Original Article

# Responses of salivary cortisol and α-amylase to official competition

MOHAMMAD A. AZARBAYJANI[1], HOSEYN DALVAND[1], HOSEYN FATOLAHI[1], SEYED A. HOSEINI[2], PARVIN FARZANEGI[3], STEFAN R. STANNARD[4]

[1]*Department of Sport Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Sport Physiology, Bushehr Branch, Islamic Azad University, Bushehr, Iran*
[3]*Department of Sport Physiology, Sari Branch, Islamic Azad University,Sari, Iran*
[4]*School of Sport and Exercise, Massey University, New Zealand*

**ABSTRACT**

Azarbayjani MA, Dalvand H, Fatolahi H, Hoseini SA, Farzanegi P, Stannard SR. Responses of salivary cortisol and α-amylase to official competition. *J. Hum. Sport Exerc.* Vol. 6, No. 2, pp. 385-391, 2011. This study was designed to determine the relationship between salivary cortisol, $\alpha$-amylase and total protein response in the official football players during the course of a game. Nine young amateur football players agreed to participate in the study. Saliva samples were collected from each player 30 min and 5 min before the start of the competition, at half time, and then again 5 and 30 min after the end of competition. A significant increase in cortisol ($p=0.04$) in response to playing the competition was observed including a significantly higher concentration 30 min after match as compared to half time ($p=0.016$). In contrast, changes in salivary $\alpha$-amylase changes were irregular, but there was significant decline 5 min after end of match as compared to the 5 min before the beginning of match ($p<0.019$). No significant difference in total protein concentration was observed. Though salivary cortisol, $\alpha$-amylase and total protein changes were observed concomitantly, but there no significant relationship between them. We conclude that participation in competition has an accumulative effect on salivary cortisol concentration, but this was not related changes in salivary $\alpha$-amylase. **Key words**: ANTICIPATORY STRESS, FOOTBALL, SYMPATHETIC, ADRENOCORTICAL.

**Corresponding author.** Department of Sport Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.
E-mail: m_azarbayjani@iauctb.ac.ir
Submitted for publication January 2011
Accepted for publication May 2011
JOURNAL OF HUMAN SPORT & EXERCISE ISSN 1988-5202

**doi:10.4100/jhse.2011.62.19**

## INTRODUCTION

Recently, researchers studying stress and its physiological impact have focused on saliva bourne stress biomarkers because saliva sampling is rapid, non-invasive, and thus permits frequent sampling. The physiologic basis of salivary biomarkers to study stress is based on the control of sympathetic activity (Schneyer & Hall, 1966). The main response to stress is via activation of the hypothalamic-pituitary-adrenocortical and sympatho-adrenal axis (Chrousos & Gold, 1992; Lehmann et al., 1985) and this can be represented by the concentrations of related hormones in saliva.

Competition is well known to bring about a stress response, even prior to beginning the sporting event. The physical stress of the actual event itself is likely to compound the stress response, which can be observed; cortisol is one of such stress biomarker. Numerous studies have measured the salivary cortisol changes in response to sporting competition (Kim et al., 2009; Doen et al., 2006; Haneshi et al., 2007; Moreira et al., 2007). Increase of salivary cortisol after football training and competition has been observed, and is higher in the beginners (Haneshi et al., 2007) in contrast to amateur players (Moreira et al., 2007).

Concomitant reduction of saliva secretion and dry mouth has been reported after strenuous physical activity (Dawes, 1981). This phenomenon can not only be explained by a reduction in salivary secretion is related to total protein secretion in the mouth cavity (Schneyer & Hall, 1966; Dawes, 1981). Reports indicate that aside from total salivary protein, an increase in salivary $\alpha$-amylase could be considered as a suitable marker for sympathetic nervous system activity (Chatterton et al., 1996).

Yet, little is known about the effect of sport competition on the salivary $\alpha$-amylase concentration. One report has observed a significant increase of salivary $\alpha$-amylase concentration during triathlon competition (Steerenberg et al., 1997) and there is evidence of $\alpha$-amylase increase in competition when the variables of gender and previous experience are considered (Kivlighan & Granger, 2006).

Clearly, there is a paucity of data relating to the salivary total protein, $\alpha$-amylase, and cortisol concentrations during the course of competitive sport. This study was thus designed to investigate the salivary cortisol and $\alpha$-amylase acute response to official competition in football players experienced in competition at an amateur level.

## MATERIAL AND METHODS

*Participants*

Potential subjects (all male) were initially invited to an information session where the goals and procedures of the research were explained in detail. Nine of the players agreed to participate in this study and provided signed informed consent. At the time of study, none of these participants was under medication, had a history of behavioural, or sleeps disorders. The general features of the participants are given in the Table 1.

***Table 1.*** *The personal features of the participants.*

| Characteristics | Mean±SD |
|---|---|
| Age (year) | 24±2.79 |
| Weight (Kg) | 70.5±8.46 |
| Height (Cm) | 175.34±7.12 |
| Body mass index (Kg/m$^2$) | 22.94±2.47 |

*The football official competition*
The competition included a football match officially recognized by the Regional Amateur League, and was conducted according to the International Football Federation regulations. The match began at 2 PM, ended at 3:50 PM, and included a 15 period between halves.

*Saliva sampling*
Three milliliters of un-stimulated total saliva was collected via passive drooling, 30 and 5 minutes before, between the 2 halves, 5 and 30 min after the end of the match. The participants drank 200 ml water to guard against hydration-related dry mouth 30 min before the first sampling. After collection, the samples immediately were kept in the ice and within 2 hours frozen at -20 ºC. Subjects were asked to avoid drinking caffeine 24 hours before participation in the study and also not to eat 2 h before sampling. They were banned of undertaking any physical activity 48 h before the first sampling.

*Biochemical assay*
DIAMETRA kits assayed salivary C, and sAA. The competitive immune enzymatic colorimetric method was used for assaying the salivary C; and the kinetic colorimetric method was applied for measurement of sAA activity. The total protein was measured by Bradford method using the Comasi blue G 250 dye. ELISA reader (State Fox model 2100 Awareness, USA) detected all tests.

The sensitivity of the DIAMETRA assaying kits used for the salivary C, total protein, and sAA was 0.05 ng/ml, 1 mg/dl and 2.5 U/ml respectively.

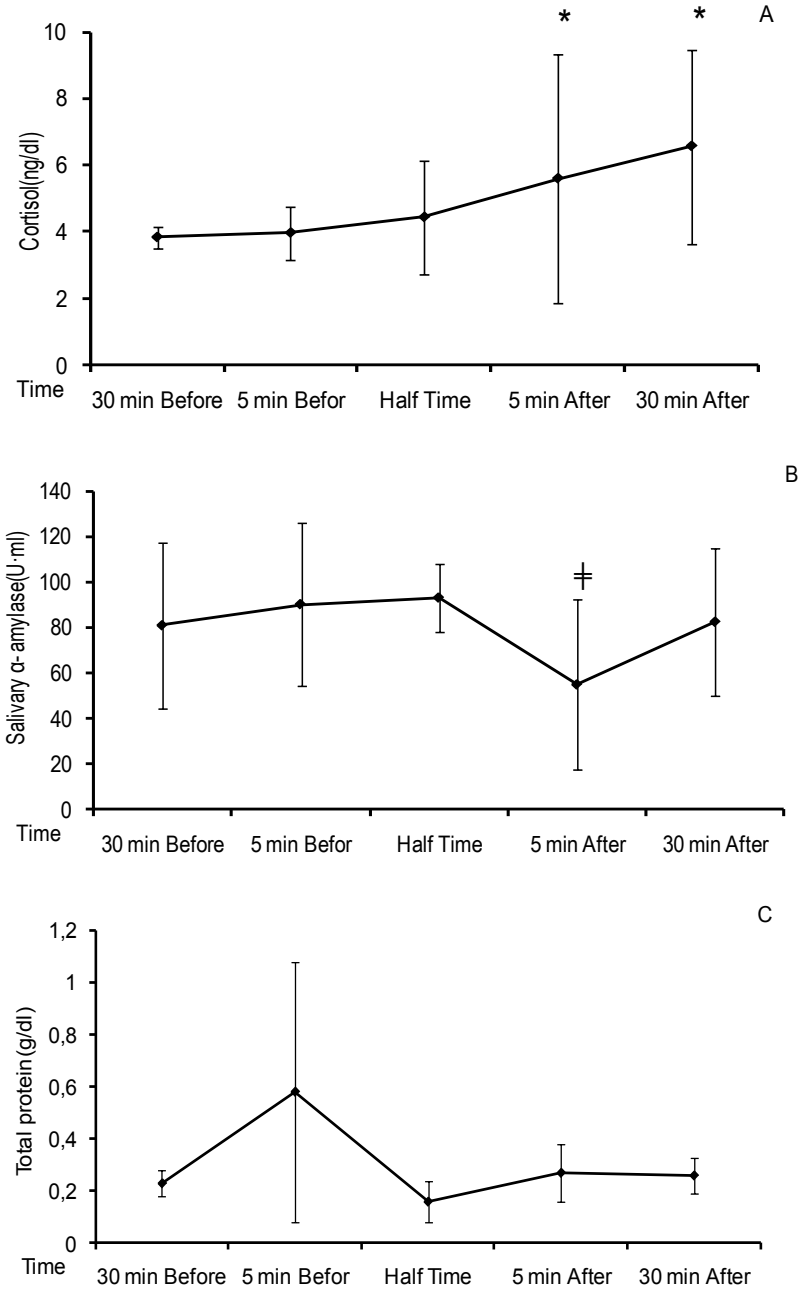
***Statistical analysis***
First, the K-S test was used for certainly about the normal distribution of the obtained data to establish the use of parametric statistical analyses. For salivary cortisol, $\alpha$-amylase and total protein data, analysis of variance with repeated measurement was used for the similar groups to investigate main and interactive effects. In case of observing significant difference, a post hoc paired *t*-test and for establishing the origin of significance for the associated groups, a modified P Bonferoni test was used.

The Pearson r coefficient was used in determining the relationship between the measured variables. The value of $p<0.05$ was considered significant for all tests.

## RESULTS

Participating at official football playing had significant effect on salivary cortisol ($F_{2,28}=4.11$, $p=0.01$) in a way that its concentration at the beginning of competition started increasing and this process continued till 30 min after the end of competition. Post hoc testing showed that cortisol concentration had a significant increase 5 min and 30 min after the end of competition compared to the level between the two half times ($p=0.016$) (Figure 1, section A). In addition, participating in the official football competition had significant effect on $\alpha$-amylase concentration ($F_{2, 28}=2.78$, $p=0.04$). Post hoc testing showed a significant reduction of $\alpha$-amylase concentration between 5 min after the end of competition and 5 min before competition ($p=0.019$), (Figure 1, section B). Analysis of variance revealed that participating in football competition has significant effect on the total protein concentration ($F_{2,28}=2.057$, $p=0.003$). After correction using the Bonferoni test, it was found that the observed difference is statistically significant (Figure 1, section C).

The results of Pearson r coefficient demonstrated that, in none of the five-time sampling, was there is significant relationship between the salivary cortisol, $\alpha$-amylase and total protein concentrations.




**Significant difference compared to the level between the two halves.*
*ǂ Significant difference compared to the levels 5 min before the beginning of competition.*

***Figure 1.*** *Cortisol (Section A), $\alpha$-amylase (Section B) and Total protein (Section C) response to official competition.*

## DISCUSSION

*The salivary cortisol*

In the present study, we have shown that the salivary cortisol concentration in response to competition increases in an incremental fashion such that it reached to the highest level 30 minutes after the cessation of the event. This dynamic response to competition is in agreement with the data obtained about the cortisol changes in the competitive motorcycle riders (Filaire et al., 2007). However, changes in this biomarker did not correspond to changes in either total salivary protein or $\alpha$-amylase concentrations.

The reason for the increase in cortisol could be explained thus: Previous studies (Filaire et al., 2007) suggest that excitement prior to and during competition affects the physiological HPA axis resulting in an increase of cortisol secretion (Filaire et al., 2007).

It has been confirmed that cortisol response to physical activity is depended to the intensity (Port, 1991) and duration of activity (Snegovskaya & Viru, 1993). In past, the threshold of physical activity which causes cortisol secretion has been determined to require 50% to 60% of maximal oxygen uptake, but a recent report indicates about 85% of maximal oxygen uptake is necessary (Butki et al., 2001). However, longer duration low intensity activity can produce an increase of cortisol concentration (Brandenberger & Follenius, 1975). On this basis, increase of cortisol following a football competition is no unexpected in this study. That Cortisol concentration increased even thought the game had finished is likely due to the duration and intensity of the physical activity on the cortisol concentration, however it might also be explained by a post exercise lowering of blood glucose as no food was administered after the match (Hawley et al., 1997).

*Salivary $\alpha$-amylase*

The effect of cathecolamins on saliva secretion is such that the use of beta blockers significantly reduces the total protein and $\alpha$-amylase concentration (Nederfors & Dahlöf, 1992). In contrast to our data, others have shown a significant increase of amylase after competition by frequent sampling method (Calvo et al., 1997). Bicycling at an intensity of 60% oxygen uptake leads to stimulation of $\alpha$-amylase (Bishop et al., 2000) and $\alpha$-amylase responses to 20 min training with intensity of 50% oxygen uptake has occurred (Allgrove et al., 2008). The catecholalmine response to exercise is more rapid and acute than that of cortisol, so this may explain the dissonance between cortisol and amylase responses. To enable a better understanding of this relationship, further studies with tight control on intensity and duration are required. Nevertheless, it is likely that the decrease in $\alpha$-amylase following cessation of exercise may be due to rapid reductions in the catecholamines.

An interesting question is why $\alpha$-amylase did not show sudden reduction between two half times. It is possible that an inability to relax during the half time break on top of the fact that the players are in fatigued condition, means that the secondary stressors and cause of sympathetic activity stimulation persist. In contrast, at the end of competition the players are in full recovery mode.

## CONCLUSIONS

Results of this study indicated that effect of competition on cortisol is cumulative and its concentration increases by time. It also indicates that salivary cortisol and $\alpha$-amylase changes are independent to each other.

## REFERENCES


1. 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(6):653-61. doi:10.1080/02640410701716790 [Back to text]
2. BISHOP NC, BLANNIN AK, ARMSTRONG E, RICKMAN M, GLEESON M. Carbohydrate and fluid intake affect the saliva flow rate and Iga response to cycling. *Med Sci Sports Exerc*. 2000; 32(12):2046-51. [Abstract] [Back to text]
3. BRANDENBERGER G, FOLLENIUS M. Influence of timing and intensity of muscular exercise on temporal pattern of plasma cortisol levels. *J Clin Endocrinol Metab*. 1975; 40(5):845-9. doi:10.1210/jcem-40-5-845 [Back to text]
4. BUTKI BD , RUDOLPH DL , JACOBSEN H . Self-efficacy, state anxiety, and cortisol responses to treadmill running . *Percept Mot Skills*. 2001; 92(3 Pt 2):1129-38. [Abstract] [Back to text]
5. CALVO F, CHICHARRO JL, BANDRES F, LUCIA A, PEREZ M, ALVAREZ J, MOJARES LL, VAQUERO AF, LEGIDO JC. Anaerobic threshold determination with analysis of salivary amylase. *Can J Appl Physiol*. 1997; 22(6):553-61. [Abstract] [Back to text]
6. CHATTERTON RT JR, VOGELSONG KM, LU YC, ELLMAN AB, HUDGENS GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol*. 1996; 16(4):433-48. doi:10.1111/j.1475-097X.1996.tb00731.x [Back to text]
7. CHROUSOS GP, GOLD PW. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. *JAMA.* 1992; 267(9):1244-52. [Abstract] [Back to text]
8. DAWES C. The effects of exercise on protein and electrolyte secretion in parotid saliva. *J Physiol.* 1981; 320:139-48. [Abstract] [Back to text]
9. DOAN BK, NEWTON RU, KRAEMER WJ, KWON YH, SCHEET TP. Salivary Cortisol, Testosterone, and T/C Ratio Responses during a 36-hole Golf Competition. *Int J Sports Med.* 2007; 28(6):470-9. [Abstract] [Back to text]
10. FILAIRE E, FILAIRE M, LE SCANFF C. Salivary cortisol, heart rate and blood lactate during a qualifying trial and official race in motorcycling competition. *J Sports Med Phys Fitness.* 2007; 47(4):413-7. [Abstract] [Back to text]
11. HANEISHI K, FRY AC, MOORE CA, SCHILLING BK, LI Y, FRY MD. Cortisol and stress responses during a game and practice in female collegiate soccer players. *J Strength Cond Res*. 2007; 21(2):583-8. [Abstract] [Back to text]
12. HAWLEY JA, SCHABORT EJ, NOAKES TD, DENNIS SC. Carbohydrate-loading and exercise performance. An update. *Sports Med.* 1997; 24(2):73-81. [Abstract] [Back to text]
13. KIM KJ, CHUNG JW, PARK S, SHIN JT. Psychophysiological Stress Response during Competition between Elite and Non-elite Korean Junior Golfers. *Int J Sports Med*. 2009; 30(7):503-8. doi:10.1055/s-0029-1202338 [Back to text]
14. KIVLIGHAN KT, GRANGER DA. Salivary a-amylase response to competition: Relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology*. 2006; 31(6):703-14. doi:10.1016/j.psyneuen.2006.01.007 [Back to text]
15. LEHMANN M, SCHMID P, KEUL J. Plasma catecholamines and blood lactate accumulation during incremental exhaustive exercise. *Int J Sports Med*. 1985; 6(2):78-81. [Back to text]
16. MOREIRA A, ARSATI F, DE OLIVEIRA LIMA ARSATI YB, DA SILVA DA, DE ARAÚJO VC. Salivary cortisol in top-level professional soccer players. *Eur J Appl Physiol*. 2009; 106(1):25-30. doi:10.1007/s00421-009-0984-y [Back to text]

17. NEDERFORS T, DAHLÖF C. Effects of the beta-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. *Arch Oral Biol*. 1992; 37: 579-584. doi:10.1016/0003-9969(92)90141-T [Back to text]
18. PORT K. Serum and saliva cortisol responses and blood lactate accumulation during incremental exercise testing. *Int J Sports Med*. 1991; 12(5):490-4. [Abstract] [Back to text]
19. SCHNEYER CA, HALL HD. Autonomic pathways involved in sympathetic-like action of pilocarpine on salivary composition. *Proc Soc Exp Biol Med*. 1966; 121(1):96-100. [Back to text]
20. SNEGOVSKAYA V & VIRU A. Steroid and pituitary hormone response to rowing exercise: Relative significance of exercise intensity and duration and performance. *Eur J Appl Physiol Occup Physiol*. 1993; 67(1):59-65. doi:10.1007/BF00377706 [Back to text]
21. STEERENBERG PA, VAN ASPEREN IA, VAN NIEUW AMERONGEN A, BIEWENGA A, MOL D, MEDEMA GJ. Salivary levels of immunoglobulin A in triathletes. *Eur J Oral Sci.* 1997; 105(4):305-9. [Abstract] [Back to text]