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Review

Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science

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Abstract

Objectives: This paper discusses the use of saliva analysis as a tool for monitoring steroid, peptide, and immune markers of sports training. *Design*: Salivary gland physiology, regarding the regulation and stimulation of saliva secretion, as well as methodological issues including saliva collection, storage and analysis are addressed in this paper. The effects of exercise on saliva composition are then considered. *Method*: Exercise elicits changes in salivary levels of steroid hormones, immunoglobulins, antimicrobial proteins and enzymes. Cortisol, testosterone and dehydroepiandrosterone can be assessed in saliva, providing a non-invasive option to assess the catabolic and anabolic effects of exercise. Validation studies using blood and salivary measures of steroid hormones are addressed in this paper. Effects of acute exercise and training on salivary immunoglobulins (SIgA, SIgM, SIgG) and salivary antimicrobial proteins, including α-amylase, lysozyme and lactoferrin, are also discussed. *Results*: Analysis of cortisol and testosterone in saliva may help detect the onset of non-functional overreaching and subsequently may help to prevent the development of overtraining syndrome. Assessment of salivary immunoglobulins and antimicrobial proteins has been shown to successfully represent the effects of exercise on mucosal immunity. Increases in SIgA and antimicrobial proteins concentration and/or secretion rate are associated with acute exercise whereas conversely, decreases have been reported in athletes over a training season leaving the athlete susceptible for upper respiratory tract infections. *Conclusions*: The measurement of physiological biomarkers in whole saliva can provide a significant tool for assessing the immunological and endocrinological status associated with exercise and training.
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Keywords: Saliva analysis; Hormones; Exercise training; Performance

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1. Introduction

Saliva collection and analysis is rapidly developing as a tool for the assessment of physiological biomarkers of sports training.[1,3] The use of saliva for monitoring steroid, peptide, and immune markers in sport and exercise has made saliva sampling and analysis very attractive to several researchers and clinicians. Saliva can provide a useful, non-invasive alternative to the collection of serum and plasma, because it can be collected rapidly, frequently and without stress.[1–3] Furthermore, saliva collection requires less medical training and can be performed on the sports field. Therefore, the use of saliva is becoming very popular in the field of sport and exercise science.

This review provides an overview of established procedures and promising future implications of saliva analysis for monitoring performance and immune function in sports, through assessment of hormones, antimicrobial proteins and physiological stress parameters.

2. Physiology of saliva

Saliva composition: Saliva is a colourless, dilute liquid composed of 98% water with density between 1002 and 1012 g/L and pH around 6.64.[1,2] Saliva composition consists of hormones, peptides, electrolytes, mucus, antibacterial compounds and various enzymes.[3] The total concentrations of most of these compounds are much lower in saliva when compared with serum or plasma; however, they can provide a reliable reference for their respective blood concentrations.[4,5] Steroid hormones detectable in saliva include cortisol, androgens including testosterone and dehydroepiandrosterone (DHEA), oestrogens and progesterone as well as aldosterone. Furthermore, saliva is rich in organic constituents which include proteins, albumin, urea, uric acid, lactate, creatinine and immunoreactive insulin.[3] Inorganic compounds are also present in saliva; Na^+, K^+ and Ca^{2+} are the main cations and Cl^- and HCO_3^- the main anions.[3] Furthermore, markers of mucosal immunity are detectable in saliva which include immunoglobulins, including IgA, IgM and IgG,[6] α-amylase, lysozyme and lactoferrin.[7]

Anatomy of salivary glands: Salivary glands are duct-containing exocrine glands which produce and secrete a large volume of fluid containing organic and inorganic components to the outside of the body. Around 20% of total plasma volume (which approaches ∼750 ml) is translocated every day through the salivary glands, with saliva secretion in a healthy human estimated to range from 750 to 1500 ml per day.[3,7] There are 3 major pairs of salivary glands and more than 600 minor mucus glands contained within the oral cavity; the submaxillary glands contribute ∼65% of total unstimulated saliva secretion, the parotid glands contribute ∼23%, the sublingual glands contribute ∼4% and the minor mucus glands contribute ∼8%.[8]

Salivary glands are innervated by the parasympathetic and sympathetic nerves of the autonomous nervous system. The duct system of the salivary glands terminates into the mouth and saliva secretion aids the lubrication and initial digestion of food.[3] The lobules on each gland are rich in blood vessels and nerves which enter the glands at the hilum and progressively diverge into branches. The arterial capillaries that supply the parotid glands originate from the external carotid artery, whereas the sublingual glands are supplied by branches from both the sublingual and submental arteries and the submandibular glands are fed from the facial and lingual arteries.[3]

Saliva secretion and regulation: Saliva secretion is strongly affected by the neural control of the autonomic nervous system which in turn indirectly regulates salivary flow rate.[3] Salivary flow rate depends primarily on the type of autonomic receptor being activated, whereby, in turn, this can affect saliva composition.[9] Parasympathetic cholinergic nerve stimulation seems to induce vasodilation of the capillaries supplying the salivary glands, thereby increasing blood flow; hence, elevated blood flow to the glands is associated with a higher saliva secretion rate.[9] Even though the nervous control of saliva secretion is strongly influenced by parasympathetic cholinergic stimulation, the sympathetic adrenergic nerve stimulation also seems to affect saliva secretion. Saliva secreted from sublingual and minor mucus glands is regulated by stimulation of sympathetic nerves, whereas saliva secretion from the parotid and submandibular glands is regulated by stimulation of the parasympathetic innervation.[9] Even though normal saliva secretion results from the cooperative action of both parasympathetic and sympathetic innervation, it is the stimulation of parasympathetic innervation that provides the main stimulus for increased saliva secretion.[1]

Saliva secretion evoked by parasympathetic stimulation is characterized by a watery flow of saliva, which is low in organic and inorganic compounds.[7] In contrast, sympathetic stimulation results in a low volume of salivary output which is rich in organic content such as total protein, especially α-amylase, as well as particular inorganic salts such as Ca^{2+}, K^+ and HCO_3^-.[3] Consequently, elevated levels of α-amylase in saliva can serve as potential indicator of increased sympathetic nerve activity.[7] Under resting conditions in healthy individuals, unstimulated saliva is secreted into the mouth at a rate of 0.30–0.65 ml/min, whereas stimulation of saliva flow (induced by chewing or tasting citric acid) can increase saliva flow rate to 1.5–6.0 ml/min.[10,11] Since physical exercise elicits increased sympathetic activation, it is expected that strenuous exercise could reduce saliva flow rate and modify the salivary components. Indeed, during prolonged exercise above the lactate threshold, salivary flow rate was reported to decrease.[12] Factors associated with lower secretion of saliva when exercising at high intensities (i.e. >60% VO_{2max}) include sympathetic nerve activation, dehydration and hyperventilation (causing evaporative loss of saliva). Yet, parasympathetic withdrawal is probably more important than sympathetic activation in causing reduced saliva flow

rate during exercise.[7] Furthermore, dehydration, induced by a 24 h period without food and water, has been shown to decrease resting parotid saliva flow rate in adults.[13]

Some studies have suggested that salivary flow rate is influenced by aging, with the elderly displaying lower rates of unstimulated salivary flow compared to younger individuals.[14] However, stimulated parotid saliva flow rates do not seem to differ between age groups. In regards to gender, females are reported to have lower unstimulated whole saliva flow rates compared with males due to smaller salivary glands.[15] A circadian rhythm is also reported for salivary flow rate, with flow rate being relatively high in the early morning and decreasing as the day progresses; the highest rates of saliva flow are reported at 04:00 and the lowest at 20:00 h.[16,17]

3. Saliva collection

Pre-sampling guidelines: It is important to follow some guidelines prior to saliva collection in order to minimize error variance and chances of methodological errors.[18,19] Food or drink intake as well as exercise must be avoided at least 2 h prior to sampling, due to variations in saliva secretion which in turn will negatively affect the results. Food or drink high in sugar content, caffeine or acidity can stimulate saliva flow, and acidity will lower mouth pH levels, both leading to compromised antibody–antigen binding and enzyme activity thus leading to invalid immunoassay results.[18] In addition, alcohol consumption 24 h prior to sampling should be avoided as it may cause increased saliva secretion.[20] Blood contamination of saliva by microinjuries or abrasions in the oral cavity also becomes a problem which may significantly affect the results. Blood steroid hormone concentrations are generally an order of magnitude higher than that found in saliva (e.g. plasma cortisol is typically 200–800 nmol/l but in saliva is only 3–30 nmol/l), therefore blood leakage into the mouth might lead to increased concentrations of steroids, which is in proportion to the leakage of blood into saliva.[21] Blood contamination of saliva can be established when levels of plasma proteins such as albumin or transferrin exceed certain threshold levels in saliva.[21] To diminish any possibilities of blood contamination, participants should be instructed not to undertake any dental surgery 48 h prior to sampling as well as not to brush their teeth 45 min prior to collection. The wearing of gum shields should also be avoided where possible.

Before sampling, participants should wash their mouth with water, at least 10 min before collection, and then to swallow the first amount of saliva accumulated into their mouth before saliva collection is begun by passive drool.[19] The time of sampling has to be carefully monitored and registered for estimation of saliva flow rate. Samples have to be collected at the same time of day to control for diurnal variation, which may significantly affect the concentration of several salivary hormones.[19] Finally, frequent sampling, especially in the case of hormones, can give more accurate results, as they represent more closely the relationship between salivary and plasma hormones.[22]

Collection method and sample storage: Two methods are the most popular in the literature for the collection of saliva: the cotton swabs to absorb saliva and the collection of whole saliva in a vial by passive drool.[18] The advantages of cotton swabs are the minimisation of the risk by gingival bleeding[23] as well as the comfort and acceptability they are rated for, especially in studies involving children or elderly.[7] However, it seems that the collection of whole saliva by passive drool is the most reliable option, as cotton or polyester-based materials tend to increase sample acidity[18] and to give false concentrations of saliva components.[24] An explanation for this could be that placing the cotton swabs in different places in the oral cavity could preferentially stimulate different salivary glands and affect saliva composition.[7] An obvious advantage of the passive drool method is the estimation of saliva flow rate by timed collection of saliva in a pre-weighed vial.

The use of stimulated or unstimulated saliva has also been questioned, whereby some saliva compounds are affected by stimulation of saliva and some do not seem to be influenced. Salivary steroid hormones are not actually affected by variations in flow rate as they are transported from blood to saliva by passive diffusion. However, Dabbs[25] reported that stimulation of saliva by gum chewing led to a transient rise in testosterone concentrations in the first few minutes of sampling. Conversely, salivary IgA is strongly affected by variations in flow rate and a diluting or a concentrating effect in its absolute concentrations may take place with increased or decreased saliva flow rate, respectively (see SIgA).

In regards of storing the saliva samples two freezing options are generally used. Saliva can be stored for up to a year at a domestic freezer (−20 °C), and possibly several years at a laboratory-based freezer (−80 °C).[19] After collection saliva should be frozen as soon as possible, however if unavailable, saliva can be stored at room temperature for up to 6 h.[19] Storing saliva at >−5 °C will not freeze the samples and generally is not recommended due to bacterial growth which may degrade salivary components and may interfere with antibody binding.[26]

4. Salivary measures in sport and exercise science

It is well known that acute and chronic exercise elicits changes in levels of hormones and immunological compounds.[3,6,7] Older studies have had used collection of blood to detect hormonal and immune markers in response to exercise.[48–51] More recently, several studies have utilized saliva measures as to assess the levels of these compounds in response to exercise and training.[38–42,52–57,77–97] Salivary measures of steroid hormones can provide a reference for their respective blood concentrations and they can give useful information on how steroid hormones are modified in response to exercise and training. Immune markers are

Table 1
Relationships between salivary and blood measures of cortisol, free testosterone and dehydroepiandrosterone (DHEA) at rest, during and post-exercise.

	Rest	During exercise	Post-exercise	Protocol (number of subjects)	Reference
Cortisol	$r = 0.52$, $P = 0.05$	–	$r = 0.62$, $P = 0.01$	Multiple-set resistance exercise ($n = 28$)	4
	–	$r = 0.86$, $P < 0.001$	–	Incremental cycling ($n = 50$)	36
	$r = 0.89$, $P < 0.01$	$r = 0.72$, $P < 0.01$	$r = 0.93$, $P < 0.01$	Cycling at 75% of VO_{2max} for 30 min ($n = 8$)	30
Testosterone	$r = 0.70$–0.97, $P < 0.05$	–	–	$n = 40$–67	5 and 60
DHEA	$r = 0.68$, $P < 0.001$	–	$r = 0.70$, $P < 0.001$	Multiple-set resistance exercise ($n = 28$)	4

detectable in saliva as well and assessment of salivary antimicrobial proteins can indicate the load of exercise training and subsequently influence the risk of respiratory infections. In this regard, some important steroid, peptide and immune markers within the field of sport and exercise science will be discussed.

Cortisol: Cortisol is a steroid hormone and an important member of the glucocorticoid family. It is secreted from the adrenal cortex, via the hypothalamic–pituitary–adrenal (HPA) axis and increases in response to stressors including physical exertion. Increased cortisol levels are associated with anxiety,[27] depression states[28] and intensive physical exercise.[29,30] Cortisol is considered the main hormone responsible for catabolic processes, as it reduces protein synthesis, increases protein degradation[31] and inhibits the inflammatory process and immunity.[32] Cortisol levels increase in proportion to exercise intensity but the secretory limit is also dependant on exercise duration. A "threshold intensity" of ≥60% of maximal oxygen uptake (VO_{2max}) in exercise lasting at least 20–30 min has been reported and above this relative intensity large elevations in blood cortisol levels can occur.[33,34]

Measuring cortisol in saliva can successfully provide a reference for blood cortisol levels. Significant correlations have been reported between blood and salivary cortisol concentrations at rest (Table 1).[22,35] During submaximal exercise, Port[36] reported a significant correlation of salivary and blood cortisol, where it was suggested that the rise in cortisol coincided with the onset of blood lactate accumulation. However, in this study, the blood-saliva relationship was not consistent during the maximal effort. Post-exercise, significant correlations were found between salivary and blood concentrations of cortisol, following intense standardized exercise protocols,[4,29,30,37] a 30-s Wingate test[38,39] and competitive training matches.[40–42] The peak cortisol increase is reported to occur at 20 min post-exercise in blood[43] and at around 30 min post-exercise in saliva.[30,44]

One important characteristic of saliva is that it represents the free concentration of steroid hormones in blood and thus, the biologically active portion of the hormone. Studies have shown that cortisol changes in response to exercise are more pronounced in saliva compared with blood.[29,45,46] Gozansky et al.[47] provided evidence to support that salivary cortisol represents the biologically active, free fraction of blood cortisol, as in this study salivary cortisol measures displayed a greater relative increase in response to exercise and intravenous administration of corticotrophin-releasing hormone compared with blood cortisol measures. Therefore, cortisol in saliva may provide a better measure for the assessment of dynamic hypothalamic–pituitary–adrenal axis activity. Further evidence comes from Crewther et al.[39] who validated the use of salivary cortisol measures in response to a short, high-intensity cycle bout, also displaying that the hormonal changes to exercise are greater in saliva than corresponding blood measurements. However, monitoring cortisol responses must be done via separate assessment for each individual due to great variability within athletic populations.[38,40] Taking these factors into consideration, together with the fact that salivary measures of steroids are unaffected by variations in flow rate, we suggest that salivary cortisol measures can provide an enhanced measure of plasma and/or total cortisol concentrations in response to exercise.

Cortisol monitoring in sports can indicate the stress response to physical exertion as in exercise or training. The increased exercise-induced cortisol response is usually characterized by a hormonal adaptation to exercise.[31] Monitoring the hormonal response to exercise can provide a useful indicator of the onset of overreaching or overtraining; a robust rise in resting cortisol levels is reported as an approach to assess excessive training fatigue or the onset of overreaching.[49] Conversely, an adrenocortical dysfunction or "exhaustion" of the HPA axis to exercise is reported in overreached athletes, which is manifested by a markedly lower than normal cortisol response post-exercise.[48–50] Aldercreutz et al.[51] proposed the use of free testosterone/cortisol ratio as a diagnostic or preventive test to detect overtraining, as it gives an indication of the anabolic/catabolic balance in response to training. A decrease in testosterone/cortisol ratio by >30% may indicate "insufficient regeneration" and the onset of non-functional overreaching which, if not diagnosed correctly, may subsequently develop into overtraining syndrome.[50] Acute increases in salivary cortisol and decreases in the testosterone/cortisol ratio were found following a rugby match, which were restored within 4 h.[52] Similarly, salivary cortisol concentration displayed acute increases following a competitive kickboxing match,[53] a wrestling competition[54] and after a treadmill Bruce test in active male participants.[55]

In regards to training, increased concentrations of salivary cortisol, testosterone and decreases in the testosterone/cortisol ratio were reported during 3 weeks of intensive training and competition in elite rugby players.[56] Similarly, increased concentrations of salivary cortisol were found during a period of intensive training and competition in basketball players.[57] Since salivary measures of steroids

were suggested to provide a more sensitive marker of changes in steroid hormones than blood,[39,45,47] then their use may provide additional information in terms of understanding the stress responses of exercise and training.

Testosterone: Testosterone is the primary steroid hormone within the androgen family and its secretion is regulated by the hypothalamic–pituitary–gonadal axis.[59] It is synthesized and secreted from the Leydig cells of the testes in males and from the ovaries in females with a smaller amount also produced by the adrenal glands.[59] Testosterone exerts anabolic actions upon muscle tissue, as it contributes to muscle growth by increasing protein synthesis and decreasing protein degradation, thus enhancing muscle strength-related performance.[58] Indirect anabolic actions of testosterone include stimulating the secretion of other anabolic hormones such as growth hormone. Most of the circulating testosterone is bound to albumin and sex-binding globulins (∼95–98%) while a small amount (∼2–5%) remains free or unbound in the circulation.[59] Subsequently, the unbound concentrations reflect the biologically available fraction of testosterone in the circulation available to target tissues.

In males, significant correlations were reported between salivary and blood concentrations of testosterone at rest.[5,60–62] Salivary measures of testosterone can provide a reliable indicator of serum or plasma concentrations, as salivary testosterone concentrations were found to be strongly correlated with measures of bioavailable testosterone.[63] In regards to acute exercise, Crewther et al.[39] showed that the relative peak increase in salivary testosterone to a short high-intensity sprint exceeded the relative increases in total and free testosterone in plasma, suggesting that salivary measures are more sensitive for assessing the hormonal response to exercise. A circadian rhythm is also evident in salivary testosterone levels with the highest concentrations in the morning (08:00 h) and the lowest in the evening (20:00 h).[64]

Increases in testosterone levels are evident following resistance exercise, where acute increases in anabolic hormones are essential for muscular adaptation and muscle growth.[65] Several studies suggested that a relationship exists between measures of salivary testosterone and strength-related performance capacity.[38,39,58,66–68] Monitoring salivary testosterone levels in sports is a practical way to determine the preferred volume and intensity of exercise and this in turn should lead to enhanced functional gains.[67] Beaven et al.[67] showed that the maximal salivary testosterone levels of each individual were linked to increased gains in strength; in this study a consistent protocol-dependent testosterone response was evident and significantly higher strength gains were observed when the protocol was based on each individual's maximal, salivary testosterone response post-exercise. Therefore, monitoring acute and chronic individualised salivary testosterone responses may assist in the optimisation of training prescription to further enhance muscle-related performance capacity.

Dehydroepiandrosterone: Dehydroepiandrosterone (DHEA) is one of the major steroid hormones secreted from the adrenal glands, which serves as a precursor of other steroid hormones including testosterone. Similarly to the abovementioned steroid hormones, DHEA is also found in saliva secretions. Granger et al.[69] validated the use of salivary DHEA, as strong linear relationships were evident between serum and saliva levels of DHEA in both males and females. Regarding anabolic hormonal activity in response to exercise, a gender difference between salivary and serum testosterone concentrations was reported[62]; in females very low testosterone concentrations are usually observed compared with males, which is mainly attributed to the fact that testosterone derived from the ovaries is subsequently converted to estradiol and progesterone. Therefore, in female athletes salivary testosterone concentrations may not be a reliable indicator of the anabolic response of exercise. For that reason Fillaire and Lac[70] proposed the use of salivary DHEA as a substitute for salivary testosterone measurements to assess training responses in elite female handball players; significant correlations were found between salivary testosterone and salivary DHEA both at rest and following a training match.

Salivary IgA: Secretory IgA is the most abundant antimicrobial protein found in mucus secretions, including saliva in the mouth. As part of innate immunity, salivary IgA (SIgA) is considered the best indicator of mucosal immunity as it acts as the first line of defence by neutralizing and preventing viral pathogens entering the body via mucosal surfaces,[71] which are mainly responsible for infections of the upper respiratory tract. A "J-shaped" relationship has been presented by Nieman[72] between the level of physical activity and susceptibility to upper respiratory tract infections (URTI), where a high training load is often related to increased incidence of URTI. Decreases in SIgA levels have been shown to be associated to increased incidence of upper respiratory tract infections in elite professional athletes[73–77] and college football players.[78] An immediate decrease in SIgA is seen after a bout of acute, prolonged strenuous exercise which usually recovers within 24 h post-exercise.[7] Strenuous long-term training is associated with chronic suppression of mucosal immunity lasting 7 days or more.[7] During this "open window" period of depressed mucosal immunity athletes are more susceptible to URTI, which will in turn negatively affect training and performance.[79]

The acute effects of exercise on SIgA response are not consistent in the literature, with the majority of studies reporting decreases in SIgA post-exercise levels,[80–85] although some studies have reported no change[86–88] or even increases in SIgA concentration following acute exercise.[89–91] The overall intensity of the exercise bout seems to influence the post-exercise response of SIgA, whereas short duration, high-intensity exercise was reported to induce increases in SIgA secretion rate.[91] Conversely, strenuous and prolonged bouts of acute exercise compromise SIgA levels; a 21% decrease in SIgA concentration and a 25% decrease in SIgA secretion rate were reported in trained runners following a competitive

marathon race.[84] Generally, increases are seen in response to short bouts (<30 min) of high intensity exercise (>80% VO_{2max}), whereas no change or falls are seen with very prolonged exercise (>2 h).[7]Hard and demanding training may compromise mucosal immunity. Engaging in heavy training involving bouts of high intensity or high volume[92] for prolonged periods of time[74] may cause decreases in SIgA concentrations thus leaving the athletes susceptible to picking up infections. Both cross-sectional studies[80] and longitudinal studies have been conducted to assess the chronic effects of intensive training on mucosal immunity.[74–78,82,93–96] A cross-sectional study by Tomasi et al.[80] was the first to demonstrate the chronic suppression of mucosal immunity when engaging in long-term exercise training; lower SIgA levels were evident in elite cross-country skiers compared with recreational athletes. Longitudinal studies are most popular for monitoring the effects of a training program on SIgA levels in athlete groups. Engaging in long-term, strenuous training resulted in decreased levels of SIgA in triathletes,[74] swimmers,[76,93,94] kayakers,[82,95] distance runners[96] and football players.[78] Taking the evidence together, the cohort of highly trained athletes experience immunodepression during periods of heavy training which is manifested by decreased levels of SIgA.[6,7]

Monitoring SIgA levels can be useful in terms of identifying excessive training load as well as determining the risk of respiratory infection in elite athletes. It has been shown that decreased concentration and secretion rate of SIgA are associated with increased salivary cortisol levels,[57] which may indicate that elevated cortisol levels may serve as a precursor for suppression of mucosal immunity. Assessment of mucosal immune status has conventionally involved monitoring salivary immunoglobulins (SIgA, SIgM, SIgG) as well as albumin and saliva osmolality.[6] Reporting of SIgA levels can be done in terms of absolute concentrations or SIgA secretion rate as well as in relation to protein (usually albumin) to correct for any changes on saliva volume, and in relation to osmolality to assess effects of dehydration.[78] An absolute concentration of less than 40 mg/L[76] or secretion rate of less than 40 μg/min[78] have been reported to relate to increased URTI incidence in athletes. The most physiologically significant method of reporting seems to be the SIgA secretion rate because it takes flow rate into account and represents the actual amount of SIgA available in the secreted saliva for defence against pathogens.[7,78] Increases in saliva flow rate, which are often seen by stimulation of saliva flow (i.e. immediately after eating or drinking), may lead to a diluting effect and an obvious decrease in SIgA levels; similarly, decreases in saliva flow rate (anxiety, high-intensity exercise) may lead to a concentrating effect and apparent increases in SIgA concentrations, both leading to (most probably) false impressions of SIgA concentration.[7] Inadequate dietary practices and dehydration also seem to decrease SIgA levels and subsequently compromise mucosal immunity.[97] Monitoring mucosal immune status can inform guidelines for nutritional support and management of training load and so may minimise physical stress and subsequently the risk for URTI (reviews[6,7,98]).

Salivary IgM and IgG: Other immunoglobulins present in the oral mucosa are salivary immunoglobulin M (SIgM) and salivary immunoglobulin G (SIgG). SIgM and SIgG, together with SIgA all contribute to mucosal immunity in the defence against URTI. In contrast to SIgA, few studies have examined changes of other salivary immunoglobulins in response to acute exercise and training. SIgG levels have been found to be unchanged in response to acute exercise in both athletes and healthy participants,[6] whereas SIgM displays a parallel decrease with SIgA levels and is usually restored within 24 h.[7] Acute decreases in SIgM concentrations were evident following a 2-h exhaustive cycling in competitive cyclists,[81,99] during a competition in female hockey players[100] and following brief supramaximal interval cycling in recreational athletes.[101] In response to exercise training, SIgM concentration has been found to decrease after each training session in elite swimmers.[93,95]

Alpha-amylase: Salivary α-amylase is the most dominant enzyme in the pancreatic juice and saliva, responsible for degradation of starch and glycogen to maltose. Saliva contains a number of components with antimicrobial action which contribute to innate mucosal immunity, with α-amylase being one of them. Salivary α-amylase inhibits bacterial adherence and growth to epithelial surfaces.[102] Similarly to cortisol, salivary α-amylase has also been proposed recently as a biomarker of body stress and sympathetic nervous system activity.[7] Ehlert et al.[103] showed increased sympathetic activity via significant increases in salivary α-amylase activity after injection of yohimbine (an alpha-2 adrenergic receptor antagonist) and Klein et al.[104] supported these results reporting increased salivary α-amylase levels after consumption of caffeine which is known to stimulate sympathetic activation. As exercise induces physiological strain, the use of salivary α-amylase as a measure of sympathetic activity under exercise conditions has been proposed.[105] Chatterton et al.[106] reported increased salivary α-amylase, epinephrine and norepinephrine concentrations after aerobic exercise, further supporting the findings of Rohleder et al.[107] for the use of salivary α-amylase as a marker of increased exercise-induced plasma catecholamines levels and increased sympathetic adrenal activity. Subsequently, elevated levels of α-amylase in saliva can serve as an indication that saliva secretion was primarily induced by adrenergic stimulation.

The utilization of salivary α-amylase monitoring in response to exercise can be similar to that of cortisol, as both serve as salivary biomarkers reflecting stress response to exertion.[7,107–112] Yet, salivary α-amylase activity has been reported to be a more sensitive marker of exercise-induced stress than cortisol, as it is produced locally in the salivary glands controlled by the autonomous nervous system, instead of being transported from blood to saliva in the case of cortisol.[108] As physical exercise causes activation of the sympathetic nervous system, it is expected that sali-

vary α-amylase will display increases in response to exercise. Kivlighan and Granger[105] reported higher levels of salivary α-amylase compared with cortisol in response to a maximal cycle ergometer exercise task. In this study, salivary α-amylase reactivity explained a bigger part of the individual differences in dominance and team bonding compared with cortisol reactivity, suggesting the use of salivary α-amylase in response to psychological approach to competition. Furthermore, α-amylase levels in saliva following a progressive treadmill test were found to be highly correlated with anaerobic threshold, supporting the use of salivary α-amylase as a valid indicator of the anaerobic threshold.[109] In accordance to this, de Oliveira et al.[110] found acute increases in salivary α-amylase and total protein levels following incremental cycling, and moreover in this study, a strong relationship between measures of salivary α-amylase and blood lactate was evident. Furthermore, an elevated concentration of salivary α-amylase was found in young athletes immediately following a Taekwondo competition.[111] Salivary α-amylase activity is increased in response to acute exercise and the magnitude of the increase is mostly dependent on exercise intensity.[7] Acute increases in salivary α-amylase secretion rate and concentration were evident following intensive exercise of either shorter[91] or longer duration,[112] whereas prolonged submaximal exercise did not affect salivary α-amylase activity or secretion rate.[113] Even though salivary α-amylase responses have received increasing interest in response to acute exercise,[110–112] no studies to date assessed the chronic effects of training on salivary α-amylase concentration or secretion rate. Since salivary α-amylase responses express the sympathetic nervous system activity to exertion, it is speculated that decreased levels of salivary α-amylase concentration and/or secretion rate may exist in elite athletes or over a heavy training period, which might demonstrate the sympathetic withdrawal (or even the parasympathetic activation) of the nervous system which is likely to happen during non-functional overreaching.[114] However, research is needed to validate this. Taking the evidence together it is suggested that the stress response to (acute) exercise may be assessed by monitoring both salivary cortisol and salivary α-amylase, for a more precise prescription of training and recovery cycles in athletes.

Lysozyme: Lysozyme is an enzyme found in mucus secretions which contributes to innate mucosal immunity. Salivary lysozyme has antimicrobial properties and its catalytic activity facilitates destruction of bacteria by breaking down the polysaccharide wall of the bacterial cell.[7] It has been shown that salivary lysozyme concentration and secretion rate are negatively influenced by psychological stress and furthermore, salivary lysozyme levels were found to be lower in students waiting for examination compared to after completion of all exams.[115]

Only a few studies have examined the effects of exercise (acute or chronic) on salivary lysozyme concentration. Similarly to α-amylase, the salivary lysozyme response to exercise also seems to depend on exercise intensity. Increases in salivary lysozyme secretion rate were evident in response to acute bouts of intense exercise of both shorter and longer duration, whereas no change was seen at a lower submaximal load.[91] In accordance, acute increases in salivary lysozyme concentration were evident following a progressive rowing test.[116] Regarding exercise training, salivary lysozyme responses are similar to those of the other antimicrobial proteins. A study conducted at the Australian Institute of Sport has found lower salivary lysozyme concentrations (∼50%) in elite rowers compared to sedentary controls over a 5-month training season.[116] Assessing acute and/or chronic salivary lysozyme responses, along with the other antimicrobial proteins, may provide a physiological indication of the innate mucosal immune defence against URTI during periods of heavy training.

Lactoferrin: Lactoferrin is an antimicrobial protein present in mucus secretions including saliva and, together with the other antimicrobial proteins, contributes to innate mucosal immunity. Lactoferrin has both anti-inflammatory and antimicrobial properties, such as preventing bacterial growth by sequestering ferric iron from the bacteria and subsequently acting against a number of viruses responsible for respiratory infections.[7]

Lactoferrin responses in regards to exercise or training have not received much interest to date. Plasma lactoferrin concentration was found to increase immediately after completing a 160-km triathlon race.[117] Similarly, more recently serum lactoferrin concentration demonstrated an acute increase following a ∼30-min running exercise and this was associated with antibacterial activity in host defence.[118] However, investigations assessing the effects of acute exercise on salivary lysozyme concentration are scarce. In regards to training, salivary lactoferrin responses were found to follow the same pattern of the other antimicrobial proteins; lower salivary lactoferrin concentrations were found in elite rowers compared with non-exercising controls over a training season.[116] In accordance, the secretion rate and absolute concentration of salivary lactoferrin were reduced over a competitive training season in basketball players.[57] Both studies provide evidence to highlight the fact that intense exercise over a prolonged period compromises markers of mucosal immunity, including salivary lactoferrin, and may subsequently increase the risk of URTI.

5. Other potential measures in saliva

Growth hormone: Human growth hormone (hGh) or somatostatin is a polypeptide hormone secreted from the anterior pituitary glands. hGh secretion is regulated by the hypothalamus with the growth hormone-releasing hormone inducing release of hGh and somatotropin inhibiting its secretion. hGh has anabolic and lipolytic effects upon muscle tissue; it is popular for increasing muscle mass through muscle hypertrophy and sarcomere hyperplasia. It is secreted

in a pulsatile manner with 6–12 spurts throughout the day following a circadian rhythm. A number of stimulating factors influence hGh release including sleep, age, gender and dietary intake, with the most significant stimuli of which are sleep and physical exercise. hGh secretion peaks at around 1 h after the onset of sleep (around 01:00 h); however, great variability exists between individuals. Physical exercise of both resistance and endurance type is suggested to stimulate hGh release. Resistance exercise training induces increases in hGh and increases in muscle mass, however recent findings suggest that the increases are mostly attributed to insulin like growth factor I.[119] High intensity exercise (above the lactate threshold) for a minimum of 10 min elicits a large release of hGh, and exercise training above the lactate threshold may magnify the pulsatile resting release of hGh throughout the day.[120]

Resting salivary concentrations of hGh were reported to partly represent their respective circulating concentrations. A study by Rantonen et al.[35] reported that salivary measures of hGh were significantly correlated to those of serum at rest following an overnight fast in 51 healthy subjects. In this study salivary hGh concentrations were found to be 1000-fold lower than serum concentrations, yet this did not affect the relationship. However, no studies exist reporting relationships between salivary and blood measures of hGh in response to exercise. Assessing hGh responses may determine the anabolic effects of exercise on muscle growth.[119] Furthermore, salivary hGh assessments may be useful in paediatric exercise science, as they provide a non invasive method for determining hGh in children and its response following exercise. Therefore, studies assessing relationships between salivary and blood measures of hGh are lacking and further research is needed.

Insulin-like growth factor I: Insulin-like growth factor I (IGF-I) is an anabolic hormone of similar structure to insulin. Production is stimulated by hGh as IGF-I is a primary mediator to the effects of hGh, whereas exercise stimulates the hGh/IGF-I axis.[121] IGF-I plays an important role in childhood growth as well as total systemic body growth in adults. Physical exercise stimulates IGF-I release whereby this increase in IGF-I concentrations post-exercise was found to be more apparent in saliva; Antonelli et al.[122] reported a rise in salivary IGF-I (sIGF-I) levels following physical exercise in well-trained athletes but this increase was not apparent in plasma. However in this study, a relationship was reported between plasma measures of IGF-I and sIGF-I post-exercise. Taking these collectively, this study suggests that salivary measures of IGF-I may be a more sensitive approach to assess IGF-I activity, which may be attributed to the fact that salivary measures could display more accurately the unbound fractions of this hormone in the circulation. However, studies are scarce assessing sIGF-I concentrations in response to exercise and further research is needed in this regard.

Insulin: Insulin is a peptide hormone responsible for the active glucose uptake by the cells and energy metabolism in the body. Diminished secretion of insulin leads to hyperglycaemia, glucosurea and type 2 diabetes mellitus. The structure of salivary insulin is similar to that of pancreatic insulin leading to the speculation that pancreatic insulin is probably transported into saliva.[123,124] However, the presence of insulin mRNA *in vitro* cultured parotid glands led to the assumption that insulin may be actively synthesised in the parotid glands of the mouth.[125] During the past decades it has been shown that a positive linear relationship exists between plasma and salivary immunoreactive insulin in diabetic patients following an oral glucose tolerance test.[126–128] However, this relationship was not examined in response to physical exercise. Assessing salivary insulin responses in response to exercise may provide a non-invasive method in determining the metabolic demands of exercise and training.

6. Summary

Scientists in the area of sports have focused on the collection of blood for measurements of hormones, peptides and immunological compounds to study training adaptations and the body's responses to acute exercise or training. However, evidence is constantly accumulating suggesting that saliva collection can be a useful alternative to blood for the assessment of some of these compounds. The measurement of whole saliva composition can provide a stress-free, non-invasive method to assess immunological and endocrinological status associated with exercise as well as to examine the loads of training and subsequently the risk for overreaching and/or upper respiratory tract infections. Assessing steroid hormones in saliva can provide a reference for the circulating blood concentrations where this in turn can aid in prescribing exercise training. Salivary cortisol can provide an indicator of the body's stress response to acute exercise or training, whereas assessment of the salivary testosterone/cortisol ratio should indicate the anabolic/catabolic adaptations of training. Monitoring anabolic hormones such as testosterone and DHEA in saliva may assist in designing enhanced training programs to maximise strength adaptations. Analysis of immunological compounds, such as immunoglobulins and other antimicrobial proteins in saliva can identify the risk of respiratory infections, and subsequently prevent the onset of non-functional overreaching and overtraining. Evidence is constantly increasing to support the use of saliva as a non-invasive tool for monitoring biomarkers in the field of sport and exercise science. Therefore it is concluded, that the assessment of salivary composition can provide a feasible and reliable tool for monitoring several steroid hormones, markers of stress and immune makers in sports and exercise, and thus saliva collection and analysis has essential and significant applications in exercise testing and prescription.

Practical implications

- Saliva analysis is a relatively new tool for assessing biological markers of training (hormones, immunoglobulins and antimicrobial proteins).
- Salivary cortisol can provide an indicator of the body's stress response to acute exercise or training, whereas assessment of the salivary testosterone/cortisol ratio indicates the anabolic/catabolic adaptations of training. Monitoring anabolic hormones such as testosterone and DHEA in saliva may assist in designing better training programs to maximise strength adaptations.
- Analysis of immunological compounds, such as immunoglobulins and other antimicrobial proteins in saliva can identify the risk of respiratory infections.
- Saliva can provide a useful, non-invasive alternative to blood collection because it can be collected rapidly, frequently and without stress. Another advantage is that it requires no medical training and can be performed in the sports field.

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