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# Estrogen Treatment and Body Fat Distribution Are Involved in Corticotropin and Cortisol Response to Corticotropin-Releasing Hormone in Postmenopausal Women

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**To assess the effect of transdermal estrogen substitution on the hypothalamic-pituitary-adrenal (HPA) axis responsiveness/sensitivity and the impact of the antrophometric characteristics on these parameters, 20 postmenopausal women seeking treatment for the relief of postemenopausal symptoms were studied. They received transdermal 50 μg/d estradiol for 12 weeks (estrogen replacement therapy [ERT]). Patients were classified as low waist-to-hip ratio (WHR) (peripheral fat distribution women; n = 12) and high WHR (central fat distribution women; n = 8) according to the cut-off value of 0.85. Plasma hormone and lipid concentration were assessed at baseline and after 12 weeks of treatment. Results were compared with a group of 8 placebo-treated patients who served as controls. Corticotropin (ACTH) and cortisol (F) were expressed as fasting values, area under the curve (AUC), and time course over 90 minutes after corticotropin-releasing hormone (CRH) intravenous (IV) bolus (1 μg/kg body weight [BW]). Adrenal sensitivity to CRH stimulus was expressed as time course over 90 minutes and AUC of the F/ACTH molar ratio. The plasma F levels in response to ACTH stimulation did not change after ERT; however, a highly significant improvement of adrenal sensitivity was observed ($P < .01$). In fact, estrogen treatment significantly decreased the amount of ACTH produced after CRH stimulation, both as absolute time course and AUC ($P < .01$). No significant change was observed in controls. Considering body fat distribution, the high WHR group showed higher ACTH ($P < .01$), lower F/ACTH values, and superimposable F plasma values compared with the low WHR group. Estrogen treatment induced a significant ACTH reduction after CRH ($P < .01$) only in the high WHR group, whereas cortisol response was similar in both groups both before and after treatment. A significant negative correlation was found between WHR and adrenal sensitivity before treatment. ERT significantly improved adrenal sensitivity only in the low WHR group ($P < .01$). These data suggest that different mechanisms can prevail in the control of the HPA axis in menopause. Estrogens could exert different effects on the hypothalamic-pituitary axis, as well as on adrenal function, and these changes seem to be partially dependent on the pattern of body fat distribution.**



IT IS WELL KNOWN THAT aging is associated with critical changes in the functional organization of several neuroendocrine systems. However, it remains unclear whether these changes are a cause or a consequence of the aging process.

The hypothalamic-pituitary-adrenal (HPA) axis is 1 of the most important mediators of the neuroendocrine adaptative response of the organism to the external milieu.[1,2] Aging in animals is associated with a progressive alteration of the hippocampal and hypothalamic feedback regulation of HPA.[3] In humans, a progressive reduction of adrenal androgens (AA), such as dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) has been observed, while basal blood levels of cortisol (F) and adrenocorticotropin (ACTH) do not change.[4-7] Some investigators have shown that stimulation with corticotropin-releasing hormone (CRH) and ACTH bolus results in an unchanged production of adrenal steroids,[1,4,8-10] whereas others have found an age-related increased response after acute stimulation; moreover in the healthy older subjects, an impairment of dexamethasone suppression has been documented.[2,11]

The bulk of these studies suggest that functions of the HPA axis are possibly involved in the aging process. Furthermore, studies of both rats and humans have shown a greater sensitiv-

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ity of the adrenal glands to ACTH in females compared with males, suggesting a gender-related difference in the activity of the HPA axis.[12-14]

Conflicting results have also been reported on the effects of estrogen replacement therapy (ERT) on adrenal steroidogenesis, both in ovariectomized perimenopausal and postmenopausal women. Some investigators have reported that oral conjugated estrogens increased DHEA production after ACTH acute administration, suggesting an estrogen-mediated increased sensitivity of the adrenals[15]; other investigators reported that oral or intravenous (IV) estradiol administration did not alter the sensitivity and/or responsiveness of the adrenals to ACTH in producing androgens,[16,17] nor did it reverse the blunted production of AA.[18]

The aim of the present study was to assess the effect of physiologic transdermal estrogen substitution on HPA axis responsiveness/sensitivity and to distinguish whether something other than estrogen interfered with the HPA activity in postmenopausal women.

## SUBJECTS AND METHODS

After screening, 20 healthy white postmenopausal women, aged 47 to 63 years (52.8 ± 0.95; mean ± SE), who were attending the Gynecology Department of our University for the relief of menopausal symptoms, were submitted to the study protocol. Women were between 1 and 7 (2.3 ± 0.42; mean ± SE) years postmenopause; none had undergone hysterectomy or bilateral oophorectomy. Prior to beginning the study, assessment of plasma follicle-stimulating hormone (FSH >50 IU/L) and 17-$\beta$ estradiol (E2 <15 pg/mL) concentrations, mammography, transvaginal ultrasound examination for ovarian and endometrial thickness, and cervical cytology were performed. All were found to be normal or compatible with menopausal status. No patient was undergoing treatment known to affect lipid or glucose metabolism, nor had any taken steroids within the previous 6 months. None smoked more than 10 cigarettes/d or drank more than 300 g alcohol/wk. Diabetes or impaired glucose tolerance, breast cancer, liver or kidney parameter alterations, history of major thromboembolism, thyroid disease, and uncontrolled or $\beta$ blocker-treated hypertension (systolic blood pressure > 160 mm Hg or diastolic pressure > 90 mm Hg) were considered as exclusion criteria. The study was approved by the Ethics Committee of our university. Informed consent was obtained from each woman.

Estrogen patches delivering transdermal 50 $\mu$g 17-$\beta$ estradiol daily were administered to each subject (Epiestrol 50; Roerig Farmaceticals, Latina, Italy) for 12 weeks. Patches were changed twice weekly. The dosage of the transdermal compound was chosen as published data had demonstrated these values to be within the range of physiologic levels of estrogens during the menstrual cycle.[19]

After the end of the study, a progesterone-induced withdrawal was obtained. Placebo transdermal patches were administered to 8 women as a control group of the same study protocol.

Women attended the Obstetrics and Gynecology Unit before (time a) and again 12 weeks after the start of the study (time b). Plasma FSH, luteinizing hormone (LH), E2, estrone (E1), F, DHEAS, androstenedione (A), 17-hydroxyprogesterone (17-OHP), free testosterone (T), sex hormone-binding globulin (SHBG), and insulin-like growth factor-I (IGF-I) were assayed in basal samples.

After an overnight fast, IV catheters were placed in both forearms at 8 AM, 30 minutes before CRH bolus, with 1 catheter being used for IV injection and the other for blood sampling. Human CRH (Inalco, Milan, Italy) was injected as an IV bolus at a dose of 1 $\mu$g/kg body weight (BW). Blood samples were drawn in basal conditions (-30 and 0 minutes) and 15, 30, 60, and 90 minutes after stimulus. Immediately after collection, the blood samples were cooled and centrifuged at 4°C, and plasma samples were stored at -80°C until assayed. All samples from the same patient were analyzed in the same assay. An ultrasonographic pelvic evaluation was performed for the monitoring of endometrium and ovaries.

Anthropometric measurements (height and weight) were obtained to calculate body mass index (BMI) (kg/m$^2$). Patients with a BMI of 25 or greater were defined as obese; this cut-off point was approximately at the median. Body fat distribution was defined by the waist-to-hip ratio (WHR), W circumference being obtained as the minimum value between the iliac crest and the lateral costal margin and the H circumference as the maximum value over the buttocks. ACTH assay was performed by radioimmunoassay method with reagents obtained from the Nichols Institute (San Juan Capistrano, CA); the sensitivity of this assay in our laboratory was approximately 0.95 pg/mL. The other hormones were measured using commercial radioimmunoassay (RIA) kits (Radim, Pomezia, Italy). The intra-assay and the interassay coefficient of variation was below 8% and 15%, respectively, for all hormones.

ACTH and F were expressed as fasting values during a 90-minute time course after CRH bolus and were also expressed as area under the curve (AUC) after CRH, calculated by trapezoidal rule. Adrenal sensitivity to CRH stimulation was measured over a 90-minute time course, and values were expressed as AUC of the F/ACTH molar ratio.

The patients were classified as low WHR (peripheral fat distribution women) and high WHR (central fat distribution women) according to the cut-off value of 0.85.

Data were analyzed using SPSS (Statistical Package for Social Science; Release 5.0; Chicago, IL) on an IBM-compatible computer.

Kolmogorov-Smirnov test was performed to assess differences in the general shapes of the distributions. According to the nonparametric distribution of the study population, data were analyzed as median and range, but expressed as mean and standard error (SE).

A value of $P \leq .05$ was considered to be significant. Differences between baseline and treatments were analyzed by 1-way analysis of variance (ANOVA) and Scheffé test for post hoc comparisons. The Wilcoxon and Mann-Whitney paired and unpaired tests were used for nonparametric comparisons. Simple and multiple correlation analyses were performed by Spearmann and stepwise methods.

## RESULTS

All patients recruited completed the study protocol. No difference in glomerular filtration was observed in relation to treatment. No patient developed skin irritation, bleeding disturbances, or mastalgia. Blood pressure remained stable, and no changes in dietary habits were reported during ERT.

Table 1 shows the antrophometric and hormonal profiles of the study population.

Transdermal estrogen treatment significantly reduced FSH, LH, and IGF-I ($P < .01$) and increased $E_2$ ($P < .05$), DHEAS ($P < .01$) and SHBG ($P < .001$) plasma concentrations; on the contrary, ACTH, F, androstenedione, 17-OHP, free T, and E1 plasma concentrations remained unchanged. Moreover, a significant increase of WHR ($P < .05$), without variation of BMI value, was observed. No baseline difference was found between the studied and control groups; placebo treatment did not induce any variation in the above-mentioned parameters.

In Fig 1, the ACTH and F plasma concentrations after CRH injection are shown in relation to ERT and placebo. In a 90-minute time course study, the level of ACTH produced in response to CRH significantly decreased after ERT when com-

**Table 1. Anthropometric and Hormonal Profiles of All Studied Populations**

| | Treated (n = 20) | | | Controls (n = 9) | | |
|---|---|---|---|---|---|---|
| | A | B | A *v* B | A | B | A *v* B |
| BMI (kg/m$^2$) | 26.4 ± 1.4 | — | NS | 26.1 ± 1.2 | — | NS |
| WHR | 0.81 ± 0.02 | 0.85 ± 0.02 | .05 | 0.83 ± 0.02 | 0.84 ± 0.06 | NS |
| ACTH (pg/mL) | 17.9 ± 1.4 | 12.1 ± 1.5 | NS | 16.7 ± 1.8 | 14.5 ± 2.1 | NS |
| Cortisol (ng/mL) | 73.6 ± 5.6 | 80.5 ± 5.8 | NS | 77.6 ± 4.5 | 78.5 ± 5.1 | NS |
| 17-$\beta$ Estradiol (pg/mL) | 9.9 ± 1.5 | 56.4 ± 8.3 | .05 | 11.3 ± 1.8 | 15.2 ± 2.1* | NS |
| FSH (IU/L) | 72.1 ± 5.7 | 42.8 ± 3.6 | .01 | 66.8 ± 5 | 72 ± 3.5† | NS |
| LH (IU/L) | 25.4 ± 1.6 | 19.1 ± 1.4 | .01 | 28.2 ± 1.1 | 30.1 ± 1.2† | NS |
| Estrone (pg/mL) | 19.7 ± 3.7 | 25.1 ± 2.3 | NS | 17.6 ± 2.7 | 16.6 ± 3.1 | NS |
| Androstenedione (ng/mL) | 1.6 ± 0.1 | 1.5 ± 0.1 | NS | 1.4 ± 0.2 | 1.3 ± 0.1 | NS |
| 17-OHP (ng/mL) | 1.0 ± 0.1 | 0.9 ± 0.1 | NS | 1.1 ± 0.1 | 1 ± 0.2 | NS |
| DHEAS (ng/mL) | 779.5 ± 111.9 | 909.2 ± 126.7 | .01 | 813.5 ± 87.9 | 790.4 ± 95.7* | NS |
| Free T | 3.2 ± 0.4 | 2.5 ± 0.3 | NS | 3.5 ± 0.2 | 3.2 ± 0.3 | NS |
| IGF-I (ng/mL) | 184.37 ± 38.00 | 149.29 ± 50.3 | .01 | 182.41 ± 27.87 | 185.22 ± 42.41 | NS |
| SHBG (nmol/L) | 41.9 ± 4.1 | 59.2 ± 5.8 | .001 | 38.9 ± 4.1 | 41.6 ± 4.3† | NS |

NOTE. Values are expressed as mean ± SE. A, pretreatment values; B, posttreatment values.
Abbreviation: Free T, free testosterone index.
Controls *v* Treated: NS, not significant; *$P \leq .05$; †$P \leq .01$.

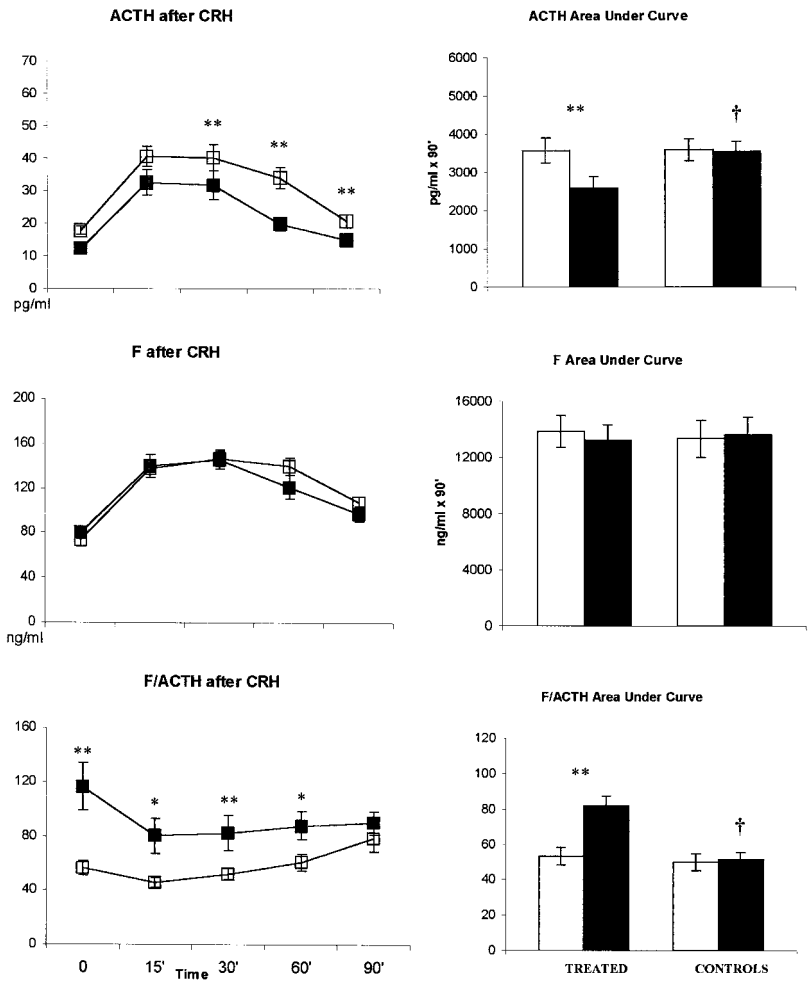


**Fig 1. Mean ± SE ACTH and cortisol (F) plasma values and adrenal sensitivity (F/ACTH) before and after estrogen treatment in postmenopausal women expressed as time course over 90 minutes and as AUC after CRH bolus. Data shown are before treatment (□, line and area) and after 12 weeks of unopposed transdermal estrogen therapy (■, line and area) in treated women and after placebo in the control group. Intragroup significance: line, □ *v* ■; **P* < .05; ***P* < .01; area, □ *v* ■; **P* < .05; ***P* < .01. Intergroup significance: ■ *v* ■; †*P* < .01; ††*P* < .01.**

pared with pretreatment values ($P < .01$ from 30 to 90 minutes and in the AUC values). However, F plasma levels did not change following treatment and remained similar to values before ERT treatment. No differences regarding the abovementioned parameters were observed before treatment between the study and the control groups. Placebo treatment failed to induce any variation in F and ACTH levels. Interestingly, ERT significantly increased the F/ACTH molar ratio for most of the time points (basally, 30 minutes: $P < .01$; 15 and 60 minutes: $P < .05$) and for the AUC values ($P < .01$), thus indicating an enhanced adrenal sensitivity to ACTH.

Based on WHR values, 12 patients (60%) were classified as low WHR and 8 (40%) as high WHR. In Table 2, the effect of ERT on antrophometric and hormonal profiles is shown according to body fat distribution. DHEAS and free T plasma values were higher in the high WHR group both before and after ERT ($P < .01$ and $P < .05$) (Table 2). Estrogen therapy induced a significant DHEAS increase only in the high WHR group without any change in free T values. The low WHR group had higher SHBG and lower IGF-I concentration ($P < .01$) both before and after therapy compared with the high WHR. However, estrogen treatment induced both a significant SHBG increase and an IGF-I decrease in the 2 study groups.

Women with high WHR had significantly higher ACTH levels before treatment ($P < .01$) and superimposable F plasma concentrations compared with subjects with low WHR. In both groups, ERT induced a significant decrease of basal ACTH concentrations, whereas it failed to affect F plasma levels.

The effect of CRH bolus on ACTH and F plasma levels before and after ERT in relation to body fat distribution is shown in Fig 2.

In women with high WHR, the ACTH response to CRH was significantly greater than that of the low WHR group; no difference in F plasma levels was observed. Therefore, the high WHR group exhibited significantly lower F/ACTH molar ratio compared with the other group.

After estrogen administration, a highly significant reduction in ACTH levels in response to CRH was observed in the high WHR group (AUC; 0, 15, 30, 60, minutes, data not shown; $P < .01$), whereas in the low WHR population, there was a slight, but not significant, decrease of such response. However, ACTH AUC values remained significantly higher in the high WHR group. Cortisol response was similar in both groups and in relation to treatment.

Figure 2 also shows the analysis of F/ACTH ratio, which indicates that ERT induced an increase in adrenal sensitivity in the low WHR group, while, although there was an improvement in the high WHR group, this value did not reach statistical significance.

Figure 3 shows the significant negative correlation between WHR and F/ACTH ratio ($r = -.51$; $P < .02$) before treatment.

## DISCUSSION

The present report investigates the ACTH and cortisol levels in response to CRH in postmenopausal women. In addition, the effect of transdermal estradiol treatment and the relationship of such responses to body fat distribution were studied.

Results of our study show that 12 weeks of transdermal estradiol administration in postmenopausal women caused a decreased production of the ACTH response to CRH without any change in F plasma concentration. This, in turn, causes an increased F/ACTH ratio, which can be interpreted as an increased sensitivity of adrenal to ACTH circulating concentrations.

These data indirectly agree with those of other investigators,[20-23] who demonstrated a decreased adrenal sensitivity to ACTH during aging without any change in F level. In our study, although estrogen therapy did not induce any variation in total F plasma levels, the effect of ERT on the unbound F concentration was not supported by cortisol-binding globulin (CBG) plasma levels.

In fact, an increase of the unbound F fraction in theory partially explains the changes in ACTH response to CRH. Even if our study lacks the evaluation of CBG, which could be of use for the evaluation of the unbound cortisol fraction, some considerations should be taken into account: in other studies, the increase in CBG levels[24,25] during gestation resulted in increased binding,[26] which contributed to the elevation in total cortisol concentration. Moreover, the ratio of bound to free cortisol

**Table 2. Effect of Treatment on Anthropometric and Hormonal Profiles According to Body Fat Distribution**

| | Low WHR (n = 12) | | Intragroup Significance *P* | High WHR (n = 8) | | Intragroup Significance *P* | Intergroup Significance *P* | |
|---|---|---|---|---|---|---|---|---|
| | A | B | | A | B | | A | B |
| BMI (kg/m$^2$) | 28.4 ± 2.0 | — | | 24.8 ± 0.6 | — | | .01 | NS |
| WHR | 0.77 ± 0.01 | 0.82 ± 0.02 | .01 | 0.88 ± 0.01 | 0.88 ± 0.02 | NS | .001 | NS |
| ACTH (pg/mL) | 15.1 ± 1.8 | 10.7 ± 1.9 | NS | 22.0 ± 1.6 | 14.2 ± 2.1 | .01 | .01 | .01 |
| F (ng/mL) | 75.6 ± 9.4 | 84.8 ± 6.8 | NS | 70.5 ± 1.7 | 74.0 ± 10.3 | NS | NS | NS |
| 17-$\beta$ Estradiol (pg/mL) | 11.6 ± 2.3 | 52.0 ± 8.7 | .01 | 7.2 ± 0.7 | 65.3 ± 18.6 | .01 | NS | NS |
| Estrone (pg/mL) | 20.5 ± 6.2 | 26.8 ± 3.2 | NS | 18.5 ± 0.6 | 21.7 ± 2.0 | NS | .05 | NS |
| Androstenedione (ng/mL) | 1.72 ± 0.2 | 1.59 ± 0.2 | NS | 1.5 ± 0.3 | 1.2 ± 0.2 | NS | NS | NS |
| 17 OHP (ng/mL) | 1.1 ± 0.2 | 0.9 ± 0.1 | .01 | 0.8 ± 0.1 | 0.9 ± 0.2 | NS | NS | NS |
| DHEAS (ng/mL) | 560.7 ± 95.8 | 622.8 ± 91.7 | NS | 1107.7 ± 194.1 | 1338.7 ± 212.7 | .01 | .01 | .01 |
| Free T | 2.39 ± 0.4 | 1.67 ± 0.2 | NS | 4.1 ± 0.7 | 3.7 ± 0.5 | NS | .05 | .01 |
| IGF-I (ng/mL) | 192.00 ± 50.2 | 155.5 ± 65.2 | .02 | 179.8 ± 27.9 | 135.8 ± 27.71 | .05 | NS | NS |
| SHBG (nmol/L) | 53.2 ± 4.3 | 70.2 ± 7.1 | .01 | 24.9 ± 1.0 | 42.7 ± 6.8 | .05 | .001 | .05 |

NOTE. Values are expressed as mean ± SE. A, pretreatment values; B, posttreatment values.

Abbreviation: Free T, free testosterone index; NS, not significant.

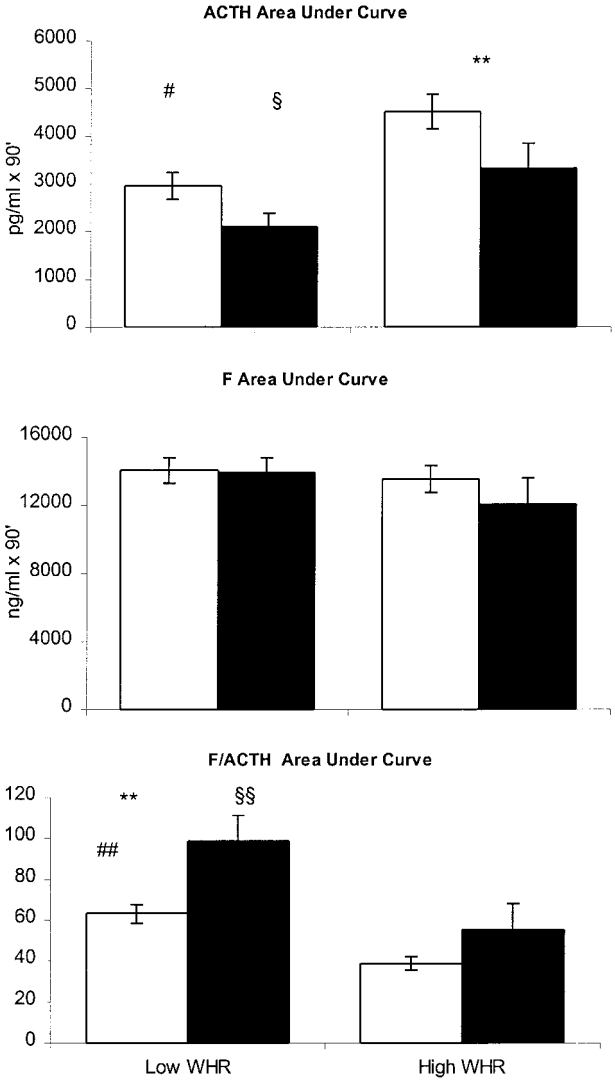


**Fig 2. Mean ± SE ACTH and cortisol (F) plasma values and adrenal sensitivity (F/ACTH) before and after estrogen treatment in postmenopausal women expressed as AUC after CRH bolus. Data are shown before (□) and after (■) 12 weeks of unopposed transdermal estrogen in low and high WHR groups. Intragroup significance: □ *v* ■; ***P* < .01. Intergroup significance: area, (□) high WHR *v* low WHR: #*P* < .05; ##*P* < .01; (■) high WHR *v* low WHR: §*P* < .05; §§*P* < .01.**

is unchanged,[26] and free cortisol levels increase in proportion to total cortisol concentration. More recent data[25,27,28] suggest that ACTH concentrations are increased, thus indicating a lack or an adjustment of the set point in the cortisol negative feedback mechanism, which controls ACTH secretion.[29,30] However, in pregnancy, while some of the changes are induced by estrogen, several other factors may contribute to modulating cortisol homeostasis, such as progesterone, which competes with cortisol for CBG,[26] chorionic gonadotropin,[27-31] adrenal hyperresponsivnes to ACTH, and shunt of cortisol into the fetoplacental compartment. However, increased levels of free cortisol have been described in pregnancy despite a CBG increase.[26]

It should be emphasized that this study reports on the effects of pure estrogen, and that our findings cannot be compared with those in pregnancy due to the different circulating estrogen concentrations and the different exposure in terms of time-dependent induction.

In this matter, Lapidus et al[32] failed to find in postmenopausal women any relationship between CBG and WHR; Laufer et al,[33] using a transdermal therapeutic system delivering estradiol, did not find any variation in CBG plasma concentration. Baumann et al,[34] by using a supraphysiologic dose of ethinyl estradiol in normal women (100 $\mu$g/d), conclude that estrogen does not increase integrated free cortisol. Moreover, because CBG and SHBG are 2 liver-derived proteins, an estrogen-induced increase of CBG is to be expected, as observed in our study for SHBG. Therefore, it seems unlikely that the decreased ACTH response to CRH after therapy observed in all, as well as in high WHR women, could be due to an increased inhibitory feedback at the pituitary level, as a consequence of an increase of the free cortisol concentration.

Previous data suggest that estrogens may affect adrenocortical function, particularly in estrogen-deficient postmenopausal women, leading to the hypothesis that hypoestrogenism of menopausal women may be responsible, at least in part, for adrenopause.[15] Those studies were, however, conducted using supraphysiologic doses of estrogens, which may not accurately reflect the adrenocortical response to physiologic endogenous $E_2$ levels (early follicular phase). To overcome this problem, transdermal daily delivery of 50 $\mu$g of 17-$\beta$ estradiol was used, achieving an $E_2$ plasma concentration of 56 pg/mL.

In different experimental conditions, the impact of $E_2$ therapy on adrenal function was evaluated by pharmacologic stimulatory tests, which were not able to detect subtle changes in adrenocortical sensitivity.[4,35,36] In our study, a more physiologic stimulus on the adrenal gland was performed by CRH IV administration. Although after treatment the ACTH secretion was significantly reduced, cortisol secretion was unchanged, thus demonstrating that the adrenal reserve was not impaired. Interestingly, after ERT, DHEAS plasma concentration increased significantly.

It is likely, as suggested by many investigators,[37,38] that aging, as well as reduction in estrogen level due to menopause,

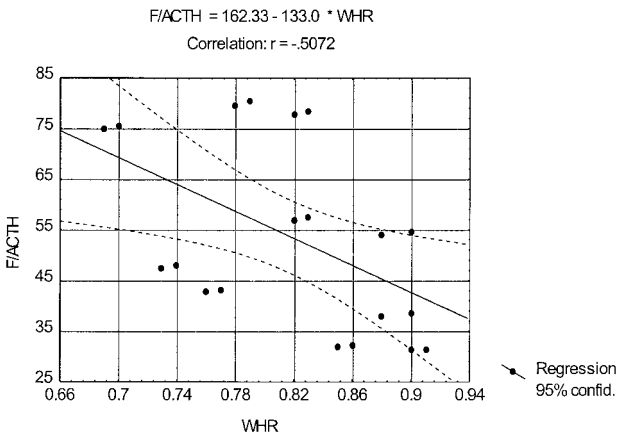


**Fig 3. Linear correlation between adrenal sensitivity (F/ACTH) and WHR before treatment.**

could be related to a decrease in ACTH adrenal receptors and/or a reduction in their binding affinity.

The mechanism by which estrogens reduce the ACTH pituitary response to CRH is complex. Laatikainen[39] found in menopause a blunted opioidergic tone, probably as a consequence of estrogenic deprivation, that could result in a reduction of $\beta$-endorphin circulating levels, which in turn, may induce a decreased CRH production by hypothalamic neurons.

The hypothesis of a correlation between the estrogen milieu and opioid tone is reinforced by the observation that $\beta$ endorphins gradually increase during pregnancy in a manner directly related to estrogen plasma concentration.[40]

Related to this, Pasquali et al[41,42] have shown in fertile women a strict linear correlation between body fat distribution and opioid tone; women with a prevalence of central body fat distribution (high WHR) exhibited higher circulating $\beta$-endorphins levels compared with those with a peripheral fat distribution (low WHR). Another observation indicated that the administration of CRF resulted in a significantly higher increase of $\beta$ endorphins in abdominal than in peripheral fat distribution women.[43] All of these findings seem to indicate that the hyperactivity of the opioid system in obesity is probably limited only to the abdominal obese phenotype, at least in women.

Related to this, in our study, women with abdominal fat distribution (high WHR) have an increased pituitary sensitivity to CRH stimulus, as shown by a higher ACTH response to bolus, evaluated by a time course or as AUC, when compared with women with prevalent peripheral body fat distribution (low WHR).

Estrogen treatment over 3 months significantly decreased pituitary sensitivity to CRH stimulus in high WHR women, but failed to exert this effect in the low WHR group. However, in the low WHR group, there was a higher adrenal sensitivity (F/ACTH ratio) to pituitary stimulus compared with the high WHR one. Estrogen therapy augmented such parameters only in the low WHR group. The relationship between body fat distribution and pituitary adrenal function in postmenopausal women has been studied previously. Grenman et al[44] did not find a significant association between WHR and fasting serum concentration of cortisol in 25 obese women. Hauner et al[45] demonstrated a positive, but weak correlation, between cortisol and WHR in a sample of 40 obese women. Kaye and Folsom[46] suggested, studying a group of 88 women, that neither cortisol nor ACTH levels were associated with body fat distribution. In our study, neither ACTH nor cortisol were correlated to WHR; however, WHR appears to influence adrenal sensitivity (expressed as F/ACTH ratio) because of the negative linear correlation between these 2 parameters ($r = -.51$; $P < .02$).

In normal conditions, $\beta$-endorphin secretion is regulated by the same mechanisms controlling pro-opiomelanocortin processing,[40] and CRF administration has been found to increase $\beta$ endorphins concomitantly with ACTH and $\beta$ lipotropin. Theoretically, it could also be possible that the increased circulating $\beta$-endorphin levels[41] originate both from the pituitary, as well as from the intestinal tract.

The results of our study suggest that, before estrogen, women with abdominal fat distribution exhibit higher circulating $\beta$-endorphin levels compared with those with peripheral fat distribution, and this could induce a higher pituitary and a lower adrenal sensitivity. This result could explain the negative linear correlation between WHR and F to ACTH ratio. It seems possible that the supposed increased opioid activity in the present study is part of a more complex neuroendocrine alteration, which appears to be present in individuals with abdominal obesity, and includes hyperactivity of the HPA axis, hyperglycemia, hyperinsulinemia, and a subsequent increased risk of cardiovascular disease. The absence of significant estrogen probably induced an increase of adrenal sensitivity in the high WHR compared with low WHR women, whereas a significant decrease of ACTH-stimulated response is due to a prevalent central driving of opioid tone in the abdominal fatty women compared with a peripheral driving presumed in the low WHR group.

It is interesting to note that the results of posttreatment observation seem to be dependent on the improvement of $E_2$ plasma levels, because WHR values remained unchanged in the high WHR group after therapy.

In conclusion, data of the present study indicate that different mechanisms may contribute to the control of the HPA axis in menopause. Estrogen may exert different effects on the hypothalamic-pituitary, as well as on the adrenals, and it appears that these changes could be partially dependent on the pattern of body fat distribution.

## REFERENCES

1. Born J, Ditschuneit I, Schreiber M, et al: Effects of age and gender on pituitary-adrenocortical responsiveness in humans. Eur J Endocrinol 132:705-711, 1995

. Ferrari E, Magri F, Dori D, et al: Neuroendocrine correlates of the aging brain humans. Neuroendocrinology 61:464-470, 1995

3. Cizza G, Gold PW, Chrousos GP: Aging is associated in the 344/N Fischer rat with decreased stress responsivity of central and peripheral catecholaminergic systems and impairment of the hypothalamic-pituitary-adrenal axis. Ann N Y Acad Sci 771:491-511, 1995

4. Pavlov EP, Harman SM, Chrousos GP, et al: Responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. J Clin Endocrinol Metab 62:767-772, 1986

5. Parker L, Gral T, Perrigo V, et al: Decreased adrenal androgen sensitivity to ACTH during aging. Metabolism 30:601-604, 1981

6. Ohashi M, Fujio N, Kato K, et al: Aging is without effect on the pituitary-adrenal axis in men. Gerontology 32:335-339, 1986

7. Liu CH, Laughlin GA, Fischer UG, et al: Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: Evidence for a reduced 17,20-desmolase enzymatic activity. J Clin Endocrinol Metab 71:900-906, 1990

8. Sapolsky RP, Krey LP, McEwen BS: Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. J Neurosci 5:1222-1227, 1985

9. Friedman M, Green MF, Sharland DE: Assessmentof hypothalamic-pituitary-adrenal function in the geriatric age group. J Gerontol 24:292-297, 1969

10. Waltman C, Blackman MR, Chrousos GP, et al: Spontaneous and glucocortcoid-inhibited adrenocorticotropic hormone and cortisol secretion are similar in healthy young and old men. J Clin Endocrinol Metab 73:495-502, 1991

11. Dilman VM, Ostroumova MN, Tsyrlina EV: Hypothalamic mechanism of ageing and of specific age pathology. II. On the sensitivity threshold of hypothalamo-pituitary complex to homeostatic stimuli in adaptative homeostasis. Exp Gerontol 14:175-181, 1979

12. Horrocks PM, Jones AF, Ratcliffe WA, et al: Pattern of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. Clin Endocrinol 32:127-134, 1990

13. Roelfsema F, van den Berg G, Frolich M, et al: Sex-dependent alteration in cortisol response to endogenous adrenocorticotropin. J Clin Endocrinol Metab 77:234-240, 1993

14. Kitay JI, Coyne MD, Newsom W, et al: Relation of the ovary to adrenal corticosterone production ad adrenal enzyme activity in the rat. Endocrinology 77:902-908, 1965

15. Lobo RA, Gobelsmann U, Brenner PF, et al: The effects of estrogen on adrenal androgens in oophorectomized women. Am J Obstet Gynecol 142:471-478, 1982

16. Anderson DC, Yen SSC: Effects of estrogens on adrenal 3B-hydroxy-steroid dehydrogenase in ovariectomized women. J Clin Endocrinol Metab 43:561-570, 1976

17. Rose DP, Fern M, Liskowski L, et al: Effect of treatment with estrogen conjugates on endogenous plasma steroids. Obstet Gynecol 49:80-82, 1977

18. Slayden SM, Crabbe L, Bae S, et al: The effect of 17 B estradiol on adrenocortical sensitivity, responsiveness, ad steroidogenesis in postmenopausal women. J Clin Endocrinol Metab 83:519-524, 1998

19. Cucinelli F, Paparella P, Soranna L, et al: Differntial effect of transdermal estrogen plus progestagen replacement therapy on insulin metabolism in postmenopausal women: Relation to their insulinemic secretion. Eur J Endocrinol 140:215-223, 1999

20. Hauger RL, Thrivikraman KV, Plotsky PM: Age related alterations of hypothalamic-pituitary adrenal axis function in male Fisher 344 rats. Endocrinology 134:1528-1536, 1994

21. Dohm G: The prepubertal and pubertal growth of the adrenal (adrenarche). Beitr Pathol 150:357-377, 1973

22. Wilkinson CW, Peskind ER, Raskind MA: Decreased hypothalamic-pituitary-adrenal axis sensitivity to cortisol feedback inhibition in human aging. Neuroendocrinology 65:79-90, 1997

23. Parker L, Odell W: Control of adrenal androgen secretion. Endocr Rev 1:392-410, 1980

24. DeMoor P, Steeno O, Brosens I, et al: Data on transcortin activity in human plasma as studied by gel filtration. J Clin Endocrinol Metab 26:71-78, 1966

25. Wilson EA, Fina AE, Rayburn W, et al: Corticosteroid-binding globulin and estrogens in maternal and cord blood. Am J Obstet Gynecol 135:215-218, 1979

26. Rosenthal HE, Slaunwhite WR, Sandberg AA: Transcortin: A corticosteroid binding protein of plasma. X. Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. J Clin Endocrinol Metab 29:352-367, 1969

27. Rees LH, Burke CW, Chard T, et al: Possible placental origin of ACTH in normal human pregnancy. Nature 254:620-622, 1975

28. Carr BR, Parker CR, Madden JD, et al: Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. Am J Obstet Gynecol 139:416-422, 1981

29. Jolivet L, Blanchier J, Gautray JP, et al: Blood cortisol variations during late pregnancy and labor. Am J Obstet Gynecol 119:775-783, 1974

30. Nolten WE, Lindheimer MD, Rueckert PA, et al: Diurnal patterns and regulation of cortisol secretion in pregnancy. J Clin Endocrinol Metab 51:466-472, 1980

31. Gennazzani AR, Fraioli F, Hurlimann J, et al: Immunoreactive ACTH and cortisol plasma levels during pregnancy. Detection and partial purification of corticotrophin-like placental hormone: The human chorionic corticotrophin (HCC). Clin Endocrinol (Oxf) 4:1-14, 1975

32. Lapidus L, Linstedt G, Lundberg PA, et al: Concentrations of sex–hormone binding globulin and corticosteroid binding globulin in serum in relation to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. Lin Chem 32:146-152, 1986

33. Laufer LR, De Fazio JL, Lu IKH, et al: Estrogen replacement therapy by transdermal estradiol administration. Am J Obstet Gynecol 1:533-554, 1983

34. Bauman G: Estrogens and the hypothalamo-pituitary adrenal axis in man: Evidence for normal feedback regulation by corticosteroids. J Clin Endocrinol Metab 57:1193-1197, 1983

35. Sapolsky RM, Krey LC, McEwen BS: Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. Proc Natl Acad Sci USA 81:6174-6177, 1984

36. Reul JMHM, Rothuizen J, DeKloet ER: Age-related changes in the dog hypothalamic-pituitary-adrenocortical system: Neuroendocrine activity and corticosteroids receptors. J Steroid Biochem Mol Biol 40:63-69, 1991

37. Roth G: Hormone receptor changes during adulthood and senescence: Significance for aging research. Fed Proc 38:1910-1014, 1979

38. Wishnow R, Lifrak E, Chen C: Mode of action of Vibrio cholerae enterotoxin in cultured adrenal tumor cells. J Infect Dis 133:108-114, 1976 (suppl)

39. Laatikainen TJ: Corticotropin-releasing hormone and opioid peptides in reproduction and stress. Ann Med 23:489-496, 1991

40. Raisanen I: Plasma levels and diurnal variation of $\beta$-endorphins, $\beta$-lipotropin and corticotropin during pregancy and early puerperium. Eur J Obstet Gynecol Reprod Biol 27:13-20, 1988

41. Pasquali R, Casimirri F, Cantobelli S, et al: B-endorphin response to exogenous corticotrophin-releasing hormone in obese women with different patterns of body fat distribution. Int J Obes Relat Metab Disord 17:593-596, 1993

42. Pasquali R, Cantobelli S, Casimirri F, et al: Hypothalamic–pituitary-adrenal function in obese women with different pattern of body fat distribution. 74th Annual meeting of Endocrine Society, San Antonio, TX, June 24-27, 1992

43. Zellisen PMJ, Kopperschaar HPF, Erkelens DW, et al: $\beta$-endorphins and adrenocortical function in obesity. Clin Endocrinol 35:369-372, 1991

44. Grenman S, Ronnemaa T, Irjala K, et al: Sex steroids, gonadotropin, cortisol, and prolactin levels in healthy, massively obese women: Correlation with abdominal fat cell size and effect of weight reduction. J Clin Endocrinol Metab 63:1257–1261, 1986

45. Hauner H, Ditschuneit HH, Pal SB, et al: Fat distribution, endocrine and metabolic profile in obese women with and without hirsutism. Metabolism 37:281-286, 1988

46. Kaye SA, Folsom AR: Is serum cortisol associated with body fat distribution in postmenopausal women? Int J Obes 15:437-439, 1991