



## Human salivary alpha-amylase reactivity in a psychosocial stress paradigm

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### Abstract

Biological indicators for stress reactions are valuable markers in psychophysiological research and clinical practice. Since the release of salivary enzyme alpha-amylase was reported to react to physiological and psychological stressors, we set out to investigate human salivary alpha-amylase changes employing a reliable laboratory stress protocol to investigate the reactivity of salivary alpha-amylase to a brief period of psychosocial stress.

In a within-subject repeated-measures design, 24 healthy adults were exposed to the TSST and a control condition on separate days with randomized sequence. Salivary alpha-amylase, salivary cortisol and heart rate were repeatedly measured before, during and after both conditions.

Significant differences between psychosocial stress and the rest condition in alpha-amylase activity [ $F(3.74,86.06)=4.52$ ;  $P=0.003$ ], cortisol levels [ $F(4.21,88.32)=12.48$ ;  $P<0.001$ ] and heart rate [ $F(1,22)=81.15$ ;  $P<0.001$ ] were observed, with marked increases before and after stress.

The data corroborate findings from other studies that showed increased levels of alpha-amylase before and after psychological stress. We discuss the role of salivary alpha-amylase as a promising candidate for a reliable, noninvasive marker of psychosocial stress.

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### 1. Introduction

The use of salivary biomarkers has gained increased popularity over the past decade in psychological and biomedical research. While the measurement of free cortisol in saliva has proven

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useful to assess functioning and reactivity of the hypothalamic–pituitary–adrenal (HPA) axis (e.g., Kirschbaum and Hellhammer, 1994), a suitable marker of the sympathoadrenal medullary (SAM) activity in saliva has not been yet found (Schwab et al., 1992; Kennedy et al., 2001). Salivary alpha-amylase is a candidate substance to indicate autonomic activity since secretion from human salivary glands occurs in response to neurotransmitter stimulation and salivary glands are innervated by both sympathetic and parasympathetic nerves (Garrett, 1999). Generally, it is considered that sympathetic stimulation [via norepinephrine (NE)] leads to high levels of protein concentrations, e.g., alpha-amylase, whereas high rates of fluid output occur in response to parasympathetic cholinergic stimulation [via acetylcholine (ACh); Baum, 1993]. With regard to the secretion of alpha-amylase, the two branches of the autonomic nervous system do not act independently; results from animal studies suggest that both parasympathetic and sympathetic activation lead to an increase in alpha-amylase levels. However, examination of the two branches separately may not be considered a physiological approach to an *in vivo* situation. In studies combining sympathetic and parasympathetic stimulation, marked elevations in amylase levels have been found (Kyriacou et al., 1988). This methodological approach led to the notion, that amylase secretion is primarily mediated by activation of  $\beta_1$  adrenoceptors (e.g., Schneyer and Hall, 1991). Three major glands (parotid, submandibular and sublingual) and numerous minor glands produce about 500 to 1500 ml of saliva daily. Saliva plays a role in speech and swallowing through its lubricating action, in tasting through its solubilizing action, and in initial enzymatic digestion through one of its major constituents, alpha-amylase (Humphrey and Williamson, 2001). Amylase is produced by the serous acinar cells of the parotid and submandibular glands. It is one of the principal salivary protein appearing as a number of isoenzymes. Amylase accounts for 10–20% of the total salivary gland-produced protein content and is mostly synthesized by the parotid gland. It is a calcium-containing metalloenzyme that hydrolyzes the  $\alpha$ 1,4 linkages of starch to glucose and maltose (Zakowski and Bruns, 1985). Alpha-amylase is not only responsible for an initiation of digestion in the oral cavity but it is also considered to play an

important role in binding to oral bacteria (Scannapieco et al., 1993).

Due to the insights gained in animal and human research, it was concluded that marked elevations of alpha-amylase concentrations are indicative for autonomic activation. For example, there is evidence that alpha-amylase levels increase in response to physical stressors, such as treadmill exercise (Gilman et al., 1979a), exposure to a high-pressure chamber (Gilman et al., 1979b), running (Nexo et al., 1988; Steerenberg et al., 1997), bicycle exercise (Chatterton et al., 1996; Walsh et al., 1999) or cold exposure (Chatterton et al., 1996).

Salivary alpha-amylase levels were also found to respond to psychological stress (Bosch et al., 1996, 1998; Skosnik et al., 2000) or relaxation interventions (Morse et al., 1981a; Morse et al., 1983a,b). While it seems clear that alpha-amylase levels rise following physical stress, the response to a psychological stressor appears to be more inconsistent. This might be due to the psychological nature of the stressors employed or other methodological details. For example, most of these studies measured alpha-amylase levels too infrequently for recording short-term responses to a brief stressor. Since the reported findings on the effects of psychological stress are not consistent in their conclusions, we set out to examine alpha-amylase activity in a standardized psychosocial stress test (Kirschbaum et al., 1993) in a repeated-measures design. Elucidating a detailed course of alpha-amylase activity due to psychological stimuli may further establish the use of salivary alpha-amylase as an indicator of human stress reactions.

## 2. Methods

### 2.1. Participants

Subjects were recruited at the University of Zurich and the Swiss Federal Institute of Technology, Zurich. They received a screening questionnaire, containing exclusion criteria designed to reduce confounding factors that have been shown to affect physiological dependent measures. All subjects were medication-free and refrained from smoking, physical exercise, meals, alcoholic beverages

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