SALIVARY α-AMYLASE

KINETIC ENZYME ASSAY KIT

For Research Use Only
Not for use in Diagnostic Procedures

Item No. 1-1902, (Single) 96-Well Kit;
1-1902-5, (5-Pack) 480 Wells

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Intended Use

The Salimetrics® α-Amylase Kinetic Enzyme Assay Kit is specifically designed and validated for the kinetic measurement of salivary α-Amylase activity. It is not intended for diagnostic use. It is intended only for research use in humans and some animals. Salimetrics has not validated this kit for serum or plasma samples.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Technical advances that make the assessment of biomarkers in saliva possible have enabled researchers to non-invasively study biosocial processes related to stress in naturalistic contexts. Much of the attention has focused on the activity of the limbic hypothalamic-pituitary-adrenal (LHPA) axis as indexed by individual differences and intra-individual change in salivary cortisol. Recently, it was suggested that the nearly exclusive focus of this endeavor on salivary cortisol may not enable researchers to adequately operationalize the psychobiology of the stress response (1). Physiologists have known for decades that the stress response has at least two principal components. One involves corticotropin-releasing hormone, activation of the LHPA axis, and the secretion of glucocorticoids (e.g., cortisol) into circulation. The second involves activation of the locus coeruleus /autonomic (sympathetic) nervous system and the release of catecholamines (e.g., norepinephrine) into the blood stream (2). Theorists argue that, to advance our understanding of how biological, social, and behavior processes interact to determine risk versus resilience, the next generation of studies will need to employ analytical models that operationalize both the behavioral and biological sides of the equations using multi-method and trait measurement approaches (1). Unfortunately, our ability to do so has been restricted because, in contrast to the highly sensitive, accurate, and valid measurement of LHPA products in saliva (i.e., cortisol, dehydroepiandrosterone), the non-invasive measurement of autonomic (sympathetic) nervous system activity in saliva (i.e., catecholamines) has been problematic (3).

In an attempt to overcome this problem, we conducted an extensive computerized literature search for potential surrogate markers of autonomic (sympathetic) nervous system activation that could be measured accurately in saliva. α-Amylase, the most abundant salivary enzyme in humans, has been identified as a biomarker that appears to fill this role. Best known for its function as a digestive enzyme that breaks down dietary starch, α-Amylase has also been studied for its ability to bind to oral bacteria and to tooth enamel. It is believed to play a key role in the establishment and maintenance of the oral microflora to form dental plaques (4,5). Secretion of α-Amylase from the salivary glands is controlled by autonomic nervous signals,

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and substantial literature reveals that salivary α-Amylase (sAA) is a correlate of sympathetic activity under conditions of stress. Studies show that levels of sAA increase under a variety of physically (i.e., exercise, heat and cold) and psychologically (i.e., written examinations) stressful conditions (6) in human subjects. Interestingly, studies show that cortisol levels often do not correlate with α-Amylase during stress, (1,6,7) suggesting that individual differences in α-Amylase represent a response to a stress signal independent of the LHPA axis.

Early studies on sAA showed that its concentrations are predictive of plasma catecholamine levels, particularly norepinephrine (NE), and are highly correlated with NE changes in response to stress (6). However, more recent studies call this relationship into question (7). The literature does show that stress-related increases in sAA can be inhibited by the adrenergic blocker propranolol (8,9) and also that beta-adrenergic agonists are capable of stimulating α-Amylase release without increasing salivary flow (10,11). This link suggests that the same stimuli that increase autonomic (sympathetic) arousal may activate sympathetic input to the salivary glands. The sAA response to stress is complex, however, and it appears also to involve the parasympathetic system to a lesser degree (7). A recent article has emphasized the contribution of the parasympathetic system to sAA secretion, pointing out in particular that autonomic reflex activity from the oral cavity, which can increase the parasympathetic signaling to the salivary glands, may have the potential to obscure the effects of central sympathetic nervous system (SNS) activity (12). However, a subsequent study has found that sAA responses significantly predict responses to the Trier Social Stress Task (TSST) for NE but not for epinephrine (E). The relationship between sAA and NE was stronger than the relationship between NE and E responses, indicating the predictive power of sAA is well within the expected range for different SNS markers (13).

Although further work is necessary to understand better the underlying physiological factors that influence sAA secretion, studies have already shown that sAA measurements may be employed as a non-invasive measure of autonomic nervous system activation and are related to a variety of behavioral, social, health, and cognitive phenomena in human subjects (14-16).

Test Principle

This method utilizes a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose (17). The enzymatic action of α-Amylase on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotometrically measured at 405 nm. The amount of α-Amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm. For ease of use, the reaction is read in a 96-well microtiter plate with controls provided.

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Safety Precautions
Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

The α-Amylase Substrate contains:

- Potassium thiocyanate. Do not ingest. May produce irritating fumes if exposed to bleach.

- 0.01% sodium azide as a preservative. Do not ingest. Upon contact with acid, sodium azide forms toxic hydrazoic acid. Explosive metal azides may form in copper or lead plumbing. Disposal requires large volumes of water to prevent the buildup of azide.

We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory practices when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using appropriate absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

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General Kit Use Advice

- This is a kinetic assay that involves taking readings at two different time points. Accurate timing of reagent addition and plate reading is critical for correct assay results. Until experience is gained, we recommend that samples be processed one strip at a time. See Technical Bulletin on the Salimetrics website for Running Multiple Amylase Strips: www.salimetrics.com/documents/amylase-tb.pdf

- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.

- Controls should be assayed at least once on each day of testing. Volume supplied in the kit is sufficient for testing multiple times on multiple days.

- Do not mix components from different lots of kits.

- Protect the α-Amylase Substrate from exposure to direct sunlight.

- When using a multichannel pipette, α-Amylase Substrate should be added to wells at the same time, using the dispensing mode to avoid introducing bubbles into the wells.

- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date.

Specimen Collection

Avoid sample collection within 60 minutes after eating a major meal or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Collect whole saliva by unstimulated passive drool. Donors may tilt the head forward, allowing the saliva to pool on the floor of the mouth, and then passing the saliva through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com or upon request.

Samples visibly contaminated with blood should be recollected. Record the time and date of specimen collection.

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Note: The technique used to collect saliva (various swabs, passive drool), the collection point duration, and the oral fluid type (whole saliva vs. specific glandular saliva) all have an effect on estimates of salivary α-Amylase (sAA) activity. Recent studies have stressed that consistency in collection methods is important in order to avoid introducing unsystematic error into study data (18,19). Typically, α-Amylase concentrations in saliva from the parotid glands in the cheeks are higher than those found in pooled whole saliva from the floor of the mouth. We find that saliva collected by placing a swab underneath the tongue on the floor of the mouth yields results similar to those from whole saliva collected by passive drool. We recommend this location for studies measuring α-Amylase along with other analytes.

Alternatively, if measuring α-Amylase alone, a swab may be used to collect samples of parotid saliva by placing it next to the cheek opposite the 2nd upper molar, where the duct from the parotid gland opens into the mouth. Unstimulated flow from the parotid glands is lower than from the submandibular glands in the floor of the mouth; if collecting parotid saliva, we recommend extending the collection time period in order to ensure the collection of sufficient amounts of saliva.

Although one study has reported that response patterns of sAA during the Trier Social Stress Task were consistent regardless of whether the Amylase concentration (U/mL) or the Amylase output (U/min) was examined, (11) there is still a concern that the effects of saliva flow rate on levels of sAA may lead to problems in the interpretation of data (18,12). Salimetrics currently advises that researchers should note the time period needed to collect the desired amount of saliva, in order to estimate the flow rate (mL/min). Assay results (U/mL) may then be multiplied by the flow rate in order to express the results as output per unit of time (U/min), which may be used for comparison in the data analysis.

If an absorbent device from the SalivaBio Oral Swab family (SOS, SCS, SIS) is used to collect saliva for determination of sAA levels, the volume of saliva collected by the swab can be determined by weighing the device along with the storage tube before and after collection. (An approximate value of 1.0 g/mL may be assumed for the density of the saliva.) If the length of time the swab is in the mouth is also recorded, the flow rate can then be estimated. The device must be removed from the mouth before it reaches its capacity, however, since after that point the estimate of flow rate will not be accurate (18). This ceiling effect is especially a concern for smaller devices, such as the SIS swab, which can reach saturation fairly quickly. A preliminary study may be necessary to determine the optimum collection period, and it may be difficult to find a collection period that will work for all participants.

Sample Handling and Preparation

After collection it is important to keep samples cold, in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC for up to 6 months.) For long term storage, refer to the Salimetrics Collection and Handling Advice Booklet.

Do not add sodium azide to saliva samples as a preservative, as it may cause interference when testing samples for multiple analytes. (Sodium azide may be used only if testing for α-Amylase alone.)

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging removes mucins and other particulate matter which may interfere with the assay and affect results. Samples should be at room temperature before making dilutions. Pipette clear sample into appropriate dilution tubes. Re-freeze saliva samples as soon as possible after running assay. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

Saliva samples must be diluted for this assay. See Procedure for details.

Materials Supplied with Single Kit

	Item	Quantity/Size
1	**α-Amylase Substrate** Liquid preparation of 2-chloro-p-nitrophenol linked with maltotriose. Contains: Substrate, buffer, 0.01% sodium azide preservative.	1 bottle/45 mL
2	**α-Amylase Controls** High, Low, in a saliva-like matrix. Ready to use. Do not dilute. Contain: α-Amylase, buffer, preservative.	2 vials/100 µL each
3	**α-Amylase Diluent** Contains: phosphate buffer, preservative.	1 bottle/30 mL
4	**Microtiter Plate** Break apart. Use number of strips desired.	1/96-well
5	**Reagent Warming Trough** Trough may be reused for several partial plate runs.	1

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Materials Needed But Not Supplied

- Precision pipette to deliver* 8 µL

- Precision multichannel pipette to deliver* 320 µL

- Vortex

- Plate reader with 405 nm filter

- Computer software for data reduction

- Small disposable polypropylene tubes for dilution of samples

- Pipette tips

- Timer

- 37°C incubator (A Microtiter plate 37°C incubator/rotator is recommended for heating of the α-Amylase Substrate.)

- Plate rotator with 0.08-0.17 inch orbit capable of operating at 500 rpm & 37°C (only if NOT using a Microtiter plate 37°C incubator/rotator)

- Centrifuge capable of 1500 x g

  * without employing "blow-out" mechanism

Reagent Preparation

- Bring all reagents to room temperature and mix before use. Minimum warm-up times of 1.5 hours is recommended.
- See Step 4 of procedure for other α-Amylase Substrate considerations before beginning assay.

Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Controls and saliva samples should be assayed in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ctrl-H	Ctrl-H										
B	Ctrl-L	Ctrl-L										
C	SMP-1	SMP-1										
D	SMP-2	SMP-2										
E	SMP-3	SMP-3										
F	SMP-4	SMP-4										
G	SMP-5	SMP-5										
H	SMP-6	SMP-6										

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the bag.

Step 3: Set your plate reader to incubate at 37ºC, and to read in center measurement kinetic mode initially at one minute, then again two minutes later. Choose the 405 nm filter with no reference filter. For plate readers without these options, incubation can take place in a plate incubator/rotator with manual movement of the plate into and out of the plate reader for the 1 minute and 3 minute readings. Kit validation was performed under these conditions.

Step 4: Heat the α-Amylase Substrate to 37ºC in the trough provided. (For ease of use we recommend using a ***preheated*** 37ºC microtiter plate incubator.)   Be sure the α-Amylase Substrate has reached 37ºC before use. A minimum warm up time of 20 minutes, from room temperature, in a preheated microtiter plate incubator is recommended. (If using any other incubator it can take an hour or more to reach 37ºC.) Keep trough covered to prevent evaporation.

Step 5: Saliva samples are to be diluted with the α-Amylase Diluent provided. Prepare a 1:10 dilution of the saliva by pipetting 10 μL of saliva into 90 μL α-Amylase Diluent. Mix well. Further dilute by pipetting 10 μL of the 1:10 dilution into 190 μL α-Amylase Diluent (1:20). Final dilution is 1:200. The remainder of the 1:10 dilution may be set aside in case a different final dilution is necessary.

Step 6: Add 8 µL of controls and/or diluted saliva samples to individual wells. ***We strongly recommend reverse pipetting to avoid introducing any bubbles into the well.***

Step 7: Add 320 µL of the preheated (37ºC) α-Amylase Substrate to each well simultaneously using a multichannel pipette. Discard pipette tips to avoid reagent contamination. ***Do not return any of the α-Amylase Substrate left in the tips to the bulk tray once you have dispensed it into the wells. This could contaminate the bulk tray contents and affect any subsequent testing.*** Any well containing bubbles at the time of reading must be repeated.

Step 8: If reading kinetically in a programmable 37ºC plate reader, immediately place plate in reader and start reader. ***Wells are very full. Program plate reader to mix slowly or liquid could spill into the plate reader.***

Otherwise, follow these steps:

- Start timer ***immediately*** and mix (500-600 RPM) at 37ºC.

- Transfer plate to reader in time to read the Optical Density (OD) at ***exactly*** 1 minute, and then return to mixing at 37ºC. ***Save*** 1 minute OD readings.

- Transfer plate again and read the OD at ***exactly*** 3 minutes. ***Save*** 3 minute OD readings.

Calibration

This procedure is standardized using the millimolar absorptivity of 2-chloro-p-nitrophenol under the test conditions described.

Quality Control

The Salimetrics' High and Low α-Amylase Controls should be run at least once on each day of testing. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

Subtract the one minute reading from the three minute reading and multiply by the conversion factor (see below). The conversion factor takes the 1:200 sample dilution into account for the controls and pre-diluted samples.

It is convenient to set up a spreadsheet to subtract the ODs and multiply by the conversion factor. Results are expressed in U/mL.

$$\frac{\Delta Abs./min \times TV \times DF}{MMA \times SV \times LP} = \text{U/mL of } \alpha\text{-Amylase activity in sample}$$

Where:
- $\Delta Abs./min$ = Absorbance difference per minute
- TV = Total assay volume (0.328 mL)
- DF = Dilution factor
- MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
- SV = Sample volume (0.008 mL)
- LP = Light path = 0.97 (specific to plate received with kit)

$$\frac{\Delta Abs./2 \times 0.328 \times 200}{12.9 \times 0.008 \times 0.97} = \Delta Abs. \times 328^* = \text{U/mL } \alpha\text{-Amylase activity}$$

Example: If change in absorbance (OD change over 2 minutes) was 0.3, then 0.3 x 328 = 98.4 U/mL

*If using a Tecan plate reader and data capture by AssayZap software, multiply by 0.0328.

NOTE: *Multiply value by 0.01667 to convert to SI Units (nKat/L)*

See Limitations below.

Limitations

- Samples that exceed 400 U/mL (linearity limit at 1:200 dilution) or have an OD value > 3.0, should be rerun at a dilution of 1:800. Multiply the results by 4.

- Samples that yield values less than 2.0 U/mL (at a 1:200 dilution) may not result in a reliable value. These samples should be repeated at a 1:10 dilution, dividing the results by 20.

- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.

- Do not add sodium azide to saliva samples as a preservative, as it may cause interference when testing samples for multiple analytes. (Sodium azide may be used only if testing for α-Amylase alone.)

- Any quantitative results indicating abnormal α-Amylase levels should be followed by additional testing and evaluation.

- Cigarette use can be associated with lower α-Amylase scores returned by this assay because acid aldehydes in cigarette smoke are capable of changing the function and/or structure of the α-Amylase enzyme (20).

- Caffeine and other exogenous substances with sympathomimetic properties may be associated with higher α-Amylase levels (21).

- Other potential confounding factors include increased production of saliva and salivary components from activities such as eating and drinking (22,23). Additionally, naturally occurring α-Amylase inhibitors have been identified in a number of foods (24,25).

Salivary α-Amylase Example Ranges*

Group	N	Mean (U/mL)	Absolute Range (U/mL)
Adult	75	92.4	3.1 - 423.1

*To be used as a guide only. Each laboratory should establish its own range.

Salivary α-Amylase Kinetic Enzyme Assay Kit Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 10 replicates each.

Saliva Sample	N	Mean (U/mL)	Standard Deviation (U/mL)	Coefficient of Variation (%)
H	10	474.6	11.8	2.5
M	10	108.8	7.2	6.7
L	10	17.7	1.3	7.2

The inter-assay precision was determined from high and low α-Amylase samples run in 8 individual runs.

Saliva Sample	N	Mean (U/mL)	Standard Deviation (U/mL)	Coefficient of Variation (%)
H	8	166.0	6.0	3.6
L	8	10.6	0.6	5.8

Recovery

Five saliva samples containing different levels of an endogenous α-Amylase were spiked with known quantities of α-Amylase and assayed.

Saliva Sample	Endogenous (U/mL)	Added (U/mL)	Expected (U/mL)	Observed (U/mL)	Recovery (%)
1	72.18	65.19	137.37	134.73	98.1
2	123.97	77.09	201.06	224.43	111.6
3	103.28	10.01	113.29	109.37	96.5
4	29.99	6.72	36.71	40.96	111.6
5	42.01	3.14	45.15	39.44	87.4

Sensitivity
The lower limit of sensitivity is governed by the change in absorbance. Samples that yield values less than 2.0 U/mL (at a 1:200 dilution) may not result in a reliable value. These samples should be repeated at a 1:10 dilution, dividing the results by 20.

Sample Dilution Recovery
Two samples were serially diluted with α-Amylase Diluent and assayed.

Saliva Sample	Dilution Factor	Expected (U/mL)	Observed (U/mL)	Recovery (%)
1			40.96	
	1:2	20.48	19.35	94.5
	1:4	10.24	9.76	95.3
	1:8	5.12	4.57	89.3
	1:16	2.56	2.13	83.2
2			1943.16	
	1:3	647.72	649.21	100.2
	1:9	215.91	224.64	104.0
	1:27	71.97	78.08	108.5

References

1. Granger, D.A., Kivlighan, K.T., El-Sheikh, M., Gordis, E., & Stroud, L.R. (2007). Salivary alpha-amylase in biobehavioral research: Recent developments and applications. *Ann N Y Acad Sci, 1098*, 122-44.

2. Chrousos, G.P. & Gold, P.W. (1992). The concepts of stress and stress system disorders: Overview of physical and behavioral homeostasis. *JAMA, 267*(9), 1244-52. Erratum in *JAMA* (1992), *268*(2), 200.

3. Kirschbaum, C., Read, G.F., & Hellhammer, D.H. (1994). *Assessment of hormones and drugs in saliva in biobehavioral research*. Göttingen: Hogrefe & Huber.

4. Scannapieco, F.A., Torres, G., & Levine, M.J. (1993). Salivary α-amylase: Role in dental plaque and caries formation. *Crit Rev Oral Biol Med, 4*(3/4), 301-7.

5. Rogers, J.D., Palmer, R.J., Jr., Kolenbrander, P.E., & Scannapieco, F.A. (2001). Role of Streptococcus gordonii amylase-binding protein A in adhesion to hydroxyapatite, starch metabolism, and biofilm formation. *Infect Immun, 69*(11), 7046-56.

6. Chatterton, R.T., Jr., Vogelsong, K.M., Lu, Y.C., Ellman, A.B., & Hudgens, G.A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol, 16*(4), 433-48.

7. Nater, U.M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology, 34*(4), 486-96.

8. Speirs, R.L., Herring, J., Cooper, W.D., Hardy, C.C., & Hind, C.R. (1974). The influence of sympathetic activity and isoprenaline on the secretion of amylase from the human parotid gland. *Arch Oral Biol, 19*(9), 747-52.

9. van Stegeren, A., Rohleder, N., Everaerd, W., & Wolf, O.T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. *Psychoneuroendocrinology, 31*(1), 137-41.

10. Gallacher, D.V. & Petersen, O.H. (1983). Stimulus-secretion coupling in mammalian salivary glands. *Int Rev Physiol, 28*, 1-52.

11. Rohleder, N., Wolf, J.M., Maldonado, E.F., & Kirschbaum, C. (2006). The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology, 43*(6), 645-52.

12. Bosch, J.A., Veerman, E.C., de Geus, E.J., & Proctor, G.B. (2011). α-Amylase as a reliable and convenient measure of sympathetic activity: Don't start salivating just yet! *Psychoneuroendocrinology, 36*(4), 449-53.

13. Thoma, M.V., Kirschbaum, C., Wolf, J.M., & Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol Psychol, 91*(3), 342–48.

14. Granger, D. A., Kivlighan, K. T., Blair, C., El-Sheikh, M., Mize, J., Lisonbee, J.A., Buckhalt, J. A., et al. (2006). Integrating the measurement of salivary alpha-amylase into studies of child health, development, and social relationships. *J Soc Pers Relat, 23*(2), 267-90.

15. Segal, S.K., & Cahill, L. (2009). Endogenous noradrenergic activation and memory for emotional material in men and women. *Psychoneuroendocrinology, 34*(9), 1263-71.

16. Susman, E.J., Dockray, S., Granger, D.A., Blades, K.T., Randazzo, W., Heaton, J.A., & Dorn, L.D. (2010). Cortisol and alpha amylase reactivity and timing of puberty: Vulnerabilites for antisocial behaviour in young adolescents. *Psychoneuroendocrinology, 35*(4), 557-69.

17. Wallenfels, K., Foldi, P., Niermann, H., Bender, H., Linder, D. (1978). The enzymic synthesis, by transglucosylation of a homologous series of glycosidically substituted malto-oligosaccharides, and their use as amylase substrates. *Carbohyd Res, 61*(1), 359-68.

18. Beltzer, E.K., Fortunato, C.K., Guaderrama, M.M., Peckins, M.K., Garramone, B.M., & Granger, D.A. (2010). Salivary flow and alpha-amylase: Collection technique, duration, and oral fluid type. *Physiol Behav, 101*(2), 289-96.

19. Harmon, A.G., Towe-Goodman, N.R., Fortunato, C.K., & Granger, D.A. (2008). Differences in saliva collection location and disparities in baseline and diurnal rhythms of alpha-amylase: A preliminary note of caution. *Horm Behav, 54*(5), 592-96.

20. Weiner, D., Levy, Y., Khankin, E.V., Reznick, A.Z. (2008). Inhibition of salivary amylase activity by cigarette smoke aldehydes. *J Physiol Pharmacol, 59*(Suppl 6), 727-37.

21. Klein, L.C., Bennett, J.M., Whetzel, C.A., Granger, D.A., & Ritter, F.E. (2010). Caffeine and stress alter salivary α-amylase activity in young men. *Human Psychopharmacol, 25*(5), 359-67.

22. Nater, U.M., Rohleder, N., Scholtz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology, 32*(4), 392-401.

23. Mackie, D.A. & Pangborn, R.M. (1990). Mastication and its influence on human salivary flow and alpha-amylase secretion. *Physiol Behav, 47*(3), 593-95.

24. Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M., & Chou, C.J. (2008). Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *J Med Chem, 51*(12), 3555-61.

25. Hara, K., Ohara, M., Hayashi, I., Hino, T., Nishimura, R., Iwasaki, Y., Ogawa, T., et al. (2012). The green tea polyphenol epigallocatechin gallate precipitates salivary proteins including alpha-amylase: Biochemical implications for oral health. *Eur J Oral Sci, 120*(2), 132-39.

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