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Effects of sugarless chewing gum as a stimulant on progesterone, cortisol, and testosterone concentrations assessed in saliva

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ABSTRACT

Sugarless chewing gum is a frequently used stimulant to collect saliva samples for hormone analyses. This study tested the effect of sugarless chewing gum on cortisol, testosterone, and progesterone concentrations measured in saliva samples collected from 8 individuals at different times of the day (morning, evening) and under different collection conditions (gum, no gum) as well as in a saliva pool and water, either untreated or treated with chewing gum. Sugarless chewing gum raised all progesterone concentrations by 20 to 40 pg/mL, corresponding to a twofold increase, relative to no-gum controls and attenuated salivary testosterone and cortisol concentrations. It is recommended that the use of sugarless chewing gum as a stimulant should be avoided with saliva samples.

1. Introduction

Over the past 20 years, salivary hormone assessment has become an increasingly popular method of assessing individual differences and situationally induced changes in endocrine function, particularly of the hypothalamic–pituitary–adrenal (HPA) axis (cortisol [C]) and the hypothalamic–pituitary–gonadal (HPG) axis (testosterone [T], progesterone [P], estradiol, [E]). Although collecting saliva samples is as simple as having research participants drool into suitable vessels, researchers frequently try to speed up the collection process by using a variety of devices that stimulate saliva flow, such as dental rolls, citric acid, or chewing gum. However, it has also become increasingly clear that some of these stimulants may distort the determination of salivary hormone concentrations (van Anders, 2010; Gröschl & Rauh, 2006) because they appear to contain substances that either mimic specific steroids, leading to increased hormone concentrations, or bind to steroid hormones, thereby making them unavailable for binding to an assay's antibodies and thus leading to reduced hormone concentrations, introducing or interacting with enzymes that metabolize steroids, changing the pH level of the sample, or a variety of other ways. The issue is further complicated by the fact that some stimulants do not appear to bias the assessment of one hormone, but may at the same time severely interfere with the valid assessment of another hormone.

For instance, having participants collect their saliva samples by chewing on cotton rolls yields essentially the same results as passive drooling into a collection vessel in the case of C, but markedly increased concentrations for the gonadal steroids T, P, and E (Shirtcliff et al., 2001) (but see Kidd et al., 2009 for evidence that cotton can also interfere with salivary C assessment). Granger et al. (2004) demonstrated that citric acid interferes with the assessment of salivary T, whereas Kidd et al. (2009) found this stimulant to be safe for the determination of C levels. Dabbs (1991) tested the effect of various collection methods on salivary T measurements and, like Shirtcliff et al. (2001), found cotton to elevate testosterone levels. He also observed that using regular chewing gum led to increased salivary hormone concentrations, whereas T concentrations in samples collected with sugarless chewing gum did not differ from concentrations in samples collected by passive drooling.

Because sugarless chewing gum is a frequently used stimulant for collecting samples that are later analyzed not only for T, but also for other hormones for which possible interference effects of the use of this device are unknown (e.g., P Liening et al., 2010), the present study was designed to re-evaluate the utility of sugarless chewing gum in the valid assessment of salivary T concentrations and to explore the effects of this stimulant on salivary C and P concentrations.

2. Method

2.1. Samples

Saliva samples were obtained from 8 healthy adults (7 women, 1 man) on two consecutive weekdays in the morning, after getting up, and late in the evening, before going to bed. This yielded a total of 32 samples. On one of the days, participants used a strip of Orbit ® Spearmint sugarless chewing gum to stimulate saliva flow for the collection

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of each sample. On the other day, participants collected saliva by unstimulated, passive drooling. The order of stimulated and unstimulated saliva collection days was counterbalanced across participants, and 7.5 mL was collected on both days. Samples were collected in polystyrene tubes and frozen after collection. They were subjected to three freeze–thaw cycles with subsequent centrifugation to obtain a clear, watery supernatant amenable to precise pipetting and measured in duplicate.

2.2. Additional testing

To examine whether possible effects of sugarless chewing gum on hormone concentrations represent a main effect of the ingredients of the chewing gum or an interaction between the chewing gum with the ingredients of saliva, four pools were created, with two each (a) from an in-house saliva pool collected via passive drooling by a male subject in the morning to obtain high hormone reference levels and (b) from deionized water stripped of steroid content with a Millipak 20 filter (Millipore, USA). Orbit ® Spearmint sugarless chewing gum (1 strip per 7.5 mL, cut into pieces) was added to one pool each from saliva and water and remained in there overnight. The other saliva and water pools were not treated with chewing gum. This corresponds to a 2 (water versus saliva) × 2 (chewing gum versus no chewing gum) treatment design. These pools were subsequently processed like the saliva samples collected from participants and analyzed with 10 (T, C) or 7 (P) replicates for each of the four pools using the same assay protocols.

2.3. Assays

All hormone assays were conducted using solid-phase Coat-A-Count ^{125}I radioimmunoassays for T (TKTT), P (TKPG), and C (TKCO) provided by Siemens Healthcare Diagnostics GmbH (Eschborn, Germany), following validated protocols for the assessment of these hormones (Campbell et al., 1999; Schultheiss et al., 2003; Wirth et al., 2006) (a 24-h preincubation at room temperature was added for T and C). Sensitivity was determined as the lower limit of detection at B_0-3SD. Intraassay coefficient of variation (CV%) was calculated from participants' 32 samples (see Table 1). According to validation data supplied by the manufacturer, none of the assays cross-reacts with estrogens and gestagens contained in oral contraceptives. Participant sample C concentrations were log-transformed for statistical analyses to correct for a leftward skew of distributions.

3. Results

3.1. Participant sample analyses

Table 1 shows assay quality control data and descriptive and inferential statistics for participants' salivary hormone concentrations, broken down by time of the day and gum use. Effects of time of the day on salivary hormone concentrations were evident for C and (marginally) for T, but not for P. The use of chewing gum as a collection device had a highly reliable effect on salivary concentrations of P, adding 20 to 24 pg/mL to the morning and evening P concentrations observed in the no-gum condition. As Fig. 1 illustrates, P concentrations were higher in the gum condition than in the no-gum condition for almost every single pair of measurements, although the magnitude of this effect varied from participant to participant (rank order stability, estimated by Pearson's correlations, between the gum and the no-gum conditions was .61 for samples collected in the morning and .52 for samples collected in the evening). The interaction between time of the day and gum use was significant for C, reflecting the fact that morning C concentrations were nonsignificantly reduced, 31%, $t(7) = 1.53$, $p = .17$, and evening C concentrations significantly increased, 141%, $t(7) = -3.49$, $p < .05$, in the gum condition relative to the no-gum condition. For salivary T, gum use also appeared to be associated with a greater reduction in morning concentrations than in evening concentrations, but neither the main effect of treatment nor the Treatment × Time interaction became significant.

3.2. Saliva and water pool analyses

Results from additional testing of saliva and water pools treated or untreated with chewing gum revealed a main effect of medium on P, with saliva having higher P concentrations than water, $F(1, 24) = 121.35$, $p < .0000005$, and a main effect of treatment on P, with gum-treated pools having higher P concentrations than untreated pools, $F(1, 24) = 128.06$, $p < .0000005$. As Fig. 2 (panel A) shows, the treatment with sugarless chewing gum added about 40 pg/mL to P concentrations measured in water and saliva, and the slope for the treatment effect was the same for water and saliva (for the Medium × Treatment interaction, $F(1, 24) = 0.58$, *ns*).

In the case of C, there was a clear-cut main effect of medium, with the saliva pool (2.51 ng/mL) having a significantly higher hormone concentration than the water pool (0.00 ng/mL), $F(1, 36) = 840.59$, $p < .0000005$, and a significant Medium × Treatment interaction, $F(1, 36) = 20.71$, $p < .0001$. While gum treatment did not affect C concentrations in water (which was not reliably detectable in either condition), the gum treatment led to lower C in the saliva pool (2.35 ng/mL) compared to the no-gum condition (2.66 ng/mL), $t(18) = 4.52$, $p < .0005$, corresponding to a 12% reduction of measured salivary C (see Fig. 2, panel B).

Similarly, for T, there was a main effect of medium, with the saliva pool having a significantly higher hormone concentration than the water pool, $F(1, 36) = 5{,}002$, $p < .0000005$, and a significant Medium × Treatment interaction, $F(1, 36) = 605.61$, $p < .0000005$. Without gum, water sample T concentrations (1 pg/mL) could not be differentiated from the assay's lower limit of detection (4 pg/mL) and salivary T concentrations were at 125 pg/mL. Relative to these levels, T assayed in water was significantly increased (18 pg/mL), $t(18) = 21.02$, $p < .0000005$, and T assayed in saliva was significantly reduced (78 pg/mL), $t(18) = 18.97$, $p < .0000005$, corresponding to a 38% reduction of measured salivary T (see Fig. 2, panel C).

4. Discussion

Findings obtained in participant samples and in comparisons of salivary and water pools treated with chewing gum or left untreated revealed a sizable and consistent increase in P concentrations due to

Table 1
Assay quality parameters and mean (SEM) salivary hormone concentrations.

			Morning		Evening				
Hormone	Sensitivity	CV%	No gum	Gum	No gum	Gum	F_{Gum}	F_{Time}	$F_{G\times T}$
Cortisol (ng/mL)	0.02	7.49	7.56 (1.46)	5.23 (1.03)	0.68 (0.12)	1.64 (0.58)	0.30	65.06***	9.70*
Testosterone (pg/mL)	1.16	13.13	41.59 (19.03)	29.57 (14.13)	14.07 (6.10)	13.93 (3.97)	0.99	3.61H	2.60
Progesterone (pg/mL)	0.03	11.74	24.13 (3.94)	44.19 (5.35)	13.96 (2.97)	37.63 (4.42)	50.55***	3.21	0.45

Note. For cortisol, all statistical analyses were done on log-transformed data, but descriptive statistics are provided for the untransformed data. For *F* tests, *df*s = 1,7.
H $p < .10$.
* $p < .05$.
*** $p < .0005$.

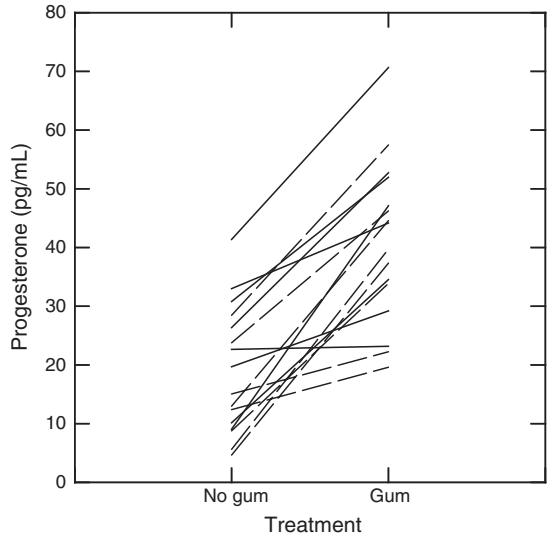

Fig. 1. Progesterone concentrations in participants' saliva samples as a function of stimulation condition and time of the day (solid lines: morning; striped lines: evening).

the use of sugarless chewing gum. The use of gum added a constant load of about 40 pg/mL to both saliva and water pools and an average of about 22 pg/mL to samples collected in the morning and in the evening from research participants. This is clearly a nontrivial increase of P concentrations assessed in saliva if one considers that follicular-phase concentrations in women, like levels in men, are usually between 20 and 30 pg/mL. The use of sugarless chewing gum as a device to stimulate saliva flow approximately doubles these values, which may explain the unusually high salivary P concentrations observed in studies using this protocol to collect saliva samples (e.g., Liening et al., 2010). As suggested by the variation of the detected net increases between saliva and water pool measurements and participant samples as well as the large variability in the effects of gum on participant samples (Fig. 1), the use of chewing gum on salivary P concentrations does not add a constant amount of P to endogenous P concentrations. Rather, although chewing gum appears to elicit an increase of salivary P concentrations in almost everyone, the effect varies considerably from person to person and by testing occasion. Thus, sugarless chewing gum is likely to alter the rank order of salivary P concentrations in a tested population and therefore prevents the valid assessment of individual differences in current hormone concentrations.

Findings from the analyses of participants' salivary T and C concentrations and particularly from the analysis of gum-treated saliva and water pools indicate that, despite earlier reports to the contrary (e.g., Dabbs, 1991), the use of sugarless chewing gum also appears to affect the assessment of these hormones. In contrast to the consistently concentration-increasing effect of gum on salivary P, however, chewing gum had a reducing effect on the normal-to-high concentrations of T and C (nonsignificant in the participants' samples, significant in the saliva pool) and an increasing effect on the assessment of T (but not of C) in water and of C (but not of T) in participants' low-concentration evening samples. The concentration-reducing effect of chewing gum on participants' morning T and C cannot be explained by an effect of chewing gum on flow rate and thus a greater dilution of these hormones, because previous studies have consistently failed to demonstrate a systematic effect of flow rate on salivary hormone concentrations (e.g., Riad-Fahmy et al., 1982). Moreover, this explanation also could not account for the parallel effects observed in a saliva pool that was treated with chewing gum *after* collection, nor is it consistent with the *increase* observed in participants' evening samples. It thus appears that sugarless chewing gum makes some of the regular (high) concentrations of endogenous T and C unavailable for antibody binding while, somewhat paradoxically, introducing at a low concentration and perhaps in

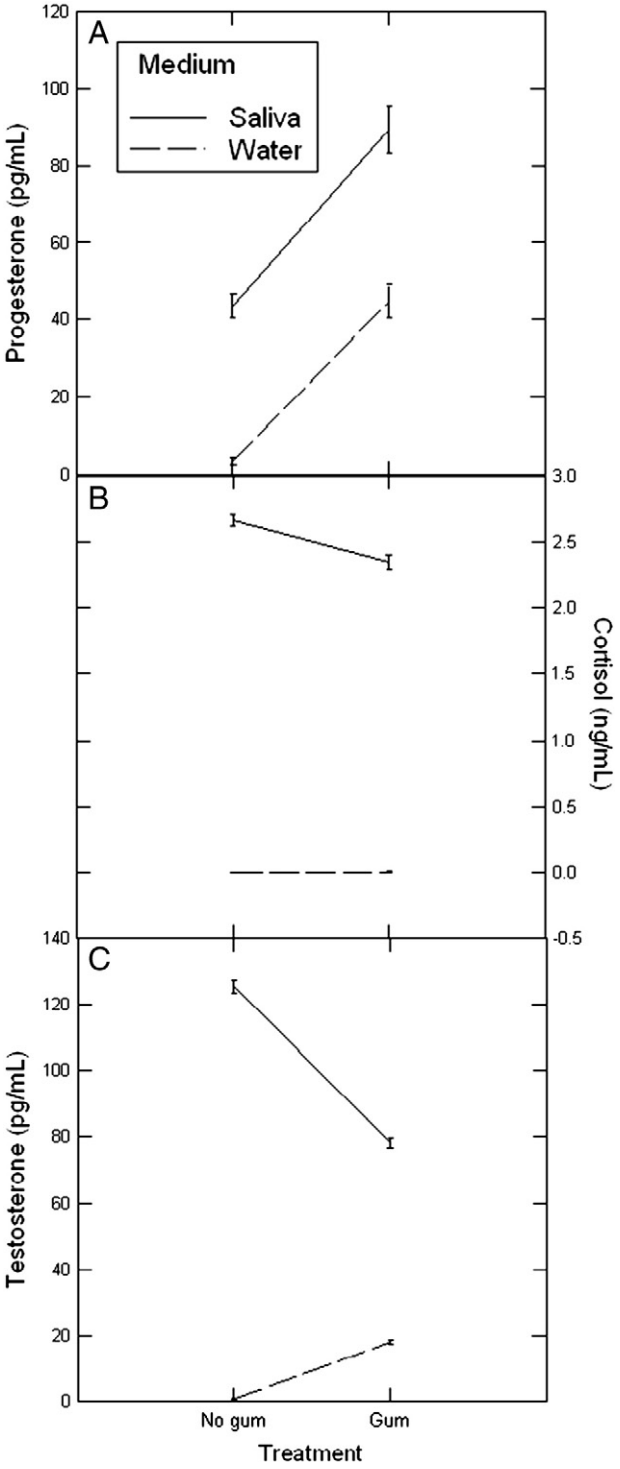

Fig. 2. Progesterone (A), cortisol (B), and testosterone (C) concentrations (±SEM) measured in pools of saliva or deionized water, treated or not treated with Orbit ® Spearmint sugarless chewing gum.

interaction with the saliva matrix steroid-like substances to the assay that bind to the antibodies. The latter effect is suggested by the increased C concentrations in participants' evening samples collected with chewing gum (but not by the gum-treated water pool), the substantial T concentration measured in gum-treated water pools, and the T-raising effects of Orbit ® Spearmint sugarless chewing gum reported for low-T female saliva samples, but not for high-T male samples (van Anders, 2010).

Overall, these findings suggest that the use of sugarless chewing gum is not suited for the collection of saliva samples that will later be assayed for P and may also be problematic, despite earlier reports to the contrary (e.g., Dabbs, 1991), for the assessment of salivary T and C (see also van Anders, 2010 for similar conclusions regarding the assessment of salivary E). The inconsistencies between the present results and those of earlier studies concerning the use of chewing gum in the collection of samples later assayed for T and C suggest that published research reports on the suitability of a given sample collection technique should be viewed with caution. They may apply only to the specific make of the collection device (e.g., a particular sugarless chewing gum, made according to a specific recipe at a specific time), but not to others which appear to fall into the same category (e.g., other sugarless chewing gums). More generally, it may be prudent to test whatever collection technique one intends to use for the hormone(s) one wants to measure against the control condition of unstimulated saliva collection before proceeding to use them in actual studies testing substantive research questions[1]. In the absence of clear-cut evidence that a stimulant does not alter salivary steroid concentrations, passive drooling is the safe method of choice.

One limitation of the present study is the focus on only one make of sugarless chewing gum. Perhaps other types of sugarless chewing gum introduce less, no, or even more bias into salivary hormone assessments and perhaps in other directions than those observed in the present study. Another limitation is the small number of participants who provided saliva samples under gum and no-gum conditions in the morning and the evening and the fact that only one male participant was included. This shortcoming was offset to some extent by the systematic treatment of saliva (collected from a male) and water pools with chewing gum and the observations obtained from these additional analyses, which largely corroborated the results observed in participants' samples.

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[1] The unintended side effects of collecting saliva samples with chewing gum may not only apply to physiological measures, but also extend to the psychological domain. A growing literature suggests that chewing gum has an effect on mood and cognition, particularly on alertness (e.g., Johnson et al., 2011; Smith, 2009). Thus, the collection of saliva samples with chewing gum may also bias concurrently or subsequently collected psychological measures.