Published in final edited form as:

Metabolism. 2013 December ; 62(12): . doi:10.1016/j.metabol.2013.08.014.

Gender determines ACTH recovery from hypercortisolemia in healthy older humans

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Abstract

Objective—Available clinical data raise the possibility that stress-adaptive mechanisms differ by gender. However, this notion has not been rigorously tested in relation to cortisol-mediated negative feedback.

Materials/Methods.—Degree of ACTH inhibition during and recovery from an experimental cortisol clamp was tested in 20 healthy older subjects (age 60 ± 2.2 y). Volunteers received oral placebo or ketoconazole (KTCZ) to inhibit adrenal steroidogenesis along with i.v. infusions of saline or a low vs high physiological dose of cortisol in a prospectively randomized double-blind, placebo-controlled design. ACTH and cortisol concentrations were measured every 10 min during the feedback-clamp phase and thereafter (recovery or escape phase). Corticosteroid-binding globulin (CBG) was measured, and free cortisol concentrations were calculated.

Results.—Gender did not determine mean ACTH concentrations during the saline or cortisol feedback-clamp phases per se. However, women had markedly impaired ACTH recovery after stopping both low- and high-dose cortisol infusions compared with men ($P = 0.005$, KTCZ/low-dose cortisol arm; and $P = 0.006$, KTCZ/high-dose cortisol arm). Decreased ACTH recovery in women was accompanied by lower total and free cortisol concentrations, pointing to heightened feedback inhibition of hypothalamo-pituitary drive of ACTH secretion as the main mechanism.

Conclusions.—In summary, gender or a factor related to gender, such as sex steroids or body composition, determines recovery of ACTH secretion from cortisol-enforced negative feedback. Attenuated ACTH recovery in post-menopausal women may have relevance to sex differences in stress-related adaptations.

Keywords

Aging; Feedback; Cortisol; Human

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Conflict of interest

The authors have no conflicts to declare.

1. Introduction

Time-delimited stress-adaptive responses of the corticotropic axis are critical to sustain life and regulate key metabolic processes [1]. Hypothalamo-pituitary responses are activated and restrained by feedforward and feedback signals, which together mediate homeostatic ACTH fluctuations in patho-physiological states [2-5]. Albeit crucial to multicomponent regulation in neuroendocrine systems, feedback regulation is less well studied than feedforward drive [6,7]. Moreover, how gender, sex steroids, puberty, hypogonadism and aging supervise feedback effects is largely unknown [8-10]. In particular for the stress axis, whereas gender and sex steroids regulate ACTH secretion strongly in animals, investigations in human subjects are both limited and discrepant [11].

In the rodent, female sex (and estradiol) and male sex (and testosterone) respectively potentiate and attenuate stress responses [1,12,13]. However, in the human, inferences on the role of gender in modulating hypothalamic-pituitary-adrenal (HPA) responses are inconsistent. In part, inconsistencies may reflect the dose and type of feedback signal applied (e.g. long-lived synthetic glucocorticoids or physiological cortisol), as well as confounding interactions among gender, age and the degree and type of stress [5,14-16]. In an initial effort to address these uncertainties, the present clinical investigation examines, in a given age group, how gender impacts (a) feedback inhibition of ACTH secretion by varying systemic cortisol concentrations; and (b) feedforward recovery of hypothalamo-pituitary drive of ACTH secretion during subsequent hypocortisolemia. These issues are important, since the short-term consequences of blunted cortisol/ACTH feedback include elevated glucocorticoid secretion and heightened ACTH responsiveness to CRH and AVP, while the long-term consequences of excessive ACTH/cortisol feedforward include elevated blood pressure, suppressed immune function, impaired insulin action, visceral-fat accumulation, decreased muscle protein and bone mass, and possibly reduced memory and hippocampal neurogenesis [17-20]. Thus, understanding how gender regulates ACTH secretion in older adults should provide insights into stress-adaptive mechanisms in individuals at increased risk of frailty, injury and disability.

2. Materials and methods

2.1. Overview

The basic postulate tested was that older men and post-menopausal women differ in the degree of feedback inhibition of ACTH secretion by, and subsequent escape of ACTH secretion from, controlled intravenous cortisol exposure and withdrawal. This was an institutional review board-approved, prospectively randomized, double-blind, and placebo-controlled crossover study. The investigational protocol was also reviewed by the U.S. Food and Drug Administration and assigned an investigator new drug number, inasmuch as ketoconazole (KTCZ) was used off-label to block adrenal steroidogenesis reversibly [21,22].

2.2. Subjects

Participants were recruited from the community within the allowable age range of 50–80 years. Ten men and ten women were enrolled. Women were postmenopausal by 2 yr or more. No volunteers were receiving hormone replacement. Subjects maintained conventional work and sleeping patterns and reported no recent (within 10 days) transmeridian travel, significant (> 2 kg) weight change, psychosocial stress, substance abuse, neuropsychiatric medications or illness, or systemic disease. There were no concurrent or historical neuropsychiatric diagnoses or related drug treatments, and no disorders of eating, behavior, anxiety, depression or psychosis, when assessed as in earlier cortisol feedback studies [23]. A complete medical history, physical examination, and

screening tests of hematological, renal, hepatic, metabolic and endocrine function were normal.

Volunteers were admitted to the Mayo Center for Translational Science for 4 separate inpatient overnight study sessions, each separated by at least 10 days. The study was double blind, using oral placebo vs KTCZ capsules, and i.v. saline vs cortisol infusions. (We did not try to infuse cortisol under oral placebo, since the levels would then be hard to interpret under high uninhibited cortisol pulses). Overnight infusions and next-morning sampling were chosen to examine gender-related regulation of the strong diurnal rise in ACTH in the cortisol-feedback paradigm. Lights were extinguished at 2000 h. Subjects received either oral placebo tablets or KTCZ pills (600 mg in men and 400 mg in women) at 1800 h. KTCZ was given with a nondairy snack to obviate dyspepsia. The dose was selected in pilot studies to achieve comparable suppression of cortisol in the two sexes. KTCZ (at one-half the dose) or placebo was given again at 2200 h and 0600 h to continue to block adrenal steroidogenesis. Concurrently, either saline (100 mL) or cortisol (as hydrocortisone succinate in saline 100 mL) at a total dose of 2.5 mg/m² (low dose) or 10 mg/m² (high dose) was infused continuously i.v. over 8 h until 0200 h. This created 4 separate cortisol clamps (treatment arms): 1) placebo + saline addback; 2) KTCZ + saline addback; 3) KTCZ + low-dose cortisol (LDC) infusion; and 4) KTCZ + high-dose cortisol (HDC) infusion (Fig. 1). Blood samples were obtained for later measurement of plasma ACTH (1.0 mL) and serum cortisol (0.5 mL) every 10 min for 14 h from 2200 h until 1200 h the next day. Subjects slept through the sampling until 0600 h. Fourteen h of sampling was to monitor ACTH secretion during the last 4 h of the constant 8-h infusion clamp, and for 10 additional h after stopping i.v. cortisol or saline abruptly at 0200 h. At 1200 h, 25 mg hydrocortisone succinate was administered as an i.v. bolus for safety reasons.

2.3. Hormone assays

Plasma ACTH was assayed via a highly sensitive and specific, solid-phase sequential immunoassay (Siemens Healthcare Diagnostics, Deerfield, IL). The detection threshold was 5 ng/L (to convert to SI units, divide by 4.5). The intra-assay coefficient of variations (CV) were 6.4%, 2.7%, and 3.2% at 5.7 ng/L, 28 ng/L, and 402 ng/L, respectively. Cortisol was assayed via competitive binding immunoassay (Beckman Coulter, Inc., Fullerton CA). The detection limit was 0.4 µg/dL (to convert to SI units, multiply by 27.6) and intraassay CVs were 13%, 9.4%, and 6.6% at 1.6 µg/dL, 2.8 µg/dL, and 30 µg/dL, respectively. All samples from a given subject were assayed in the same batch. Sex steroids were assayed by mass spectrometry, and LH, FSH, TSH and prolactin by immuno-chemiluminescence [24,25].

2.4. CBG assay

Serum corticosteroid binding globulin (transcortin, RD192234200R, BioVendor, Asheville, NC) was quantified by ELISA. Microplate wells precoated with a polyclonal CBG antibody are used to capture the analyte, and a biotin-labeled monoclonal human CBG antibody conjugated with streptavidin-horseradish peroxidase is used for the indicator reaction in substrate solution (3',5,5'-tetramethylbenzidine), as described by the vendor [26,27]. The product is a yellow color, wherein absorbance at 450 nm is proportional to the concentration of CBG. Assay range is 3–200 ng/mL with interassay and intraassay coefficients of variation of 2% and 7%, respectively. Dilution linearity and recovery were 98.5% and 100.3%. All samples were run in the same assay.

Measurements of albumin, CBG and total cortisol allowed calculation of free cortisol by Coolens' equation, as modified by Mazer [28,29].

2.5. Statistical analysis

Mean plasma ACTH (and serum cortisol) concentrations served as the primary endpoints. The effects of gender (2 factors) and saline/cortisol feedback-intervention type (4 factors) on the natural logarithm of the outcome measures were evaluated by two-way analysis of covariance (ANCOVA). Log transformation was used to satisfy the Shapiro-Wilks test of normality. To adjust for intrasubject correlations, the mean ACTH (and cortisol) concentration during the placebo/saline arm served as the model covariate. When the covariate was not significant, 2-way ANOVA with partially repeated measures was employed. Post hoc analysis utilized Tukey's Honestly Significantly Different (HSD) test. Responses were segmented into the initial clamp phase (last 4 h of the continuous 8-h saline/cortisol infusion) and the subsequent (10-h) recovery phase. A secondary outcome during the infusion clamp was the feedback slope defined by regressing median 4-h ACTH concentrations on corresponding median 4-h cortisol concentrations in each volunteer based upon his/her four treatments. Slopes in women and men were compared using the Mann-Whitney U test. Similar outcomes were obtained by unpaired t test of the absolute value of the slopes. Another secondary outcome was the slope of the rising and falling portion of a two-segment piecewise regression model relating ACTH concentrations to time over the 10-h recovery period [30]. This permitted objective determination of a 'breakpoint' in time, and thereby separate slopes for the initial rise (recovery before the breakpoint) and delayed fall (recovery after breakpoint) in ACTH concentrations, when cortisol infusions were stopped. Three-way ANCOVA (gender, breakpoint, infusion type) was applied to assess mean ACTH levels in relation to all 3 factors. Escape from suppression (recovery) was confirmed by nonlinear curve-fitting of time-varying (median) ACTH concentrations to a 4-parameter sigmoidal function with 95% confidence intervals. Experiment-wise $P < 0.05$ was construed as significant. Linear regression was used in the group of 20 subjects to test the relationship between ACTH responses and age, T or E₂ levels. For ease of visualization, although P values reflect analysis after log transformation, hormonal data are presented as the mean $\pm$ SEM, median or 95% confidence intervals.

3. Results

All 20 subjects completed all 4 randomized infusions and 14-h sampling sessions. Anorexia and dyspepsia were adverse events. Men and women had comparable age, body mass index (BMI), and screening 0800 h albumin, cortisol, prolactin and TSH concentrations. Testosterone and estradiol were lower and SHBG, LH and FSH higher in women than men, as expected: Table 1. Screening (0800 h) ACTH (ng/L) was 21 ± 1.4 in men and 10.6 ± 0.8 in women ($P = 0.023$), whereas cortisol ($\mu\text{g/dL}$) was 12.5 ± 1.1 in men and 12.5 ± 1.2 in women ($P = 0.97$). To visualize the hormone time patterns, Fig. 2 presents median 14-h ACTH and cortisol concentration profiles for all 4 treatment arms. A validating hypothesis was that KTCZ/saline administration should induce significant hypocortisolemia, thereby unleashing ACTH secretion. This was confirmed by comparing (mean $\pm$ SEM) cortisol and ACTH concentrations over the entire 14-h period in the KTCZ/saline and placebo/saline groups; viz. cortisol: 6.7 ± 0.2 vs 7.9 ± 0.3 $\mu\text{g/dL}$ ($P = 0.007$); and ACTH: 26.7 ± 3.2 vs 16.5 ± 1.9 ng/L ($P = 0.001$) [$N = 20$]. Men and women also did not differ significantly with respect to peak plasma ACTH concentrations over the 14 h: viz., placebo/saline: 43 ± 6.1 (men) and 35 ± 8.2 ng/L (women); or during KTCZ/saline: 77 ± 18 (men) and 62 ± 21 ng/dL (women). Mean and peak cortisol concentrations likewise did not differ by gender over 14 h under the 2 validating clamps (see profiles in Fig. 2, right).

3.1. Saline/cortisol clamp phase

This period was defined as the 8-h interval from the start of saline/cortisol infusion to its discontinuation (1800 h–0200 h), with blood sampling over the last 4 h (2200 h–0200 h).

Linear regression analysis ($N = 20$) disclosed that mean 4-h ACTH concentrations in the control session (placebo/saline arm) significantly determined mean 4-h ACTH concentrations during each of the other 3 main treatment arms, viz. KTCZ/saline [$R^2 = 0.81$; $P < 0.001$], KTCZ/low-dose cortisol (LDC) [$R^2 = 0.84$; $P < 0.001$] and KTCZ/high-dose cortisol (HDC) [$R^2 = 0.54$; $P < 0.001$]. This validated the use of placebo/saline ACTH means as covariates in ANCOVA. By ANCOVA and post hoc Tukey's HSD test, sex did not determine mean ACTH concentrations during any of the 3 KTCZ saline/cortisol clamps (gender $P = 0.98$, gender*treatment $P = 0.29$) (Fig. 3). Gender independence of ACTH concentrations attained during clamped saline/cortisol feedback was confirmed by regression of mean 4-h ACTH on mean 4-h cortisol concentrations in each of the 20 subjects to estimate feedback slopes. Statistical comparison of slopes revealed no significant difference in ACTH-cortisol regression slopes between men and women (-0.57 ± 0.19 [$N = 10$] vs -0.26 ± 0.04 [$N = 10$], $P = 0.45$; Mann-Whitney U test). This was confirmed by unpaired t-test of log-transformed absolute slopes.

3.2. Post-infusion ACTH recovery

Recovery was defined as the 10-h interval between discontinuing the saline/cortisol infusion and cessation of blood sampling (0200 h–1200 h). During 10-h recovery, mean ACTH concentrations on the control day (placebo/saline arm) predicted those on each of the other 3 treatment days, viz. KTCZ/saline ($R^2 = 0.63$; $P < 0.001$), KTCZ/LDC ($R^2 = 0.63$; $P < 0.001$) and KTCZ/HDC ($R^2 = 0.57$; $P < 0.001$), thus justifying the use of ANCOVA (*Methods*). Two-way ANCOVA showed (1) a main treatment/infusion effect ($P < 0.001$), in which the mean ACTH concentration (ng/L) during recovery in the two genders ($N = 20$) was significantly lower in the KTCZ/HDC arm (16 ± 2.2) than in both the KTCZ/saline (31 ± 3.7) and the KTCZ/LDC (29 ± 3.2) arms, but no different from that in the control (19 ± 2.1) arm; and (2) a strong sex effect ($P < 0.001$), in which women as a group had lower 10-h mean ACTH concentration responses across the 3 KTCZ interventions than men (19.2 ± 1.8 ng/L vs 32.3 ± 3.5 ng/L, $P < 0.001$). Post hoc infusion-specific sex comparisons by Tukey's HSD test disclosed significantly lower 10-h mean ACTH recovery in women than men in the KTCZ/HDC arm only (9.0 ± 0.87 ng/L vs 23 ± 3.5 ng/L, $P = 0.045$ by Tukey's HSD test). There was a similar trend-level effect in KTCZ/LDC: mean ACTH in women 18 ± 2.0 and in men 37 ± 6.4 ng/L ($P = 0.062$).

Visual inspection of the 10-h ACTH recovery plots suggested a possibly biphasic sex-dependent pattern, characterized by rapid post-infusion rebound ACTH secretion and delayed ACTH suppression (Fig. 2). To test this hypothesis objectively, two-segment piecewise linear-regression analysis was applied to 10-h ACTH (and cortisol) concentrations in each of the 4 treatment arms (Appendix Figure 1). The breakpoint so defined identifies the time of a significant change in slope of ACTH recovery on time from positive (initially rising ACTH) to negative (delayed suppression of ACTH). Mean ACTH concentrations in the combined genders ($N = 20$ subjects) were significantly higher before than after the breakpoint only in the KTCZ/LDC arm (35 ± 4.2 ng/L vs 23 ± 3.2 ng/L; $P < 0.001$), and KTCZ/HDC arm (21 ± 3.4 ng/L vs 10 ± 1.7 ng/L; $P < 0.001$, by 2-way ANCOVA), thus verifying sequential escape and suppression of ACTH (Fig. 4). Post-breakpoint mean cortisol concentrations were higher than pre-breakpoint values in the KTCZ/LDC arm (8.7 ± 0.3 μ g/dL vs 7.1 ± 0.3 μ g/dL; $P = 0.03$), and conversely in the KTCZ/HDC arm (5.6 ± 0.3 μ g/dL vs 10.1 ± 0.5 μ g/dL; $P < 0.001$): Appendix Table 1 (*left columns*). Three-way ANCOVA corroborated that the breakpoint in time serves as a boundary for rising and falling ACTH concentrations during 10-h recovery ($P < 0.001$ main effect). Sex-specific comparisons showed that gender determines pre- and post-breakpoint ACTH levels ($P < 0.001$); and infusion type does the same ($P < 0.001$). The precise sex effects are discussed in the next two paragraphs (pre- and post-breakpoint).

3.3. Pre-breakpoint ACTH and cortisol by gender

By 2-way ANCOVA and Tukey's HSD test, the mean ACTH concentration during the pre-breakpoint (initial) recovery phase in post-menopausal women was significantly lower than that in older men (overall $P < 0.001$) in both the KTCZ/LDC arm (15 ± 1.3 women vs 30 ± 5.8 men; $P = 0.005$), and the KTCZ/HDC arm (5.9 ± 0.5 ng/L women vs 14 ± 2.9 ng/L men; $P = 0.006$). This is shown in Fig. 4. In contrast, gender did not determine time of the breakpoint ($P = 0.70$ KTCZ/LDC, $P = 0.80$ KTCZ/HDC). Further ANCOVA demonstrated lower peak (single maximal) ACTH concentrations before the breakpoint in women than men in the KTCZ/HDC arm (22 ± 1.4 women vs 41 ± 6 ng/L men; $P = 0.020$). In contrast, there were no gender differences in mean albumin or CBG and total or free cortisol concentrations in any of the 4 treatment arms before the breakpoint: Table 2.

3.4. Post-breakpoint ACTH and cortisol by gender

Two-way ANCOVA revealed that post-breakpoint mean ACTH concentrations were significantly lower in women than men (overall $P < 0.001$) in both the KTCZ/LDC arm (25 ± 2.4 ng/L vs 45 ± 7 ng/L; $P = 0.024$), and the KTCZ/HDC arm (12 ± 0.9 ng/L vs 31 ± 5.2 ; $P < 0.001$) (Fig. 4). The post-breakpoint mean total and free cortisol concentrations were both lower in women than men in the KTCZ/HDC arm: total cortisol 4.2 ± 0.32 (women) vs 6.4 ± 0.4 μ g/dL (men) ($P = 0.023$); and free cortisol 0.45 ± 0.02 (women) and 0.62 ± 0.035 (men) μ g/dL ($P < 0.05$). Cortisol levels also were lower in women than men under KTCZ/LDC ($P < 0.05$) (Table 2). Thus, lower ACTH escape in women was not due to relative hypercortisolemia in women.

3.5. Four-parameter sigmoid model: gender effect

The gender differences in initial ACTH recovery were verified independently of the breakpoint concept by means of a simplified 4-parameter sigmoidal model of time-varying ACTH and cortisol concentrations. The nonlinear model was superior to a linear fit at $P < 0.01$ by F-ratio testing. This model identified objective baseline (minimum) and plateau (maximum) values for hormone-concentration recovery in the low and high-dose cortisol treatment groups (Fig. 5) (*color*). Statistical 95% C.I. for the fits are shown by interrupted lines. No ACTH recovery was detected in the placebo/saline or KTCZ/saline interventions, as expected. Baseline ACTH concentrations exhibited no gender differences. Plateau ACTH recovery was lower in post-menopausal women than older men in both the KTCZ/LDC and KTCZ/HDC arms ($P < 0.05$): Appendix Table 1 (*right columns*). Plateau cortisol concentrations were also lower in women than men in the KTCZ/LDC arm (8.2 μ g/dL, 95% CI = 7.8 – 8.6 μ g/dL in women vs 9.3 μ g/dL, 95% CI = 8.9 – 9.6 μ g/dL in men) and the KTCZ/HDC arm (4.2 μ g/dL, 95% CI = 4.1 – 4.3 μ g/dL in women vs 6.4 μ g/dL, 95% CI = 6.1 – 6.7 μ g/dL in men).

4. Discussion

Investigations of gender differences in ACTH regulation in healthy humans have been conflicting, possibly due to different experimental paradigms, selection criteria, ages, sampling schedules, stress contexts, comorbidities, and glucocorticoid feedback signals used (e.g. cortisol vs dexamethasone) [2,3,31–33]. Moreover, no human studies have specifically addressed how gender impacts the recovery of ACTH secretion after experimentally controlled stress. In contrast, in the rat, estradiol exerts a repertoire of net stimulatory effects on the HPA axis, such as muting glucocorticoid negative feedback [34], increasing CRH gene expression in the paraventricular nucleus [4], and potentiating adrenal responsiveness to ACTH [35]. Testosterone has inhibitory effects on the rat CRH/AVP-ACTH-adrenal axis. However, available data in humans are discrepant [11,15,21,36], and possibly confounded by stressor specificity and age dependence of sex differences [15,37,38]. In this regard, Otte

et al. in a metaanalysis reported an almost 3-fold greater age-related increase in certain ACTH/cortisol responses in women than men [5]. Based upon these issues, we evaluated ACTH-feedback control in the context of withdrawal and addback of the naturally occurring glucocorticoid, cortisol, in healthy comparably aged older individuals. This required a several step paradigm comprising (i) reversible blockade of adrenal steroidogenesis at the level of CYP11A (cholesterol side-chain cleavage enzyme) using KTCZ in the absence of metyrapone availability [21,22]; (ii) i.v. infusion of saline or either of two doses of cortisol (clamp phase); and (iii) abrupt cessation of the infusions to allow ACTH escape (recovery phase). The clamp phase yielded cortisol concentration-dependent inhibition of ACTH secretion, thus validating the experimental design. The degree of overnight ACTH inhibition during the cortisol clamps was comparable in men and women. Specifically, there were gender-comparable mean and peak ACTH concentrations during the cortisol clamps ($P = 0.98$), and gender-similar clamped cortisol-concentration feedback slopes onto ACTH levels ($P = 0.45$). This main outcome during controlled cortisol clamps may help clarify past ambiguity on the role of gender in glucocorticoid feedback inhibition, wherein studies reported augmented [39], equal [40,41], and attenuated [42,43] feedback sensitivity in women compared with men. Published inconsistencies may in part reflect heterogeneity of age, sex hormone levels, confounding by body composition and/or type of feedback effector, viz., natural cortisol or synthetic glucocorticoids. Indeed, glucocorticoids differ in plasma half-lives, potency, brain permeation, CNS export and CBG binding [3,4,21].

Abrupt cessation of i.v. cortisol infusions permitted estimation of ACTH recovery after feedback suppression. Recovery putatively reflects the degree of amplification or suppression of normal diurnal feedforward onto ACTH as the experimental feedback effect is withdrawn. This aspect of ACTH dynamics has been only poorly studied. To capture quantitative ACTH dynamics, the effect of gender on ACTH recovery was analyzed in three ways. First, early (pre-breakpoint) and delayed (post-breakpoint) recoveries of mean ACTH concentrations were compared by gender. Women manifested markedly impaired early recovery (pre-breakpoint) compared with men after both low and high-dose cortisol infusion, despite sex-comparable total and free cortisol levels during early recovery. This could be due to heightened feedback inhibition in women, inasmuch as ACTH recovery was not only lower but also delayed after high-dose cortisol infusion. Second, peak (maximal) ACTH concentrations achieved during recovery were also lower in women than men, despite comparable total and free cortisol levels by sex. Third, delayed (post-breakpoint) ACTH recovery was decreased in women compared with men in relation to mean ACTH and according to a linear, regression and nonlinear sigmoidal (4-parameter) model. Notably, impaired late (post-breakpoint) ACTH recovery in women was associated with simultaneously lower total and free cortisol in females than males. Thus, by multiple criteria, ACTH escape from cortisol suppression is attenuated in older women compared with older men. This sex difference might be due to impaired hypothalamic CRH/AVP drive in women and/or heightened direct corticotrope inhibition by cortisol. Implications of this sex difference could include attenuated metabolic and stress responses in women compared with men [32,44].

In animals, sex differences in the mechanistic regulation of the HPA axis are substantially explicable by ambient sex-steroid concentrations. In particular animal models show an activational role for estrogens and an inhibitory role for androgens [1,17,33]. Whether this is true in humans is less clear. Although no significant effects of T or E₂ could be detected by exploratory linear regression in the present cohorts, the study was not powered for this possibility. Because ACTH was relatively suppressed in women compared with men, the present data suggest the possibility that a higher E₂/T ratio in women impairs ACTH recovery from cortisol suppression.

Caveats include the relatively limited size of the study sample (20 adults in total) and the narrow age range (60 ± 2 y). Thus, these outcomes require confirmation in larger cohorts and over a wider age range. However, power analysis for the negative inference of no gender effect on steady-state clamped cortisol feedback onto ACTH was 85% ($\beta = 0.15$) for two-sided alpha ($P = 0.05$) at an effect size of one standard deviation by unpaired two-sample comparisons. Additionally, the conclusions of this study apply to healthy individuals [45]. Possible confounding issues could include a) incomplete inhibition of adrenal steroidogenesis by KTCZ; however, ACTH secretion was twofold higher in the KTCZ/saline compared with placebo/saline context; b) possible dependence of outcomes on time of day (here, overnight and morning), therefore ultimately requiring daytime and evening comparisons; c) an unknown role for body composition (not assessed here) in influencing the effects of free cortisol in negative feedback; however, there are no direct experimental data in humans showing concentration-dependent effects of cortisol-binding globulin (CBG) on negative feedback, or an interaction between body composition and free cortisol feedback per se. Although free cortisol seems to be higher in women than men in young adults, CBG levels do not differ by gender in older subjects (present data and [46]); d) 3 weeks of KTCZ administration can increase CBG concentrations [47], but whether this occurs after single-day KTCZ administration or differs by gender is not known; and e) future studies should ultimately attempt to mimic pulsatility of cortisol feedback.

In conclusion, according to the present preclinical model, whereas gender does not measurably modulate ACTH suppression under constant intravenous cortisol feedback in older individuals, sex strongly determines the magnitude of overnight ACTH recovery after removal of inhibition by stopping a constant cortisol infusion. These mechanisms may have relevance to understanding gender differences in stress reactivity in this age group. The roles of sex steroids and body composition in this sex differences will require further study, possibly under conditions illustrated here by cortisol concentration-dependent feedback clamps.

Acknowledgments

We thank Jill Smith for support of manuscript preparation; Sandra Cabral for data analysis and graphics; the Mayo Immunochemical Laboratory for assay assistance; and the Mayo research nursing staff for implementing the protocol. Supported in part via DK073148 and DK050456 (Metabolic Studies Core of the Minnesota Obesity Center) from the National Institutes of Health (Bethesda, MD). The project described was supported by Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCTS). Contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Appendix

Abbreviations

ACTH	adrenocorticotrophic hormone
E₂	estradiol
T	testosterone
KTCZ	ketoconazole
CRH	corticotropin-releasing hormone
AVP	arginine vasopressin

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Fig. 1.

Schema of experimental design. Ten healthy older men and ten postmenopausal women each received successive oral doses of either KTCZ (steroidogenesis inhibitor) or placebo at regular intervals during 4 separate randomly ordered inpatient study sessions. To create a feedback clamp, saline or cortisol as a low dose (LDC) or a high dose (HDC) was infused continuously i.v. over 8 h (1800–0200 h). This paradigm yielded 4 intervention types: placebo/saline (control), KTCZ/saline, KTCZ/LDC, and KTCZ/HDC. The infusion was stopped abruptly at 0200 h to permit ACTH recovery. In each session, 10-min blood sampling was conducted over a total of 14 h, divided into an initial (4-h) feedback clamp phase (last 4 h of the 8-h saline or cortisol infusion, viz. 2200–0200 h), and a later (10-h) ACTH recovery phase (2200–1200 h). Time is represented in clock hours.

Fig. 2.

Fourteen-h profiles of median gender-categorized concentrations of ACTH (*left panel*) and cortisol (*right panel*). Analyses were divided into an earlier 8-h feedback clamp phase, with sampling over the last 4 h of this interval (Fig. 1) and a later 10-h recovery phase. Each panel shows an individual interventional group (placebo/saline, KTCZ/saline, KTCZ/LDC, KTCZ/HDC). Data from men (*closed circles*) and women (*open circles*) are displayed separately. To convert ACTH ng/L to pmol/L multiply by 0.2202, and cortisol μg/dL to nmol/L multiply by 27.6.

Fig. 3.

Absence of gender effect on mean ACTH (*top*) and cortisol (*bottom*) concentrations in older men and women during the last 4 h of the 8-h KTCZ-saline/cortisol feedback clamp in the 4 interventions. Overall infusion effects were significant by ANCOVA at $P < 0.001$ for both ACTH and cortisol. Paired bar graphs surmounted by brace brackets with unique (unshared) boldface capital letters are significantly different independently of gender. Individual P values by post hoc testing under each bracket show the lack of gender effects within intervention. Data are mean $\pm$ SEM ($N = 10$ men, $N = 10$ women). Post hoc testing was by Tukey's HSD multiple-comparison test.

Fig. 4.

Effects of gender on 10-h mean ACTH concentrations during the post-infusion recovery phase segmented into before and after the objective breakpoint in each of the 4 interventions. Different (unshared) upper-case letters within KTCZ/LDC as well as within KTCZ/HDC signify significantly different mean ($\pm$ SEM) ACTH concentrations measured pre- vs post-breakpoints. Different lower-case letters in the KTCZ/HDC arm denote significant gender effects on mean ACTH concentrations both before and after the breakpoint. Analysis is based upon ANCOVA and Tukey's post hoc HSD testing.

Fig. 5.

Time course of median ACTH (top) and cortisol (bottom) concentrations during recovery phase in the KTCZ/LDC arm (left) and KTCZ/HDC arm (right) in older men (blue) and women (red) evaluated via a 4-parameter sigmoidal-curve model. The analysis yields estimates of the minimum (floor) and maximum (plateau) concentration along with 95% confidence bands (*interrupted lines*). Time is shown in min. Zero time (x-axis) denotes 0200 h.

Linear-Segmental Analysis of ACTH Recovery

Older Men

Older Women

Appendix Figure 1.

Linear-segmental regression of 10-h individual ACTH concentrations on time during recovery in 10 men (*left*) and 10 women (*right*) in each of the 4 treatment arms. ACTH recovery overtime is segmented by a calculated breakpoint into a pre- (ACTH escape) and post- (return to baseline) phase. Time is shown in min with zero denoting 0200 h.

Table 1

Subject characteristics at screening.

Subject characteristics	Older men	Older women	P value
Number (N)	10	10	
Age (years)	60 ± 2.2	60 ± 2.2	0.90
BMI (kg/m ²)	28 ± 0.7	26 ± 1	0.08
Albumin (g/L)	44 ± 0.5	43 ± 0.7	0.29
Cortisol (µg/dL) *	12 ± 1.1	12 ± 1.2	0.97
Estradiol (pmol/L)	84 ± 8.4	42 ± 5.5	<0.001
FSH (IU/L)	5.4 ± 0.5	74 ± 7.5	<0.001
LH (IU/L)	2.9 ± 0.5	26 ± 4.4	<0.001
Prolactin (pmol/L)	287 ± 23	326 ± 26	0.27
SHBG (nmol/L)	37 ± 5.4	59 ± 4.7	0.01
Testosterone (nmol/L)	18 ± 2.3	0.5 ± 0.1	<0.001
TSH (mIU/L)	1.8 ± 0.2	2.3 ± 0.5	0.21
ACTH (ng/L)	21 ± 1.4	10.6 ± 0.8	0.023
Free cortisol (µg/L) *	1.0 ± 0.27	0.92 ± 0.27	0.38
CBG (µmol/L)	0.44 ± 0.039	0.47 ± 0.052	0.71

Data are the mean ± SEM, single measures at 0800 hr.

* To convert to SI units (nmol/L) multiply by 27.6.

Table 2

Measured and calculated cortisol levels.

Condition	Men		Women	
	Measured	Calculated	Measured	Calculated
	Total cortisol ($\mu\text{g/dL}$)	Free cortisol ($\mu\text{g/dL}$)	Total cortisol ($\mu\text{g/dL}$)	Free cortisol ($\mu\text{g/dL}$)
0800 h baseline	12.5 $\pm$ 3.2	1.04 $\pm$ 0.27	12.5 $\pm$ 3.7	0.92 $\pm$ 0.27
Placebo/saline	4.3 $\pm$ 0.28	0.21 $\pm$ 0.014	4.0 $\pm$ 0.26	0.17 $\pm$ 0.011
KTCZ/saline	3.3 $\pm$ 0.25	0.15 $\pm$ 0.011	3.3 $\pm$ 0.23	0.14 $\pm$ 0.010
KTCZ/LDC-clamp	5.2 $\pm$ 0.22	0.27 $\pm$ 0.012	6.7 $\pm$ 0.31	0.34 $\pm$ 0.016
KTCZ/LDC-recovery	9.3 $\pm$ 0.33	0.62 $\pm$ 0.035	8.2 $\pm$ 0.39	0.45 $\pm$ 0.021
KTCZ/HDC-clamp	14 $\pm$ 2.6	1.30 $\pm$ 0.24	16 $\pm$ 3.5	1.49 $\pm$ 0.33
KTCZ/HDC-recovery	6.4 $\pm$ 0.40	0.36 $\pm$ 0.023	4.2 $\pm$ 0.32	0.17 $\pm$ 0.013

Data are mean $\pm$ SEM.Boldface denotes $P < 0.05$ lower in women than men; all other P values were >0.10 for sex difference.

CBG median (range) men: 0.439 (0.369–0.551).

CBG median (range) women: 0.496 (0.371–0.556) $\mu\text{mol/L}$.Assumed K_a for cortisol-CBG was 5.23×10^7 L/mol, and for albumin 2.7×10^3 L/mol [29].

Appendix Table 1

Time-dependent ACTH recovery by gender.

Linear Recovery phase	ANCOVA Mean ($\pm$ SEM)		Nonlinear Curve-fit mean (95% CI)	
	Men	Women	Mean ACTH concentration (ng/L)	
A. Mean ACTH concentration (ng/L)			Men	Women
Pre-breakpoint			Sigmoid floor	
KTCZ/LDC	30 $\pm$ 5.8	15 $\pm$ 1.3	KTCZ/ LDC	11 (6.7-15)
KTCZ/HDC	14 $\pm$ 2.9	5.9 $\pm$ 0.9*	KTCZ/ HDC	4.8 (2.7-5.9)
Post-breakpoint			Sigmoid plateau	
KTCZ/LDC	45 $\pm$ 7.0	25 $\pm$ 2.4*	KTCZ/ LDC	39 (37-41)
KTCZ/HDC	31 $\pm$ 5.2	12 $\pm$ 0.9*	KTCZ/ HDC	27 (26-28)
B. Maximum ACTH concentration (ng/L)				
KTCZ/LDC	71 $\pm$ 9.6	51 $\pm$ 7.6		
KTCZ/HDC	41 $\pm$ 6.0	20 $\pm$ 1.4*		

Comparison of ACTH (10-h) recovery in men and women using ANCOVA of mean line-segment values (*left*) and 95% confidence intervals of nonlinear curve fitting (4-parameter sigmoid curve, *right*). ANCOVA evaluated gender effects on mean and maximal (peak) ACTH concentrations during recovery both pre- and post-breakpoint. Nonlinear modeling assessed recovery floor (minimum) and plateau (maximum). Significant gender differences are denoted by * ($P < 0.05$).

* $p < 0.05$ for the gender contrast.