

Human Glucocorticoid Feedback Inhibition Is Reduced in Older Individuals: Evening Study*

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ABSTRACT

We have previously shown that when tested in the morning, older men and women, pretreated with metyrapone to block endogenous cortisol synthesis, exhibit delayed suppression of plasma ACTH in response to cortisol infusion. To confirm this finding and to determine whether aging-related changes in feedback responsiveness are exaggerated near the time of the circadian nadir in adrenocortical secretion, we performed a similar study in the evening. Healthy young (20–35 yr, n = 22) and old (>65 yr, n = 21) men and women were administered metyrapone orally (750 mg) at 1600 and 1900 h, followed by a cortisol infusion of 0.06 mg/kg/h for 150 min. Blood samples

were taken at 15-min intervals for 4 h following infusion onset for measurement of plasma ACTH, cortisol, 11-deoxycortisol, and corticosteroid binding globulin. When corrections were made for differences in circulating cortisol concentrations achieved among age and gender subgroups, feedback inhibition of ACTH was found to be significantly greater in young than in old subjects of both genders. Our studies support the hypothesis that glucocorticoid responses to stress in aging individuals are likely to be prolonged due to blunted and delayed inhibition of ACTH secretion, thus increasing the total exposure to glucocorticoids. (*J Clin Endocrinol Metab* 86: 545–550, 2001)

AGING IN RODENTS has been found to result in prolonged glucocorticoid responses to stress and impaired feedback inhibition of ACTH secretion by glucocorticoids (1–3). These age-related modifications of hypothalamic-pituitary-adrenal (HPA) function have been linked to deficits in learning and memory (4–6). Until recently, it has not been clear whether similar changes occur in the human HPA axis during aging. Several groups have reported age-associated increases in resting cortisol levels, especially near the nadir of the cortisol circadian rhythm (7–16). The magnitude of pituitary-adrenal responses to ovine corticotropin-releasing factor (CRF) has generally not been found to change with aging in humans (17, 18). However, when human CRF has been used to provoke the HPA axis, greater ACTH and cortisol responses have been found in older individuals (19, 20), both with and without concurrent administration of arginine vasopressin (19).

Impairment of glucocorticoid feedback inhibition of ACTH secretion, one of the primary age-related changes in HPA function reported in animal studies, has been supported by some, but not all, human studies. Pavlov *et al.* (17) concluded that the finding of a trend toward increased ovine CRF-stimulated levels of plasma ACTH and cortisol in older subjects in the face of age-related increases in resting cortisol was “consistent with the hypothesis that aging is associated

with decreased pituitary sensitivity to negative feedback regulation by glucocorticoids.”

Studies of age-related changes in feedback inhibition using dexamethasone have reported age-related impairments (11, 20–26) or no change (18, 27–29). In these studies, dexamethasone was administered orally, cortisol was generally measured 9 or more hours after dexamethasone administration, and ACTH responses were measured only by Waltman *et al.* (18). These studies do not provide information regarding the time course of the rapid effects of glucocorticoid feedback inhibition involved in the regulation of ACTH secretion (30). Furthermore, the use of dexamethasone to evaluate relative differences in feedback sensitivity is complicated by the fact that the effects of dexamethasone, unlike the endogenous glucocorticoids, are mediated primarily at the pituitary rather than the central nervous system (30–33), and the affinities of corticosteroid receptors for dexamethasone differ considerably from those for cortisol (34).

We have previously demonstrated directly that responsiveness to glucocorticoid negative feedback is decreased in the elderly by showing that older men and women, pretreated with metyrapone, exhibit delayed and blunted suppression of plasma ACTH in response to infusion of cortisol in the morning (35). These findings have been supported by another group using a different experimental approach (36). In light of findings that alterations of glucocorticoid circadian rhythms and stress responsiveness in depression, chronic stress, and aging seem to be particularly affected during the evening hours near the nadir of the glucocorticoid rhythm (11–12, 16, 37–40), we have now used our protocol of oral metyrapone administration, followed by cortisol infusion, to investigate the hypothesis that age-related changes in glu-

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corticoid feedback inhibition are further accentuated in the evening.

Subjects and Methods

Experimental subjects

The study was approved by the Human Subjects Review Committee of the University of Washington, and written informed consent was obtained from all subjects. A total of 22 young (28 ± 1 yr old) and 21 older (74 ± 1 yr old) subjects of both genders underwent two study conditions in random order. All young women were studied within days 5–12 of the start of their menstrual cycle. All older women were postmenopausal. No subjects were taking oral contraceptives or hormone replacement at the time of the study. Subjects were nonsmoking community volunteers in excellent general health. Exclusionary criteria included history of psychiatric disorders, alcoholism, or drug abuse, presence of current major depressive episode or neurological diagnoses likely to affect cognitive function, and unstable medical problems including unstable heart disease, uncontrolled hypertension, diabetes in poor control, respiratory disease complicated by hypoxia or hypercapnia, infectious illness, or unstable thyroid dysfunction. Subjects who had undergone a major life event such as a change in residence within the past month or death of a close family member within the past 6 months were excluded. All subjects were free of medications known to affect the HPA axis or cognitive function. Complete blood counts and blood chemistries were performed on samples from all subjects. All were within 25% of ideal body weight (1983 Metropolitan Life Insurance tables). Studies were conducted in a clinical research unit at the VA Puget Sound Health Care System in Seattle.

Materials and methods

Each subject was studied in an active drug condition and a placebo condition in random order. Subjects were blind with respect to study condition. In the experimental condition, subjects were administered 750 mg metyrapone (Metopirone, Ciba-Geigy & Co., Whitehouse Station, NJ) p.o. at 1600 h and again at 1900 h, followed by a cortisol infusion of 0.06 mg/kg/h for 150 min. Metyrapone suppresses endogenous cortisol by inhibiting the conversion of 11-deoxycortisol to cortisol by 11 β -hydroxylase. In the control condition, subjects received placebo tablets at 1600 and 1900 h, followed by measurement of baseline hormone levels. Details of the protocols were: subjects fasted from 1000 h on each study day and were maintained at bed rest throughout the study. At 1830 h, an iv catheter was placed in an antecubital vein of each arm and kept patent with a slow infusion of normal saline. One iv catheter was used for blood sampling and the other for infusion. At 1855 and 1900 h, baseline blood samples were drawn for measurement of ACTH, cortisol, and 11-deoxycortisol. Measurement of 11-deoxycortisol was used to affirm the efficacy of the metyrapone in blocking cortisol synthesis. The placebo condition was ended after the withdrawal of the second baseline blood sample, and no infusion was made.

In the metyrapone condition beginning at 1900 h, cortisol (hydrocortisone phosphate injection; Merck, Summit, NJ) was infused at 0.06 mg/kg/h (166 nmol/kg/h) in 300 mL normal saline over 150 min. This was followed by slow infusion (50 mL/h) of normal saline for 90 min. Blood sampling (10 mL/sample) occurred every 15 min for 240 min following baseline.

Plasma ACTH was measured by immunoradiometric assay kits from Nichols Institute Diagnostics (San Juan Capistrano, CA). The detection limit of the assay is 3 pg/mL (0.7 pmol/L), and the intra- and interassay coefficients of variation are 7.2 and 12.3%, respectively.

Cortisol was measured by RIA in unextracted plasma. Samples were diluted with phosphate buffer and heated for 20 min at 80 C to denature binding globulins. Cortisol antiserum was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA). Cross-reactivity of the antiserum with 11-deoxycortisol is 10%. Assay values for cortisol were corrected by subtracting 10% of the 11-deoxycortisol values for each sample. The detection limit for cortisol is 10 pg/mL (28 pmol/L); the intra- and interassay coefficients of variation are 8.1 and 13.8%, respectively.

Plasma 11-deoxycortisol was measured by RIA in unextracted plasma, to which steroid binding globulin inhibitor was added. Antiserum, standards, and 125 I-labeled 11-deoxycortisol were obtained from

ICN Biomedicals, Inc., and a modification of the commercial protocol was used. Cross-reactivity of the antiserum with cortisol is 0.14%. Minimum sensitivity of the assay is 0.5 ng/mL (1.4 nmol/L). The intra- and interassay coefficients of variation were 9.1 and 14.4%, respectively.

Plasma corticosteroid binding globulin (CBG) was measured by radial immunodiffusion (The Binding Site, San Diego, CA) as described previously (35). Minimum sensitivity of the assay is 8 mg/L (154 nmol/L). The intra- and interassay coefficients of variation were 3.1 and 5.0%, respectively.

Metyrapone treatment was judged to be effective for a given subject if the treatment resulted in at least a 2-fold increase in the plasma 11-deoxycortisol to cortisol ratio and an increase in plasma ACTH concentration. A total of 35 of 43 subjects (8–9 in each age-gender combination) met these criteria and were included in the data analysis.

Postmetyrapone (or postplacebo) baseline values for ACTH, cortisol, and 11-deoxycortisol were calculated as the means of the concentrations in the two plasma samples collected just prior to starting the cortisol infusion (or in the placebo condition, just prior to terminating the session).

Results

Plasma ACTH concentration was significantly suppressed in both young and old subjects by 60 min after the beginning of the cortisol infusion (as determined by the difference in ACTH concentration between postmetyrapone baseline and each subsequent time point by single group *t* test; Fig. 1). When each gender group was analyzed separately, young men showed a more rapid suppression than old men (significant difference from ACTH baseline at 75 min *vs.* 90 min), but older women exhibited a more rapid decline in ACTH than young women (45 *vs.* 90 min; Fig. 2). The postmetyrapone baseline levels of ACTH and the rate of decrease of ACTH concentration were higher in the young women, but the variability was also high in this group. The same relationships occurred if percentage change in ACTH concentration rather than absolute concentration change was analyzed, except that differences between young and older women were reduced. The percentage change in plasma ACTH differed significantly from zero at 45 min for the older women and at 60 min for the young women (single group *t* test).

Comparison of peak plasma cortisol concentrations achieved as a result of the cortisol infusion with cortisol

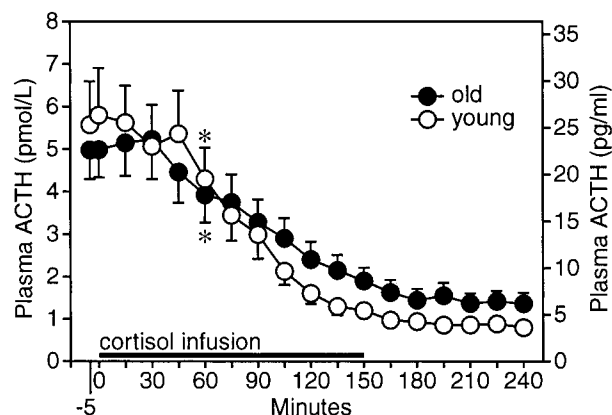


FIG. 1. Plasma ACTH concentration in young ($n = 18$) and old ($n = 17$) metyrapone-treated subjects during and after cortisol infusion. *, Time for each age group at which the fall in ACTH concentration below the postmetyrapone baseline first differs significantly from zero by one group *t* test.

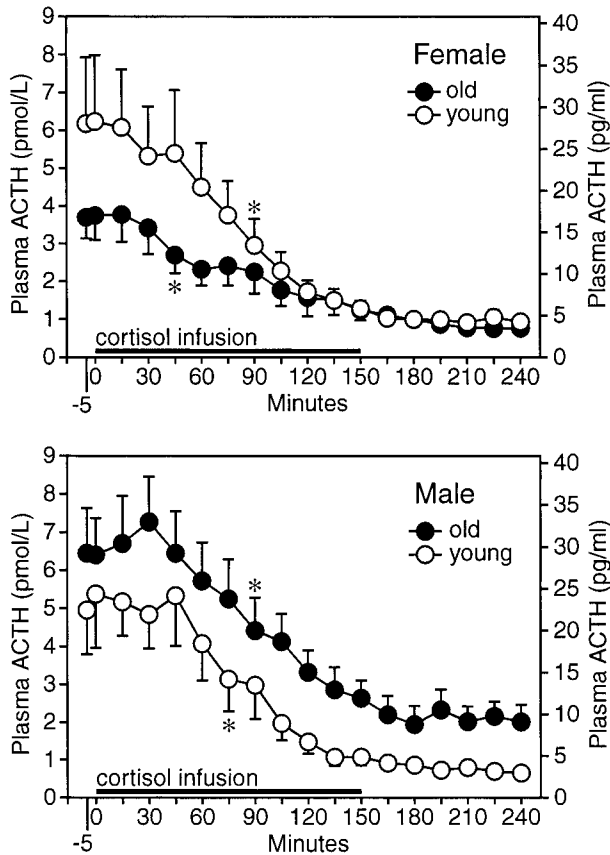


FIG. 2. Plasma ACTH concentration in metyrapone-treated young and old women (top) and young and old men (bottom) during and after cortisol infusion [0.06 mg/kg/h (166 nmol/kg/h)]. *, Time for each age group at which the fall in ACTH concentration below the post-metyrapone baseline first differs significantly from zero by one group *t* test. Young women, *n* = 9; old women, *n* = 9; young men, *n* = 9; old men, *n* = 8. *Flags* indicate SEM.

responses elicited in clinical test procedures shows that the cortisol levels reached in this study were below the range of peak values normally observed in response to the standard short Synacthen test or insulin tolerance test, and the rate of increase in circulating cortisol was markedly slower (2–3 nmol/L/min compared with 7–10 nmol/L/min for short Synacthen test and insulin tolerance test; Refs. 41–43). Comparison of plasma cortisol levels between young and old subjects showed that greater plasma cortisol levels were achieved as a result of the cortisol infusion in old subjects than in young subjects and that women achieved consistently higher plasma cortisol levels than men (Fig. 3). Two-way ANOVA for repeated measures revealed significant main effects of age and gender, but no significant age-gender interaction (age: $F = 6.20$, $P < 0.02$; gender: $F = 10.03$, $P < 0.005$; age-gender interaction: $F = 0.00$, $P = 0.99$). Baseline cortisol concentrations did not differ among the age-gender groups in either the placebo or metyrapone pretreatment condition.

In view of the possibility that age differences in total plasma cortisol concentration were due to differences in bound cortisol rather than in biologically active free cortisol, plasma CBG was measured in two samples from each subject in each experimental session. There was no effect of age on plasma CBG concentration, but despite the exclusion of sub-

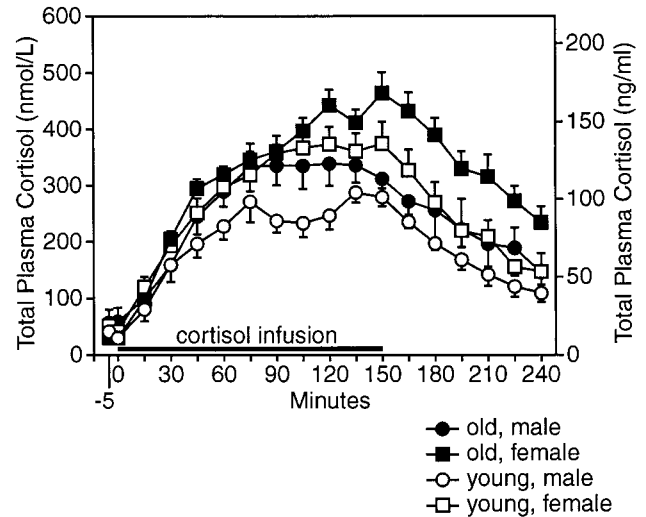


FIG. 3. Total plasma cortisol concentration in metyrapone-treated subjects during and after cortisol infusion [0.06 mg/kg/h (166 nmol/kg/h)]. *Flags* indicate SEM.

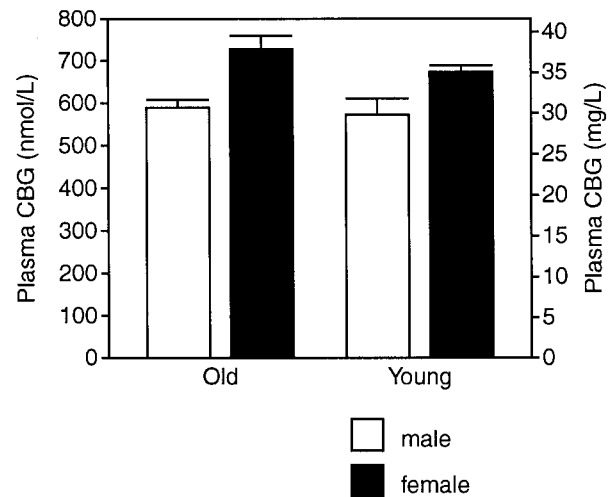


FIG. 4. Plasma CBG concentration in metyrapone-treated subjects. CBG was measured in the first (time, -5 min) and last (time, +240 min) sample from each subject, and the mean of the two determinations for each subject was used to calculate group means and SEM.

jects taking oral contraceptives or estrogen replacement therapy, CBG concentrations were higher in women than in men (age \times gender ANOVA: age, $F = 1.76$, $P = 0.19$; gender, $F = 19.24$, $P < 0.001$). This gender difference was also significant within each age group (*t* tests: young women *vs.* young men, $t = 2.55$, $P < 0.05$; old women *vs.* old men, $t = 3.72$, $P < 0.005$; see Fig. 4). Free cortisol concentration for each subject for each time point was estimated by the method of Coolens *et al.* (44). A nearly significant age-related difference in free cortisol was found, similar to that described above for total cortisol (age \times gender ANOVA for repeated measures: age, $F = 3.67$, $P = 0.06$; gender, $F = 1.16$, $P = 0.29$).

To produce a measure of feedback responsiveness adjusted for differences in free cortisol concentration achieved among groups, we calculated the ratio of the change in ACTH (relative to baseline) to the change in calculated free cortisol concentration at each time point during the period of

dynamic ACTH changes (from 75–150 min). To take into account the time lag between the increase in plasma cortisol and the subsequent fall in ACTH levels, the change in ACTH concentration at a given time point was divided by the change in plasma free cortisol concentration 30 min earlier to provide a “feedback responsiveness index” (Fig. 5). The ratio of ACTH suppression to increment of free cortisol increase was significantly higher in the young subjects ($F = 5.22$, $P < 0.05$, by ANOVA for repeated measures). When compared by t tests at individual time points, this ratio was significantly greater in young than in old subjects at 105, 120, 135, and 150 min (time of ACTH sample in the ratio). The same relationship held for each of the gender groups analyzed separately.

There was no significant effect of age or gender and no significant interaction of the two on plasma 11-deoxycortisol concentrations (ANOVA for repeated measures). There was a tendency for 11-deoxycortisol levels to be higher in young subjects than in old subjects (data not shown).

Discussion

The results of this evening study confirm our earlier results from a morning study, indicating that feedback inhibition of plasma ACTH concentration by cortisol is diminished in older individuals. This age-related reduction in glucocorticoid feedback inhibition was determined by measurement of: 1) the initial time at which plasma ACTH concentration decreased significantly in response to cortisol infusion; and 2) the relative magnitude of the decline in ACTH concentration for a given increment in plasma cortisol concentration. The latency for a significant decrement in ACTH concentration was significantly longer in older men than in young men. Women exhibited an apparent moderate reversal of age relationships in that older women reached significant levels of ACTH inhibition earlier than young women in the evening study. This apparent reversal, however, was at least partially due to the higher plasma cortisol levels (*i.e.* a greater feedback signal) achieved in older women. When results are expressed as the ratio of the fall in ACTH concentration at

each time point to the increase in free plasma cortisol generated 30 min earlier by cortisol infusion, consistent and pronounced decrements in ACTH feedback responsiveness to cortisol in older individuals are found when compared with young subjects in both the morning and the evening (Fig. 6).

It is not clear whether increased plasma cortisol levels achieved in women and in older subjects, in general, as a result of the cortisol infusion are due entirely to the measured differences in CBG concentration or partially to differences in cortisol metabolism or distribution volume among groups. However, the consistent rates of decline in plasma cortisol concentration after the termination of the infusion suggest that metabolic clearance does not differ appreciably with age or gender.

One of the questions to be addressed by this study was that of human nycthemeral differences in feedback sensitivity to cortisol inhibition. The answer is complex. When the latency to significant decreases in ACTH concentration are compared between morning and evening subjects, it is clear that a significant feedback-elicited decline in plasma ACTH occurs more rapidly in the evening than in the morning. This finding holds for every gender-age group, except the young males (see Table 1). However, when the magnitude of the decrease in ACTH concentration to a given increase in plasma free cortisol is compared between morning and evening, the effectiveness or potency of feedback inhibition is greater in the morning for young subjects and not different for old subjects (Fig. 6). Our interpretation of these data is that the “threshold” change in cortisol concentration necessary to initiate feedback inhibition is lower in the evening than in the morning, but once inhibition is initiated, the magnitude of the inhibition for a given change in level of circulating cortisol is greater in the morning. Although an age-related difference in feedback inhibition was found in both morning and evening studies, the magnitude of the difference was much greater in the morning for both genders (Fig. 7).

Suppression of plasma cortisol by dexamethasone has also been found to be impaired as a function of increasing age (11, 20–26), although other studies have failed to find a relationship (18, 27–29). Dexamethasone differs from the endogenous glucocorticoid cortisol in its affinities for corticosteroid receptor subtypes (34) and the HPA axis level at which feedback inhibition primarily occurs (30–33). Direct comparisons in human subjects have indicated discordant feedback responses to cortisol and dexamethasone (45, 46), and, thus, use of dexamethasone may not accurately characterize the

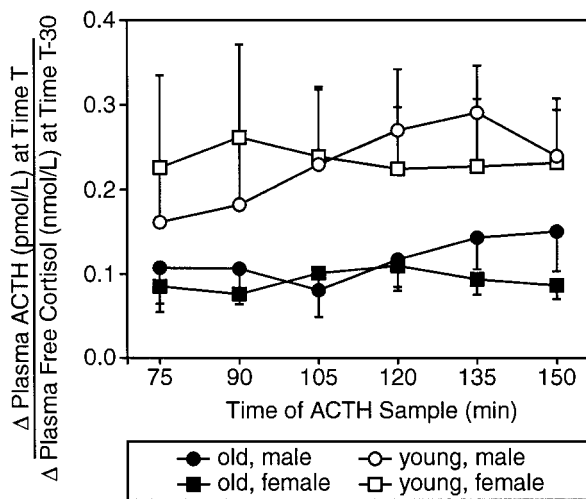


FIG. 5. Index of responsiveness of ACTH to feedback inhibition by cortisol. The index is calculated by dividing the change in plasma ACTH concentration from baseline at each time point by the change in plasma free cortisol achieved 30 min earlier. *Flags* indicate SEM.

TABLE 1. Time (in minutes after the onset of the cortisol infusion) at which the change in plasma ACTH concentration is significantly different from zero (the postmetyrapone baseline) by one group t test, $P < 0.05$

	Morning ^a	Evening
Young women	195	90
Young men	60	75
Old women	165	45
Old men	120	90

^a Method of analysis and number of subjects differ from the previous description of findings from the morning study (12).

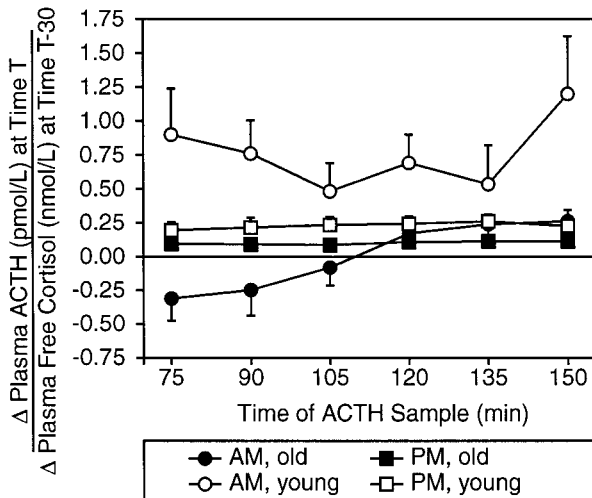


FIG. 6. Comparison of index of responsiveness of ACTH to feedback inhibition by cortisol between morning and evening studies. For the morning study, $n = 16$ for young subjects and $n = 15$ for old subjects. Flags indicate SEM.

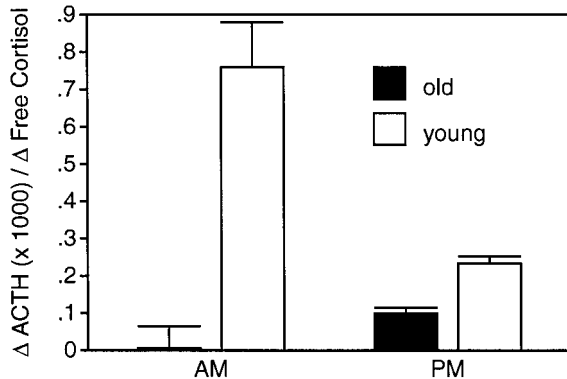


FIG. 7. The mean index of responsiveness across all time points between 75 and 150 min was calculated for each subject. Means and SEM for those values are indicated for young and old subjects in the morning and evening studies. ACTH and free cortisol concentrations are expressed in nmol/L.

effects of aging on feedback inhibition by endogenous glucocorticoids.

Gender differences and gender-age interactions in HPA responses to challenge have been found by several groups (e.g. Refs. 47–49), but relatively few studies have investigated gender differences in human glucocorticoid feedback inhibition directly. Gallucci *et al.* (50) found that young women showed more sustained elevations of cortisol to released ACTH than young men, “suggesting relatively greater resistance to cortisol feedback” in women. Studies using dexamethasone have found that women exhibit significantly greater (20), slightly greater (26), or no different (29) cortisol suppression than men. In this study, we found neither gender differences nor age-gender interactions in the ratio of ACTH inhibition to circulating free cortisol concentration (Fig. 5). However, data from our previous morning study indicate resistance to glucocorticoid feedback inhibition in both young and older women in comparison with same age-group men (35).

In conclusion, this study of age-related responsiveness to

glucocorticoid feedback inhibition in the evening confirms our earlier morning study in showing deficits in suppression of ACTH secretion in older subjects. Differences in results between the two studies indicate that not only gender, but time of day, influences the expression of age-related reductions in feedback sensitivity. These studies support the assertion that in humans, as well as in rodents, glucocorticoid responses to stress in aging individuals are likely to be prolonged due to blunted and delayed inhibition of ACTH secretion, thus heightening the probability that integrated levels of glucocorticoid exposure are elevated with increasing age.

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References

- Seeman TE, Robbins RJ. 1994 Aging and hypothalamic-pituitary-adrenal responses to challenge in humans. *Endocr Rev.* 15:233–260.
- Sapolsky RM. 1992 Stress, the aging brain, and the mechanisms of neuron death. Cambridge, MA: MIT Press.
- Sapolsky R, Armanini M, Packan D, Tombaugh G. 1987 Stress and glucocorticoids in aging. *Endocrinol Metab Clin North Am.* 16:965–980.
- Meaney MJ, Aitken DH, van Berkel C, Bhatnagar S, Sapolsky RM. 1988 Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science.* 239:766–768.
- Yau JL, Olsson T, Morris RG, Meaney MJ, Seckl JR. 1995 Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. *Neuroscience.* 66:571–581.
- Angelucci L. 1994 The hypothalamus-pituitary-adrenocortical axis: epigenetic determinants changes with aging, involvement of NGF. *Neurochem Int.* 25:53–59.
- Parnetti L, Mecocci P, Neri C, et al. 1990 Neuroendocrine markers in aging brain: clinical and neurobiological significance of dexamethasone suppression test. *Aging.* 2:173–179.
- Belanger A, Candas B, Dupont A, et al. 1994 Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year old men. *J Clin Endocrinol Metab.* 79:1086–1090.
- Dori D, Casale G, Solerte SB, et al. 1994 Chrono-neuroendocrinological aspects of physiological aging and senile dementia. *Chronobiologia* 21:121–126.
- Copinschi G, Van Cauter E. 1995 Effects of ageing on modulation of hormonal secretions by sleep and circadian rhythmicity. *Horm Res.* 43:20–24.
- Ferrari E, Magri F, Dori D, et al. 1995 Neuroendocrine correlates of the aging brain in humans. *Neuroendocrinology.* 61:464–470.
- Van Cauter E, Leproult R, Kupfer DJ. 1996 Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab.* 81:2468–2473.
- Deuschle M, Gotthardt U, Schweiger U, et al. 1997 With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sci.* 61:2239–2246.
- Nicolson N, Storms C, Ponds R, Sulon J. 1997 Salivary cortisol levels and stress reactivity in human aging. *J Gerontol A Biol Sci Med Sci.* 52:M68–M75.
- Deuschle M, Weber B, Colla M, Depner M, Heuser I. 1998 Effects of major depression, aging and gender upon calculated diurnal free plasma cortisol concentrations: a re-evaluation study. *Stress.* 2:281–287.
- Raff H, Raff JL, Duthie EH, et al. 1999 Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density. *J Gerontol A Biol Sci Med Sci.* 54:M479–M483.
- Pavlov EP, Harman SM, Chrousos GP, Loriaux DL, Blackman MR. 1986 Responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. *J Clin Endocrinol Metab.* 62:767–772.
- Waltman C, Blackman MR, Chrousos GP, Riemann C, Harman SM. 1991 Spontaneous and glucocorticoid-inhibited adrenocorticotropin hormone and cortisol secretion are similar in healthy young and old men. *J Clin Endocrinol Metab.* 73:495–502.
- Born J, Ditschuneit I, Schreiber M, Dodt C, Fehm HL. 1995 Effects of age and gender on pituitary-adrenocortical responsiveness in humans. *Eur J Endocrinol.* 132:705–711.
- Heuser IJ, Gotthardt U, Schweiger U, et al. 1994 Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. *Neurobiol Aging.* 15:227–231.

21. **Georgotas A, McCue RE, Kim OM, et al.** 1986 Dexamethasone suppression in dementia, depression, and normal aging. *Am J Psychiatry.* 143:452–456.
22. **Weiner MF, Davis BM, Mohs RC, Davis KL.** 1987 Influence of age and relative weight on cortisol suppression in normal subjects. *Am J Psychiatry.* 144:646–649.
23. **O'Brien JT, Schweitzer I, Ames D, Tuckwell V, Mastwyk M.** 1994 Cortisol suppression by dexamethasone in the healthy elderly: effects of age, dexamethasone levels, and cognitive function. *Biol Psychiatry.* 36:389–394.
24. **Oxenkrug GF, Pomara N, McIntyre IM, Branconnier RJ, Stanley M, Gershon S.** 1983 Aging and cortisol resistance to suppression by dexamethasone: a positive correlation. *Psychiatry Res.* 10:125–130.
25. **Branconnier RJ, Oxenkrug GF, McIntyre I, Pomara N, Harto NE, Gershon S.** 1984 Prediction of serum cortisol response to dexamethasone in normal volunteers: a multivariate approach. *Psychopharmacology (Berl).* 84:274–275.
26. **Keitner GI, Ryan CE, Kohn R, Miller IW, Norman WH, Brown WA.** 1992 Age and the dexamethasone suppression test: results from a broad unselected patient population. *Psychiatry Res.* 44:9–20.
27. **Tourigny-Rivard M-F, Raskind M, Rivard D.** 1981 The dexamethasone suppression test in an elderly population. *Biol Psychiatry.* 16:1177–1184.
28. **Anseau M, von Frenckell R, Simon C, Sulon J, Demey-Ponsart E, Franck G.** 1986 Prediction of cortisol response to dexamethasone from age and basal cortisol in normal volunteers: a negative study. *Psychopharmacology (Berl).* 90:276–277.
29. **Huizenga NATM, Koper JW, de Lange P, et al.** 1998 Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. *J Clin Endocrinol Metab.* 83:47–54.
30. **Dorin RI, Ferries LM, Roberts B, Qualls CR, Veldhuis JD, Lisansky EJ.** 1996 Assessment of stimulated and spontaneous adrenocorticotropin secretory dynamics identifies distinct components of cortisol feedback inhibition in healthy humans. *J Clin Endocrinol Metab.* 81:3883–3891.
31. **de Kloet R, Wallach G, McEwen BS.** 1975 Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology.* 96:598–609.
32. **Kovács KJ, Makara GB.** 1988 Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin hypersecretion. *Brain Res.* 474:205–210.
33. **Miller AH, Spencer RL, Pulnera M, Kang S, McEwen BS, Stein M.** 1992 Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. *Biol Psychiatry.* 32:850–869.
34. **de Kloet ER.** 1991 Brain corticosteroid receptor balance and homeostatic control. *Front Neuroendocrinol.* 12:95–164.
35. **Wilkinson CW, Peskind ER, Raskind MA.** 1997 Decreased hypothalamic-pituitary-adrenal axis sensitivity to cortisol feedback inhibition in human aging. *Neuroendocrinology.* 65:79–90.
36. **Boscaro M, Paoletta A, Scarpa E, et al.** 1998 Age-related changes in glucocorticoid fast feedback inhibition of adrenocorticotropin in man. *J Clin Endocrinol Metab.* 83:1380–1383.
37. **Raskind M, Peskind E, Rivard MF, Veith R, Barnes R.** 1982 Dexamethasone suppression test and cortisol circadian rhythm in primary degenerative dementia. *Am J Psychiatry.* 139:1468–1471.
38. **Dahl RE, Ryan ND, Puig-Antich J, et al.** 1991 24-Hour cortisol measures in adolescents with major depression: a controlled study. *Biol Psychiatry.* 30:25–36.
39. **Abelson JL, Curtis GC.** 1996 Hypothalamic-pituitary-adrenal axis activity in panic disorder. 24-Hour secretion of corticotropin and cortisol. *Arch Gen Psychiatry.* 53:323–331.
40. **Magri F, Locatelli M, Balza G, et al.** 1997 Changes in endocrine circadian rhythms as markers of physiological and pathological brain aging. *Chronobiol Int.* 14:385–396.
41. **Darmon P, Dadoun F, Frachebois C, et al.** 1999 On the meaning of low-dose ACTH(1–24) tests to assess functionality of the hypothalamic-pituitary-adrenal axis. *Eur J Endocrinol.* 140:51–55.
42. **Long TD, Ellingrod VL, Kathol RG, Christensen LE, Resch DS, Perry PJ.** 2000 Lack of menstrual cycle effects on hypothalamic-pituitary-adrenal axis response to insulin-induced hypoglycaemia. *Clin Endocrinol (Oxf).* 52:781–787.
43. **Inder WJ, Ellis MJ, Evans MJ, Donald RA.** 1995 A comparison of the naloxone test with ovine CRH and insulin hypoglycaemia in the evaluation of the hypothalamic-pituitary-adrenal axis in normal man. *Clin Endocrinol (Oxf).* 43:425–431.
44. **Coolens J-L, Van Baelen H, Heyns W.** 1987 Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem.* 26:197–202.
45. **Gispén-de Wied CC, D'Haenen H, Verhoeven WM, et al.** 1993 Inhibition of the pituitary-adrenal axis with dexamethasone and cortisol in depressed patients and healthy subjects: a dose-response study. *Psychoneuroendocrinology.* 18:191–204.
46. **Gispén-de Wied CC, Jansen LM, Wynne HJ, et al.** 1998 Differential effects of hydrocortisone and dexamethasone on cortisol suppression in a child psychiatric population. *Psychoneuroendocrinology.* 23:295–306.
47. **Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH.** 1999 Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med.* 61:154–162.
48. **Petrie EC, Wilkinson CW, Murray S, Jensen C, Peskind ER, Raskind MA.** 1999 Effects of Alzheimer's disease and gender on the hypothalamic-pituitary-adrenal axis response to lumbar puncture stress. *Psychoneuroendocrinology.* 24:385–395.
49. **Seeman TE, Singer B, Wilkinson CW, McEwen B.** Gender differences in age-related changes in HPA axis reactivity. *Psychoneuroendocrinology.* In press.
50. **Gallucci WT, Baum A, Laue L, et al.** 1993 Sex differences in sensitivity of the hypothalamic-pituitary-adrenal axis. *Health Psychol.* 12:420–425.