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BRIEF COMMUNICATION

Chewing gum has large effects on salivary testosterone, estradiol, and secretory immunoglobulin A assays in women and men

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Summary Salivary assays are increasingly prevalent in behavioral research, and chewing gum is a widely used sialogogue. Methodological investigations into sialogogues have provided mixed results, and few of these have incorporated multiple analytes, gums, and genders. To test effects of gum on salivary testosterone (T), estradiol (E), and immunoglobulin A (IgA) assays, participants (86 women; 91 men) provided two saliva samples, the first of which was unstimulated. Participants were randomly assigned to one of the following seven conditions for the second sample, which was provided after the first: No Gum or one of six sugar-free gums with one of two flavors and three brands. This design avoided the confounding of time and condition by comparing endogenously vs. exogenously induced changes in analytes. Chewing gum significantly decreased production time for the second saliva samples by 3—6 min, and had very large effects on assay results, leading to lower IgA and higher T and E in men and women. Variability was large and differed by gender/sex. Implications include strong gum-assay immunoreactivity, the importance of gender/sex in methodological investigations, and that immunoreactivity can differ in degree and direction depending on analytes.

Salivary measurement has facilitated growth in human behavioral neuroendocrinology (cf. Lipson and Ellison, 1989). Salivary assays are validated (e.g. Baxendale et al., 1982; Dabbs et al., 1995; cf. Shirtcliff et al., 2002), show high test—retest reliability (Dabbs, 1993), and are methodologically advantageous. Still, concerns exist (e.g. Granger et al., 2007), e.g. with sialogogues that speed production but may interact with assays (e.g. Lipson and Ellison, 1989). Cotton is used but can inflate gonadal hormone readings (e.g. Shirtcliff et al., 2001). Chewing gum is more inactive than, e.g. candy (Lipson and Ellison, 1989), but some have found gum-assay immunoreactivity (Lipson and Ellison, 1989; Granger et al., 2004; Shirtcliff et al., 2000) though others have not (e.g. Dabbs, 1991). Some evidence indicates that time since chewing matters (e.g. Granger et al., 2004) but

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other evidence does not (Dabbs, 1991). These studies have provided important though mixed results.

Given widespread use of sugar-free gum as sialogogues, verifying inertness is an important consideration. Here, I examine effects of gum on salivary assays of testosterone (T), estradiol (E), and immunoglobulin A (IgA) in women and men, which is important given no previous studies with IgA and mixed findings with T and E. Including measures of gonadal steroids and an immune marker also allows for considering analyte-specific immunoreactivity. This study employs a rigorous design and analysis, controlling for sample production such that time and stimulant are not confounded: participants provide one unstimulated sample and then a sample that is either unstimulated or gum-stimulated. As such, this study provides novel and important data about gum-assay immunoreactivity, taking gender/sex into account, that applies to contemporary gums and widely used assays. It remains important to note that results can be assay- or lab-specific (Lipson and Ellison, 1989), though previous methodological inquiries have been understood to generalize beyond the assays employed (e.g. Granger et al., 2004) as might results from this study.

1. Methods

1.1. Participants

Participants were part of a larger study approved by the institutional review board (IRB), designed specifically to include this methodological examination. Participants were 86 women (mean age = 20 years, SD = 3 years) and 91 men (mean age = 21 years, SD = 3 years), recruited via ads (receiving financial compensation) and the Psychology Subject Pool (receiving credit). The data from women using hormonal contraceptives who volunteered despite selection criteria and two women who reported being menopausal were not analysed as per IRB approval; as a result, women's *n* by condition was altered. I use gender/sex throughout this paper despite the focus on hormones, because differences cannot knowingly be attributed to biology or gender socialization.

1.2. Procedure and chewing gum

Participants provided two saliva samples in our lab between 13:00 and 18:00 to control for diurnal rhythmicity (Axelsson et al., 2005). To control effects of producing two samples in succession and compare endogenous to exogenously induced changes, sample 1 (S1) was unstimulated and sample 2 (S2) was stimulated via one of seven randomly assigned conditions: No Gum (i.e. no sialogogue), Orbit Spearmint, Extra Spearmint, Dentyne Spearmint, Orbit Peppermint, Extra Peppermint, Dentyne Peppermint. Gums containing aspartame were selected from popular options to ensure a wide selection. I did not include Trident Original as analyses already indicate immunoreactivity (Shirtcliff et al., 2000).

1.3. Samples and assays

Saliva (3—4 mL) was collected in 17 mL polystyrene tubes by spitting after participants rinsed their mouths with water before S1. Tubes were frozen until assay. Assays were conducted between 08/2008 and 09/2008 at the Core Biomarkers Lab at Yerkes Primate Research Center at Emory University via radioimmunoassay (T, E) and ELISA (IgA). Assays were from widely used commercially prepared kits from Salimetrics, LLC (E, IgA) (State College, PA) and Diagnostic Systems Laboratories (T) (Webster, TX). Assay ranges were: E, 1—32 pg/mL; T, 2—500 pg/mL; IgA, 2.5—600 μg/mL. Inter-assay coefficients of variation were: E, 7.8% at 0.107 μg/dL, 5.48% at 1.071 μg/dL, and 10.21% at 0.20 μg/dL; T, 19.16% at 5.03 pg/mL, 15.08% at 170.81 pg/mL, and 16.40% at 25.31 pg/mL; IgA, 14.85% at 28.30 pg/mL and 11.68% at 197.06 pg/mL. Intra-assay coefficients of variation were: E, 10.35% at 0.22 μg/dL; T, 3.41% at 26.89 pg/mL; IgA, 9.96% at 278.10 pg/mL. Outliers (over 3 SD from the mean) were excluded from relevant analyses.

There were several outliers (over 3 SD from the mean as well as visually) who were excluded from analyses with those variables; some of these included extreme outliers that likely resulted from blood contamination. Outlier numbers were similar for men (42/819) and women (49/774). For men, there were four T1 outliers, two T2 outliers, three T% outliers, eight E1 outliers (including four too high to be read), eight E2 outliers (including three too high to be read), three E% outliers, four IgA1 outliers, four IgA2 outliers, and six IgA% outliers. For women, there were 12 T1 outliers, two T2 outliers, two T% outliers, 12 E1 outliers (including six too high to be read), 10 E2 outliers (all too high to be read), two E% outliers, three IgA1 outliers, two IgA2 outliers, and four IgA% outliers. Outliers were spread throughout the conditions, including No Gum; outlying values thus did not result from any gums and eliminating the outliers should not have introduced any confounds into the analyses. Though there appears to be many outliers, not all of which can be explained by possible blood contamination, the number of outliers per sample and per hormone (e.g. four T1 outliers from 91 men) is generally in line with previous studies (though the number of E outliers is higher); outlier number appears higher likely because there were more samples and more analytes measured in this study than other recent comparable studies.

2. Results

Since my aim was to accept the null hypothesis, statistical considerations were directed towards avoiding Type II (falsely concluding inertness) rather than Type I error. Accordingly, I conducted multiple independent and paired *t*-tests. Analyses were conducted with percent changes (i.e. [S2 − S1]/S1), which are more sensitive in the face of large variation since they take individual changes into account (van Anders and Watson, 2007). I also conducted analyses with absolute values for comparison purposes. There was a significant interaction between Gender/Sex and Gum, *multivariate* $F(18,204) = 1.65$, $p = 0.050$, with women's T% larger than men's, so analyses were separate by gender/sex.

One-sample *t*-tests confirmed no significant difference from zero in the No Gum condition for women's T%, $t(9) = -0.60$, *ns*, E%, $t(9) = 0.25$, *ns*, or IgA%, $t(11) = 1.23$, *ns*, or men's T%, $t(10) = 0.48$, *ns*, E%, $t(9) = -1.60$, *ns*, or IgA%, $t(9) = 1.04$, *ns*. In contrast, there were significant differences from zero for women's and men's gum-stimulated analytes (see Fig. 1). Women's T was significantly increased

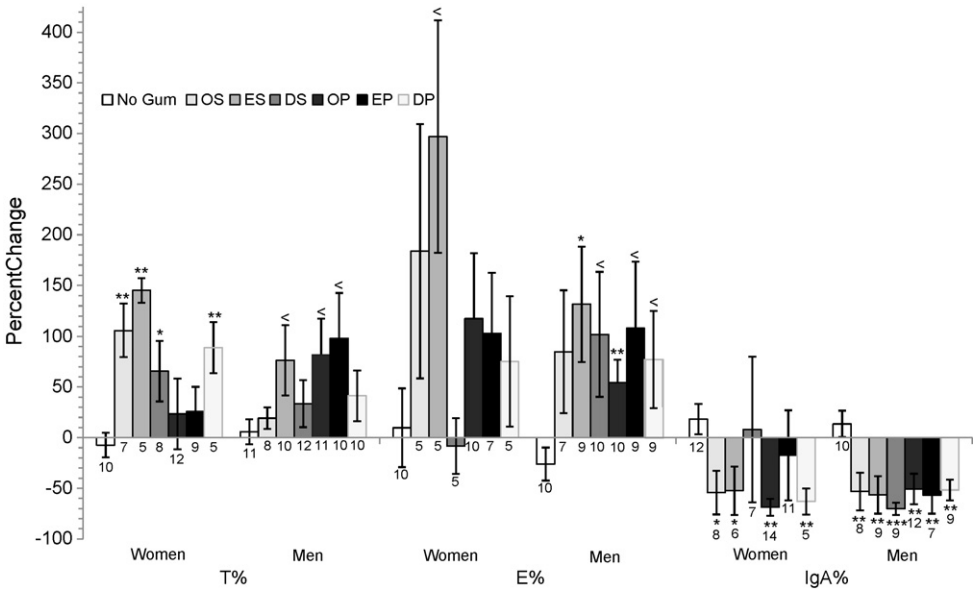

Figure 1 Mean percent changes in women's and men's T (testosterone), E (estradiol), and IgA (immunoglobulin A) by condition with standard error bars. Notations indicate within-analyte differences from No Gum condition at '<' = $p < 0.10$, '*' = $p < 0.05$, '**' = $p < 0.01$, '***' = $p < 0.001$. 'O' = Orbit, 'E' = Extra, 'D' = Dentyne; 'S' = Spearmint, 'P' = Peppermint. Numbers above or below the bars indicate *n*'s per group. Percent change in analyte.

by four gums, with magnitudes ranging from 65 to 145%. There was a statistical trend for women's E to be increased by one gum, and women's IgA was significantly decreased by four gums with magnitudes ranging from −50 to −68%. Men's E was increased significantly by two gums, and potentially three more, with magnitudes of 54 to 131%. There was a statistical trend for men's T to be increased by three gums, with magnitudes of 76 to 98%. Men's IgA was significantly

Table 1 Means and standard errors in brackets for absolute values of samples 1 and 2 of testosterone (T) (pg/mL), estradiol (E) (pg/mL), and immunoglobulin A (IgA) (μg/mL) in women and men.

	T1	T2	E1	E2	IgA 1	IgA 2
No Gum						
Women	20.51 (8.22)	19.51 (12.00)	5.52 (3.37)	5.28 (5.55)	53.36 (32.94)	54.07 (32.45)
Men	117.76 (46.77)	110.29 (33.68)	5.38[a] (3.34)	3.09[a] (2.32)	51.83 (28.13)	67.24 (28.38)
Orbit Spearmint						
Women	14.42[b] (8.02)	29.29[b] (9.50)	2.63 (3.68)	6.09 (5.66)	47.69 (32.27)	19.67 (26.56)
Men	91.73 (31.89)	107.27 (36.75)	3.43 (1.59)	4.93 (2.69)	61.85[b] (22.26)	25.59[b] (26.77)
Extra Spearmint						
Women	16.79[b] (3.24)	41.78[b] (16.88)	2.24[a] (.61)	8.63[a] (5.30)	56.51[b] (8.68)	23.07[b] (22.40)
Men	85.77 (41.60)	115.41 (36.87)	3.89[a] (1.88)	6.53[a] (3.57)	64.81 (53.48)	30.20 (35.81)
Dentyne Spearmint						
Women	17.78[a] (10.46)	29.61[a] (18.68)	3.36 (1.90)	6.35 (8.31)	32.26 (22.48)	22.82 (25.51)
Men	96.32 (35.76)	113.85 (46.40)	3.05 (2.05)	4.06 (3.05)	62.81[a] (46.72)	27.44[a] (32.52)
Orbit Peppermint						
Women	32.90 (15.66)	25.97 (17.15)	3.46 (1.96)	5.41 (4.94)	51.08[b] (30.96)	20.87[b] (26.75)
Men	61.85[b] (29.18)	105.67[b] (49.85)	4.67[b] (3.56)	7.06[b] (4.87)	58.23[b] (20.70)	25.79[b] (21.79)
Extra Peppermint						
Women	26.04 (14.37)	27.05 (9.08)	5.60 (3.77)	8.08 (7.16)	44.19[b] (24.54)	17.01[b] (27.18)
Men	81.40[b] (44.47)	129.90[b] (44.34)	3.73 (3.09)	6.82 (4.52)	66.60[a] (51.16)	14.92[a] (12.53)
Dentyne Peppermint						
Women	19.13[b] (7.07)	34.12[b] (9.50)	3.31 (.87)	5.53 (4.35)	46.70 (42.59)	44.88 (30.62)
Men	90.31 (43.83)	106.32 (35.49)	5.16 (3.70)	6.76 (4.87)	47.17 (41.39)	36.47 (42.00)

Notes: 'a' indicates a trend for a statistical difference between samples 1 and 2 at $p < 0.10$. 'b' indicates a significant difference between samples 1 and 2 at $p < 0.05$. Analyses are independent *t*-tests.

decreased by all gums, with magnitudes of −51 to −70%. Analyses with absolute values (see Table 1) showed similar effects of gum with increased T and E, and decreased IgA in men and women.

2.1. Sample length

Researchers use gum to speed saliva production, so I conducted analyses to compare sample 2 production lengths between the No Gum and Gum conditions. There was a significant overall effect of gum on sample production length, $F(6,159) = 5.27$, $p < 0.001$. Participants in each of the Gum conditions (means ranged from 4.74 min, SD = 3.39 min, to 7.90 min, SD = 5.13 min) were significantly quicker at producing the saliva samples than participants in the No Gum condition (mean = 10.89 min, SD = 5.16 min), all p's < 0.05.

To confirm that these were not just pre-existing group differences, I conducted the same analysis on sample 1 production lengths. There was an overall significant effect of gum, $F(6,145) = 2.60$, $p = 0.020$, but this could not explain the above effects (i.e. that gum made saliva production quicker) since participants in only one of the six Gum conditions (Dentyne Peppermint, mean = 7.74 min, SD = 3.69 min) produced significantly quicker saliva samples than the No Gum condition (mean = 11.87 min, SD = 5.69 min), $p = 0.009$. Instead, these overall differences appeared to reflect that this group had more rapid productions at times 1 and 2 than most of the other conditions (an effect that might be expected to occur on the basis of chance). Further, samples 1 and 2 in the No Gum conditions took similar lengths of time to produce, while samples 2 in the Gum conditions were more rapidly produced. Thus, results from this study support researchers' observations that gum does significantly decrease the length of time participants take to produce saliva samples; in this study, chewing gum reduced the time to produce a second saliva sample by 3–6 min. Given this study's design, however, these data cannot demonstrate whether gum speeds up a first saliva sample.

3. Discussion

Given chewing gum's widespread use in biobehavioral salivary research, data from this paper provide important information on gum-assay immunoreactivity for T, E, and IgA in men and women. Findings indicated that chewing gum significantly speeds up sample production by 3–6 min, which may be helpful when time is a critical consideration. Results also indicated extremely large effects of gums (50–150%) on women's and men's T, E, and IgA relative to a No Gum condition, and show for the first time that this is not an effect of producing two samples or passage of time. Further, gum immunoreactivity was not consistent between analytes, increasing T and E readings, but decreasing IgA readings, and gum led to a larger decrease in women's relative to men's T.

Researchers have conducted methodological investigations into the effects of gum on salivary assays (Lipson and Ellison, 1989; Dabbs, 1991; Shirtcliff et al., 2000; Granger et al., 2004), finding some evidence that sugar-free gums, including the most widely used Trident Original flavor, affect both salivary T and E, but findings have been mixed. As such, the present study provides some of the first clear evidence of very strong gum immunoreactivity for T, IgA, and E using a design that controlled for sample production. Differences with reports of no immunoreactivity (e.g. Dabbs, 1991) may have resulted from the present study's use of larger sample sizes, conservative statistics that minimize Type II error (incorrectly concluding inertness), and/or difference in sweetener (e.g. aspartame vs. saccharin).

A limitation of this study is that it was not designed to examine why chewing gum interacts with assay results. Still, one possibility is that the gums affect analyte production. A more plausible possibility is that the gums interact with the assays. Another limitation is the prohibitive cost of conducting a study that would include all salivary analytes or chewing gums, though I attempted to include more analytes and gums than comparable studies. Additionally, it remains important to note that results can be assay- or lab-specific (Lipson and Ellison, 1989), though findings may and often do generalize to other assays (e.g. Granger et al., 2004).

Results from this study showed very large and non-scaled gum-assay immunoreactivity, but there are additional implications. For example, gender/sex is important in methodological investigations, as immunoreactivity differed for women and men. Additionally, assay immunoreactivity can and does differ in degree *and* direction depending on analytes, as gum increased T and E values, but decreased IgA values. More broadly, however, results from this study indicate that there is a need for more methodological investigations to support evidence-based best practices.

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Conflict of interest

The author has no conflicts of interest to disclose.

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