# Chapter 13
# Saliva in Sport Sciences

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## 13.1 Introduction and General Concepts

Saliva is a biological sample that due to its easy and simple collection can have a wide potential application in sport sciences to evaluate different biomarkers. There are previous excellent reviews in which the possible applications of saliva in sport sciences have been indicated (Lindsay and Costello 2017; Papacosta and Nassis 2011). In this chapter we will review the main biomarkers of potential use in sport that can be measured in saliva grouped in biomarkers of stress, immune system and inflammation, muscle damage, anaerobic metabolism and oxidative status (Fig. 13.1). For each biomarker we will provided general ideas and updated information that can help to gain knowledge about its interpretation and possible applications in sport sciences.

It is important to indicate that as Lindsay and Costello (2017) pointed out, there are four main forms to express the results of salivary biomarkers in exercise studies:

1. Absolute concentration (i.e. g or mmol/L) or activities in case of enzymes (i.e. IU/L)
2. Corrected by Flow rate (i.e. g or mmol/min), that is obtained by multiplying the unit of point 1 by the volume obtained by unit of time, for example during 1 min of sampling. By this correction the changes in flow rate are explored, that can be due to psychological causes and related to parasympathetic withdrawal. As will be later mentioned, this has been used in case of IgA, in which a study reported a 50% of decrease in secretion rate but no changes in absolute concentration after a 160 km run.

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A. Tvarijonaviciute et al. (eds.), *Saliva in Health and Disease*,
https://doi.org/10.1007/978-3-030-37681-9_13

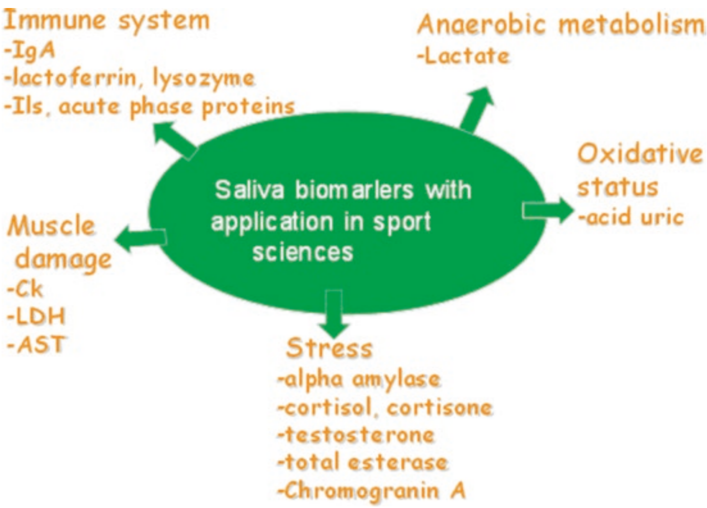


**Fig. 13.1** Main biomarkers that can be measured in saliva with application in sport sciences, grouped by the biological information that can provide

3. Corrected by protein content (i.e. g or mmol/mg protein). This has been used in some reports but there are authors who indicated that it is not adequate to correct the values by concentration (Blannin et al. 1998)
4. Concentration relative to saliva osmolality (mg/mOsm). This has been recommended for IgA measurements (Sari-Sarraf et al. 2007), although further studies should be made about the real utility of this correction.

## 13.2 Biomarkers of Stress

### *13.2.1 Alpha Amylase*

Salivary alpha-amylase (sAA; EC 3.2.1.1) is secreted by the parotid gland in response to adrenergic activity and increases in psychological and physical stress situations. Although it can be measured by concentration, usually the measurement of sAA activity is preferred for the evaluation of sAA in exercise. The activity is expressed without any correction by total protein or flow, since this can lead to errors in its interpretation (Contreras Aguilar et al. 2017).

Koubuchi and Suzuki (2014) reviewed the situations in sport science that can increase amylase and indicate that sAA increases tended to be more pronounced at exercise intensities >70% $VO_2$max in healthy young individuals. Another evidence of the relation between sAA and exercise intensity is the fact that amylase in saliva might be used as method for determining the anaerobic threshold in exercise. Being the anaerobic threshold defined as the oxygen consumption above which aerobic energy production is supplemented by anaerobic mechanisms, causing a sustained increase in lactate and metabolic acidosis (Calvo et al. 1997).

Although further research about possible factors that can influence sAA in exercise and also possible applications of sAA should be performed in the future, this enzyme was higher in response to competition in experienced compared to novice athletes, and was positively associated with performance and interest in team-bonding (Kivlighan and Granger 2006). However, increases in alpha-amylase correlated with a decrease in physical activity has been described in older adults (Strahler et al. 2010). Regarding possible new applications of this enzyme, recently the increases in sAA has been found after administration of probiotic supplement in rugby players suggesting a possible role as a host defence peptide (Pumpa et al. 2019).

### *13.2.2 Cortisol*

Cortisol is secreted by the adrenals after activation of the hypothalamus-hypophysis due to any stressor stimulus. Cortisol in saliva is only in its free form, whereas in serum it is in its free form and also bound to proteins. Higher responses to exercise in cortisol in saliva than in serum have been described. This is because an exponential increase in free cortisol occurs when the binding capacity of the corticosteroid-binding globulin, which is the protein that binds cortisol in serum, is exceeded (Del Corral et al. 2016).

Usually it is described that salivary cortisol increases after exercise. However, in some sports, such as soccer, conflicting results have been obtained. Since some authors indicated an increase of salivary cortisol of 78% after a soccer game compared to basal values taken before, (Torphe and Sunderland 2012), whereas other did not find significant changes (Moreira et al. 2009). These differences could be possible related with different performances or training of the individuals of the study. Also the period of time in which the saliva is collected can influence, since for example it has been described that immediately after a rugby match there is an increase in cortisol, that returns to normal values in 4 h (Elloumi et al. 2003).

It is also important to consider the circadian influence of the cortisol. Therefore, in trials, it is recommended to do comparisons of cortisol with a control day, in which the same population is sampled at the same time without doing the exercise. However, in short duration studies (less than 60 min) cortisol can be compared with baseline (Del Corral et al. 2016).

In general cortisol in saliva can be used for:

– *Evaluate training.* Usually intensive training produces a short-term effect with increase in cortisol in saliva (Li et al. 2012). However, in situations of overtraining cortisol can be decreased (Filaire et al. 2013). For this purpose, it is very interesting the evaluation of alpha-amylase and cortisol together. For example, in cases of overtraining in tennis players, there is an asymmetry between the two markers, with a rise in the alpha-amylase awakening response and a higher alpha-amylase activity output, but a decrease in the overall output of salivary cortisol. Therefore, in these cases the ratio of sAA over cortisol increases, being this ratio in correlation with a decrease in the performance (Filaire et al. 2013). The increase in alpha-amylase in correlation with a decrease in physical activity found in older adults would be in line with these findings (Strahler et al. 2010).

– *Establish the optimal recovery time after an intense exercise.* This could be established by the time in which cortisol reaches its basal values before the exercise. For example, after a rugby match there is an increase in salivary cortisol that returned to basal values after 4 h and reach values lower than the basal concentrations, even until the day 5 after the match. It is possible that this long time is required to restore the break-down of homeostasis induced by the very hard mental and physical strain associated with a rugby match (Elloumi et al. 2003). In general, it could be indicated that decreased values of cortisol in comparison with basal levels would be indicative of overtraining or that the body is recovering from intensive efforts.
– As other possible applications of the cortisol, interestingly, a higher difference between values of cortisol from saliva collected at the morning, and the values of cortisol in saliva collected after a training test, is related with winning games in an international rugby union competition (Crewther et al. 2018).

In veterinary science, in horses that participated in exercise competitions, increases of cortisol in saliva were detected, possible due to the exposure to a novel environment compared to home. However, there was no consistent relationship between baseline salivary cortisol concentrations and competition scores (Munk et al. 2017).

## *13.2.3 Others Biomarkers of Stress*

### 13.2.3.1 Cortisone

It is a metabolite of the cortisol being at higher concentration than cortisol in saliva and also increases more than cortisol after a physical exercise. In addition, compared to cortisol in saliva, it is more correlated with plasma total and plasma free cortisol (Del Corral et al. 2016).

### 13.2.3.2 Testosterone

In the section of cortisol, it was indicated that cortisol decreases until the day 5 after the rugby match were observed. However, for salivary testosterone measured for the same period of time, only slight decreases were observed just after the game, with further increases that resulted in values higher than the basal ones, at day 5 (Elloumi et al. 2003). Since in this trial cortisol was decreased until day 5, a high ration testosterone/cortisol could reflect a situation of recovery of the body.

#### 13.2.3.3 Total Esterase

An assay for total esterase, being carbonic-anhydrase-VI the esterase that contribute more to the activity of the assay, has been developed. Total esterase in saliva increased after an indoor football match and positively correlated with salivary alpha-amylase (Tecles et al. 2016).

#### 13.2.3.4 Chromogranin A

It has been described increased in chromogranin A, measured by western blott, after an incremental maximal exercise in swimmers, being this increase highly correlated with alpha-amylase and salivary lactate (Bocanegra et al. 2012). In another study in swimmers chromogranin A correlated positively with adrenalin (and therefore with the sympathetic activity), total proteins in saliva and with the intensity during a 21 week training season (Diaz-Gomez et al. 2013).

## 13.3 Biomarkers of the Immune System and Inflammation

### *13.3.1 Immunoglobulin A (IgA)*

IgA is involved in the defense of the mucosal surfaces. There are two main controversies about its relation with exercise because:

– some authors did not find decreases in IgA after an acute exercise, for example a football game (Torphe and Sunderland 2012) and in general, low-moderate intensity exercises does not produce changes in IgA (Walsh et al. 1999). However, high intensity exercise such as a marathon, 50 km ski race or more than 2 h of cycling (Nieman et al. 2002; Tomasi et al. 1982; Walsh et al. 2002) produce decreases in salivary IgA, although these decreases are of lower magnitude in well trained individuals (Mackinnon and Hooper 1994). This data could indicate that IgA seems to decrease only in cases in which exercise is excessive or the athlete is not enough trained.

– another controversy is if the IgA decrease produced after exercise can be related or not with certain immunosuppression and decrease in performance. This is because some authors indicate that decreases in IgA are associated with an increase risk of infections (Svendsen et al. 2016). Maybe the cause of the controversy could be explained because that although an excessive exercise can decrease IgA, this decrease can represent a risk of infections if: (1) the excessive exercise is maintained, (2) the athlete is prone to infection due to predisposition to a pro-inflammatory response and a dysregulated anti-inflammatory cytokine response to intense exercise.

IgA has been described to be affected by the secretion rate, since in an experiment consisting in running 160 km, there were no changes in total concentration in salivary IgA after the run, but a decrease of 50% was observed when IgA was expressed by secretion rate (amount/minute) (Nieman et al. 2003). Although it is interesting to consider the secretion rate, it is not recommended to correct IgA by total protein to take in consideration the possible effect of dehydration, since the changes in total protein are not related to evaporative loss of saliva water and they just increase after exercise (Walsh et al. 1999). Also it can be important in the interpretation of IgA to consider the time after exercise in which it is measured, since it has been described decreases in IgA few hours after exercise that are no longer observed after 2 days (Canto et al. 2018).

In general there are two major potential applications of IgA in exercise:

– *To identify situations of excessive training*, fact that could help to trainers to decrease or adjust the intensity of the training performed (Shephard and Shek 1998). Overall it could be stated that a lack of decrease in IgA would be a marker of adequate training sessions. Even there are some intensive training systems that can increase IgA in saliva and therefore augment the oral mucosal immunity (Antualpa et al. 2018).
– *To detect possible situations of risks of infection*, possible due to excessive exercise or due to the lack of IgA by other causes such as genetic condition. For example, risk of upper respiratory tract infection (URS) has been found to occur due to decrease of IgA concentrations after an intensive endurance training. In these situations, dietary supplementation with bovine colostrum, probiotics and selected antioxidants can reduce the incidence or severity of URS in some athletes (Gleeson and Pyne 2016). In this line, marathon runners who did not receive a polysaccharide-based multiingredient supplement for 15 days prior a race, had a decrease of salivary IgA corrected by total protein after the race, while runners with this supplementation did not show changes in salivary IgA and had a better immunoregulation (Roca et al. 2019)

### *13.3.2 Lactoferrin and Lysozyme*

Both are antimicrobial compounds and would have a similar role than salivary IgA, in protecting mucosal surfaces from external pathogens.

For both analytes controversial results have been published. In the case of lactoferrin increases in serum have been reported after high and middle intensity exercise (5000 steps running at 180 and 130 steps/min respectively) (Inoue et al. 2004). But no changes in lactoferrin in saliva were found after a marathon. Interestingly, a correlation between salivary lactoferrin and blood lymphocyte counts were found in this study before the race (Canto et al. 2018).

Also there is some controversy regarding the response of lysozyme after exercise. Decreases in lysozyme have been reported in athletes over a training (Papacosta and Nassis 2011) or after a marathon (Canto et al. 2018). However increases in this protein have been found after exercise with moderate intensity, being further increased after exercise with high intensity (Ligtenberg et al. 2015). Higher levels of lysozyme 2 days after a race were detected in marathon runners who developed a lower respiratory tract infection in the 2 weeks after the race in comparison with those without infection, this could be related with the protective function of this enzyme (Canto et al. 2018).

### *13.3.3 Interleukines (IL) and Acute Phase Proteins*

Both are biomarkers of inflammation. Although it could be interesting to monitor inflammation associated to exercise, there are relatively few studies about that. In one of these studies, it was found that serum and saliva IL-6 showed different patters after an acute strenuous exercise. Serum IL-6 significantly rose in the late recovery after the exercise, while the salivary levels showed a modest and not significant increase immediately after the termination of the test. In this report a relation between IL-6 and lactate in saliva was found and it was postulated that IL-6 can be produced predominantly by type II fibers. These authors indicated the need of studies to clarify possible interferences with ILs measurements due to different sample collection methods and the importance of using validated assays (Minetto et al. 2005a, b). Also, the acute phase protein haptoglobin has been described to increase in saliva after exercise, opening a new interesting field about the response of the acute phase proteins to exercise and its possible practical applications as biomarkers of inflammation (Mateo et al. 2019).

In the future other markers of immune system such as adenosine deaminase could be evaluated in exercise and their possible correlations with other biomarkers studied.

## 13.4 Biomarkers of Muscle Damage

### *13.4.1 Creatine Kinase (CK), Lactate Dehydrogenase (LDH) and Aspartate Aminotransferase (AST)*

These are enzymes that are inside muscle fibers and that increase in serum after a muscle damage. CK and LDH can increase in saliva after an indoor football game. However, there are some limitations in the use of these enzymes:

– A high interindividual variability of results, with some individuals showing no increases in these analytes (Barranco et al. 2018).
– A poor correlation between saliva and plasma. This low correlation could be due to a delay in the pass from blood to saliva. In addition, for CK, a threshold level in the movement from plasma to saliva is possible, since this protein appears in lower concentration in saliva compared with serum. Curiously, in animals such as horses or dogs, a correlation of CK in serum and plasma has been reported (Tvarijonaviciute et al. 2017; Contreras Aguilar et al. 2019).
– Lack of specificity since these enzymes are increased in saliva in periodontal disease, that in case of AST can increase in this condition until five fold (Todorovic et al. 2006). Also salivary LDH is increased in oral cancers (Shetty 2012). In addition the CK total and CK-MB are increased in saliva of patients with myocardial infarction or heart muscle damage (Mirzah-Dizgah and Jafari-Sebet 2011; Mirzaii-Dizgah et al. 2012), although this fact make possible that CK, together with troponin in saliva, could be used to detect cardiac damage in athletes.

Overall more studies are needed in order to assess and clarify if measurements of CK and LDH in saliva could be potentially used to evaluate possible muscle stress or damage in cases of intensive exercise. Also it would be interesting not only to evaluate the changes in these enzymes in acute muscle stress, but also in situations of chronic muscle stress or true muscle damage.

## 13.5 Biomarkers of Anaerobic Metabolism

The measurement of lactate in saliva corrected by total protein has been studied for the assessment of fatigue induced during repeated explosive effort sequences.

A correlation between lactate in saliva and blood has been described after intensive exercise. However the values of this correlation differ between authors. In a study ranged between 0.5 in athletes to 0.38 in non-athletes (Tekus et al. 2012). Other studies reported similar correlations but only in non-trained and when results were corrected by total protein (Franco-Martinez et al. 2019). On the other hand, higher correlations (around 0.9) were described by Segura et al. (1996). These authors indicated that the stimulation with citric acid produced higher correlations between lactate in serum and saliva. Interestingly, blood and saliva lactate are highly correlated with the total protein in saliva, therefore it has been suggested that total protein in saliva could be potentially used a marker of salivary anaerobic threshold, which is the phase in which a subject pass from the aerobic to anaerobic metabolism (Bortolini et al. 2009; Justino et al. 2018).

## 13.6 Biomarkers of Oxidative Status

Saliva has an antioxidant system integrated by various metabolites and enzymes. Uric acid (UA) is one of the most important antioxidant molecules in saliva, contributing around 70% of the total salivary antioxidant capacity (Battino et al. 2002) and being able to chelate transition metals and to react with biological oxidants. Also UA is the biomarker more sensitive to oxidative damage. Since in a previous report UA was the only analyte showing a significant change when a panel of oxidative stress biomarkers in saliva was measured after an acute session of resistance exercise, it was concluded that uric acid should be one of the most important biomarkers to be analyzed in saliva in order to evaluate oxidative stress (Deminice et al. 2010).

## 13.7 Concluding Remarks

- Coaches or practitioners can have advantages of the measurement of certain analytes in saliva in order to get data from their athletes, specially to evaluate and detect possible situations of stress or immunodeficiency that could predispose to a lower performance or even lesions.
- There is a high variability in the components of saliva between different subjects (Gleeson et al. 2011). Therefore individual player monitoring is highly recommended, and ideally resting values of the same individual should be used as reference for comparative purposes.
- Some analytes in saliva such as IL-6 or muscle enzymes can have different response than in serum. For example, after an exercise, serum IL-6 significantly rose in the late recovery while saliva concentration showed a modest and not significant increase immediately after the termination of the test. This could suggest, in the case of IL-6 that there are specific mechanisms responsible for IL-6 release in saliva, which are independent of the systemic compartment and the circulating pools, being unlikely that this molecule diffuses though the acinar cells of the salivary glands from the bloodstream in to saliva, and that serum levels can influence salivary concentration as occurs for steroid hormones (Minetto et al. 2005a, b).
- Further studies in order to detect new biomarkers by “omics” techniques will be of interest in order to increase the range of analytes in saliva that can be applied for evaluation of the physiological responses to sport.
- It would be recommended in the future to clarify various aspects in the different analytes, such as: their origin, how the results should be given, possible gender influence, if the individual should be used as control instead the use of reference ranges, influence of sampling material and circadian rhythms, haemolysis effect or ideal storage conditions.

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