Changes in Salivary Immunoglobulin A (IgA) following Match-play and Training among English Premiership Footballers

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SUMMARY

Decreased salivary immunoglobulin A (sIgA), a component of mucosal immunity, is associated with intensive physical activity: suggesting that sIgA may be used for the monitoring of mucosal immunity with footballers. We investigated changes in sIgA in elite footballers, in response to training and match-play. There was a decrease in sIgA following training, with a return to pre-training levels after 18 hours of rest. This return to resting levels was not observed following competitive match-play. Overnight rest was sufficient for mucosal IgA recovery following training but not following two successive matches, suggesting that sIgA may be used to monitor training in multi-sprint sports.

INTRODUCTION

The risk of upper respiratory tract infections (URTI) has been observed to be greater in endurance athletes, who engage in prolonged, heavy exercise, than in the general population. Reviewers of relevant research findings have concluded that transient suppression of several innate and adaptive immune functions account for endurance athletes' increased susceptibility to URTI [1-4]. In several studies, heavy, prolonged exercise has been found to be associated with decreased salivary IgA levels [5-9]. Also, decreased salivary IgA levels have been observed to increase the risk of infections [10].

Thus far, the association between exercise and immunity has been studied more extensively among endurance athletes [11-14] than in non-endurance team sports participants [4]. However, evidence is now emerging that non-endurance sports, involving heavy physical exertion for brief periods, are also associated with transiently decreased salivary IgA levels in participants. For example, in 'American football' players, intensive exercise training has been shown to reduce salivary IgA concentration and secretion rate [15]. It is likely that the salivary IgA levels may be comparably affected by heavy physical exertion that football (soccer) players experience during training and match-play. However, data on the association between physical exercise and salivary IgA levels in footballers is very limited.

Although football is considered a multi-sprint sport rather than an endurance exercise, the modern game is becoming increasingly more physically demanding in terms of athletic stamina. During a 90-minute game, elite footballers on the average cover distances of 10 to 12 km [16], although this distance varies with the players' positions in defence, midfield, or attack. During the game, physical exercise is carried out at intensities of 80% to 90% of maximum heart rate and 70% to 80% of maximum oxygen uptake [17]. Due to the nature of the game, footballers expend energy in bursts for performing physical tasks such as jumping, kicking, tackling, turning, throwing, changing pace, and sustaining forceful contractions to maintain balance and control of the ball [18].

We hypothesized that the heavy physical exercise associated with professional footballers' training and match-play would transiently reduce their salivary IgA levels. To test this hypothesis we studied the salivary IgA/total protein ratio before and after intense physical exertion in footballers of two teams. Each team was studied in one of two different physical exertion scenarios commonly faced by English Premier League (EPL) clubs. These scenarios were: (a) a demanding pre-season training session, and (b) two consecutive league matches played within a period of approximately 48 hours.

MATERIALS AND METHODS

Participants

Two different EPL teams, team-A and team-B, were studied in order to examine the influence of physical activity on salivary IgA/protein ratio. Team-A; participated in a study examining a pre-season training session and team-B participated in a match-play study. For the training session study, 24 players were selected from a squad not confined to regular first team players. Their median (range) age was 26 (18-39) years. This cohort was comprised of 16 players who were regularly selected for international duty, and a significant proportion of these had recently participated in a major Federation Internationale de Football Association (FIFA) international tournament in the month of June, two months earlier. Nine footballers participated in the match-play study; their median (range) age was 25 (22-36) years. Participants were regular first team players from all outfield play positions and the goal keeper. All participants (both studies) were of typical height and weight for EPL footballers and all were free from concurrent or recent (within that last month) upper respiratory infections or allergies based on self reporting.

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Influence of Training
The effect of the first training session, following the summer off-season break, on salivary IgA/protein ratio was studied in a successful EPL club was used to study. Samples were collected on the morning prior to training session, 20 minutes after session and the following morning at 08:00, this was 18 hours after the training session. The training session took place in the first week of August one week before the start of the football season. Sessions consisted of football specific training activities typical of pre-season training.

Influence of match play
The affect of playing two football matches within a relatively short period on salivary IgA/protein ratio was studied with a struggling EPL football team. The matches were played at a time approximately 75% of the way through the football season. The team was 19th, out of 20 teams, in the league (relegation position) and the two games were played against the two teams occupying the first and second positions within the league.

The study team played two consecutive EPL matches within a period of 53 hours. The first fixture was at home on a Sunday afternoon at 15:00, the second was on the following Tuesday evening at 19.45. This second game was played at an away venue, 200 kilometres from the home ground of the studied team.

During the course of the home-game, all nine players played for at least 60 minutes from kick-off, whilst three of the nine players were substituted at times 63, 74 and 82 minutes into the game. All nine players were in the starting line up for the away-game, and all played for at least 50 minutes. Three players were substituted at times 54, 55 and 68 minutes. The home game was recorded using the Prozone (Prozone, Leeds, UK) match analysis system. This technology was used to record data on distances travelled on the pitch and running velocities of each player.

Saliva samples were collected from players at four time points: sample one 20 minutes after the home game, sample two 08:00 the following morning, sample three 20 minutes after the away game, and sample four 08:00 the following morning. These timings are summarised in Table II.

Sample analysis
Saliva samples were collected using Omni-Sal (SDS Inc, Delaware, US) collection devices, according to the manufacturer's simple instructions that accompany each device. This device employs an absorbent pad that is placed under the tongue. The pad is attached to a thin plastic holder, resembling a lollipop stick, with a built-in saturation indicator. All subjects were shown how to use the devices by demonstration and all devices were packaged with instructions for use.

Total protein was assayed using the Bradford method and IgA was assayed using an immunoturbidometric assay (DiaSorin, Wokingham, UK), performed on a Cobas Mira autoanalyser (Roche, Basal, Switzerland). Both assays gave within-assay imprecision of less than 5% and between-assay imprecision of less than 7%. Amounts of IgA in saliva were normalised to protein content, and results are expressed as mg IgA per g of protein. Non-parametric statistical tests were employed as we could not reliably test for normality for each sample group based on the relatively low sample number (n=9) in each group. Statistical comparisons of IgA/protein ratio between time-points were performed using a Wilcoxon signed rank test. Minitab®, version 14 (Minitab Ltd, Coventry, UK), statistical software was used for all statistical computations. A p-value of <0.05 was considered significant.

RESULTS

Influence of Training
There was a significant difference in salivary IgA/protein ratio across the pre, post 20-minutes and post 18 hour periods (p=0.005). The median (range) IgA/protein ratios for the pre, post 20-minutes and post 18 hour time periods were 126.6 (75.3 - 208.7), 82.5 (38.2 - 244.8) and 116.7 (55.3 - 195.8) respectively. These data are summarised in Figure 1.

There was a significant difference in salivary IgA/protein ratios between the pre training and the post 20 minute samples (p=0.002). By contrast there was clearly no significant difference observed between the pre-training group and the post 18 hours group (p=0.122). There was also no statistically significant difference between the post training session samples 20-minutes and 18-hours, (p=0.059). Although this may be considered statistically borderline, the statistical power for this comparison was 80% to detect differences of 11 µg/g, IgA per protein.

Influence of match play
The home fixture resulted in a score-draw, whilst the away game resulted in a defeat. Based on objective match analysis software each player covered distances typical 19, of elite football match play. The median (range) distance covered by players (outfield only) for the first half of the home game was 5.3 (5.1 – 6.4) km, and after 90 minutes, for the five outfield players that played to the final whistle, the median (range) distance covered was 10.8 (9.6 – 12.3) km. This was as recoded and calculated by Prozone.

The median (range) IgA/protein ratio was 129.3 (54.3 - 221.1) for samples collected 20 minutes after the home game and 130.4 (86 - 234.8) mg/g for samples collected the following day (home post 15 hours). There was no significant difference between the home post 20 minutes and the home post 15 hour samples. Similarly there was no significant difference between the 20-minutes and 10 hours post away game samples. The median (range) IgA/protein ratio for 20 minutes post away game was 66.0 (23.7 - 98.1) and 92.9 (22.6 - 129.4) mg/g 10 hours post away game. The difference between the post home game samples and post away game samples was highly significant ($p < 0.0001$). Match play salivary IgA results are summarised in Figure 2.

DISCUSSION

We investigated the relationship between salivary IgA (IgA/protein ratio) and football training and match-play involving elite-level footballers. IgA to total protein (a component of saliva that is constitutively secreted) ratio was

Table I: Summary of study participants drawn from the two English Premier League football teams, (A) and (B), participating in the two studies to investigate the influence of training and match-play on salivary IgA

Team	Study	Number of participants	Age range
A	Training session	24	18-39 years
B	Match-play	9	22-36 years

Table II: Saliva sample collection times in relation to the two English Premier League (EPL) games played within a period of just over two days (53 hours kick-off to kick-off). Sample collection times are relative to final whistle.

Game	Kick-off	Sample	Time of collection
Home	Sunday 15:00	Post home game 20-minutes	20 minutes after final whistle
		Post home game 15-hours	08:00 on the morning (Monday) following the home game
Away	Tuesday 19:45	Post away game 20-minutes	20 minutes after final whistle
		Post away game 10-hours	08:00 on the morning (Wednesday) following the away game

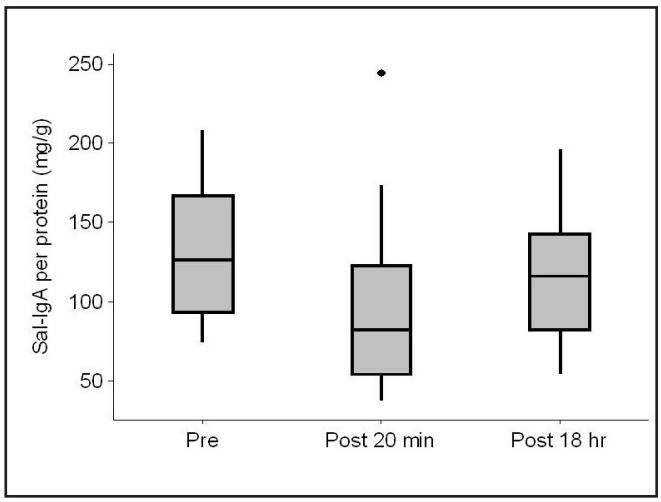

Fig. 1: Median, inter quartile range [box], and range [whisker] for salivary immunoglobulin A to protein ratio (Sal-IgA). Single dot represents outlier. Samples were collected from footballers of a single EPL squad (n=24) at periods before (Pre) and after (post 20 min, and post 12 hr) a football specific training session.

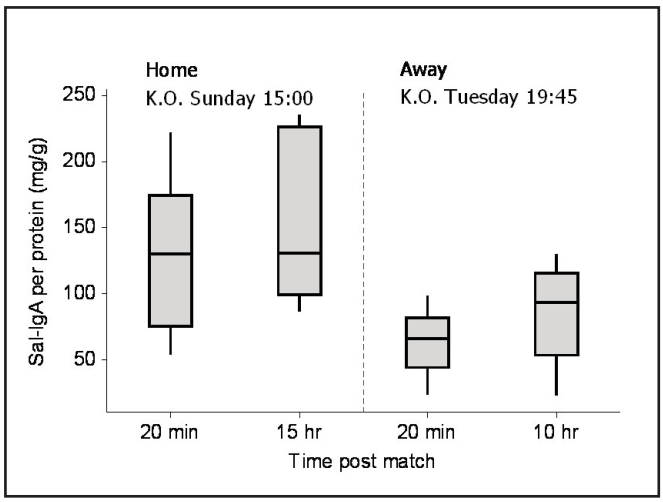

Fig. 2: Median, inter quartile range [box], and range [whisker] for salivary immunoglobulin A to protein ratio (Sal-IgA). Samples were collected from footballers from first team of one club (n=9) at periods after a home game (played on a Sunday afternoon) and an away game (played on a Tuesday evening). KO (kick off) is the start time for each match.

here considered to be a reflection of the salivary content of IgA. There was an initial change in salivary IgA in response to the demanding training session; with a fall in salivary IgA between the pre and post 20 minutes periods, which returned to pre-training values following overnight rest. Our match-play investigation, involving two successive competitive football matches, showed a significant fall in salivary IgA following the second match, as compared to pre-match values for this match.

The two matches studied were played within a short period of just over 48 hours of one another. Based on physiological stresses on professional footballers it has been suggested that greater attention should be placed on organisers to minimise, or avoid, congested schedules and to allow time for recovery (72 hours) following competitive games 20. In our study two matches were played against opposition of the highest standard (first and second in the league), and under considerable pressures to perform based on league position of the team under investigation, which was nineteenth out of [20] positions in the league table. The timing of the game within the course of the season was also critical, as it was within this last quarter of the season. This is the time when relegation positions are confirmed. Conversely, the overall league winner and the various runners-up positions are finalised within this period. There are significant economic rewards or penalties for teams occupying either relegation or "European" positions at the end of a season. These end-of-season positions potentially influence the market value of individual players. The intensity of physical exertion, and the various

pressures (professional and financial) associated with a competitive match cannot be recreated on the training field, regardless of how physically demanding a training session may be. We suggest that the observed changes in salivary IgA reflect this difference between match-play and training. The immediate fall in salivary IgA was not sustained, and overnight rest (18 hours) was enough to restore salivary IgA to pre-training salivary IgA levels. This was in contrast to overnight rest following two competitive matches. The 10 hours of rest following the second game (the away game with the evening kick off) did not seem sufficient for salivary IgA to return to the pre-match levels.

Variations in testosterone and cortisol in saliva have been studied in relation to team sport activity, including football [21-26]. Cortisol, in particular, has been related to psychological stress associated with competitive sport [27-29]. However, we were unable to measure cortisol or testosterone. This was a deficiency in our study, as biochemical markers of stress may have contributed to quantitatively assessing stress responses to the relatively rare sporting scenario described, i.e. considering league position of teams involved and timing of the games.

This study demonstrated changes in salivary IgA among elite sportsmen; training and competing at the highest level. Unfortunately, the number of participants was relatively low; a limitation of the investigation. This is a reflection of the nature of elite football and the relative scarcity of players at this level. However, further studies are necessary, looking at other football clubs within this stratum of the sport, in order to confirm our findings. Caution should be taken when interpreting these changes in salivary IgA in the light of non-elite sports persons. The effects of excessive exercise on salivary IgA may not necessarily follow the pattern, described here, when conducted in the general population. Our results highlight the need for further studies, with participants from non-elite sportsmen populations, in order to assess the effects of high levels of physical activity on mucosal immunity.

CONCLUSION

Overnight rest was enough to reverse a decrease in salivary IgA observed following a soccer specific training session, but not following two consecutive matches played within a short period. These changes in salivary IgA reflect the expected pattern of pressures and stresses associated with training and match-play. This supports the notion that salivary IgA, an established marker of overtraining in endurance sports, may also be used to monitor training within the context of football and other similar sports involving heavy physical excursion for brief periods.

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