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Sexual orientation and salivary alpha-amylase diurnal rhythms in a cohort of U.S. young adults

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ABSTRACT

Sexual minorities in the United States are at elevated risk of prejudice, discrimination, and violence victimization due to stigma associated with their sexual orientation. These stressors may contribute to physiological stress responses and changes in the regulation of the sympathetic nervous system (SNS). To date, no studies have examined the associations among minority sexual orientation, recent stressful events, and diurnal salivary alpha-amylase (sAA) patterns. The present study included 1663 young adults ages 18–32 years (31% men, 69% women) from the Growing Up Today Study, a prospective cohort of U.S. youth. Participants provided five saliva samples over the course of one day to estimate diurnal sAA patterns. Sexual orientation groups included completely heterosexual with no same-sex partners (CH; referent), mostly heterosexual/completely heterosexual with same-sex partners, and gay/lesbian/bisexual (LB or GB). Sex-stratified multilevel models were fit to evaluate the association of sexual orientation with diurnal patterns of log sAA. The association of recent stressful events was also evaluated. Among women, sexual minorities scored significantly higher than CH on perceived stress and number of stressful events in the past month ($p < 0.05$). Among men, sexual minorities scored higher than CH on perceived stress but not recent stressful events. In multivariable models, recent stressful events were not associated with sAA patterns, but significant sexual orientation group differences in sAA diurnal rhythm were observed among women though not among men. Compared to CH women, LB showed a blunted awakening response and elevated sAA levels across the day, both indicators consistent with SNS dysregulation. Findings suggest dysregulation of stress physiology in LB women, but not other sexual minority women or men, relative to same-sex heterosexuals. Observed dysregulation may relate to exposure among LB women to chronic stressors associated with sexual orientation stigma, although these relations and differences by sex warrant further study.

1. Introduction

Sexual orientation-related physical and mental health disparities have been well-documented in the United States. Sexual minorities report greater prevalence of depressive and anxious symptoms, post-traumatic stress disorder (PTSD), disordered eating, and other adverse health outcomes compared with heterosexual populations (Institute of Medicine, 2011). Both stress and attachment paradigms suggest that health inequities adversely affecting sexual minorities result from social stigmatization enacted through discrimination, harassment, abuse, and violence (Minority Stress Theory) (Meyer, 2003; Rosario et al., 2002) and from less secure attachment during child or adolescent development as a consequence of poor family dynamics (e.g., parental rejection) (Attachment Model for LGB Individuals) (Rosario, 2015; Rosario

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et al., 2014a, b). Dysregulation of physiological stress response pathways, such as the hypothalamic-pituitary-adrenocortical (HPA) axis, can indicate early signs of negative health effects of acute and chronic stressors (Almeida et al., 2009; Miller et al., 2007). Some evidence indicates that sexual minorities exhibit more dysregulated HPA axis response when faced with acute social evaluative stressors in laboratory settings (Hatzenbuehler et al., 2009; Juster et al., 2015). Interestingly, one study restricted to lesbian, gay, and bisexual young adults found that stronger family but not peer support was associated with less cortisol reactivity in response to social evaluative stressors in a laboratory setting.(Burton et al., 2014) In terms of research on diurnal cortisol patterns as indicators of chronic HPA axis dysregulation, two studies, including one on which the present study is based using the same salivary samples (Austin et al., 2016), have not found sexual orientation group differences (Austin et al., 2016; Juster et al., 2013). Another study restricted to gay men found black compared to white men experienced a flatter diurnal cortisol rhythm, an indicator of HPA axis dysregulation in the black gay men, which the authors suggested may be due to chronic stress of racial discrimination.(Cook et al., 2017) However, a small study restricted to gay men and lesbians that explored whether there might be a relationship between discrimination and diurnal cortisol did not find a statistically significant association (Juster and Bockting, 2017).

Another physiological stress response pathway that can become dysregulated through both acute social evaluative stressors and chronic stressors is the autonomic nervous system (ANS) (Lucini et al., 2005, 2002). The ANS maintains homeostasis through dynamic interactions between its sympathetic and parasympathetic branches. Sympathetic nervous system (SNS) activation occurs in response to stressors and other environmental challenges and mobilizes physiological resources to respond to these environmental demands (Cacioppa et al., 1998; Lucini et al., 2002). The parasympathetic nervous system serves an opposing set of functions that promote growth and restoration when the organism is at rest and facilitates a return to homeostasis following stressors (Berntson et al., 1997; Porges, 2007). Dysregulation of this system has been linked to elevated glucocorticoid sensitivity, inflammatory cytokine production, and other perturbations that have been associated with a variety of inflammatory diseases, cardiovascular disease, and cancer (Pongratz and Straub, 2014; Thoma et al., 2012a).

Salivary alpha-amylase (sAA), an enzyme synthesized and secreted from the salivary glands (Baum, 1993), is an established biomarker of SNS activity (Nater and Rohleder, 2009; Thoma et al., 2012b). A benefit of sAA is that its collection is relatively noninvasive for study participants compared to SNS activity biomarkers collected through serum or cerebrospinal fluid, such as epinephrine and norepinephrine (Thoma et al., 2012a). sAA levels have a distinct diurnal profile pattern, decreasing shortly after awakening and increasing throughout the course of the day (Nater et al., 2007). sAA dysregulation is a valid and reliable marker of stress-reactive physiological changes, which can manifest as a blunted awakening response (i.e., less of a decline in sAA level 30 min after awakening) and higher output of sAA throughout the day (Nater et al., 2006; Nater and Rohleder, 2009). For instance, sAA dysregulation has been associated with higher glucocorticoid sensitivity and inflammatory cytokine production (Thoma et al., 2012a). Chronic psychosocial stress has been linked to changes in the diurnal rhythm of sAA. For example, adults who report high levels of job strain exhibit a blunted awakening response than do those with low levels of job strain (Karhula et al., 2016). Children who report victimization by peers have higher levels of sAA in response to a peer-oriented social challenge than those who do not report peer victimization (Rudolph et al., 2010); a similar pattern has been observed among adults who experienced trauma and maltreatment as children in response to a laboratory-based stressor and trauma reminders (Kuras et al., 2017; Yoon and Weierich, 2016). Dysregulated sAA also can manifest as higher sAA output across the day (Nater et al., 2007). Elevated sAA diurnal output has been found in adolescents and adults with trauma histories and PTSD (Nater et al., 2007; Skoluda et al., 2017; Thoma et al., 2012a) and in adults with generalized social anxiety disorder (van Veen et al., 2008).

Sexual minorities consistently have been found to experience more trauma and victimization than same-sex heterosexual peers (Katz-Wise and Hyde, 2012; Roberts et al., 2010, 2012). Furthermore, some research with adolescents suggests that harassment based on a stigmatized identity compared to general harassment not related to identity results in worse decrements to mental and physical health (Russell et al., 2012). The disproportionate exposure to stressors among sexual minorities and potentially to more potent stressors may produce lasting alterations to the SNS. To date, however, no studies have examined diurnal sAA patterns across sexual orientation groups.

We undertook the present study to address this gap in the literature, examining patterns in diurnal sAA across sexual orientation groups in a national cohort of young adult women and men in the United States. We hypothesized that women and men sexual minorities, compared to same-sex heterosexuals, would experience a blunted awakening response (meaning less of a decline in sAA level after awakening) and elevated sAA levels across the day, both of which are considered indicators of SNS dysregulation. Further, we hypothesized that greater level of stress exposure would explain these patterns and that there may be a synergistic effect between sexual orientation and stressor exposure due to potentially more potent identity-based exposures experienced by sexual minorities.

2. Methods

2.1. Study sample

Study participants were from the Growing Up Today Study (GUTS), a national, prospective cohort of 27,324 youth (ages 9–16 years at enrollment in 1996 for GUTS1 cohort and 2004 for GUTS2 cohort). The GUTS cohort consists of children of women in the Nurses' Health Study 2, a prospective cohort of over 116,000 U.S. women, and surveys have been administered annually or biennially since the cohort's inception. The sample is primarily white (94%) and has a limited socioeconomic range, as all the participants' mothers have a four-year nursing degree.

The current study is based on a subset of GUTS youth who participated in the 2011–2014 GUTS Saliva Substudy; only those GUTS respondents who had completed a previous GUTS survey for the 2010-11 wave were eligible. The substudy, which has been reported previously (Austin et al., 2016), was designed to examine the association between sexual orientation and stress response physiology. All sexual minority participants were invited to participate, and a random subsample of heterosexuals were also invited. Youth were excluded if they were currently pregnant or pregnant in the past six months, if they reported any history of cancer treatment or diagnosis of diabetes, or if they reported past-month use of oral or inhaled steroids. A total of 6980 participants were invited to the GUTS Saliva Substudy by email and were screened for eligibility, of whom 1966 (28%) agreed to participate. Of these participants, 287 did not return at least one usable saliva sample and an additional 16 were missing data on wakeup time, resulting in a total analytic sample 1663 individuals. The 303 excluded, compared with the 1663 included, were more likely to be heterosexual, white, and older ($p < 0.05$), but not different by sex ($p > 0.05$).

2.2. Survey measures

2.2.1. Sexual orientation

Sexual orientation was assessed on the GUTS 2005, 2007, 2010–2011, and 2013 survey waves with two widely used measures. The first asked participants to report the sex of any past or present sexual partners (female[s], male[s], female[s] and male[s], or no sexual contact). The second measure asked participants to report which of the following best describes them: Completely heterosexual (attracted to persons of the opposite sex); mostly heterosexual; bisexual (equally

attracted to men and women); mostly homosexual; completely homosexual, gay, or lesbian (attracted to persons of the same sex); or unsure (Remafedi et al., 1992). Due to sample size limitations, participants were combined into three sexual orientation groups for analysis: (1) completely heterosexual with no same-sex partners (CH), (2) mostly heterosexual or completely heterosexual with same-sex partners (MHCH), and (3) lesbian, gay, or bisexual (LB for women and GB for men). Mostly heterosexual or completely heterosexual with same-sex partners were combined to create an additional category for analyses since prior research indicates that these individuals have elevated health risks compared to completely heterosexuals with no same-sex partners (Roberts et al., 2010, 2012). Bisexual individuals were combined with their same-sex lesbian or gay peers because of insufficient power to conduct separate analyses.

2.2.2. Brief questionnaire

The GUTS Saliva Substudy included a brief questionnaire that participants completed on the day of saliva collection. This questionnaire included a modified version of the Stressful Life Events Screening Questionnaire (Goodman et al., 1998) assessing self-reported lifetime exposure to 13 types of traumatic experiences (e.g., life-threatening events, death of a loved one). Four questions about stressful life events pertaining to financial hardship and divorce or separation were added to fully capture a variety of stressful life experiences and the time frame was modified to focus on experiences in the past 30 days.

The brief questionnaire was administered on a single day, the day when saliva collection occurred. The brief questionnaire included measures accounting for factors that may impact diurnal rhythms (Adam and Kumari, 2009) including measures of sleep quality and duration (previous night's hours of sleep, usual hours of sleep time, time of falling asleep on night before data collection, time of awakening on day of data collection), and mood during each saliva collection throughout the day ("Do you feel worried, anxious, or fearful right now?" and "Do you feel happy, excited, or content right now?" with response options from (1) Not at all to (4) Extremely). The "worried" and "happy" mood items were coded as ordinal time-varying covariates in the model, with higher scores indicating greater levels of worry or happiness at each time point. Participants also completed the four-item Perceived Stress Scale (PSS-4) (Cohen et al., 1983), which addresses perceived stress-related feelings and thoughts in the previous month (e.g., "In the last month, how often have you felt you were unable to control the important things in your life?" with response options from (0) Never to (4) Very often) (Cohen et al., 1983; Cohen and Williamson, 1988). The Cronbach's alpha for the PSS-4 in our sample was 0.76.

Participants were asked to report whether they had, on the day of saliva collection, any alcoholic beverages (yes/no), smoked any cigarettes (yes/no) or engaged in any vigorous physical activity (increasing heart rate and sweat; yes/no) and to list any drugs or medications taken. Women were asked if they had in the previous month used or were currently using any form of hormonal contraception (yes/no). Additional covariates included participant age (in years) and non-compliance with the wakeup sample (yes/no; non-compliance defined as the first saliva sample not being taken within 15 min of waking up).

2.3. Salivary sample collection and assays

Participants received saliva collection tubes and brief questionnaire through the mail with instructions. Samples were collected from participants on a single weekday (Monday–Thursday), and participants were asked to provide five passive-drool saliva samples over the course of the day: at awakening, 45 min, 4 h, and 10 h after awakening, and at bedtime. Participants were instructed not to brush their teeth before the first (awakening) sample was collected and not to eat, drink, chew, or engage in vigorous physical activity for at least 30 min before each subsequent sample. Participants were instructed to record the time of collection for each sample and whether or not they had brushed their teeth, eaten, drunk, chewed, or exercised in the previous 30 min. Filled tubes were stored in baggies in a refrigerator in the participant's home until sampling was complete and then were returned via two-day delivery in a postage-paid mailer along with an ice pack. sAA has been found to be moderately stable across a 24-month period (Skoluda et al., 2017). All saliva samples were collected between August 2011 and February 2014, and participants received $25 upon return of saliva samples. This study was approved by the Brigham and Women's Hospital Institutional Review Board.

Saliva samples were sent to the Channing Division of Network Medicine (CDNM) biorepository, aliquoted, stored at −20 °Centigrade, and assayed by Rohleder Lab at Brandeis University along with quality control (QC) samples provided by the CDNM to obtain three distinct QC pools of saliva. Aliquots of QC pools were distributed randomly among the participant samples and were blinded with alias IDs. Each batch of samples analyzed contained two QC pool samples from at least two of the three available pools and each pool was represented in at least one-third of the batches analyzed. At Rohleder Lab, sAA was measured using a procedure developed in-house with commercially available reagents from Roche Diagnostics and described in prior research (Thoma et al., 2012b). Rohleder Lab provided CDNM with final data from study samples and unblinded QC samples, and QC results were compiled and analyzed across the study. Coefficients of variation for the study met the biorepository standard of 15% or below, and batch adjustment was performed to adjust for any potential batch effects (Rosner et al., 2008).

2.4. Statistical analyses

Separate multilevel models of log-transformed sAA values were fit to examine the diurnal patterns in men and women. The models included individual-level, day-level, and sample-level covariates, a linear, quadratic, and cubic effect of time since waking, and an indicator variable for non-compliance with the wake-up sample. Models also included an estimate of the awakening response, defined as the difference between the wake-up sample and the second sample as well as random individual-level intercept (wake-up level) and slope terms (time since waking). All continuous covariates were centered around the mean. The primary predictor in the model was sexual orientation. Thus the main effect of sexual orientation and interactions between sexual orientation and time since waking and with awakening response were included. Variables from the brief questionnaire were examined for association with sexual orientation of sAA, and those with a significant association were included in the final models. As models predicted log-transformed values of sAA, parameter estimates were transformed (as 100*(exp(β)-1) for interpretation as percentage increase or decrease in mean sAA value. The effect of recent stressful events was also examined by including a main effect term in the model and an interaction between recent stressful events and sexual orientation. All analyses were sex-stratified so that models for women could account for hormonal contraception use and were conducted using SAS version 9.4 (SAS, 2013).

3. Results

As shown in Table 1, mean ages for all groups ranged between 24.4 and 25.1 years, and sexual minority women were slightly older than same-sex CH. Among women, sexual minorities scored significantly higher than CH on perceived stress and number of stressful events in the past month, as well as on substance use and antidepressant medication use. Among men, sexual minorities scored higher than CH on perceived stress.

In multivariable models, significant sexual orientation group differences in sAA diurnal rhythm were found among women (Table 2) but not among men (Table 3). In women, compared to CH, LB showed a blunted awakening response ($p = 0.01$), with an sAA level 42.1% higher than that of CH at the second time point. The slope of the diurnal

Table 1
Participant characteristics for young adult women and men in the Growing Up Today Study who completed saliva sampling in 2011–2014 by sexual orientation identity (n = 1663).

	Women (N = 1148)				Men (N = 515)			
	Completely heterosexual (CH) (n = 755)	Mostly heterosexual or CH w/ SS partners (n = 323)	Bisexual or lesbian (n = 70)	*P*	Completely heterosexual (CH) (n = 360)	Mostly heterosexual or CH w/ SS partners (n = 95)	Bisexual or gay (n = 60)	*P*
Age in years, *M* (SD)	24.4 (3.5)	25.1 (3.1)	24.9 (3.6)	0.01	24.8 (3.5)	25.1 (3.3)	24.7 (3.1)	0.68
Sleep, *M* (SD)								
Hours of sleep in previous night	7.8 (1.3)	7.7 (1.3)	7.7 (1.5)	0.24	7.3 (1.4)	7.4 (1.4)	7.5 (1.3)	0.69
Hours of sleep on typical night	7.7 (0.9)	7.7 (1.0)	7.8 (0.9)	0.60	7.4 (0.9)	7.5 (0.9)	7.4 (0.8)	0.63
Waking time (HH:MM)	7:58 AM (1:36)	7:59 AM (1:37)	8:13 AM (1:38)	0.45	7:57 AM (1:46)	8:03 AM (1:44)	8:13 AM (2:11)	0.55
Health-related behaviors, *n* (%)								
Any alcohol use	164 (22.0)	103 (32.4)	15 (21.4)	0.001	94 (26.7)	32 (34.4)	22 (37.9)	0.11
Any cigarette smoking	14 (1.9)	17 (5.3)	5 (7.1)	0.002	17 (4.7)	7 (7.4)	5 (8.3)	0.39
Antidepressant uses	69 (9.1)	38 (11.8)	11 (15.7)	0.13	14 (3.9)	6 (6.3)	6 (10.0)	0.11
Hormonal contraception use (women only), *n* (%)	387 (51.5)	171 (53.1)	22 (31.4)	0.003	n/a	n/a	n/a	
Perceived Stress Scale[a], *M* (SD)	2.2 (0.7)	2.4 (0.7)	2.5 (0.7)	< .001	2.2 (0.7)	2.5 (0.8)	2.5 (0.8)	< .001
Eating before sample, *N (%)*								
Sample 1 (Wake up)	0 (0)	0 (0)	0 (0)	n/a	0 (0)	0 (0)	0 (0)	n/a
Sample 2 (45 min post-wake up)	35 (4.6)	15 (4.6)	0 (0)	0.18	18 (5.0)	5 (5.3)	3 (5.0)	0.99
Sample 3 (4 h post-wake up)	43 (5.7)	17 (5.3)	2 (2.9)	0.60	17 (4.7)	3 (3.2)	2 (3.3)	0.74
Sample 4 (10 h post-wake up)	48 (6.4)	16 (5.0)	4 (5.7)	0.67	20 (5.6)	5 (5.3)	2 (3.3)	0.77
Sample 5 (Before bed)	21 (2.8)	10 (3.1)	0 (0)	0.34	8 (2.2)	2 (2.1)	0 (0)	0.51
Exercise before sample, *N* (%)								
Sample 1 (Wake up)	0 (0)	0 (0)	0 (0)	n/a	0 (0)	0 (0)	0 (0)	n/a
Sample 2 (45 min post-wake up)	12 (1.6)	13 (4.0)	3 (4.3)	0.04	4 (1.1)	3 (3.2)	1 (1.7)	0.36
Sample 3 (4 h post-wake up)	23 (3.1)	4 (1.2)	0 (0)	0.08	8 (2.2)	0 (0)	1 (1.7)	0.34
Sample 4 (10 h post-wake up)	18 (2.4)	4 (1.2)	0 (0)	0.22	7 (1.9)	2 (2.1)	0 (0)	0.54
Sample 5 (Before bed)	7 (0.9)	4 (1.2)	0 (0)	0.62	3 (0.8)	1 (1.1)	0 (0)	0.75
Stressful events in past month, *n* (%)								
None	640 (84.8)	260 (80.5)	48 (68.6)	0.002	299 (83.1)	72 (75.8)	48 (80.0)	0.26
1 +	115 (15.2)	63 (19.5)	22 (31.4)		61 (16.9)	23 (24.2)	12 (20.0)	
Compliant with wakeup sample, *n* (%)	725 (96.7)	305 (94.4)	66 (94.3)	0.19	350 (97.8)	89 (98.9)	59 (98.3)	0.78

Notes: P-values based on Chi-square tests for categorical variables and ANOVA for continuous variables.
Compliant with wake up sample = saliva sample taken within 15 min of waking. All numbers are rounded to nearest zero.
[a] Perceived stress scale scores range from 0 to 4, with higher scores indicating more perceived stress.

trajectory was also significantly greater in LB compared to CH women (p = 0.02) resulting in higher mean sAA as time progressed since waking (Fig. 1). Recent stressful life events were not associated with sAA patterns, and interactions between sexual orientation and recent stressful life events were not statistically significant; therefore, these terms were not included in final models predicting sAA patterns.

4. Discussion

To our knowledge, our study is the first to examine patterns in diurnal sAA by sexual orientation group and the largest study to examine diurnal sAA patterns in young adults more generally. We found support for our hypotheses for women, in which LB compared to CH women experienced a blunted awakening response and also elevated sAA levels across the day. Both of these findings for LB women are consistent with ANS dysregulation. For men, however, our hypotheses were not borne out: No sexual orientation-related differences were observed in awakening response or levels of sAA across the day. Also contrary to our hypotheses, recent stressful life events were not associated with sAA patterns in either women or men, nor were interactions between sexual orientation and recent stressful life events statistically significant.

Investigation into sexual orientation-related disparities in stress physiology is an emerging area of research. Studies focused on acute social evaluative stressors in laboratory settings have found sexual orientation-group differences in HPA axis functioning as indicated through cortisol response to acute psychosocial stressors (Hatzenbuehler et al., 2009; Juster et al., 2015). In addition, one study of lesbian, gay, and bisexual young adults found that stronger family but not peer support predicted less cortisol reactivity in response to a study-administered social evaluative stressor (Burton et al., 2014). In contrast, two studies, including one from the same cohort used in the present study, have investigated sexual orientation group patterns in diurnal cortisol patterns, and neither study found sexual orientation group differences (Austin et al., 2016; Juster et al., 2013). The present study extends the literature by offering for the first time evidence of

Table 2
Multilevel model of log(amylase) including random effects for wakeup amylase and time since waking and fixed effects of amylase awakening response and time since waking squared and cubed among young adult women in GUTS Saliva Study (n = 1113).

Variable	Estimate	SE	p-value	Interpretation (% increase/decrease per unit change of predictor)[a]
Effects on wakeup amylase level (main effects)				
Intercept	4.004	0.047	< .0001	**54.8 nmol/L mean wakeup amylase**[b]
Age in years	0.068	0.007	< .0001	**7.1% higher for each year older**
Hours sleep, previous night	−0.001	0.020	0.97	−0.1%
Hours sleep, typical night	−0.003	0.025	0.91	−0.3%
Waking time	−0.000	0.000	0.82	0.0%
Any cigarette smoking	0.150	0.128	0.24	16.2%
Any alcoholic drinks	−0.140	0.052	0.01	**−13.1% lower if alcohol**
Perceived stress scale	0.024	0.032	0.44	2.5%
Hormonal contraception use	0.047	0.045	0.29	4.9%
Noncompliance (wakeup sample)	−0.002	0.113	0.98	−0.2%
Antidepressant	0.036	0.074	0.63	3.7%
Sexual orientation (Ref = CH)				
MH or CHwSS	0.004	0.068	0.96	0.4%
Bisexual or Lesbian	−0.139	0.128	0.28	−13.0%
Effects on amylase level(main effects of time varying covariates)				
Eating (before each sample)	0.136	0.066	0.04	**14.6% higher if ate before sample collection**
Exercise (before each sample)	0.159	0.096	0.10	17.2% higher if exercise before Sample collection
Effects on time since waking (interactions with linear time effect)				
Intercept	0.059	0.008	< .0001	**6.1% higher per hour since wakeup**
Sexual orientation (Ref = CH)				
MH or CHwSS	−0.001	0.005	0.86	−0.1%
Bisexual or Lesbian	0.022	0.009	0.02	**2.3% higher if Lesbian or Bisexual**
Effects on time since waking squared (quadratic time effect)				
Intercept	−0.005	0.001	< .0001	**−0.5% per hour squared since wakeup**
Effects on time since waking cubed (cubic time effect)				
Intercept	0.000	0.000	< .0001	**0.0% per hour cubed since wakeup**
Effects on amylase awakening response (interactions with awakening response)				
Intercept	−0.854	0.039	< .0001	**−57.5% lower if sAA-AR**
Noncompliance (wakeup sample)	0.115	0.137	0.40	12.2%
Sexual orientation (Ref = CH)				
MH or CHwSS	0.068	0.068	0.32	7.1%
Bisexual or Lesbian	0.351	0.133	0.01	**42.1% higher if bisexual or lesbian**
Random Effects	Variance estimate	SE	p-value	
Awakening amylase	0.548	0.036	< .0001	
Time since waking	0.000	0.000	0.01	

Notes: Mean-centered variables are age, hours sleep in previous night, hours sleep on a typical night, waking time, perceived stress score, level of worry during each saliva sample.
Sexual orientation categories: (1) Bisexual or Lesbian (2) MH = Mostly Heterosexual or CHwSS = Completely Heterosexual with same-sex partners; (3) CH = Completely Heterosexual with no same-sex partners (Ref).
sAA-AR: Salivary alpha-amylase awakening response.

[a] In interpreting effect sizes, use of a logarithmic outcome allows coefficients to be interpreted as percentage change in the outcome per unit change in the independent variable, after applying the following transformation: $B_{\%change} = [\exp(B_{raw})] - 1$. The "Interpretation" column of the table includes the percentage change based on this transformation.

[b] Mean wakeup amylase among CH, non-smoking, no alcohol, no hormonal contraception use, no antidepressant use, compliant participants with mean age sleep, waking time, and stress, no eating and no alcohol before sample collection.

SNS dysregulation by sexual orientation group.

Contrary to our hypotheses, we found sexual orientation-related perturbations in sAA diurnal patterns among only women. The observed sex difference in orientation-related ANS dysregulation is intriguing, but mechanisms that might lead to this pattern are far from clear. There may be at least three plausible explanations for this finding. One, the bisexual-to-lesbian/gay ratio of the composition of LB group (where bisexual women were predominant) was much higher than that of the GB group (where gay men were predominant), meaning that the LB and GB groups differed not only by sex but also by sexual orientation subgroup composition. Emerging evidence suggests that bisexuals may be at distinctly elevated risk for both adverse exposures and negative health sequelae (Conron et al., 2010; Dyar et al., 2018 (Epub ahead of print)). Two, while more LB women reported recent stressful life events than any other group defined by sex and sexual orientation in our sample, recent stressful experiences were not associated with sAA diurnal patterns and so are not likely to explain sex differences in our main findings. Interestingly, Nater and colleagues administered a measure of past-month stressors similar to ours and also did not find associations with diurnal sAA in women or men (sexual orientation not reported); however, they did find chronic stressors to be positively associated with sAA dysregulation, specifically higher sAA output throughout the day (Nater et al., 2007). It is possible that the LB women in our sample experienced higher levels of chronic stressors and traumatic events over the life course that were not captured in our assessment of recent stressful life events. For example, population-based studies consistently document higher rates of exposure to childhood abuse and maltreatment as well as other forms of interpersonal violence among sexual minority women as compared to heterosexual women (Corliss et al., 2002; Roberts et al., 2010). In studies that have examined both men and women, sexual minority women have higher rates of exposure to child maltreatment and interpersonal violence than

Table 3
Multilevel model of log(amylase) including random effects for wakeup amylase and time since waking and fixed effects of amylase awakening response and time since waking squared and cubed among young adult men in GUTS Saliva Study (n = 487).

Variable	Estimate	SE	p-value	Interpretation (% increase/decrease per unit change of predictor)[a]
Effects on wakeup amylase level (main effects)				
Intercept	3.758	0.069	< .0001	**42.9 nmol/L mean wakeup amylase**[b]
Age in years	0.085	0.011	< .0001	**8.9% higher for each year older**
Hours sleep, previous night	−0.012	0.031	0.70	−1.2%
Hours sleep, typical night	0.033	0.041	0.43	3.3%
Waking time	0.000	0.000	0.002	**0.002% higher per minute waking up late**
Any cigarette smoking	0.235	0.157	0.14	26.5%
Any alcoholic drinks	0.142	0.079	0.07	15.3% higher if alcohol
Perceived stress scale	−0.014	0.052	0.79	−1.4%
Noncompliance (wakeup sample)	0.200	0.252	0.43	22.1%
Antidepressant	0.298	0.166	0.07	34.8% higher if taking antidepressant
Sexual orientation (Ref = CH)				
MH or CHwSS	0.109	0.131	0.40	11.5%
Bisexual or Gay	−0.007	0.156	0.97	−0.7%
Effects on amylase level (main effects of time-varying covariates)				
Eating (before each sample)	0.087	0.111	0.43	9.1%)
Exercise (before each sample)	0.154	0.180	0.39	16.7%
Effects on time since waking (interactions with linear time effect)				
Intercept	0.120	0.015	< .0001	**12.8% higher per hour since wakeup**
Sexual orientation (Ref = CH)				
MH or CHwSS	−0.008	0.009	0.34	−0.8%
Bisexual or Gay	−0.006	0.010	0.54	−0.6%
Effects on time since waking squared (quadratic time effect)				
Intercept	−0.010	0.001	< .0001	**−1.0% per hour squared since wakeup**
Effects on time since waking cubed (cubic time effect)				
Intercept	0.000	0.000	< .0001	**0.02% per hour cubed since wakeup**
Effects on amylase awakening response (interactions with awakening response)				
Intercept	−0.605	0.061	< .0001	**−45.4% lower if sAA-AR**
Noncompliance (wakeup sample)	−0.073	0.30	0.81	−7.1%
Sexual orientation (Ref = CH)				
MH or CHwSS	−0.152	0.130	0.24	−14.1%
Bisexual or Gay	−0.059	0.158	0.71	−5.8%
Random Effects	Variance estimate	SE	p-value	
Awakening amylase	0.657	0.066	< .0001	
Time since waking	0.000	0.000	0.36	

Notes: Mean-centered variables are age, hours sleep in previous night, hours sleep on a typical night, waking time, perceived stress score, level of worry during each saliva sample.
Sexual orientation categories: (1) Bisexual or Gay (2) MH or CHwSS = Mostly Heterosexual or Completely Heterosexual with same-sex partners; (3) CH = Completely Heterosexual with no same-sex partners (Ref).
sAA-AR: Salivary alpha-amylase awakening response.
[a] In interpreting effect sizes, use of a logarithmic outcome allows coefficients to be interpreted as percentage change in the outcome per unit change in the independent variable, after applying the following transformation: $B_{\%change} = [\exp(B_{raw})] - 1$. The "Interpretation" column of the table includes the percentage change based on this transformation.
[b] Mean wakeup amylase among CH, non-smoking, no alcohol, no hormonal contraception use, no antidepressant use, compliant participants with mean age sleep, waking time, and stress, no eating and no alcohol before sample collection.

do men (Roberts et al., 2010). Disproportionate exposure to traumatic events, particularly in childhood, may be driving the sex differences in sAA regulation in early adulthood among the sexual minorities in our sample. This possibility warrants greater investigation in future research both in its own right and because it may be related to the child's attachment (Rosario, 2015). Three, research on gender differences in attachment, which plays a critical role in healthy child and adolescent development, may offer insights on our findings. Rosario has elaborated an attachment paradigm specific to sexual minorities that draws from the strong empirical evidence that societal social stigma undermines the development of secure attachment for sexual minorities at vulnerable periods of child and adolescent development (Rosario, 2015; Rosario et al., 2014a,b). Furthermore, theory and research suggest that during young adulthood (the developmental period in which salivary sample data were collected from GUTS participants) an anxious attachment style is more common in women compared to men and an avoidant attachment style becomes more prevalent among men as they age (Del Giudice, 2009, 2010, 2011). A recent study with young adults found anxious but not avoidant attachment style was associated with cortisol reactivity in response to a social stressor administered in a lab-based setting (Smyth et al., 2015), and another study with adolescent girls found anxious attachment to be associated with perturbations in diurnal cortisol rhythms (Oskis et al., 2011). We are not aware of studies examining attachment and sAA, but further research is warranted to explore whether attachment style differences may play a role in sex differences in sAA dysregulation observed in the present study.

These findings must be considered in light of several notable limitations. First, power limitations are a concern given the relatively small sample of individuals in each orientation and sex subgroup who provided saliva for this study, particularly among men. Additionally, the low response rate may have introduced bias in our findings. Future research should include larger samples to allow for analyses of more sexual orientation subgroup patterns, for instance to distinguish patterns in bisexuals from those of lesbian or gay peers. We gathered saliva samples on only a single day. Future studies may be strengthened by including more days of salivary sample collection from participants.

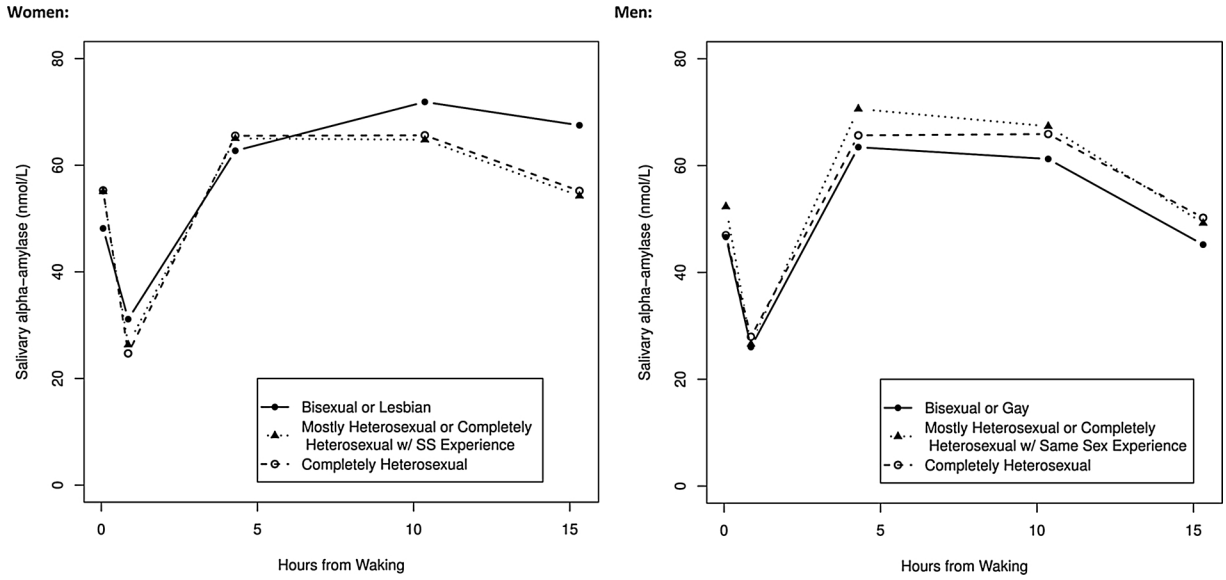

Fig. 1. Estimated model-based mean salivary alpha-amylase (sAA) values as a function of time since awakening, by sexual orientation among women and men participants ages 18–32 years in the Growing Up Today Study (GUTS) Saliva Substudy. Notes: Estimates based on model presented in Tables 2 and 3. Saliva sample times: Sample 1 = 0 h from waking; Sample 2 = 45 min from waking; Sample 3 = 4 h from waking; Sample 4 = 10 h from waking; Sample 5 = before bed.

Compliance with sampling protocols was not monitored electronically, although models were adjusted for sampling-time noncompliance. Failure to adhere to the protocol would depress effect sizes. Despite this threat, we found sexual orientation effects among the women. Participant ages ranged from 18 to 32 years, limiting generalizability to young adulthood. Finally, as participants were predominantly white, raised in largely middle-class households, and members of an ongoing cohort of children of U.S. nurses, these findings may not be generalizable to other young adult populations.

5. Conclusion

Substantial evidence now documents pervasive sexual orientation-related disparities in stigma, harassment, and discrimination and also in negative mental and physical health outcomes adversely associated with minority sexual orientation (Institute of Medicine, 2011). Sexual minority stress theory (Meyer, 2003; Rosario et al., 2002) posits that one pathway through which stressors may manifest in poorer health is through dysregulation of the physiologic stress response (Hatzenbuehler et al., 2009). Studies investigating hypothesized sexual orientation group disparities in stress physiology have focused most attention on HPA axis dysregulation. Those examining cortisol response to acute social evaluative stressors have found some evidence for greater dysregulation in sexual minorities than in heterosexuals (Hatzenbuehler et al., 2009; Juster et al., 2015); whereas, those focusing on diurnal cortisol have not (Austin et al., 2016; Juster et al., 2013). The HPA axis, however, is just one important system of stress physiology. Our study offers the first evidence of sexual orientation group differences in ANS dysregulation, particularly in women. Further research into the possible harmful effects of sexual minority stigma and discrimination on stress physiology and subsequent disease outcomes is urgently needed. Likewise, investigation into the perhaps beneficial effects of improvements in societal acceptance of sexual orientation diversity should be undertaken to broaden our understanding of the potential role of society in both causing and mitigating health inequities.

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Contributors

SB Austin, M Rosario were responsible for study conceptualization. SB Austin, M Rosario, KA McLaughlin, AL Roberts, S Missmer, and L Anatale-Tardiff were responsible for data collection. SB Austin, M Rosario, KA McLaughlin, AL Roberts, K Yu, V Sarda, S Missmer, L Anatale-Tardiff, and EA Scherer were responsible for data analysis, interpretation, and article preparation.

Conflict of interest

The authors have no conflicts of interest to declare.

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