Effect of Estrogen on Serum DHEA in Younger and Older Women and the Relationship of DHEA to Adiposity and Gender

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This case-controlled study consisted of 2 parts. The objective of part 1 was to determine the relationship between DHEA, body mass index (BMI), and age in young males, young females, and postmenopausal (PM) females. Part 2 examined the effects of estrogen on DHEA by analyzing the relationship between DHEA and age in young females on and off oral contraceptives (OCs) and PM females on and off estrogen or hormone replacement therapy (ERT/HRT). The study was performed at the Obstetrics and Gynecology Clinic, Texas Tech Health Sciences Center-Amarillo, Exercise Physiology Laboratory at Southeastern Louisiana University, and Woman's Health Research Institute, Woman's Hospital, Baton Rouge, LA. Part 1 groups consisted of: (1) young males between the ages of 18 to 40 years; (2) normally cycling females off OCs, ages 18 to 40 years; and (3) PM females older than 40 years not receiving ERT/HRT. Part 2 groups consisted of: (1) normally cycling females on OCs, ages 18 to 40 years;, (2) normally cycling females off OCs, ages 18 to 40 years; (3) PM females 50 years or older not receiving ERT/HRT; and (4) PM females 50 years or older receiving ERT/HRT. The main outcome measure was serum DHEA concentrations. For part 1, there were significant ($P < .05$) inverse relationships between DHEA and age for young males; young females, off OCs; PM females, no ERT/HRT $r = -.44$, $-.26$, and $-.25$, respectively. There were no significant relationships between DHEA and BMI for any of the groups. DHEA concentrations were significantly higher in young males than young females even after accounting for age. For part 2, DHEA concentrations were significantly higher in young females off OCs compared with young females on OCs, and significantly higher in PM women off ERT/HRT than those on ERT\HRT. There were significant inverse relationships between DHEA and age for young females and PM females on and off ERT/HRT. From these findings, we conclude that there is an inverse relationship between DHEA and age for young males, young females off OCs, and PM females, no ERT/HRT. No relationship between BMI and DHEA was observed in these same 3 groups. These results agree with previous findings in young men, but differ from previous findings in obese young females. The data also suggest that estrogen treatment (OCs and ERT/HRT) suppresses DHEA concentrations in premenopausal and PM females, and that DHEA declines with age in PM females regardless of estrogen treatment.

MUCH RECENT ATTENTION has been given to dehydroepiandrosterone (DHEA), an adrenal androgen, due to accumulating evidence that DHEA administration may have multiple benefits. These benefits may include cardioprotection, insulin sensitivity, antiobesity effects, reduced bone turnover, increased activity of the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis, and improved immune function,[1-3] however, a gender difference may exist for some of these benefits. There is evidence that oral administration of DHEA will reduce body fat in experimental animals,[4-6] and similar results on body fat have been repeated in men, but not in women.[7,8] However, in women, but perhaps not in men, DHEA may be associated with insulin sensitization.[1]

Plasma levels of DHEA and the hormone's sulfated form, DHEAS, decline with age,[9-12] and ageing is typically associated with changes in body composition. Some data exist concerning the relationship between serum levels of DHEA/DHEAS and body composition. In 2 studies of premenopausal obese women, inverse correlations between DHEA and body mass index (BMI) were found[13,14]; however, in the later of the 2 studies, DHEAS was shown not to be related to BMI in this obese population. Another study of premenopausal females with relatively low BMI (mean, 22) found no significant relationship between serum DHEAS levels and BMI or percent body fat.[15]

There are a few studies that have focused on estrogen and adrenal androgens with conflicting findings. Lobo et al[16] found that estrogen replacement therapy (ERT) administration increased the DHEA response to ACTH stimulation, whereas Slayden et al[17] found no effect after ACTH stimulation. Moreover, there is evidence that hormone replacement therapy (HRT) affects DHEA responses to stress, and we have reported that HRT enhances DHEA increases in response to exercise.[18] Abraham and Maroulis[19] reported increased adrenal androgen levels in women on ERT. However, Casson et al[20] recently reported suppressed adrenal androgens from oral micronized estradiol treatments. Some of these discrepancies may to be due to the dose and/or the route of administration of oral estrogen.

The purpose of part 1 of the investigation was to determine whether there is a relationship between DHEA and BMI, as well as DHEA and age among young males, young females, and postmenopausal (PM) females. The purpose of part 2 was to compare the effect of oral contraceptives (OCs) on the relationship between DHEA concentrations and age in young females against age-matched normally cycling controls and to compare the effect of ERT/HRT on the same relationship in

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Table 1. Mean (±SE) Age, BMI, and DHEA Concentrations in Young Males, Young Females, and Older Females

Group (age)	No.	Age (yr)	BMI (kg/m^2)	DHEA (ng/mL)
Young males (18–40 yr)	52	24.90 (0.63)	27.06 (0.54)	11.66 (0.82)*†
Young females (18–40 yr)	69	26.80 (0.59)	30.02 (1.08)	8.06 (0.53)
Older females (>40 yr)	72	57.63 (0.88)	27.87 (.080)	4.20 (0.23)*‡

*P = .0001 compared with young females.
†P = .0001 compared with older females.
‡P = .0001 compared with young males.

older PM females against nontreated age-matched controls. One of the advantages of the current study over previous reports is the larger sample size.

These data were collected in an effort to elucidate the role of endogenous DHEA in different populations and to determine whether age-induced reduction in DHEA is affected by estrogen status. Because previous studies suggest a gender difference exists for DHEA and because estrogen administration has been shown to alter endogenous adrenal androgens in some studies, we hypothesized that estrogen status might alter serum DHEA levels. Moreover, because DHEA is a better indicator of acute adrenal function than DHEAS, we expected morning resting values of DHEA to be the most reliable indicator of adrenal androgen production.

SUBJECTS AND METHODS

The study was approved by the Institutional Review Boards of Southeastern Louisiana University, Hammond LA, Woman's Hospital, Baton Rouge, LA, and Texas Tech Health Sciences Center, Amarillo, TX. All subject volunteers were recruited from the 3 institutions and the surrounding communities and gave written informed consent to complete the study. As part of the medical screening, subjects with any disorder that would interfere with endocrine function (eg, diabetes mellitus, liver disease) were excluded from the study.

Subjects

In part 1, 193 subjects were subgrouped into (1) young males (ages 18 to 40 years, n = 52); (2) young premenopausal females off OCs (ages 18 to 40 years, n = 69); and (3) PM females not on ERT/HRT (older than 40 years, n = 72). In the PM females, menopause occurred naturally, except for 4 subjects between the ages of 40 and 50 years in which menopause was surgically induced. BMI was calculated from height and weight that was obtained at the time of phlebotomy.

In part 2, 350 female subjects were subgrouped into (1) young females (ages 18 to 40 years, n = 58) on OCs; (2) normal cycling females off OCs (ages 18 to 40 years, n = 114); (3) older PM females (age 50 years or older, n = 76) on ERT/HRT; and (4) older PM females (age 50 years or older, n = 102) not on ERT/HRT. PM females were on a variety of standard dose estrogen regimens, although conjugated equine estrogens were the most common form. The active estrogen in all of the oral contraceptives used by the subjects was ethinyl estradiol. The subjects in this study used almost exclusively a 30- to 35-μg dose. Subjects in the OC and ERT/HRT groups had been on OCs or ERT/HRT for at least 3 months and were not taking any other medications that could alter hormone concentrations. Conversely, women on no treatment had been without any estrogen (OCs or ERT/HRT) for the same time interval. Some of the subjects in part 1 of the study qualified for part 2.

Protocol

Blood samples were drawn from all subjects into a 10-mL separator tube between 8:00 and 10:00 AM after an overnight fast and without regard for stage of the menstrual cycle in reproductive age women. Samples were centrifuged and serum aliquoted and frozen until it was assayed. DHEA was determined by radioimmunoassay (RIA) using reagents from Diagnostic Systems Laboratory (Webster, TX). Intraassay coefficient of variation for DHEA was less than 5%. Interassay coefficient of variation for the high control pool was 6.64% and for the low control pool, 7.64%.

Statistics. In part 1, Pearson correlation coefficients were used to determine whether relationships existed between DHEA and BMI or age for young men, young women, and older women. Independent *t* tests were used to compare serum DHEA concentrations of the different subgroups. Analysis of covariance (ANCOVA) was conducted to compare DHEA levels of different subgroups and account for differences produced by age using DHEA as the dependent measure and age as the covariate. In part 2, Pearson correlation coefficients were used to determine whether relationships existed between DHEA and age for young females on and off OCs and PM females on and off ERT/HRT. Independent *t* tests were used to compare serum DHEA concentrations of different subgroups. ANCOVA was conducted to compare DHEA levels of different subgroups and account for differences produced by

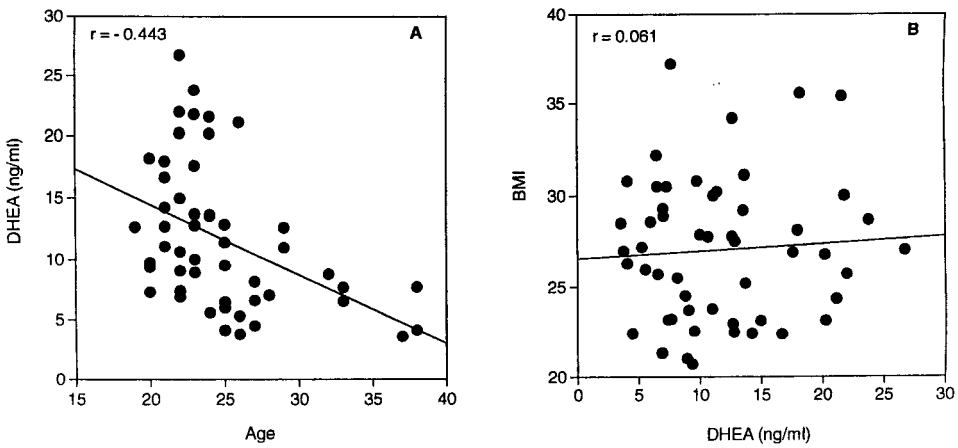

Fig 1. (A) Relationship between DHEA and age for young males (18 to 40 years, n = 52). (B) Relationship between DHEA and BMI for young males (18 to 40 years, n = 52). Data are from part 1 of the study.

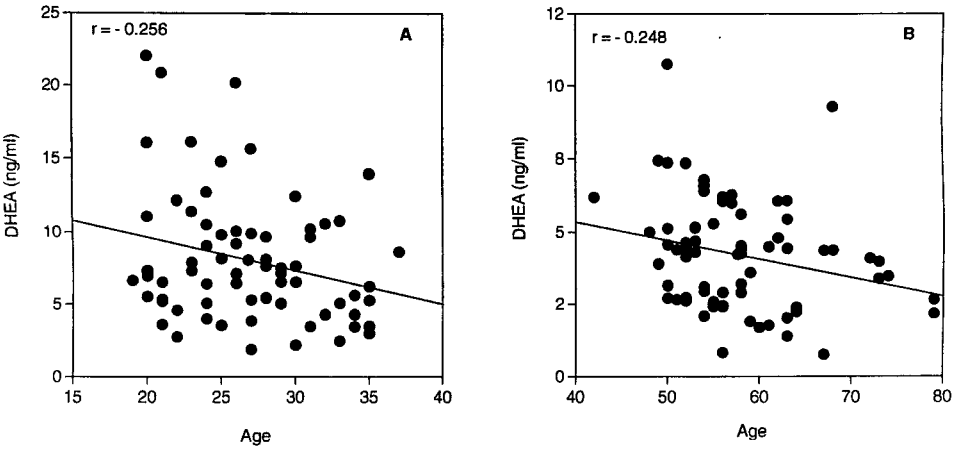

Fig 2. (A) Relationship between DHEA and age for young females, no OCs (18 to 40 years, n = 69). (B) Relationship between DHEA and age for PM females, no ERT/HRT (older than 40 years, n = 72). Data are from part 1 of the study.

age using DHEA as the dependent measure and age as the covariate. The alpha level was set at $P < .05$.

RESULTS

The data for part 1 are presented in Table 1 and represent the mean (± SE) values for age, BMI, and DHEA concentrations in young males and younger and PM females. There were significant inverse correlations for DHEA and age for young males (r = -.44, Fig 1), young females, no OCs, (r = -.26, Fig 2), and PM females, no ERT/HRT (r = -.25, Fig 2). As expected, the DHEA concentrations in PM females were significantly lower than those of younger males and young females not on OCs, and DHEA concentrations in young males were significantly higher than those in young females, no OCs.[9,10] To confirm that the gender-associated difference in DHEA was not due to age within the subgroup, we conducted an ANCOVA using age as a covariate. The adjusted mean DHEA concentrations for young males and young females were also significantly different (males, mean DHEA = 11.66 ng/mL, adjusted mean = 11.27 ng/mL; females, mean DHEA = 8.06 ng/mL, adjusted mean = 8.36 ng/mL). There were no significant relationships between DHEA and BMI for any of the groups (Figs 1 and 3).

The data for part 2 are presented in Table 2 and represent the mean (± SE) values for age and DHEA concentrations. There was a significant inverse correlation for DHEA and age in young females, no OCs (r = -.31, Fig 4); there was not a significant relationship between DHEA and age for young females taking OCs (Fig 4) and may represent an estrogen effect in this subgroup. There was a significant inverse correlation for DHEA and age in PM females, no ERT/HRT (r = -.32, Fig 5), and PM females on ERT/HRT (r = -.25, Fig 5).

DHEA concentrations were significantly higher in young females not on OCs compared with females on OCs. To confirm that the estrogen-associated difference in DHEA was not due to age within the subgroup, we conducted an ANCOVA using age as a covariate. The adjusted mean DHEA concentrations for young females on and off OCs were also significantly different (females, off OCs, mean DHEA = 7.57 ng/mL, adjusted mean = 7.81 ng/mL; females on OCs, mean DHEA = 5.25 ng/mL, adjusted mean = 4.79 ng/mL). Additionally, DHEA concentrations were higher in PM females not on ERT/HRT than those on ERT/HRT. An ANCOVA with age as a covariate showed significantly different adjusted means (PM females, no ERT/HRT, mean DHEA = 3.51 ng/mL, adjusted mean = 3.50 ng/mL; PM females, ERT/HRT, mean DHEA =

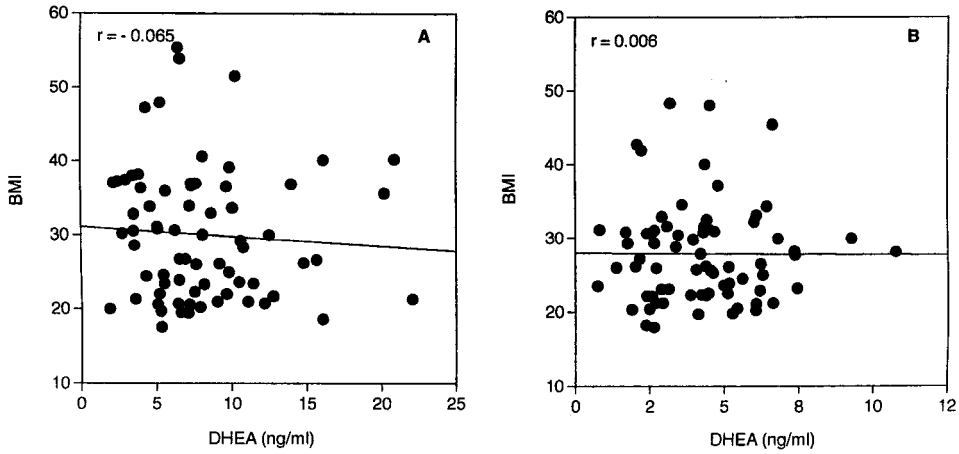

Fig 3. (A) Relationship between DHEA and BMI for young females, no OCs (18 to 40 years, n = 69). (B) Relationship between DHEA and BMI for PM females, no ERT/HRT (older than 40 years, n = 72). Data are from part 1 of the study.

Table 2. Mean (±SE) Age and DHEA Concentrations in Young Females on OCs, Young Females Not Taking OCs, Older Females on ERT/HRT, and Older Females Not Taking ERT/HRT

Group (age)	No.	Age (yr)	DHEA (ng/mL)
Young females, OC	58	25.66 (.73)	5.25 (0.53)*
Young females, no OC	114	28.70 (0.53)	7.57 (0.45)*†
Older females, ERT or HRT	76	60.70 (1.05)	2.69 (.23)*
Older females, no ERT or HRT	102	60.15 (.89)	3.51 (.20)*‡

*P = .0001 compared with young males (Table 1).
†P = .001 compared with young females, OC.
‡P = .0035 compared with older females, ERT or HRT.

2.69 ng/mL, adjusted mean = 2.71 ng/mL). DHEA was significantly lower in younger and PM females, on and not on OCs and ERT/HRT, respectively, than in younger males in part 1.

DISCUSSION

There are only a few studies that have examined the relationship between DHEAS and body composition, with even less data available concerning the relationship between DHEA and body composition, especially in older women. One improved aspect of this study over the few existing DHEA/body composition studies was the large sample size. Moreover, this is the first study to examine the effect of estrogen on the relationship between DHEA and age in females.

The data from this study show the presence of an inverse relationship between DHEA and age in young males and females, as well as older PM females. In older PM women, the data suggest DHEA is related to age regardless of estrogen treatment, however, this relationship between DHEA and age was not found in younger women on OCs, but was found in younger women not taking OCs. The suppression of DHEA by estrogen appeared to be greater in young women compared with older women. Mean DHEA concentrations were 30% less in young women on OCs compared with age-matched untreated controls, whereas DHEA levels in PM women on ERT/HRT were 25% less than those in age-matched untreated controls. The greater suppressive effect could account for the lack of a relationship between DHEA and age in younger women on OCs and may be related to the greater estrogenic potency of these preparations.

The data in the present study concur with other studies that DHEA concentrations are related to age in younger and older females without estrogen treatment.[9,10,20] The mechanism for age-associated changes in DHEA may be related to alterations in steroid enzymology in the zona reticularis of the adrenal cortex.[21]

There was not a relationship between DHEA and BMI in any of the populations examined in the study. Previous research has shown an inverse relationship for DHEA and BMI, but a positive correlation between DHEAS and BMI in obese young women.[14] This was explained by the difference in metabolic clearance of the 2 hormones.[14] DHEA is able to diffuse into fat cells with a positive tissue/plasma gradient, whereas DHEAS cannot and therefore is metabolized in adipose tissue at a lower level than DHEA. Thus, with an increase in storage fat, there is more DHEA catabolism and less DHEA. Moreover, the metabolic clearance rate of DHEA is 2 to 5 times greater in obese and insulin-resistant states,[22] and the metabolic clearance rate of DHEA increases with insulin infusion.[23] We found no relationship between DHEA and BMI in younger males and older PM females. Thus, the disparity between previous reports and the present study may be due to the fact that the majority of our subjects were not obese (mean BMI = 27.06 for our subjects compared with 41.8 for previous subjects[14]), however, subjects in the present study did represent the broad spectrum of BMIs (47.8% greater than 30 BMI) across the premenopausal population with an age range of 18 to 40 years.

Other related investigations have examined the effects of estrogen on adrenocortical (DHEA) responses to ACTH stimulation, Lobo et al[16] administered conjugated equine estrogens (.625 mg/d Premarin [Wyeth Ayerst, Philadelphia, PA]) for 4 weeks, which produced a 25% increase in serum DHEA in response to ACTH in oophorectomized women, and even greater responses to ACTH were seen after administration of 2.5 mg of conjugated equine estrogen for 4 weeks. Additionally, adrenal androgen levels in these women on 2.5 mg were similar to those of matched ovulating controls. Using a similar approach, Slayden et al[17] found that transdermal estradiol ther-

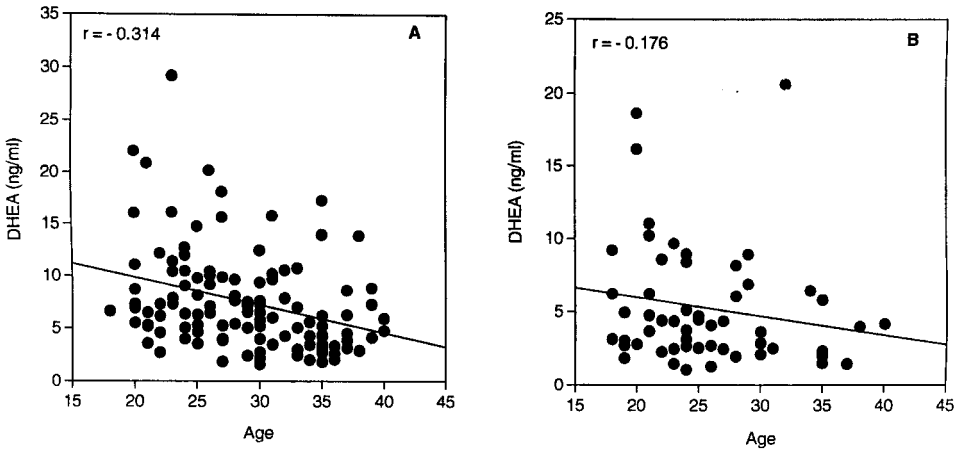

Fig 4. Comparison of estrogen treatment on DHEA/age relationship. (A) Relationship between DHEA and age for young females, no OCs (18 to 40 years, n = 114). (B) Relationship between DHEA and age for young females on OCs (18 to 40 years, n = 58). Data are from part 2 of the study.

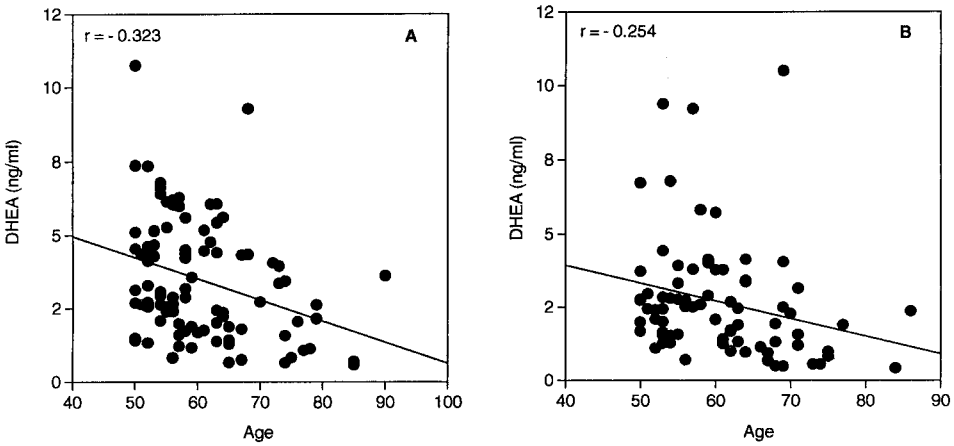

Fig 5. Comparison of estrogen treatment on DHEA/age relationship. (A) Relationship between DHEA and age for older PM females, no ERT/HRT (50 years or older, n = 102). (B) Relationship between DHEA and age for older PM females on ERT/HRT (50 years or older, n = 76). Data are from part 2 of the study.

apy did not change cortisol or DHEA basal levels, nor did it affect maximally stimulated serum levels after continuous incremental ACTH infusion. These findings differed from Lobo et al[16] possibly due to the route of administration, with transdermal estrogen not having a first hepatic pass and resulting in lower serum levels of estrogens. Oral estrogens produce increases in cortisol binding globulin and sex hormone-binding globulin (SHBG), as well as renin, which may result in adrenocortical response. In a related study, we showed that exercise sufficient to activate the hypothalamic-pituitary-adrenal axis (as evidenced by increased cortisol levels) elicits a greater DHEA response in women on oral estrogens than in women not on HRT/ERT.[18] It is impossible to explain the disparate results among these 3 previous studies because there were a number of different variables that could have affected the outcome, such as different forms, doses, and routes of administration of estrogen.

In a smaller study than the present investigation, 10 postmenopausal women who had been treated with hormone replacement for at least 2 months were found to have higher DHEA basal levels than those not treated.[19] Our findings do not agree with that study, however, our study was larger and included 76 women on ERT/HRT and 102 off ERT/HRT. It has been shown previously that treatment of postmenopausal women with 2 mg/d of oral micronized estradiol for 12 weeks reduces DHEAS levels (although DHEA levels did not decline as much as DHEAS).[20] The cause of this was suggested to be a direct effect of estrogen on the adrenal or may be due to a secondary effect of estrogen-induced suppression of gonadotropin production leading to reduced androgen production by the postmenopausal ovarian stroma.[20] This is consistent with the findings in the present study of suppressed DHEA levels in older women on ERT/HRT and younger women on OCs.

In summary, the data indicate the presence of an inverse relationship between DHEA and age for young males; young females, no OCs; and older PM women, but no relationship between DHEA and BMI in all 3 groups. These results agree with previous findings in young men, but differ from previous findings in obese young women. These findings suggest that further studies are necessary to determine whether there is a difference in metabolic factors in obese versus nonobese subjects that affect DHEA concentrations. The data also suggest that oral estrogen treatment (OCs and ERT/HRT) suppresses DHEA concentrations in premenopausal and postmenopausal women and that DHEA is related to age in postmenopausal women regardless of estrogen treatment. The lower DHEA levels in women treated with estrogen appears to be responsible for the lack of a relationship between DHEA and age in younger females on OCs. Our data support the contention that oral estrogen suppresses serum adrenal androgen levels, and we agree with Casson et al[20] that adrenal androgen supplementation should be considered in these individuals. Additional research should be conducted to elucidate the mechanisms that explain the effects of estrogen on DHEA.

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