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Sexual Orientation Modulates Endocrine Stress Reactivity

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ABSTRACT

BACKGROUND: Biological sex differences and sociocultural gender diversity influence endocrine stress reactivity. Although numerous studies have shown that men typically activate stronger stress responses than women when exposed to laboratory-based psychosocial stressors, it is unclear whether sexual orientation further modulates stress reactivity. Given that lesbian, gay, and bisexual (LGB) individuals frequently report heightened distress secondary to stigma-related stressors, we investigated whether cortisol stress reactivity differs between LGB individuals and heterosexual individuals in response to a well-validated psychosocial stressor.

METHODS: The study population comprised 87 healthy adults (mean age, 25 years) who were grouped according to their biological sex and their gendered sexual orientation: lesbian/bisexual women (n = 20), heterosexual women (n = 21), gay/bisexual men (n = 26), and heterosexual men (n = 20). Investigators collected 10 salivary cortisol samples throughout a 2-hour afternoon visit involving exposure to the Trier Social Stress Test modified to maximize between-sex differences.

RESULTS: Relative to heterosexual women, lesbian/bisexual women showed higher cortisol stress reactivity 40 min after exposure to the stressor. In contrast, gay/bisexual men displayed lower overall cortisol concentrations throughout testing compared with heterosexual men. Main findings were significant while adjusting for sex hormones (estradiol-to-progesterone ratio in women and testosterone in men), age, self-esteem, and disclosure status (whether LGB participants had completed their "coming out").

CONCLUSIONS: Our results provide novel evidence for gender-based modulation of cortisol stress reactivity based on sexual orientation that goes beyond well-established between-sex differences. This study raises several important avenues for future research related to the physiologic functioning of LGB populations and gender diversity more broadly.

Keywords: Cortisol, Gender diversity, Sex differences, Sexual orientation, Stress reactivity, Trier Social Stress Test

Being male or female drives variability in biobehavioral responsivity to stress (1). Numerous human studies demonstrate that men show higher free cortisol (the major stress hormone in humans) concentrations in response to psychosocial stressors than women of reproductive age (2–6). However, few systematic studies have investigated within-sex variations such as gender socialization that could influence these sex differences (7). Biological sex refers to a multidimensional construct that includes differences in genes, anatomy, gonads, and hormones, whereas sociocultural gender refers to a spectrum of implicit and explicit dissimilarities among men and women in socially constructed roles, identities, and orientations (8–10). We propose that sexual orientation can help differentiate between sex-based versus gender-based factors that modulate endocrine stress reactivity.

Gendered sexuality comprises a complex set of sexual behaviors, identities, and orientations with important health inequalities (11). In particular, lesbian, gay, and bisexual (LGB) populations are at greater risk for psychiatric (12) and physical (13) diseases compared with heterosexual individuals. These disparities are hypothesized to represent forms of minority stress, referring to the cumulative stress individuals from stigmatized minority groups experience (12). Despite the ubiquity of minority stress theory in the psychosocial literature on LGB health, there is a paucity of research using paradigms and measures from stress biology.

Biological stress responses include the hypothalamic-pituitary-adrenal (HPA) axis production of cortisol that mobilize energy necessary to adapt to environmental demands (14). Interactions between the HPA axis and sex hormones (e.g., testosterone, estrogen) are believed to influence biological sex differences in stress response patterns. Such individual differences in stress reactivity are considered important measurable sources of various pathogenic vulnerabilities (15) to psychiatric morbidities (16) that ultimately differ between the sexes. The Trier Social Stress Test (TSST) (17), a widely used

stress-induction paradigm, elicits robust activations of the HPA axis (6,18).

Numerous studies employing the TSST show that men generally manifest higher free cortisol increases than women depending on their menstrual cycle phase, contraceptive use, and pregnancy status (2,6,19). Despite this strong evidence for biologically determined age and sex differences in stress responsivity, few TSST studies have assessed if elusive gender-based factors further modulate within-sex variations. In one exceptional study (20), women showed higher cortisol stress reactivity when supported by their male partner, whereas men showed lower cortisol reactivity when supported by their female partner, suggesting that social contexts can influence endocrine stress reactivity.

To date, only one published study has examined HPA axis functioning using a modified TSST emphasizing discrimination among LGB individuals exposed to varying degrees of stigma (21). Results indicated that LGB young adults who lived in high structural stigma states (e.g., states without policies that protect members of sexual minorities from hate crime, support employment nondiscrimination, and support same-sex marriage) as adolescents showed blunted cortisol responses after the TSST compared with LGB young adults from low-stigma states. This finding suggests that the stress of growing up in environments that target members of sexual minorities for social exclusion may result in HPA axis dysfunction, consistent with research showing hypocortisolism among individuals exposed to extreme adversity (22–24). Because this study focused on understanding LGB within-group differences in HPA axis functioning without a heterosexual comparison group, it is unknown whether there are sexual orientation differences in stress reactivity after exposure to the TSST.

The present study explored whether LGB individuals differ in stress reactive cortisol compared with heterosexual individuals of the same sex. If cortisol stress reactivity is based strictly on biological sex, men should respond more strongly to the TSST, whereas women should not, regardless of sexual orientation. By contrast, if stress reactivity is modulated by sociocultural gender as hypothesized, we would expect within-sex differences based on sexual orientation. In a sexual minority stress framework (12), these differences could be manifested as either hypercortisolemic or hypocortisolemic profiles because both are considered pathogenic and might represent various stages of disease trajectories (25,26). To increase the power of our experimental design, we used a modified version of the TSST that amplifies sex differences in cortisol stress reactivity (27,28), while accounting for confounders that included sex hormones, age, disclosure status, and self-esteem.

METHODS AND MATERIALS

Participants

There were 87 participants 18–45 years old (mean age, 24.61 ± .61 years [± SE]) identifying as lesbian or gay (8 women and 20 men), bisexual (13 women and 5 men), or heterosexual (20 women and 21 men) recruited from Montreal as part of a broader study (29). Owing to fewer lesbians and bisexual men, to equalize groups we combined lesbian/gay and bisexual individuals (20 women and 26 men) and contrasted them to heterosexual individuals (20 women and 21 men). The main exclusionary criteria were medicinal use of synthetic steroid hormones, major health problems, and severe mental illness. Table 1 stratifies sample descriptive information for several characteristics. The Supplemental Methods and Materials section in Supplement 1 provides detailed information for the general protocol, questionnaire descriptions, endocrine measures, and statistical analyses.

Sexual Orientation and Gender Roles

Sexual orientation was assessed and cross-validated using three methods: 1) response to separate advertisements recruiting either lesbian/gay, bisexual, or heterosexual participants; 2) asking participants their identified sexual orientation in an open-ended manner; and 3) administration of a modified five-item Klein Sexual Orientation Scale (30). This instrument uses a 7-point Likert scale to assess "sexual attractions," "sexual behavior," "sexual fantasies," "lifestyle preference," and "sexual identity" along a continuum of sexual experiences "in your life up to now." The sample's responses showed very strong internal consistency ($\alpha = .98$). Sexual orientation was finally coded as "sexual minority" (lesbian, gay, or bisexual) or "heterosexual" on confirmation using all three methods.

As described in the Supplemental Methods and Materials section of Supplement 1, gender roles were contrasted according to sex and sexual orientation to explore potential reversals that would support our view that sexual orientation is gendered. We calculated masculinity-femininity *t* ratios that strongly differed between the sexes ($p < .0001$): men self-endorsed greater masculinity (e.g., "dominant"), whereas women self-endorsed greater femininity (e.g., "tender"). Gay/bisexual men self-endorsed more femininity relative to less masculinity than did heterosexual men; however, no such associations were found when contrasting women. This calculation partially supports the notion that sexual orientation is a gendered construct for men but not for women in our sample, which was predominantly composed of bisexual women.

Disclosure Status

Disclosure of one's sexual orientation is generally a major stressor in the lives of LGB individuals (11). Using the current sample, we previously reported that LGB individuals who had fully disclosed to family and friends had lower diurnal cortisol concentrations as well as lower self-rated psychiatric symptoms of anxiety, depression, and burnout than LGB individuals who had not disclosed their sexual orientation (29). Disclosure was measured using a brief four-item inventory for LGB individuals ($n = 46$) that asked their age for four key milestones that included 1) self-recognition, 2) self-identification, 3) disclosure to friends, and 4) disclosure to family of same-sex attraction as described elsewhere in detail (29).

Participants were coded as "disclosed" ($n = 31$) if they provided ages for all four items, "nondisclosed" ($n = 14$) if one or more of the items were not answered or refuted with open-ended statements, and "controls" ($n = 41$) if heterosexual. One LGB participant did not provide this information and was excluded from the study. Given the importance of this

Table 1. Sample Descriptive Statistics According to Sexual Orientation and Sex

Information	Sample	Lesbian/Bisexual Women	Heterosexual Women	Gay/Bisexual Men	Heterosexual Men	*p*
n	87	20	20	26	21	—
Demographic						
Age (years), mean (SE)	24.61 (.61)	24.10 (1.34)	25.45 (1.13)	23.77 (.98)	25.33 (1.47)	.685
Race/ethnicity						
White, %	70.1	75.0	55.0	73.1	76.2	.147
Black, %	5.7	15.0	10.0	0	0	.147
Asian, %	12.6	0	10.0	23.1	14.3	.147
Hispanic, %	6.9	0	20.0	3.8	4.8	.147
Arab, %	4.6	10.0	5.0	0	4.8	.147
Occupation						
Workers, %	34.5	40.0	35.0	23.1	42.9	.490
Students, %	65.5	60.0	65.0	76.9	57.1	.490
Working/studying hours/week, mean (SE)	28.02 (1.82)	28.40 (3.63)	28.5 (4.38)	29.96 (3.24)	24.91 (3.54)	.789
Sexual Orientation[a]						
Sexual attractions, mean (SE)	3.51 (.26)	4.75 (.33)	1.40 (.15)	5.96 (.30)	1.29 (.10)	<.001
Sexual behaviors, mean (SE)	3.34 (.26)	4.25 (.44)	1.30 (.11)	6.00 (.29)	1.114 (.8)	<.001
Sexual fantasies, mean (SE)	3.61 (.26)	5.15 (.25)	1.65 (.25)	5.81 (.32)	1.29 (.12)	<.001
Lifestyle preferences, mean (SE)	3.37 (.27)	5.15 (.44)	1.115 (.08)	5.54 (.33)	1.10 (.07)	<.001
Sexual identity, mean (SE)	3.52 (.27)	5.05 (.43)	1.15 (.08)	6.04 (.26)	1.19 (.09)	<.001
Socioeconomic						
Postsecondary education, %	95.3	95.0	90.0	100.0	95.0	.575
Personal annual income, CAD, mean (SE)	16,000 (.17)	14,500 (.34)	19,000 (.53)	14,000 (.19)	16,800 (.33)	.737
Household annual income, CAD, mean (SE)	32,100 (.32)	37,000 (.68)	25,000 (.54)	27,100 (.52)	39,000 (.75)	.311
Health and Well-Being						
General						
Medication use, %	18.4	25.0	15.0	15.4	14.3	.105
Oral contraceptive use, %	16.1	20.0	50.0	—	—	.017
Minor physical condition, %	34.5	50.0	40.0	23.1	28.6	.238
Psychiatric history						
None, %	28.7	15.0	40.0	26.9	33.3	.522
Past history, %	8.0	15.0	5.0	11.5	33.3	.522
Family history, %	35.6	35.0	45.0	30.8	33.3	.522
Both past and family history, %	27.6	35.0	10.0	30.8	33.3	.522
Subjective dimensions[b]						
Self-rated health, mean (SE)	3.72 (.09)	3.50 (.21)	3.95 (.14)	3.69 (.17)	3.75 (.20)	.419
Self-rated physique, mean (SE)	3.37 (.10)	3.15 (.22)	3.70 (.18)	3.27 (.14)	3.40 (.28)	.284
Self-rated diet, mean (SE)	3.30 (.11)	3.25 (.19)	3.40 (.25)	3.34 (.19)	3.15 (.25)	.832
Behavioral						
Tobacco smoking						
Smokers, %	11.5	5.0	10.0	11.5	19.0	.376
Social smokers, %	14.9	10.0	5.0	19.2	23.8	.376
Nonsmokers, %	73.6	85.0	85.0	69.2	57.1	.376
Alcohol consumption (weekly)						
0 or infrequently, %	25.3	25.0	50.0	18.1	4.8	.134
1–5, %	40.2	45.0	40.0	26.9	52.4	.134
6–10, %	26.4	25.0	10.0	38.5	28.6	.134
≥11, %	8.0	5.0	0	11.5	14.3	.134
Illicit drug use						
None, %	66.7	60.0	85.0	61.5	61.9	.334

Table 1. Continued

Information	Sample	Lesbian/Bisexual Women	Heterosexual Women	Gay/Bisexual Men	Heterosexual Men	*p*
Occasional (monthly or annually), %	24.1	35.0	5.0	30.8	23.8	.334
Regular (daily or weekly), %	9.2	5.0	10.0	7.7	14.3	.334
Interpersonal						
Single, %	72.1	60.0	80.0	80.8	65.0	.315
Children, %	4.7	10.0	5.0	0	5.0	.463
Siblings, %	86.0	95.0	85.0	80.8	85.0	.578
Parents alive, %	94.2	95.0	90.0	92.3	100.0	.559
Infrequent family gatherings, %	36.1	30.0	30.0	57.7	20.0	.169
Nonreligious/spiritual, %	78.8	76.5	84.2	84.0	68.4	.569

CAD, Canadian dollar.

[a]Sexual orientation was ascribed using subscales from the Klein Sexual Orientation Scale including 1 (other sex only), 2 (other sex mostly), 3 (other sex somewhat more), 4 (both sexes equally), 5 (same sex somewhat more), 6 (same sex mostly), and 7 (same sex only).

[b]Self-rated health, physique, and diet included 1 (poor), 2 (fair), 3 (good), 4 (very good), and 5 (excellent).

developmental process in the lives of LGB individuals and informed by our previous findings related to diurnal cortisol and psychiatric symptoms (29), we controlled for disclosure status as a proxy of sexual minority stress in our main cortisol analyses.

Self-Esteem

Personality traits represent important covariates to include in investigations assessing sexual orientation and disclosure (31) and can further modulate HPA axis functioning (32). Our group is particularly interested in the role self-esteem or global worth has on stress responsivity. Previous studies reveal that participants with low self-esteem experience increased cortisol levels in response to repeated exposure to the TSST (33), induced failure during single exposure to a mentally challenging task (34), and exposure to the Montreal Imaging Stress Test (35,36).

As reported in our Supplemental Methods and Materials section in Supplement 1, self-esteem is also associated with gender roles. Self-esteem was positively correlated with masculinity for women ($p = .030$) and men ($p = .002$) together but only significantly so among gay/bisexual men ($p = .010$) when delineated further according to subgroups. Given these preliminary associations with gender roles and documented influence on cortisol stress reactivity, we controlled for self-esteem in main analyses.

Stress Reactivity Paradigm

Exposure to a modified version (27,28) of the TSST occurred during a 2-hour afternoon visit to our laboratory (17). After a 10-min anticipation phase, participants were led to a separate room where they were asked to perform a 5-min mock job interview followed by 5 min of mental arithmetic in front of an unseen, ostensible behavioral expert seated behind a one-way mirror. The participant and the "behavioral expert" communicated via an intercommunication device, and the participant's performance was recorded by a video camera. A seminal meta-analysis (18) posits that laboratory-based stressors eliciting social-evaluative threat include evaluative audiences, negative social comparisons, or recorded performance that maximize HPA axis reactivity.

Previous studies by our group demonstrated that placing the evaluative audience behind a one-way mirror ("panel-out") further maximizes between-sex differences in cortisol stress reactivity. Specifically, men exposed to this type of the TSST show no significant differences in HPA axis reactivity compared with the standard performance in front of the audience ("panel-in") (27). In contrast, heterosexual women exposed to the "panel-out" condition show decreased cortisol stress reactivity compared with heterosexual women in the "panel-in" condition (28). Another study of heterosexual women in the "panel-out" condition also reported comparable decreased cortisol reactivity (37). Taken together, these studies confirm that heterosexual women show significantly reduced reactivity in the "panel-out" condition perhaps because of a minimization of visual social-evaluative threat that appears to be more salient for them. We used this modified TSST in the present study to maximize sex or gender differences in stress reactivity.

Visit Order

The order of visits was counterbalanced randomly to manipulate experienced novelty of the testing environment (38). In the first group (morning/afternoon; $n = 49$), participants received a blood draw in the morning during their first visit and were exposed to the TSST in the afternoon during their second visit about 1 week later; this order was reversed for the second group (afternoon/morning; $n = 37$). Because the second group arrived for the first time to our laboratory when exposed to the TSST, we expected that they would be more distressed than the first group, who had already familiarized themselves with the setting. Given that novelty to testing environments can be appraised as stressful (38), preliminary analyses assessed whether visit order modulated endocrine functioning.

Endocrine Measures

To determine cortisol concentrations before and after TSST exposure, 10 saliva samples were collected at 10-min intervals. The first of these saliva samples was reused to assay salivary sex hormones used as sex-specific covariates: estradiol and progesterone (transformed into an estradiol-to-progesterone ratio) for women and testosterone for men. Our

goal here was not to explore sex hormones as determinants of sexual orientation (39,40) but rather to account for potential confounding of stress reactive cortisol secondary to biological sex–based differences in our sample. Refer to the Supplemental Methods and Materials section of Supplement 1 for details on saliva collection, storage, assaying, and justification for controlling for salivary estradiol-to-progesterone ratios in women and testosterone in men.

Statistical Analyses

To assess group differences in descriptive information reported in Table 1, we employed univariate analysis of variance, Tukey post-hoc analysis, and χ^2 tests. With the exception of preliminary analyses reported in the Supplemental Results section of Supplement 1, our main analyses were stratified by sex given our focus on within-sex (gendered sexual orientation) differences in stress reactive cortisol. Mixed-design repeated-measures analysis of covariance was run with sexual orientation entered as the between-subject factor and time entered as the within-subjects factor for 10 repeated measures of cortisol. Main analyses adjusted for predetermined covariates included sex hormones (estradiol-to-progesterone ratio for women and testosterone for men), age, disclosure status, and self-esteem. Greenhouse-Geisser corrections are reported whenever Mauchly tests denoted violations in sphericity.

RESULTS

Sample Characteristics

See Supplement 1 for complete Supplemental Results concerning sample characteristics reported in Table 1. To summarize, groups differed according to sexuality only as expected (all $p < .001$); heterosexual women were more likely than lesbian/bisexual women to be using oral contraceptives ($p = .017$).

Subjective Distress

Sex and sexual orientation groups did not differ for psychological distress 1) within the last month, 2) on arrival to the laboratory for testing, or 3) in response to the TSST. See the Supplementary Methods and Materials and Supplemental Results sections of Supplement 1 for more information.

Preliminary Analyses

Potential confounders of cortisol stress reactivity were first scrutinized in preliminary analyses using repeated-measures analysis of covariance as a function of visit order (morning/afternoon, $n = 49$; afternoon/morning group, $n = 37$), menstrual cycle status (follicular, $n = 20$; luteal, $n = 20$), and oral contraceptive use (users, $n = 14$; nonusers, $n = 26$). No between-subject differences were detected as a function of visit order for cortisol among women ($p = .86$) or men ($p = .10$). Among women, no between-group differences were found as a function of menstrual cycle ($p = .60$) or oral conceptive use ($p = .44$). Despite these nonsignificant effects, we included reproductive covariates among women to account conservatively for any potential interactions in confirmatory analyses.

See Supplement 1 for all preliminary cortisol analyses focused on between-sex differences. To summarize, we confirmed a group difference in stress reactive cortisol whereby heterosexual men showed stronger cortisol reactivity than heterosexual women; however, this association was nonexistent when contrasting LGB individuals.

Main Analyses

To explore within-sex variations, main analyses were split by sex for repeated-measures analysis of covariance with cortisol concentrations entered as the within-subjects factor and sexual orientation entered as the between-subjects factor. To account for individual differences that can confound cortisol stress reactivity, we controlled for sex hormones (estradiol-to-progesterone ratios for women and testosterone for men), age, disclosure status, and self-esteem.

Among women (Figure 1A), within-subject results revealed a time by sexual orientation interaction effect [$F_{2.64,87.06} = 3.265$, $p = .030$, $\eta^2_P = .090$]. We decomposed this post-hoc using separate one-way analysis of variance: lesbian/bisexual women had significantly higher cortisol concentrations 40 min after TSST exposure compared with heterosexual women [$F_{1,34} = 5.157$, $p = .030$, $\eta^2_P = .132$]. No between-subjects results were detected for sexual orientation ($p = .465$), age ($p = .824$), self-esteem ($p = .496$), or disclosure status ($p = .701$). By contrast, a significant covariation effect for estradiol-to-progesterone ratio [$F_{1,33} = 5.598$, $p = .04$, $\eta^2_P = .145$] was detected and is decomposed further in the Supplemental Results section of Supplement 1. Briefly, increased estradiol and progesterone concentrations were associated with increased HPA axis systemic output only among lesbian/bisexual women (Supplement 1). Additional confirmatory analyses controlling for oral contraceptive use and menstrual status showed no change in statistical significance.

Among men (Figure 1B), between-subjects results revealed an overall group difference as a function of sexual orientation [$F_{1,40} = 4.157$, $p = .048$, $\eta^2_P = .094$], with gay/bisexual men showing comparatively lower overall cortisol concentrations than heterosexual men throughout testing. To explore these differences across time further, we ran post-hoc tests using one-way analysis of variance: groups differed significantly 10 min [$F_{1,41} = 4.315$, $p = .044$, $\eta^2_P = .095$] and 20 min [$F_{1,41} = 4.16$, $p = .048$, $\eta^2_P = .092$] after TSST exposure. No main or interaction effects were found for time ($p = .362$), time by testosterone ($p = .763$), time by age ($p = .098$), time by self-esteem ($p = .197$), time by disclosure status ($p = .712$), or time by sexual orientation ($p = .527$). Between-subjects covariation effects were significant for disclosure status [$F_{1,40} = 4.511$, $p = .040$, $\eta^2_P = .0101$], trending for age [$F_{1,40} = 3.933$, $p = .054$, $\eta^2_P = .090$], but were not significant for self-esteem ($p = .202$) or testosterone concentrations ($p = .399$).

DISCUSSION

The present study assessed whether LGB individuals differ from heterosexual individuals in terms of endocrine stress

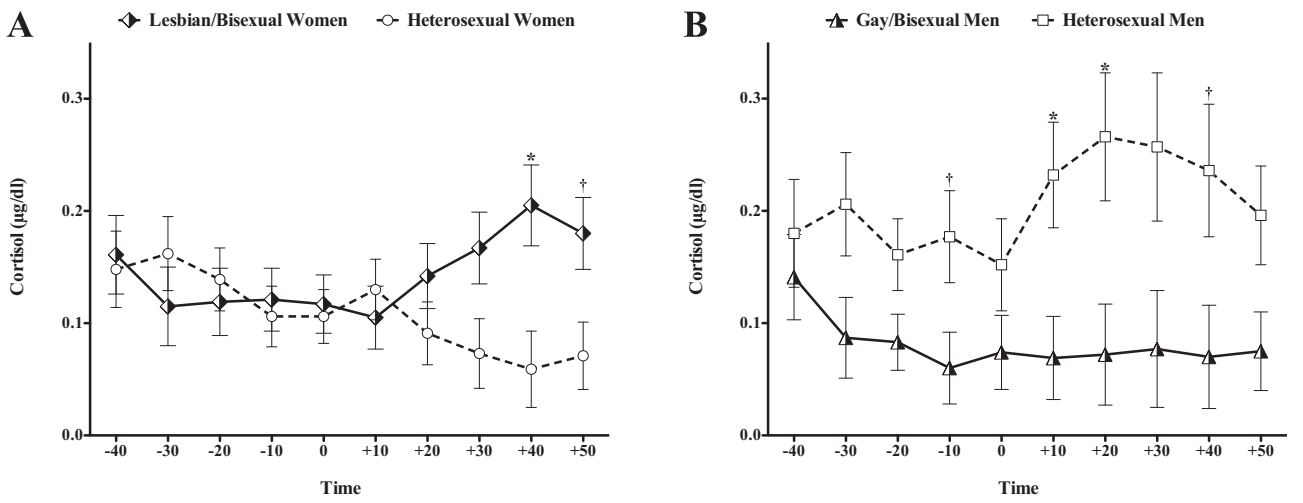

Figure 1. Estimated mean (± SE) salivary free cortisol concentrations in response to the Trier Social Stress Test among **(A)** women ($n = 40$) and **(B)** men ($n = 46$) as a function of sexual orientation. Values are adjusted for sex hormones (estradiol-to-progesterone ratio for women and testosterone for men), age, self-esteem, and disclosure status. $^{*}p < .05$; $^{\dagger}p < .10$.

reactivity. Our results reveal that sexual orientation modulates free cortisol dynamics in distinct gender-based patterns. Although lesbian/bisexual women had higher concentrations of stress reactive cortisol 40 min after exposure to a stressor compared with heterosexual women, gay/bisexual men showed overall lower cortisol concentrations compared with heterosexual men as well as specifically 10 min and 20 min after stress exposure while controlling for important confounders such as sex hormone concentrations. These findings using objective biomarkers are in stark contrast to the lack of group differences in subjective distress. To the best of our knowledge, this is the first study to examine sexual orientation and biological sex differences in HPA axis reactivity to a laboratory-based stressor.

Our novel findings contribute significantly to ongoing debates in the stress literature on the relative influence of biological sex versus sociocultural gender as modulators of endocrine stress reactivity. Kirschbaum *et al.* (3) first demonstrated >2 decades ago that men mount a 2-fold greater cortisol response compared with women; women show further attenuation when using oral contraceptives (41) and during the high estrogen (follicular) phase of their menstrual cycles (42). These differences in bioactive cortisol activity (43) are partly due to the estrogen-induced changes in cortisol-binding globulin (42). Despite few subsequent studies that have assessed sex hormones as part of the TSST paradigm, these early studies provided compelling evidence that sex hormone variations might modulate age-specific sex differences in HPA axis reactivity. By incorporating a gender-based approach, we found that sexual orientation modulates within-sex variations in endocrine stress reactivity beyond variations attributable strictly to reproductive functioning.

Among women, we found that lesbian/bisexual women manifested peak cortisol concentrations late during recovery from the TSST. Specifically, peak levels were attained 40 min after the TSST rather than the typical peak at 10–20-min (18). Although speculative, this delayed peak could be indicative of ruminative processes; this would be consistent with reports by Hatzenbuehler *et al.* (44–46), who showed that lesbians and gay men are more ruminative than heterosexual individuals in response to stigma-related stressors. Rumination is also associated with delayed cortisol recovery after a stressor (47). Ruminative cognitive-behavioral processes were not assessed in our study, and this represents an avenue for future inquiry. Distinct temporal patterns in peak HPA axis reactivity might be driven by elusive processes related perhaps to biological sex in interaction with sociocultural gender that we did not identify here. For example, estradiol and progesterone were positively associated with HPA axis output only among lesbian/bisexual women (Supplement 1), representing an avenue to explore further in future studies.

In contrast to findings among women and consistent with a gender-based reversal in male-typical HPA axis reactivity, we observed lower overall cortisol concentrations throughout testing among gay/bisexual men relative to heterosexual men. From a sexual minority stress perspective (12) and in light of findings from Hatzenbuehler and McLaughlin (21) showing a blunted cortisol response among LGB young adults exposed to high structural stigma environments as adolescents, this observation suggests that gay/bisexual men may be displaying HPA axis down-regulation. There is an expanding literature on hypocortisolism as it relates to severe stressors early in development (22,24) or in the face of traumatic experiences (48), which are ubiquitous among gay/bisexual men (11). The functional significance of this blunted cortisol stress reactivity to the TSST must be delineated further because it is unclear whether this profile represents adaptive or maladaptive processes among gay/bisexual men.

Alternatively, lower cortisol responses among gay/bisexual men in our study may indicate a psychoneuroendocrine resistance to social-evaluative threat. A previous report using the current sample (29) revealed that compared with heterosexual men, gay/bisexual men showed unexpectedly lower depressive symptoms and lower allostatic load levels representing the multisystemic "wear and tear" of chronic stress indexed with numerous neuroendocrine, immune, metabolic,

and cardiovascular biomarkers (49–52). Perhaps young gay/bisexual men who are able to overcome stigma successfully develop adaptive coping strategies that protect against stress reactivity, physiologic dysregulations (allostatic load), and psychopathology. Despite this possibility and the consistent direction of our findings that triangulate methodologies, we cannot conclude that gay/bisexual men are demonstrating resilient pathways owing to our cross-sectional design. Only longitudinal studies would allow insights into the developmental mechanisms involved in differential cortisol reactivity among diverse sexual orientations and trajectories toward vulnerability or resilience to stress-related pathophysiology.

The present study has some limitations. Beyond our cross-sectional design, our study was limited by a small self-selected sample and restricted generalizability. By combining the LGB sample to increase power, we may have obscured important nuances between different LGB subgroups. In particular, given that 62% of the LGB women in our sample were bisexual, the current findings may be more specific to bisexual women. Bisexual individuals are believed to experience the highest risk of psychopathology compared with individuals in other sexual orientation subgroups because of multiple sources of stigma (53). In addition, very few bisexual men participated, rendering our results more applicable to gay men. Differences among specific LGB groups intersect according to age cohorts, race and ethnicity, socioeconomic status, culture, and geographic location (11), which should be explored further in the stress reactivity literature more broadly. For instance, known race and ethnicity differences in stress reactivity may be amplified for members of double minorities, who may experience distinct forms of gender-based stress.

In the context of our nonprobability sample, our results do not generalize to international LGB communities. Specifically, Montreal is a liberal city, and Canadian social policies are progressive. This environment could have inadvertently generated a selection bias in recruitment of participants and may not generalize to the realities of more marginal LGB communities in more conservative locations. Cross-cultural studies could provide important comparisons to increase generalizability.

Methodologic differences are also important to consider when contrasting TSST permutations. Hatzenbuehler and McLaughlin (21) used a modified TSST in which participants discussed an event where they were rejected or discriminated against because of their sexual orientation. This variation underlines identity-relevant aspects that differ from the performance-relevant aspects of the traditional TSST consisting of a mock job interview and mental arithmetic that we employed. Cross-study comparisons are difficult. It was previously shown that men may be more distressed by achievement-based stressors such as mathematics, whereas women may be more sensitive to affiliation-based stressors such as social rejection (16). Future studies could use several stressor paradigms to delineate sex and gender nuances among diverse sexual orientations further.

Special mention of reproductive factors is warranted. Despite our adjustment for sex hormone concentrations for both sexes, this does not preclude potential interaction with other unmeasured biomarkers of HPA axis regulation (e.g., cortisol-binding globulin, adrenocorticosteroid hormone), which should be assessed further in subsequent studies. Our reuse of stored saliva may represent a conservative underestimate because sex hormones are much more sensitive to collection and storage procedures than other salivary analytes such as cortisol that do not degrade as rapidly (54).

A covariation effect for estrogen and progesterone concentrations between women provides food for thought for future inquiry. As described in the Supplemental Results section of Supplement 1, we found that HPA axis systemic output throughout testing was positively correlated to both estradiol and progesterone concentrations only among lesbian/bisexual women. This finding suggests that time-dependent HPA axis patterns may diverge among sexual orientations in synergy with sex hormone modulation. Although beyond the scope of the present analysis, repeated measurement of sex hormones collected throughout the TSST would provide insights into dynamic interactions beyond covariation effects.

In conclusion, our novel findings underline the importance of measuring sexual orientation in studies of endocrine stress reactivity. Although our findings are preliminary and further research is required, this study is responsive to recent calls for greater attention to the ways in which stress related to minority status and other social exposures affect physiologic functioning among LGB populations (13). Incorporating biological stress paradigms into psychosocial studies of LGB populations opens up new avenues for interdisciplinary research identifying the causes of health disparities related to sexual orientation. More generally in the context of stress reactivity research, this study provides new evidence that sociocultural gender modulates cortisol dynamics otherwise attributed primarily to biological sex differences.

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