

Original research article

Restoring testosterone levels by adding dehydroepiandrosterone to a drospirenone containing combined oral contraceptive: I. Endocrine effects^{☆,☆☆,★}

Y. Zimmerman^{a,*}, J.-M. Foidart^b, A. Pintiaux^b, J.-M. Minon^c,
B.C.J.M. Fauser^d, K. Cobey^e, H.J.T. Coelingh Bennink^a

^aPantarhei Bioscience, Zeist, the Netherlands

^bDepartment of Gynecology–Obstetrics, University Hospital CHR Citadelle, Site Sainte Rosalie in Liège, Liège, Belgium

^cDepartment of Laboratory Medicine, University Hospital CHR Citadelle, Liège, Belgium

^dDepartment of Reproductive Medicine & Gynecology, University Medical Center Utrecht, Utrecht, the Netherlands

^eDepartment of Psychology, School of Natural Sciences, University of Stirling, Stirling, United Kingdom

Received 7 November 2013; revised 23 October 2014; accepted 2 November 2014

Abstract

Objectives: Combined oral contraceptives (COCs) decrease testosterone (T) levels. This study investigated restoration of T and other androgen concentrations during COC use by ‘co-administration’ of dehydroepiandrosterone (DHEA).

Study design: In this randomized, double-blind, placebo-controlled study in 99 new COC starters (18–35 years old with body mass index range 18–34 kg/m²), a COC containing 30 mcg ethinylestradiol (EE) and 3 mg drospirenone (DRSP) was used for 3 cycles, followed by 6 cycles of the same COC combined with either 50 mg/day DHEA or placebo. Total T, albumin, sex hormone-binding globulin (SHBG), DHEA-sulfate (DHEA-S), Δ 4-androstenedione (AD), 3 α -androstane diol glucuronide (ADG) and estradiol (E₂) were measured, whereas free T and the free T index (FTI) were calculated. Assessments took place at baseline (no COC use), after the run-in period (COC use alone) and during the treatment period (DHEA or placebo).

Results: During COC use alone, androgen levels decreased, especially total T by 62% and free T by 86%, and SHBG increased by 243%. Total T increased with DHEA compared to placebo (change from end of run-in period to end of treatment period — 1.3 \pm 1.2 nmol/L vs. 0.0 \pm 0.4 nmol/L; p<.0001) — and was restored to baseline levels. Free T and the FTI increased significantly (p<.0001), but the free T level was still 53% below baseline levels. DHEA-S, AD and ADG increased significantly to levels above baseline (p<.0001 for each). DHEA had no effect on SHBG, albumin and E₂.

Conclusions: An EE/DRSP containing COC strongly suppressed endogenous androgen concentrations in all users. The addition of 50 mg DHEA to a COC regimen containing EE/DRSP restored total T to baseline levels, but free T levels were restored by only 47% as most of the T remains bound to SHBG.

Implications: When using a COC that increases SHBG considerably, a daily dose of 50 mg DHEA is insufficient to normalize free T levels completely.

© 2015 Elsevier Inc. All rights reserved.

Keywords: Free testosterone; SHBG; DHEA; Androgens

[☆] Source of funding: The study was financially supported by Pantarhei Bioscience.

^{☆☆} Conflict of interest: Y.Z. is an employee of Pantarhei Bioscience (PRB), the company developing the Androgen Restored Contraceptive concept for contraception. J.M.F. has no conflict of interest in the course of this study. A.P. shares expertise as a lecturer, member of advisory boards and/or consultant, with Bayer, Amgen, Gedeon Richter and Teva/Theramex, without personal gain. J.M.M. has nothing to declare. K.C. has nothing to declare. B.F. has received fees and grant support from the following companies (in alphabetic order): Andromed, Ardana, Euroscreen, Ferring, Genovum, Merck (MSD), Merck Serono, Organon, Ovascience, Pantarhei Bioscience, PregLem, Schering, Schering Plough, Serono, Uteron Pharma, Watson Pharmaceuticals and Wyeth. H.C.B. is the CEO and a shareholder of PRB. After publication of the paper, PRB will make the clinical study report available upon request. The authors alone are responsible for the content and the writing of the paper.

^{*} Clinical Trial Registration Number: ISRCTN06414473.

^{*} Corresponding author at: PO Box 464, 3700 AL, Zeist, the Netherlands. Tel.: +31 30 6985020; fax: +31 30 6985021.

E-mail address: yz@pantarheibio.com (Y. Zimmerman).

1. Introduction

Combined oral contraceptives (COCs) reduce circulating androgen levels by inhibition of ovarian and adrenal androgen synthesis. In particular, the level of testosterone (T), the most potent female androgen, decreases by a mean of 31% [1]. In addition, the estrogenic component of COCs, ethinylestradiol (EE), stimulates hepatic sex hormone-binding globulin (SHBG) production considerably in a dose-dependent manner [1]. Because SHBG binds and inactivates T, there is further suppression of the free T concentrations (up to 61%) and the free androgen or T index (FAI or FTI), a measure of bioavailable T, is significantly reduced [1,2].

T deficiency in women has been associated with a broad range of undesired effects [3,4], some of which, including mood disturbances and interference with sexual function, have been reported as side effects of COCs [5–8]. Normalizing total T and other androgen levels in women using a COC may be achieved by the ‘co-administration’ of the natural human adrenal androgen dehydroepiandrosterone (DHEA), because it is orally bioavailable [9] and partially metabolized into T [10–12].

In the present prospective study, the effect of daily ‘co-administration’ of 50 mg DHEA in new COC users was investigated. In this paper, the effect on androgen and androgen-related endocrine parameters will be reported. In a second paper, the clinical effects on sexual function, mood, skin and safety will be reported [13].

2. Materials and methods

2.1. Study population

Participants had to be healthy, sexually active, aged between 18 and 35 years, and have a body mass index (BMI) between 18 and 35 kg/m². All participants must not have taken a hormonal contraceptive for at least 3 months prior to the start of the study medication. In addition, subjects were required to have a regular menstrual cycle of 25–35 days before the start of the study. Major exclusion criteria were contraindications for COC use; concomitant medication that might interfere with the metabolism of contraceptive steroids or DHEA; hyperandrogenism (free T levels ≥ 31.0 pmol/L), severe acne, severe hirsutism and polycystic ovarian syndrome based on ovarian ultrasound and/or the Luteinizing hormone/follicle-stimulating hormone ratio.

The study was approved by an independent ethics committee and was conducted in accordance with the Declaration of Helsinki and the ICH guideline for Good Clinical Practice. All participants gave written informed consent. The study has been registered at the International Standard Randomised Controlled Trial Number Register (no. ISRCTN06414473).

2.2. Study design and procedures

This randomized, double-blind, placebo-controlled, comparative, parallel-group study was conducted from November 2007 until November 2009 at the University Hospital Centre

Hospitalier Régional (CHR) Citadelle, in Liège, Belgium. Prior to randomization, evidence of ovulation during a spontaneous menstrual cycle was documented by a midluteal progesterone level of >4.77 nmol/L (1.5 ng/mL). Eligible participants were randomized to a 30 mcg EE and 3 mg drospirenone (DRSP) COC with ‘co-administration’ of DHEA or placebo in a ratio of 1:1. Although all COCs suppress androgens [1], the EE/DRSP COC was chosen, because at the time of study it was one of the most prescribed contraceptive pills in Western Europe [14]. Blinded study medication was packed per subject number according to a computer-generated randomization list that was only known to an independent biostatistician.

The study consisted of a 3-cycle run-in period with COC use alone, followed by a 6-cycle treatment period in which participants continued COC use in combination with either DHEA or a placebo. Each treatment cycle consisted of 28 days. During all treatment cycles, participants took one tablet of the EE/DRSP COC from day 1 to day 21 followed by a pill-free period of 7 days. During the 6-cycle treatment period, DHEA or placebo was used continuously, including during the pill-free period.

Study visits took place before the start of the COC during the luteal phase of the cycle (baseline), once every 3 cycles thereafter on cycle days 22–28 (cycle 3, cycle 6 and cycle 9) and at a follow-up 7–14 days after the last intake of study medication. During these visits, blood samples were taken in the morning after an overnight fasting period before administration of study medication.

The primary endpoint was the change in total T levels between the end of the run-in treatment period (cycle 3) and the end of the treatment period (cycle 9). Secondary objectives included the change in the following laboratory parameters during COC use only (baseline to end of the run-in period at cycle 3) and during the treatment period (end of the run-in period to cycle 6 and cycle 9): Total T, albumin, SHBG, free T, FTI, DHEA-sulfate (DHEA-S), $\Delta 4$ -androstenedione (AD), 3 α -androstane diol glucuronide (ADG) and estradiol (E₂).

2.3. Study medication

A dose of 50 mg DHEA was calculated to compensate for the loss of T induced by a COC, based on previous dose-finding studies with DHEA in elderly people [10], along with the magnitude of the decrease of T levels during COC use [1]. The DHEA and placebo tablets were manufactured by Unither Pharmaceuticals (Le Haillan, France) and were identical in appearance. The commercially available COC Yasmin® (Bayer Healthcare, Berlin) was used.

2.4. Analytical assays and measurements

Serum samples were sent to the laboratory of CHR Citadelle in Liège, Belgium, for immediate analysis (albumin, SHBG, DHEA-S, E₂) and for storage at -80 °C until further analysis at the end of the study (T, AD, ADG). Total T (nmol/L) was measured by ImmuChem Double Antibody TESTOSTERONE ¹²⁵I RIA Kit (ICN Biomedicals/MP Biomedicals). AD (nmol/L) and ADG (nmol/L) were analyzed using the Androstenedione-

RIA-CT and A-Diol Glucuronide-RIA-CT radioimmunoassay kits, respectively (DIAsource ImmunoAssays S.A.). Electrochemiluminescence immunoassays (Modular E170 analyzer; Roche) were applied for measuring SHBG (nmol/L), DHEA-S (μmol/L) and E₂ (pmol/L). Analysis of albumin was performed by an automated bromocresol green method on Modular system. The intra-assay and interassay coefficients of variance for the assays are shown in Supplemental Table 1.

Free T (pmol/L) was calculated based on total T, SHBG and albumin (g/L) concentrations [15,16]. The FTI (or FAI) was calculated using total T and SHBG, according to the formula: $FTI=100 \times [T]/[SHBG]$.

2.5. Statistical analysis

A sample size calculation was carried out, which was based on the primary objective of the study to test the null hypothesis that 50 mg DHEA added to an EE/DRSP COC is able to significantly increase total T levels from the end of the run-in period to the end of the treatment period compared to placebo. Assuming that T levels would decline by at least one third of their value (33%) at the end of the run-in-period, and that initial T levels would be completely restored (100%) after 6 cycles in the DHEA group and remain unchanged in the placebo group, at the 5% significance level and with a power of 90%, 37 participants were to be needed in each group to demonstrate a difference (total 74). Considering a 30% adjustment of sample sizes due to discontinuation and nonevaluable cycles, a minimum of 96 women were to be included in the study. Therefore, it was decided to randomize in total 100 participants (50 per treatment group).

Data analyses were based on an intention-to-treat approach. A log-transform was used to normalize the distribution of the variables if necessary. Within each treatment group (DHEA and

Table 1
Baseline characteristics of the study population (n=99).

Parameter	DHEA (n=49)	Placebo (n=50)
Age, years	23.9±3.7	22.6±3.9
BMI, kg/m ²	22.8±4.0	21.7±2.5
Ethnic origin		
Caucasian, n (%)	46 (94)	47 (94)
Black, n (%)	2 (4)	2 (4)
Asian, n (%)	1 (2)	1 (2)
Other, n (%)	0	0
Endocrine parameters		
Total T (nmol/L)	1.4±0.7	1.4±0.6
SHBG (nmol/L)	59.3±21.1	58.0±23.1
Albumin (g/L)	46.2±2.3	46.8±2.3
Free T (pmol/L)	19.6±14.6	19.4±12.7
FTI	3.1±2.9	3.1±2.8
DHEA-S (μmol/L)	4.9±1.8	5.2±1.9
Δ4-AD (nmol/L)	8.5±3.1	7.7±2.2
3α-androstenediol (nmol/L)	14.7±6.8	14.1±5.7
E ₂ (pmol/L)	532.9±332.1	516.3±257.6

Data expressed in mean±standard deviation. n, number of subjects.

placebo), changes between study visits were assessed for each variable using a paired t test. When comparing these changes between the two groups (DHEA vs. placebo), the unpaired t test with unequal variances was used. Results were considered to be significant at the 5% level (p<.05). All calculations were carried out using SAS (Version 9.1 for Windows) and S-PLUS (Version 7.0) statistical packages.

3. Results

3.1. Study population characteristics

A total of 142 women were screened, of which 99 were randomized and treated. The baseline characteristics of the

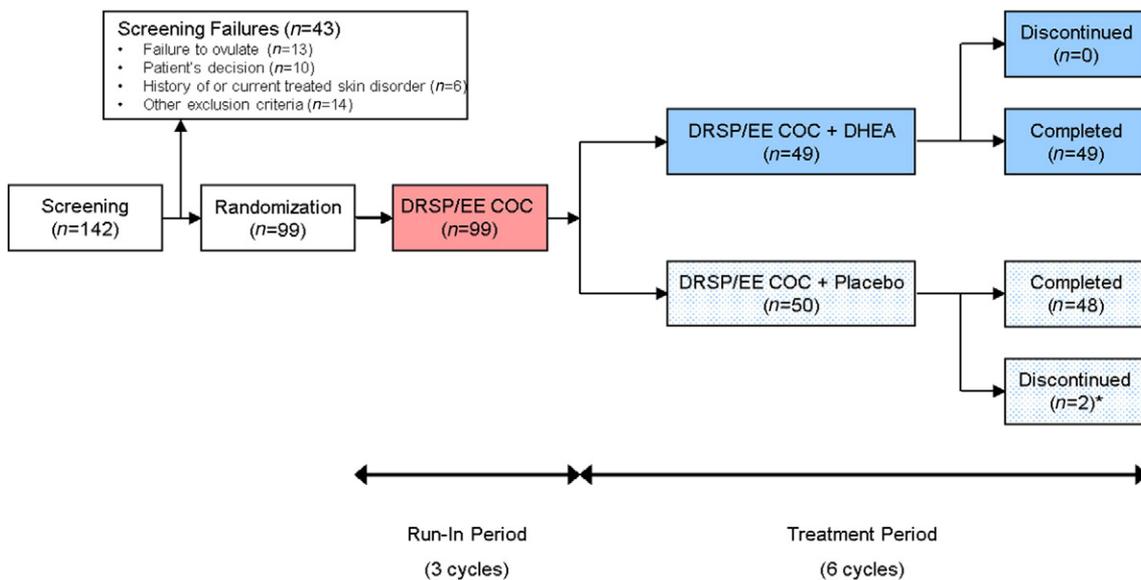


Fig. 1. Study design and subject disposition. *One discontinued after 6 cycles for logistical reasons and the second one after 4 cycles at her own initiative because of hirsutism; both in the placebo group.

study population were comparable between groups (Table 1). Only two women, both in the placebo group, discontinued early (Fig. 1), one after 6 cycles for logistical reasons and one after 4 cycles at her own initiative because of hirsutism. All available data were included in the analyses.

3.2. Endocrine parameters

The effect of COC use alone on androgens and parameters related to androgen metabolism during the run-in period are shown in Table 2. At the end of the run-in period, a statistically significant decline of 62% was found for total T ($p < .0001$) (Table 2 and Fig. 2). A large and statistically significant decrease of free T (86%) and the FTI (91%) was also observed (both $p < .0001$) (Table 2 and Fig. 2). SHBG levels increased significantly (243%; $p < .0001$) (Table 2 and

Fig. 2). COC use alone resulted in statistically significant decreases in all the other parameters (Table 2).

The effect of 50 mg DHEA plus COC compared to placebo plus COC on androgens and parameters related to androgen metabolism is shown in Table 2. Total T significantly increased in the DHEA group compared to the placebo group (change from end of run-in period to end of treatment period: 1.3 ± 1.2 vs. 0.0 ± 0.4 nmol/L; $p < .0001$) (Table 2 and Fig. 2). Statistically significant increases from the end of the run-in period to the end of the treatment period in the DHEA compared to the placebo group were also observed for free T, the FTI, DHEA-S, AD and ADG (Table 2; $p < .0001$ for each). The change from the end of the run-in period to the end of the treatment period was not statistically significantly different between the DHEA and the placebo groups for SHBG, albumin and E_2 (Table 2).

Table 2

Effect of COC use alone and of 50 mg DHEA daily 'co-administration' compared to placebo on androgens and parameters related to androgen metabolism in women using a COC containing 30 mcg EE and 3 mg DRSP.

Variable	Baseline	End of run-in period (cycle 3: COC only)	Relative change from baseline	Treatment	Treatment period (cycle 6)	End of treatment period (cycle 9)	Change from end of run-in to end of treatment period ^a	Change from baseline to end of treatment period	Relative change from baseline
Total T (nmol/L)									
All ($n=99$)	1.4±0.6	0.6±0.4*	-62%	DHEA ($n=49$)	2.1±1.5	1.7±1.1	1.3±1.2**	0.3±1.1**	20%
				Placebo ($n=48$)	0.8±1.5	0.6±0.4	0.0±0.4	-0.8±0.6	-52%
SHBG (nmol/L)									
All ($n=99$)	58.4±22.4	199.3±64.5*	243%	DHEA ($n=49$)	196.2±64.6	195.1±64.4	-7.4±69.6	134.9±57.5	234%
				Placebo ($n=48$)	211.8±59.8	206.9±68.1	13.3±61.5	140.7±76.3	255%
Albumin (g/L)									
All ($n=99$)	46.5±2.3	43.9±2.1*	-6%	DHEA ($n=49$)	43.9±2.4	43.8±2.0	0.1±1.6	-2.5±2.0	-5%
				Placebo ($n=48$)	43.7±2.4	43.9±1.9	-0.2±2.1	-3.7±5.5	-6%
Free T (pmol/L)									
All ($n=99$)	19.5±13.6	2.8±2.4*	-86%	DHEA ($n=49$)	10.6±7.9	9.3±8.0	6.8±7.8**	-10.3±13.1**	-53%
				Placebo ($n=48$)	3.6±5.8	3.0±2.8	-0.2±2.8	-15.2±9.2	-79%
FTI									
All ($n=99$)	3.1±2.9	0.3±0.3*	-91%	DHEA ($n=49$)	1.2±0.9	1.1±1.0	0.8±1.0**	-2.1±2.5**	-66%
				Placebo ($n=48$)	0.4±0.6	0.3±0.3	-0.0±0.4	-2.5±1.7	-88%
DHEA-S (µmol/L)									
All ($n=99$)	5.0±1.9	3.7±1.9*	-26%	DHEA ($n=49$)	10.1±4.9	9.3±4.9	5.9±4.5**	4.4±4.4**	88%
				Placebo ($n=48$)	3.6±1.7	3.8±2.1	-0.3±0.9	-1.4±1.4	-27%
Δ4-AD (nmol/L)									
All ($n=99$)	8.1±2.7	5.4±2.1*	-33%	DHEA ($n=49$)	12.2±6.3	10.2±5.8	5.1±6.0**	1.7±6.0**	22%
				Placebo ($n=48$)	6.3±2.2	5.5±2.5	-0.3±2.1	-2.2±2.5	-27%
3α-androstanediol (nmol/L)									
All ($n=99$)	14.4±6.2	8.6±4.2*	-40%	DHEA ($n=49$)	22.8±13.2	19.5±9.3	10.9±8.3**	4.9±9.0**	34%
				Placebo ($n=48$)	9.1±2.6	8.0±3.0	-0.8±2.9	-6.1±5.2	-42%
E₂ (pmol/L)									
All ($n=99$)	524.9±294.0	119.3±95.4	-77%	DHEA ($n=49$)	117.1±107.3	140.2±98.2	11.3±142.2	-392.7±330.9	-75%
				Placebo ($n=48$)	122.8±88.4	139.7±97.5	34.5±126.0	-372.8±281.8	-71%

Data expressed in mean±standard deviation. COC, combined oral contraception; n , number of subjects.

^a Primary study objective.

* $p < .0001$ between no COC (baseline) and COC use only (cycle 3) (in bold font).

** $p < .0001$ between-group difference (in bold font).

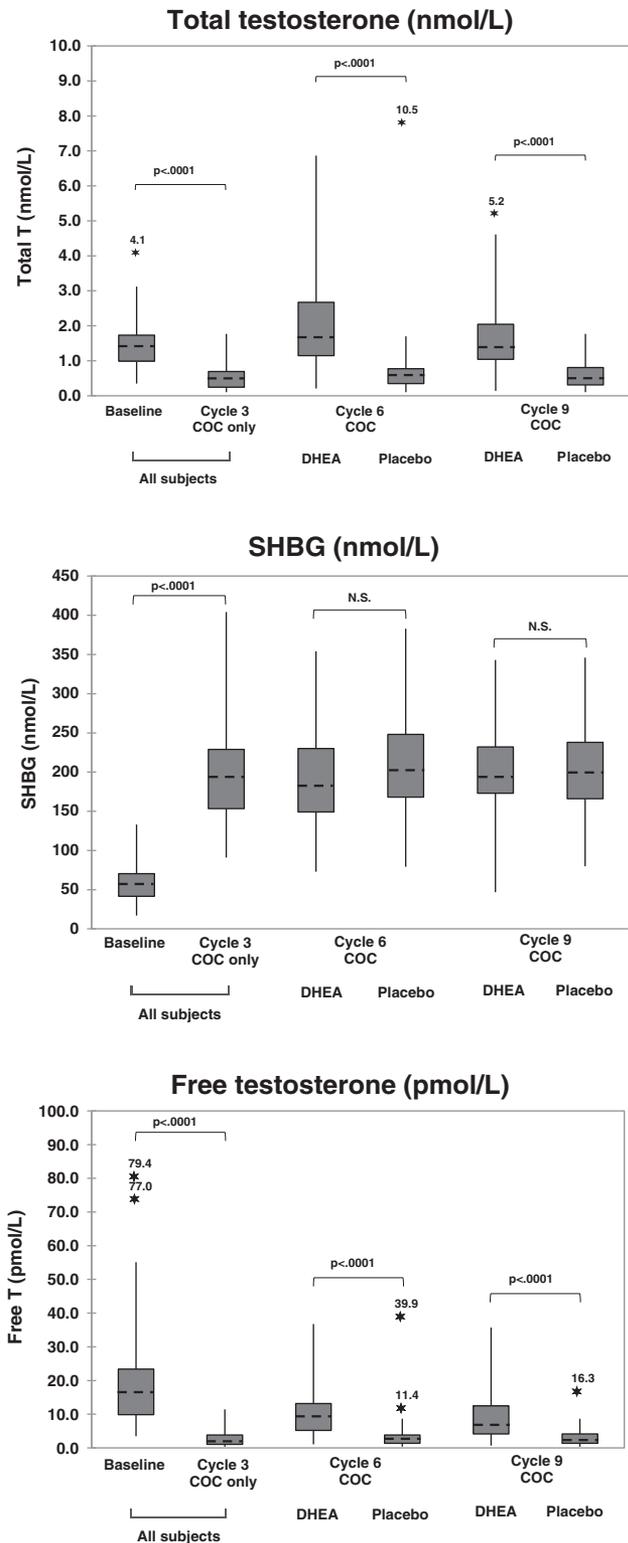


Fig. 2. Effect of COC use alone and of 50 mg DHEA daily ‘co-administration’ on total T (nmol/L), SHBG (nmol/L) and free T (pmol/L) in women using a COC containing 30 mcg EE and 3 mg DRSP. In these box plots, half of the data (percentile 25–75) is represented by the boxes. Dark dashed lines in the boxes indicate the median. T-bars from the boxes extend to the minimum and maximum. Stars are outliers, defined as values more than three times the height of the box.; N.S., not significant; T, testosterone.

The change and the relative change from baseline to the end of the treatment period are also shown in Table 2. Total T levels were restored to baseline levels in the DHEA group and remained low in the placebo group; the levels were significantly increased from baseline to the end of the treatment period in the DHEA group compared to placebo [0.3 nmol/L (relative change: 20%) vs. -0.8 nmol/L (-52%); $p < .0001$] (Table 2 and Fig. 2). In the placebo group, the free T levels and the FTI remained as low as at baseline, and in the DHEA group, they were still lower than at baseline, but were statistically higher than placebo [free T: -10.3 pmol/L (-53%) vs. -15.2 pmol/L (-79%); FTI: -2.1 (-66%) vs. -2.5 (-88%); both $p < .0001$] (Table 2 and Fig. 2). The increased levels of SHBG observed during the run-in period were maintained at the same level in both treatment groups and there was no statistically significant difference between the groups [134.9 (234%) vs. 140.7 (255%)] (Table 2 and Fig. 2). DHEA-S, AD and ADG levels were restored to levels above baseline in the DHEA group but not in the placebo group (between group difference $p < .0001$ for each; Table 2). There were no statistically significant between group differences for albumin and E_2 ; albumin levels remained relatively unchanged in both groups (relative change: -5% and -6%) and E_2 levels decreased from baseline in both groups (-75% and -71%).

4. Discussion

To our knowledge this is the first study aiming at restoring the levels of T during COC use. For this purpose we could have used e.g. a T-gel or a T-patch, but we have chosen to try to achieve normalization of T levels by daily ‘co-administration’ of the oral T-precursor DHEA, since our goal is to develop a new oral contraceptive combination.

The study was designed to create optimal conditions to evaluate first the effect of a COC only. Therefore only women who had not used hormonal contraception for at least 3 months were included in the study and there was a 3-cycle run-in period with COC use alone prior to the DHEA/placebo treatment period. The observed qualitative changes of the (precursor) androgens and SHBG after COC use alone are in agreement with previous studies [1,2,17–21], but the quantitative suppression of total T and free T by 62% and 86%, respectively, is more pronounced than reported in the literature previously (mean of 31% and 61%, respectively) [1]. Importantly, all COC users experienced a decrease of their total and free T.

By administering 50 mg/day DHEA, total T was restored completely to baseline levels. However, the decrease of the biologically active free T levels was restored by only 47%. We hypothesize that this is caused by the increased binding of T due to the strong rise of SHBG (243%) caused by the COC. The progestin DRSP does not counteract the effect of the estrogen (EE) on SHBG and may even increase SHBG further due to its antiandrogenic properties. Also the radioimmunoassay used to measure total T itself may be sensitive to high SHBG levels [22]. Levels of total T, as well as its measurement, are critically

influenced by the concomitant rise of SHBG, which occurs with most, but not all COCs; therefore, total T levels during COC use may be unreliable to judge the androgen status. Studies aiming at restoration of T homeostasis during COC use need to take these issues into account [22]. Although androgens including DHEA are known to decrease SHBG [23,24], in the current study, no significant decrease of SHBG was observed in the DHEA group, which may be due to the relatively low 50 mg dose of DHEA used. The precursor androgens DHEA-S, AD and ADG were restored to levels above baseline. This is not considered to be clinically relevant since these circulating androgen precursors are biologically inactive [25]. Overall, to normalize free T completely when using a DRSP containing COC, a higher dose of DHEA may be required.

Accurate measurement of free T in blood of females is difficult [26,27] due to the very low concentrations (35–700 pmol/L; [24]), especially in women using COCs [1]. Preferred methods for direct measurement of free T are the mass action equation and equilibrium dialysis [3,28]. These methods are time-consuming and have several drawbacks, such as requirement of a large volume sample, dilution effects, complicated correction of volume changes and inherent limitations of tracer analog method [29]. Furthermore, the detection level of the available LC-MS/MS method was not low enough. Therefore, in this study free T was obtained by calculation based on total T, SHBG and albumin concentrations [15,16], which has previously been demonstrated to be a reliable method and comparable to direct measurements [28,30].

A small but significant lowering of albumin by COC use only has been observed in some [2,31] but not all [32] earlier studies. In the current study, neither the COC use only nor the addition of DHEA affected albumin levels. Because COCs inhibit follicular development, E₂ levels decreased significantly during both treatments. Since oral DHEA is not only metabolized by the liver to T, but also to E₂ [33], it is important to note that E₂ levels did not increase with the low 50 mg dose of DHEA used in the current study.

In conclusion, this study shows that an EE/DRSP containing COC strongly suppresses endogenous androgen concentrations in all users. Total T levels were restored completely to baseline levels by adding 50 mg DHEA, but this was partly due to the strong increase of SHBG and thereby of biologically inactive SHBG-bound T, whereas the biologically active free T levels were restored by only 47%. The clinical effects of adding DHEA to this COC will be reported in a separate paper [13].

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.contraception.2014.11.002>.

Acknowledgments

We acknowledge the excellent contribution of the entire study staff at the Hospital CHR Citadelle, Site Sainte Rosalie in Liège, Belgium, ensuring a remarkable study compliance and high quality of the study data. We are very grateful to Prof. A. Albert and his team at the Department of Biostatistics, University Hospital of Liège in Belgium, who performed the

statistical analysis of the data. We are also thankful to Louise Beekman who performed the monitoring of the study with enormous dedication. We would like to thank Amanda Prowse, PhD (Appletree Medical Writing), for her editorial assistance in the preparation of the manuscript.

References

- [1] Zimmerman Y, Eijkemans MJ, Coelingh Bennink HJ, Blankenstein MA, Fauser BC. The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Hum Reprod Update* 2014;20:76–85.
- [2] Coenen CM, Thomas CM, Borm GF, Hollanders JM, Rolland R. Changes in androgens during treatment with four low-dose contraceptives. *Contraception* 1996;53:171–6.
- [3] Bachmann G, Bancroft J, Braunstein G, Burger H, Davis S, Dennerstein L, et al. Female androgen insufficiency: the Princeton consensus statement on definition, classification, and assessment. *Fertil Steril* 2002;77:660–5.
- [4] Traish A, Guay AT, Spark RF. Are the Endocrine Society's Clinical Practice Guidelines on Androgen Therapy in Women misguided? A commentary. *J Sex Med* 2007;4:1223–34 [discussion 34–5].
- [5] Gingnell M, Engman J, Frick A, Moby L, Wikström J, Fredrikson M, et al. Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill—a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. *Psychoneuroendocrinology* 2013;38:1133–44.
- [6] Oddens BJ. Women's satisfaction with birth control: a population survey of physical and psychological effects of oral contraceptives, intrauterine devices, condoms, natural family planning, and sterilization among 1466 women. *Contraception* 1999;59:277–86.
- [7] Sanders SA, Graham CA, Bass JL, Bancroft J. A prospective study of the effects of oral contraceptives on sexuality and well-being and their relationship to discontinuation. *Contraception* 2001;64:51–8.
- [8] Wallwiener M, Wallwiener L-M, Seeger H, Mueck AO, Zipfel S, Bitzer J, et al. Effects of sex hormones in oral contraceptives on the female sexual function score: a study in German female medical students. *Contraception* 2010;82:155–9.
- [9] Arlt W, Justl H-G, Callies F, Reincke M, Hübler D, Oettel M, et al. Oral dehydroepiandrosterone for adrenal androgen replacement: pharmacokinetics and peripheral conversion to androgens and estrogens in young healthy females after dexamethasone suppression. *J Clin Endocrinol Metab* 1998;83:1928–34.
- [10] Legrain S, Massien C, Lahlou N, Roger M, Debuire B, Diquet B, et al. Dehydroepiandrosterone replacement administration: pharmacokinetic and pharmacodynamic studies in healthy elderly subjects. *J Clin Endocrinol Metab* 2000;85:3208–17.
- [11] Meno-Tetang GM, Blum RA, Schwartz KE, Jusko WJ. Effects of oral prasterone (dehydroepiandrosterone) on single-dose pharmacokinetics of oral prednisone and cortisol suppression in normal women. *J Clin Pharmacol* 2001;41:1195–205.
- [12] Morales AJ, Nolan JJ, Nelson JC, Yen SS. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J Clin Endocrinol Metab* 1994;78:1360–7.
- [13] Zimmerman Y, Foidart J-M, Pintiaux A, Minon J-M, Fauser BCJM, Cobey K, et al. Restoring testosterone levels by adding dehydroepiandrosterone to a drospirenone containing combined oral contraceptive: II Clinical effects. *Contraception* 2015;91:134–42.
- [14] Mansour D. Welcome developments in contraception. *Trends Urol Gynaecol Sex Health* 2009;14:4–6.
- [15] Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 1982;16:801–10.
- [16] Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999;84:3666–72.

- [17] Ågren UM, Anttila M, Mäenpää-Liukko K, Rantala M-L, Rautiainen H, Sommer WF, et al. Effects of a monophasic combined oral contraceptive containing norgestrel acetate and 17beta-oestradiol in comparison to one containing levonorgestrel and ethinylestradiol on markers of endocrine function. *Eur J Contracept Reprod Health Care* 2011;16:458–67.
- [18] Duijkers IJ, Klipping C, Grob P, Korver T. Effects of a monophasic combined oral contraceptive containing norgestrel acetate and 17 beta-oestradiol on ovarian function in comparison to a monophasic combined oral contraceptive containing drospirenone and ethinylestradiol. *Eur J Contracept Reprod Health Care* 2010;15:314–25.
- [19] Haring R, Hannemann A, John U, Radke D, Nauck M, Wallaschofski H, et al. Age-specific reference ranges for serum testosterone and androstenedione concentrations in women measured by liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab* 2012;97:408–15.
- [20] Wiegatz I, Jung-Hoffmann C, Kuhl H. Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. *Contraception* 1995;51:341–6.
- [21] Wiegatz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, et al. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 2003;67:25–32.
- [22] Heijboer AC, Zimmerman Y, de Boer T, Coelingh Bennink H, Blankenstein MA. Peculiar observations in measuring testosterone in women treated with oral contraceptives supplemented with dehydroepiandrosterone (DHEA). *Clin Chim Acta* 2014;430:92–5.
- [23] Granger LR, Roy S, Mishell Jr DR. Changes in unbound sex steroids and sex hormone binding globulin-binding capacity during oral and vaginal progestogen administration. *Am J Obstet Gynecol* 1982;144:578–84.
- [24] Speroff L, Fritz M. *Clinical gynecologic endocrinology and infertility*. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
- [25] Burger HG. Androgen production in women. *Fertil Steril* 2002;77 (Suppl 4):S3–5.
- [26] Groenestegge WM, Bui HN, ten Kate J, Menheere PCCA, Oosterhuis WP, Vader HL, et al. Accuracy of first and second generation testosterone assays and improvement through sample extraction. *Clin Chem* 2012;58:1154–6.
- [27] Taieb J, Mathian B, Millot F, Patricot M-C, Mathieu E, Queyrel N, et al. Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography–mass spectrometry in sera from 116 men, women, and children. *Clin Chem* 2003;49:1381–95.
- [28] Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. *J Clin Endocrinol Metab* 2004;89:525–33.
- [29] Burtis CA, Ashwood ER, & Bruns DE, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. St Louis Saunders: Elsevier; 2012.
- [30] Rinaldi S, Geay A, Déchaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. *Cancer Epidemiol Biomarkers Prev* 2002;11:1065–71.
- [31] Carlstrom K, Karlsson R, Von Schoultz B. Diurnal rhythm and effects of oral contraceptives on serum dehydroepiandrosterone sulfate (DHEAS) are related to alterations in serum albumin rather than to changes in adrenocortical steroid secretion. *Scand J Clin Lab Invest* 2002;62:361–8.
- [32] Hammond GL, Langley MS, Robinson PA, Nummi S, Lund L. Serum steroid binding protein concentrations, distribution of progestogens, and bioavailability of testosterone during treatment with contraceptives containing desogestrel or levonorgestrel. *Fertil Steril* 1984;42:44–51.
- [33] Legrain S, Girard L. Pharmacology and therapeutic effects of dehydroepiandrosterone in older subjects. *Drugs Aging* 2003;20:949–67.