Revista Brasileira de Prescrição e Fisiologia do Exercício
ISSN 1981-9900 *versão eletrônica*
Periódico do Instituto Brasileiro de Pesquisa e Ensino em Fisiologia do Exercício
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RELATIONSHIP BETWEEN SALIVARY BIOMARKERS AND PERFORMANCE IN LONG-DISTANCE RUNNING

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ABSTRACT

Purpose: The aim of the present study was to evaluate the salivary cortisol and alpha amylase levels in response to long-distance running according performance variables as accumulated distance, total time and velocity of running in athletes. Methods: Eight sportsmen (aged 21 ± 2 years; Body Mass Index 20 ± 1 kg/m²) completed a total of 259 km in 17 hours, throughout 3 days. The distance, total time run, and velocity during running were computed, and saliva samples were collected in the morning and at night for 3 days. Pearson correlations between variables were performed. Results: The range of distances was ≈ 7 to 54 km, and total time per run was ≈ 32 to 198 minutes, and the velocity of run was 13 to 16.5 km/h. Pearson correlations showed positive relationships between salivary cortisol and distance (r=0.81, p<0.01), total time running (r=0.77, p<0.02) and velocity during run (r=0.89, p<0.002). In addition, no correlations were observed to salivary alpha amylase. Conclusions: Based on this result, the accumulated distance, total time and velocity of running exhibits positive correlations to salivary cortisol, but not to salivary alpha amylase. This data shows that an endocrine response is continuously demanded in long distance running.

Key words: Running. Hormone. Saliva. Athletic performance.

RESUMO

Objetivo: O objetivo do presente estudo foi avaliar os níveis de cortisol e alfa amilase salivar em resposta a corrida de longa distância de acordo com variáveis de desempenho como distância acumulada, tempo total e velocidade de corrida em atletas. Métodos: Oito atletas (idade entre 21 ± 2 anos, índice de massa corporal de 20 ± 1 kg / m²) completaram um total de 259 km em 17 horas, ao longo de 3 dias. A distância, tempo total e velocidade durante a corrida foram computados, e amostras de saliva foram coletadas no período da manhã e noite, durante 3 dias. Para análises estatísticas, correlações de Pearson entre as variáveis foram realizadas. Resultados: A variação de distâncias foi ≈ 7-54 km, tempo total foi ≈ 32 a 198 minutos e a velocidade de execução foi entre 13 e 16,5 km / h. Correlações de Pearson mostraram relações positivas entre cortisol salivar e a distância (r = 0,81, p <0,01), tempo total de corrida (r = 0,77, p <0,02) e velocidade durante a corrida (r = 0,89, p <0,002). Além disso, não foi observado correlações com a alfa-amilase salivar. Conclusões: Com base nesse resultado, a distância acumulada, o tempo total e velocidade exibem correlações positivas com cortisol salivar, mas não com a alfa-amilase salivar. Estes dados mostram que uma resposta endócrina é continuamente exigida na corrida de longa distância.

Palavras-chave: Corrida. Hormônios. Saliva. Desempenho Esportivo.

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INTRODUCTION

The maintenance of physical exercise in several intensities requires neuro-immuno-endocrine modifications in order to maintain physical exercise and recovery, given that these changes are fundamental to different systems, such as the cardiovascular, respiratory and musculoskeletal (Fragala et al., 2011).

Cytokines, neurotransmitters and hormones, such as cortisol, are involved in the homeostatic regulation during exercise and the interactions between these factors are essential during exercise. An important hormone is cortisol, a catabolic hormone secreted in response to physiological stress and some studies have shown an increase in cortisol secretion due to consumption of oxygen greater than or equal to 60% (Viru, 1992; Hackney, 1996).

At the same time, alpha-amylase (α-amylase) concentrations in humans rises ahead of body homeostasis variations, mediated by physical exercise and/or body temperature alterations, and are correlated with sympathetic activity upon stress conditions (Granger et al., 2007; Nater and Rohleder, 2009).

Interestingly, there are indications that cortisol levels are not related to α-amylase during stress suggesting that individual differences in this enzyme represents a response to stress signals independent of the hypothalamic-pituitary-adrenal axis (HPA axis).

According McKune et al. (2014) there are no differences in HPA responses, characterized by salivary cortisol, after a session of repeatedly running downhill, however salivary α-amylase results indicate a change in the sympathoadrenal system response.

In addition, Costa et al., (2012) observed that 2 hours of running at 75% maximal oxygen consumption resulted in a 42% decrease in salivary immunoglobulin A (IgA) concentration during recovery, but increased the salivary α-amylase concentration by 81% and it remained elevated throughout recovery.

On the other hand, is important to note that immune responses, mediated by immunoglobulin, as a result of physical exercise are positively associated with cortisol plasmatic concentration and all immunometabolic alterations occur in order to maintain the health and homeostasis of runners (Mckune et al., 2005).

It is clear in several studies that the salivary proteins respond after exercise, however, the association between salivary proteins (cortisol and α-amylase) with specific training variables, such as distance, velocity and total time, in long-distance running is unclear, and we hypothesized that one of these proteins, or both, may be associated with performance in long-distance running, through the influence and/or regulation of specific training variables.

Thus, the main goal of this study is to evaluate the salivary cortisol and alpha amylase levels in response to long-distance running according performance variables as accumulated distance, total time and velocity of running in athletes.

MATERIALS AND METHODS

Participants and Experimental protocol

Eight professional male runners (aged 21 ± 2 years; Body Mass Index 20 ± 1 kg/m^2) from one state-level running team participated in this study. This study was conducted in accordance with the Helsinki Declaration (written consent obtained for the testing protocols after having been informed of the aims and risks of the study).

To take part in this study the subjects were required to present the following characteristics: (I) be able to run 5 kilometers in < 20 minutes; (II) to be training at least four times per week; (III) to be equal to or older than 17 years and less than 35 years in age; (IV) and to not be involved in any supplementation or medical treatment. Saliva was collected at 7:00AM (before starts the run) and at 22:00PM in 24 hours after of the start and throughout three days of official competition.

Performance Analysis

The distance and total time run were computed by the team coach during each stage of competition. Afterward, distance (km) / total time (h), velocity during run was determined throughout the competition.

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Salivary biochemistry parameters

Saliva samples were collected in special tubes (salivates) for determination of α-amylase by the enzymatic colorimetric method by the manufacturer (LABTEST®, Brazil), and salivary cortisol levels were quantified using an enzyme-linked immunosorbent assay (ELISA), obtained from Diametra (Segrate, Milan, Italy). The salivates were centrifuged for 10 minutes at 3000 rpm in order to sediment the saliva in the vial, and the saliva that was used for analysis after the stock at -80ºC.

For biochemistry parameter adjustments, protein concentration was analyzed using the Bradford assay (Bio-Rad, Hercules, California, USA), with bovine serum albumin as a reference. All samples were measured in triplicate. The intra-assay coefficient of variation (CV) for the measurements of cortisol and α-amylase activity was 3 and 4%, respectively.

Statistical Analysis

The descriptive analyses consisted of the mean and standard deviation. For all measured variables, the estimated sphericity was verified according to Mauchly's W test, and the Greenhouse-Geisser correction was used when necessary. The data normality was verified using the Shapiro-Wilk test.

RESULTS

In *Table 1* are shown the metabolic profile of the athletes during the running by description, and by mean and standard deviation of salivary cortisol and α-amylase according period of the day. In addition, in *Table 2* was describing the individual performance during the run by total distance run, total time and average velocity. Pearson correlations with pooled values (cumulative cortisol concentration during competition) showed positive relationships between salivary cortisol and distance ($r=0.81$, $p<0.01$), total time of run ($r=0.77$, $p<0.02$) and velocity during run ($r=0.89$, $p<0.002$), but no correlations were observed with salivary α-amylase.

Our main results given the relationship between salivary cortisol and total distance (km), total time (minutes) and velocity during run (km/h) are shown in Figure 1 according to the pattern of each run. Alpha amylase data exhibited no statistically significant differences (data not shown).

Table 1 - Salivary cortisol and α-amylase concentration during the time running according the period of the day.

Cortisol (ng·µg protein^{-1})	**1º Day**	**2º Day**	**3º Day**	**Pooled**
Morning	4.5 ± 1.8	6.0 ± 4.9	5.0 ± 3.4	15.5 ± 6.8
Night	3.8 ± 1.2	2.8 ± 1.0	3.7 ± 1.6	10.4 ± 2.9
α-Amylase (U·µg protein^{-1})				
Morning	6.3 ± 2.5	7.2 ± 2.8	8.4 ± 4.7	21.9 ± 5.7
Night	8.0 ± 1.8	5.7 ± 2.0	7.8 ± 1.9	21.5 ± 3.3

Table 2 - Individual description, followed the sum, of the characterization variables of the running.

Participants	**Total Running Distance (km)**	**Total Time (minutes)**	**Average Velocity Running (km·h^{-1})**
1	53.9	198.6	16.3
2	19.7	80.5	14.7
3	37.2	132.8	16.8
4	41.5	173.5	14.4
5	13.2	59.4	13.4
6	41.3	163.2	15.2
7	7.1	32.3	13.2
8	44.7	175.0	15.3
*Sum / **Mean	258,8 *	1015,4*	14.9 **
Dp			1.3

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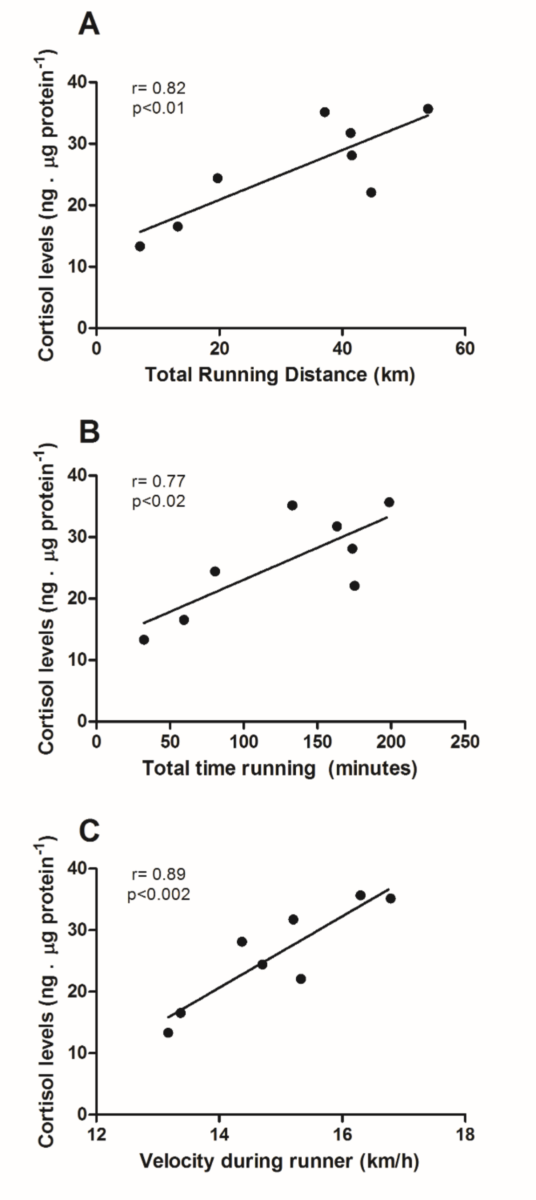

Figure 1 - Relationship between salivary cortisol levels and racing variables

DISCUSSION

Through stress conditions, the HPA axis and sympathetic adrenal-medullary activation increase, and consequently, circulatory stress hormones such as catecholamines and cortisol are released.

The purpose of the present study was to evaluate the salivary cortisol and alpha amylase levels in response to long-distance running and performance variables, as accumulated distance, total time and velocity of running, and according to the findings there is a correlation between salivary cortisol with distance and total time run suggesting that there is an endocrine response and regulation for long-distance running.

It is already well established that elite endurance athletes show tissue damage and are most likely to suffer upper respiratory tract infections during and/or after intensive training, and these alterations are able to activate innate and adaptive immunity in order to maintain health and homeostasis.

According McKune et al. (2005) ultra-endurance exercise alters immunoglobulin

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concentration by enhancing the immune response, including isotype switching, interactions with the innate immune system, and a secondary antibody response that observes an increase in subclasses and total immunoglobulin G (IgG) and decrease in immunoglobulin M (IgM). These positive regulations in immunoglobulin are associated with cortisol plasmatic concentration that is able to promote B cell immunoglobulin isotope switching from IgM to IgG.

IgG is one of the most important serum immunoglobulins, and appears during the first and second immune responses, by activating the complement system and macrophages, and by protecting the body from infections and other germ activities (Vidarsson, Dekkers and Rispens, 2014).

Djken et al. (2000) also observed that intense physical training promotes an increase in cortisol as well as in immunoglobulins, such as IgA, IgM and IgG, in active and sedentary subjects suggesting that there is a beneficial response of the immune system to the organism, mediated by cortisol, in order to maintain health, homeostasis and activity.

In study to analyze the effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men, Allgrove et al., (2008) observed modifications in salivary cortisol and α-amylase in exercise at 75%VO_{2max} and in the incremental exhaustion trial suggesting that there is an association between short-duration and high intensity exercise to salivary immunoglobulin secretion and this effect may be linked to alterations in sympathetic activity and not with an HPA axis. Interestingly, this data complemented our findings because we verified a significant increase in the cortisol after exercise and relating to training variables in runners when oxygen consumption during training is similar. However, no correlations were identified with α-amylase, and this result may be associated with the duration and effort time being considered in the present study as long-duration.

On the other hand, in recent study by Filaire et al., (2013) they observed that there is a decrease in α-amylase at waking in adolescent female tennis players after a 2-week rest (W0) and an increased awakening response and a higher α-amylase activity output after 4 months (W16) where there was an increase in load training. At the same time, the players showed an increase in awakening responses in W0 and W16, but had a low overall output of salivary cortisol and a blunted response to waking at W16. These findings suggest that increasing the training load induced asymmetry activation between the two stress systems, and in addition, amylase scores were higher at W16 than at W0 after controlling for cortisol.

In the present study, our results contribute to the consideration of the relationship between salivary cortisol with accumulated distances and total running time in athletes. However, the wide range of distances run (7-54), and variation in running time (32-198 minutes) represents a different external load, and may, least in part, limit our data. In addition, a lack of information about aerobic power, capacity, intensity and the athlete's physical fitness also limit our data. Future studies are needed to better understand the response of salivary cortisol in athletes.

In summary, we conclude that there is a positive relationship between salivary cortisol and accumulated distance and total running time in athletes, but not with salivary α-amylase, showing that the endocrine response is constantly demanded in long distance running.

Conflict of Interest

Barbara de Moura Mello Antunes, José Gerosa Neto, Camila Yuri Haraguchi, Rodrigo Xavier Neves, Helton de Sá Souza, Romulo Araujo Fernandes and Fábio Santos Lira declare that they have no conflict of interest

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ISSN 1981-9900 *versão eletrônica*

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Recevied for publication in 06/13/2016
Accepted in 10/30/2016