Original Research

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

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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. Anecsdotal 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 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. (*Clin Ther.* 2015;37:515–522)

Key words: 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

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Accepted for publication January 12, 2015.
http://dx.doi.org/10.1016/j.clinthera.2015.02.015
0149-2918/$ - see front matter

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 populations[11]; radiotherapy in the head and neck region[12]; 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 al[16] 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] Schultheiss[21] 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 al[22] found that chewing gum during laboratory stress was associated with reduced perceived and lower salivary cortisol. Gray et al[23] 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

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