HHS Public Access

Author manuscript

Curr Opin Behav Sci. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Curr Opin Behav Sci. 2019 August ; 28: 58–65. doi:10.1016/j.cobeha.2019.01.007.

Salivary Biomarkers in Psychoneuroimmunology

Christopher G. Engeland[1,2,*], **Jos A. Bosch**[3], **Nicolas Rohleder**[4]

[1]The Department of Biobehavioral Health, The Pennsylvania State University, University Park PA, 16801 [2]The College of Nursing, The Pennsylvania State University, University Park PA, 16801 [3]Department of Medical Psychology, Academic Medical Centers (UMC) Amsterdam, Amsterdam, The Netherlands [4]Chair of Health Psychology, Institute of Psychology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Abstract

As molecular biology advances, an increasing number of proteins are becoming detectable at very low levels in different biological tissues. In this regard, saliva holds vast promise. Unlike blood, saliva can be sampled 1) non-invasively; 2) across all ages (newborn to elderly); 3) in the field; 4) by study participants; and 5) many times per day. With respect to psychoneuroimmunology (PNI), physiological measures of stress such as cortisol have been well characterized. Alpha amylase provides another physiological index of stress; it is a measure of autonomic nervous system activation and is quantifiable in saliva. Other salivary measures, such as inflammatory biomarkers and immunoglobulin A (IgA), provide valuable information pertaining to the effects of stress on inflammation, mucosal immunity, and oral health. Importantly, due to various methodological issues and a lack of strong correlation between saliva and blood measures, investigators should proceed with caution in drawing conclusions from measures of salivary inflammation that pertain to systemic immunity or generalized health.

Keywords

inflammation; IgA; alpha amylase; cytokine; stress; health; cortisol; C-reactive protein; saliva; immunoglobulin

Introduction

The increased availability of salivary biomarkers has become extremely valuable to behavioral scientists, and allowed the field of stress research to make fast progress. Before salivary biomarkers, human psychobiology was investigated mainly by measurement of stress hormones in urine and blood, and by inference through indicators of physiological

*Corresponding author Christopher G. Engeland cge2@psu.edu.

Declarations of interest: None.

function, such as assessment of skin conductance or heart rate. Due to logistic difficulties or high costs associated with most blood-based measures, many early studies limited the assessment of biomarkers to a single time point, which had limitations and hampered the ability to understand the impact of stress under different (often dynamic) contexts and conditions. A typical example is the early finding that depression was associated with increased cortisol secretion; this had to be revised when salivary measurement of cortisol permitted researchers to sample more frequently, showing that depression is associated with an aberrant diurnal pattern of cortisol secretion (i.e., a flattened diurnal cycle) characterized by increased levels at some time points and lower levels at other times [1].

This increased opportunity to use saliva for non-invasive biomarker assessment has greatly increased our understanding of how acute and chronic stress, including psychiatric conditions, manifests in humans in the form of a distinct biological signature. Acute stress coincides with broadly altered functioning in a large number of physiological systems, most notably the brain, whereas research has mostly focused on activation of the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). When a threat to an organism is perceived, both systems are activated rapidly via endocrine (i.e., cortisol [HPA], epinephrine and norepinephrine [SNS]) and neural (SNS) pathways.

Despite being activated simultaneously, each of these systems has a different temporal response pattern, with SNS effects being apparent almost immediately, and cortisol secretion being delayed by about 20 minutes. This difference in temporal dynamics between SNS hormones (mostly epinephrine) and the HPA hormone cortisol appears to serve biologically relevant functions delineating an adaptive stress response [2]. At the level of the immune system, neuro-endocrine effects of acute stress appear to have immune-activating properties. For example, sympathetic activation causes the quick release of antibodies into saliva and the release (mobilization) of effector-type lymphocytes (e.g., NK cells, differentiated cytotoxic T cells) into the blood, whereby subsequent cortisol release may promote migration of these cells from the blood into peripheral tissues. In addition to these synergistic effects, there are indications that the sequential release of SNS and HPA hormones can have antagonistic effects. For example, there is evidence that the SNS first activates inflammatory mechanisms, which are later downregulated by cortisol. In this case, the difference in temporal patterns is thought to allow for a precautionary upregulation of a system in preparation for injury, followed by a down-regulation in the absence of an infectious stimulus. However, if systems are activated too often, or fail to activate, maladaptive regulation may be observed; this is denoted as allostatic load [3].

While blood-based biomarkers initially helped us understand these response patterns, the addition of saliva-based measures of HPA axis and SNS activity have allowed tighter sampling schedules and, hence, a better description of response patterns, thus having a major impact on this line of work.

Saliva-based measures of stress system activity

The HPA axis is characterized by pronounced circadian activity, with increases in the morning (cortisol awakening response [CAR]) and declines throughout the day. One-time

measurements of cortisol in blood have led to inconsistent findings, because timing of blood samples was typically not adjusted to the axis' circadian rhythm. Years of research using salivary cortisol have allowed researchers to understand that chronic stress affects parameters of the diurnal slope which becomes flatter, and after long stress exposure results in a state of hypocortisolism and loss of rhythmicity [4]. When assessing diurnal cortisol slopes, current recommendations are to use date from more than one, ideally three or more, sampling days, to use the wake-up cortisol level as a starting point [5,6] and to employ multilevel models for data analysis [7]. Despite adhering to these guidelines, a significant proportion of cortisol variability is explained by day-to-day fluctuations [8].

Salivary alpha amylase (sAA) has been used as a proxy measure of autonomic nervous system (ANS) activity during acute and chronic stress. sAA is an enzyme secreted by acinar cells in saliva glands, in which innervation by sympathetic nerves via noradrenaline signaling induces protein release. As sAA is the predominant protein in saliva, and can be easily and relatively cheaply assessed, it rapidly became popular as a measure of sympathetic activation [9–11]. Although stress-induced increases in sAA poorly correlate with other SNS markers [12], this is a generalized issue with the assessment of SNS markers and is not regarded as a disqualifying property. Moreover, recent pharmacological work has suggested that sAA might be a marker of central noradrenergic activity [13]. The assessment and interpretation of sAA is not as straightforward as initially thought. For example, levels can vary depending on sample timing, saliva collection method, and stressor type, and it is recommended that saliva flow rate is taken into account as a potentially confounding factor. The field would greatly benefit from further methodological studies that help standardize the use of sAA in research. These cautionary notes notwithstanding, recent work has for example shown that sAA stress responses are higher in individuals with self-reported early adversity [14]* and in people with lower reported self-compassion [15]. Hence, the addition of sAA to the methodological repertoire permits non-invasive assessment of ANS function, although sAA is a less direct proxy for the SNS than cortisol is for the HPA axis.

Autonomic activation occurs with a much lower threshold (i.e., it is observable even with mild stressors; [16]) than HPA activation. As a result, sAA tends to respond to acute stressors that are not powerful enough to activate the HPA axis, such as restriction of cell phone use in college students [17]. In a recent study, women with a history of trauma showed sAA, but not cortisol, reactivity to a trauma reminder during a clinical interview; those increases were positively associated with neural reactivity of trauma-related brain areas [18]*. The authors concluded that sAA might be a more reliable marker of trauma-related reactivity and hypervigilance than measures of cortisol.

Alterations of sAA circadian patterns have also been reported in *chronic* stress. In a one-year longitudinal study of caregivers to brain cancer patients, amylase diurnal rhythms, which are typically characterized by a sharp decline in the first hour after waking and increase thereafter, became flatter over time [19]. Altered diurnal sAA patterns have also been found in female sexual minorities (non-heterosexual women; [20]*), and in police officers with lower subjective social status [21]. These patterns of sAA alteration support strong associations with the ANS. As discussed below, questions still needing attention are the relative contribution of sympathetic and parasympathetic components, and methodological

requirements in regard to sampling protocols [22]. However, amylase diurnal rhythms appear to be more stable than those of cortisol [23].

When analyzing the effects of acute and chronic stress on health, research on stress systems alone has not proven useful for understanding pathophysiological mechanisms. Investigating the combined effects of both stress systems on target systems, such as immunity and inflammation, has the potential to fill this important gap. Inflammation, while necessary for fighting off infections and healing tissues, has a strong potential to damage the organism [24]. A slow but steady increase in systemic inflammation is a common part of the aging process, denoted as *inflammaging* (see [25] for review). While such slow increases of low-grade inflammation occur with aging, they are most likely not a simple consequence of becoming older, but caused by accumulation of environmental impacts on the immune system, some of which can be psychological in nature. Both acute and chronic stress exposure promote inflammation in the absence of acute infection or tissue damage [26], thereby providing a potential link between stress and adverse health outcomes. In addition, stress can weaken immune defenses through negative effects on other components of the immune system, e.g., adaptive immunity [27]. Assessing inflammation at multiple time points and in natural settings would be highly informative. Salivary biomarkers allow for this and provide a better understanding of the links between stress and inflammation, which is critical in PNI research.

Factors that modulate inflammation in saliva

Throughout our entire lifespan, oral health modulates salivary inflammation. For instance, very young children (birth to 71 months) with early childhood caries exhibited elevated salivary inflammation (based on cytokine levels) compared to age-matched controls [28]. Moreover, oral health tends to worsen with age, which limits the usefulness of salivary inflammation within PNI. By adulthood, 80% of the population has gingivitis [29] and almost half of individuals over 30 years of age have some degree of periodontal disease [30], both of which relate to higher levels of salivary cytokines and C-reactive protein (CRP) [31–33]. Other oral conditions are also associated with elevated salivary inflammation, such as oral lesions (personal observations, [34] [35]) and oral squamous cell carcinoma [36]. The local production of cytokines that occurs under these types of conditions likely accounts, at least in part, for the weak (or lack of) correlation often reported for measures of inflammation between saliva and blood (see [37] for review).

A number of systemic and inflammatory diseases or conditions have also been associated with elevated inflammation in saliva. These include, but are not limited to, acute myocardial infarction [38], heart failure [39], cardiovascular disease [40], metabolic syndrome [41], and rheumatoid arthritis [42]. Finally, a large number of factors not specifically related to disease states have been associated with higher salivary inflammation, including BMI [43], obesity [44,45], short sleep duration [46], obstructive sleep apnea [47], smoking [48], and even cell phone use, assumedly due to heat production [49]. As a result, inflammatory cytokines do not serve as reliable indicators of systemic inflammation, as they often do not correlate well with blood values (cytokine values are often higher in saliva than in blood, assumedly due to multiple sources of local production; personal observations; see [29] for review).

In contrast to cytokines, there is no local production of CRP in the mouth as it is produced by the liver. As a result, CRP levels are much more dilute in saliva than in blood, e.g., 1600x more dilute [50]. However, similar to cytokines, CRP levels are higher in saliva under conditions of poor oral health (e.g., gingivitis), likely due to 1) higher infiltration of CRP from blood and 2) the presence of blood in the mouth from damaged tissues. As a result, some studies report significant correlations for CRP between saliva and blood, whereas others do not (see [51] for review).

Theoretically, individuals with good oral health should have salivary levels of inflammation that closely relate to levels of systemic inflammation. However, this has yet to be demonstrated in empirical studies and, as discussed above, many factors outside of oral health can seemingly affect salivary inflammation.

How might researchers determine good versus poor oral health? Ideally an oral examination should be conducted by a trained clinician, but this is seldom possible. Fortunately, even a rudimentary assessment can allow for the identification of individuals with poor oral health. Some examples of simple questions are: Do you have good oral health? y/n; Rate your oral health on a scale of 1–5; Do your gums sometimes bleed when you brush or floss? Y/n. The inclusion of such questions is recommended for research studies involving salivary inflammation.

Utility of salivary inflammation in psychoneuroimmunology

As reviewed by Slavish et al. (2015), saliva may have good utility for detecting inflammatory change from baseline, for instance in response to *acute* stressors [37]. In this regard, salivary levels of the classic pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) were found to increase fairly consistently in response to acute stress [37]. Moreover, these changes generally appeared to mirror, possibly with some time lag, similar changes in blood. Note that these findings were based on a relatively small number of studies and more work is needed in this area.

Some recent studies have demonstrated elevations in salivary inflammation in response to acute stressors, such as university examinations [52]) and public speaking [53]). In one study, the Trier Social Stress Test (TSST) and the retrieval of angry memories were each shown to increase salivary levels of IL-1β, IL-6, and TNF-α in healthy young adults. Distracting individuals related to lower salivary IL-1β levels after memory retrieval but not after the TSST, suggesting that emotional reactivity plays a role in salivary inflammation [54]*.

A number of recent studies have assessed the efficacy of interventions aimed at reducing stress on salivary inflammation. Two separate studies found that mindfulness-based stress reduction lowered salivary inflammation after four or six weeks of training [55,56]. Intriguingly, a single 20-minute session of yoga breathing reduced salivary inflammation levels compared to controls [57].

Although cytokine measures in saliva are seemingly not reliable indicators of systemic inflammation, a few studies have found relations between salivary inflammation and longer-

term measures such as *chronic* stress. For instance, Martinez et al. (2018) studied 111 individuals living in thirty low-income Mexican-origin families with at least one immigrant [58]**. They found that household fear of deportation and family conflict (both chronic stressors) strongly related to elevated levels of inflammation in saliva [58]**. While salivary CRP (sCRP) levels do not appear to change readily to acute stress [37], a recent study reported that negative emotionality related to higher sCRP levels, whereas effortful control related to lower sCRP levels [59]. Finally, female partners of military veterans with traumatic brain injury (TBI) who reported higher blame/anger exhibited higher salivary inflammation [60]. The implications of such findings on systemic inflammation, or other parameters of health, are so far unclear.

To sum, there appears to be utility in assessing inflammatory change (from baseline) in saliva across multiple time points. It is well established that short-term stress can cause temporary increases in inflammation which in time return to baseline. Assessment of this inflammatory response and its recovery period appears viable in saliva, which can be done more easily (e.g., in the field) and in a non-invasive manner compared to blood. One additional point: given that such measures are increasingly being utilized in PNI studies, both the stability and replicability of salivary inflammatory measures need to be delineated in future research.

Stress and salivary secretory immunoglobulin A (S-IgA)

Most infections (~95%) start at mucosal surfaces such as the lining of the respiratory and gastrointestinal tracts. These surfaces are protected by various antimicrobial proteins, which are secreted by local glands and form a first line of immune defense. Among these proteins, secretory immunoglobulin A (S-IgA) represents the main agent of adaptive immunity [61,62]. S-IgA can bind to antigens, such as microbes and toxins, in a highly selective manner which prevents such antigens from attaching to or penetrating mucosal surfaces. There are several compelling reasons to study salivary S-IgA in behavioral medicine. First, the salivary glands are strategically located at the portal of entry to the respiratory and gastrointestinal tracts, and play a key role in the maintenance of oral health [63,64]. Second, salivary S-IgA may serve as a representative model of IgA-secreting B-cells across different mucosal sites [65]. In this manner, salivary S-IgA may provide a general overview of the entire secretory immune system.

A third reason is that the release of S-IgA is under strong neuroendocrine control [66]. The autonomic nerves that innervate the salivary glands robustly impact salivary S-IgA secretion, whereby activation of the sympathetic nerves enhances S-IgA output [66,67]. This mechanism is thought to explain why acute stress generally increases salivary S-IgA [68–72]. Conversely, protracted stress is typically associated with decreased levels of S-IgA, possibly through cortisol’s inhibitory effects on S-IgA concentrations [68,69,73–75].

S-IgA is released into saliva in a two-step process [63]. First, IgA-producing B lymphocytes, which have migrated into the glandular tissues, produce and release IgA. The IgA molecule is then shuttled through the glandular cell, via binding to the transporter molecule Secretory Component (SC), and subsequently released into saliva as S-IgA (i.e., the IgA-SC complex).

This shuttling by SC also takes place when no IgA is bound, in which case only SC is released into saliva [63]. Hence, S-IgA and SC serve as indicators of IgA production and glandular transport capacity, respectively. By measuring both S-IgA and SC it can be estimated if stress-related S-IgA reduction is attributable to a decrease in IgA release by B-lymphocytes or a reduction in glandular transport capacity. The reductions in S-IgA concentrations during chronic stress appear to be due to lower availability of IgA; transport capability remains stable or increases in individuals who report high protracted stress levels [74]**,[76].

Human S-IgA is secreted as two distinct isotypes; IgA1 and IgA2. Differentiating between these isotypes is important as concentrations of S-IgA1, but not S-IgA2, are associated with an increased vulnerability to upper respiratory tract infections [77]. Interestingly, both acute and protracted stress appear to have a larger impact on S-IgA1 levels than on S-IgA2 [74,78].

Very little research on S-IgA has studied antibody release in response to antigen [62,79,80]. This is an area of future research, now that oral vaccines have been well-validated and are used more routinely [81]. Research on salivary S-IgA should also be developed with more attention to methodological issues. For example, flow rate should preferably be measured in addition to concentration (ug/ml) so that S-IgA output (ug/minute) can be estimated; this allows one to determine if changes in concentrations are secondary to flow rate (e.g., dry mouth during stress) [63].

In general, for optimal results in measuring salivary biomarkers (other than cortisol), we recommend avoiding absorbent materials, collecting unstimulated saliva samples (e.g., by passive drool), and recording flow rates (either by volume or weight per minute of collection) [63,82,83]. In addition, some published studies report using commercial assay kits that have not been validated for use in saliva; this may produce erroneous values. For a broader discussion of these methodological issues, see recent reviews [16,51]; see Box 1 for a summary of these issues.

Immunosenescence and telomeres

Another way that stress can alter immune function is through promoting cell senescence. Similar to chronological aging, prolonged psychological stress has been related to telomere shortening; in this manner, stress appears to contribute directly to cellular aging. Thus, oral tissues can provide insights into biological aging. The problem with using saliva samples for the determination of telomere length is that that the results are highly variable due to mixed cell types (i.e., saliva contains both leukocytes and buccal cells) [84]. A buccal (cheek) swab, which is equally non-invasive, will often yield better (less variable) results for research. For a recent review on psychological stress and telomeres see [85]*.

Future perspectives

See Box 2 for a summary of key points from the above review. As technology advances, other immune-related measures in saliva are increasingly being reported in PNI research. Such markers include cytokines with different primary functions than inflammation (e.g.,

IL-7, IL-12p70, IL-23, interferons), growth factors, adiponectin, anti-microbial proteins (e.g., endogenous lysozymes), and full characterizations of the oral microbiome. In addition to basic research, salivary biomarkers hold immense promise in the field of medicine. Saliva is increasingly being used to determine responses to vaccination, and has become important in the early detection of various cancers through the measurement of circulating tumor cells and tumor DNA fragments (i.e., liquid biopsies). For a recent review of the diagnostic potential of saliva in disease (i.e., to inform clinical decisions and as a predictor of post-treatment outcomes) see [86]*.

Acknowledgements

The authors thank Brooke C. Mattern who helped with the referencing and formatting of this manuscript.

References

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Miller GE, Rohleder N, Stetler C, Kirschbaum C: Clinical depression and regulation of the inflammatory response during acute stress. Psychosom Med 2005, 67:679–687. [PubMed: 16204423]
2. Sapolsky Robert; Romero MMAU: How Do Glucocorticoids Influence Stress Responses ? Preparative Actions *. Endocr Rev 2000, 21:55–89. [PubMed: 10696570]
3. McEwen B: Protective and damaging effects of stress mediators. New Englend J Med 1998, 338:171–179.
4. Miller GE, Chen E, Zhou ES: If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull 2007, 133:25–45. [PubMed: 17201569]
5. Segerstrom SC, Boggero IA, Smith GT, Sephton SE: Variability and reliability of diurnal cortisol in younger and older adults: implications for design decisions. Psychoneuroendocrinology 2014, 49:299–309. [PubMed: 25137484]
6. Kraemer HC, Giese-Davis J, Yutsis M, O'Hara R, Neri E, Gallagher-Thompson D, Taylor CB, Spiegel D: Design decisions to optimize reliability of daytime cortisol slopes in an older population. Am J Geriatr Psychiatry 2006, 14:325–333. [PubMed: 16582041]
7. Hruschka DJ, Kohrt BA, Worthman CM: Estimating between- and within-individual variation in cortisol levels using multilevel models. Psychoneuroendocrinology 2005, 30:698–714. [PubMed: 15854786]
8. Ross KM, Murphy MLM, Adam EK, Chen E, Miller GE: How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology 2014, 39:184–193. [PubMed: 24119668]
9. Bosch JA, Brand HS, Ligtenberg TJ, Bermond B, Hoogstraten J, Nieuw Amerongen AV: Psychological stress as a determinant of protein levels and salivary-induced aggregation of Streptococcus gordonii in human whole saliva. Psychosom Med 1996, 58:374–382. [PubMed: 8827800]
10. Rohleder N, Nater UM, Wolf JM, Ehlert U, Kirschbaum C: Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? Ann N Y Acad Sci 2004, 1032:258–263. [PubMed: 15677423]

11. Nagy T, van Lien R, Willemsen G, Proctor G, Efting M, Fülöp M, Bárdos G, Veerman ECI, Bosch JA: A fluid response: Alpha-amylase reactions to acute laboratory stress are related to sample timing and saliva flow rate. Biol Psychol 2015, 109:111–119. [PubMed: 25976524]
12. Thoma MV, Kirschbaum C, Wolf JM, Rohleder N: Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. Biol Psychol 2012, 91:342–348. [PubMed: 22954623]
13. Warren CM, van den Brink RL, Nieuwenhuis S, Bosch JA: Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase. Psychoneuroendocrinology 2017, 78:233–236. [PubMed: 28232237]
14*. Kuras YI, McInnis CM, Thoma MV, Chen X, Hanlin L, Gianferante D, Rohleder N: Increased alpha-amylase response to an acute psychosocial stress challenge in healthy adults with childhood adversity. Dev Psychobiol 2017, 59:91–98. [PubMed: 27577885] One of the first papers relating early adversity, a major predictor of altered adult life stress-reactivity as well as mental and physical disease, to increased alpha-amylase stress response.
15. Breines JG, McInnis CM, Kuras YI, Thoma MV, Gianferante D, Hanlin L, Chen X, Rohleder N: Self-compassionate young adults show lower salivary alpha-amylase responses to repeated psychosocial stress. Self Identity 2015, 14:390–402. [PubMed: 26005394]
16. Bosch JA: The use of saliva markers in psychobiology: mechanisms and methods. Monogr Oral Sci 2014, 24:99–108. [PubMed: 24862598]
17. Hunter JF, Hooker ED, Rohleder N, Pressman SD: The Use of Smartphones as a Digital Security Blanket: The Influence of Phone Use and Availability on Psychological and Physiological Responses to Social Exclusion. Psychosom Med 2018, 80:345–352. [PubMed: 29521885]
18*. Yoon SA, Weierich MR: Salivary biomarkers of neural hypervigilance in trauma-exposed women. Psychoneuroendocrinology 2016, 63:17–25. [PubMed: 26398002] First paper showing relationships between brain imaging indicators of a critical PTSD symptom cluster with salivary alpha amylase reactivity to trauma stimuli.
19. Rohleder N, Marin TJ, Ma R, Miller GE: Biologic cost of caring for a cancer patient: Dysregulation of pro- and anti-inflammatory signaling pathways. J Clin Oncol 2009, 27:2909–2915. [PubMed: 19433690]
20*. Austin SB, Rosario M, McLaughlin KA, Roberts AL, Sarda V, Yu K, Missmer S, Anatale-Tardiff L, Scherer EA: Sexual orientation and salivary alpha-amylase diurnal rhythms in a cohort of U.S. young adults. Psychoneuroendocrinology 2018, 97:78–85. [PubMed: 30015008] One of the first papers relating amylase diurnal rhythms with stigmatization related to sexual orientation.
21. Habersaat S, Abdellaoui S, Geiger AM, Urben S, Wolf JM: Low subjective social status in the police is linked to health-relevant changes in diurnal salivary alpha-amylase activity in Swiss police officers. Stress 2018, 21:11–18. [PubMed: 29037115]
22. Bosch JA, Veerman ECI, de Geus EJ, Proctor GB: A-Amylase As A Reliable And Convenient Measure Of Sympathetic Activity: Don’t start salivating just yet! Psychoneuroendocrinology 2011, 36:449–453. [PubMed: 21295411]
23. Out D, Granger DA, Sephton SE, Segerstrom SC: Disentangling sources of individual differences in diurnal salivary alpha-amylase: reliability, stability and sensitivity to context. Psychoneuroendocrinology 2013, 38:367–375. [PubMed: 22819683]
24. Couzin-Frankel J: Inflammation bares a dark side. Science 2010, 330:1621. [PubMed: 21163993]
25. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A: Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 2018, 14:576–590. [PubMed: 30046148]
26. Rohleder N: Stimulation of systemic low-grade inflammation by psychosocial stress. Psychosom Med 2014, 76:181–189. [PubMed: 24608036]
27. Segerstrom SC, Miller GE: Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull 2004, 130:601–630. [PubMed: 15250815]
28. Sharma V, Gupta N, Srivastava N, Rana V, Chandna P, Yadav S, Sharma A: Diagnostic potential of inflammatory biomarkers in early childhood caries - A case control study. Clin Chim Acta 2017, 471:158–163. [PubMed: 28579141]

29. Albandar JM: Periodontal diseases in North America. Periodontol 2000 2002, 29:31–69. [PubMed: 12102702]
30. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ: Prevalence of periodontitis in adults in the united states: 2009 and 2010. J Dent Res 2012, 91:914–920. [PubMed: 22935673]
31. Liukkonen J, Gürsoy UK, Pussinen PJ, Suominen AL, Könönen E: Salivary Concentrations of Interleukin (IL)-1β, IL-17A, and IL-23 Vary in Relation to Periodontal Status. J Periodontol 2016, 87:1484–1491. [PubMed: 27541079]
32. Lira-Junior R, Öztürk VÖ, Emingil G, Bostanci N, Boström EA: Salivary and Serum Markers Related to Innate Immunity in Generalized Aggressive Periodontitis. J Periodontol 2017, doi:10.1902/jop.2017.170287.
33. Balaji A, Chandrasekaran S, Subramanium D, Fernz A: Salivary Interleukin-6 - A pioneering marker for correlating diabetes and chronic periodontitis: A comparative study. Indian J Dent Res 2017, 28:133. [PubMed: 28611321]
34. Mozaffari HR, Ramezani M, Mahmoudiahmadabadi M, Omidpanah N, Sadeghi M: Salivary and serum levels of tumor necrosis factor-alpha in oral lichen planus: a systematic review and meta-analysis study. Oral Surg Oral Med Oral Pathol Oral Radiol 2017, 124:e183–e189. [PubMed: 28823317]
35. Tvarijonaviciute A, Aznar-Cayuela C, Rubio CP, Ceron JJ, Lopez-Jornet P: Evaluation of salivary oxidate stress biomarkers, nitric oxide and C-reactive protein in patients with oral lichen planus and burning mouth syndrome. J oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol 2017, 46:387–392.
36. Lee LT, Wong YK, Hsiao HY, Wang YW, Chan MY, Chang KW: Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2018, 47:699–707. [PubMed: 29174861]
37. Slavish DC, Graham-Engeland JE, Smyth JM, Engeland CG: Salivary markers of inflammation in response to acute stress. Brain Behav Immun 2015, 44:253–269. [PubMed: 25205395]
38. Ebersole JL, Kryscio RJ, Campbell C, Kinane DF, McDevitt J, Christodoulides N, Floriano PN, Miller CS: Salivary and serum adiponectin and C-reactive protein levels in acute myocardial infarction related to body mass index and oral health. J Periodontal Res 2017, 52:419–427. [PubMed: 27549083]
39. Dekker RL, Lennie TA, Moser DK, Miller CS, Ebersole JL, Chung ML, Campbell CL, Bailey A, Tovar EG: Salivary Biomarkers, Oral Inflammation, and Functional Status in Patients With Heart Failure. Biol Res Nurs 2017, 19:153–161. [PubMed: 27605566]
40. Gohel V, Jones J, Wehler C: Salivary biomarkers and cardiovascular disease: a systematic review. Clin Chem Lab Med 2018, 56:1432. [PubMed: 29630504]
41. Chauhan A, Yadav SS, Dwivedi P, Lal N, Usman K, Khattri S: Correlation of Serum and Salivary Cytokines Level With Clinical Parameters in Metabolic Syndrome With Periodontitis. J Clin Lab Anal 2016, 30:649–655. [PubMed: 26899213]
42. Silvestre-Rangil J, Bagan L, Silvestre FJ, Martinez-Herrera M, Bagan J: Periodontal, salivary and IL-6 status in rheumatoid arthritis patients. A cross-sectional study. Med Oral Patol Oral Cir Bucal 2017, 22:e595–e600. [PubMed: 28809379]
43. Naidoo T, Konkol K, Biccard B, Dudose K, McKune AJ: Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children. Cardiovasc J Afr 2012, 23:501–506. [PubMed: 23108518]
44. Dogan GE, Toraman A, Sebin SO, Dogan C, Gungor A, Aksoy H, Asutay H: Salivary IL-6 and IL-10 levels in subjects with obesity and gingivitis. Am J Dent 2016, 29:261–265. [PubMed: 29178738]
45. Abduljabbar T, Al-Sahaly F, Kellesarian SV, Kellesarian TV, Al-Anazi M, Al-Khathami M, Javed F, Vohra F: Comparison of peri-implant clinical and radiographic inflammatory parameters and whole salivary destructive inflammatory cytokine profile among obese and non-obese men. Cytokine 2016, 88:51–56. [PubMed: 27560655]
46. Reinhardt ÉL, Fernandes PACM, Markus RP, Fischer FM: Short sleep duration increases salivary IL-6 production. Chronobiol Int 2016, 33:780–782. [PubMed: 27070477]

47. Nizam N, Basoglu OK, Tasbakan MS, Lappin DF, Buduneli N: Is there an association between obstructive sleep apnea syndrome and periodontal inflammation? Clin Oral Investig 2016, 20:659–668.

48. Sgambato JA, Jones BA, Caraway JW, Prasad GL: Inflammatory profile analysis reveals differences in cytokine expression between smokers, moist snuff users, and dual users compared to non-tobacco consumers. Cytokine 2018, 107:43–51. [PubMed: 29217402]

49. Siqueira EC, de Souza FTA, Ferreira E, Souza RP, Macedo SC, Friedman E, Gomez MV, Gomes CC, Gomez RS: Cell phone use is associated with an inflammatory cytokine profile of parotid gland saliva. J oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol 2016, 45:682–686.

50. Ouellet-Morin I, Danese A, Williams B, Arseneault L: Validation of a high-sensitivity assay for C-reactive protein in human saliva. Brain Behav Immun 2011, 25:640–646. [PubMed: 21236331]

51. Slavish DC, Graham-Engeland JE, Smyth JM, Engeland CG: Salivary markers of inflammation in response to acute stress. Brain Behav Immun 2015, doi:10.1016/j.bbi.2014.08.008.

52. La Fratta I, Tatangelo R, Campagna G, Rizzuto A, Franceschelli S, Ferrone A, Patruno A, Speranza L, De Lutiis MA, Felaco M, et al.: The plasmatic and salivary levels of IL-1beta, IL-18 and IL-6 are associated to emotional difference during stress in young male. Sci Rep 2018, 8:3031. [PubMed: 29445205]

53. Auer BJ, Calvi JL, Jordan NM, Schrader D, Byrd-Craven J: Communication and social interaction anxiety enhance interleukin-1 beta and cortisol reactivity during high-stakes public speaking. Psychoneuroendocrinology 2018, 94:83–90. [PubMed: 29775877]

54*. Newton TL, Fernandez-Botran R, Lyle KB, Szabo YZ, Miller JJ, Warnecke AJ: Salivary cytokine response in the aftermath of stress: An emotion regulation perspective. Emotion 2017, 17:1007–1020. [PubMed: 28287751] One of the few studies to examine (and find) a relationship between emotional reactivity and levels of salivary inflammation.

55. Walsh E, Eisenlohr-Moul T, Baer R: Brief mindfulness training reduces salivary IL-6 and TNF-alpha in young women with depressive symptomatology. J Consult Clin Psychol 2016, 84:887–897. [PubMed: 27281371]

56. Lengacher CA, Reich RR, Paterson CL, Shelton M, Shivers S, Ramesar S, Pleasant ML, Budhrani-Shani P, Groer M, Post-White J, et al.: A Large Randomized Trial: Effects of Mindfulness-Based Stress Reduction (MBSR) for Breast Cancer (BC) Survivors on Salivary Cortisol and IL-6. Biol Res Nurs 2018, doi:10.1177/1099800418789777.

57. Twal WO, Wahlquist AE, Balasubramanian S: Yogic breathing when compared to attention control reduces the levels of pro-inflammatory biomarkers in saliva: a pilot randomized controlled trial. BMC Complement Altern Med 2016, 16:294. [PubMed: 27538513]

58**. Martinez AD, Ruelas L, Granger DA: Household fear of deportation in relation to chronic stressors and salivary proinflammatory cytokines in Mexican-origin families post-SB 1070. SSM - Popul Heal 2018, 5:188–200.Study has strong results showing higher chronic stress relates to higher salivary inflammation using real-life chronic stressors (fear of deportation and family conflict) in healthy Mexican-origin families with at least one immigrant.

59. Nelson BW, Byrne ML, Simmons JG, Whittle S, Schwartz OS, O'Brien-Simpson NM, Walsh KA, Reynolds EC, Allen NB: Adolescent temperament dimensions as stable prospective risk and protective factors for salivary C-reactive protein. Br J Health Psychol 2018, 23:186–207. [PubMed: 29034558]

60. Saban KL, Mathews HL, Collins EG, Hogan NS, Tell D, Bryant FB, Pape TLB, Griffin JM, Janusek LW: The Man I Once Knew: Grief and Inflammation in Female Partners of Veterans With Traumatic Brain Injury. Biol Res Nurs 2016, 18:50–59. [PubMed: 25636402]

61. Boyaka PN: Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems. J Immunol 2017, 199:9–16. [PubMed: 28630108]

62. Brandtzaeg P: Mucosal immunity: induction, dissemination, and effector functions. Scand J Immunol 2009, 70:505–515. [PubMed: 19906191]

63. Brandtzaeg P: Secretory immunity with special reference to the oral cavity. J Oral Microbiol 2013, 5.

64. Pabst O, Cerovic V, Hornef M: Secretory IgA in the Coordination of Establishment and Maintenance of the Microbiota. Trends Immunol 2016, 37:287–296. [PubMed: 27066758]

65. Heaney JLJ, Phillips AC, Carroll D, Drayson MT: The utility of saliva for the assessment of anti-pneumococcal antibodies: investigation of saliva as a marker of antibody status in serum. Biomarkers Biochem Indic Expo response, susceptibility to Chem 2018, 23:115–122.

66. Proctor GB, Carpenter GH: Salivary secretion: mechanism and neural regulation. Monogr Oral Sci 2014, 24:14–29. [PubMed: 24862591]

67. Teeuw W, Bosch JA, Veerman ECI, Amerongen AVN: Neuroendocrine regulation of salivary IgA synthesis and secretion: implications for oral health. Biol Chem 2004, 385:1137–1146. [PubMed: 15653426]

68. Bosch JA, de Geus EEJ, Ring C, Nieuw Amerongen AV, Stowell JR: Academic examinations and immunity: academic stress or examination stress? Psychosom Med 2004, 66:625–627. [PubMed: 15272113]

69. Bosch JA, Ring C, de Geus EJC, Veerman ECI, Amerongen AVN: Stress and secretory immunity. Int Rev Neurobiol 2002, 52:213–253. [PubMed: 12498106]

70. Birkett M, Johnson L, Gelety C: Investigation of Sex Differences In sIgA Response to the Trier Social Stress Test. Stress Health 2017, 33:158–163. [PubMed: 27075235]

71. Laurent HK, Stroud LR, Brush B, D'Angelo C, Granger DA: Secretory IgA reactivity to social threat in youth: Relations with HPA, ANS, and behavior. Psychoneuroendocrinology 2015, 59:81–90. [PubMed: 26036453]

72. Nyklicek I, Bosch JA, Amerongen AVN: A generalized physiological hyperreactivity to acute stressors in hypertensives. Biol Psychol 2005, 70:44–51. [PubMed: 16038773]

73. Kang LJ, Koleva PT, Field CJ, Giesbrecht GF, Wine E, Becker AB, Mandhane PJ, Turvey SE, Subbarao P, Sears MR, et al.: Maternal depressive symptoms linked to reduced fecal Immunoglobulin A concentrations in infants. Brain Behav Immun 2018, 68:123–131. [PubMed: 29032226]

74**. Engeland CG, Hugo FN, Hilgert JB, Nascimento GG, Junges R, Lim H-J, Marucha PT, Bosch JA: Psychological distress and salivary secretory immunity. Brain Behav Immun 2016, 52:11–17. [PubMed: 26318411] The first study to differentiate between IgA availability and transport capacity while accounting for IgA1 and IgA2 subclasses; these are important steps for determining the mechanisms through which stress affects susceptibility to infection (see reference 71: commentary).

75. Phillips AC, Carroll D, Evans P, Bosch JA, Clow A, Hucklebridge F, Der G: Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. Brain Behav Immun 2006, 20:191–197. [PubMed: 16055305]

76. Jensen SE: Psychological stress and infectious illnesses: One step closer to understanding the mechanisms. Brain Behav Immun 2016, 52:9–10. [PubMed: 26518297]

77. Gleeson M, Hall ST, McDonald WA, Flanagan AJ, Clancy RL: Salivary IgA subclasses and infection risk in elite swimmers. Immunol Cell Biol 1999, 77:351–355. [PubMed: 10457203]

78. Bosch JA, de Geus EJ, Kelder A, Veerman EC, Hoogstraten J, Amerongen A V: Differential effects of active versus passive coping on secretory immunity. Psychophysiology 2001, 38:836–846. [PubMed: 11577907]

79. Whittaker AC: The Vaccination Model in Psychoneuroimmunology Research: A Review. Methods Mol Biol 2018, 1781:309–326. [PubMed: 29705854]

80. Valdimarsdottir HB, Stone AA: Psychosocial factors and secretory immunoglobulin A. Crit Rev Oral Biol Med 1997, 8:461–474. [PubMed: 9391755]

81. Milligan R, Paul M, Richardson M, Neuberger A: Vaccines for preventing typhoid fever. Cochrane database Syst Rev 2018, 5:CD001261. [PubMed: 29851031]

82. Thomadaki K, Bosch J, Oppenheim F, Helmerhorst E: The diagnostic potential of salivary protease activities in periodontal health and disease. Oral Dis 2013, 19:781–788. [PubMed: 23379269]

83. Proctor GB, Carpenter GH: Chewing stimulates secretion of human salivary secretory immunoglobulin A. J Dent Res 2001, 80:909–913. [PubMed: 11379894]

84. Lin J, Smith DL, Esteves K, Drury S: Telomere length measurement by qPCR - Summary of critical factors and recommendations for assay design. Psychoneuroendocrinology 2019, 99:271–278. [PubMed: 30343983]

85*. Shalev I, Hastings WJ: Psychosocial stress and telomere regulation. In Genes, brain, and emotions: From resilience to psychopathology Edited by In Miu AC, Homberg JR & LK (Eds). Oxford University Press; [date unknown].Good current review summarizing how psychological stress relates to telomere length, mitichondrial function, and cellular aging.

86*. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT: Saliva diagnostics - Current views and directions. Exp Biol Med (Maywood) 2017, 242:459–472. [PubMed: 27903834] Good recent review summarizing the current/future utility of salivary diagnostics for both biomedical researchers and medical clinicians.

Highlights:

- Salivary cortisol and amylase have advanced the understanding of stress biology
- Salivary inflammation appears to reliably increase following stress
- Salivary S-IgA may provide a good general overview of the secretory immune system
- Saliva may give unique insights into the effects of stress on health in PNI

Box 1 –

Tips to the researcher using salivary biomarkers

- Use a collection method that is proven to work for the salivary parameter to be measured
- To reliably assess diurnal activity of cortisol and salivary alpha-amylase, collect at least three daily profiles, and use the wake-up concentration as anchor for daily slope
- Avoid absorbent collection materials and quantify salivary flow rate
- Ensure assay kits are validated for use with saliva
- Assessing oral health (even 1–2 rudimentary questions) may be helpful in research involving salivary inflammation

Box 2 –

Summary of key points

In saliva:

- Alpha-amylase is a good proxy for autonomic activity and reactivity and might reflect central noradrenergic activity
- Inflammation is not a reliable indicator of systemic inflammation
- Inflammatory responses to stress appear to occur reliably; in this manner, salivary cytokines may be useful measures in PNI research
- S-IgA levels tend to drop under conditions of stress, especially S-IgA1