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Revision

Salivary markers of stress and physical activities: a systematic review

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ABSTRACT

In recent years, stress levels during exercise are measured using different salivary markers. The aim of the study was to conduct a systematic review of the main salivary stress markers related to physical exercise and their effects on health of MEDLINE, LILACS, IBECS, BVS and SciELO databases between 2011 and 2018. The descriptors used in the search were "Biochemical" and "Marker"; "Stress" and "Physical" and "Exercise"; "Salivary chromogranin-A"; "Salivary Alpha-Amylase"; "Salivary IgA"; and "Salivary cortisol", in three languages.-After assessment of eligibility criteria, of the 13405 studies identified, 12 were included in the final review and, although saliva has clear advantages over blood by easy to collect and non-invasive, the use of these markers in the response to stress remains incomplete due to the different effects observed, considering that the information available in the literature involves different types of participants as study subjects and a range of protocols.
Keywords: Biomarkers; Saliva; Stress; Physical activity; Psychological stress.

Marcadores salivares de estrés y actividades físicas: una revisión sistemática

RESUMEN

En los últimos años, se han medido los niveles de estrés durante el ejercicio mediante el análisis de diferentes marcadores salivales. El objetivo del estudio fue realizar una revisión sistemática de los principales marcadores salivares de estrés relacionados con el ejercicio y sus efectos en la salud en bases de datos MEDLINE, LILACS, IBECS, BVS y SciELO, de 2011 a 2018. Los descriptores utilizados en la investigación fueron "bioquímicos" y "marcadores"; "estrés" y "físico" y "ejercicio"; "cromogranina A salivar"; "alpha-amilase salivar"; "IgA salivar"; "cortisol salivar", en tres idiomas. Después de la evaluación de los criterios de elegibilidad, de los 13405 estudios identificados, 12 fueron incluidos en la revisión final y, aunque la saliva presentaba claras ventajas sobre la sangre por la fácil recolección y no invasión, el empleo de estos marcadores en la respuesta al estrés sigue incompleto por los distintos efectos observados, considerando los diferentes tipos de participantes como sujetos del estudio y la gama de protocolos.
Palabras clave: Biomarcadores; Saliva; Estrés; Actividad física; Estrés psicológico.

Marcadores salivares de estresse e atividades físicas: uma revisão sistemática

RESUMO

Nos últimos anos, os níveis de estresse durante o exercício foram mensurados pela análise de diferentes marcadores salivares. O objetivo do estudo foi realizar uma revisão sistemática dos principais marcadores salivares de estresse relacionados ao exercício físico e seus efeitos na saúde em bases de dados MEDLINE, LILACS, IBECS, BVS e SciELO, no período de 2011 a 2018. Os descritores utilizados na pesquisa foram "bioquímicos" e "marcadores"; "estresse" e "físico" e "exercício"; "cromogranina A salivar"; "alfa-amilase salivar"; "IgA salivar"; e "cortisol salivar", em três idiomas. Após a avaliação dos critérios de elegibilidade, dos 13405 estudos identificados, 12 foram incluídos na revisão final e, embora a saliva apresente claras vantagens sobre o sangue pela fácil coleta e não invisibilidade, o emprego desses marcadores na resposta ao estresse após o exercício permanece incompleto pelos distintos efeitos observados, considerando os diferentes tipos de participantes como sujeitos do estudo e a gama de protocolos.
Palavras-chave: Biomarcadores; Saliva; Estresse; Atividade física; Estresse psicológico.

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Introduction

High-intensity exercise provokes physiological and mental responses involving the sympathetic nervous system (SNS), in addition to activating the hypothalamic-pituitary- adrenal (HPA) system and the sympathetic-adrenal-medullary (SAM) system.[1,2] Exercise is a prototype of physical stress, and high-performance training is a systematic process involving variations in workloads (volume vs. intensity).[1,2]

Thus, optimal performance and tolerance to large workloads reflect appropriate training adaptation. One of the most widely used measures to assess this adaptation is blood lactate response during an incremental exercise test.[3] Anaerobic threshold (AT) is defined as the exertion level at which the body switches from aerobic to anaerobic metabolism in order to meet energy demands, thereby initiating lactate accumulation.[4] Quantifying exercise intensity at the AT has been used to assess cardiovascular or pulmonary health by determining ventilator parameters, and to prescribe and assess training.[5]

Stress is normal adaptation to environmental demands, involving a series of body changes or reactions at physical, mental, cognitive or behavioral levels. These changes are characterized by activation, acceleration or implementation of new resources, in order to respond to a certain demand.[6]

However, stress is a state in which homeostasis is disturbed by real or perceived challenges in the external environment,[7] and stress response is the body's response to restore homeostasis. During stress response, two biological systems are activated: the SNS and HPA system.[8]

In this respect, stress levels during exercise are commonly measured using heart rate, oxygen uptake (VO_2max) and blood biomarkers, invasive collection measures that already cause a degree of stress, in contrast to the salivary stress biomarkers used to evaluate autonomous nervous system activity, physical condition levels and individual reaction to training.[5]

The results of studies[9] and demanding competitions[10-12] indicate that the simultaneous measurement of psychological, physical and hormonal parameters offers a unique possibility of achieving a more comprehensive assessment of stress responses. In particular, SAM activation, with the secretion of two catecholamines and noradrenalin, clearly involved in homeostasis and psycho-physical demands, has been considered an objective marker of individual stress.[13] Acute stress increases HPA axis activity with a subsequent rise in salivary cortisol levels (sCort).[13,14] Salivary immunoglobulin A (IgA) and salivary chromogranin-A levels (sCgA) may be a sensitive and quantitative index of SAM activity.[15,16]

Among the main types of diagnostic samples, saliva is non-invasive and perhaps the most easily collected. It is secreted from three large glands (parotid, submandibular and sublingual), in a process regulated by the autonomous nervous system. Sympathetic and parasympathetic innervation in the salivary glands stimulates salivary secretion; sympathetic stimulation is responsible for the rise in salivary protein secretion, while a parasympathetic stimulus increases salivary flow.[17,18]

Given that stress symptoms are induced by SAM and HPA axis reactions, saliva is considered good material for assessing stress conditions. Additionally, the use of salivary biomarkers to evaluate stress in human beings has attracted considerable attention in recent years, particularly the investigation of sCort, salivary α-amylase (sAA), CgA and IgA.[18]

For example, cortisol is secreted by the suprarenal gland in serum and saliva under physical and psychological stress. Circulating cortisol is also dependent on exercise intensity, rising at intensities above 60% of maximum oxygen uptake (VO_2max), and related to HPA axis activation.[18]

Thus, since it is difficult to measure catecholamine in saliva and seems not to reflect SAM activity,[19] markers such as sAA, sCgA and sIgA have been used to assess SAM activity. Levels of these salivary markers may reflect changes in sympathetic activity under a variety of stressful conditions.[20]

sAA is one of the most abundant proteins in saliva, and studies have demonstrated the feasibility of quantifying AT in saliva,[21,22] given that the secretion pattern of sAA is indirectly related to changes in catecholamine levels that occur due to the increase in SAM activity during incremental submaximal and maximal exercise. The results of studies showed high correlations between aAA and catecholamines and since then, sAA has been suggested as an indirect marker of SAM activity in exercise and stressful situations.[23]

sCgA, in turn, is co-released by the submandibular gland. A number of studies have shown similar sCgA and sAA secretion dynamics during stressful situations.[16,12] Physical stress has an impact on sCgA levels analogous to the effect described by a number of studies in relation to sAA levels.[18,24]

Likewise, sIgA has been suggested as a potential stress marker in humans. However, several studies demonstrate a negative correlation between sIgA levels and mental stress. Since sIgA is strongly affected by oral contamination and salivary flow, its half-life is too long to assess psychological stress in real time.[18] Studying the factors that can modulate the secretion of this biomarker is important in controlling stress levels during physical activity.

Recent years have seen an increase in the number of studies involving salivary biomarkers that can be used to assess autonomous nervous system activity as a response to stress and physical conditioning level. Saliva is considered good material for assessing stress conditions since it is simple to collect, non-invasive and sampling and handling do not require trained personnel. Conduct a systematic review of salivary stress markers related to physical exercise and their effects on health, involves different types of participants as study subjects and a range of protocols.

Method

The present study is based on a systematic review of articles published between 2011 and 2018 on the following databases: MEDLINE/PubMed (Medical Literature Analysis and Retrieval System Online), LILACS (Literatura Latino Americana e do Caribe em Ciências da Saúde), IBECS (Índice Bibliográfico Espanhol de Ciências da Saúde), BVS (Biblioteca Virtual em Saúde) and SciELO (Scientific Electronic Library Online).

The descriptors used in the search were Biochemical" and "Marker"; "Stress" and "Physical" and "Exercise"; "Salivary chromogranin-A"; "Salivary Alpha-Amylase"; "Salivary IgA"; and "Salivary cortisol", in three languages.

The following inclusion criteria were established: 1) written in Spanish, English or Portuguese; 2) published between 2011 and 2018; 3) conducted with men and/or women older than 18 years; 4) quantitative studies; 5) studies that examined the physical and/or psychological effects resulting from physical activity in the quantification of biological markers; 6) analysis of salivary markers.

The Quality Assessment Tool for Quantitative Studies of Effective Public Health Practice Project (McMaster University, Canada) was applied to assess methodological quality, using "strong", "moderate" or "weak" for each study.[25,26] Excluded were studies that did not contain biological tests or adequately describe statistical assessments or those where the design, study site, outcome, sample, results or risk of bias were unclear. The search strategy was carried out independently by two researchers, the results obtained compared and disagreements settled by consensus.

Results and Discussion

A total of 13405 articles were found initially, and after those that did not meet eligibility criteria following assessment of titles, abstracts and duplicates were removed, 12 studies were included in the final review (Figure 1).

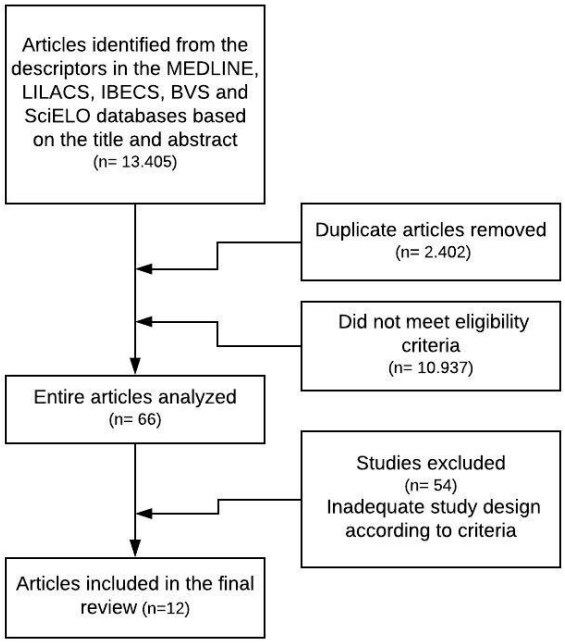

Figure 1. Flowchart with the article selection stages of the systematic review

Studies with human beings have observed different effects related to stress in salivary biomarker stimulation after exercise. The increased concentration (sCgA, total proteins, sCort, salivary inflammatory cytokines) or enzymatic activity (sAA) of the markers assessed in physical exercise contexts have been prioritized in both sedentary individuals and athletes (Table 1 and 2). By contrast, no changes in the individual responses of sIgA were observed in several studies[27,28] and an increase[29,30] or decrease[31,32] in others, when this marker was quantified according to the study design and samples used in assessment.

Most studies that assessed stress biomarkers in sedentary individuals revealed improved health. Gallina et al.[33] correlated sCgA and sAA levels in 21 men with cardiovascular and psychological parameters before and after a standard ergometric test and during the recovery phase. With respect to baseline, there was a significant increase in peak sCgA ($p < 0.001$; median of 64%) and sAA (median of 86%) at the end of exercise. However, during the recovery phase, sAA levels fell sharply, while sCgA remained elevated ($P < 0.001$). These data suggest that sCgA is a reliable marker for assessing SAM activation. Additionally, the relation between sCgA and exercise intensity highlights the potential of this non-invasive parameter in monitoring the adrenergic response during intense physical stress.

In the same year, Usui et al.[32] examined whether cortisol, IgA, CgA and salivary inflammatory cytokines levels may be affected by prolonged strenuous exercise. Ten young men were submitted to an ergometric test at 75% of maximum VO_2 for 60 minutes or sat still during two experimental sessions (exercise and resting conditions) with at least a seven-day interval between them, and saliva samples were obtained at 60-minute intervals during the sessions to measure salivary markers. The results demonstrate that a single session of strenuous exercise caused an increase in CgA and salivary inflammatory cytokine levels and a decline in salivary IgA after exercise.

In another study, saliva samples were collected from 20 healthy men immediately before and after walking and at intervals of 20 and 40 minutes in order to investigate the effect of walking in the forest on salivary stress markers, cortisol and salivary CgA. On another day, controls sat in a circle in an office and samples were

Table 1. Studies with changes in stress biomarkers after exercise in sedentary individuals.

Study	Biomarker studied*	Effect observed	Study design/Sample	Impact on Health
Gallina et al.[33]	sCgA sAA	↑sCgA ↑sAA	Follow-up of 21 healthy men before and after a standard treadmill stress test and at intervals during the recovery phase (average age was 24 ± 2 years, ranging from 21 to 30 years)	Improvement before and during a standard ergometric test and the recovery phase based on assessment of salivary markers
Usui et al.[32]	sCort IgA CgA Salivary inflammatory cytokines	↑sCort, ↑sCgA ↑Salivary inflammatory cytokines ↓sIgA	Ten young male university students (average age was 23 ± 3 years)	A single session of strenuous exercise caused an increase in sCort, sCgA and salivary inflammatory cytokin levels and a decline in salivary IgA after exercise.
Toda et al.[34]	sCort sCgA	↑sCgA No change in sCort level	Twenty healthy men were assessed before and after walking (average age was 67.6 ± 2.8 years, ranging from 64 to 74 years)	A walk in the forest caused satisfaction/stimulation, despite being a physical stress factor.
Alghadir, Gabr and Aly[35]	sCort testosterone LDH	↑ sCort, ↑Testosterone ↑LDH	The study assessed the effect of 4 weeks of moderate aerobic exercise on 16 young male adults. (average age was 22.8±2.14 years, age range of 15-25 years)	This suggests a positive effect of physical exercise on stress hormones after four weeks of training.
Strahler et al.[36]	sAA sCort	sAA remained unchanged ↓sCort	115 sedentary men were monitored by simulating a validated stress situation (graduated exercise on an ergometric bicycle). (45.7 ± 10.1 years, ranging from 19 to 64 years)	The physical activity level of men showed a slight impact on stress responses.
Rutherfurd-Markwick et al.[29]	sAA sIgA	↑ sAA ↑ sIgA	Samples were collected from 12 women and 8 men in response to exercise and at rest. (average age of the two groups = 27.4 ± 5.9 years)	This suggests a different physiological response to stress according to sex, with a greater effect on women.

sAA: salivary α-amylase; sCort: salivary cortisol; sCgA: salivary chromogranin A; IgA: salivary immunoglobulin A; LDH: lactate dehydrogenase; TP: total proteins. * Most studies that assessed stress biomarkers in sedentary individuals measured using an enzyme-linked immunosorbent assay (ELISA) for measurements. Exception: Strahler et al.36, sAA levels were measured by kinetic colorimetric test with reagents from Roche. Rutherfurd-Markwick et al.29, sCort and sAA was determined, respectively, by radioimmunoassay and the Infinity Amylase Liquid stable reagent (Thermoscientific, Worthing, UK).

Table 2. Studies with changes in stress biomarkers after exercise in athletes.

Study	Biomarker studied*	Effect observed	Study design/Sample	Impact on Health
Diaz et al.[37]	TP sAA sCgA	↑ TP ↑ AA ↑ sCgA	Eleven professional male swimmers (average age was 21.5±2.16 years)	Marker concentrations were different from controls immediately before pre-competition warmup and 5 minutes after the event.
Bocanegra et al.[38]	bLAC sLAC sAA sCgA	Similar results between sAA and sCgA in relation to bLAC. Lactate accumulation in saliva followed the same pattern observed in blood.	Twelve professional male athletes, one incremental test consisting of eight series of 100 m. (average age was 21.2 ± 1.8 years)	The first demonstration of sCgA as a new marker of exercise intensity in well-trained men.
Peñailillo et al.[31]	sCort testosterone sIgA	sCort remained unchanged ↓Testosterone ↓IgA	Assessed the effect of a soccer match on marker concentrations of 16 students (young adults, 26 ± 3.5 years)	A decline in IgA and testosterone was observed, the latter related to distance traveled (time/movement analysis).
Edmonds et al.[27]	sAA sIgA	↑sAA No significant change in sIgA.	Eight Paralympic swimmers were monitored during the weeks preceding an important competition. (average age was 20.6 ± 4,8 years)	The result indicates the need for a well-managed swimming program to cope with stress.
Sinnott-O'Connor et al.[30]	sIgA sAA sCort	↑ sIgA ↑ sAA ↑ sCort,	Four Paralympic swimmers provided twice monthly saliva samples during the training phases and competition (19 ± 4 years)	These results show that elite athletes exhibit a significant response to stress in important competitions, despite the marked decline in training load.
Pritchard et al.[28]	sIgA sCort	No significant change in sIgA levels. ↑ sCort,	Saliva samples were collected from a team of 45 players of an elite Australian soccer team at three different times (start of the pre-season, start of the season and end of the season). (mean age was 22.8 ± 3.5 years)	The data found show that caffeine consumption, infection and recent use of antibiotics affect salivary control but not sIgA.

sAA: salivary a-amylase; sCort: salivary cortisol; sCgA: salivary chromogranin A; IgA: salivary immunoglobulin A; LDH: lactate dehydrogenase; TP: total proteins. * Edmonds et al.27 and Sinnott-O'Connor et al.30 measured biomarkers levels using an Lateral Flow Immunoassay (IPRO Interactive, Oxfordshire, UK) in combination with IPRO Reader for the quantitative measurement. This method of saliva analysis has previously been validated against ELISA analysis. Diaz et al.37 and Bocanegra et al.38 assessed sAA and sCgA using Western blotting. Pritchard et al.28 measured sCgA and sAA levels using ELISA.

collected at the same times. The samples collected after walking showed a significant increase in CgA levels, but there was no significant change in cortisol levels. However, controls exhibited a statistically significant decline in both levels. These results suggest that walking in the forest is a physical stress factor, although this activity can be stimulating and satisfying.[34]

Alghadir, Gabr and Aly[35] assessed the effect of four weeks of moderate aerobic exercise on sCort, testosterone and salivary dehydrogenase lactate (LDH) levels in 16 healthy young adults who engaged in moderate intensity exercise (treadmill walking) three times a week. After four weeks of training, saliva cortisol, testosterone and LDH levels showed a significant increase in cortisol, free testosterone and LDH activity, in addition to a significant decline in the relation between testosterone and cortisol levels.

Likewise, Strahler et al.[36] found that factors such as lifestyle, regular exercise and physical conditioning affected salivary stress markers (sCort and sAA) in 115 bankers and insurance agents, using graduated exercise on an ergometric bicycle, with an initial workload of 60 watts and a 25-watt increase every 3 min until exhaustion. Assessment of standardized physical aptitude used the blood lactate test to determine the anaerobic threshold of sedentary and inactive individuals. Before the intervention, sCort and sAA levels were similar in all the groups and, after stress induction, individuals with moderate and high aptitude exhibited slightly lower sCort levels. There was no significant effect of aptitude status on sAA. The physical aptitude level in the male population showed a small impact on neuroendocrine and autonomic stress responses to a socio evaluative stressor.

In one of the few studies that assessed the effect of stress according to sex, Rutherfurd-Markwick et al.[29] analyzed salivary markers in 12 women and 8 men (average age of the two groups = 27.4 ± 5.9 years) in response to 60 minutes of stationary cycling at 70% peak power output, and participants seated in silence for 60 minutes, with saliva collection at 15 and 45 minutes. Alpha-amylase activity, sIgA secretion rate and sCort levels increased between resting and exercise in the group of women compared to their male counterparts. These data suggest a different physiological response to stress according to sex and illustrated the role of steroid hormones (not quantified in the study) in prompting a response to the SAM and HPA axes (sAA and sCgA).

By contrast, Diaz et al.[37] investigated the TP, AA and salivary CgA response in a competition involving 11 professional swimmers, who were examined on the first day of competition and a training day two weeks later. Salivary TP, AA and CgA concentrations were different from controls immediately before pre-competition warmup, with higher salivary TP, sAA and CgA levels before the event on the day of competition and 5 minutes after the event. By contrast, no significant changes in salivary protein concentrations were observed upon awakening and 60 minutes later. These results demonstrate a rise in salivary protein activity and concentration during the stress of sport competition.

Corroborating these findings, 12 professional male athletes underwent a salivary lactate (sLAC), sAA and sCgA test to determine blood lactate accumulation (bLAC)[38]. Inflection points in bLAC, sLAC, sAA and sCgA concentration were found in all the subjects. Lactate accumulation in saliva followed the same pattern observed in blood, with a high correlation between the two (r = 0.91). Similar results were obtained between sAA (r = 0.81) and sCgA (r = 0.82) in relation to bLAC. These results reinforce the use of saliva to determine lactate threshold and provides the first evidence of sCgA as a new marker of exercise intensity in well-trained men.

In another study with professional athletes, Peñailillo et al.[31] assessed the effect of a soccer match on sCort, testosterone and sIgA levels (before and 10 minutes after the match) in nine professional soccer players. sCort remained unchanged after the game. However, pre- and post-test testosterone levels correlated with post-game sIgA concentrations (r = 0.80; P = 0.005 and r = 0.89; P = 0.001, respectively), both of which declined. Changes in testosterone concentrations also correlated (r = 0.85; P = 0.004) with distance travelled based on performance assessment (time/movement analysis).

One of the first studies with a homogeneous athletic population was conducted by Edmonds et al.[27] in order to assess sAA and sIgA in eight Paralympic swimmers (2 women and 6 men) submitted to a chronic training load and monitored for 14 weeks. Swimming training in the present study revealed no significant differences in

sIgA measures, although sAA increased from the seventh week onward. sAA levels rose during the competition, indicating the need for a well-managed program to deal with this situation.

In another study carried out with four Paralympic swimmers (1 man and 3 women aged 19 years), Sinnott-O'Connor et al.[30] assessed the relation between training load (TL) and the saliva biomarkers sIgA, sAA and cortisol during the training phase, subdivided into normal, intensified and taper (twice-monthly samples for 16 weeks), and competition phase (daily samples during the 10 days). There was a significant increase in sIgA simultaneous to a rise in TL during intensive training and a decline in sIgA, sAA and salivary cortisol in the taper phase, with a 49.5% decrease in TL, although the three markers remained above basal levels. During competition, even with a drastic reduction in TL in the taper phase, sCort, sIgA and AA rose. These results indicate that even with a decline in TL, elite athletes exhibit a significant stress response in important competitions, a similar result to that observed by Edmonds et al. (2015), and a relevant finding in devising post-competition protocols that optimize recovery and performance.

Pritchard et al.[28] assessed pace and lifestyle factors that could affect sIgA and sCort quantification in professional Australian soccer players, aged between 19 and 31 years with similar environmental stressor levels and psychological pressure related to competitiveness, training regimen and competition. The data found show that caffeine consumption, infection and recent use of antibiotics affect sCort, but not sIgA. The aforementioned study performed a number of correlations, demonstrating the importance of developing a protocol to accurately measure sCort and sIgA that considers the effects of pace and lifestyle on quantifications.

Changes in sIgA responses observed in the studies assessed, including non-significant changes,[27,28] increased[29,30] or decreased concentrations,[31,32] reflect the study design and sample selection based on inclusion criteria, since these effects could be related to a specific infectious process not considered in the screening of study subjects.[28] This demonstrates the need for more detailed analyses to investigate the relation between stress response, physical activity and recurring infection.

The evidences in the studies analyzed could corroborate the use of salivary biomarkers according to the experimental protocol and a more consistent number of individuals in order to predict psycho-physical effects in response to stressors in physical activities. Assessment of stress response using salivary markers is relevant since it is a non-invasive approach and shows the need for changes in individual post-competition recovery protocols aimed at optimizing performance.

Conclusion

Understanding the role of salivary markers (cortisol, sAA, CgA and IgA) in the response to stress remains incomplete, considering that the information available in the literature involves different types of participants as study subjects and a range of protocols, even though sIgA, sAA and sCort have been applied in a number of studies. A literature search of the issue shows the current status of knowledge, demonstrating that most studies recruit men to analyze salivary biomarkers as a response to exercise (stress) and that some of the mixed cohort studies did not conduct a sex-specific analysis, although the sex-related differences and role of steroid hormones are described. As such, these gaps need to be addressed in future studies in order to clarify the relations between other salivary markers and physical activity and health.

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References

1. Cano Sokoloff N, Misra M, Ackerman KE.Exercise, Training, and the Hypothalamic-Pituitary-Gonadal Axis in Men and Women. Front Horm Res. 2016;47:27-43.
2. Leal-Cerro A, Gippini A, Amaya MJ, Lage M, Mato JA, Dieguez C, et al. Mechanisms underlying the neuroendocrine response to physical exercise. J Endocrinol Invest. 2003;26:879-85.
3. Beneke R, Leithäuser RM, Ochentel O. Blood lactate diagnostics in exercise testing and training. Int J Sports Physiol Perform. 2011;6(1):8-24.
4. Svedahl K, Macintosh BR. Anaerobic threshold: the concept and methods of measurement. Can J Appl Physiol. 2003; 28:299-323.
5. Scharhag-Rosenberger F, Meyer T, Gässler N, Faude O, Kindermann W. Exercise at given percentages of VO^2 max: Heterogeneous metabolic responses between individuals. J Sci Med Sport. 2010;13:74-9.
6. Edwards JP, Walsh NP, Diment PC, Roberts R. Anxiety and perceived psychological stress play an important role in the immune response after exercise. Exerc Immunol Rev. 2018;24:26-34.
7. Ali N, Pruessner JC. The salivary alpha amylase over cortisol ratio as a marker to assess dysregulations of the stress systems. Physiol Behav. 2012;106(1):65-72.
8. Chrousos GP, Kino T, Charmandari E. Evaluation of the hypothalamic-pituitary-adrenal axis function in childhood and adolescence. Neuroimmunomodulation. 2009;16(5):272-83.
9. Perroni F, Tessitore A, Cibelli G, Lupo C, D'Artibale E, Cortis C, et al. Effects of a simulated firefighting on the responses of salivary cortisol, alpha-amylase and psychological variables. Ergonomics. 2009;52:484-91.
10. Fernandez-Fernandez J, Boullosa DA, Sanz-Rivas D, Abreu L, Filaire E, Mendez-Villanueva A. Psychophysiological stress responses during training and competition in young female competitive tennis players. Int J Sports Med. 2015;36(1):22-8.
11. Filaire E, Alix D, Ferrand C, Verger M. Psychophysiological stress in tennis players during the first single match of a tournament. Psychoneuroendocrinology. 2009;34:150-7.
12. Kivlighan KT, Granger DA. Salivary alpha-amylase response to competition: relation to gender, previous experience, and attitudes. Psychoneuroendocrinology. 2006;31:703-14.
13. Strahler J, Skoluda N, Kappert MB, Nater UM. Simultaneous measurement of salivary cortisol and alpha-amylase: Application and recommendations. Neurosci Biobehav Rev. 2017;83:657-77.
14. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34(2):163-71.
15. Allgrove JE, Gomes E, Hough J, Gleeson M. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. J Sports Sci. 2008;26:653-61.

16. Lippi G, De Vita F, Salvagno GL, Gelati M, Montagnana M, Guidi GC. Measurement of morning saliva cortisol in athletes. Clin Biochem. 2009;42(9):904-6.
17. Malon RS, Sadir S, Balakrishnan M, Córcoles EP. Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics. Biomed Res Int. 2014;2014:962903.
18. Obayashi K. Salivary mental stress proteins. Clin Chim Acta. 2013;21:196-201.
19. Kennedy B, Dillon E, Mills PJ, Ziegler MG. Catecholamines in human saliva. Life Sci. 2001;69(1):87-99.
20. Viru A, Viru M. Cortisol - essential adaptation hormone in exercise. Int J Sports Med. 2004;25:461-4.
21. Bortolini MJ, De Agostini GG, Reis IT, Lamounier RP, Blumberg JB, Espindola FS. Total protein of whole saliva as a biomarker of anaerobic threshold. Res Q Exerc Sport. 2009;80:604-10.
22. Koibuchi E, Suzuki Y. Exercise upregulates salivary amylase in humans. Exp Ther Med. 2014;7(4):773-7.
23. Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, et al. Stress-induced changes in human salivary alpha-amylase activity -- associations with adrenergic activity. Psychoneuroendocrinology. 2006;31(1):49-58.
24. Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. N Engl J Med. 2003;348:1134-49.
25. National Collaborating Centre for Methods and Tools. Quality assessment tool for quantitative studies Acessed: 10/09/2019. Available: https://www.nccmt.ca/resources/search/14.
26. Thomas BH, Ciliska D, Dobbins M, Micucci S. A Process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. Worldviews Evid Based Nurs. 2004;1(3):176-84.
27. Edmonds R, Burkett B, Leicht A, McKean M. Effect of Chronic Training on Heart Rate Variability, Salivary IgA and Salivary Alpha- Amylase in Elite Swimmers with a Disability. PLoS One.2015;10:1-12.
28. Pritchard BT, Stanton W, Lord R, Petocz P, Pepping GJ. Factors Affecting Measurement of Salivary Cortisol and Secretory Immunoglobulin A in Field Studies of Athletes. Front Endocrinol (Lausanne). 2017;8:1-7.
29. Rutherfurd-Markwick K, Starck C, Dulson DK, Ali A. Salivary diagnostic markers in males and females during rest and exercise. J Int Soc Sports Nutr. 2017;14:1-8.
30. Sinnott-O'Connor C, Comyns TM, Nevill AM, Warrington GD. Salivary Biomarkers and Training Load During Training and Competition in Paralympic Swimmers. Int J Sports Physiol Perform. 2018;13:839-43.
31. Peñailillo L, Maya L, Niño G, Torres H, Zbinden-Foncea H. Salivary hormones and IgA in relation to physical performance in football. J Sports Sci. 2015;33:2080-7.
32. Usui T, Yoshikawa T, Ueda S, Katsura Y, Orita K, Fujimoto S. Effects of Acute Prolonged Strenuous Exercise on the Salivary stress Markers and Inflammatory Cytokines. Jpn J Phys Fitness Sports Med. 2011;60(3):295-304.
33. Gallina S, Di Mauro M, D'Amico MA, D'Angelo E, Sablone A, Di Fonso A, et al. Salivary chromogranin A, but not alpha-amylase, correlates with cardiovascular parameters during high-intensity exercise. Clin Endocrinol. 2011;75:747-52.
34. Toda M, Den R, Hasegawa-Ohira M, Morimoto K. Effects of woodland walking on salivary stress markers cortisol and chromogranin A. Complement Ther Med. 2013;21:29-34.
35. Alghadir AH, Gabr SA, Aly FA. The effects of four weeks aerobic training on saliva cortisol and testosterone in young healthy persons. J Phys Ther Sci. 2015;27:2029-33.
36. Strahler J, Fuchs R, Nater UM, Klaperski S. Impact of physical fitness on salivary stress markers in sedentary to low-active young to middle-aged men. Psychoneuroendocrinology. 2016;68:14-9.
37. Diaz MM, Bocanegra OL, Teixeira RR, Soares SS, Espindola FS. Response of salivary markers of autonomic activity to elite competition. Int J Sports Med. 2012;33:763-8.
38. Bocanegra OL, Diaz MM, Teixeira RR, Soares SS, Espindola FS. Determination of the lactate threshold by means of salivary biomarkers: chromogranin A as novel marker of exercise intensity. Eur J Appl Physiol. 2012;112:3195-203.