus élevées dans le groupe d'élite 1 h avant et avant la compétition par rapport au jour de repos ($p < 0,05$). Les concentrations de sAA du groupe amateur étaient significativement plus élevées lors de la pré-compétition, après la compétition et 1 heure après la compétition par rapport au jour de repos ($p < 0,05$). Les concentrations de DHEA des deux groupes ont nettement diminué à 1 heure après la compétition par rapport au jour de repos ($p < 0,05$). Aucune différence significative dans les concentrations de DHEA n'a été observée entre les deux groupes ($p > 0,05$). Le rapport de la DHEA/cortisol a montré une différence significative entre les cinq sessions de mesure dans le groupe amateur ($p < 0,05$), alors qu'aucun changement significatif n'a été observé dans le groupe d'élite ($p > 0,05$). Les concentrations totales de protéines du groupe d'élite ont augmenté de manière significative au cours de trois sessions, avant la compétition, après la compétition et une heure après la compétition par rapport au jour de repos ($p < 0,05$). En outre, les concentrations totales de protéines à la pré-compétition et une heure après la compétition étaient significativement plus élevées chez les élites ($p < 0,05$).

Conclusion. – Nos résultats ont confirmé que l'augmentation optimale de l'activité surrénale se produit avant une compétition, ce qui peut améliorer la performance athlétique. Cependant, une augmentation similaire après la compétition suggère que l'organisme s'efforce de maintenir l'homéostasie. Par conséquent, les réponses des marqueurs de stress lors d'une compétition soulignent le besoin de relaxation pour les athlètes avant compétition.

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Introduction

Taking part in a competition has been confirmed to cause physiological and psychological changes [1,2]. These changes may influence competitive stress, and some researchers have reported that long-term exercise can lead to changes in the emergence of stress in adult athletes.

Competitive stress causes performance drop by altering homeostasis. This phenomenon signifies that physiological and psychological changes alter performance by affecting the concentration of some hormones and other brain functions [3]. The interaction between functional ability and competitive stress affecting physiological and psychological changes is an important matter in athletes.

Table 1 General characteristics of the participants in the study.

Groups	Number	Age (year)	Height (m)	Weight (kg)	BMI (kg/m ²)
Elite	n = 9	11.11 ± 1.52	148.11 ± 15.03	41.23 ± 11.78	18.75 ± 1.72
Amateur	n = 9	11.22 ± 1.69	151.11 ± 10.21	42.77 ± 9.14	18.51 ± 2.45

The data are given based on mean values and standard deviations.

One way to measure competitive stress is to examine salivary composition changes. Sampling from saliva is very popular because it is non-invasive [3]. Researchers have shown that salivary composition changes in response to stress [4–8] and had a strong association with their serum concentrations [9–11]. In response to physical activity [12], such as during competitions [1,13], the activities of the Hypothalamic Pituitary Adrenal (HPA) and the Sympathetic Adrenal Medullary (SAM) axes increase to maintain homeostasis [14,15]. In addition, significant association between the salivary sAA protein and training outcomes was suggested to underline its potential as non-invasive marker of training status in professional athletes [16]. Activation of the HPA axis results in secretion of cortisol, which increases in response to competitive stress [1,2,8]. In response to golf [17] and soccer [18] competitions, the concentrations of salivary cortisol in starters and amateur individuals have been reported to increase; salivary cortisol concentrations are also higher during competition than during an exercise session. Other findings suggest that cortisol levels are higher in winning athletes than in losers of competitions [19]. Dehydroepiandrosterone (DHEA) is another marker secreted by the adrenal cortex. The DHEA-to-cortisol ratio can be calculated by measurement [20], indicating catabolic/anabolic balance. DHEA notably demonstrates adrenal cortex activation. Therefore, investigating the changes in DHEA concentrations is beneficial to understand the catabolic/anabolic balance in the adrenal cortex.

In addition, the SAM axis activity in response to competitive stress leads to the increase in salivary alpha amylase (sAA) activity [1,7,21]. The measurement of sAA activity is significant because this response to stress apparently occurs much quicker than that of cortisol [22,23] and presents a close association with lactic acid threshold [24]. However, contradictory findings result from differences in protocols used when studying sAA activity in response to competitions and physical activities. To confirm such discrepancies, two studies have notably reported almost identical results by using the same methodology [25,26]. Salivary total protein can also be used as an evidence of net changes in salivary proteins, particularly for sAA, to determine whether the changes are in response to athletic activities or consequences of an increase in saliva concentrations. Numerous studies have considered the changes in sAA, cortisol, and DHEA in response to sports competitions and physical activities. However, their conclusions are debatable, and in some cases, little information is available. The mechanism involved in these changes is especially important in individual sports, such as skating, which needs high concentrations of the aforementioned biomarkers. Therefore, the present study designed to analyse the effect of competition on stress

salivary biomarkers (sAA, cortisol, DHEA) in elite or amateur female adolescent inline skaters.

Method

Participants

A total of 18 female adolescent skaters, including 9 elite skaters (who have previously participated in national and international competitions and have an average experience of 4 years) and 9 amateur skaters (who volunteered as first-time participants in an official competition), participated in this study. The University ethics committee approved the experimental protocol with ethics reference number 9801.290. Each participant was informed of the study's purposes before they signed their written consent. After the participants were identified, they were invited to an informational session, in which the research goals and procedures were explained in detail. None of the participants reported any record of hormonal or behavioural deficiencies, and all of them had normal menstrual cycles (28–30 day). None of the participants was under medical treatment or using oral contraceptives during the study. The general characteristics of the subjects are presented in Table 1.

Skating competitions

The skating competitions in this study took place during the National Competition and were conducted under the supervision of the Skating Federation. All conditions, rules, and regulations were in effect consistent with the international sport federation. These competitions were held in three categories: 300 m individual time trial, 500 m team speed skating, and 1000 m team speed skating. All stages of each competition lasted 2 h (09:00 am to 11:00 am).

Salivary sampling method

Unstimulated saliva samples (5 mL) were collected at rest day, 1 h before competition, immediately before competition, 1 h after competition, and immediately after competition (Fig. 1). Prior to saliva collection, subjects

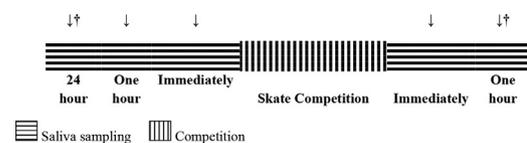


Figure 1 The experimental design of the study. Arrows (↓) indicate where saliva samples were collected.

washed their mouths and drank 500 mL of water to prevent thirst and maintain hydration. After sampling, all specimens were kept in ice containers, transferred to the laboratory, stored at -20°C , and then centrifuged for 15 min.

Biochemical assay

Salivary concentrations of free cortisol, sAA, and DHEA were determined using commercial DIAMETRA ELISA kit (AKSA Medical, Italy) and an ELISA reader device (ELISA Reader Stat Fax Model 3200 Awareness Technology, USA). The competitive immune enzymatic colorimetric method was performed for cortisol and DHEA concentrations. Kinetic colorimetric method was conducted for sAA activity. Salivary total protein concentrations were determined using Bradford method and Coomassie Blue G 250. The assay accuracies for the evaluation of cortisol, DHEA, sAA, and total protein were 0.05 ng/mL, 0.045 ng/mL, 2.5 U/mL, and 1 mg/dL, respectively. Inter-assay values for cortisol, sAA, and DHEA were 0.7%, < 1.5%, and 8.9%, respectively. In addition, intra-assay values for cortisol, sAA, and DHEA were 9.5%, < 1.5%, and 4.8%, correspondingly.

Data analysis

Kolmogorov–Smirnov test results indicated that the data were normally distributed. To differentiate the elite and amateur groups, independent *t*-tests were performed randomly. Before conducting the test by using the “Explore” command, drawing box plots, outliers were determined, and temporarily omitted using the “Select Cases” command. The results were then analyzed using the Levene test significance level. To evaluate the within-group trend change among the five measurements in both elite and amateur groups, repeated-measures ANOVA was conducted. Prior to this analysis, the sphericity of the data was assessed by Mauchly’s test of sphericity. In case of significance in the sphericity test, Greenhouse–Geisser correction was used to correct the degree-of-freedom values. In case of significance in ANOVA, Bonferroni correction was used to locate the difference position. To improve the precision of the data analysis, power analysis and effect size were also considered in all the cases. The significance level for the entire study was $P \leq 0.05$. Statistical analyses were performed using SPSS 18 (PASW Statistics 18).

Results

Salivary Cortisol

Cortisol concentrations in the elite and amateur groups are presented in Fig. 2. Significant differences in free cortisol concentrations ($F_{1.77, 14.09} = 17.46$, $P = 0.0001$) within the elite group were observed throughout the five measurement stages. Bonferroni correction showed that free cortisol concentrations 1 h before and pre-competition were significantly higher, with 13.7- and 4.5-fold increases, respectively, than that during rest day in the elite group. No significant changes were observed in the amateur group among the five measurement stages. Free cortisol

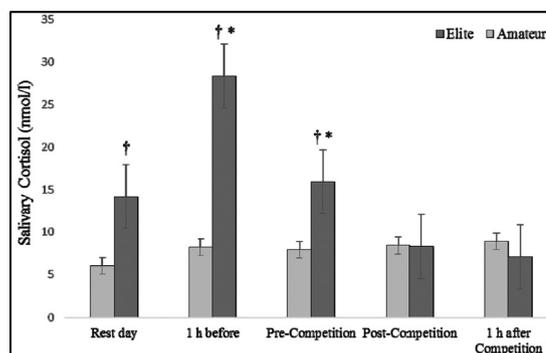


Figure 2 Comparison of the salivary Cortisol changes in the five stages of measurements between elite and amateur groups. *Indication of significant difference with respect to the rest day in the elite group. †Indication of significant difference between elite and amateur groups.

concentrations during rest day ($t_{11.48} = 2.89$, $P = 0.014$), 1 h before the competition ($t_{8.86} = 4.67$, $P = 0.001$), and pre-competition ($t_{16} = 3.28$, $P = 0.005$) in the elite group were higher than those in the amateur group.

Salivary α -amylase activity

The sAA activity concentrations were significantly higher in the elite group at 1 h before and at pre-competition than that at rest day, with 9.12- and 16.2-fold increases, respectively. In the amateur group, the sAA activity concentrations were significantly higher at pre-competition, post-competition, and 1 h after the competition compared than that at rest day, with 12.7-, 4.9-, and 18.9-fold increases, respectively ($F_{4.32} = 3.51$, $P = 0.018$). Moreover, the sAA activity 1 h before the competition ($t_{15} = 2.55$, $P = 0.022$) in the elite group was higher than that in the amateur group. However, post-competition ($t_{15} = -3.68$, $P = 0.002$) and 1 h after the competition ($t_{15} = -3.11$, $P = 0.007$) concentrations were significantly higher in the amateur group (Fig. 3) than in the elite group.

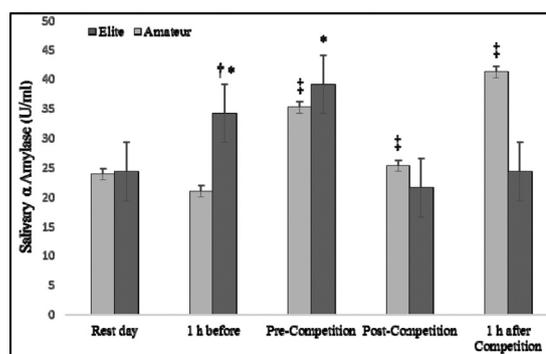


Figure 3 Comparison of the salivary α -amylase activity changes in the five stages of measurements between elite and amateur groups. ‡Indication of significant difference with respect to the rest day in the amateur group. *Indication of significant difference with respect to the rest day in the elite group. †Indication of significant difference between elite and amateur groups.

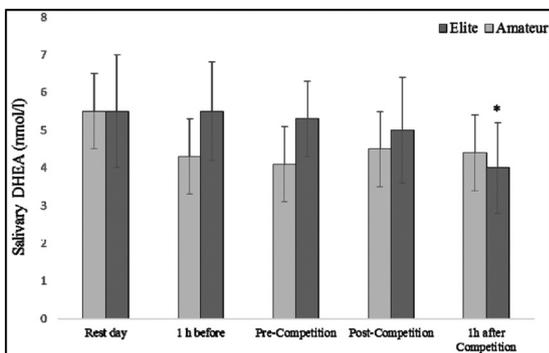


Figure 4 Comparison of the salivary DHEA changes in the five stages of measurements between elite and amateur groups. *Indication of significant difference with respect to the rest day in the elite group.

Salivary DHEA

No significant changes in DHEA concentrations were observed at all measurement stages in the elite group except at 1 h after competition, with a 1.3-fold decrease compared with that at rest day. DHEA concentrations in the amateur group slightly fluctuated at the five measurement stages except at 1 h after competition, with a 1.4-fold decrease compared with that at rest day. No significant differences in DHEA concentrations were observed between the two groups (Fig. 4).

DHEA to cortisol ratio

Significant differences in DHEA-to-cortisol ratio ($F_{1,19, 9.58} = 4.47, P = 0.006$) were observed among the five measurement stages in the amateur group, whereas no significant changes were observed in that of the elite group. This ratio decreased at 1 h before competition compared with that at rest day in the amateur group. The DHEA-to-cortisol ratio values at rest day and 1 h before the competition in the amateur group were higher than those in the elite group (Fig. 5).

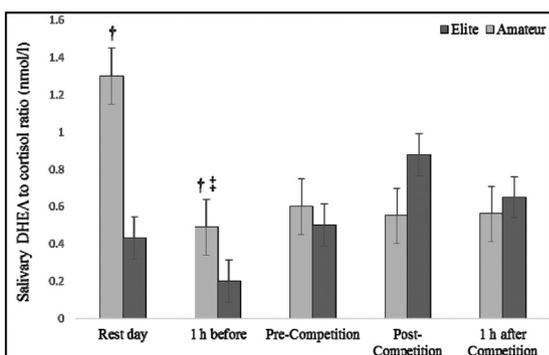


Figure 5 Comparison of the DHEA/Cortisol changes in the five stages of measurements between elite and amateur groups. †Indication of significant difference with respect to the rest day in the amateur group. †‡Indication of significant difference between elite and amateur groups.

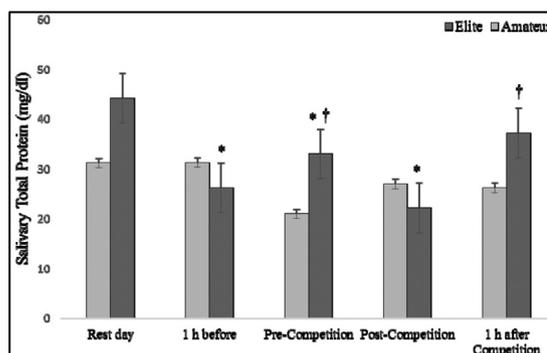


Figure 6 Comparison of the salivary total protein changes in the five stages of measurements between elite and amateur groups. *Indication of significant difference with respect to the rest day in the elite group. †Indication of significant difference between elite and amateur groups.

Total protein

The total protein ($F_4, 32 = 6.18, P = 0.001$) in the elite group exhibited significant decrease at 1 h before, pre-competition, and post-competition compared with that at rest day. Additionally, the total protein concentrations at pre-competition ($t_{13} = 4.13, P = 0.001$) and 1 h after competition ($t_{15} = 4.07, P = 0.001$) were significantly higher in the elite group than those in the amateur group (Fig. 6).

Discussion

The results of this study revealed that the baseline free cortisol concentrations in the elite group were consistently higher than those in the amateur group. Nevertheless, some researchers believe that the existence of this competitive stress improves the performance [27]. The present study also showed that the adrenal cortex activity in the elite group was higher than that in the amateur group. This point is very challenging and contradicts some previous findings that reported higher cortisol concentrations in amateurs and less-experienced groups [17,18]. Changes in adrenal cortex activation biomarkers do not only indicate the amount of competitive stress but also notably represent the amount of effort that an organism exerts to maintain homeostasis. Our previous study showed that the highest concentration of salivary cortisol was observed 30 min after an official competition in amateur soccer players [1]. In the current study, the increased adrenal cortex activity indicated the amplification of the anabolic paths 1 h after the competition. This confirmation is a reminder that elite athletes need relaxation methods prior to sports competitions.

Exercise circumstances for all participants were the same in the present study; hence, we should explore mechanisms underlying these results in the elite and amateur groups. Haneishi et al. showed that the adrenal cortex activity in elite groups, probably because of the compatibility, is lower than that in amateur groups; however, their study included an exercise and unofficial competition [18]. By contrast, another study reported that after an official judo competition, cortisol concentrations were higher in the winners than in the losers [19]. Researchers have also demonstrated

hypertrophy of the adrenal glands during the compatibility process, thereby increasing the adrenal cortex activity [28]. This higher adrenal cortex activity is probably ascribed to understanding the importance of the competition in the elite group with respect to the amateur group who participated for the first time in an official competition. The performance of the elite group also showed that they made greater effort. Hence, stress management [17] and increased self-confidence in the elite group [17,29] may improve their performances. Furthermore, an optimal level of stress can enhance the performance level [27].

Our results showed that the sAA activity in the elite group was higher than that in the amateur group, probably because the former used mechanisms that improved their performance. Labudda et al. reported that participants with better decision-making forms demonstrated higher sAA activity during the task [30]. An optimal amount of fight-or-flight activity is also notably necessary for performance. Momentary sAA activity in response to stress can validate this phenomenon. The momentary sAA activity may justify the reciprocal connection of sAA and cortisol. Nater et al. stated that individuals with high chronic stress exhibit increased momentary sAA activity [31]. Therefore, the higher cortisol concentrations in the elite group can also be an appropriate justification for higher sAA activity. The sAA activity in the elite group increased gradually. In addition, sAA is considered as a useful tool for evaluating the SNS activity [32] which is highly sensitive to stress, and its changes are more remarkable than those in salivary cortisol after the same mental stress event [33]. However, after the start of the competition and increased sympathetic nerve activity, the sAA activity decreased until the end of the competition [26,34]. Moreover, the sAA activity changes in the amateur group were erratic.

Overall, the most important mechanisms to consider are the physiological reasons of these biomarkers' secretion and the nature of their appearance in saliva. Along these discussions and in reference to the nature of these biomarkers' appearance and emergence in saliva, the results, agreements, contradictions, and future challenges are mentioned. From the psychological stimulus type point of view, presentation conditions, such as that of being in the laboratory or that of being in real situations, as well as the participants' perception of the stimulus, are also very important. This point signifies that sAA in male athletes is active in response to a 20 min exercise with intensity of 50% of VO_{2max} [35], and during an official football competition [1], with 70% to 85% of maximum heart rate [12]. In addition, from the physiological point of view, the intensity, duration, diurnal cruses, peripheral consumption, and (to some extent) the compatibility of participants can affect hormonal responses [28]. Participants' gender, age, and their influence on the size of salivary glands and consequently the salivary flow rates also play a part in hormonal responses. However, these factors do not affect the unstimulated whole saliva [36]. Measurement of free hormones and the form of their appearance in saliva is also another influential factor.

Two biomarkers in the saliva can explain this observation. Cortisol is known as an HPA axis change indicator; by contrast, sAA is produced by serous acinar cells of the parotid and submandibular glands [37]. Moreover, sAA is one of the principal salivary protein appearing while the amount

of isoenzymes and indicators for SAM system changes [38]. Simultaneously, part of the cortisol stabilizes in tissues, and some of its released amount, which has not stabilized in the tissues, enters the saliva (free cortisol). Under direct influence of the autonomic nervous system, sAA then enters the saliva. However, the sAA before production and secretion also exists in salivary glands and can be produced and stored [39]. Another factor that might have influenced this observation is the role of diurnal cruses. Cortisol is at the highest concentration level in the morning, whereas sAA activity reaches its peak in the afternoon [31].

We hypothesized that inconsistencies exist in the changes among stress salivary biomarkers in elite and amateur female athletes at real competitions. However, our results showed that in most of the measurement stages, free cortisol and DHEA concentrations, as well as sAA activity, were higher in the elite group. The lower DHEA-to-cortisol ratio in the elite group resulted from the significantly higher cortisol concentrations with respect to the amateur group. This discovery contradicts previous reports that suggest the existence of lower cortisol concentrations in elite groups [17,18]. Physiological factors related to skating competitions, such as intensity, duration, and protocol, may also lead to these differences.

The effect of salivary flow rate reduction is also an important factor [40]. In case of increased saliva concentration caused by dehydration, measured biomarkers' concentrations must demonstrate rising trends. However, in the present study, measured biomarkers presented a declining trend toward the end of the measurement stages. This point exists because sAA comprises almost half of the salivary proteins [41]; moreover, in the present study, the highest sAA activity occurred at 1 h before the competition while the total protein decreased. Hence, factors that may have interfered must be considered. For instance, sAA activity changes can be the result of sympathetic activity's increase and epinephrine's influence on the decrease in amylase production and salivary flow rate [26,34]. Challenges regarding the influence of salivary flow rate on salivary composition also exist. Some studies showed that sAA activity is not affected by the salivary flow rate [42]. Lack of salivary flow rate measurement was one of the limitations of the present research and should be given proper consideration in future studies. Previous studies also showed that sAA activity in response to stress is acute and momentary, whereas the response of cortisol is dilatory [22,23]. Therefore, the role of cortisol in chronic stresses should be considered to imply the role of amylase in immediate changes. Two points should be considered here. First, the researcher is usually not aware that the utilized stimulus leads to chronic or acute stress. Second, the effects of these stimuli vary in different people. Notably, individuals with chronic stress yield high momentary responses to stimuli [31].

Conclusion

Undoubtedly, the non-invasive nature of utilizing salivary composition in research has offered considerable approaches as biomarkers. Investigating these biomarkers and the consistency of their changes with respect to important criteria, such as lactic acid and heart rate changes,

validates their use. For instance, lactic acid measurements from a lactometer, heart rate monitoring, or the use of valid criteria, such as rate of perceived exertion, can obtain information on athletic stress. In conclusion, an increase of HPA and SAM were observed before competition in elite skaters while the SAM increases in amateurs only before competition and remained activated after the competition. Moreover, investigation of sAA variation alone may not be enough. For every physiological phenomenon, a potential or an actual evolutionary explanation should be investigated to understand how sAA secretion helps maintain homeostasis or how this activity is categorized from an evolutionary perspective. Salivary biomarkers can be interesting parameters in stress studies in addition to being easy to obtain and measure. The mechanism underlying changes in salivary biomarkers related to psychological stress remains unclear and should be examined in future studies. Therefore, further research is needed to investigate the stressor mediators involved in sporting events, as well as the roles of the parasympathetic and sympathetic nervous systems in biomarker changes.

Disclosure of interest

The authors declare that they have no competing interest.

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