Published in final edited form as:

Clin Ther. 2015 March 1; 37(3): 515–522. doi:10.1016/j.clinthera.2015.02.015.

New Techniques for Augmenting Saliva Collection: Bacon Rules and Lozenge Drools

Jeremy C. Peres, MS^{#1}, Jacob L. Rouquette, MS^{#1}, Olga Mio evi , BS², Melissa C. Warner¹, Paul D. Slowey, PhD³, and Elizabeth A. Shirtcliff, PhD²

¹Department of Psychology, University of New Orleans, New Orleans, Louisiana

²Department of Human Development and Family Studies, Iowa State University, Ames, Iowa

³Oasis Diagnostics Corporation, Vancouver, Washington

These authors contributed equally to this work.

Abstract

Purpose—Saliva is a reliable, noninvasive, and cost-effective alternative to biomarkers measured in other biological fluids. Within certain populations, saliva sampling may be difficult because of insufficient saliva flow, which may compromise disease diagnosis or research integrity. Methods to improve flow rates (eg, administering citric acid, chewing gum, or collecting cotton) may compromise biomarker integrity, especially if the methods involve the presence of a collection aid in the oral cavity. Anecdotal strategies (eg, looking at pictures of food or imagining food) have not been evaluated to date. In this study, we evaluate whether 2 novel collection techniques improve saliva flow or interfere with assay of common biomarkers (ie, cortisol, dehydroepiandrosterone, and testosterone). We evaluate an over-the-counter anhydrous crystalline maltose lozenge intended to increase saliva production for patients with xerostomia long after the lozenge dissolves. We then evaluate whether the smell of freshly cooked bacon stimulates a pavlovian-type reflex.

Methods—Saliva was collected from 27 healthy young adults (aged 20–34 years; 12 men) on a basal day and a lozenge day, providing 5 samples at 15-minute intervals. Twenty participants then returned for the bacon day condition, providing 2 saliva samples with an interval of 15 minutes between samples. Collection times required to generate 2 mL of saliva across collection strategies were recorded, and then saliva samples were assayed for cortisol, dehydroepiandrosterone, and testosterone.

Findings—Repeated analysis of variance measures revealed that both the lozenges and bacon significantly decreased collection time compared with the passive drool collection on the basal day. No significant effects were found related to the quantification of cortisol, testosterone, or dehydroepiandrosterone when comparing lozenge or bacon to the basal day. In addition, bivariate

Address correspondence to: Elizabeth Shirtcliff, PhD, Department of Human Development and Family Studies, 2061C Palmer Building, Iowa State University, Ames, IA 50011. birdie@iastate.edu.

CONFLICTS OF INTEREST

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

correlations revealed that concentrations from time-matched control samples correlated significantly with concentrations from the lozenge and bacon conditions.

Implications—These results indicate that both the lozenge and smelling bacon improve saliva collection times and that neither technique interferes with salivary hormone concentrations. This study reveals new methods to augment saliva collection strategies.

Keywords

saliva; bacon; cortisol; testosterone; DHEA; interference

INTRODUCTION

Saliva is a reliable, noninvasive, and cost-effective biological measure and diagnostic tool in research and clinical settings.^{1–6} There are many salivary biomarkers (eg, lipid soluble hormones, enzymes, and immunoglobulins) that can be targeted and analyzed by researchers and clinicians for diagnostic purposes.^{6,7} In many cases, saliva sampling is a good alternative to the use of other biological fluids (eg, blood, urine, and cerebrospinal fluid) and offers important advantages, especially when point-of-care sampling is required.⁸ The benefits (ie, ease of use, minimal invasiveness, reliability, and tolerability) are sufficient for many biomarkers.^{9,10}

Successful measurement of analyte concentrations in saliva is typically dependent on the participants providing an adequate quantity of saliva, especially when multiple biomarkers are of interest or when timely collection is needed. The inability to collect an adequate quantity of saliva may exclude some participants from successfully completing saliva sampling protocols. A simple method to increase salivary flow rate without affecting biomarker assessment or quantification would be a valuable tool to decrease the rate of unsuccessful saliva sampling and improve research and diagnostic protocols.

There are wide individual differences in saliva flow rates. In the extreme case, decreased salivary flow rates are associated with dry mouth (xerostomia). Dry mouth is related to demographic factors, such as age; medications (eg, diuretics, anticholinergics, antihistamines, and antihypertensives), which are especially relevant in geriatric populations¹¹; radiotherapy in the head and neck region¹²; autoimmune diseases attacking the salivary glands; and stress and anxiety.¹³ A reduction in xerostomic effects can significantly increase the success rate in salivary sampling and can also improve collection times in those producing saliva in the normal range for healthy individuals.

Methods to increase the saliva flow and saliva collection of participants have been explored. With mixed success, techniques to stimulate saliva flow include use of citric acid, chewing gum, drink mix crystals, Jell-O, and marshmallows.^{14–17} These techniques have the potential drawback that they each involve introducing substances into the oral cavity and therefore have the potential to compromise sample integrity. For instance, Schwartz et al¹⁶ found that drink mix crystals artificially increased the estimated concentration of cortisol due to reduced sample pH. The most common saliva collection aid, cotton, has been found to compromise assay of a range of biomarkers.¹⁸ Cotton and related absorbent materials also

have a potential drawback of requiring a degree of saturation before the saliva can be successfully extracted from the cotton fibers after collection.¹⁹ Chewing gum has been found to artificially inflate salivary testosterone measurements in the first few minutes after chewing.²⁰ Schultheiss²¹ found that sugarless gum raised salivary progesterone concentrations while attenuating cortisol and testosterone concentrations. Other investigations have found that chewing gum may moderate stress responsivity. For instance, Scholey et al²² found that chewing gum during laboratory stress was associated with reduced perceived and lower salivary cortisol. Gray et al²³ similarly found that chewing gum during a stressful task reduced subjective measures of stress but heightened cortisol levels. However, others have failed to find this attenuation of perceived stress.²⁴ Increased alertness as a result of chewing gum has also been indicated.^{22,24} The shortcomings of the available methods to increase saliva flow and collection volume create frustration for investigators who would otherwise benefit from the use of saliva as a diagnostic tool. In addition, these confounding findings regarding stress responsivity and analyte interference further reinforce that caution is necessary when saliva stimulants are used, especially when introduced into the oral cavity.

The purpose of the present study is to explore strategies to increase salivary flow rates for sample collection without compromising the integrity of biomarkers. We evaluated an over-the-counter dietary supplement in lozenge form[†] composed of anhydrous crystalline maltose. The intended use for the product is to increase saliva production and provide relief from oral dryness. The efficacy of this product as a clinical treatment for persistent dry mouth suggests administration produces a significant increase in salivation and a decrease in dry mouth symptoms.^{25,26} The lozenge is designed to work long after it dissolves and so does not necessitate use of the lozenge during saliva collection. Whether the lozenge has the ability to improve salivary flow rates within normal participants providing a saliva sample after the lozenge is completely dissolved has not previously been investigated.

Our strategy to increase saliva flow in the research setting involves providing instructions to imagine a favorite food,²⁰ looking at pictures of delicious foods,²⁷ or making jaw movements that simulate chewing food.²⁸ Beyond the pavlovian logic,²⁹ such strategies are only anecdotally related to improved salivary flow rates. We sought to empirically identify the utility of one such strategy by presenting participants with the sight and smell of freshly cooked bacon to initiate a pavlovian-type saliva flow reflex. The advantage of using the bacon strategy is that there is no introduction of material into the oral cavity and therefore less potential to compromise sample integrity. Beyond measuring saliva collection times, the present study sought to confirm that these collection strategies do not compromise common stress-responsive salivary biomarkers, namely, cortisol, testosterone, and dehydroepiandrosterone (DHEA).

METHODS

This study was approved by the institutional review board at the University of New Orleans. Saliva was collected via passive drool from 27 young adults (12 men) ranging in age from

[†]Trademark: Maxisal (Amarillo Biosciences Inc, Amarillo, TX).

20 to 34 years (mean [SD] 25.28 [3.77] years) on 3 separate days: a basal day, a lozenge day, and a bacon day. All 27 of the participants participated in both the basal and lozenge days. Twenty young adults (10 men) were additionally invited to participate in the bacon day. Exclusionary criteria included use of anticholinergics, antihistamines, antihypertensives, or diuretics or self-reported vegetarianism or dislike of bacon. Participants were also asked ahead of time to refrain from eating or drinking for 1 hour before arrival at the laboratory and to have water plenty of water that day to avoid dehydration.

On each day, participants arrived at the laboratory in the morning and then rinsed their mouths with water and waited 5 minutes. With assistance from a researcher, participants then expectorated 2 mL of saliva through a short plastic straw into a collection vial and completed a short questionnaire.

On the basal day, participants provided 5 saliva samples. Saliva sampling began at a target time of 9:45 AM, and the 4 subsequent samples were collected 15-minute intervals thereafter, with the final sample starting at approximately 10:45 AM. The exact time of day for each sample collection was recorded across all days.

On the lozenge day, participants returned to the laboratory and were administered one lozenge 25 minutes before sample collection, ensuring enough time for the lozenge to dissolve in the mouth and induce its effects before expectoration. Participants again provided a total of 5 samples that were time matched to the basal day collection times.

On the bacon day, bacon was prepared via microwave 5 minutes before the participants arrived to allow the aroma to permeate the laboratory. Bacon was placed in front of the participants for 5 minutes before expectoration began at a target time of 9:45 AM, and a second sample was commenced 15 minutes after the first sample began. Although 5 samples were used on the lozenge day to test for analyte interference and how long any interference might last, 2 samples were deemed sufficient for comparison on the bacon day because, unlike in the lozenge condition, there were no substances introduced into the oral cavity.

After collection, saliva samples were immediately frozen and stored at -80°C . On the day of assay, samples were thawed and then centrifuged (1500g for 15 minutes) to remove particulate matter. Samples were then transferred into appropriate wells via pipette and assayed with commercially available enzyme immunoassays (www.salimetrics.com) following the manufacturer's recommendations without modification. All samples from the same individual were assayed on the same plate. All samples were tested in duplicate and the mean used for analyses. Duplicates that varied by more than 7% error based on the coefficient of variation were repeat tested. Distributions were examined for normality and winsorized.³⁰

To test whether different conditions improved saliva collection times, a series of repeated-measures ANOVA procedures were used with sample collection time as the dependent variable and 2 repeated-measures predictors, including sample number (first, second, and so on) and condition (basal, lozenge, or bacon). Next, to test whether collection strategies interfere with salivary hormone concentrations, another set of parallel repeated-measures

ANOVAs were conducted with hormone concentration as the dependent variable and again sample number and condition as the independent predictors. Last, we also conducted a series of bivariate correlations to test the reliability of the hormone concentrations.

RESULTS

Although the target times of day of the sample collections started at 9:45 ^{AM} with subsequent samples provided in 15-minute intervals, the descriptive statistics describing the actual recorded times of the first samples taken that day include a mean (SD) time of 9:45 (1) minutes for basal, a mean time of 9:45 (1) minutes for lozenge, and a mean (SD) time of 9:48 (4) minutes for bacon. As noted above, assay duplicates with CVs >7 were retested. These CVs ranged from 0.013 to 6.92, with a mean (SD) of 2.14 (1.71).

Do Collection Strategies Improve Saliva Collection Times?

A repeated-measures ANOVA was conducted to compare the lozenge and basal conditions. Figure 1 illustrates that, compared with the basal day, sample collection times were significantly faster on the lozenge day compared with the basal day ($F_{1,26} = 5.48$, $P = 0.027$). There was no main effect of sample collection across the 5 samples ($F_{4,104} = 1.19$, $P = 0.32$). Last, there was not a significant interaction of day by collection ($F_{4,104} = 0.86$, $P = 0.49$), indicating that saliva flow during the lozenge day was consistently faster than during the basal day.

To focus on the bacon collection technique, a parallel repeated-measures ANOVA was run in which we specifically tested whether sample collection times on the bacon day were distinct from the basal day. There was a significant effect of condition on sample collection times ($F_{1,19} = 11.15$, $P = 0.003$) such that sample collection times on the bacon day were substantially faster than the basal samples (Figure 2). There was no main effect across samples ($F_{1,19} = 3.51$, $P = 0.08$), and there was no significant interaction of condition by collection time ($F_{1,19} = 0.14$, $P = 0.71$).

Next, we were interested in determining which of the methods bacon or lozenge best improved sample collection times, again using a parallel repeated-measures ANOVA with only bacon and lozenge as predictors of collection times. There was no significant effect across conditions ($F_{1,19} = 0.70$, $P = 0.41$), no main effect across samples ($F_{1,19} = 2.00$, $P = 0.17$), and no interaction between condition and sample number ($F_{1,19} = 0.21$, $P = 0.65$).

Do Collection Strategies Interfere with Salivary Hormone Concentrations?

This series of analyses parallel the repeated-measures ANOVAs above, with the exception that hormone concentrations were the dependent variable (s) of interest to determine whether conditions interfere with measuring biomarkers. As above, independent predictors included condition (basal and lozenge) and sample (samples 1–5). The results confirm that hormone concentrations were not significantly different across conditions for cortisol ($F_{1,25} = 2.41$, $P = 0.13$), testosterone ($F_{1,23} = 3.24$, $P = 0.09$), or DHEA ($F_{1,21} = 0.18$, $P = 0.68$). There were significant main effects found across the 5 samples for cortisol ($F_{4,100} = 40.09$, $P < 0.0005$), testosterone ($F_{4,92} = 3.16$, $P = 0.018$), and DHEA ($F_{4,84} = 3.15$, $P = 0.018$), indicating hormone concentrations for each hormone decreased throughout the morning

session, which was expected given the natural diurnal rhythm of each of these hormones. Last, there were not significant interactions of condition by sample for cortisol ($F_{4,100} = 0.35$, $P = 0.85$), testosterone ($F_{4,92} = 0.29$, $P = 0.89$), or DHEA ($F_{4,84} = 1.20$, $P = 0.32$).

Next, to compare hormone concentrations across bacon and basal conditions, another repeated-measures ANOVA was run with independent predictors, including condition (basal and bacon) and sample (first or second sample). There was not a significant effect of condition on concentration for cortisol ($F_{1,20} = 3.12$, $P = 0.09$), testosterone ($F_{1,18} = 0.20$, $P = 0.66$), or DHEA ($F_{1,20} = 1.70$, $P = 0.21$). There were significant main effects found across samples for cortisol ($F_{1,20} = 3.12$, $P < 0.001$) and DHEA ($F_{1,20} = 1.70$, $P = 0.04$) but not for testosterone ($F_{1,18} = 0.37$, $P = 0.55$). There were no significant interactions of condition by sample for cortisol ($F_{1,20} = 0.36$, $P = 0.55$), testosterone ($F_{1,18} = 0.48$, $P = 0.50$), or DHEA ($F_{1,20} = 0.08$, $P = 0.78$).

Last, to compare hormones across bacon and lozenge conditions, a final repeated-measure ANOVA was run with independent predictors, including condition (lozenge and bacon) and sample (first or second sample). Here, there was a significant effect of condition for testosterone ($F_{1,18} = 4.62$, $P = 0.045$) such that testosterone was higher in the bacon condition compared with lozenge but not for cortisol ($F_{1,20} = 0.65$, $P = 0.43$) or DHEA ($F_{1,19} = 0.01$, $P = 0.93$). There were significant main effects across samples for cortisol ($F_{1,20} = 11.73$, $P = 0.003$) but not for testosterone ($F_{1,18} = 2.43$, $P = 0.14$) or DHEA ($F_{1,19} = 1.64$, $P = 0.22$). There were no significant interactions of condition by sample for cortisol ($F_{1,20} = 0.13$, $P = 0.73$), testosterone ($F_{1,18} = 0.00$, $P = 0.99$), or DHEA ($F_{1,19} = 0.161$, $P = 0.69$).

Do Collection Strategies Result in Reliable Hormone Levels?

Bivariate correlations were conducted to compare detected biomarker levels from the time-matched samples across the basal, lozenge, and bacon conditions. As can be seen in the Table, hormone concentrations across collection methods were consistently correlated with basal for cortisol (mean $r = 0.71$ for lozenge; mean $r = 0.46$ for bacon), testosterone (mean $r = 0.90$ for lozenge; mean $r = 0.89$ for bacon), and DHEA (mean $r = 0.66$ for lozenge; mean $r = 0.61$ for bacon) with the exception of the fifth basal and lozenge samples for DHEA ($r = 0.40$, $P = 0.07$), which had trend-level significance.

DISCUSSION

Salivary collection methods that do not compromise biomarker integrity can greatly benefit clinical and research protocols. We confirm that 2 methods improve saliva collection and do not appear to compromise biomarker integrity: lozenges and bacon.

We found that lozenges significantly improve saliva flow and do not interfere with salivary hormones across 5 repeated saliva collections. This finding is notable given that the lozenge had been completely dissolved, but collection times remained significantly faster even more than an hour after the lozenge dissolved. Similarly, the lozenge did not interfere with hormones within minutes or more than an hour after use of the lozenge.

Regarding the bacon day, we also found an improvement in saliva flow compared with basal levels. There was not a significant difference between the lozenge and bacon collection conditions, indicating that both strategies resulted in similar improvements in saliva flow rates. One potential advantage of the bacon strategy is that there is no introduction of material into the oral cavity and therefore sample integrity is very high. One drawback of using bacon, however, is that it requires a means to prepare it and so would not be useful for ecologic momentary assessments^{31,32} or samples collected outside the home or laboratory setting. Although nonsignificant, flow rate on the second bacon-day sample was somewhat faster than the first bacon-day sample. This finding may suggest that we did not capture the peak saliva flow increase from the bacon effect. The benefits of bacon may be especially protracted, increasing saliva flow rates for even longer than 15 minutes. Including a wider time range and more samples on the bacon day would have provided better insight into when the saliva flow rate peaks.

Our results indicate that lozenge and the aroma of bacon significantly increase saliva flow compared with basal levels without interfering with sample integrity, specifically concerning the quantification of salivary biomarkers, including cortisol, testosterone, and DHEA. When comparing the measured concentrations of these hormones across sample conditions, we found that samples did not significantly differ across conditions. Furthermore, almost all time-matched samples significantly correlated with each other across conditions. The one exception regarding differences in hormone concentrations across conditions was testosterone being significantly higher on the bacon day compared with the lozenge day. These data suggest that smelling bacon may artificially increase testosterone levels in participants. Future studies will need to confirm these findings. In general, these analyses substantiate that both lozenge and the smell of bacon did not artificially change the concentrations of these hormones available for quantification in saliva.

Our study is not without limitations. First, the order of collection days was not counterbalanced. The lozenge is designed to affect saliva flow for several days, so it was always administered after passive drool to reduce the potential for carryover effects. Nonetheless, saliva collection times may be slower on the basal day because of the novelty of the expectoration experience. Although typically a small effect, the first sample collection has been found to be slower than subsequent times.³³ It is unlikely that experience explains away the present study because 5 samples were administered and experience effects would have been apparent within the first day and culminated in a day by collection interaction; this was not apparent because sample collection times were stable within each session, including the basal day. Second, because participants were informed that this was a study investigating saliva flow, the placebo effect may also be a confounding factor. Third, when saliva flow enhancers such as lozenge or bacon are used, investigators must be aware that interference with cortisol, DHEA, or testosterone are not apparent, but other biomarkers may be affected; for example, secretory IgA salivary concentration is inversely related to saliva flow,³⁴ and so increasing flow rate may compromise biomarker integrity in this instance. Fourth, a potential confounding factor that may increase individual differences in the efficacy of the bacon condition is how delicious the participants consider bacon. If one has an aversion to bacon, then it may have little to no effect on saliva flow and may even increase collection times. This may not be a large problem because more than 75% of

participants indicated that they “liked bacon”. It has been anecdotally suggested that the smell of cooking bacon may engage a primal craving for the fat and protein-rich calories even in vegetarians.³⁵ Future studies could investigate this assertion further by comparing the saliva flow rates of vegetarians and nonvegetarians to see whether positive bacon associations are necessary to benefit from pavlovian-type responses. Future studies could also further disentangle the differences between other types of flavorful aromas from a variety of other foods. Because viewing pictures of food is already a common method used in salivary research, perhaps examining the utility of scratch-and-sniff pictures might reveal saliva flow rate benefits to this aroma-based method. An additional consideration regarding the use of foods in future research is whether being exposed to appetitive aromas without being able to consume those foods might be frustrating, possibly affecting mood and stress levels as the literature on chewing gum suggests.^{22–24} We considered this in post hoc analyses using repeated-measures ANOVAs; we found that mean mood ratings (provided at the time of each sample) of sadness, happiness, anger, and anxiety did not significantly differ across days ($P < 0.136$), suggesting that this was not the case in our sample. However, future research could attempt to investigate this more directly and in other relevant contexts, such as stress responsivity to laboratory tasks.

CONCLUSION

Both lozenge and the aroma of bacon significantly increase saliva flow and decrease saliva collection time. Many psychological and endocrinologic studies can use either of these 2 techniques for augmenting saliva collection.

ACKNOWLEDGMENTS

Mr. Peres and Mr. Roquette were responsible for sample collection, statistical analyses, figure creation, and writing. Mr. Roquette was responsible for the literature search. Ms. Mio e vi and Ms. Warner contributed to sample collection and data analysis. Dr. Slowey contributed to the study design and writing. Dr. Shirtcliff coordinated and supervised throughout the entire study.

This research was supported by phase I (R43AT006634) and phase II (R44 AT006634) Small Business Innovative Research Award to Dr. Slowey. Dr. Slowey is the CEO of Oasis Diagnostics, and Dr. Shirtcliff sits as a volunteer on the board of advisors.

REFERENCES

1. Malamud D. Saliva as a diagnostic fluid. *BMJ*. 1992; 305:207–208. [PubMed: 1290500]
2. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis. *J Clin Periodontol*. 2000; 27:453–465. [PubMed: 10914885]
3. Granger DA, Kivlighan KT, Fortunato C, et al. Integration of salivary biomarkers into developmental and behaviorally-oriented research: problems and solutions for collecting specimens. *Physiol Behav*. 2007; 92:583–590. [PubMed: 17572453]
4. Zhang L, Farrell JJ, Zhou H, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. *Gastroenterology*. 2010; 138:949–957. e7. [PubMed: 19931263]
5. Slowey PD. Commercial saliva collections tools. *J Calif Dent Assoc*. 2013; 41:97–99. 102–105. [PubMed: 23505755]
6. Slowey, PD. Saliva collection devices and diagnostic platforms.. In: Streckfus, C., editor. *Advances in Salivary Diagnostics*. Springer-Verlag; Heidelberg, Germany: 2015.
7. Slavkin HC. Toward molecularly based diagnostics for the oral cavity. *J Am Dent Assoc*. 1998; 129:1138–1143. [PubMed: 9715016]

8. Punyadeera, C.; Slowey, PD. Nanobiomaterials in Clinical Dentistry. Vol. 2013. Elsevier; Oxford, United Kingdom: Saliva as an emerging biofluid for clinical diagnosis and applications of MEMS/NEMS in salivary diagnostics.; p. 453–468.<http://espace.library.uq.edu.au/view/UQ:294926>. [December 11, 2014]
9. Cruz HM, da Silva EF, Villela-Nogueira CA, et al. Evaluation of saliva specimens as an alternative sampling method to detect hepatitis B surface antigen. *J Clin Lab Anal.* 2011; 25:134–141. [PubMed: 21438008]
10. Sakamoto W, Fujii Y, Kanehira T, et al. A novel assay system for myeloperoxidase activity in whole saliva. *Clin Biochem.* 2008; 41:584–590. [PubMed: 18237554]
11. Turner MD, Ship JA. Dry mouth and its effects on the oral health of elderly people. *J Am Dent Assoc.* 2007; 138(suppl 1):15S–20S. [PubMed: 17761841]
12. Dirix P, Nuyts S, Poorten VV, et al. The influence of xerostomia after radiotherapy on quality of life. *Support Care Cancer.* 2008; 16:171–179. [PubMed: 17618467]
13. Kassan SS, Moutsopoulos HM. Clinical manifestations and early diagnosis of Sjögren syndrome. *Arch Intern Med.* 2004; 164:1275–1284. [PubMed: 15226160]
14. Watanabe S, Dawes C. The effects of different foods and concentrations of citric acid on the flow rate of whole saliva in man. *Arch Oral Biol.* 1988; 33:1–5. [PubMed: 3190508]
15. Dabbs JM Jr. Salivary testosterone measurements: collecting, storing, and mailing saliva samples. *Physiol Behav.* 1991; 49:815–817. [PubMed: 1881989]
16. Schwartz EB, Granger DA, Susman EJ, et al. Assessing salivary cortisol in studies of child development. *Child Dev.* 1998; 69:1503–1513. [PubMed: 9914636]
17. Clements AD, Parker CR, Dixon WE, Salley B. Marshmallows used as saliva stimulant do not affect cortisol concentrations: finally a palatable alternative for toddler saliva collection. *Dev Psychobiol.* 2007; 49:702–707. [PubMed: 17943982]
18. Shirtcliff EA, Granger DA, Schwartz E, Curran MJ. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. *Psychoneuroendocrinology.* 2001; 26:165–173. [PubMed: 11087962]
19. Harmon AG, Hibell LC, Rumyantseva O, Granger DA. Measuring salivary cortisol in studies of child development: watch out—what goes in may not come out of saliva collection devices. *Dev Psychobiol.* 2007; 49:495–500. [PubMed: 17577235]
20. Granger DA, Shirtcliff EA, Booth A, et al. The “trouble” with salivary testosterone. *Psychoneuroendocrinology.* 2004; 29:1229–1240. [PubMed: 15288702]
21. Schultheiss OC. Effects of sugarless chewing gum as a stimulant on progesterone, cortisol, and testosterone concentrations assessed in saliva. *Int J Psychophysiol.* 2013; 87:111–114. [PubMed: 23220228]
22. Scholey A, Haskell C, Robertson B, et al. Chewing gum alleviates negative mood and reduces cortisol during acute laboratory psychological stress. *Physiol Behav.* 2009; 97:304–312. [PubMed: 19268676]
23. Gray G, Miles C, Wilson N, et al. The contrasting physiological and subjective effects of chewing gum on social stress. *Appetite.* 2012; 58:554–558. [PubMed: 22123610]
24. Johnson AJ, Jenks R, Miles C, et al. Chewing gum moderates multi-task induced shifts in stress, mood, and alertness: a re-examination. *Appetite.* 2011; 56:408–411. [PubMed: 21232569]
25. Fox PC, Cummins MJ, Cummins JM. Use of orally administered anhydrous crystalline maltose for relief of dry mouth. *J Altern Complement Med.* 2001; 7:33–43. [PubMed: 11246934]
26. Fox PC, Cummins MJ, Cummins JM. A third study on the use of orally administered anhydrous crystalline maltose for relief of dry mouth in primary Sjögren's syndrome. *J Altern Complement Med.* 2002; 8:651–659. [PubMed: 12470447]
27. Shirtcliff EA, Granger DA, Likos A. Gender differences in the validity of testosterone measured in saliva by immunoassay. *Horm Behav.* 2002; 42:62–69. [PubMed: 12191648]
28. Shirtcliff EA, Granger DA, Schwartz EB, et al. Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity. *Horm Behav.* 2000; 38:137–147. [PubMed: 10964528]
29. Pavlov, I. Conditioned Reflexes. Oxford University Press; New York, NY, US: 1927.

30. Tukey, JW. Exploratory Data Analysis. Addison-Wesley; Reading, MA: 1977.
31. Adam EK, Hawkley LC, Kudielka BM, Cacioppo JT. Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. *Proc Natl Acad Sci U S A*. 2006; 103:17058–17063. [PubMed: 17075058]
32. Almeida DM, McGonagle K, King H. Assessing daily stress processes in social surveys by combining stressor exposure and salivary cortisol. *Biodemography Soc Biol*. 2009; 55:219–237. [PubMed: 20183906]
33. Shirtcliff EA, Essex MJ. Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Dev Psychobiol*. 2008; 50:690–703. [PubMed: 18726897]
34. Kugler J, Hess M, Haake D. Secretion of salivary immunoglobulin a in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. *J Clin Immunol*. 1992; 12:45–49. [PubMed: 1551941]
35. Barclay, E. [December 9, 2014] Why bacon is a gateway to meat for vegetarians.. NPR.org. <http://www.npr.org/blogs/health/2011/02/02/133304206/why-bacon-is-a-gateway-to-meat-for-vegetarians>.

Figure 1.
Sample Collection Times for Maxisal (Lozenge) and Basal (Passive Drool) Conditions.

Figure 2.
Sample Collection Times for Basal (Passive Drool), Maximal (Lozenge), and Bacon Conditions.

Table 1

Paired-Samples Correlations of Hormones Across Conditions.

Sample	Cortisol		Testosterone		DHEA	
	Maxisal Lozenge	Bacon	Maxisal Lozenge	Bacon	Maxisal Lozenge	Bacon
Association With Basal Day (Passive Drool)						
Sample 1: 9:45 AM	.77 ***	.46 *	.90 ***	.84 ***	.74 ***	.53 *
Sample 2: 10:00 AM	.79 ***	.46 *	.90 ***	.94 ***	.72 ***	.68 ***
Sample 3: 10:15 AM	.70 ***		.89 ***		.77 ***	
Sample 4: 10:30 AM	.62 **		.89 ***		.66 ***	
Sample 5: 10:45 AM	.67 ***		.90 ***		.40	
Association With Lozenge						
Sample 1: 9:45 AM		.55 **		.87 ***		.54 *
Sample 2: 10:00 AM		.49 *		.90 ***		.43

*
p<.05**
p<.01***
p<.001.