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Maintaining physiological testosterone levels by adding dehydroepiandrosterone to combined oral contraceptives: I. Endocrine effects☆,☆☆

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Abstract

Objective: To determine whether adding dehydroepiandrosterone to combined oral contraceptives (COCs) maintains physiological levels of free testosterone.

Study design: A randomized, double-blind, placebo-controlled, two-way crossover study conducted in 81 healthy women (age range: 20–35 years; Body mass index (BMI) range: 18–35 kg/m^2) using oral contraceptives. Androgens, sex hormone-binding globulin (SHBG), estradiol (E2) and estrone (E1) were measured, and free testosterone and the free testosterone index were calculated. Subjects discontinued oral contraceptive use for at least one menstrual cycle before being randomized to receive five cycles of ethinyl estradiol (EE) combined with either levonorgestrel (EE/LNG group) or drospirenone (EE/DRSP group) together with either dehydroepiandrosterone (DHEA) (50 mg/day orally) or placebo. Subsequently, all subjects crossed over to the other treatment arm for an additional five cycles.

Results: Both COCs decreased the levels of all androgens measured. Significant decreases ($p<.05$) were found with EE/LNG and EE/DRSP for total testosterone (54.5% and 11.3%, respectively) and for free testosterone (66.8% and 75.6%, respectively). Adding DHEA to the COCs significantly increased all androgens compared to placebo. Moreover, including DHEA restored free testosterone levels to baseline values in both COC groups and total testosterone levels to baseline in the EE/LNG group and above baseline in the EE/DRSP group. SHBG concentrations were significantly higher with EE/DRSP compared to EE/LNG ($p<.0001$). The addition of DHEA did not affect the levels of SHBG.

Conclusions: Taking COCs reduces total and free testosterone levels and increases SHBG concentrations. By coadministration with DHEA, physiological levels of total and free testosterone are restored while using EE/LNG. With EE/DRSP, only the free testosterone level is normalized by DHEA coadministration.

Implications: A daily oral dose of 50-mg DHEA maintains physiological free and total testosterone levels in women who are using an EE/LNG-containing COC.

Keywords: Oral contraception; Free testosterone; SHBG; DHEA; Androgens

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1. Introduction

In women, testosterone (T) is believed to play an important role in sexual function; specifically, T is associated with sexual desire, the ability to achieve orgasm, and the frequency of sexual intercourse [1–3]. Thus, T therapy may represent an effective strategy for treating decreased sexual desire related to low free T levels that is common among postmenopausal women, in women approaching their late reproductive years and in women with ovarian and/or pituitary dysfunction [4–12].

The majority of circulating T is either tightly bound to sex hormone-binding globulin (SHBG) or weakly bound to albumin; only 0.5–3.0% circulates as free T [13,14]. Because T binds to albumin with relatively low affinity, the pools of free and albumin-bound T are defined together as bioavailable T [13,14]; this is also represented using the free T index (FTI), a calculated value of bioavailable T. Combined oral contraceptives (COCs) significantly lower the levels of androgens, including free T, in women [14]. With respect to T, this reduction is caused by several processes, including the suppression of ovarian and adrenal androgen production, the suppression of peripheral T synthesis and the stimulation of SHBG production in the liver; the last of these effects is due to the estrogenic component of COCs, ethinyl estradiol (EE), which stimulates hepatic SHBG production.

Although studies of the effects of COCs on mood and sexual function have yielded conflicting results [15–21], the reduction in free T levels is a plausible mechanism by which COCs can negatively affect sexual function, at least in some women [2,22–24].

Based on these findings, we hypothesized that the negative effects of COCs on sexual function may be improved by maintaining physiological levels of androgens, particularly free T, in these women. Physiological levels of androgens may be achieved by adding dehydroepiandrosterone (DHEA) — a naturally occurring androgenic hormone produced by the adrenal gland — to COCs. Because oral DHEA is partially metabolized by the liver into T [25–28], it could in principle be incorporated as a prodrug into a COC pill, thereby maintaining T levels in women who use these COCs. Previous studies found that adding a daily dose of 50-mg DHEA to a drospirenone (DRSP)-containing COC significantly increased total T levels; however, free T was restored to only 47% of normal, presumably due to the EE-induced increase in T-binding SHBG [29].

Although most COCs contain EE, the progestin component in COCs varies. Different progestins have different intrinsic antiestrogenic and androgenic potencies. For example, levonorgestrel (LNG) has antiestrogenic effects on SHBG levels, thereby counteracting the EE-induced increase in SHBG; in contrast, DRSP does not interfere with the effect of EE on SHBG [5]. In addition, LNG has intrinsic androgenic effects, whereas DRSP exhibits antiandrogenicity [14]. Thus, DRSP-containing COCs causes a more robust reduction of androgenicity than LNG-containing COCs.

Here, we studied the endocrine effects of including DHEA in COCs containing 30-mcg EE in combination with either 150-mcg LNG or 3-mg DRSP. Specifically, we measured androgen levels and androgen-related endrocrine parameters (including T, SHBG and free T) and clinical parameters. In this paper, we present the endocrine effects; in our companion paper, we present the clinical effects, including sexual function, dermatological findings and safety (e.g., effects on lipids and skin) [30].

2. Materials and methods

2.1. Study population

The following criteria were used to determine eligibility for inclusion in the study: prior to screening, subjects had to have used a contraceptive pill for at least 3 months and had to have been in a stable, satisfactory, heterosexual relationship for at least 3 months; 20–35 years of age; body mass index (BMI) of 18–35 kg/m^2; at least one regular menstrual cycle (lasting 24–35 days) prior to the last start of COC use; total T level <5 nmol/L; and willing to interrupt COC use for a period of at least 4 weeks (one menstrual cycle).

The study was approved by the medical ethics committee of the Academic Medical Center (AMC) (Amsterdam, the Netherlands) and was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization/Good Clinical Practice guidelines. All participants provided written informed consent. The study was registered at the ISRCTN registry (no. ISRCTN03247616).

2.2. Study design and procedures

This randomized, double-blind, placebo-controlled, two-way crossover study was conducted at the AMC (Amsterdam, the Netherlands). The primary objective, the results of which are reported in our companion paper [30], was to determine and compare the effects of DHEA versus placebo on sexual function in subjects using a COC containing 30-mcg EE with either 3-mg DRSP (EE/DRSP group) or 150-mcg LNG (EE/LNG group). The secondary objective, which we report here, was to evaluate the effect of DHEA versus placebo on endrocrine parameters in these two COC groups.

In accordance with the study protocol, each subject discontinued using their contraceptive pill for one regular cycle (the baseline cycle period; Fig. 1). After this cycle, the subjects were assigned randomly to either the EE/DRSP or EE/LNG group. The subjects then took their assigned COC together with either DHEA or placebo for five 28-day cycles (each cycle consisted of 21 days on the COC followed by 7 days off the COC; DHEA or placebo was taken throughout the cycle). After the first five cycles (Treatment Period 1), the subjects then crossed over (i.e., subjects who took DHEA

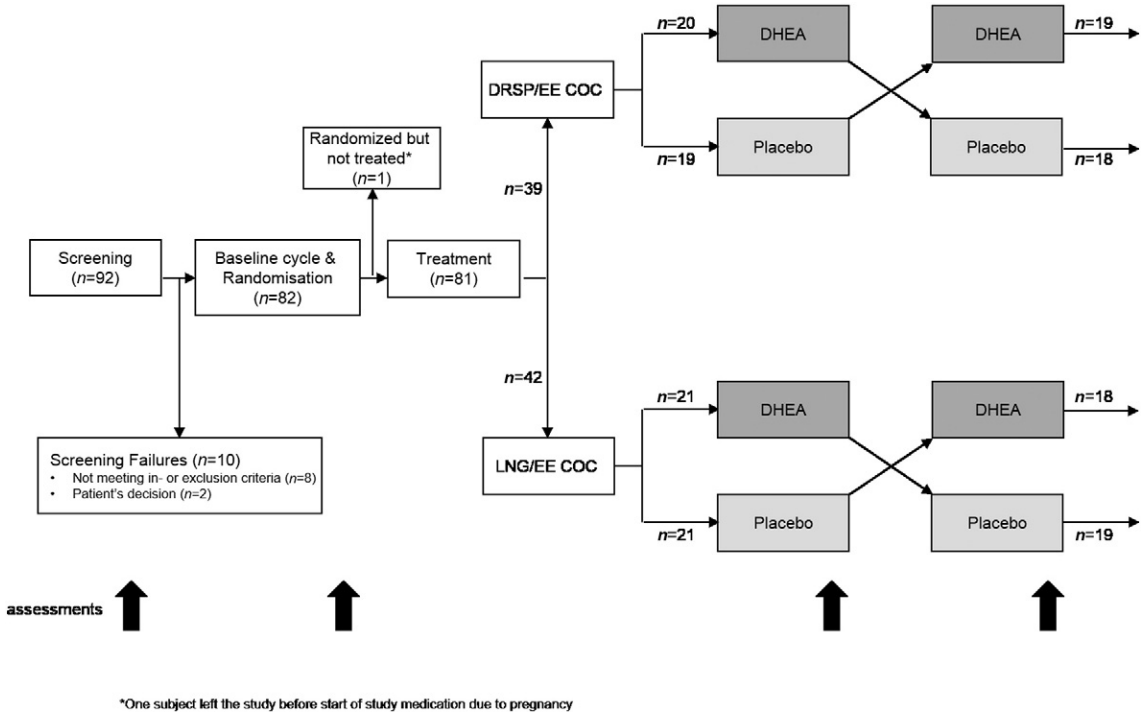

Fig. 1. Study design and subject disposition.

in Treatment Period 1 switched to taking placebo, and vice versa) for an additional five cycles (Treatment Period 2). The study design is depicted in Fig. 1.

2.3. Endocrine parameters

Plasma samples for measuring endocrine parameters were taken at screening (during the subject's prior COC use), between days 14 and 17 during the pretreatment cycle (baseline) and between days 14 and 17 in the fifth cycle in each treatment period. Plasma samples were analyzed at the AMC laboratory using radioimmunoassay to measure total extracted T, SHBG, DHEA, DHEA-sulfate (DHEA-S), Δ4-androstenedione (AD), estradiol (E2), and E1; albumin was measured using a bromocresol assay. The lower limits of quantification were as follows: total T, 0.3 nmol/L; SHBG, 5 nmol/L; DHEA, 1.5 nmol/L; DHEA-S, 0.1 μmol/L, AD, 0.4 nmol/L; and E2, 40 pmol/L. Because a method for directly measuring free T was not available at our laboratory when the study was performed, free T was calculated based on total T, SHBG and albumin concentration [31,32]. In addition, FTI was calculated using the following formula: FTI= 100×[T]/[SHBG], where T is total T [32].

2.4. Study medication

DHEA was manufactured by Akzo Laboratories (Diosynth B.V., Oss, the Netherlands) in accordance with Good Manufacturing Practices. The DHEA and placebo tablets were manufactured by Unither Pharmaceuticals (Le Haillan, France) and were identical in appearance. Blinded study medication was packed per subject number according to a computer-generated randomization list that was only known to an independent biostatistician. The subjects received either a COC containing 30-mcg EE+3-mg DRSP (Yasmin; Bayer Healthcare, Berlin, Germany) or a COC containing 30-mcg EE+150-mcg LNG (Microgynon; Bayer Healthcare).

2.5. Statistical analysis and sample size calculation

Based on the primary objective of achieving a within-subject effect size of 0.5 with respect to sexual function, with a significance level of 5% and a power of 80%, a minimum of 36 subjects was required [33]. Thus, because two different COCs (EE/DRSP and EE/LNG) were used in this study, a minimum of 72 evaluable subjects in total was required.

The data were analyzed based on an intention-to-treat approach. Unless indicated otherwise, all summary results are expressed as the mean±SD. A log-transform was used to normalize skewed distributions, and all subsequent calculations were performed on the log-transformed data. When variables could not be normalized, nonparametric tests were used. The one-way analysis of variance or the Kruskal–Wallis test was used to compare values in the four randomized groups, whereas the chi-square test or Fisher's Exact Test was used for categorical variables. Crossover data recorded at the end of Treatment Periods 1 and 2 were analyzed as described by Altman [34]. Specifically, for each

COC, the period effect and treatment–period interaction effect were tested using the unpaired Student *t* test or the Kruskal–Wallis test; in the absence of such effects, the treatment effect was tested using a paired Student *t* test or the Wilcoxon signed-rank test on the combined sequence data. The COC effect was assessed by comparing the overall levels in each COC group using an unpaired Student *t* test or the Kruskal–Wallis test. The same analysis was repeated on data that were adjusted for baseline values.

The paired Student *t* test or Wilcoxon signed-rank test was used to analyze the within-subject changes between the following measurement pairs: screening versus baseline; Treatment Period 1 versus baseline; and Treatment Period 2 versus baseline. Differences were considered to be significant at $p<.05$. A Bonferroni correction was applied to the crossover tests ($p<.01$). All analyses were performed using SAS (Version 9.3 for Windows, SAS Institute, Cary, NC, USA) and S-PLUS (Version 8.1 for Windows, Tibco Software, Palo Alto, CA, USA) statistical packages.

3. Results

3.1. Study population

A total of 92 women who were using a COC were screened; 82 of these women who met all of the study criteria were randomized, and 81 entered the study. Seven women withdrew from the study due to withdrawal of consent with no further explanation (*n*=2), high T levels (*n*=1), severe acne (*n*=1), headache (*n*=1), termination of her relationship with partner (*n*=1) or personal reasons (*n*=1). Thus, a total of 74 women completed the study (Fig. 1).

The baseline characteristics of the study population are summarized in Table 1. The majority of the subjects (91%) were of Caucasian descent. The EE/DRSP and EE/LNG groups were similar with respect to age, BMI, ethnicity, type of COC used prior to the study and endocrine parameters (Table 1). The majority (73%) of subjects had been taking an LNG-containing COC at the time of screening (Table 1).

Table 1
Baseline characteristics of the study population (*n*=74)

Parameter	EE/DRSP *n*=37		EE/LNG *n*=37		p-value (EE/DRSP vs. EE/LNG)
	DHEA-P[1] *n*=18	P-DHEA[2] *n*=19	DHEA-P[1] *n*=19	P-DHEA[2] *n*=18	
Age, years	25.0±3.9	24.5±3.0	24.2±4.0	22.4±3.2	0.16
BMI, kg/m^2	23.3±2.7	22.2±2.6	22.8±3.6	23.3±2.7	0.63
Ethnic origin, *n* (%)					0.17
Caucasian	14 (77.8)	19 (100.0)	17 (89.5)	17 (94.4)	
Asian	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	
Other	3 (16.7)	0 (0.0)	2 (10.5)	1 (5.6)	
OC type used at screening, *n* (%)					0.081
LNG+EE	11 (61.1)	12 (63.2)	14 (73.7)	17 (94.4)	
CYP+EE	2 (11.1)	2 (10.5)	2 (10.5)	1 (5.6)	
DRSP + EE	2 (11.1)	1 (5.3)	2 (10.5)	0 (0.0)	
DSG+EE	1 (5.6)	2 (10.5)	0 (0.0)	0 (0.0)	
GSD+EE	1 (5.6)	1 (5.6)	0 (0.0)	0 (0.0)	
LYN+EE	1(5.6)	0 (0.0)	0 (0.0)	0 (0.0)	
DSG	0 (0.0)	0 (0.0)	1 (5.3)	0 (0.0)	
NOR + EE	0 (0.0)	1 (5.3)	0 (0.0)	0 (0.0)	
Endocrine parameters					
Total T (nmol/L)	1.4±0.7	1.1±0.5	1.4±0.6	1.4±0.7	0.24
SHBG (nmol/L)	87.0±54.3	85.4±31.2	84.0±55.5	72.2±36.1	0.65
Albumin (g/L)	44.3±3.2	43.0±4.4	44.8±2.9	45.3±2.7	0.62
Free T (pmol/L)	21.8±11.9	12.9±5.9	21.9±13.5	25.0±22.4	0.19
FTI	2.1±1.1	1.8±2.3	2.4±1.8	2.5±1.9	0.32
DHEA (nmol/L)	34.8±12.9	40.3±13.6	43.6±18.3	41.0±17.3	0.52
DHEA-S (μmol/L)	5.0±2.8	5.8±2.3	5.8±2.9	5.6±1.9	0.53
AD (nmol/L)	7.4±3.0	7.3±2.1	8.3±2.9	8.2±3.4	0.63
E2 (pmol/L)	212±254	229±261	182±187	306±252	0.28
E1 (nmol/L)	0.4±0.2	0.5±0.2	0.5±0.2	0.5±0.3	0.38

Data are expressed as mean±standard deviation or *n* (%).
CYP, cyproterone; DSG, desogestrel; E2, 17ß-estradiol; GSD, gestodene; n, number of subjects; NOR, norethisterone; OC, oral contraceptive; P, placebo.
[1] DHEA-P, DHEA in Treatment Period 1 and placebo in Treatment Period 2.
[2] P-DHEA, placebo in Treatment Period 1 and DHEA in Treatment Period 2.

3.2. Endocrine parameters

Nearly all endocrine parameters increased significantly at the end of the baseline (COC-free cycle) period compared to their respective values at screening. In contrast, SHBG levels decreased significantly and albumin concentration was unchanged (data not shown).

An analysis of the crossover data for all parameters revealed no significant period and/or treatment×period interaction effect; therefore, the data in the two treatment periods were combined. Table 2 summarizes the change relative to baseline after five cycles with 50 mg/day DHEA or placebo. After five cycles, all parameters (except SHBG, albumin and E2) differed significantly between DHEA and placebo in both COC groups (Table 2).

3.2.1. Total T

Significantly higher total T levels were measured in the DHEA treatment period compared with their respective placebo treatment (Fig. 2A). In the placebo treatment period, total T decreased significantly compared to baseline ($p<.05$ and $p<.0001$ in the EE/DRSP and EE/LNG groups, respectively) (Table 2; Fig. 2A). In contrast, in the DHEA treatment period, total T levels increased significantly compared to baseline in the EE/DRSP group ($p<.0001$) but not in the EE/LNG group ($p=.15$) (Table 2; Fig. 2A).

3.2.2. SHBG

The SHBG levels were similar between the DHEA and placebo treatment periods in both the EE/DRSP ($p=.081$) and EE/LNG ($p=.40$) groups (Table 2; Fig. 2B). However, compared to baseline, SHBG levels increased in both the EE/DRSP and EE/LNG groups, regardless of whether the subjects received DHEA or placebo (Table 2; Fig. 2B). The SHBG levels were significantly higher in the EE/DRSP compared to the EE/LNG group ($p<.0001$; Table 2).

3.2.3. Free T

Our analysis revealed significant differences in free T levels between the DHEA and placebo treatment periods in both the EE/DRSP and EE/LNG groups ($p<.0001$ for each group) (Table 2 and Fig. 2C). In the placebo treatment period, free T decreased significantly compared to baseline in both the EE/DRSP and EE/LNG groups ($p<.0001$). In the DHEA treatment period, free T levels were similar to baseline values in both the EE/DRSP and EE/LNG groups. However, free T levels were significantly higher in the EE/LNG+DHEA group than in the EE/DRSP + DHEA group

Table 2
Endocrine parameters at baseline and after five cycles of EE/DRSP or EE/LNG with 50 mg/day DHEA or placebo[1]

Parameter	COC group (n=34–37)	Baseline	Placebo		DHEA		p-value (DHEA vs. placebo)[2]
			Absolute change relative to baseline	Mean percent change relative to baseline	Absolute change relative to baseline	Mean percent change relative to baseline	
Total T (nmol/L)	EE/DRSP	1.24±0.61	−0.14±1.12*	−11.3%	1.78±1.56*	+143.5%	<0.0001
	EE/LNG	1.43±0.63	−0.78±0.59*	−54.5%	0.25±0.85	+17.5%	<0.0001
SHBG (nmol/L)	EE/DRSP	86.2±43.4	164±87.2*	+190.3%	145±75.8*	+168.2%	0.081
	EE/LNG	78.3±46.8	18.1±47.6*	+23.1%	14.9±49.0*	+19.0%	0.40
Albumin (g/L)	EE/DRSP	43.6±3.86	−0.69±4.17	−1.6%	0.12±4.08	+0.3%	0.081
	EE/LNG	45.0±2.77	−1.55±4.89	−3.4%	−1.12±3.57	−2.5%	0.32
Free T (pmol/L)	EE/DRSP	17.2±10.2	−13.0±11.8*	−75.6%	−3.33±10.8	−19.4%	<0.0001
	EE/LNG	23.2±17.7	−15.5±16.8*	−66.8%	−3.76±19.5	−16.2%	<0.0001
FTI	EE/DRSP	1.91±1.83	−1.43±1.89*	−74.9%	−0.53±1.87	−27.7%	<0.0001
	EE/LNG	2.46±1.82	−1.71±1.70*	−69.5%	−0.51±1.86	−20.7%	<0.0001
DHEA (nmol/L)	EE/DRSP	37.6±13.4	−8.19±22.9*	−21.8%	25.0±37.2*	+66.5%	<0.0001
	EE/LNG	42.4±17.6	−13.7±17.0*	−32.3%	9.10±30.3	+21.5%	<0.0001
DHEA-S (μmol/L)	EE/DRSP	5.40±2.56	−1.19±3.15*	−22.0%	6.62±6.44*	+122.6%	<0.0001
	EE/LNG	5.72±2.45	−1.12±2.94*	−19.6%	6.25±7.86*	+109.3%	<0.0001
AD (nmol/L)	EE/DRSP	7.34±2.57	−1.92±4.07*	−26.2%	13.4±13.09*	+182.6%	<0.0001
	EE/LNG	8.26±3.07	−3.27±2.76*	−39.6%	4.37±11.6*	+52.9%	<0.0001
E2 (pmol/L)[3]	EE/DRSP	221±254	−177±258*	−80.1%	−155±304*	−70.1%	NA
	EE/LNG	242±227	−202±227*	−83.5%	−203±228*	−83.9%	NA
E1 (nmol/L)	EE/DRSP	0.48±0.23	−0.18±0.27*	−37.5%	0.18±0.34*	+37.5%	<0.0001
	EE/LNG	0.48±0.23	−0.12±0.25*	−25.0%	0.20±0.34*	+41.7%	<0.0001

Data expressed as mean±standard deviation.
n, number of subjects; NA, not applicable.
[1] Analysis of the crossover data did not reveal any significant period and/or treatment×period interaction effect, therefore, the data in the two treatment periods were combined.
[2] Comparison of absolute value at end of treatment.
[3] Measured E2 values were below the detection threshold in 89–100% of samples obtained during the treatment period; these values were set to the lower limit of detection (40 pmol/L).
* $p<.05$ versus baseline.

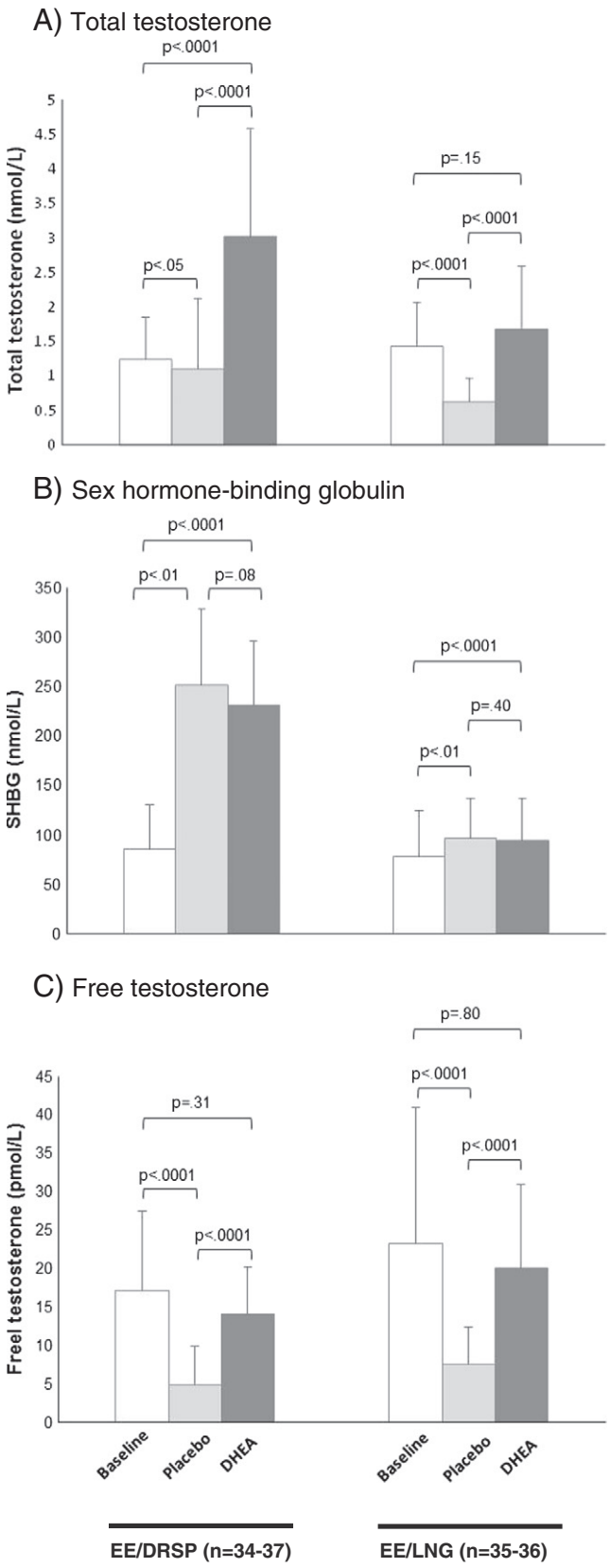

Fig. 2. The absolute values of endocrine parameters (mean±standard deviation) measured at baseline and after five cycles of EE/DRSP or EE/LNG with 50-mg/day DHEA or placebo.

(p<.01) (Table 2 and Fig. 2C). Similar results were obtained when we calculated FTI rather than free T (see Table 2); therefore, FTI levels are not discussed further.

3.2.4. DHEA, DHEA-S and AD

Compared to the placebo treatment period, DHEA, DHEA-S and AD were all significantly increased in the DHEA treatment period in both the EE/DRSP and EE/LNG groups (p<.0001; Table 2). In the placebo treatment period, the DHEA, DHEA-S and AD levels were decreased significantly compared to baseline levels (p<.0001). In the DHEA treatment period, all three androgens were increased significantly compared to baseline in the EE/DRSP group (p<.01); in the EE/LNG group, DHEA-S and AD levels were increased significantly compared to baseline (p<.01), whereas DHEA levels were unchanged. The DHEA and DHEA-S levels were similar between the EE/DRSP and EE/LNG groups, where the AD levels were significantly higher in the EE/DRSP group (p<.01; Table 2).

3.2.5. E1 and E2

E1 levels were significantly increased in the DHEA treatment period in both COC groups compared to placebo (p<.0001; Table 2). In the placebo treatment period, both E1 and E2 were decreased significantly compared to baseline in both COC groups (p<.001). In the DHEA treatment period, E1 levels increased compared to baseline in both COC groups (p<.01) (Table 2). Compared to their respective baseline levels, E2 levels were significantly decreased in both the EE/DRSP + DHEA group (p<.005) and the EE/LNG+DHEA group (p<.0001).

4. Discussion

Based on observations in the current study, we conclude that adding 50 mg daily of DHEA to a COC containing 30-mcg EE along with either 3-mg DRSP or 150-mcg LNG maintained physiological levels of free T in healthy women, thereby preventing the significant reduction in free T levels associated with the use of COCs. Importantly, in the women receiving EE/DRSP, free T levels were restored to baseline, but the inclusion of DHEA significantly increased total T levels relative to baseline.

These results differ from our previous study using the same DRSP-containing COC, in which free T levels were restored to only 47% of baseline, and total T levels returned to — but did not exceed — baseline levels [21]. Changes in the levels of SHBG (which binds tightly to circulating T) cannot explain this difference in results, as both studies found that SHBG levels increased to a similar extent in the COC-only groups, and the addition of DHEA did not affect the levels of SHBG. On the other hand, the difference in results with respect to T levels between these two studies may be explained — at least in part — by the use of different T assays, which are affected differently by high levels of SHBG [35]; additional studies are needed to address this issue. Ideally, free T concentration should not be calculated

but should be measured directly using liquid chromatography assays or equilibrium dialysis [8,36].

Androgens — particularly T — have become increasingly appreciated as important hormones in both postmenopausal and premenopausal women [4,9–12,37,38]. The crossover design of our study allowed us to measure the effect of COC use on androgen levels during five cycles and then compare these results with the effect of COC combined with DHEA in the same group of patients. In the placebo treatment period (i.e., with the COC alone), all ovarian, adrenal and precursor androgens measured were decreased significantly, which is consistent with previous reports [14]. The addition of DHEA for five cycles significantly increased the levels of both total T and free T, E1 and the precursor androgens DHEA, DHEA-S and AD. The increases in precursor androgen levels relative to baseline are not considered to be clinically relevant, as these prohormones are biologically inactive in the circulation [39] and are relatively safe at the levels measured in our subjects. The increase in E1 levels was relatively small (an increase of approximately 40% above baseline) and is also not considered to be a safety concern, particularly in light of the huge increase in E1 associated with the new COCs that contain E2 [40–42].

Because COCs inhibit follicular development, in the placebo treatment periods, E2 levels decreased significantly relative to baseline in both COC groups. DHEA is metabolized by the liver to produce both T and E2 [43]; however, it is important to note that E2 levels were not increased during the DHEA treatment period in either COC group. This finding suggests that a daily dose of 50-mg DHEA is sufficient to maintain physiological levels of T but does not affect E2 levels.

In conclusion, we confirm that the COCs EE/DRSP and EE/LNG have strong suppressive effects on androgen levels. Moreover, adding a daily dose of 50-mg DHEA to the COC can maintain androgen levels in women using an LNG-containing COC. In addition to the improved endocrine outcome reported here, we report in our companion paper that adding DHEA also improves the clinical outcome, particularly with respect to some aspects of sexual function [30].

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