



HAL
open science

Salivary α -amylase as a marker of sleep disorders: A theoretical review

Marine Thieux, Aurore Guyon, Laurent Seugnet, Patricia Franco

► To cite this version:

Marine Thieux, Aurore Guyon, Laurent Seugnet, Patricia Franco. Salivary α -amylase as a marker of sleep disorders: A theoretical review. *Sleep Medicine Reviews*, 2024, 74, pp.101894. 10.1016/j.smrv.2023.101894 . hal-04804185

HAL Id: hal-04804185

<https://hal.science/hal-04804185v1>

Submitted on 23 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Salivary α -amylase as a marker of sleep disorders: a theoretical review

Marine Thieux¹, Aurore Guyon², Laurent Seugnet¹ and Patricia Franco^{1,2}

1 Centre de Recherche en Neurosciences de Lyon (CRNL), INSERM, Lyon, France

2 Pediatric Sleep Unit and CRMR Narcolepsie-Hypersomnies rares, Department of Pediatric Clinical Epileptology, Sleep Disorders and Functional Neurology, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Lyon, France

Corresponding author: Marine Thieux (marine.thieux@chu-lyon.fr)

Centre de recherche en neurosciences de Lyon, Neurocampus, 95 Bd Pinel, 69500 Bron, France
+33 (0)481106500

Running title: Sleep and salivary α -amylase

Conflict of interest: The authors do not have any conflicts of interest to disclose.

Authors' contributions: AG, PF, LS, and MT participated in the conception of the article. AG, LS, and MT screened the articles. MT wrote the first version of the manuscript. All authors participated in the revisions of the manuscript and approved the final version.

Acknowledgments: This review was part of MT Ph.D. thesis that was funded by the French Society for Sleep Research and Medicine (SFRMS). We thank Véréna Landel for her help in manuscript preparation.

Summary

Sleep disorders are commonplace in our modern societies. Specialized hospital departments are generally overloaded, and sleep assessment is an expensive process in terms of equipment, human resources, and time. Biomarkers would usefully complement current measures in the screening and follow-up of sleep disorders and their daytime repercussions. Among salivary markers, a growing body of literature suggests that salivary α -amylase (sAA) may be a cross-species marker of sleep debt. However, there is no consensus as to the direction of variation in sAA with sleep disorders. Herein, after describing the mechanisms of sAA secretion and its relationship with stress, studies assessing the relationship between sAA and sleep parameters are reviewed. Finally, the influence of confounding factors is discussed, along with methodological considerations, to better understand the fluctuations in sAA and facilitate future studies in the field.

Keywords: saliva, biomarkers, sleepiness, sleep disorders

Abbreviations: AHI: apnea-hypopnea index; ANS: autonomous nervous system; BMI: body mass index; EEG: electroencephalogram; ESS: Epworth sleepiness scale; HPA: hypothalamic-pituitary-adrenal (axis); HF: high-frequency; HR: heart rate; KSS: Karolinska sleepiness scale; LF: low-frequency; NA: noradrenaline; NREM: non-rapid eye movement; ODI: oxygen desaturation index; OSA: obstructive sleep apnea; PNS: parasympathetic nervous system; PSG: polysomnography; PVT: psychomotor vigilance task; REM: rapid eye movement; sAA: salivary α -amylase; sCort: salivary cortisol; SD: sleep deprivation; SNS: sympathetic nervous system; SR: sleep restriction; SSS: Stanford sleepiness scale; SWS: slow-wave sleep.

1. Introduction: why salivary α -amylase?

Whether it is related to a pathology (e.g. insomnia, breathing disorder) or to an environmental condition (e.g. work- or socially-related), sleep disorders are commonplace in our modern societies. To assess these disorders, subjective (i.e. scales and questionnaires) and objective (e.g. sleep monitoring *via* polysomnography, PSG) methods are available. Although the former are attractive because of their ease of use and low cost, they are sensitive to inter- and intra- individual variability and do not represent reliable tools [1]. The latter, on the other hand, can only be performed in a few sleep laboratories, require considerable time and resources, and are far from real-life conditions. There is thus a need to develop more readily accessible markers to screen for sleep disorders and their diurnal repercussions.

Among several biomarkers, salivary α -amylase (sAA) concentrations are thought to be modulated by sleep characteristics, which has recently led more researchers to study their potential use as an objective measure of sleep disorders. This article aims to provide a theoretical review of the relationship between sAA and sleep, considering the mechanisms of sAA secretion, its relationship with stress, and the influence of other confounding factors.

1.1. Generalities

First described by Leuchs [2], sAA is one of the most abundant proteins in saliva (i.e. 10-20% of total proteins). This calcium-requiring metallo-enzyme, which belongs to the broader group of glycosyl hydrolases, has as main function the digestion of macromolecules such as starch and sugars [3–5]. The breakdown of carbohydrate chains into smaller units depends on its enzymatic activity, which requires the presence of Ca^{2+} ions (i.e. 1 to 10 per protein molecule) [2,6,7]. The cleavage of glycosidic bonds occurs randomly in different parts of the chain and produces maltose, maltotriose, and glucose units [3,4]. sAA is also involved in the maintenance of mucosal immunity in the oral cavity *via* inhibition of bacterial adhesion and

growth [6,8–10]. sAA is expressed in the salivary, lacrimal, and mammary glands, and depends on the AMY1 gene, while its pancreatic isoform depends on 2 other genes (i.e. AMY2 A and B), all located on chromosome 1p21 [3,7,11].

1.2. Secretion mechanisms and diurnal variation

In humans, salivary secretions are dependent on the autonomous nervous system (ANS). The primary salivary centers (i.e. superior and inferior salivary nuclei) receive afferent stimulation (e.g. mastication, taste) from cranial nerves (i.e. V, VII and IX). From these primary centers, (i) parasympathetic nerves project to the salivary glands *via* the parasympathetic ganglion using acetylcholine interacting with muscarinic receptors (i.e. M3 and M1) and (ii) sympathetic nerves project from the superior thoracic cord to the salivary glands *via* superior cervical ganglion using noradrenaline (NA) interacting with α 1- and β 1- adrenergic receptors (Figure 1) [12]. Although the parasympathetic nervous system (PNS) is mostly responsible for salivary flow and the sympathetic nervous system (SNS) for protein secretion, the PNS is also involved in protein secretion from some salivary glands (e.g. sublingual and minors), whereas the activation of the SNS can lead to a modulation of saliva volume [13]. More specifically, sAA is produced locally in the oral cavity: its secretion is mainly dependent on the stimulation of adrenergic receptors in the acinar cells of the parotid glands [5,8,14–21]. As stated above, while its release depends primarily on the SNS, the PNS may also be involved; thus, sAA levels reflect the balanced effect of the SNS and PNS, and can be considered as a peripheral biomarker of the global ANS activity [12,16,18,22,23].

In adults, the diurnal variation in sAA is characterized by a decrease during the first hour following awakening (called “awakening response”), followed by a steady increase until the late afternoon, that mirrors that of salivary cortisol (sCort; Figure 2) [5,8,14,17,24–26]. The circadian rhythm of sAA secretions involve the hypothalamic paraventricular nucleus which

receives inputs from the suprachiasmatic nuclei and projects *via* the spinal cord to the salivary glands [27].

1.3. Stress response

Early studies on sAA examined its relation to changes in the ANS related to physical, psychological, emotional, or cognitive stress [14,17,19]. These results consistently showed an increase in sAA levels after exposure to a stressful stimulus (e.g. physical activity, examination, skydiving, exposure to extreme temperatures, emotional pictures or videos) [7,8,17,18]. Thus, it was suggested that sAA and sCort can serve as surrogate markers for the two main systems involved in stress responses; while sCort reflects the activity of the hypothalamic-pituitary-adrenal (HPA) axis, the sAA reflects the activity of the ANS (i.e. particularly the sympatho-adrenal system) [7,8,18–20,28–31]. Mainly under hormonal control, the cortisol reaches the saliva *via* passive intracellular diffusion, which takes approximately 25-30 minutes post-stimulation. It is slower than the neural response that causes NA release, leading to sAA secretion 10-15 minutes post-stimulation [27,32]. This temporal shift may explain the reported lack of correlation between these 2 markers [14,19].

The reciprocal interactions between stress responses and sleep are well established, and are already present in children [33,34]. The activity of the SNS (i.e. especially the sympatho-adrenal system) and HPA axis are closely related to the sleep-wake cycle, due to their dependence on the hypothalamic suprachiasmatic nuclei [31,35,36]. Under physiological conditions, they are inhibited during early night sleep (i.e. rich in slow-waves) and reactivated during late night sleep (i.e. dominated by REM sleep) [31]. Indeed, sleep architecture influences ANS activity: the cardiac sympatho-vagal balance shows different profiles depending on sleep stages. From wakefulness to NREM sleep, there is an increase in blood pressure and heart rate (HR) markers of PNS activity: the high-frequency (HF) index evolves alongside the delta activity and reaches a maximum during slow-wave sleep (SWS), increasing with the depth of

sleep. From NREM to REM sleep, there is an increase in markers of SNS predominance: the low-frequency (LF) index evolves in opposition to the HF index and reaches a maximum during REM sleep [37,38]. Upon awakening in the morning, there is a decrease in the HF component of HR paralleled by a rise in the LF component of blood pressure [39]. While acute stress can affect subsequent sleep architecture and ANS activity [31], acute or chronic sleep disorders (e.g. increased sleep pressure or circadian shift) may result in an increased activity and reactivity of stress systems, leading first to a modulation of the release of stress mediators (i.e. glucocorticoids, catecholamines, and cytokines) whose regulation also depends on the negative feedback from the PNS, and then to secondary endocrine, immune, metabolic, and/or cardiovascular repercussions [31,33,47–49,34,40–46]. Thus, if sAA is a relevant measure of the ANS activity, sleep disorders and their repercussions (e.g. sleepiness, attentional lapses) should be associated with its modulations. The overarching goal of this theoretical review is to determine whether sAA can be used as a marker of sleep disorders.

2. Literature review: is sAA a good marker of sleep disorders?

2.1. Method

2.1.1. Literature search and selection criteria

In June 2022, PubMed was searched for all articles reporting the relationship between sAA and sleep, without a language restriction. The search was structured as follows: saliva AND amylase AND sleep. The references of all relevant articles were checked to identify additional sources. The Connected Papers website (<https://www.connectedpapers.com>) was also used to check all the extra studies related to the articles included in our review, using the keyword “amylase”. Based on the title, only those related to sleep were included.

By screening titles, 66 articles were selected. After full-text screening, 34 human studies and 3 animal studies were conserved for this review. Among the excluded articles, 10 were

reviews or methodological papers and the 19 others did not assess the relationship between sleep and sAA. Nonetheless, some of their findings are discussed in the introduction and discussion sections.

2.1.2. Data synthesis

The data are presented as narrative syntheses for studies in which sleep was experimentally manipulated (i.e. total sleep deprivation, SD, or sleep restriction, SR), those conducted in ecological situations, in patients with sleep pathologies, and in animals. A summary of the methods and results of the studies in humans is presented in Supplementary Materials.

2.2. Results

Numerous studies have examined the relationship between sleep and sAA, using different parameters, leading to conflicting results (Table 1).

2.2.1. Experimental manipulations of sleep

2.2.1.1. Sleep deprivation

In an initial study, after 28h of SD, sAA activity was increased in adults compared with circadian-matched controls. However, this change in sAA activity -significant at the group level- was only found in 5 out of 9 participants when looking at the individual level. This increase was reported to be independent of that of sCort: sCort levels were not changed after 28h of SD. A parallel study in flies yielded comparable results, making sAA a potential cross-species biomarker of sleep debt [50]. These results were then replicated in a study comparing SD (i.e. one night), SR (i.e. 3h of sleep from 0:45 to 3:45 a.m.), and no manipulation (i.e. 7h of sleep from 0:45 to 7:45 a.m.) in 10 healthy adults. Decreased sleep duration was associated with increased sAA concentrations, measured at 8:00 a.m. (i.e., approximately 15 minutes after awakening in the no manipulation condition and 4h15 after awakening in the SR condition) [51]. However, 3 studies failed to show a modulation of sAA after SD. The first, carried out in

a sample of 12 healthy adults who were continuously kept awake for 27h, showed that the levels of sAA measured at 8.00 a.m. returned to the same levels as those measured at the same time at the beginning of the experiment [52]. Two other studies showed no difference in sAA levels measured during the afternoon in 26 [53] and 218 adults [54] divided into two groups, with and without a night of SD. Non-homogeneous sampling times as well as inter- vs intra- individual comparisons can certainly explain some of the variability in these results.

There are also inconsistencies in the direction of the relationship between sAA and the repercussions of SD (e.g. sleepiness and cognitive performances). Three studies conducted by Pajcin and colleagues showed positive associations between sAA and cognitive performance, at least during the first 24h of SD. During 50h of SD, sAA values assessed in 11 healthy adults exhibited a diurnal profile similar to that of vigilance levels assessed by the psychomotor vigilance task (PVT) and a driving simulator, with higher sAA levels associated with better performance. Unlike performances, which were significantly worse on the 2nd day, sAA levels did not differ between the 1st and 2nd day. The authors explained the inconsistency between these results and those of previous reports -proposing that sAA may be considered as a measure of sleep pressure- by the individual variability in sAA activity after SD and by less frequent saliva sampling in other studies (i.e. traditionally two points, at baseline and after SD) [55]. In another study, 12 healthy adults were given caffeine during the same SD protocol. As in the first study, caffeine-mediated changes in the diurnal profile of sAA were similar to variations in vigilance, with higher sAA levels associated with better performance on the PVT during the first 24h. However, these associations were not consistent across the entire protocol: only response speed at the PVT remained associated with sAA levels on day 2 [56]. In a third study, this team assessed the relationship between sAA and cognitive performance in 10 healthy non-shift working men during a simulated night-shift protocol composed of 28h SD followed by sleep opportunities from 10:00 a.m. to 4:00 p.m. over the next 4 days. As in previous studies,

higher levels of sAA were associated with better cognitive performance (i.e. faster PVT responses, reduced variability in a driving simulation, and faster processing speed) on the 1st night, but not on the subsequent days [57]. These results are in agreement with observations realized without sleep manipulation: increased levels of sAA before an fMRI target detection task were associated with decreased reaction times on the first trial, with no significant correlation for subsequent trials [58]. However, conflicting results were reported in a study comparing 11 controls with 11 subjects with an adenosine deaminase polymorphism (i.e. characterized by higher levels of endogenous adenosine and slow waves during sleep) during 40h of SD. The group with the mutation had higher sleep pressure, reflected by higher subjective sleepiness, enhanced α power during wakefulness, and slow oscillations (i.e. 0.75 to 1.5 Hz) in non-rapid eye movement (REM) sleep compared to controls. They also had higher levels of sAA (i.e. collected at 2h-intervals throughout prolonged wakefulness and averaged per 6h-intervals) and reduced vigilance levels (i.e. assessed by the PVT and attention tasks) [59].

2.2.1.2. Sleep restriction

In an overnight SR study of 108 healthy young adults, an increase in sAA levels (i.e. measured between 9:00 and 12:00 a.m.) was reported in the SR group (i.e. participants were awakened during the night when they had slept 40% of their usual sleep time, verified by wrist actigraphy) compared to the rested group who had a full night sleep [22]. Nonetheless, other studies report contradictory results.

For instance, after 2 nights of SR (i.e. 3h with sleep opportunity from 3:00 to 6:00 a.m., monitored by wrist actigraphy) a decrease in sAA levels in the afternoon following the SR (i.e. starting at 1:30 p.m. with a clear reduction at 4:00 p.m., without changes at 11:00 a.m. or 6:30 p.m.) was shown in a group of 17 young healthy men. In parallel, this SR was associated with an increase in subjective sleepiness (i.e. assessed with the Stanford sleepiness scale, SSS) and

subjective stress, while memory performance decreased. The relationship between these factors and sAA levels was not assessed [60]. Rabat and colleagues conducted a longer SR protocol (i.e. 7 nights of 4h with sleep opportunity between 2:00 and 6:00 a.m., monitored by PSG) in 12 healthy men. They showed that this longer lack of sleep (i.e. after 7 days of SR) was associated with a modulation of sleep architecture (i.e. a higher percentage of SWS), an increase in subjective and objective sleepiness (i.e. increased scores on the Karolinska sleepiness scale, KSS, and decreased sleep latencies on the maintenance of wakefulness test at 10:00 a.m.), and deficits in sustained attention (i.e. decreased response speed reflecting impulsivity and increased attentional lapses at 8:30 a.m. and 6:30 p.m.). Finally, they showed a significant decrease in morning sCort and sAA levels upon awakening on day 7 of SR [61]. In another protocol, daytime sleepiness, cognitive performance, and sAA were assessed after 5 nights of SR (i.e. 3h per night with sleep opportunities from 4:00 to 7:00 a.m. and a 30 min early afternoon nap, monitored by PSG) in 11 healthy young adults. After this SR, subjective sleepiness and attention deficits increased while sAA levels decreased (i.e. at 8:30 a.m., 90 minutes after awakening) [62]. In both studies, sAA levels were associated with cognitive performance. In the former, negative correlations were observed between sAA levels and Go-noGo errors (i.e. between morning sAA levels at baseline and morning errors at baseline, at day 7 of SR and day 3 of recovery; and between morning sAA levels at day 2 of recovery and evening errors at day 3 of recovery). In the latter, sAA levels were positively correlated with PVT response speed and negatively correlated with attention lapses and Go-noGo errors. Furthermore, sAA levels did not always return to baseline after recovery [61,62].

2.2.2. Ecological situations

This section refers to studies without experimental manipulation of sleep and / or carried out outside research laboratories. Closer to everyday life, they are of major interest for the study of biomarkers that will eventually be used in naturalistic settings.

2.2.2.1. *In adults*

In a large cohort study (i.e. 454 adults), low sleep efficiency and short sleep duration were associated with ANS markers indicating lower levels of parasympathetic activation and/or higher levels of sympathetic tone. Indeed, subjects with good sleep efficiency (i.e. $\geq 85\%$ on 7-day actigraphy) had decreased sAA levels during the day and increased HF HR variability (i.e. representing parasympathetic influences), compared with those with poor sleep efficiency. While sleep duration appears to have an impact on HR and its variability, there was no clear association with sAA levels in this study [63]. Similarly, a restful self-reported sleep assessed at awakening over 4 days in 77 young healthy adults was related to decreased sAA levels (i.e. daily area under the curve) [64]. These results were replicated in cancer survivors with sleep complaints. In the 19 subjects who practiced mind-body bridging meditation, self-reported improvement in sleep over the previous week paralleled a decrease in sAA levels (i.e. 30 min post-awakening), which was not found in the 18 subjects who did not undergo this protocol. However, while sAA and sleep moved in the same direction, the correlations between them were weak [65]. In the same line, Unno and collaborators conducted 3 studies assessing the relationship between low-caffeine green tea consumption (compared to standard green tea), sleep (i.e. monitored with a single-channel electroencephalogram located at Fpz-M1), and sAA levels. In 7 elderly people, consumption of low-caffeine green tea for two weeks was associated with improved sleep and decreased sAA levels in the morning within the first hour after awakening (i.e. no change in the evening). This decrease in sAA levels was associated with increased sleep duration - particularly NREM sleep- and decreased arousals [66]. In 20 middle-aged adults who consumed low-caffeine green tea for 7 days, diminished sAA levels (i.e. in the evening after work, and a trend in the morning, 30 min post-awakening), and diminished self-reported fatigue was observed compared to those who consumed standard green tea. In addition, there was a positive correlation between the proportion of NREM sleep (i.e. N2 + N3) and the

consumed quantity of low-caffeine green tea. There was a correlation between sleep duration (i.e. total period of sleep and total sleep time) and sAA levels in the morning, only in the group who consumed standard green tea [67]. Finally, in a 3rd study, 20 students were assigned to a group taking theanine or placebo during their daily life at university and during professional practice (i.e. 7 baseline days followed by 10 stressful days). In both groups during practice, no direct association was found between sleep time and sAA. However, subjects with high sAA levels following awakening (i.e. > 50 U/ml) had a shorter sleep duration. No variation in sAA levels between morning and evening was found in the baseline condition [68].

In addition, the sAA awakening response – as an indicator for the morning activation pattern of the ANS- was also studied in relation to sleep characteristics. In 48 healthy young adults, the awakening time was positively associated with the sAA awakening response (i.e. the delta between sAA at awakening and 30 min later) assessed over 7 days: an early awakening time was associated with greater sAA awakening response [69]. These results contrast with those of Nater and colleagues, where post-awakening sAA was not related to the time of awakening (nor to sleep duration or quality – unspecified measures); but participants were required to awaken before 9:00 a.m. and those who woke up with an alarm clock had slightly higher levels of sAA on awakening (compared to those who woke up on their own) [14]. Finally, a seasonal comparison of sAA measurements in 45 healthy individuals showed higher levels 10 min post-awakening (with no significant difference for 0, 20-, and 30-minutes post-awakening) and a less pronounced awakening response (i.e. during the 10 minutes following awakening) during winter compared with summer. Self-reported sleepiness (i.e. using the KSS) was also greater in winter, but no significant correlation was observed with sAA. The higher sAA levels were associated with a greater amount of melatonin, and the less deepened awakening response was associated with shorter self-reported sleep duration [27]. Overall, a decrease in sleep efficiency,

quality, and / or duration seems to lead to an increase in sAA measures, but these results remain contradictory.

A study of 12 healthy young adults compared sAA levels on awakening after a night sleep with or without noise disturbance. If noise did not influence sAA levels, neither did it affect self-reported sleep quality. In addition, the classical diurnal rhythm of sAA was not observed (i.e. absence of awakening response) [70]. Another study conducted on 50 healthy young adults assessed the relationship between sleep characteristics (i.e. self-reported sleep quality, duration, latency, awakenings, as well as duration assessed by wrist actigraphy in a subsample of 29 participants) and sAA over 5 days at the beginning and end of the semester (i.e. low-stress vs high-stress condition). In the high-stress condition, poorer subjective sleep quality was associated with lower sAA levels at awakening and shorter sleep duration was associated with lower awakening response. Finally, a longer sleep latency was associated with a steeper diurnal slope the following day, reflecting a greater increase in ANS activity. None of the diurnal sAA parameters predicted sleep characteristics the following night [71].

2.2.2.2. *In the pediatric population*

While associations between sleep characteristics assessed by actigraphy (i.e. sleep efficiency, duration and awakenings) and sAA measures (i.e. awakening levels and diurnal slope) are not yet present in 47 toddlers aged 12-24 months [15], they appear to exist somewhat later in childhood and adolescence. In a cohort of 55 children and adolescents (i.e. 8-16 years), increased morning sAA levels were positively associated with subjective sleep disorders (i.e. assessed with the 10-items sleep-wake problems subscale of the school sleep habits survey), with no association with objective sleep characteristics (i.e. assessed by wrist actigraphy over the 7 days of the study) [72]. Likewise, increased morning sAA levels in 76 older adolescents (i.e. first semester of college) were associated with decreased self-reported sleep quality in the last few months, but unrelated to sleep duration or short-term objective sleep characteristics

(i.e. assessed by wrist-actigraphy over 3 or 4 nights) [25]. Another study of 276 children during a laboratory stress task revealed that the lower the sleep efficiency (i.e. assessed by actigraphy during 3 to 14 nights), the higher the overall level of afternoon sAA throughout the protocol [28]. Finally, a recent study by Pradhan and colleagues conducted on 168 adolescents showed a negative association between subjective sleep duration and late morning sAA levels (i.e. 11:00-11:30 a.m.), which were positively correlated with subjective levels of sleepiness (i.e. using the Epworth sleepiness scale, ESS). Interestingly, participants with a pathological ESS score (i.e. >10) showed higher levels of sAA compared to those with a normal score [73].

2.2.3. Sleep pathologies

Overall, obstructive sleep apnea (OSA) syndrome appears to be associated with increased sAA levels. Indeed, 40 adults with moderate to severe OSA showed higher morning sAA levels (i.e. at 7 a.m. within 30 min after waking up) compared to 18 controls or patients with mild OSA [74]. These results are concordant with a study conducted in 72 children with OSA, which showed higher levels of sAA at bedtime (i.e. around 22:00 p.m., before PSG) and in the morning (i.e. within 30 min of PSG ending) compared to 74 children without OSA [75]. In these studies, sAA levels were positively associated with the apnea-hypopnea index (AHI) [74,75], the oxygen desaturation index (ODI) [75], and negatively with the lowest pulse oxygen saturation [74]. In another study, the sAA levels were assessed in the evening before PSG and the morning after (i.e. around 22:00 p.m. and around 7:00 a.m., within 30 min after waking up). The ratio between the two measurements was higher, while the absolute difference was lower, in 15 children with severe OSA compared to 26 children with mild OSA and 26 controls, with no difference between children with OSA (i.e. mild and severe combined) and controls. Children with severe OSA showed lower levels of sAA at night and higher levels in the morning, but there was no significant difference between sAA levels at each sampling time between the 3

groups. In this study, the sAA ratio/subtraction was also positively correlated with the AHI and the ODI [76].

However, these results are not consensual. No difference in sAA levels (i.e. measured between 08:30 and 9:00 a.m., about 1h post-awakening, and at 7:00 p.m.) was reported in 27 children with OSA (i.e. 13 with mild and 14 with moderate to severe OSA) compared with 7 healthy controls. Also, sAA levels measured at midday (i.e. before lunch) were significantly lower in children with OSA than in controls [77]. Finally, morning sAA levels (i.e. measured between 7:00 and 9:00 a.m., immediately after waking-up) were not different between 25 adults with nocturnal bruxism compared to 20 controls. The relationship with sleep parameters (e.g. quality, fragmentation) was not assessed [78].

2.2.4. Animal studies

In animals, studies have consistently shown an increase in sAA levels with the perturbation of sleep. Following the first study in drosophila [50], two studies have examined the relationship between sleep and sAA in rats and mice. In the first one, 3 groups of 6 rats were randomly assigned to a sleep condition consisting of SD, SR (i.e. 4h of sleep per night), or control condition (i.e. uncontrolled sleep) for 7 days. The activity of sAA on the 8th day was higher in the SD rats than in the SR or control condition. Surprisingly, there was no difference between the latter two conditions, suggesting that a few hours of recovery may be sufficient to offset the deleterious impact of sleep debt [79]. Pharmacological manipulation of sleep in mice by injection of stilbene glucoside resulted in an increase in sleep duration by increasing NREM sleep and decreasing arousal and REM sleep. This modulation was responsible for a decrease in sAA concentrations, which were negatively associated with sleep duration [80].

3. Discussion

Although salivary secretions “*lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears*” [81], their ease of use, low cost, and non-invasive collection method have made them the tools of choice for studies in the pediatric population and in natural settings. Indeed, samples can be collected close to each other without inducing additional stress, anxiety, or pain, and without relying on the assistance of trained staff, thus offering clear advantages over conventional physiological measurements of the ANS (e.g. skin conductance, HR measurements, or plasma adrenaline/NA) [5,8]. However, although an increase in sAA levels with sleep disorders appears in the majority of studies, conflicting results persist in the literature. The underlying mechanisms of the relationship between sAA and sleep, as well as methodological considerations that might explain this heterogeneity need to be explored to improve the potential of sAA as a peripheral measure of sleep disorders.

3.1. What to know before using sAA as a marker of sleep debt? A brief look at the underlying mechanisms

3.1.1. The intensity of the sleep disorders matters

Given the relative stability of sAA diurnal rhythms, it has been proposed that a moderate and/or acute sleep disruption might not be sufficient to induce changes in sAA secretions the following day, and that a chronic (e.g. over several weeks, sleep pathology) or intense sleep disruption (e.g. SD protocol) might be required to generate greater ANS activation, thereby increasing sAA levels [25]. Supporting this hypothesis, some studies have revealed that while sAA measures were not related to objective sleep parameters, increased sAA levels were associated with self-reported reduced sleep quality and frequently disrupted sleep in healthy children and adolescents, potentially revealing a more chronic impairment than that measured by actigraphy over a period not exceeding one week [25,72]. Furthermore, in children with sleep-related breathing disorders the increased instability of the autonomic activity related to sleep fragmentation is reflected by the upregulation of sAA [75,76] (i.e. except in [77]). Studies

in the adult population are scarcer: only 3 studies investigated sAA in patients with sleep disorders. While one study conducted in patients with bruxism did not report changes in sAA compared to healthy controls [78], patients with OSA showed increased sAA levels post-awakening compared with controls (or patients with mild OSA) [74] and cancer survivors with subjective sleep improvement showed decreased sAA levels post-awakening [65]. Finally, even if inconsistent (e.g. [53–55]), changes in sAA in adults have been more massively reported after SD than SR or control condition [51]. These observations are reinforced by those of animal studies where changes in sAA only appear when rats are in the SD condition, whereas SR gives similar results to the control condition. According to the authors, a few hours of sleep per night would be sufficient to limit the disruption of salivary concentrations [79]. Thus, a lesser disturbance might not influence sAA or only shift the secretion rhythm, such that the observed modulation attributed to sleep disorders may be solely attributable to a measurement made at a different phase of the rhythm [82]. Indeed, a slight circadian shift occurring during long SR protocols and contributing to the observed changes in sAA cannot be ruled out on the basis of the current literature, which uses only a few sampling time points. This hypothesis would partly explain the discrepancies in the literature.

3.1.2. The sleep architecture matters

In humans, increases in sleep duration, especially increases in NREM sleep and decreases of arousals, have been associated with decreased sAA levels the following day [66–68,73]. Accordingly, drug-induced increases in sleep duration in mice, through increases in NREM sleep and decreases in REM sleep and nighttime arousals, result in decreased sAA levels [80]. These observations suggest a modulation of sAA through sleep architecture characterized by a decrease during NREM sleep, and an increase during wakefulness and REM sleep (i.e. also applicable to sleep fragmentation). Thus, SD would increase sAA levels by increasing arousal, whereas SR over several days, would promote slow-waves rebound during sleep opportunities

leading to a normalization (or even a decrease) of sAA levels (e.g. as observed in [61,62]). In rats, the absence of difference between the SR and control conditions suggests that a few hours of recovery may be sufficient to compensate for the deleterious impact of sleep debt [79]. Several studies conducted in ecological settings also seem to be in favor of a disruption of sleep quality or efficiency associated with increased sAA levels [28,63–65,72], potentially due to more fragmented sleep. Accordingly, as mentioned above, sleep fragmentation due to OSA induces higher sAA levels, reflecting the SNS stimulation [74–76]. This hypothesis could also explain why intense sleep disturbance is necessary to observe changes in sAA levels, as only a pathology or extreme situation leads to massive changes in the sleep architecture.

In addition to these sleep-related concerns, methodological aspects will be briefly discussed below in order to provide some elements to explain the heterogeneity of the data obtained in the literature. The interested reader can refer to earlier extensive reviews conducted on these specific topics (e.g. [7,8,16,18]).

3.2. How to explain this heterogeneity?

3.2.1. Towards an optimal method of analysis

3.2.1.1. Saliva collection

Different collection methods have been used in the literature; therefore, it is important to emphasize that the collection method itself may influence the biomarker concentrations. First, collecting saliva from different areas of the mouth can lead to the collection of secretions from different glands that are not equally involved in the secretion of specific proteins (e.g. sAA is mainly generated in the parotid glands) [5,8]. In addition, recommendations differ as to whether the collection method should be passive (i.e. unstimulated or resting) or active (i.e. stimulated) [8,16]. Some advocate a passive method because saliva stimulation (e.g. by chewing) increases salivary flow and protein secretion by certain glands (i.e. saliva is primarily produced by the

submandibular glands in the passive condition, and by the parotid glands in the active condition) [16]. Since sAA is a digestive enzyme that responds to gustatory stimulation, the influence of mechanical stimulation (e.g. moving the cotton in the mouth or chewing) on its secretion induces autonomic reflexes that distort the basal studied effects [16]. In practice, these aspects are rarely considered. Stimulation of saliva before or during sampling, as well as the duration of sampling, are rarely reported. Finally, a further distinction can be made between techniques based on absorbent materials or those based on passive drool into collection tubes. The use of absorbent material is generally preferred by participants, but has the disadvantage that it stimulates saliva production even in the absence of chewing, and that the sponge does not fully release the collected protein and saliva [8,16].

3.2.1.2. Number and timing of samples

In the present literature review, the number and timing (i.e. on a daily or even seasonal basis) of collections varied considerably, and many studies were performed using a small number of samples. However, as sAA follows a diurnal variation (Figure 2), these parameters have a decisive influence on the collected concentrations. While some studies suggest that only 2 samples are needed (i.e. waking and evening) to characterize the diurnal slope, and 3 to measure the total secretion of sAA [26], other research emphasizes the value of several samples, particularly at awakening (e.g. directly upon awakening, 30- and 60- min later, and then covering the rest of the day, ideally during more than one day) [8,83].

3.2.1.3. Units of measure

The units of measure are also a factor of variability. The majority of studies use sAA enzymatic activity (U/ml) as a measure of protein concentration. However, although correlated, these two measures are not completely interchangeable [16]. In addition, sAA concentration (U/ml) reflects the combined effect of salivary flow rate (i.e. mainly under the control of the PNS) and protein secretion (i.e. mainly under the control of the SNS) [16]. It is therefore

necessary to determine whether the influence of the PNS on flow rate also has an impact on the influence of the SNS on sAA concentration: considering the secretion rate (U/min) in conjunction with the concentration (U/ml) would provide a more accurate measure of the ANS activity [16,22].

3.2.2. Confounding factors

Apart from these considerations, various elements are likely to influence the secretion of sAA. Among these confounding factors, it is possible to distinguish between “traits”, which refer to the characteristics of an individual, and “states”, which refer to the characteristics of a situation (i.e. which may become traits if chronic).

3.2.2.1. Trait factors

Among the trait factors, demographic characteristics such as age, sex, body mass index (BMI), genetic and psychological factors, or those influencing circadian rhythm can affect sAA measurements.

Age: Absent at birth, sAA emerges in the first years of life [15], with dietary diversification and dentition, increasing continuously to reach adult levels between 1 and 3 [8,18] or even 5-6 years [7]. In middle school, pubertal status and age have been reported to be positively correlated with sAA activity [7]. While studies are scarce in children, they are contradictory in adults. Advancing age may be characterized by a flattening of the diurnal slope [14,18], associated with a lack of awakening response and higher overall concentrations in older subjects [18,54]; an increase in the evening [84] or no change [8].

Sex: While some studies suggest no sex difference [8,14,84], others report higher sAA activity in women, only in the evening [85]. The menstrual cycle, hormonal contraception, and pregnancy do not appear to influence daily sAA levels in adolescents and adults (with the

exception of the last trimester of pregnancy, which may result in increased levels, but with contradictory results) [18].

BMI: In children, a higher BMI z-score has been associated with a decrease in awakening sAA levels and with a faster increase during the day [29]. In adults, BMI was not associated with sAA measures [14,84].

Oral health: Due to its role in protecting the oral mucosa, higher sAA activity is associated with good oral health [7].

Genetic: Although the intra-individual profiles of adults are relatively stable over time [7], the large inter-individual variability must be considered [26] as measured values can range over 2 orders of magnitude in some studies (e.g. [14]). Concentrations are positively influenced by individual copy number variations of the AMY1 gene, that codes for sAA and varies from 1 to 15 (or 2 to 17): barely detectable in some individuals, sAA can account for more than 50% of salivary proteins in others. Moreover, for the same concentration, the enzymatic activity can show large inter-individual variability and vice-versa [11,86,87].

Somatic, psychological, and psychiatric disorders: Fluctuations in sAA levels in different psychiatric conditions, in response to ANS dysregulation, have been previously described in detail (for review, [5]). Similarly, somatic conditions may be associated with modulations of sAA levels, with various orientations [8,18,19,88].

Circadian rhythms: As sAA fluctuates according to a diurnal rhythm, factors that influence the circadian rhythm such as chronotype, shift work, or light exposure can modulate sAA concentrations. Indeed, light exposure increases the modulation amplitude of sAA over 24 hours compared to constant darkness (i.e. increasing the night-day contrast) [52], while shift work results in a flattening of the diurnal slope (i.e. compared to daytime-only work) [89].

3.2.2.2. State factors

Among the state factors, those that influence the ANS, such as physical activity, physiological or psychological stress, and those associated with saliva production, such as smoking, medication, food or beverage consumption, can influence sAA measurements as sAA is not passively transported in saliva but actively produced by stimulation of sympathetic and parasympathetic nerves [8].

Physical activity: Exercise acutely increases sAA levels (i.e. with no difference between morning and afternoon exercise sessions), especially when performed at high intensity [8,18] whereas minor physical activity (i.e. in intensity and duration, such as running to catch a bus) in the hour before the collection does not influence sAA levels [14]. Regular physical activity does not appear to influence sAA levels, but high-intensity chronic activity, compared to sedentary behavior, may decrease basal sAA levels [84].

Physiological, psychological, cognitive, or affective stress: Exposure to a stressful event causes an increase in sAA levels about 10 minutes after exposure, returning to baseline levels after 30-45 minutes [8,17]. Results for chronic stress are still controversial, suggesting an increase [14] or no change in baseline sAA levels [90].

Smoking: Cigarette smoke is thought to immediately inhibit sAA activity, resulting in lower levels after smoking. Studies on chronic smokers are controversial, showing an increased awakening response, decreased or increased sAA levels, or no influence of smoking [8,14,18,84,85,91].

Medication: Any form of medication that influences salivation (e.g. diuretics, antidepressants, antihistamines, analgesics) can impact sAA levels [18]. More specifically, adrenergic agonists or antagonists influence sAA levels [8,18,20,21,92]. However, the influence of their chronic consumption is not known [84].

Food and beverage consumption: While some studies report no impact of food and beverage consumption in the hour prior to sample collection on sAA measurements [14], some authors recommend the avoidance of food and beverage consumption at least 30 minutes before salivary sampling to avoid the modulation of sAA levels, which may be stimulated by chewing and food contact with the oral cavity [8]. The impact of diet type must also be considered. As sAA has a primary function in the degradation of macromolecules, a high-carbohydrate diet (e.g. vegetarianism or veganism) may result in a higher concentration of sAA [8,18]. Indeed, an elevated AMY1 gene copy number (i.e. which is associated with higher sAA levels) has been observed in subjects whose ancestors ate a starchy diet [11]. In addition, chronic alcohol consumption, through its impact on saliva, salivary glands, and stress response systems, tends to decrease baseline sAA levels, although studies are not consensual [8,64,84]. Finally, caffeine and green tea consumption appear to acutely increase sAA levels [8,18], whereas the effect of habitual caffeine consumption is unclear, resulting in either an increase or no change in sAA levels [14,64,85].

3.2.3. About the stress factor: disturbed sleep, increased stress

As sAA is a marker of physiological and psychological stress [17], it is questionable whether sAA reflects the effect of sleep disorders independently of stress itself. For example, one study reported no effect of sleep on sAA levels other than an impact of waking up with an alarm clock compared to waking naturally [14], which makes it unclear whether the increase in sAA levels is the result of a stress response or a sleep disturbance. Inadequate sleep may also potentiate the physiological effects of stress [46]: under high-stress, poor sleep has been associated with lower sAA levels at awakening and a more rapid increase during the day, which was not the case under low-stress [71]. Disentangling the respective influence of each of these factors and their interactions would provide a better understanding of the determinants of sAA fluctuations. Furthermore, it is questionable whether sleep disorders can be equated with stress.

The modulation of cortisol levels (i.e. decreased amplitude, increased evening levels, and shift in the quiescence period), as well as the modification of the immune response and general state following intense or chronic sleep restriction, have led some authors to equate sleep disorders with biological stress state [31,93,94]. Nevertheless, some experimental conditions (e.g. acute SD or SR) sometimes show an effect of sleep disorders independent from stress. For example, Seugnet et al. have suggested that stress is unlikely to play a role in modifying sAA activity during a sustained period of wakefulness, by showing an elevated sAA activity after SD without change in sCort in humans [50]. Still, using the association between sAA and sCort activities to address their independence is also limited by the temporal shift of their secretion, as discussed in the introduction section. It is also possible that this dissociation is only made possible in an artificial way, *via* the experimental modalities allowing anticipation of the disturbance and recovery possibilities [31]. Thus, the independent effect of stress and sleep assessed in laboratories would not reflect their often-inextricable interaction in real-life conditions.

4. Conclusions and future directions

In conclusion, the reviewed studies seem to suggest that the increase in sAA is associated with a stimulation of the SNS, which may result from stress and/or sleep disorders (i.e. sleep fragmentation, reduced NREM sleep), and also explains the positive association between sAA and cognitive performance (at least during the first 24h of SD) that may result from the SNS stimulation as a compensatory mechanism to counteract the cognitive repercussions of SD.

Although sAA is an attractive alternative to conventional ANS measures, the lack of normative values [27], the heterogeneity of the literature regarding the influence of confounding factors, and the large number of studies conducted on between-group comparisons -while acknowledging the large inter-individual variability-, may explain some discrepancies regarding the links between sleep and sAA. Furthermore, the variability in procedures (e.g. SD,

SR over a variable duration) and measurements (i.e. from subjective to objective methods) - added to their own limitations (e.g. low reliability, far from real-life conditions)- results in a variety of situations and indices that are not always comparable.

The reviewed findings suggest that the sensitivity of sAA to massive sleep disturbances may make this biomarker an interesting tool for the screening of sleep disorders, prior to full clinical evaluation. Because of its relationship with NA, diurnal profile, and sensitivity to homeostatic pressure, sAA seems to be the ideal biomarker for sleep disorders. However, although some teams have already developed portable tools for the rapid and easy assessment of sAA concentrations [95], assessed its usefulness for the objective assessment of sleepiness in drivers [96], or suggested its use to determine appropriate times for the administration of sleep pressure countermeasures (e.g. caffeine, stimulants) [55], the methodological difficulties listed above still need to be addressed. Further controlled laboratory studies seem necessary to optimize and standardize these measures before being able to use them as a biomarker of sleep disorders in everyday life.

Practice points

- The sAA is a peripheral measure of **the autonomous nervous system activity** and is sensitive to **sleep disorders**.
 - The **intensity of sleep disorders** alters the biomarker response: a massive and / or chronic sleep impairment would be required to observe the modulation of sAA concentrations.
 - The sAA can be considered as a reflection of **sleep quality / efficiency** on the previous night: the higher the proportion of NREM sleep and the lower the fragmentation, the lower the sAA.
 - The **effects of stress** may confound the impact of sleep disorders on sAA.
 - All these effects seem to be related to the **stimulation of the sympathetic nervous system**.
-

Research agenda

- Standardized protocols are needed to avoid the **methodological pitfalls** that hinder the promising use of salivary markers.
 - Potential **confounding factors** should be accounted for.
 - **Intra-individual analyses** should be favored to counterbalance the inter-individual variability of sAA.
 - **Multiple sampling** over more than one day in addition to a serial measurement of a robust circadian marker is required to assess the effect of sleep pressure accounting for the circadian rhythm of sAA.
-

References

- [1] Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. Self-reported and measured sleep duration: How similar are they? *Epidemiology* 2008;19:838–45.
doi:10.1097/EDE.0b013e318187a7b0.
- [2] Zakowski JJ, Bruns DE. Biochemistry of human alpha amylase isoenzymes. *Crit Rev Clin Lab Sci* 1985;21:283–322. doi:10.3109/10408368509165786.
- [3] Azzopardi E, Lloyd C, Teixeira SR, Conlan RS, Whitaker IS. Clinical applications of amylase: Novel perspectives. *Surg (United States)* 2016;160:26–37.
doi:10.1016/j.surg.2016.01.005.
- [4] Movahedpour A, Asadi M, Khatami SH, Taheri-Anganeh M, Adelipour M, Shabaninejad Z, et al. A brief overview on the application and sources of α -amylase and expression hosts properties in order to production of recombinant α -amylase. *Biotechnol Appl Biochem* 2022;69:650–9. doi:10.1002/bab.2140.
- [5] Schumacher S, Kirschbaum C, Fydrich T, Ströhle A. Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?-A review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology* 2013;38:729–43. doi:10.1016/j.psyneuen.2013.02.003.
- [6] Arhakis A, Karagiannis V, Kalfas S. Salivary Alpha-Amylase Activity and Salivary Flow Rate in Young Adults. *Open Dent J* 2013;7:7–15.
doi:10.2174/1874210601307010007.
- [7] Granger DA, Kivlighan KT, El-Sheikh M, Gordis EB, Stroud LR. Salivary α -amylase in biobehavioral research: Recent developments and applications. *Ann N Y Acad Sci* 2007;1098:122–44. doi:10.1196/annals.1384.008.
- [8] Rohleder N, Nater UM. Determinants of salivary α -amylase in humans and methodological considerations. *Psychoneuroendocrinology* 2009;34:469–85.

- doi:10.1016/j.psyneuen.2008.12.004.
- [9] Scannapieco FA, Torres G, Levine MJ. Salivary α -amylase: Role in dental plaque and caries formation. *Crit Rev Oral Biol Med* 1993;4:301–7.
doi:10.1177/10454411930040030701.
- [10] Bosch JA, Turkenburg M, Nazmi K, Veerman ECI, De Geus EJC, Nieuw Amerongen A V. Stress as a determinant of saliva-mediated adherence and coadherence of oral and nonoral microorganisms. *Psychosom Med* 2003;65:604–12.
doi:10.1097/01.PSY.0000074759.71084.AB.
- [11] Peyrot des Gachons C, Breslin PAS. Salivary Amylase: Digestion and Metabolic Syndrome. *Curr Diab Rep* 2016;16. doi:10.1007/s11892-016-0794-7.
- [12] Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. *Auton Neurosci Basic Clin* 2007;133:3–18. doi:10.1016/j.autneu.2006.10.006.
- [13] Bosch JA, Ring C, de Geus EJC, Veerman ECI, Nieuw Amerongen A V. Stress and secretory immunity, 2002, p. 213–53. doi:10.1016/S0074-7742(02)52011-0.
- [14] Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology* 2007;32:392–401. doi:10.1016/j.psyneuen.2007.02.007.
- [15] Bright MA, Frick JE, Out D, Granger DA. Individual differences in the cortisol and salivary α -amylase awakening responses in early childhood: Relations to age, sex, and sleep. *Dev Psychobiol* 2014;56:1300–15. doi:10.1002/dev.21209.
- [16] 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;36:449–53. doi:10.1016/j.psyneuen.2010.12.019.
- [17] Chatterton RT, Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary α -amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 1996;16:433–48.

- doi:10.1111/j.1475-097X.1996.tb00731.x.
- [18] Strahler J, Skoluda N, Kappert MB, Nater UM. Simultaneous measurement of salivary cortisol and alpha-amylase: Application and recommendations. *Neurosci Biobehav Rev* 2017;83:657–77. doi:10.1016/j.neubiorev.2017.08.015.
- [19] Wolf JM, Nicholls E, Chen E. Chronic stress, salivary cortisol, and α -amylase in children with asthma and healthy children. *Biol Psychol* 2008;78:20–8. doi:10.1016/j.biopsycho.2007.12.004.
- [20] Van Stegeren A, Rohleder N, Everaerd W, Wolf OT. Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. *Psychoneuroendocrinology* 2006;31:137–41. doi:10.1016/j.psyneuen.2005.05.012.
- [21] Ehlert U, Erni K, Hebisch G, Nater U. Salivary α -amylase levels after yohimbine challenge in healthy men. *J Clin Endocrinol Metab* 2006;91:5130–3. doi:10.1210/jc.2006-0461.
- [22] O’Leary ED, Howard S, Hughes BM, James JE. Salivary α -amylase reactivity to laboratory social stress with and without acute sleep restriction. *J Psychophysiol* 2015;29:55–63. doi:10.1027/0269-8803/a000134.
- [23] Bosch JA, Ring C, de Geus EJC, Veerman ECI, Nieuw Amerongen A V. Stress and secretory immunity, 2002, p. 213–53. doi:10.1016/S0074-7742(02)52011-0.
- [24] Parkkila S, Parkkila A-K, Rajaniemi H. Circadian periodicity in salivary carbonic anhydrase VI concentration. *Acta Physiol Scand* 1995;154:205–11. doi:10.1111/j.1748-1716.1995.tb09902.x.
- [25] Van Lenten SA, Doane LD. Examining multiple sleep behaviors and diurnal salivary cortisol and alpha-amylase: Within- and between-person associations. *Psychoneuroendocrinology* 2016;68:100–10. doi:10.1016/j.psyneuen.2016.02.017.
- [26] Out D, Granger DA, Sephton SE, Segerstrom SC. Disentangling sources of individual

- differences in diurnal salivary α -amylase: Reliability, stability and sensitivity to context. *Psychoneuroendocrinology* 2013;38:367–75.
doi:10.1016/j.psyneuen.2012.06.013.
- [27] Danilenko K V., Kobelev E, Zhanaeva SY, Aftanas LI. Winter-summer difference in post-awakening salivary α -amylase and sleepiness depending on sleep and melatonin. *Physiol Behav* 2021;240:113549. doi:10.1016/j.physbeh.2021.113549.
- [28] Rääkkönen K, Matthews KA, Pesonen AK, Pyhälä R, Paavonen EJ, Feldt K, et al. Poor sleep and altered hypothalamic-pituitary-adrenocortical and sympatho-adrenal-medullary system activity in children. *J Clin Endocrinol Metab* 2010;95:2254–61.
doi:10.1210/jc.2009-0943.
- [29] Miller AL, Sturza J, Rosenblum K, Vazquez DM, Kaciroti N, Lumeng JC. Salivary alpha amylase diurnal pattern and stress response are associated with body mass index in low-income preschool-aged children. *Psychoneuroendocrinology* 2015;53:40–8.
doi:10.1016/j.psyneuen.2014.12.011.
- [30] Doane LD, Van Lenten SA. Multiple time courses of salivary alpha-amylase and dimensions of affect in adolescence. *Psychoneuroendocrinology* 2014;49:47–53.
doi:10.1016/j.psyneuen.2014.06.007.
- [31] Van Reeth O, Weibel L, Spiegel K, Leproult R, Dugovic C, Maccari S. Interactions between stress and sleep: From basic research to clinical situations. *Sleep Med Rev* 2000;4:201–19. doi:10.1053/smr.1999.0097.
- [32] Pérez-Valdecantos D, Caballero-García A, Del Castillo-Sanz T, Bello HJ, Roche E, Roche A, et al. Variations in salivary stress biomarkers and their relationship with anxiety, self-efficacy and sleeping quality in emergency health care professionals. *Int J Environ Res Public Health* 2021;18:1–14. doi:10.3390/ijerph18179277.
- [33] McEwen BS, Karatsoreos IN. Sleep deprivation and circadian disruption: Stress,

- allostasis, and allostatic load. *Sleep Med Clin* 2015;10:1–10.
doi:10.1016/j.jsmc.2014.11.007.
- [34] Ordway MR, Condon EM, Ibrahim BB, Abel EA, Funaro MC, Batten J, et al. A systematic review of the association between sleep health and stress biomarkers in children. *Sleep Med Rev* 2021;59:101494. doi:10.1016/j.smr.2021.101494.
- [35] Hastings MH, Reddy AB, Maywood ES. A clockwork web: Circadian timing in brain and periphery, in health and disease. *Nat Rev Neurosci* 2003;4:649–61.
doi:10.1038/nrn1177.
- [36] Hastings MH, Maywood ES, Brancaccio M. Generation of circadian rhythms in the suprachiasmatic nucleus. *Nat Rev Neurosci* 2018;19:453–69. doi:10.1038/s41583-018-0026-z.
- [37] Otzenberger H, Gronfier C, Simon C, Charloux A, Ehrhart J, Piquard F, et al. Dynamic heart rate variability: A tool for exploring sympathovagal balance continuously during sleep in men. *Am J Physiol - Hear Circ Physiol* 1998;275.
doi:10.1152/ajpheart.1998.275.3.h946.
- [38] Jurysta F, Van De Borne P, Migeotte PF, Dumont M, Lanquart JP, Degaute JP, et al. A study of the dynamic interactions between sleep EEG and heart rate variability in healthy young men. *Clin Neurophysiol* 2003;114:2146–55. doi:10.1016/S1388-2457(03)00215-3.
- [39] Van de Borne P, Nguyen H, Biston P, Linkowski P, Degaute JP. Effects of wake and sleep stages on the 24-h autonomic control of blood pressure and heart rate in recumbent men. *Am J Physiol Circ Physiol* 1994;266:H548–54.
doi:10.1152/ajpheart.1994.266.2.H548.
- [40] McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. *Metabolism* 2006;55:23–6. doi:10.1016/j.metabol.2006.07.008.

- [41] Irwin MR, Opp MR. Sleep Health: Reciprocal Regulation of Sleep and Innate Immunity. *Neuropsychopharmacology* 2017;42:129–55. doi:10.1038/npp.2016.148.
- [42] van Leeuwen WMA, Sallinen M, Virkkala J, Lindholm H, Hirvonen A, Hublin C, et al. Physiological and autonomic stress responses after prolonged sleep restriction and subsequent recovery sleep in healthy young men. *Sleep Biol Rhythms* 2018;16:45–54. doi:10.1007/s41105-017-0122-x.
- [43] van Dalen JH, Markus CR. The influence of sleep on human hypothalamic–pituitary–adrenal (HPA) axis reactivity: A systematic review. *Sleep Med Rev* 2018;39:187–94. doi:10.1016/j.smrv.2017.10.002.
- [44] Tobaldini E, Costantino G, Solbiati M, Cogliati C, Kara T, Nobili L, et al. Sleep, sleep deprivation, autonomic nervous system and cardiovascular diseases. *Neurosci Biobehav Rev* 2017;74:321–9. doi:10.1016/j.neubiorev.2016.07.004.
- [45] Huang W, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sleep, and metabolism. *J Clin Invest* 2011;121:2133–41. doi:10.1172/JCI46043.
- [46] Massar SAA, Liu JCJ, Mohammad NB, Chee MWL. Poor habitual sleep efficiency is associated with increased cardiovascular and cortisol stress reactivity in men. *Psychoneuroendocrinology* 2017;81:151–6. doi:10.1016/j.psyneuen.2017.04.013.
- [47] Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet* 1999;354:1435–9. doi:10.1016/S0140-6736(99)01376-8.
- [48] Vgontzas AN, Tsigos C, Bixler EO, Stratakis CA, Zachman K, Kales A, et al. Chronic insomnia and activity of the stress system: A preliminary study. *J Psychosom Res* 1998;45:21–31. doi:10.1016/S0022-3999(97)00302-4.
- [49] Zhong X, Hilton HJ, Gates GJ, Jelic S, Stern Y, Bartels MN, et al. Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. *J Appl Physiol* 2005;98:2024–32.

- doi:10.1152/jappphysiol.00620.2004.
- [50] Seugnet L, Boero J, Gottschalk L, Duntley SP, Shaw PJ. Identification of a biomarker for sleep drive in flies and humans. *Proc Natl Acad Sci U S A* 2006;103:19913–8. doi:10.1073/pnas.0609463104.
- [51] Figueiro MG, Rea MS. Sleep opportunities and periodic light exposures: Impact on biomarkers, performance and sleepiness. *Light Res Technol* 2011;43:349–69. doi:10.1177/1477153511404175.
- [52] Figueiro MG, Rea MS. The effects of red and blue lights on circadian variations in cortisol, alpha amylase, and melatonin. *Int J Endocrinol* 2010;2010. doi:10.1155/2010/829351.
- [53] Minkel J, Moreta M, Muto J, Htaik O, Jones C, Basner M, et al. Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. *Heal Psychol* 2014;33:1430–4. doi:10.1037/a0034219.
- [54] Schwarz J, Gerhardsson A, van Leeuwen W, Lekander M, Ericson M, Fischer H, et al. Does sleep deprivation increase the vulnerability to acute psychosocial stress in young and older adults? *Psychoneuroendocrinology* 2018;96:155–65. doi:10.1016/j.psyneuen.2018.06.003.
- [55] Pajcin M, Banks S, White JM, Dorrian J, Paech GM, Grant C, et al. Decreased salivary alpha-amylase levels are associated with performance deficits during sleep loss. *Psychoneuroendocrinology* 2017;78:131–41. doi:10.1016/j.psyneuen.2017.01.028.
- [56] Pajcin M, White JM, Banks S, Dorrian J, Paech GM, Grant CL, et al. Effects of strategic early-morning caffeine gum administration on association between salivary alpha-amylase and neurobehavioural performance during 50 h of sleep deprivation. *Accid Anal Prev* 2019;126:160–72. doi:10.1016/j.aap.2018.01.026.
- [57] Pajcin M, Banks S, Dorrian J, Gupta CC, Coates AM, Grant CL, et al. Salivary levels

- of alpha-amylase are associated with neurobehavioral alertness during extended wakefulness, but not simulated night-shift work. *Physiol Behav* 2019;204:1–9. doi:10.1016/j.physbeh.2019.02.004.
- [58] Muehlhan M, Lueken U, Siegert J, Wittchen HU, Smolka MN, Kirschbaum C. Enhanced Sympathetic Arousal in Response to fMRI Scanning Correlates with Task Induced Activations and Deactivations. *PLoS One* 2013;8. doi:10.1371/journal.pone.0072576.
- [59] Bachmann V, Klaus F, Bodenmann S, Schäfer N, Brugger P, Huber S, et al. Functional ADA polymorphism increases sleep depth and reduces vigilant attention in humans. *Cereb Cortex* 2012;22:962–70. doi:10.1093/cercor/bhr173.
- [60] Faraut B, Andrillon T, Drogou C, Gauriau C, Dubois A, Servonnet A, et al. Daytime Exposure to Blue-Enriched Light Counters the Effects of Sleep Restriction on Cortisol, Testosterone, Alpha-Amylase and Executive Processes. *Front Neurosci* 2020;13:1–15. doi:10.3389/fnins.2019.01366.
- [61] Rabat A, Gomez-Merino D, Roca-Paixao L, Bougard C, van Beers P, Dispersyn G, et al. Differential kinetics in alteration and recovery of cognitive processes from a chronic sleep restriction in young healthy men. *Front Behav Neurosci* 2016;10:1–18. doi:10.3389/fnbeh.2016.00095.
- [62] Gomez-merino D, Drogou C, Debellemanniere E, Erblang M, Dorey R, Guillard M, et al. Strategies to Limit Cognitive Impairments under Sleep Restriction: Relationship to Stress Biomarkers. *Brain Sci* 2022;12. doi:10.3390/brainsci12020229.
- [63] Castro-Diehl C, Diez Roux A V., Redline S, Seeman T, McKinley P, Sloan R, et al. Sleep duration and quality in relation to autonomic nervous system measures: The multi-ethnic study of atherosclerosis (MESA). *Sleep* 2016;39:1927–40. doi:10.5665/sleep.6218.

- [64] Strahler J, Nater UM, Skoluda N. Associations between Health Behaviors and Factors on Markers of Healthy Psychological and Physiological Functioning: A Daily Diary Study. *Ann Behav Med* 2020;54:22–35. doi:10.1093/abm/kaz018.
- [65] Lipschitz DL, Kuhn R, Kinney AY, Donaldson GW, Nakamura Y. Reduction in salivary α -amylase levels following a mind-body intervention in cancer survivors-an exploratory study. *Psychoneuroendocrinology* 2013;38:1521–31. doi:10.1016/j.psyneuen.2012.12.021.
- [66] Unno K, Noda S, Kawasaki Y, Yamada H, Morita A, Iguchi K, et al. Ingestion of green tea with lowered caffeine improves sleep quality of the elderly via suppression of stress. *J Clin Biochem Nutr* 2017;61:210–6. doi:10.3164/jcbtn.17-6.
- [67] Unno K, Noda S, Kawasaki Y, Yamada H, Morita A, Iguchi K, et al. Reduced stress and improved sleep quality caused by green tea are associated with a reduced caffeine content. *Nutrients* 2017;9. doi:10.3390/nu9070777.
- [68] Unno K, Tanida N, Ishii N, Yamamoto H, Iguchi K, Hoshino M, et al. Anti-stress effect of theanine on students during pharmacy practice: Positive correlation among salivary α -amylase activity, trait anxiety and subjective stress. *Pharmacol Biochem Behav* 2013;111:128–35. doi:10.1016/j.pbb.2013.09.004.
- [69] Skoluda N, Linnemann A, Nater UM. The role of week(end)-day and awakening time on cortisol and alpha-amylase awakening responses. *Stress* 2016;19:333–8. doi:10.1080/10253890.2016.1174850.
- [70] Michaud DS, Miller SM, Ferrarotto C, Konkle ATM, Keith SE, Campbell KB. Waking levels of salivary biomarkers are altered following sleep in a lab with no further increase associated with simulated night-time noise exposure. *Noise Health* 2006;8:30–9. doi:10.4103/1463-1741.32465.
- [71] Klaus K, Doerr JM, Strahler J, Skoluda N, Linnemann A, Nater UM. Poor night's sleep

- predicts following day's salivary alpha-amylase under high but not low stress. *Psychoneuroendocrinology* 2019;101:80–6. doi:10.1016/j.psyneuen.2018.10.030.
- [72] LaVoy EC, Palmer CA, So C, Alfano CA. Bidirectional relationships between sleep and biomarkers of stress and immunity in youth. *Int J Psychophysiol* 2020;158:331–9. doi:10.1016/j.ijpsycho.2020.10.010.
- [73] Pradhan RK, Sinha N. Level of alpha amylase activity in human saliva as a non-invasive biochemical marker of sleep deprivation. *Sleep Biol Rhythms* 2018;16:357–64. doi:10.1007/s41105-018-0165-7.
- [74] Yan YR, Zhang L, Lin YN, Wei Y, Li N, Sun XW, et al. The Association of Salivary Biomarkers With the Severity of Obstructive Sleep Apnea and Concomitant Hypertension. *Am J Med Sci* 2019;357:468–73. doi:10.1016/j.amjms.2019.03.004.
- [75] Ding H, Wang M, Hu K, Kang J, Tang S, Lu W, et al. Adenotonsillectomy can decrease enuresis and sympathetic nervous activity in children with obstructive sleep apnea syndrome. *J Pediatr Urol* 2017;13:41.e1-41.e8. doi:10.1016/j.jpuro.2016.10.009.
- [76] Park CS, Guilleminault C, Park HJ, Cho JH, Lee HK, Son HL, et al. Correlation of salivary alpha amylase level and adenotonsillar hypertrophy with sleep disordered breathing in pediatric subjects. *J Clin Sleep Med* 2014;10:559–66. doi:10.5664/jcsm.3712.
- [77] Patacchioli FR, Tabarrini A, Ghiciuc CM, Dima-Cozma LC, Prete A, Bianchini C, et al. Salivary biomarkers of obstructive sleep apnea syndrome in children. *Pediatr Pulmonol* 2014;49:1145–52. doi:10.1002/ppul.22972.
- [78] Karakoulaki S, Tortopidis D, Andreadis D, Koidis P. Relationship Between Sleep Bruxism and Stress Determined by Saliva Biomarkers. *Int J Prosthodont* 2015;28:467–74. doi:10.11607/ijp.4296.

- [79] Lasisi DTJ, Shittu ST, Meludu CC, Salami AA. Differential effects of total and partial sleep deprivation on salivary factors in Wistar rats. *Arch Oral Biol* 2017;73:100–4. doi:10.1016/j.archoralbio.2016.09.002.
- [80] Wei Q, Ta G, He W, Wang W, Wu Q. Stilbene glucoside, a putative sleep promoting constituent from *Polygonum multiflorum* affects sleep homeostasis by affecting the activities of lactate dehydrogenase and salivary alpha amylase. *Chem Pharm Bull* 2017;65:1011–9. doi:10.1248/cpb.c17-00275.
- [81] Mandel ID. The diagnostic uses of saliva. *J Oral Pathol Med* 1990;19:119–25. doi:10.1111/j.1600-0714.1990.tb00809.x.
- [82] Grant LK, Ftouni S, Nijagal B, De Souza DP, Tull D, McConville MJ, et al. Circadian and wake-dependent changes in human plasma polar metabolites during prolonged wakefulness: A preliminary analysis. *Sci Rep* 2019;9:1–14. doi:10.1038/s41598-019-40353-8.
- [83] Ghiciuc CM, Cozma-Dima CL, Pasquali V, Renzi P, Simeoni S, Lupusoru CE, et al. Awakening responses and diurnal fluctuations of salivary cortisol, DHEA-S and alpha-amylase in healthy male subjects. *Neuroendocrinol Lett* 2011;32:475–80.
- [84] Veen G, Giltay EJ, Vreeburg SA, Licht CMM, Cobbaert CM, Zitman FG, et al. Determinants of salivary evening alpha-amylase in a large sample free of psychopathology. *Int J Psychophysiol* 2012;84:33–8. doi:10.1016/j.ijpsycho.2012.01.005.
- [85] Wingenfeld K, Schulz M, Damkroeger A, Philippson C, Rose M, Driessen M. The diurnal course of salivary alpha-amylase in nurses: An investigation of potential confounders and associations with stress. *Biol Psychol* 2010;85:179–81. doi:10.1016/j.biopsycho.2010.04.005.
- [86] Mandel AL, Breslin PAS. High endogenous salivary amylase activity is associated

- with improved glycemic homeostasis following starch ingestion in adults. *J Nutr* 2012;142:853–8. doi:10.3945/jn.111.156984.
- [87] Mandel AL, Des Gachons CP, Plank KL, Alarcon S, Breslin PAS. Individual differences in AMY1 gene copy number, salivary α -amylase levels, and the perception of oral starch. *PLoS One* 2010;5. doi:10.1371/journal.pone.0013352.
- [88] Amato JN, Tuon RA, Castelo PM, Gavião MBD, Barbosa TDS. Assessment of sleep bruxism, orthodontic treatment need, orofacial dysfunctions and salivary biomarkers in asthmatic children. *Arch Oral Biol* 2015;60:698–705. doi:10.1016/j.archoralbio.2015.02.011.
- [89] Wong IS, Ostry AS, Demers PA, Davies HW. Job strain and shift work influences on biomarkers and subclinical heart disease indicators: A pilot study. *J Occup Environ Hyg* 2012;9:467–77. doi:10.1080/15459624.2012.693831.
- [90] Teixeira RR, Díaz MM, Da Silva Santos TV, Bernardes JTM, Peixoto LG, Bocanegra OL, et al. Chronic stress induces a hyporeactivity of the autonomic nervous system in response to acute mental stressor and impairs cognitive performance in business executives. *PLoS One* 2015;10:1–14. doi:10.1371/journal.pone.0119025.
- [91] Thoma M V., Kirschbaum C, Wolf JM, Rohleder N. Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol Psychol* 2012;91:342–8. doi:10.1016/j.biopsycho.2012.07.008.
- [92] Kuebler U, von Känel R, Heimgartner N, Zuccarella-Hackl C, Stirnimann G, Ehlert U, et al. Norepinephrine infusion with and without alpha-adrenergic blockade by phentolamine increases salivary alpha amylase in healthy men. *Psychoneuroendocrinology* 2014;49:290–8. doi:10.1016/j.psyneuen.2014.07.023.
- [93] El-Sheikh M, Buckhalt JA, Granger DA, Erath SA, Acebo C. The association between children's sleep disruption and salivary interleukin-6. *J Sleep Res* 2007;16:188–97.

doi:10.1111/j.1365-2869.2007.00593.x.

- [94] Besedovsky L, Lange T, Born J. Sleep and immune function. *Pflugers Arch Eur J Physiol* 2012;463:121–37. doi:10.1007/s00424-011-1044-0.
- [95] Yamaguchi M, Deguchi M, Wakasugi J, Ono S, Takai N, Higashi T, et al. Hand-held monitor of sympathetic nervous system using salivary amylase activity and its validation by driver fatigue assessment. *Biosens Bioelectron* 2006;21:1007–14. doi:10.1016/j.bios.2005.03.014.
- [96] Thieux M, Guyon A, Herbillon V, Merle L, Lachaux J, Plancoulaine S, et al. Interest of the BLAST paradigm and salivary markers for the evaluation of sleepiness in drivers. *Front Neurosci* 2022.
- [97] Proctor GB, Shaalan AM. Disease-Induced Changes in Salivary Gland Function and the Composition of Saliva. *J Dent Res* 2021;100:1201–9. doi:10.1177/00220345211004842.

Table

Table 1. Descriptive table of studies assessing the relationship between sleep and sAA in humans.

	sAA modulation		
	Increase	No variation	Decrease
Studies (N)	20 [14,22,65–69,72– 76,25,27,28,50,51,59,63,64]	7 [15,52– 54,70,77,78]	7 [55–57,60– 62,71]
Volunteers (N)			
<i>Adults: M/W</i>	952: 194/304*	288: 134/134*	122: 78/44
<i>Children: B/G</i>	499: 252/247	54: 33/21	0: 0/0
Patients with sleep disorders (N)			
<i>Adults: M/W</i>	134: 60/74	25*	0: 0/0
<i>Children: B/G</i>	187: 131/56	27: 12/15	0: 0/0
Sleep manipulation (number of studies)			
<i>Sleep deprivation</i>	3	3	2
<i>Sleep restriction</i>	2	0	4
<i>No manipulation</i>	17	4	1
Sleep measures (number of studies)			
<i>Subjective</i>	10	1#	1#
<i>Objective</i>	12	3	6

* Unreported subdivision [63,78]; # No sleep measurement during the experimentation [52,54,55,57,78]. B/G: boys/girls; M/W: men/women; sAA: salivary α -amylase

Supplementary materials

Table S1. Summary of studies in humans included in the narrative review

Authors, Year	N (%Female)	Age group	Population	Salivary samples <i>N, method, procedure</i>	Sleep manipulation	Sleep measure	Key findings
Bachmann <i>et al.</i> , (2012) [59]	22 (10)	A	H	20, AM, every 2h from 8 a.m.	SD (40h)	EEG	↑ sAA with ↑ sleep pressure (group with genetic mutation)
Bright <i>et al.</i> , (2014) [15]	47 (17)	C	H	2, AM, at awakening and 30-45' later	∅	ACT	No modification of sAA with sleep characteristics
Castro-Diehl <i>et al.</i> , (2016) [63]	454 (-)	A	-	4, AM, before a stress task, 38, 55, and 85' later	∅	ACT	↑ sAA with ↓ sleep efficiency
Danilenko <i>et al.</i> , (2021) [27]	45 (33)	A	H	8, SS, at awakening, 10, 20, and 30' later (summer, winter)	∅	SRM	↓ sAA awakening response with ↓ decreased sleep duration
Ding <i>et al.</i> , (2017) [75]	146 (45)	C	H, OSA	2 -, 10 p.m. and 30' post awakening	∅	PSG	sAA: OSA > H ↑ sAA with ↑ AHI and ↑ ODI
Faraut <i>et al.</i> , (2020) [60]	16 (0)	A	H	24, PD, at 11 a.m., 1:30, 4, and 6:30 p.m. (2 x 3 days)	SR (2 nights of 3h from 3 to 6 a.m.)	ACT	↓ sAA at 4 p.m. post SR
Figueiro & Rea (2010) [52]	12 (4)	A	H	14, AM, at 8, 9 a.m., 12, 1, 4, 5, 8, 9 p.m., 0, 1, 4, 5, 8, 9 a.m.	SD (27h)	∅	No modification of sAA levels at 8:00 a.m. after SD
Figueiro & Rea (2011) [51]	10 (5)	A	H	14, AM, at 8, 9 a.m., 12, 1, 4, 5, 8, 9 p.m., 0, 1, 4, 5, 8, 9 a.m.	SD (1 night) vs SR (3h from 1 to 4 a.m.) vs ∅	∅	↓ sAA at awakening with ↑ sleep duration (SD > SR > ∅)
Gomez-Merino <i>et al.</i> , (2022) [62]	13 (6)	A	H	12, PD, at 8 a.m. (3 x 4 days: before and after SR, after 2 and 5 nights of recuperation)	SR (5 nights of 3h from 4 to 7 a.m.)	PSG	↓ sAA after SR
Karakoulaki <i>et al.</i> , (2015) [78]	45 (25)	A	H, bruxism	1, AM, at awakening between 7 and 9 a.m.	∅	∅	sAA: bruxism = H
Klaus <i>et al.</i> , (2019) [71]	50 (31)	A	-	50, PD, 30' post-awakening, 10 a.m., 2, 6, 9 p.m. (2 x 5 days)	∅	SRM ACT	High stress condition: ↓ sAA at awakening and ↑ diurnal slope with sleep perturbation Low stress condition: no association between sAA and sleep
LaVoy <i>et al.</i> , (2020) [72]	55 (29)	C	H	1, PD, at awakening	∅	SRM ACT	↑ sAA with ↑ subjective sleep disorders ∅ association with objective measures
Lipschitz <i>et al.</i> , (2013) [65]	57 (43)	A	H (cancer survivors)	5, AM, 30' post-awakening, 12 a.m., 5, 10 p.m., and 8 a.m.	∅	SRM	↓ sAA at awakening following intervention to ↓ stress during sleep ∅ association sAA / subjective sleep disorders

Michaud <i>et al.</i> , (2006) [70]	12 (5)	A	H	24, AM, at awakening, 30', 90' later, 12 a.m., 5 p.m. and before nighttime (4 days)	Sleep disturbance with noise vs \emptyset	SRM	No modification of sAA levels with sleep disturbance
Minkel <i>et al.</i> , (2014) [53]	26 (12)	A	H	5, AM, between 5 and 5:30 p.m., 20', 5' before TSST, 5', 20', 40' later	SD (1 night) vs \emptyset	-	No modification of sAA following SD vs \emptyset
Nater <i>et al.</i> , (2007) [14]	76 (44)	A	H	15, AM, at awakening, 30', 60' later, and every hour between 9 a.m. and 8 p.m.	\emptyset (awakening before 9 a.m.)	-	\uparrow sAA at awakening and 30' later (alarm clock vs natural) No modification of sAA with sleep characteristics
O'Leary <i>et al.</i> , 2015 [22]	108 (80)	A	H	2, PD, before and after exposure to stressor (between 9 and 12 a.m.)	SR (40% TST) vs \emptyset	ACT	sAA: SR > \emptyset
Pajcin <i>et al.</i> , (2017) [55]	11 (4)	A	H	15, AM, every 3h from 9 a.m.	SD (50h)	\emptyset (EEG post SD)	No modification of sAA during SD (1 st = 2 nd day) Positive association between sAA and cognitive performance
Pajcin <i>et al.</i> , (2019) [56]	12 (4)	A	H	15, AM, every 3h from 9 a.m.	SD (50h) + caffeine	\emptyset (EEG post SD)	Positive association between sAA and cognitive performance (1 st day)
Pajcin <i>et al.</i> , (2019) [57]	10 (0)	A	H	17, AM, at 11 a.m., 5, 8 :30 p.m., 0:00, and 3 a.m. (4 days)	SD (28h) + 4 simulated night shifts (sleep from 10 a.m. to 4 p.m.)	-	Positive association between sAA and cognitive performance (1 st day SD)
Park <i>et al.</i> , (2014) [76]	67 (28)	C	H, OSA	2, PD, at 10 p.m. and 7 a.m.	\emptyset	PSG	\uparrow sAA ratio (night/day) with \uparrow OSA severity sAA levels: OSA = H
Patacchioli <i>et al.</i> , (2014) [77]	34 (19)	C	H, OSA	3, AM, 1h post-awakening (between 8:30 and 9 a.m.), at 12 a.m. and 7 p.m.	\emptyset	PSG	sAA: OSA = H
Pradhan <i>et al.</i> , (2018) [73]	168 (74)	C	H	2, SS, between 11 and 11:30 a.m. (2 years)	\emptyset	SRM	\uparrow sAA with \downarrow sleep duration
Rabat <i>et al.</i> , (2016) [61]	12 (0)	A	H	4, PD, at awakening before SR (7 a.m.), after SR (6 a.m.) and after 2 and 7 days of recuperation (7 a.m.)	SR (7 nights of 4h from 2 to 6 a.m.)	PSG	\downarrow sAA after SR Positive association between sAA and cognitive performance
Raikkonen <i>et al.</i> , (2010) [28]	276 (143)	C	H	7, AM, between 12 a.m. and 2 p.m., before and after the task, 10', 20', 30', 45' later	\emptyset	ACT	\uparrow sAA during stress task with \downarrow sleep efficiency
Schwarz <i>et al.</i> , (2018) [54]	218 (114)	A	H	5, PD, before TSST (at 1 or 4 p.m.), 28', 46', 66', 86' later	SD (1 night) vs \emptyset	\emptyset	sAA: SD = \emptyset
Seugnet <i>et al.</i> , (2006) [50]	9 (2)	A	H	2, AM, before and after SD	SD (28h)	PSG	\uparrow sAA after SD

Skoluda <i>et al.</i> , (2016) [69]	44 (25)	A	H	6, PD, at awakening, 30' later, 11 a.m., 2, 6, 9 p.m.	∅	SRM	↑ sAA (awakening response) with ↓ time of awakening
Strahler <i>et al.</i> , (2020) [64]	77 (38)	A	H	6, PD, at awakening, 30' later, 11 a.m., 2, 6, 9 p.m.	∅	SRM	↓ sAA with ↑ sleep quality
Unno <i>et al.</i> , (2013) [68]	20 (6)	A	H	20, CS, morning and evening (10 days)	∅	SRM	↑ sAA with ↓ sleep duration
Unno <i>et al.</i> , (2017) [66]	10 (9)	A	H	42, CS, morning (1 st hour post-awakening) and evening (3 weeks)	∅	EEG	↓ sAA (morning) with ↑ sleep quality
Unno <i>et al.</i> , (2017) [67]	20 (9)	A	H	10, CS, morning (30' post-awakening) and evening (before dinner) (5 days)	∅	EEG	↓ sAA (evening) and ↑ sleep quality (group with low caffeine green tea vs standard)
Van Lenten & Doane (2016) [25]	76 (58)	C	H	15, PD, at awakening, 30', 3 and 8h later, at bedtime (3 days)	∅	SRM ACT	↑ sAA (awakening) with ↓ subjective sleep quality ∅ association with objective measures
Yan <i>et al.</i> , (2019) [74]	58 (17)	A	OSA	1, AM, at awakening	∅	PSG	↑ sAA with ↑ OSA severity ↑ sAA with ↑ AHI and ↓ lowest pulse oxygen saturation

-: unknown; ∅: none; A: adults; ACT: actigraphy; AHI: apnea-hypopnea index; AM: absorbent material; C: children; CS: colorimetric system; EEG: electroencephalogram; H: healthy; ODI: oxygen desaturation index; OSA: obstructive sleep apnea, PD: passive drool; PSG: polysomnography; sAA: salivary α -amylase; SD: sleep deprivation; SR: sleep restriction; SRM: self-reported measurement; SS: stimulated spitting; TST: total sleep time; TSST: trier social stress test



