Effects of acute exercise on salivary free insulin-like growth factor 1 and interleukin 10 in sportsmen.

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Abstract

Background: Saliva analysis is rapidly developing as a tool for the assessment of biomarkers of sports training. It remains poorly understood whether a short bout of sport training can alter some salivary immune biomarkers.

Aim: To investigate the effect of acute exercise using football training session on salivary flow rate, salivary free Insulin-like Growth Factor-1 (IGF-1) and Interleukin 10 (IL-10).

Methods: Saliva samples were collected before and immediately after a football session. Salivary flow rates, salivary levels of free IGF-1 and IL-10 (using ELISA) were determined. Data was analyzed and compared using Related Samples Wilcoxon Signed Rank test (non-parametric test). Relationships between salivary flow rate and levels of free IGF-1 and IL-10 were determined using Spearman correlation test.

Results: There were 22 male footballers with a mean age of 20.46 years. Salivary flow rate reduced significantly (p = 0.01) after the training session while salivary levels of free IGF-1 and IL-10 did not show any significant change. Also, there were no correlations between salivary flow rates and salivary levels of free IGF-1 and IL-10 before and after exercise.

Conclusion: These findings suggest that acute exercise caused significant reduction in salivary flow rate but no change in the levels of salivary free IGF-1 and IL-10.

Keywords: Saliva, exercise, Insulin-like Growth Factor, interleukin 10, salivary flow rate

DOI: http://dx.doi.org/10.4314/ahs.v16i2.25

Cite as: Lasisi TJ, Adeniyi AF. Effects of acute exercise on salivary free insulin-like growth factor 1 and interleukin 10 in Sportsmen. Afri Health Sci 2016;16(2): 560-566. http://dx.doi.org/10.4314/ahs.v16i2.25

Background

Saliva analysis is rapidly developing as a tool for the assessment of physiological biomarkers of oral and systemic health.¹,² The use of saliva for monitoring hormones and immune markers in sport and exercise has made saliva sampling and analysis very attractive to several researchers.² Saliva can provide a useful, non-invasive alternative to the collection of blood because it can be collected rapidly, repeatedly and without painful invasion.¹ In addition, the collection requires minimal training and can be performed on the sports field involving many athletes. Therefore, the use of saliva is becoming more popular and fetching more applications in the field of sport and exercise science.

In the past, collection of blood was used for the detection and assessment of hormonal and immune markers following exercise.³⁴ However, several recent studies have also utilized saliva samples to assess the levels of these compounds in response to exercise and training.⁶⁻⁸ Previous studies have shown that acute and chronic exercise sessions elicited changes in the levels of hormones (such as cortisol and testosterone), immunological compounds (such as immunoglobulin A (IgA)) and cytokines (such as tumor necrosis factor (TNF)) in saliva from athletes.⁹,¹⁰ While some studies¹¹,¹² concluded that acute strenuous exercise causes reduced concentrations of salivary immune biomarkers and can be associated with a high incidence of upper respiratory tract infections, others⁸,¹³,¹⁴ reported that moderate exercise enhances immune function. As-

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Assessment of salivary immune biomarkers can indicate the effect of sports training on immune response and subsequently influence or indicate the risk of oral and respiratory infections in sportsmen and sportswomen.

Although a large number of research findings have been amassed, there is still need for a detailed understanding of the variations in immune changes observed following sports training and or exercise. One of the important areas of concern has been the impact of sporting activities on salivary biomarkers of oral and respiratory tract immunity. This is motivated by the observation of a link between exercise-induced immune suppression and common illnesses, particularly upper respiratory tract infections (URTI) and poor oral health. Insulin-like growth factor 1 (IGF-1) is a growth hormone-dependent growth factor found in its highest concentrations in plasma. It has also been reported to be produced locally from the salivary glands with the free forms measurable in saliva. Generally, Insulin-like growth factors (IGFs) are peptide hormones that play a pivotal role in growth, and cell division. Hence salivary IGF-1 may be implicated in the regeneration of oral tissues and wound healing. Similarly, Interleukin-10 (IL-10) is an important immune-regulatory cytokine produced by many cell populations. Its main physiological function appears to be the restriction and cessation of inflammatory responses. It is also involved in the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killer cells, antigen-presenting cells, mast cells, and granulocytes. Thus salivary free IGF-1 and IL-10 can serve as biomarkers of oral mucosal immunity as well as oral health status. In addition, changes in the levels of salivary free IGF-1 and IL-10 following acute moderate exercise in athletes could indicate role of these markers on the oral health of these individuals. Similarly it might indicate acute moderate exercise as one of the physiological factors that affect levels of salivary free IGF-1 and IL-10.

Despite a substantial number of studies on mucosal immunity and sporting activities, the unanswered research questions still include defining how acute exercise influences the immune response especially in saliva. This study examined the effect of acute moderate exercise on some salivary biomarkers of oral immunity in sportsmen. The objectives were to assess the effects of acute moderate exercise in form of football training session on salivary flow rate, levels of free IGF-1 and IL-10 among known sportsmen; and to determine the relationship between salivary flow rate and levels of free IGF-1 and IL-10.

Methods
Study design and participants:
This was a cohort study in which 22 apparently healthy male football players were included. This study was conducted according to international and ethical standards in sports and exercise research. The participants were football players selected from a pool that serves as a feeder team for a major football club which is in the division one professional league category in the country. All of the participants had been playing football consistently for a minimum of 6 months without any adverse health situations. Before the start of the study, the participants, coaches and clinical personnel were informed about the purpose and design of the study. All participants also signed informed consent. Bio-data of the participants was taken using a self-administered proforma. Ethical approval was obtained from the Ethics Committee of the Institution.

Saliva collection and analysis: All participants had oral examination done by one of the investigators. Participants with history of smoking and drug use were excluded. Pre-training whole saliva samples were collected before the commencement of the session and after the training exercise of 60 minutes. The training session was divided into two halves of 30 minutes each with a rest period of 5 minutes between the halves. The training session took place in the morning hours between 7:00am and 9:00am at an ambient temperature of about 25°C. Participants rinsed their mouth with clean water before baseline data collection. Unstimulated whole saliva samples were collected by expectoration into sterile tubes before and after the football training session and centrifuged at 1,000 rpm for 2 min. The supernatants were separated and stored at -20°C until use. The levels of free IGF-1 and IL-10 in undiluted saliva were determined by enzyme immunoassay kits (CLOUD-Clone Corp, USA and Diaclone SAS, France respectively). Following manufacturers’ instructions, the samples were thawed in a refrigerator for 18 hours and then centrifuged at 1,000 rpm for 2 min. The supernatants were separated and stored at -20°C until use. The levels of free IGF-1 and IL-10 in undiluted saliva were determined by enzyme immunoassay kits (CLOUD-Clone Corp, USA and Diaclone SAS, France respectively). Following manufacturers’ instructions, the samples were thawed in a refrigerator for 18 hours and then centrifuged at 8000 rpm for 15 minutes. Each test sample was diluted into 1/10,000. Then, 100 μL standard or sample was added to each well and incubated
for 2 hours at 37°C. Contents of the wells were aspirated and 100 uL prepared Detection Reagent A was added to each well and incubated for 1 hour at 37°C. The contents were aspirated and the wells were washed 3 times. Then, 100uL prepared Detection Reagent B was added to each well and incubated for 30 minutes at 37°C. Contents of the wells were aspirated and washed 5 times. Then, 90 uL substrate solution was added to each well and incubated for 20 minutes at 37°C. 50uL Stop Solution was added to each well and absorbance was read at 450nm immediately.

**Data analysis:** Physical characteristics of the participants are presented as mean ± SD while data on salivary flow rate, levels of free IGF-1 and IL-10 are presented as median with quartiles. Pre and post exercise data were compared using Related Samples Wilcoxon Signed Rank test (non-parametric test). Relationships between salivary flow rate and levels of free IGF-1 and IL-10 were determined using Spearman correlation test. P value of less than 0.05 was considered statistically significant.

**Results**

There were 22 male participants with a mean age of 20.46 ± 4.74 years (range: 15-30 years). Their mean Body Mass Index was 24.24 ± 1.98 kg/m2. The physical characteristics of the participants are shown in table 1.

| Variable     | Range    | Mean ± SD  |
|--------------|----------|------------|
| Age (years)  | 15-30    | 20.46 ± 4.74 |
| Height (m)   | 1.5-1.82 | 1.62 ± 0.08  |
| Weight (Kg)  | 50-80    | 63.55 ± 8.71 |
| BMI (kg/m²)  | 20.03-28.66 | 24.24 ± 1.98 |

Median salivary flow rates of the participants before and after the exercise were 1.00 ml/min and 0.71 ml/min respectively. Salivary flow rate was significantly reduced in the participants after 60 minutes of football training (p = 0.01). Median salivary free IGF-1 levels before and after the exercise were 3.44 µg/l and 3.28 µg/l respectively while median levels of salivary IL-10 before and after the exercise were 16.97 µg/l and 12.26 µg/l respectively (Table 2).

**Table 1: Physical characteristics of participants (n = 22)**

**Table 2: Salivary flow rates, levels of salivary IGF-1 and IL-10 before and after exercise**

| Parameter   | Before Exercise | After Exercise | P value |
|-------------|-----------------|---------------|---------|
| Flow rate (mls/min) | 1.00 (1.00) | 0.71 (0.5) | 0.01 |
|              | Range: 0.5 – 1.67 | Range: 0.39 – 1.67 |       |
| IGF-1 (µg/l) | 3.44 (3.18) | 3.28 (3.10) | 0.39 |
|              | Range:3.02 – 4.02 | Range: 2.8 – 4.22 |       |
| IL-10 (µg/l) | 16.97 (14.11) | 12.26 (10.83) | 0.29 |
|              | Range: 6.26 – 36.26 | Range: 7.4 – 35.11 |       |

Note: Data are presented as median (quartile).
There was no significant difference in the levels of salivary free IGF-1 and IL-10 before and after 60 minutes exercise in form of football training (p = 0.39 and 0.29 respectively). Also there were no significant correlations between salivary flow rates and salivary levels of free IGF-1 (p = 0.70) and IL-10 (P = 0.25) before and after exercise as shown in (Figures 1 and 2 respectively).

Figure 1: Correlation between salivary flow rate and level of salivary IGF-1 before and after exercise (n = 44)
Discussion

The main finding of this study was that acute moderate exercise significantly decreased salivary flow rate in male sportsmen but not on the salivary levels of free IGF-1 and IL-10. The reduced salivary flow rate observed in this study is similar to the reports from earlier workers\(^{18,19}\) but contrary to a study by Nasari et al.\(^{20}\) which reported no change in salivary flow rate following acute exercise. The differences in the findings could be explained by the variations in exercise protocols and populations studied. Nasari et al.\(^{20}\) included non-athlete males that performed treadmill exercise until exhaustion without indication of the duration. Typically, exercise results in dehydration which could have contributed to the reduced salivary flow rate observed in this study. The reduced salivary flow rate following acute exercise in form of football training observed in this study could predispose athletes to oral and upper respiratory tract infections. Several epidemiological studies\(^{21-23}\) have reported increased prevalence or risk of upper respiratory tract infections in athletes especially in those engaged in heavy training. Similarly, studies\(^{24-26}\) have shown poor oral health and high prevalence of oral diseases in athletes with a resulting substantial negative impact on their well-being, training and performance.

The results of this study suggested that acute moderate exercise might not affect the levels of salivary free IGF-1. Similarly, Rosa et al.\(^{27}\) reported that moderate acute exercise for a period of 60 minutes did not induce changes in salivary transforming Growth Factor (TGF-β) and interleukin 5 (IL-5) immediately after physical exercise. In contrast, Antonelli et al.\(^{28}\) reported that the concentrations of salivary free IGF-1 increased after an acute physical exercise test. Their study involved well trained male cyclists that performed ramp tests on cycle-ergometers until exhaustion. The differences in the findings may be explained by various factors including type, duration, intensity and condition of the exercise as well as saliva variables like sampling technique and analytical methods.
Exercise is a physical stressor that may affect sequence of events in immune response under different mechanisms. The immune response to any challenge is complex involving coordinated activity by many different types of cells and molecules in the body system as well as biologic fluids. Therefore, the magnitude and change of any immune parameters will depend on exercise dose, (duration and intensity) and subjects fitness level.

The results of the present study also demonstrated that acute moderate exercise of 60 minutes session of football training did not affect levels of salivary IL-10. To the best of our knowledge, no previous study has assessed effect of sport training sessions on salivary IL-10 making no data available for comparison. Most studies assessing factors influencing susceptibility to upper respiratory tract infections (URTI) in athletes involving IL-10 used blood samples. Gleeson et al. reported a high IL-10 concentration in the blood samples from 18-35 years old men and women engaged in endurance-based physical activity during the winter months in response to antigen challenge as well as low salivary immunoglobulin A (S-IgA) secretion as risk factors for development of URTI in physically active individuals.

The lack of correlation between salivary flow rates and salivary levels of IGF-1 as well as IL-10 may suggest that many other factors could affect levels of salivary IGF-1 and IL-10 that are not associated with salivary flow rate. Therefore levels of salivary IGF-1 well as IL-10 may be independent of salivary flow rate at least within the context of this study.

The present study is a cross sectional study with its limitations. Whole saliva was collected which may not reflect a true gland specific flow rate. However, the collection of whole saliva by passive drool is the most reliable option in humans. An obvious advantage of the passive drool method is the estimation of salivary flow rate by timed collection of saliva. In addition, whole saliva is what represents the physiologic fluid in terms of its function in the oral cavity. Another limitation is the lack of a control trial such as non-athletes to offer further insight into the area.

Conclusion
Acute moderate exercise in sportsmen resulted in reduced salivary flow rate while levels of salivary free IGF-1 and IL-10 were not affected. However, further studies including a control trial such as non-athletes and other measures involving some other forms of exercise to substantiate the findings as well as to offer further insight into the area are needed.

Conflict of interest
None declared.

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