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ORIGINAL RESEARCH REPORT

Circulating cortisol levels after exogenous cortisol administration are higher in women using hormonal contraceptives: data from two preliminary studies

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Abstract

Exogenous cortisol administration has been used to test the influence of glucocorticoids on a variety of outcomes, including memory and affect. Careful control of factors known to influence cortisol and other endogenous hormone levels is central to the success of this research. While the use of hormonal birth control (HBC) is known to exert many physiological effects, including decreasing the salivary cortisol response to stress, it is unknown how HBC influences circulating cortisol levels after exogenous cortisol administration. To determine those effects, we examined the role of HBC on participants' cortisol levels after receiving synthetic cortisol (hydrocortisone) in two separate studies. In Study 1, 24 healthy women taking HBC and 26 healthy men were administered a 0.1 mg/kg body weight intravenous dose of hydrocortisone, and plasma cortisol levels were measured over 3 h. In Study 2, 61 participants (34 women; 16 were on HBC) received a 15 mg hydrocortisone pill, and salivary cortisol levels were measured over 6 h. Taken together, results from these studies suggest that HBC use is associated with a greater cortisol increase following cortisol administration. These data have important methodological implications: (1) when given a controlled dose of hydrocortisone, cortisol levels may increase more dramatically in women taking HBC versus women not on HBC or men; and (2) in studies manipulating cortisol levels, women on hormonal contraceptives should be investigated as a separate group.

Keywords

Birth control, glucocorticoids, HPA axis, hydrocortisone, intravenous, oral, sex differences

History

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Introduction

Glucocorticoid (GC) effects on physiological and psychological outcomes are important from basic research and clinical perspectives. For example, a strong body of research elucidates the complex effects of GCs on learning and memory (de Kloet et al., 1999; Wolf, 2008), findings that have important implications for the origins and treatment of psychopathology (e.g. de Quervain & Margraf, 2008).

As part of this research, it is necessary to identify moderating factors, such as sex and medications, which alter cortisol's bioavailability and psychological effects. Hormonal contraceptives/hormonal birth control (hereafter abbreviated HBC) is known to affect responses to laboratory stress (Kirschbaum et al., 1999; Nielsen et al., 2013). Women taking HBC have distinct HPA axis signatures compared to those not taking HBC, including lower cortisol awakening responses (Pruessner et al., 1999); a lower salivary cortisol response to psychosocial stress (Kirschbaum et al., 1995); a blunted cortisol response to physical exercise (Bonen et al., 1991; Kirschbaum et al., 1996); greater glucocorticoid sensitivity to pro-inflammatory cytokines after acute stress (Rohleder et al., 2003); and altered circadian cortisol (Bouma et al., 2009; Pruessner et al., 1997; Reinberg et al., 1996, but refer to Wust et al., 2000). For example, women taking HBC who were administered prednisone or dexamethasone had higher serum cortisol levels (Nickelsen et al., 1989) and greater cortisol suppression (Seidegård et al., 2000). Cognitive differences have also been revealed. For example, Kuhlmann & Wolf (2005) found that the effects of hydrocortisone administration on memory retrieval differed for women on versus off HBC (also see Nielsen et al., 2011, 2013). Taken together, these findings suggest that HBC affects the bioavailability and effects of cortisol.

One approach that researchers have employed to study the effects of GCs in humans is to use environmental manipulations, such as the Trier Social Stress Task (Kirschbaum et al., 1993) or the Cold Pressor task (e.g. Bentz et al., 2013; Nielsen et al., 2013). These studies are essential for discovering the effects of endogenously generated cortisol in response to psychological stress. However, it is sometimes advantageous to study the GC effects in the absence of

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emotional arousal due to stress, and to standardize the cortisol dose. For these reasons, researchers administer synthetic cortisol (hydrocortisone) or other GCs to evaluate cortisol's influence on a host of phenomena including memory formation (e.g. Abercrombie et al., 2011; Lupien & McEwen, 1997; Wolf, 2008).

No study, to the best of our knowledge, has investigated whether HBC use alters cortisol levels achieved after exogenous cortisol administration. We hypothesized that cortisol levels after cortisol administration would differ for women on versus off HBC, as well as for women on HBC versus men. Specifically, we hypothesized that HBC would result in greater cortisol increases, based on past research demonstrating that HBC use is associated with elevated levels of other exogenously administered GCs (e.g. Seidegård et al., 2000). To test our hypotheses, we reanalyzed plasma and salivary cortisol data from two studies involving acute intravenous (IV) and oral cortisol administration, respectively (Abercrombie et al., 2011; Wirth et al., 2011). Study 1 included women on HBC and men, although Study 2 included women on HBC, women not taking HBC, and men.

Study 1 methods

Participants

Male and female participants aged 18–35, in good health and fluent in English, were recruited from the University of Wisconsin campus and the local community. Participants were screened for inclusion in the study by phone. For logistical reasons unrelated to the present hypotheses, men and women using HBC were included (Kirschbaum et al., 1999; Wirth et al., 2011). The majority (60%) of women were using a monophasic progestin/estrogen (e.g. Yasmin) while the remaining women were taking a triphasic progestin/estrogen (e.g. Ortho Tri-Cyclen). All study sessions were scheduled so that neither drug administration session fell within the HBC ''placebo'' week. Exclusion and inclusion criteria are described in more detail in Wirth et al. (2011). Fifty-four participants were enrolled in this study. Two participants served as pilots for a higher dose of hydrocortisone and are not included in the present analyses. Four individuals did not complete all parts of this study. Data from two additional participants was dropped due to failure to comply with instructions. Therefore, 46 participants were included in analyses: 22 men (Mean [SEM] Age: 21.8 [0.8]; BMI: 25.3 [0.6]) and 24 women (Age: 22.4 [0.7]; BMI: 22.9 [0.6]).

Study participants refrained from food, caffeine and vigorous exercise for 2 h prior to each study session, as these factors can affect cortisol levels (Hansen et al., 2008; Kirschbaum et al., 1992; Nicholson, 1989). Participants also refrained from alcohol intake for 24 h prior to Session 1 until completion of Session 2.

Procedures

Each participant received both hydrocortisone (i.e. synthetic cortisol; identical to the endogenous hormone) and placebo, in separate sessions 48 h apart. Participants completed hydrocortisone and placebo sessions in randomized order, and drug order was double-blinded. Blood samples were collected to assess plasma cortisol at 11 time points; three samples were collected before drug administration and seven were collected after administration.

Study sessions took place at the Clinical and Translational Research Core (CTRC) at the University of Wisconsin Hospital. All procedures received prior approval from the University of Wisconsin Health Sciences Institutional Review Board; participants provided informed consent, and were paid for their participation. Each of the two study sessions began at 1600 h to minimize circadian variation in cortisol. In each session, Participants received 0.1 mg/kg body weight IV hydrocortisone or physiological (0.9%) saline placebo, administered over 30 min using a programmed pump. Notably, this dose of hydrocortisone produced plasma cortisol levels that were somewhat higher than those caused by a moderate stressor, such as public speaking (Kirschbaum et al., 1993), but still within the physiological range (Cydulka & Emerman, 1998; Fry et al., 1991). Since the dose was calibrated to body weight, women received a significantly smaller dose than men, receiving on average [SEM] 6.29 [0.16] mg versus men's 8.15 [0.24] mg (Wirth et al., 2011). Blood samples continued to be collected at regular intervals throughout the study sessions, which lasted 5 h total, ending at 2100 h. Other study procedures, not directly relevant to this investigation, are detailed in Wirth et al. (2011).

Sample processing and cortisol analysis

Blood samples were centrifuged to extract plasma, which was aliquoted and frozen at -80°C until further analysis. All cortisol assays were performed using Coat-A-Count radioimmunoassay (RIA) kits purchased from Siemens Healthcare Diagnostics (Duluth, GA). Average inter-assay coefficient of variation (CV) across all assays was 5.9% and average intra-assay CV was 4.0%. Siemens Healthcare Diagnostics reports a lower limit of detection of 0.2 mg/dl for their Coat-A-Count cortisol RIA kits.

Study 1 data analysis

We calculated area under the curve with respect to increase (AUCi; Pruessner et al., 2003) to characterize cortisol elevations after drug infusion. AUCi only reflects the *increase* in plasma cortisol in response to the infusion, i.e. the amount of cortisol increase above each person's baseline levels. Therefore, by using AUCi rather than AUCg (or a simple peak or average of the cortisol values), we control for baseline (pre-infusion) differences in cortisol levels that we found between men and women (women having higher baseline cortisol, p's < 0.03). We calculated AUCi from the pre-infusion sample to the second-to-last sample of the study sessions, Sample 10. We did not include the final sample, Sample 11, as five participants had missing data for their final placebo session sample (also, men's cortisol levels had returned to baseline by Sample 10). A t-test was used to evaluate whether a sex difference existed in cortisol AUCi.

Study 1 results

A pairwise t-test was used to examine whether there was a difference by sex in cortisol increase, after hydrocortisone

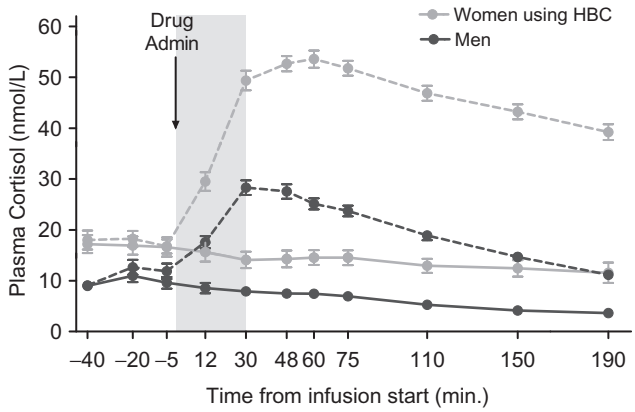

Figure 1. *Plasma cortisol after hydrocortisone (IV, 0.1 mg/kg) or placebo administration*. Plasma cortisol levels are measured in nmol/L. Dashed lines represent plasma cortisol levels during the session in which hydrocortisone (0.1 mg/kg infused over 30 min) was administered. Solid lines represent plasma cortisol levels during the session in which placebo (0.9% saline) was administered. The gray bar indicates the time of drug infusion (0–30 min). Area under the curve increase (AUCi) in plasma cortisol levels was significantly greater in women, all of whom were taking HBC, versus men following hydrocortisone administration ($t[42] = 10.65$, $p < 0.001$). This difference was not found following placebo infusion.

infusion, using AUCi as the dependent variable.[1] Results indicated a significant sex difference in AUCi, $t[42] = 10.65$, $p < 0.001$, 95% CI [2269.890–3331.874], with higher AUCi in women [M: 4016.58, SD: 1034.49] compared to men [M: 1215.70, SD: 672.99]. Notably, this difference was not found after placebo infusion, $t[42] = -0.973$, $p = 0.336$, 95% CI [−410.938 to 143.602]. Thus, women's total plasma cortisol rose significantly higher than men's following cortisol infusion, even though the sexes did not differ in cortisol AUCi on the day that they received placebo (Figure 1).

Results from Study 1 indicate a sex difference in plasma cortisol following an IV infusion of cortisol (hydrocortisone), even though the dose was tailored to each participant's body weight. Although we suspect that HBC use was at least part of the cause of this difference, since all the women in Study 1 were taking HBC, we cannot distinguish from this study alone whether the increased cortisol in women was due to sex or due to HBC use. Study 2 remedies this issue, as both women using HBC and women not using HBC were included, as well as men.

Study 2 methods

Participants

Twenty (11 women) un-medicated participants with depression symptoms and 45 (24 women) never-depressed healthy participants were enrolled in this study. Participants were recruited from the community, and screened with the Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) and the SCID (First et al., 2002). One participant was excluded due to structural brain abnormalities; one was excluded due to abnormally high salivary cortisol levels following hydrocortisone administration (possibly due to chewing the orally administered capsule); and two were excluded due to experimenter error. Altogether, then, our final sample for the present analyses includes 61 participants: 27 men and 34 women (16 taking HBC). All potential participants received a drug screen, and women were required to test negative on a pregnancy test before participation. Other inclusion and exclusion criteria are described in Abercrombie et al. (2011). Mean [SEM] age and BMI, respectively, for the three groups were as follows: women not taking HBC, 30.00 [2.57] and 25.86 [1.42]; women taking HBC, 23.06 [1.17] and 24.66 [1.12]; and men, 27.17 [1.54] and 26.48 [0.80]. Participants in the three groups did not significantly differ in age or BMI ($p > 0.05$ in all ANOVAs). The University of Wisconsin-Madison Health Sciences Institutional Review Board approved all study procedures. Participants provided written informed consent and were paid for their participation.

Of the 16 women using HBC, 63% (10 women) were prescribed a monophasic progestin/estrogen and the remaining women were taking a triphasic progestin/estrogen. We were unable to control for menstrual phase (in women not taking HBC) or pill cycle phase (for women on HBC) due to a National Institutes of Health stipulation that individuals with depressive symptoms participate in the study within 2 weeks of screening, so as not to require more than 2 weeks without mental health treatment while in the study. The same criteria were applied to individuals who did not endorse depressive symptoms, for consistency. Therefore, those not taking HBC are mixed with regard to menstrual phase; similarly, women on HBC could have been in any stage in their monthly pill cycle.

Procedure

Eligible participants completed an fMRI simulation session, two fMRI scanning sessions, and a memory testing session, to test separate hypotheses (Abercrombie et al., 2011). The fMRI scanning sessions involved cortisol or placebo administration and saliva sampling for cortisol measurement, so only those sessions are detailed here. A repeated-measures design was used in which subjects received cortisol in one scanning session and placebo in the other, with randomized order. Both sessions began between 1630 h and 1730 h, and the two sessions were spaced 48 h apart. Notably, participants were already acclimated to the scanning environment, using a mock scanning session (fMRI simulation), prior to the sessions when cortisol or placebo administration took place; hence, the fMRI environment was unlikely to cause stress due to novelty, etc. In fact, evidence suggests that if participants have even one single previous exposure to fMRI, they show no cortisol response to a subsequent fMRI experience (Tessner et al., 2006). Further details regarding the memory and fMRI procedures may be found in a previously published manuscript (Abercrombie et al., 2011). Each session lasted approximately 2.75 h. Participants refrained from eating and exercise within 90 min of all sessions. After providing an initial saliva sample, participants were administered an oral dose of 15 mg hydrocortisone (i.e. cortisol) or placebo.

[1]Using AUCg as the dependent variable, there was also a significant sex difference after hydrocortisone infusion, $t[42] = 14.24$, $p < 0.001$, CI: 3052.95 to 4061.05, with higher AUCg in women [M: 6613.74, SD: 951.24] compared to men [M: 3056.74, SD: 683.80].

Salivary cortisol measurement

Participants provided a total of six saliva samples to examine salivary cortisol levels throughout the study sessions and in their homes after the sessions, using the Salivette saliva collection device (Sarstedt, Newton, NC). One saliva sample was provided 5 min before hydrocortisone or placebo administration and five additional samples were provided 20, 95, 130, 200 and 375 min after administration. Saliva samples were stored frozen until they were assayed using a chemiluminescence assay, which has a high sensitivity of 0.16 ng/mL (IBL-International, Hamburg, Germany). Both intra- and inter-assay CVs were below 6%.

Study 2 data analysis

Similarly to data analysis in Study 1, we first tested whether there were differences between groups in baseline salivary cortisol levels – in other words, before the hydrocortisone or placebo treatment. An initial mixed ANOVA comparing the three groups' (women using HBC; women not on HBC/free-cycling women; men) baseline (Sample 1) cortisol revealed neither any significant between-group differences ($F[1,119] = 0.000$, $p = 0.994$), nor were there any differences when comparing pre-hydrocortisone Sample 1 cortisol with the pre-placebo cortisol Sample 1 within each group (Men: $F[1,52] = 0.000$, $p = 0.984$; HBC Women: $F[1,30] = 0.472$, $p = 0.497$, Control Women: $F[1,33] = 1.759$, $p = 0.194$). Also, importantly, there were no significant differences in baseline cortisol between participants with depression symptoms and those without, in any of the three groups (Men: $F[1,52] = 0.364$, $p = 0.549$; HBC Women: $F[1,30] = 3.001$, $p = 0.093$; free-cycling women: $F[1,34] = 0.390$, $p = 0.537$). As there were no baseline differences in cortisol levels, the primary analyses used area under the curve with respect to ground (AUCg; Pruessner et al., 2003). Cortisol AUCg was calculated from Sample 2 to Sample 6 (~375 min after administration; Figure 2). Despite no significant difference in baseline cortisol between participants with and without depression symptoms, we controlled for depression symptoms in the ANCOVAs.

Study 2 results

Two global ANCOVAs, one for each session, were conducted with Group as the independent variable, cortisol AUCg as the dependent variable, and controlling for depression status. As expected, the overall ANCOVA on AUCg after placebo was not significant, $F[2,60] = 2.172$, $p = 0.123$, but the overall ANCOVA on cortisol AUCg after receiving hydrocortisone was significant, $F[2,60] = 9.872$, $p < 0.001$. Notably, there was no main effect of depression status on AUCg in either ANCOVA. To follow up on this result, we used three *post-hoc* pairwise ANCOVAs comparing AUCg by group, again controlling for depression. After receiving hydrocortisone, women taking HBC had significantly higher AUCg compared to free-cycling women (i.e. women not on HBC), $F[1,33] = 4.767$, $p = 0.037$, and compared to men, $F[1,42] = 21.013$, $p < 0.001$. In the pairwise ANCOVA, free-cycling women did not have a significantly different AUCg compared to men, $F[1,44] = 1.954$, $p = 0.072$.

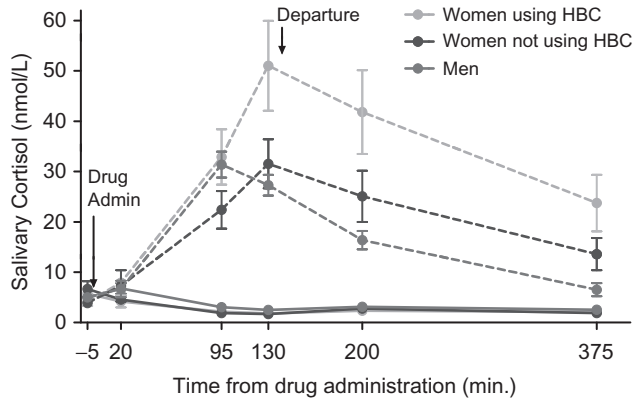

Figure 2. *Salivary cortisol after hydrocortisone (oral, 15 mg) or placebo administration.* Salivary cortisol levels are measured in nmol/L. Dashed lines represent salivary cortisol levels during the session in which hydrocortisone (15 mg capsule) was administered. Solid lines represent salivary cortisol levels during the session in which placebo capsule was administered. The final two saliva samples were collected in the evening at home after departure from the lab. On the hydrocortisone day, salivary cortisol levels at baseline did not differ between groups. However, area under the curve with respect to ground (AUCg) in salivary cortisol levels was greater in women taking HBC compared to men (M = 5107.77, 95% CI [2405.01, 7810.52], $p < 0.001$) or compared to women not on HBC (M = 3629.73, 95% CI [686.27, 6573.18], $p = 0.012$); the latter two groups did not differ. There were no group differences following placebo administration.

Tukey *post-hoc* comparisons were also conducted, which cannot control for the depression covariate, but are a more widely-used form of *post-hoc* test. In these tests, the women taking HBC had a significantly higher AUCg compared to both free-cycling women (M = 3629.73, 95% CI [686.27, 6573.18], $p = 0.012$) and men (M = 5107.77, 95% CI [2405.01, 7810.52], $p < 0.001$). The difference between free-cycling women and men was not statistically significant, $p = 0.366$.

Discussion

In two studies, we generated evidence that HBC use is associated with greater cortisol levels after an exogenous dose of cortisol. In Study 1, women (all of whom were taking HBC) had greater cortisol increases than men after IV cortisol, despite the fact that the dose of cortisol was calibrated by weight. Based on Study 2 findings, we believe this result was at least partly driven by the women's HBC use, but we cannot rule out the possible role of sex differences, e.g. in cortisol metabolism. In Study 2, women on HBC had a greater increase in cortisol after a moderate oral dose of cortisol compared with women not using HBC or men. No such differences between these groups were found in cortisol output after placebo administration; in other words, unstimulated cortisol over time did not differ as a function of sex or HBC status. Importantly, salivary cortisol was measured in Study 2, rather than plasma cortisol as in Study 1. Thus, these findings support the idea that women using HBC have greater elevations in free cortisol and potentially also total/plasma cortisol following exogenous cortisol administration.

These findings have important methodological implications. Our findings suggest that the importance of testing and/or controlling for the effects of HBC use in studies

employing cortisol administration to manipulate cortisol levels. Differences in cortisol levels after exogenous cortisol manipulation in women on versus off HBC may partially (or wholly) explain any variations in behavioral or physiological effects of exogenous cortisol observed in women on HBC. In addition, variability in outcome measures after exogenous cortisol in women on versus off HBC could create noise in data sets in which HBC use is not controlled or taken into account.

Our study alone cannot speak to potential mechanisms for our findings. However, we speculate that the effect of HBC to increase cortisol's binding proteins may be one possible mechanism (van der Vange et al., 1990). HBC-induced elevations of the blood proteins that bind cortisol could delay cortisol breakdown and prolong the elevations of the hormone in the blood. Meulenberg et al. (1987) found that HBC use was associated with higher levels of corticosteroid-binding globulins (CBGs). Another study showed that administration of low doses of oral contraceptives increased both corticosteroid- and sex hormone-binding globulins (van der Vange et al., 1990). Presuming that hormone-binding globulins are chronically higher due to HBC use, it is possible that a greater proportion of a dose of exogenous hydrocortisone is bound in participants taking HBC versus participants not on HBC. Consequently, cortisol may not be subject to the normal breakdown and clearance processes as quickly, as higher levels of binding globulins may protect the exogenous cortisol molecules from metabolism, receptor binding, etc., leading to higher, more prolonged, elevation of cortisol.

On the other hand, our findings could be explained by other factors in addition to, or instead of, greater levels of binding globulins slowing breakdown of cortisol molecules. If increased binding globulins were the only mechanism involved, we might expect to see higher total cortisol but lower salivary/unbound cortisol increases in women on HBC. However, this was not the case in Study 2, in which salivary cortisol was measured. In previous work, women taking HBC were found to have a blunted salivary (i.e. free) cortisol response to acute stress (Kirschbaum et al., 1999); Kumsta et al. (2007) found that CBG levels were negatively associated with the salivary cortisol response to the TSST, while CBGs were positively associated with total cortisol levels. Our finding in Study 2 that free/salivary cortisol is also elevated in women on HBC after cortisol administration appears to conflict with these past findings, though it is important to note that endogenous increases in cortisol due to stress are not directly comparable to increases due to exogenous administration. It is also important to note that the relationship between CBG levels and cortisol metabolism is complex. High levels of endogenous versus exogenous cortisol may yield different effects on CBG affinity for cortisol (Schlechte & Hamilton, 2008). Also, there is evidence that binding globulins become saturated less quickly in women on HBC, such that salivary cortisol measurements in women on HBC are indicative of higher total cortisol than equivalent measures in women not taking HBC (Hellhammer et al., 2009). Thus, if anything, our Study 2 salivary cortisol data might underestimate the difference in plasma cortisol between the two groups of women. Finally, HBC affects other metabolic processes as well as binding globulin levels (Cassazza et al., 2004), so there are many possibilities for mechanism(s) behind our findings. As we did not measure binding globulins in these studies, we cannot come to any conclusions yet as to mechanism(s) by which HBC use led to higher cortisol levels.

Cortisol levels after cortisol administration may also depend on the specific type of oral contraceptives that women are taking. Brien (1975) measured human blood plasma concentrations of cortisol, cortisol-resin uptake ratio, and free cortisol in women taking either estrogen/progestogen or progestogen-only HBC. Women taking progestogen-only HBC had levels of cortisol, cortisol reuptake and free cortisol that were similar to controls. However, women taking the combined pill had elevated cortisol and cortisol reuptake ratios in both morning and afternoon (although no change in free cortisol). All women in our studies were using combined HBC. Nonetheless, examining the effects of different types of HBC on the HPA axis will be an important area of future study.

Two findings unrelated to the main hypothesis deserve mention. First, in Study 1, women on HBC had higher baseline cortisol compared to men. Interpretation of this baseline difference is complicated somewhat by the fact that women in Study 1 were tested during their active pill weeks, whereas pill cycle was uncontrolled in Study 2, wherein there were no group differences in baseline levels. Nonetheless, some past research has also found baseline differences in cortisol between women on and off HBC (e.g. Nielsen et al., 2013). In fact, in Nielsen et al. (2013), elevated baseline cortisol in women on HBC was associated with a lack of cortisol response to a laboratory stressor (cold pressor), whereas there was no such association in women not taking HBC. These findings along with ours point to the importance of controlling for HBC use in studies involving laboratory stressors, not only in studies involving exogenous cortisol administration.

A second interesting pattern in our data is that in Study 2, women regardless of HBC use seemed to have a later peak in cortisol after cortisol administration compared to men. Although the relatively long time intervals between the saliva samples means we cannot pin-point the exact time of peak cortisol in any group, men's cortisol was declining by the 130-min time-point, whereas for women in both groups, 130 min was their highest cortisol measurement (Figure 2). We hesitate to draw any conclusions from this pattern, as our saliva sampling was not dense enough to examine peak timing. However, future work should examine potential sex differences in time of cortisol peak, which may or may not depend on hormonal status.

We must acknowledge several methodological limitations with the present research. First, these are *post-hoc* analyses in studies that were not specifically designed to test associations between HBC and cortisol, although we believe these remain valid and important preliminary data to be expanded on in future work. Second, menstrual phase was not controlled in cycling women in Study 2. We believe this is also a concern to be remedied in future research. Third, Study 2 contained participants with depression symptoms, as part of the original study design; however, depression status did not moderate baseline cortisol or levels of the hormone after cortisol administration. Fourth, Studies 1 and 2 used different designs,

and notably, different methods of drug administration and measurement of cortisol levels. Although these methodological differences could be thought to limit the comparability of the studies, the findings from Study 2 expand upon those from Study 1, suggesting that higher cortisol in women taking HBCs is likely due to HBCs, rather than exclusively due to a sex effect. Study 1 also addresses some of the limitations in Study 2, e.g. a less controlled dose of cortisol, and the fact that pill cycle phase was not controlled in Study 2, such that some women may have been tested during their ''placebo'' week. Obtaining similar results in two studies with different methodologies also indicates that this is a robust, replicable finding, worthy of future investigation in studies designed explicitly to test these hypotheses.

Conclusions

Data from two studies suggest that HBC use results in greater increases in cortisol levels after exogenous cortisol administration. In other words, our findings suggest that, when given a controlled dose of hydrocortisone, cortisol levels may increase more dramatically in women taking HBC versus women not on HBC or men. Though further research is needed to confirm our preliminary findings, all researchers administering cortisol to humans should be aware of this issue. If women taking HBC are included in cortisol administration studies, the potential effects of HBC use should be examined in data analyses. Our results also suggest future inquiry into the long-term effects of HBC use on cortisol and other aspects of the HPA system. Research is also needed to examine interactions among steroid binding mechanisms, sex steroids (endogenous as well as exogenous) and cortisol. Such investigations could help shed light on potential individual differences underlying HPA dysregulation in a variety of disorders as well as reactivity to acute and chronic stress.

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Declaration of interest

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