Age-related changes in the female hormonal environment during reproductive life

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Previous studies have indicated that serum levels of follicle-stimulating hormone rise with age during the female reproductive life, but the effect on other hormones is not clear. We studied the effects of age, independent of pregnancy, by comparing serum hormone levels in two groups of nulliparous, premenopausal women aged 18 to 23 and 29 to 40 years. We found that increased age during reproductive life is accompanied by a significant rise in both basal and stimulated serum follicle-stimulating hormone levels. This was accompanied by an increase in the serum level of estradiol-17β and the urine levels of estradiol-17β and 17β-estradiol-17-glucosiduronate. The serum level of estrone sulfate decreased with age. Serum and urine levels of other estrogens were unchanged. The basal and stimulated levels of luteinizing hormone were also unchanged. There was a significant decrease in basal and stimulated serum prolactin levels. Serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate decreased with age, but serum testosterone was unchanged. It is concluded that significant age-related changes in the female hormonal environment occur during the reproductive years. (AM J OBSTET GYNECOL 1987;157:312-7.)

Key words: Aging and hormones, estrogens, androgens, gonadotropins, prolactin

Although much information is available regarding the changes in the female hormonal environment at menarche and menopause, age-related hormonal changes during the intervening years have not been extensively investigated. Previous studies in regularly menstruating women have suggested that serum follicle-stimulating hormone (FSH) levels rise with increasing age, whereas luteinizing hormone (LH) levels remain unchanged.[1-3] However, apart from the gonadotropins, the effects of age on other hormones, including estrogens, during reproductive life are not clear.

Investigation in this area is complicated by our previous findings[4,5] that a first pregnancy causes long-term changes in the hormonal environment. Thus the effects of age and pregnancy must be separately determined. Previous studies[1-3] did not control for parity. To determine the effects of age during reproductive life, independent of pregnancy, we studied two groups of nulliparous women, a younger group aged 18 to 23 years and an older group aged 29 to 40 years. The following hormones were measured: basal and stimulated serum levels of prolactin, FSH, and LH; serum levels of estrone (E_1), estradiol-17β (E_2), estriol (E_3), and E_1 sulfate; urinary levels of total E_1, E_2 and E_3, together with their glucosiduronates were measured. The serum levels of the androgens testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS) were also measured.

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Methods

Subjects. Thirty-five normal healthy female volunteers aged 18 to 23 years and 29 normal healthy female volunteers aged 29 to 40 years were recruited by a mass media campaign and studied after informed consent was obtained. The mean age in years ± SD was 20.4 ± 1.33 for the younger aged group and 33.9 ± 3.03 for the older aged group. All subjects were nulliparous, had a history of regular menstrual cycles, and had never taken oral contraceptives or other hormones. None were on medication at the time of the study. After medical histories were obtained and physical examinations were performed, the subjects with conditions known to be associated with abnormal hormone levels or others that would affect the study's outcome were excluded.

Experimental protocol. All subjects were studied during the early follicular phase (days 2-4) of the menstrual cycle. On day 2, each subject collected a 24-hour

urine sample. On day 3, a blood sample was collected, and a gonadotropin-releasing hormone (GnRH) stimulation test was performed. On day 4, a perphenazine-prolactin stimulation test was done (see below). Both stimulation tests were started between 8 and 9 AM after the subjects fasted overnight. All serum and urine samples were stored at −80° C until analyzed.

GnRH stimulation test. A 19-gauge butterfly needle was inserted into a forearm vein to facilitate blood collection. The needle was kept open with a heparin-saline solution. Baseline blood samples were collected for LH and FSH assays. A 100 μg bolus of GnRH was then given intravenously. Thereafter, blood samples were collected at 30-minute intervals for 90 minutes for LH and FSH assays. Serum was separated and stored as described above.

Perphenazine-prolactin stimulation test.[6] After insertion of a 19-gauge butterfly needle into a forearm vein, a baseline blood sample was collected for prolactin determination. Perphenazine (8 mg) was then administered orally, and further blood samples were collected at 3, 4, and 5 hours after the perphenazine administration to measure prolactin levels. The serum was separated and stored as above.

Procedures for hormone assays

Analysis of LH, FSH, and prolactin. Serum levels of LH, FSH, and prolactin were measured by radioimmunoassay (RIA) with commercial kits obtained from Serono Diagnostics, Inc., Braintree, Massachusetts. The reagents for measuring serum prolactin were obtained from Hybritech, San Diego, California. The intra-assay and interassay coefficients of variation established in our laboratory were LH, 2.8% and 4.4%; FSH, 3.1% and 7.7%; prolactin, 2.8% and 6.2%.

Analysis of estrogens. Serum levels of E_1, E_2, and E_3 were measured by RIA after ether extraction as previously described.[7] The intra-assay and interassay coefficients of variation for each estrogen were E_1, 6.8% and 11.2%; E_2, 5.4% and 8.2%; E_3, 6.1% and 9.1%. The E_1 sulfate level in serum was determined by direct RIA without previous hydrolysis after ether extraction to remove the free estrogens.[8] The intra-assay and interassay coefficients of variations were 6.4% and 9.5%, respectively. Urinary levels of the estrogen conjugates, E_1 glucosiduronate, E_2 17-glucosiduronate, and E_3-16α-glucosiduronate were determined by specific RIA as previously developed in our laboratory.[9] The intra-assay and interassay coefficients of variation for each compound were E_1 glucosiduronate, 6.9% and 10.8%; E_2 17-glucosiduronate, 6.5% and 10.2%; E_3 16α-glucosiduronate, 6.6% and 11.0%. The levels of E_1, E_2, and E_3 in urine were determined after hydrolysis with β-glucuronidase (*Helix pomatia*, type H-3, Sigma Diagnostics, St. Louis, Missouri). The free estrogen was extracted with ether and measured by specific RIA for the particular estrogen as previously described.[7] The coefficients of variation were the same as those of the free estrogens in serum.

Analysis of androgens. Serum levels of testosterone were measured by RIA after ether extraction as previously described.[10] The intra-assay and interassay coefficients of variation were 4.9% and 7.9%, respectively. Serum levels of DHEA were measured by RIA after ether extraction and LH-20 chromatography as previously described.[11] The intra-assay and interassay coefficients of variation were 6.4% and 10.1%, respectively. Serum levels of DHEAS were measured by RIA with a specific antiserum as previously described.[12] The intra-assay and interassay coefficients of variation were 5.4% and 8.2%, respectively. All the measurements for each hormone were done in a single assay.

Statistical analysis. Height and weight between younger and older aged groups were compared with the use of the *t* test for differences between means. A one-way analysis of variance was used to compare basal levels of the protein hormones (FSH, LH, prolactin) and the steroid hormones between younger and older aged subject groups.

A two-way repeated measures analysis of variance was used to compare stimulated levels for FSH, LH, and prolactin between groups, with main effects "younger, older" as the "between factor" and "time" (i.e., time of sampling) as the "within" or repeated measures factor.

The square root or log transformation was used to stabilize heterogeneous variances where appropriate. Subjects with missing data on the variable of interest were omitted from the analysis. Values outside the mean ± 2 SD were considered to be outliers and were also omitted. A value for $p \leq 0.05$ was taken to indicate statistical significance. Standard errors are given for descriptive purposes but are not used in the significance tests.

Results

Subjects. The mean height in inches ± SD for the younger aged group was 65.0 ± 2.39 and for the older aged group was 65.4 ± 2.31. There was no significant difference between the two means. The mean body weight expressed as percent of ideal body weight ± SD was 106.9 ± 15.6 for the younger aged group and 112.0 ± 11.4 for the older aged group. The mean body weights were not significantly different between the two groups.

Protein hormones. The basal serum FSH level was significantly higher ($p < 0.01$) in the older aged group than in the younger aged group (Table I). The mean values for stimulated FSH (Fig. 1) were also significantly higher in the older aged group ($p < 0.05$). There was no significant difference between the groups in

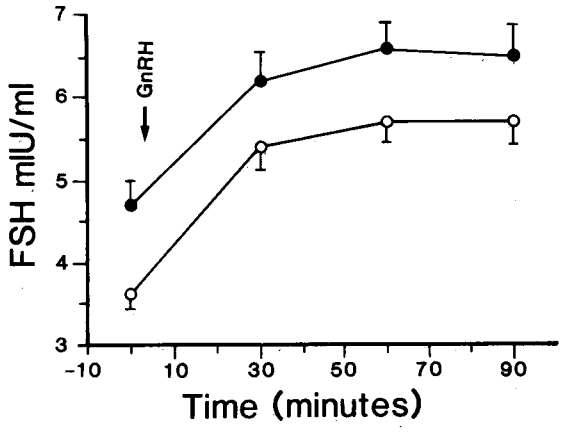

Fig. 1. Basal (time zero) and stimulated (30, 60, 90 minutes) levels of serum FSH after the intravenous administration of GnRH (100 μg) to subjects in the younger *(open circles)* and older *(closed circles)* aged groups. Both basal and stimulated values in the older aged group were significantly higher than in the younger aged group ($p < 0.01$).

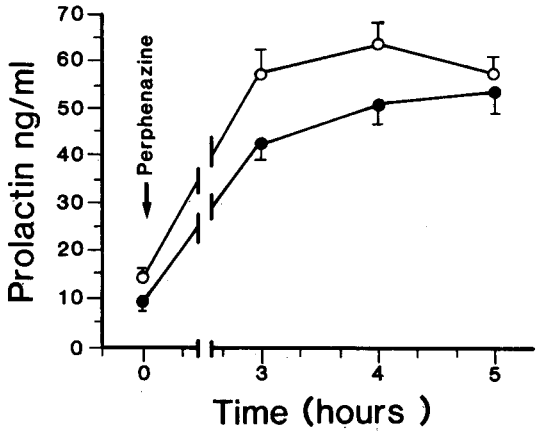

Fig. 3. Basal (time zero) and stimulated (3, 4, 5 hours) levels of serum prolactin after the oral administration of 8 mg perphenazine to subjects in the younger *(open circles)* and in the older *(closed circles)* aged groups. Both basal and stimulated values in the older aged group were significantly lower than in the younger aged group ($p < 0.01$).

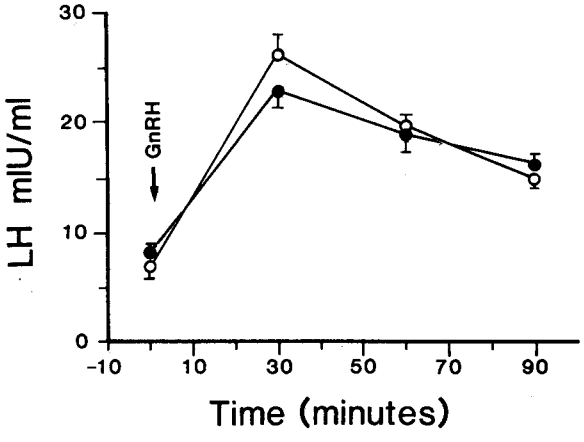

Fig. 2. Basal (time zero) and stimulated (30, 60, 90 minutes) levels of serum LH after the intravenous administration of GnRH (100 μg) to subjects in the younger *(open circles)* and older *(closed circles)* aged groups. There was no significant difference between the two groups.

either the basal serum LH or in stimulated serum LH values (Fig. 2). There was a significant ($p < 0.01$) decrease in the basal serum prolactin level in the older aged group. The stimulated prolactin values (Fig. 3) were also significantly lower ($p < 0.01$) in the older aged group.

Estrogens. The mean serum levels of E_2, E_1 sulfate, E_1, and E_3 for both groups are shown in Table II. The serum E_2 level was significantly higher ($p < 0.01$) in the older aged group, but the serum level of E_1 sulfate was significantly lower ($p < 0.01$). The serum E_1 level was lower in the older aged group, but the difference between the groups was not statistically significant. Serum E_3 levels were similar in both groups.

The mean levels of E_2, E_1, E_3, and E_2 17-glucosiduronate in urine are shown in Table III. There were significant differences in urine levels of E_2 and E_2 17-glucosiduronate between the groups, with the older aged group having higher levels than the younger aged group. There was no significant difference between the groups in levels of E_1, E_3, E_1 glucosiduronate, and E_3 16α-glucosiduronate in urine.

Androgens. Mean serum levels of testosterone, DHEA, and DHEAS are shown in Table IV. There was no significant difference between the groups for serum testosterone. However, serum DHEA and DHEAS levels were significantly lower ($p < 0.01$) in the older aged group.

Comment

This study was designed to demonstrate the effects of age on the hormonal environment in women of reproductive age. Apart from cyclic alterations of the menstrual cycle, a first pregnancy induces long-term changes in the hormonal environment.[4, 5] Thus the effects of age and pregnancy must be separately determined. In this study we have excluded effects of pregnancy that would confound the issue by using nulliparous subjects only.

A second confounding variable is differences in serum hormone levels caused by circadian rhythm and/or episodic or pulsatile secretion. Such variation could complicate the interpretation of serum hormone values from single blood samples. In this study the time

Table I. Mean basal serum levels of FSH, LH, and prolactin in the younger and the older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
FSH (mIU/ml)				
Mean	3.60	4.69	+1.09	<0.01
SE	0.22	0.42		
n	35	28		
LH (mIU/ml)				
Mean	7.20	8.09	+0.89	NS
SE	0.66	0.87		
n	35	28		
Prolactin (ng/ml)				
Mean	14.2	9.36	4.84	<0.01
SE	1.87	0.88		
n	31	28		

n = Number of subjects; difference = the difference between the two means; p = the probability that the difference between means could occur by chance; NS = not significant. SE given for descriptive purposes only; it is not used in the tests for significance.

Table II. Mean serum levels (picograms per milliliter) of E_2, E_1 sulfate, E_1, and E_3 in the younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
E_2				
Mean	61.1	74.1	+13.0	<0.01
SE	4.3	5.3		
n	34	28		
E_1 sulfate				
Mean	1043	851	−192	<0.01
SE	75	91		
n	35	27		
E_1				
Mean	72.2	62.2	−10.0	0.09
SE	5.1	5.5		
n	32	27		
E_3				
Mean	13.4	14.7	+1.30	NS
SE	0.73	0.95		
n	34	26		

See Table I for definitions.

of day at which samples were collected was controlled by obtaining all samples for basal hormone measurements in the morning (between 8 and 9 AM), thereby minimizing the effects of circadian changes. In addition, the GnRH and perphenazine stimulation tests that were used are independent of pulsatile secretion and circadian changes.

A third variable that could impact on the hormonal environment is body weight. However, this variable was discounted by finding no significant difference between the two groups of women.

Previous studies of the effects of age on serum FSH and LH levels have dealt with only basal levels. Our results in relation to basal FSH and LH levels are consistent with those reports[1-3] that suggest a significant increase in basal FSH but no change in LH. However, by using only nulliparous women in this study, we have shown that pregnancy could not have been a factor in these effects.

The GnRH stimulation test used in this study also offers additional information about age-related changes, namely, an estimate of the pituitary secretory capacity of FSH and LH. We found an increased response of FSH to GnRH stimulation. No significant change in the response of LH to GnRH stimulation was seen in the older aged group. Therefore our results suggest that both basal serum levels and pituitary secretory capacity for FSH are affected by the aging process, whereas those of LH are not affected. The mechanism for the divergent basal levels and the response

Table III. Mean levels (micrograms per 24 hours) of total E_2, total E_1, total E_3, and E_2 17-glucosiduronate in the urine of the younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
Total E_2				
Mean	3.93	5.17	+1.24	<0.01
SE	0.27	0.45		
n	35	29		
Total E_1				
Mean	6.97	7.85	+0.88	NS
SE	0.68	0.86		
n	35	28		
Total E_3				
Mean	16.9	22.5	+5.60	NS
SE	2.22	2.87		
n	34	28		
E_2 17-glucosiduronate				
Mean	1.69	1.97	+0.28	0.03
SE	.125	0.17		
n	35	28		

See Table I for definitions.

Table IV. Mean serum levels of testosterone, DHEA, and DHEAS in younger and older aged groups of nulliparous women

	Younger women (aged 18-23 yr)	*Older women (aged 29-40 yr)*	*Difference*	*p*
Testosterone (pg/ml)				
Mean	318	303	−15	NS
SE	32	28		
n	35	27		
DHEA (ng/ml)				
Mean	4.95	3.31	−1.64	<0.01
SE	.50	.35		
n	32	27		
DHEAS (μg/dl)				
Mean	290.5	195.5	−95	<0.01
SE	25.1	21.9		
n	35	27		

See Table I for definitions.

of FSH and LH is not known but might be explained by feedback regulation of gonadotropin secretion by estrogens and/or inhibin. In this study the concomitant rise in serum levels of both FSH and E_2 argues against feedback regulation by estradiol as being responsible for the increase in FSH. Conversely, inhibin preferentially inhibits FSH secretion. Therefore decreased feedback inhibition of FSH secretion by ovarian inhibin could explain the selective rise in the FSH level.

A most interesting finding in this study was the significant age-related increase in the serum level of E_2. The increase in E_2 occurred in both the serum and urine and is consistent with one previous report.[13] The increased serum E_2 level may be caused by either increased ovarian secretion or decreased metabolism of E_2, which is metabolized principally through hepatic conjugation and E_1 formation. In this study the urine levels of both total E_2 and E_2 17-glucosiduronate were increased, whereas serum levels of E_1 sulfate and E_1 were decreased, suggesting that decreased metabolism through the E_1 pathway may account for the increased levels of E_2. However, an increase in ovarian secretion of E_2 remains a possibility that cannot be excluded with our results.

This age-related increase in serum E_2 levels in nulliparous women has important implications as a risk factor for breast cancer. It is well known that nulliparity increases the risk of breast cancer.[14, 15] Bernstein et al.[16] have recently reported that nulliparous women have higher serum levels of E_2 than parous women, indicating that previous pregnancy lowers serum levels of E_2. One possible mechanism for lowering serum E_2 levels in parous women is through the prevention of the age-related rise in the serum levels of E_2, which occurs in nulliparous women. An early first pregnancy could then reduce serum E_2 levels at an earlier age than a

late first pregnancy. This is consistent with the established finding that an early first pregnancy protects against breast cancer, whereas a late first pregnancy does not.[14, 15]

A decrease in basal serum prolactin levels with increasing age has been reported in the female subject, but postmenopausal women were included, and the long-term effects of pregnancy were not taken into account.[17-19] Yu et al.[13] found no significant change in basal serum prolactin levels with age in nulliparous women. Our results clearly indicate that there is an age-related decrease in both the basal levels of prolactin and the response of prolactin to perphenazine during the reproductive years.

We have previously reported that a first pregnancy induces long-term reduction in the secretion of prolactin.[4] In this study the effects of pregnancy were excluded by using only nulliparous women. Thus both the aging process and previous pregnancy affect prolactin secretion. However, serum prolactin levels are higher in nulliparous women than in parous women,[4, 13] indicating that a pregnancy at an early age will lower serum prolactin levels at an earlier age and for a longer period than the aging process.

There was again a divergence with regard to the androgens. There was no age-related change in serum levels of testosterone, whereas serum levels of both DHEA and DHEAS decreased. Serum DHEA and DHEAS levels decrease during pregnancy.[20] We have shown that the reduction in DHEA and DHEAS levels induced by a first pregnancy persists for several years.[5] The effects of age and pregnancy are frequently concurrent and difficult to separate. However, the effects of pregnancy were discounted in this study by using only nulliparous women. Our results clearly show that serum levels of DHEA and DHEAS change in response to age, as well as to a first pregnancy.

In summary, our studies indicate that increasing age during reproductive life in the woman is accompanied by a significant change in the hormonal environment that is independent of previous pregnancy. There is an increase in both basal serum FSH levels and the response of FSH to GnRH stimulation. In turn, this is associated with an increase in serum levels of E_2, urinary levels of total E_2 and E_2 17-glucosiduronate, and a decrease in serum levels of E_1 sulfate. Other serum and urinary estrogen levels are unchanged. Basal and GnRH stimulated levels of LH are also unchanged. Serum levels of DHEAS and DHEA decrease markedly, but testosterone remains unchanged. Both basal and perphenazine-stimulated serum levels of prolactin are markedly reduced. These changes in the hormonal milieu during the reproductive years have important implications about physiologic and pathologic conditions that are affected by reproductive hormones.

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