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# **Salivary response to football matches**

**A comparative study of elite youth football players**

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### **Abstract**

An important part of the football coaching staffs' responsibilities is to keep the players healthy. In order to succeed with this, training sessions should be planned in a manner where enough recovery is achieved. Periods where matches are played more frequently have been known to elicit higher risk for injuries and illness because of the limited recovery time. Fatigue monitoring can help coaches assess the player's physical state and there are a few different methods available. A comparative study was done to compare the response of eight male elite youth players salivary Immunoglobulin A (s-IgA) and alpha-amylase after having played one match, and two matches with 72 hours in between. The results showed no statistically significant changes in s-IgA but tendencies towards lower levels after both one and two matches. A statistically significant increase was seen in alpha-amylase 24 hours post two matches ( $p = 0,012$ ) and tendency towards higher levels after one game. Some changes and tendencies could be found but future research should use bigger sample sizes in order to make broader conclusions. The research should also include match data such as high intensity running and total distance covered to help interpret results better.

## **Sammanfattning**

En viktig del i fotbollscoachers arbete är att hålla spelarna friska. För att klara av detta bör träningen vara planerad så att tillräcklig återhämtning nås. Perioder när matcher spelas med kort mellanrum har visat sig framkalla större risker för skador och sjukdomar på grund av den begränsade återhämtningstiden. Övervakning av spelares fysiska status kan hjälpa coacher att bedöma spelares fysiska status och en del olika metoder har används för detta syfte. En komparativ studie gjordes för att jämföra effekten på immunoglobulin A och alfa-amylas efter en match och med två matcher spelade med 72 timmars mellanrum, på åtta juniorfotbollsspelare på elitnivå. Resultaten visade ingen statistisk signifikant skillnad på spelarnas immunoglobulin A-värden men tendenser mot sänkta värden sågs efter både en och två matcher. En statistisk signifikant ökning av alfa-amylas sågs 24 timmar efter två matcher ( $p = 0,012$ ) och tendenser till förhöjda värden även efter en match. Några förändringar och tendenser kunde ses men framtida forskning bör innehålla större urval för att kunna göra mer generaliserande slutsatser. Forskningen bör även inkludera match-data så som mängd högintensiv löpning och total sträcka som löptes för att underlätta tolkning av resultat.

## **Foreword**

The process of making this study has been a struggle at times where finding relevant previous studies have been a challenge. The challenges of producing a scientific study have been made clearer to me and I feel that I have learned a lot from it.

I would like to thank Joe Dunbar from Ipro Interactive Ltd for his help with the testing equipment and Lennart Gullstrand for his insights as a supervisor.

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# Introduction

In football, the coaches at the elite level are striving to develop their players. Tactical, technical and physical training sessions are performed to help them to become better players and to have good performances in the matches. While at the same time scheduling training in a way to keep the players healthy. The matches are a major load physically on the players and if they are not allowed to recover properly afterwards the players may accumulate a certain degree of fatigue (Nédélec, McCall, Carling, Legall, Berthoin & Dupont, 2012; Bangsbo, Mohr & Krstrup, 2006). There are risks with players who don't reach sufficient recovery in between the physical exertions as this may in turn cause the players to become overtrained and thus being more inclined to develop injuries and illnesses (Dupont, Nédélec, McCall, McCormack, Berthoin & Wislöff, 2010; Carling, McCall, Le Gall & Dupont, 2015; Nimmo & Ekblom, 2007).

This presents a challenge to the coaches who have to carefully plan training and rest periods around the matches to avoid overtraining. This challenge may grow bigger when multiple matches are played within a few days as the time for recovery is limited. To increase the speed of recovery and reduce the risks of injury and illness some different recovery methods such as ice baths (Ihsan, Watson & Abbiss, 2016), foam rolling (MacDonald, Button, Drinkwater & Behm, 2014), stretching, carb rich nutrition (Nédélec et al., 2012) and vibrations (Nepocatych & Balilionis, 2015) have been used with varying success.

To help assess the athletes state of recovery, testing of their levels of salivary Immunoglobulin A (s-IgA) and alpha-amylase ( $\alpha$ -amylase) have been used as these factors react to physical exertion and can be seen as indicators of the athletes' physiology at that time (Nater & Rohleder, 2009; Owen, Wong, Dunlop, Groussard, Keksi, Dellal, Morgans & Zouhal, 2014). As stated previously, training or playing in an under recovered state increases the risk of injuries and illness and as such the knowledge of the players' recovery levels can help the coaches when planning training and to see if a player is recovered enough to play a match without increased risk of injury or illness.

## *Purpose*

The purpose of this study was to investigate if there are any effects on elite youth football players' salivary  $\alpha$ -amylase- and IgA-levels after one football match and after two matches played with only 72 hours to recover. A part of the purpose was also to see if there are any differences in the players s-IgA and  $\alpha$ -amylase levels after one game compared with two games played in a short time period. This was done in order to further understand how to plan training accordingly around single matches as well as in periods during the season when match frequency is high.

- What, if any, effects are detected on elite youth football player's s-IgA and  $\alpha$ -amylase levels 24 hours and 72 hours after a football match?
- What, if any, effects are found on elite youth football players' s-IgA and  $\alpha$ -amylase levels 24 hours and 72 hours after two football matches played with 72 hours in between?

- Is there a difference in s-IgA levels 24 hours and/or 72 hours after playing one match compared to playing two matches with 72 hours in between for elite youth football players?
- Is there a difference in  $\alpha$ -amylase levels 24 hours and/or 72 hours after playing one match compared to playing two matches with 72 hours in between for elite youth football players?

## Definitions

Salivary immunoglobulin-A is a protein that is produced in the salivary glands. It is a part of the immune system in that it helps to protect the body from viral pathogens which may cause infections (Papacosta et al., 2011).

Alpha-amylase is an enzyme that is used in the process of digestion, mainly in the degradation of starch and glycogen to maltose and it can be found in the saliva as well as the pancreatic juice (Papacosta et al., 2011).

The sympathetic nervous system is a part of the autonomic nervous system that reacts to physical and mental stress in a response called “fight or flight” which prepares the body for physical activity (Persson & Zakrisson, 2016).

## Background

### *Fatigue monitoring and overtraining*

Research of different kinds of fatigue monitoring with athletes have been done previously. A study from 2015 measured factors that were considered to likely be effected by fatigue such as counter movement jumps, distance of high intensity running, perceived rating of wellness and heart-rate response. The results showed that some of the chosen methods of fatigue monitoring could be effective in estimating fatigue levels. However, problems such as the potential hesitation of the players to perform explosive jumps following a match makes it a challenge to put into practice (Thorpe, Strudwick, Buchheit, Atkinson, Drust & Gregson, 2015).

A review of internal (psychological) and external load (physical) monitoring in order to detect fatigue by Halson (2014) proposed that the choice of monitoring should depend on the sport. Where different measures should be made to an individual athlete compared to a team sport athlete to fit their profile. Both practically, as it could be very time consuming to perform certain tests with a whole team of athletes, and physiologically as the demands of different sports are not always the same. Halson showed that load monitoring can be used as a way to decrease instances of injury and illness but it can be difficult to implement because of the amount time it would take the coaches to collect and analyse the data as well as the potential financial investment in testing gear that might be needed.

A method of monitoring the neuromuscular fatigue after a match of Australian rules football was researched by Wehbe, Gabbett, Dwyer, McLellan and Coad (2015). They had twelve youth players perform a six second sprint followed by one minute of active rest and then a six second sprint again on a cycle ergometer before and after a match. Peak power and peak cadence were recorded and it was concluded that even though their study showed promise this is not yet a fully applicable method of fatigue monitoring as it is the first study to test this specific method.

Another method of fatigue monitoring that is becoming more prevalent in sports is saliva testing. This method is seen, depending on what type of testing procedure that is used in order to collect and analyse data, to be a non-invasive, functional and rapid method. Also, it doesn't demand a lot of human resources and fits the tight time constraints often seen in professional sports (Papacosta & Nassis, 2011). For this study tests of salivary IgA and alpha-amylase will be used. No studies that investigate a direct link between the two factors and injuries have been found. Rather testing of IgA and amylase is done to see indications of fatigue and recovery levels in a manner that will be described later in the study.

As earlier stated, one of the main reasons for monitoring fatigue is to avoid non-functional overtraining. On the other hand, planned overtraining can be a part of a training periodization in order to attain a super compensation when it is followed by a tapering period and thereby getting an improvement in performance. However, if training volume and intensity is too high and enough recovery isn't reached then the athlete can be exposed to greater risk of injury, illness and psychological problems, this is sometimes called non-functional overtraining or overreaching (Meeusen, Duclos, Foster, Fry, Gleeson, Nieman, Raglin, Rietjens, Steinacker & Urhausen, 2012).

A study of youth athletes, where 152 of them were participating in team sports, found that 17 % of the team sport athletes had at one time experienced non-functional overtraining. Higher level youth athletes were more at risk than lower level athletes for having reached non-functional overtraining highlighting the need of fatigue monitoring in youth athletes (Matos, Winsley & Williams, 2011).

### *Alpha-amylase*

The amount of  $\alpha$ -amylase in the saliva can be seen as an indicator of the activity of the sympathetic nervous system. The amount of  $\alpha$ -amylase in the saliva increases with an increased activity of the sympathetic nervous system which is known to be heightened during and in a period after physical exertion and indicates that the body is stressed (Papacosta & Nassis, 2011). Measurements of cortisol have been made for the same purpose, to act as an indicator of a stress, but as  $\alpha$ -amylase is produced directly into the saliva rather than it being transported from blood as is the case with cortisol.  $\alpha$ -amylase is therefore seen as a more suitable measurement variable of stress from physical exertion (Rohleder & Nater, 2009). Research have shown that the size of the changes in  $\alpha$ -amylase levels looks to be related to the nature of the exercise (Bishop & Gleeson, 2009), more intense exercise yields a larger increase than submaximal training (Allgrove, Gomes, Hough & Gleeson, 2008; Bishop, Blannin, Armstrong, Rickman & Gleeson, 2000; Bishop, Walker, Scanlon, Richards & Rogers, 2006).

$\alpha$ -amylase has been found to be an indicator of sympathetic nervous system activation (Papacosta, et al., 2011; Nater & Rohleder, 2009) and a prolonged excessive activation could lead to depleted adrenal levels. Which in turn potentially could result in severe health repercussions with reactions such as depression, pain in joints and muscles and chronic fatigue (Brooks & Carter, 2013). This shows that knowledge about  $\alpha$ -amylase and in turn the nervous system activity could be beneficial not only for the athletes' performance but their overall health and testing of saliva  $\alpha$ -amylase could help in assessing this.

### *Salivary IgA*

S-IgA levels is known to decrease not only directly and up to 24 hours after physical exertion but the levels have also been seen to be depressed for more than a week after depending on the characteristics of the exertion (Papacosta et al., 2011). Because of the immunological effect of s-IgA the depressed levels are positively correlated with an increased risk of infections in the upper airways in elite athletes. Such measurements can consequently be used to avoid putting a player through a high training load when risk of illness is higher (Neville, Gleeson & Folland, 2008). Other research shows that prolonged periods of high intensity or high volume exercise seems to be the greatest risk for athletes to experience depressed levels of s-IgA and by that receiving an increased risk of getting infections (Libicz, Mercier & Bigou, 2006; Fahlman, Engels, Morgan & Kolokouri, 2001; Bishop & Gleeson, 2009; Gleeson & Pyne, 2000). A football player at the elite level can perform 150-250 short intense movements in a match with short periods of active rest in between (Bangsbo, Mohr & Krstrup, 2006) and as such it could be classified as a highly intensive sport even though the matches are long (90 minutes). Research of high intensity training in football has in fact shown depressed levels of s-IgA (Owen et al., 2014).

A study on 21 male elite football players in the English premier league also investigated the effects of a tight match schedule. During a 30-day period where five matches were played within 15 days. The results showed a decrease in s-IgA during the most frequent match period and levels returned to normal when matches were played with more time in between (Morgans, Orme, Anderson, Drust & Morton, 2014).

## Method

### *Design*

A comparative study design was used where elite youth football players' saliva was first tested for baseline levels at a well-rested state. The levels were then compared with levels 24 and 72 hours after a match. Two weeks later new data were collected for the same eight players, testing the players after 24 and 72 hours after having played two matches with 72 hours of recovery time in between matches. With the aim of having as equal post-match conditions as possible a standardized recovery session designed by club coaches (see table 1) was performed after the 24-hour samples were collected. This was followed by passive rest for the players the day after. The 72-hour post samples were collected before training.



Table 1. Recovery session.

ACTIVITY	TIME
Stationary bike (60 RPM – Gear = 0)	20 min
Foam rolling (Lower body)	12 min total
- Calves	1 min/side
- Hamstrings	1 min/side
- Glutes	1 min/side
- Adductors	1 min/side
- IT-band	1 min/side
- Quadriceps	1 min/side
Ice bath (12 degrees Celsius)	10 min

### *Subjects*

The subjects were chosen through a systematic sample method were elite level youth players who were seen as starting players by the head coach were included to make sure of their participation in the matches (Ejlertsson, 2012). The subjects (n = 8) were 16 (n = 4) or 17 (n = 4) years old and male elite youth football players from a Swedish professional club's academy. Originally ten players were tested for baseline levels but as two were not able to participate in all matches they were excluded from the study. Compliance and participation in the study were agreed on by signing a form of consent. Handling of personal information was guaranteed to be made with care and confidentially and where the option to drop out at any time was made clear.

### *Data collection*

To collect the saliva, the players were first instructed to rinse their mouth with water five minutes before testing to avoid contamination of samples. They then used IPRO Oral Fluid Collection kits where 0.5 ml of saliva was collected by having the swab in their mouth while sitting in a quiet room until a blue indicator on the swab signalled that a sufficient amount had been collected. This took between 40 seconds to one minute. The swab was then put in a buffer liquid and was subsequently mixed for one minute. In order to measure the saliva a dual s-IgA /  $\alpha$ -amylase lateral flow device (IPRO POC interactive) was used. Two drops of the saliva/buffer mix were put on a sample plate and after ten minutes it was put into the lateral flow device for analysis where the amount of s-IgA and  $\alpha$ -amylase were shown in  $\mu\text{g/ml}$ . This method has been validated with the ELISA laboratory method for both s-IgA ( $r = 0.90$ ) and  $\alpha$ -amylase ( $r = 0.87$ ) (Dunbar, Hazell & Jehanli, 2015).

### *Data processing and analysis*

The data were collected and put into an Excel-document for structure. Then the data were then analysed in SPSS version 23. Because of the small sample size and the fact that the data were not normally distributed the non-parametric "Wilcoxon signed ranks test" was used in order to see if significant changes were apparent (Ejlertsson, 2003).

### *Methodological considerations*

The s-IgA /  $\alpha$ -amylase lateral flow device was chosen to analyse the saliva tests because it is a validated method (Dunbar et al., 2015) and the material needed in order to complete the tests were available. The method is also easy to use and time efficient. These are important aspects to consider when working in professional team sports where a lot of players could be tested without demanding too much time or resources. The post-match saliva tests at 24 and 72 hours were chosen to be conducted to be able to see the potential effects and fluctuations on the saliva contents on a timeline the days following a single match. The same was then done after two other matches had been played 72 hours apart. In order to be able to compare the saliva response after the single match with the response after a more intense match period. A 48-hour post-test was not possible as the players had the day off and as such they were not available for testing. Testing after more than 72 hours would not have been useful as some of the players then participated in intense training and others had a lower loading in preparation for matches with their national teams. As different kinds of training gives varying effects on the saliva (Bishop et al., 2009; Papacosta et al., 2011) this would have eliminated the possibility to analyse what effects existed due to the previous match/matches and what effects were due to their consequent training sessions. As the purpose of this study is not only to research the effects on saliva contents after a football match, but also to give insight to the effects on players' saliva contents during periods of frequent matches. The 72-hour post-match testing fits well because this is the least amount of hours accepted to separate two football matches at the professional level. As such, data collected at this time could help in understanding the players' physiology during those match days.

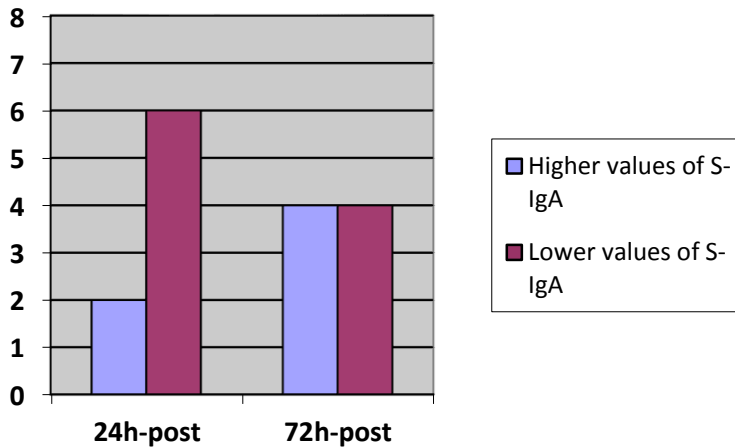
### *Ethical considerations*

The ethical considerations in this study were based on the four principles of research ethics; the information requirement, the requirement of consent, the requirement of confidentiality, the requirement of being able to discontinue the study at any time and the requirement of using the data collected for the study only (Vetenskapsrådet, 2002). To comply with the ethical requirements of consent and information the players were informed about the purpose and procedure of the study before the start. The players that were chosen for the study then had the choice to sign a form of consent where it was clear that they could cancel their participation without any negative consequences at any time which every player did. Even though the players were under 18 the non-invasive nature and the players' ability to understand the reasoning behind the study the choice was made to not include an obligatory signing of consent by the players' parents. The players' names were replaced by "Player 1", "Player 2" and so forth in order to comply to the confidentiality requirement. The data collected will be carefully handled and will not be published or used anywhere except for the parts included in this study.

# Results

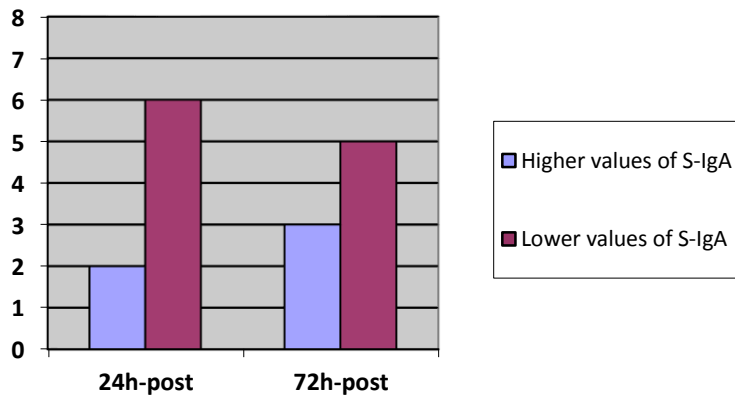
## *S-IgA results*

No statistically significant change between test values from 24 and 72 hours post one match compared to baseline ( $p = 0,208$  and  $0,575$  respectively). Tendency towards decreased values 24h post (see figure 1).



*Figure 1.* Number of players with higher or lower values of S-IgA post one match compared to baseline.

No statistically significant change between test values from 24 and 72 hours post two matches compared to baseline ( $p = 0,208$  and  $0,779$  respectively). Tendency towards decreased values 24h and 72h post (see figure 2).



*Figure 2.* Number of players with higher or lower values of S-IgA post two matches compared to baseline.

No statistically significant change between test values from 24 and 72 hours post one match compared to two matches ( $p = 1,0$  and  $0,889$  respectively). Tendency towards lower values 72h post two matches (see figure 3).

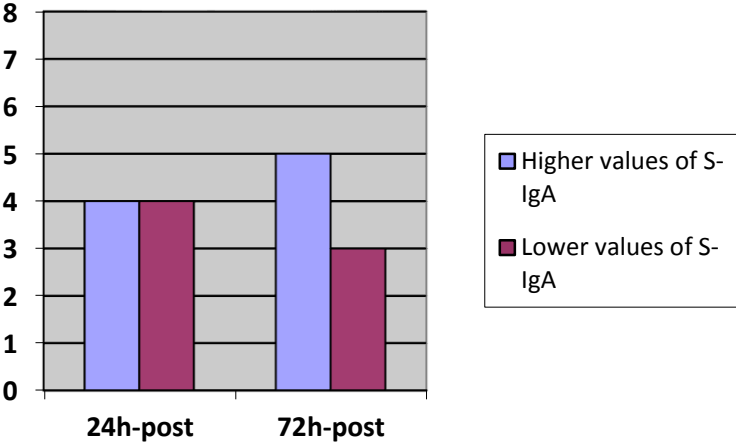


Figure 3. Number of players with higher or lower values of S-IgA post one match compared to two matches.

Standard deviation for s-IgA were shown to be between 24,4-73,9.

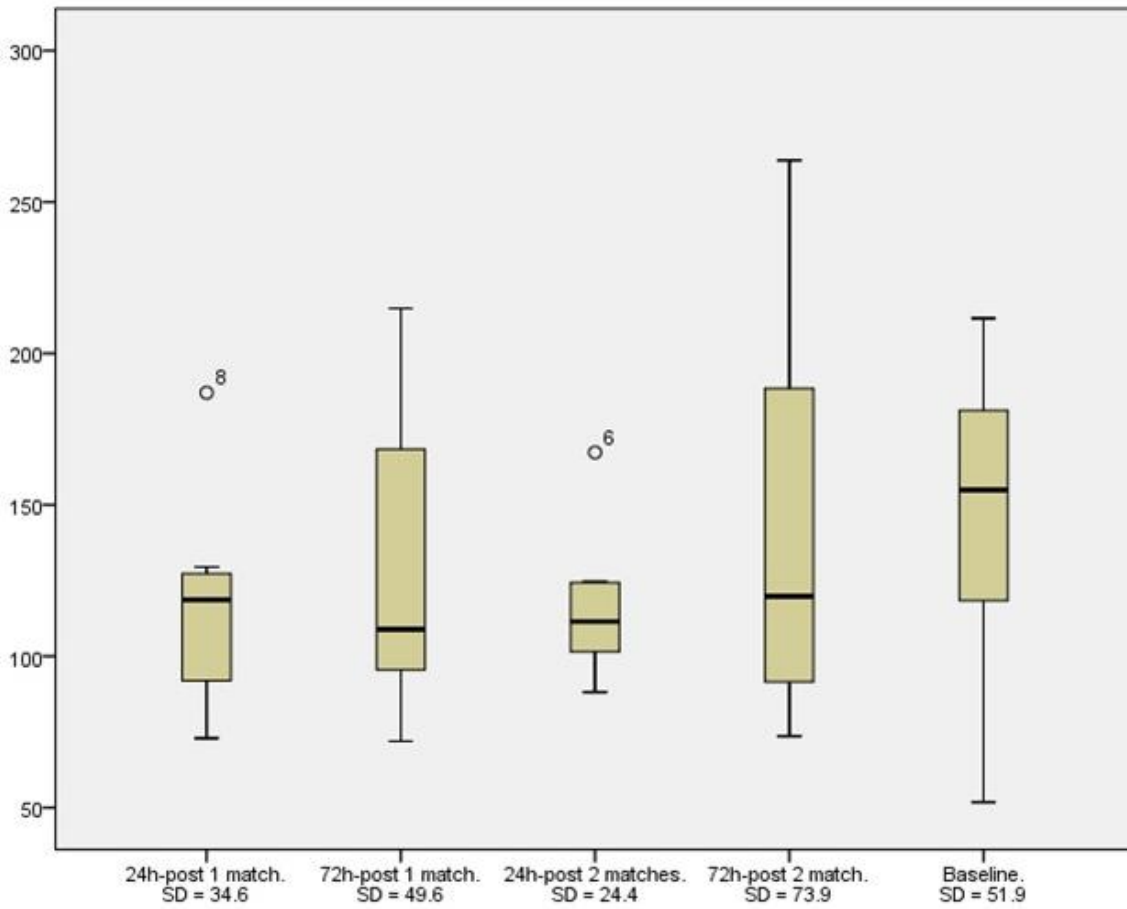


Figure 4. Boxplots showing outliers. o = outlier. SD = standard deviation

#### $\alpha$ -Amylase results

No statistically significant change between test values from 24 and 72 hours post one match compared to baseline ( $p = 0,327$  and  $0,069$  respectively). Tendency towards increased values 24h and 72h post (see figure 5).

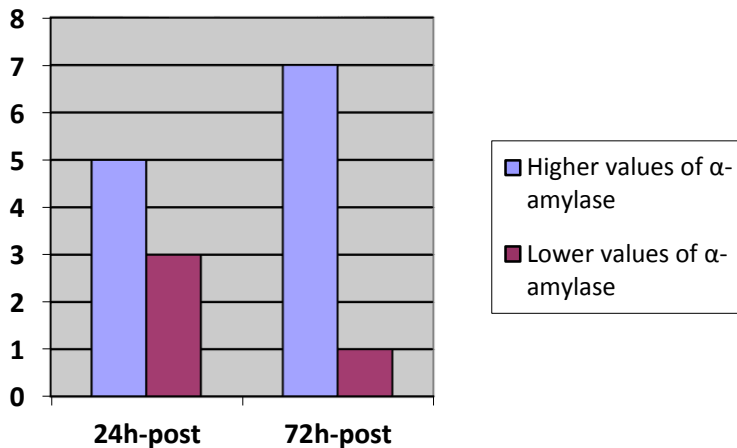


Figure 5. Number of players with higher or lower values of  $\alpha$ -amylase post one match compared to baseline.

A statistically significant increase in test values from 24 hours post two matches compared to baseline ( $p = 0,012$ ) was seen but not 72h post ( $p = 0,093$ ). Tendency towards higher values 72h post (see figure 6).

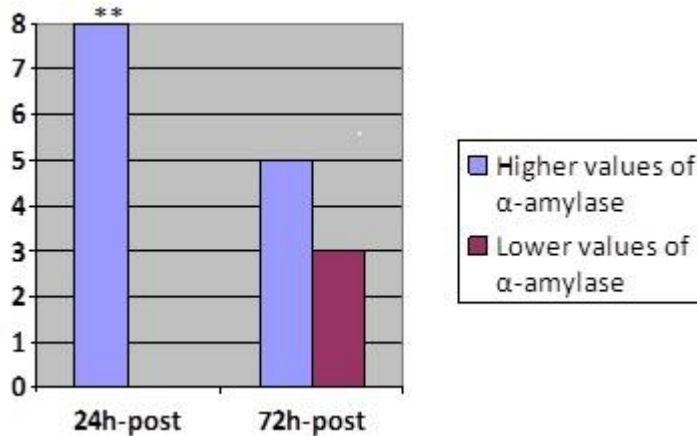


Figure 6. Number of players with higher or lower values of  $\alpha$ -amylase post two matches compared to baseline. \*\* Significant at  $p < 0,05$ . (ha standard deviation)

No statistically significant change between test values from 24 and 72 hours post one match compared to two matches ( $p = 0,161$  and  $0,484$  respectively). Tendency towards higher values after two matches 24h post. Tendency towards higher values after one match 72h post (see figure 7).

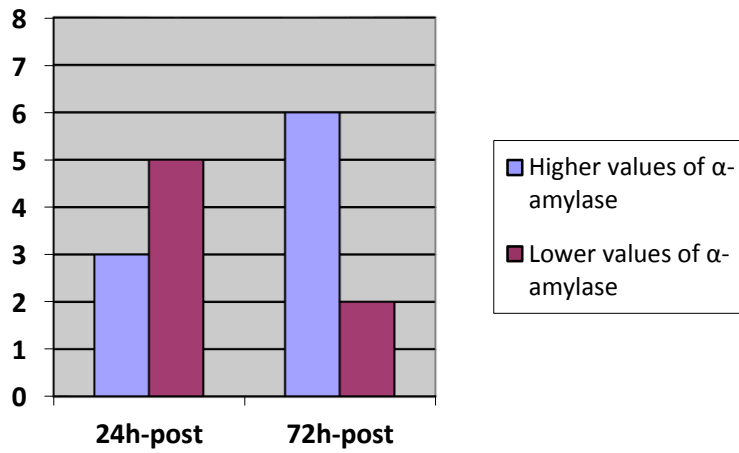


Figure 7. Number of players with higher or lower values of  $\alpha$ -amylase post one match compared to two matches.

Standard deviation for  $\alpha$ -Amylase were shown to be between 149,7-670,8.

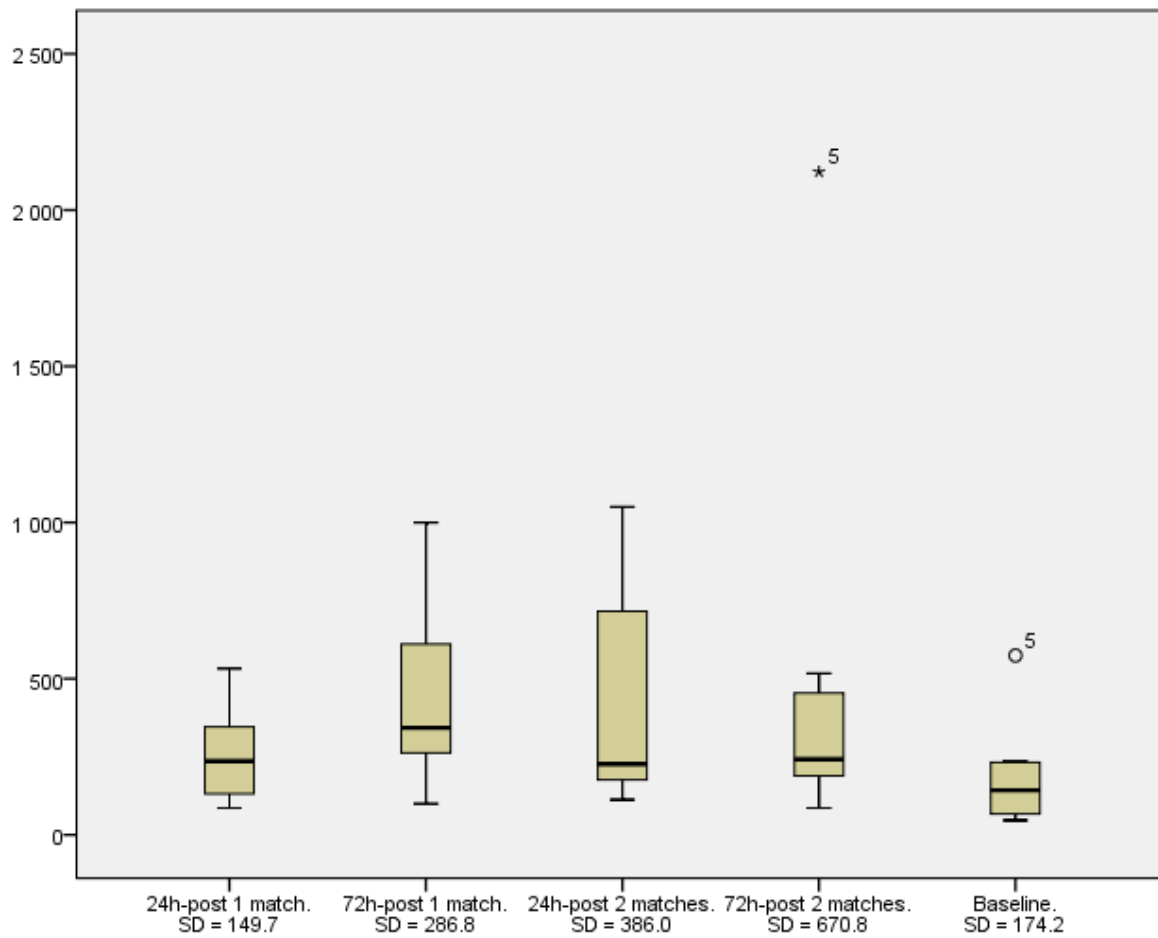


Figure 8. Boxplots showing outliers. o = outlier, ★ = extreme outlier. SD = standard deviation.

# Discussion

## Methodology discussion

The study was originally planned to include three teams of different ages; U-17, U-19 and the first team with ages ranging from 15 to 35 years old. Ten players in each team would have been tested giving a sample size of 30 instead of the current eight. As well as giving an increase in sample size this would also have made it possible to compare the saliva responses from young to adult players. This would have been interesting because the first team players are full time professionals where they could have a recovery focus all day with physios and sport scientist to aid them. The young players are still in school and as such they still face potential stressors such as homework and tests. This and the possibility that the first team players could be better conditioned to recover after matches as they have more years of experience could have made a difference in the responses. This information might have been valuable for coaches to help players in the transition between youth and first team as an example.

However, the problems with players getting sick, injured or being away with national teams as well as the limited time frame of the study made it impossible to make a sufficient amount of tests with every team. The baseline tests, 24-hour post and the 72-hour post-match tests are all crucial in answering the research questions. The fact that matches are not played with only 72 hours apart every week made the amount of potential testing opportunities limited which contributed to the challenge of having a larger sample size. Hence only the U-19 players participated in the study, but even here two players dropped out due to illness and injury. This has resulted in a total number of eight players included and as such the low sample size could be considered a weakness. Conclusions based on this study could not be said to apply to the population of elite youth football players as a whole (Bryman, 2011).

Problems with the amount of testing opportunities could have been solved had the choice been made to focus solely on the saliva response after a single game. As they are played at least once every week and as such the amount of potential testing opportunities would have increased substantially. Based on the allocated time for this study it could be argued that this would have been a preferable solution.

A strength of the study is that the reliability was increased by using a standardized procedure when testing (Bryman, 2008). The players rinsed their mouth with water before the test and sat in a quiet room while providing the saliva samples to avoid contamination of the samples. A standardized testing procedure was used when testing the samples as described in the "data collection" section. The standardized recovery protocol also contributed to the reliability in that it made certain that the players had the same physical stimulus the day after the match. Furthermore, it could be argued that it potentially made them less susceptible to injuries and therefore prevented further loss of sample size. To ensure the validity the data were collected and analyzed through a validated method using the "S-IgA /  $\alpha$ -amylase LFD" (Dunbar et al., 2015).



The ethical considerations were met but one could argue that there could be a potential problem with the consent of the players. Everyone were told that participation was on a voluntary basis and that no negative consequences would follow if they chose not to participate. But as they are all in a team they might feel forced in a way to participate due to peer pressure from other teammates or an authoritarian pressure from coaches even though the coaches were not involved at any point in the study. In hindsight this could have been solved by using a one-on-one approach where each player would be asked individually without the knowledge of the other players. This would however be a struggle when testing as the testing procedures were set in a certain standard where for example a quiet room was used and there it would be inevitable for the players to see who were participating.

## Discussion – Results

### *S-IgA results*

No significant changes were seen in s-IgA after either one or two matches compared to baseline scores and no significant changes between the salivary IgA response after one and two games were found. However, tendency towards lowered levels was seen in six out of the eight players 24 hours post both one and two games compared to baseline values. A small tendency towards slower recovery of IgA levels after two games can be seen compared to after one game. The effects on s-IgA levels 24 hours post are close to the values described by Papacosta et al. (2011). Who showed that a 21% decrease could be seen after prolonged exercise. Morgans et al. (2014) showed lower levels in response to a tight match schedule. However, those values were attained after more than two games.

As stated previously lower levels of s-IgA can have detrimental effects on the immune system, more specifically the ability to cope with pathogens (Neville, Gleeson & Folland, 2008; Wetherell & Sidgreaves, 2005). This might indicate that two matches played in a short time span is indeed related to greater risk of illness for the players because of the delayed recovery as seen in previous research (Morgans, Orme, Anderson, Drust & Morton, 2014). But the results from this study by itself are not enough to draw definite conclusions about this as no significant changes were seen.

### *$\alpha$ -Amylase results*

The values varied a lot, as an example the data collected 72 hours post two matches ranged between 86.2  $\mu\text{g/ml}$  to 2122  $\mu\text{g/ml}$ . This variability is not uncommon in measurements and is seen in previous research as well (Rohleder & Nater, 2009). The results from the  $\alpha$ -amylase tests showed a significant difference in the saliva response 24 hours post two matches compared to baseline levels ( $p = 0,012$ ). No other significant changes were seen but just as for s-IgA some tendencies could be seen. A tendency of an increase of  $\alpha$ -amylase was seen 24 hours post but especially 72 hours post one match where seven of the players showed trends toward increased levels. This can be seen as counter intuitive and against the hypothesis that a larger increase should have appeared after two games at 72 hours post as well because of the larger strain on the players. However, different teams were played and different styles of play can demand different kinds of physical output from the players. This might be an explanation as well as stresses of

everyday life which could potentially be higher at that point (Strahler, Mueller, Rosenloecher, Kirschbaum & Rohleder, 2010). But as those changes were not significant it is hard to make conclusions based on them.

### *Recommendations*

These results together with previous research shows the importance of customizing training around matches in order to avoid players getting hurt or sick. An argument could be made that recovery should not be the focus solely the day after a match, but the training 72 hours following a match should also be planned with recovery in mind. As training sessions with high intensity and excessive volume seems to have the biggest negative impact on both s-IgA and  $\alpha$ -amylase, that type of training should probably be avoided (Papacosta et al., 2011; Allgrove et al., 2008). If highly intensive training is going to be performed it should be kept short. Bishop and Gleeson (2009) found that highly intensive sessions above 80% of  $\text{VO}_2$  max performed for less than 30 minutes actually increased s-IgA. Recommendations for coaches could be that sessions following the match should consist of low intensity training such as tactical training and if high intensity training is desired it should be kept short. To help recover from the central nervous system fatigue that the amylase indicates 24h-post two matches, ice baths could be used as it has been shown to be an effective method (Ihsan et al., 2016).

A possible source of error can be seen when analysing the results. As the three different matches that were played, were played against three different teams there is a possibility that this could have affected the results. Some teams use tactics with a lot of pressing and physical play while other teams use tactics that are less physically demanding on themselves and their opponents. Another factor that has to be taken into account is the fact that the position of the player will affect the physiological response. As an example, research has shown that elite outfield players perform sprints during 1%-11% of a match and a disparity that large could potentially give different physiological responses when comparing the ones that are sprinting 1% to the ones that are sprinting 11% (Stolen, Chamari, Casagna & Wisloff, 2005). Tactics, the playing style of the opposing team and player's position are all factors that determine the degree of physical exertion. In this case there is a possibility that a larger physical exertion was performed in the single match compared to the other two matches. As such a problem occurs when comparing saliva responses and making conclusions without having knowledge of the physical workload performed during the matches. This is a problem because you can't pinpoint how much of the response is due to the exertion performed in the match and how much is because the lack of recovery between them. To be able to measure and include the workload of the players in analysis different tracking methods such as GPS:systems can be used (Carling, Bloomfield, Nelsen & Reilly, 2008).

## **Conclusion and implications**

In conclusion tendencies towards a negative impact on both s-IgA and  $\alpha$ -amylase could be seen after both a single match and two matches played in a short period of time. Tendencies towards bigger negative effects after two games compared to one were seen but the only significant change that was seen was the 24-hour post two games test compared with baseline for  $\alpha$ -amylase. Future research should use bigger sample sizes in order to make broader conclusions. It is common in human studies to see outliers which were seen in this study as well (see figure 4 and

7). Non-responders who respond in a manner that is unexpected is also usual to see. As an example from this study, two players had higher levels of s-IgA 24 hours-post two matches compared to baseline values (see figure 2) which contradicts the expected results. To minimize the outliers' ability to skew the results a bigger sample size is helpful. It should also include data of the matches that are played. As match-data such as the amount of high intensity running, amount of sprints and decelerations could help interpret results better. Because of the potentially different levels of exertion the players are experiencing depending on which team they play against and what type of tactics that are used (Bangsbo et al., 2006; Stolen et al., 2005).

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