# EDITORIAL

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# Salivary α-Amylase and Cortisol as Stress Biomarkers – Literature Review

## α-Amylaza i kortyzol jako biomarkery stresu – przegląd piśmiennictwa

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### Abstract

Studies on the human body's response to stress have shown a significant role of neurohormonal markers. The stress response is originally governed by two hormonal systems: the sympathetic nervous system (SNS) and the hypothalamic – pituitary – adrenal axis (HPA). Cortisol is well-known as a biological stress marker of the HPA activity. Initially, the cortisol level has been measured in blood serum; however, in the 1980s, the biological material used to assess its level became saliva. Since then, non-invasive saliva sampling became a method of choice in studies requiring cortisol measurement. The result of searching for a simple stress marker of sympathetic nervous sytem is α-amylase. This enzyme is produced by the salivary glands cells and therefore is an indirect product of the HPA axis, but its level is closely correlated with the sympathetic activity, and increased by the action of a physical and psychological stressor. Studies have confirmed the advantages of salivary α-amylase as a biomarker, such as reliability and a non-invasive, fast and simple sample collecting procedure (**Dent. Med. Probl. 2013, 50, 3, 271–274**).

**Key words:** hydrocortisone, salivary alpha-amylase, physiological stress.

### Streszczenie

Badania nad odpowiedzią organizmu na stres wykazały znaczącą rolę markerów neurohormonalnych. Odpowiedź na stres jest pierwotnie regulowana przez dwa systemy hormonalne: układ współczulny i oś podwzgórze–przysadka–kora nadnerczy. Kortyzol jako biomarker pobudzenia osi podwzgórze–przysadka–kora nadnerczy w odpowiedzi na stres jest znany od dawna. Początkowo stężenie kortyzolu określano na podstawie krwi. Dopiero w latach 80. XX w. materiałem do badań została ślina. Od tego czasu nieinwazyjny pobór próbek śliny stał się metodą z wyboru. Poszukiwania równie prostego biomarkera pobudzenia układu współczulnego podczas stresu doprowadziły do α-amylazy ślinowej. Enzym ten jest wytwarzany przez komórki gruczołów ślinowych, dlatego nie jest bezpośrednim produktem osi podwzgórze–przysadka–kora nadnerczy, ale jego stężenie ściśle koreluje z aktywnością układu współczulnego i zwiększa się pod wpływem bodźca fizycznego lub psychicznego. Badania dowiodły, że stężenie tego enzymu w ślinie jest wiarygodnym wskaźnikiem odpowiedzi układu współczulnego na stres, a procedura pobrania materiału do badań jest szybka, prosta, powtarzalna i nieinwazyjna (**Dent. Med. Probl. 2013, 50, 3, 271–274**).

**Słowa kluczowe:** α-amylaza ślinowa, hydrokortyzon, stres.

Stress arises from the disruption of the body homeostasis, caused by stressful factors (stressors) and causes neurohormonal changes. More than half a century ago, Selye and McKeown were the first to have defined stress as the body's response to an external stimulus [according to 1]. It is now known that stressors can be physical (eg. hunger, thirst, cold, heat, hypovolemia, mechanical trauma, surgery, heavy exercise) or psychological (fear, insecurity, depression, frustration). Stressors threaten the homeostasis of the body, and the physiological response to them is adap-

tive in nature, designed to maintain or restore homeostasis.

Studies on the human body's response to stress have shown a significant role of neurohormonal markers. The stress response is originally governed by two hormonal systems: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis (HPA) [2, 3]. The SNS is activated immediately after a stimulus and it is partially responsible for the "fight or flight" decision. Stimulated sympathetic nervous system triggers the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla, resulting in an increase in blood pressure, accelerated heart rate and respiratory rate, bronchodilatation and mydriasis. The HPA axis is the second major neuroendocrine response to stress, whose final product is cortisol.

Cortisol (hydrocortisone) is an important hormone in the stress response regulation. In the plasma, it is present in two forms – free (active) and protein-bound (inactive), and in the saliva – only in free form.

It is a long known biomarker of physical and psychological stress response. Initially, the cortisol level has been measured in blood serum; however, in the 1980s, salive came to be used as the biological material to assess its level [4, 5]. The research of Vining et al. [6] has shown that levels of hydrocortisone in blood and saliva are comparable, and since then a non-invasive saliva sampling has become the method of choice when examining the stimulation of the HPA. The level of cortisol, after stressor activation, has a characteristic slow growth profile – increases gradually from the 6–10 nmol/L (in adults), reaching a peak – 8–17 nmol/L after 20–30 min and returns to baseline after 1 h [1, 7, 8].

While the assessment of the HPA axis activity has been carried out in non-invasive collecting of saliva, the measurement of sympathetic system stimulation was still inconvenient and required expensive equipment and complex data processing techniques (impedance cardiography, ECG) or invasive procedures (collection of blood or cerebrospinal fluid). The search for a simple indicator to assess the activation of the sympathetic system has led to the study on salivary α-amylase (sAA).

This enzyme is produced by the salivary glands cells and therefore is an indirect product of the HPA axis, but its level is closely correlated with the sympathetic activity, and increased by the action of a physical and psychological stressor [1, 9]. The relationship between the sympathetic branch of the autonomic nervous system and the secretion of salivary α-amylase occurs between 2 and 6 months of age, and from that age the level of secreted α-amylase corresponds to exposure to stress [10].

Studies on the α-amylase in response to stress have been conducted for a long time in various study groups. Frequently, both salivary α-amylase and cortisol levels (or only α-amylase level) have been measured, a few studies also included chromogranin A, which is considered as a reliable indicator of psychological stress [11, 12]. In most studies, the study group consists of adults – healthy individuals of both sexes, from different professional groups (eg. medical students, psychotherapists, Air Force soldiers in Iraq). The few studies included people with various diseases (dialysis, with aggressive and chronic periodontitis) and children at different developmental ages (from infants through preschool and school children to teenagers). The papers aimed to confirm interpersonal relations of these biomarkers levels, and included mothers and their children of all ages, as well as unrelated persons, such as spouses and dating couples.

The first to discover the link between the activation of the sympathetic system and the secretion of salivary α-amylase were Batzri and Selinger [13] in 1973, who showed that beta-blockers cause the secretion of salivary α-amylase. In 1979, Gilman et al. [14] reported elevated levels of α-amylase in response to strenuous exercise. Chatterton et al. [15] have demonstrated a correlation between plasma levels of norepinephrine, released during stress, and the level of α-amylase. They suggested that salivary α-amylase may be a valid and reliable indicator of peripheral catecholaminergic activity. It has been confirmed in further studies in other centers [16, 17]. However, Nater et al. [7, 9] found no significant correlation between the levels of α-amylase and plasma catecholamines (epinephrine and norepinephrine), but only a stress-dependent increase of α-amylase. This indicates that the level of α-amylase is not a reflection of the peripheral catecholamine stimulation, but results from the central norepinephrine release. This may be due to differences in the origin of norepinephrine released centrally and peripherally. The direct influence of the sympathetic nervous system mechanisms on secretion of salivary α-amylase confirms the inhibition of enzyme secretion after the activation of emotional stressors by specific and non-specific blockers of the sympathetic nervous system [18]. Ehlert et al. [19] proved the hypothesis postulating that the increase in the level of α-amylase is correlated with centrally released norepinephrine. This hypothesis has been confirmed in a randomized, double-blind study.

Another aspect that has undermined the reliability of the α-amylase as a biomarker of SNS axis arousal, was its possible level of dependence on the

saliva secretion rate. The increase in saliva protein secretion is usually assigned to sympathetic activity, and the parasympathetic nervous system mainly plays the role in the stimulation of salivary flow rate. On this basis, Rohleder et al. [20] showed that an increase in the stress-induced α-amylase level was associated with an increase in the α-amylase production, but not with increased secretion of saliva. These results show that the salivary flow rate is not significant for measuring the level of stress-induced α-amylase.

There is no agreement between the results for different levels of α-amylase in the saliva connected with gender. Some authors indicate no difference between the sexes [1, 12], others are observe higher levels of α-amylase during stressful situations in men than in women [21].

Results in studies concerning the relation between the activity of α-amylase and age are inconsistent. It has been proven that the activity of α-amylase decreases with age [22], is not related to age [23] and increases in old age [24].

On average, the secretion of α-amylase reaches a maximum level 5 min after the actuation of stressor and returns to baseline levels after about 10 min [10, 25]. According to Murayama et al. [1] the maximum concentration is reached immediately after a stimulus is given and a return to baseline is recorded after about 20 min. The average level of α-amylase at rest (no physical activity) in adults is 20–50 U/mL, the maximum value reaches 30–150 U/mL [1, 8, 19]. Since the growth pattern of salivary α-amylase is different from the growth pattern of cortisol, the study aimed to assess the level of cortisol, and α-amylase only in addition, can miss the peak secretion of the enzyme and provide misleading results [26, 27]. Also, the site of salivary collection in oral cavity affects the activity of α-amylase [28]. It was highest in the saliva taken from the areas directly adjacent to the parotid and submandibular glands.

The study aimed at finding a relationship between the levels of salivary α-amylase and cortisol in response to stress has yielded inconclusive results. Grillon et al. [29] found a strong positive correlation between two biomarkers after using TSST (The Trier Social Stress Test). This standardized test consists of two tasks – a simulated interview with the participation of the audience and arithmetic tasks, and is used to produce a response to psychological stress [30]. Schoofs et al. [31] and van Stegeren et al. [21] found only a weak positive correlation between the two biomarkers in the following test. Nater et al. [7, 9] found no correlation to the TSST. The possible reason for this contradiction may be that no particular attention has been paid to the time periods, different when stimulating the HPA and the SNS axis. Engert et al. [32] analyzed the overall relationship between the growth profiles of α-amylase and cortisol in response to stress and – using cross-correlation – made the biomarkers‘ profiles synchronization with regard to stressor activation response including the different dynamics of each biomarker. The results confirmed the positive correlation between the biomarkers.

The level of cortisol measured in saliva is used for a long time and successfully as an indicator of activation of the hypothalamic-pituitary-adrenal cortex axis in stressful situations. The salivary α-amylase can be a reliable biomarker for the research on the second system activated under stress – the sympathetic nervous system.

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