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Clinical Research Article

Changes in Sex Steroids and Relation With Menopausal Complaints in Women Undergoing Risk-reducing Salpingo-oophorectomy

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Abstract

Context: Risk-reducing salpingo-oophorectomy (RRSO) is performed in BRCA1 or 2 mutant carriers to minimize ovarian cancer risk. Although studies have been performed investigating sex steroid levels, menopausal complaints, and sexual functioning in relation to RRSO, their exact relationship remains unknown.

Objectives: To investigate the impact of RRSO on serum sex steroid levels and their association with menopausal complaints and sexual functioning.

Methods: This prospective observational cohort study included 57 premenopausal and 37 postmenopausal women at risk of ovarian cancer and opting for RRSO. Data collection involved validated questionnaires on sexual functioning and menopausal complaints. Testosterone, androstenedione, estradiol, and estrone levels in serum determined by liquid chromatography-tandem mass spectrometry were obtained 1 day before, 6 weeks, and 7 months after RRSO.

Results: In premenopausal women, all 4 steroids were decreased both 6 weeks ($P < 0.01$) and 7 months ($P < 0.01$) after RRSO. Furthermore, in these women, decreases in estrogens were associated with a decrease in sexual functioning 7 months after RRSO ($P < 0.05$). In postmenopausal women, only testosterone was decreased 6 weeks and 7 months ($P < 0.05$) after RRSO, which was associated with an increase in menopausal complaints at 7 months post-RRSO ($P < 0.05$).

Conclusion: Our results suggest that in premenopausal women, decreases in estrogens are related to a decrease in sexual functioning and that in postmenopausal women, testosterone is decreased after RRSO, which indicates that postmenopausal ovaries maintain some testosterone production. Furthermore, in postmenopausal women, a large decrease of testosterone was associated with more menopausal complaints, indicating that future studies investigating testosterone supplementation are warranted.

Key Words: risk-reducing salpingo-oophorectomy, menopausal complaints, sexual functioning, androgens, estrogens

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; E1, estrone; E2, estradiol; ES, effect size; HFRS, Hot Flush Rating Scale; HRT, hormone replacement therapy; LC-MS/MS, liquid chromatography tandem-mass spectrometry; OC, ovarian carcinoma; RRSO, risk-reducing salpingo-oophorectomy; SFQ, Sexual Functioning Questionnaire.

Approximately 10% to 15% of all ovarian carcinomas (OC) are due to inherited predisposition [1-3]. Ovarian cancer screening has not been proven to be effective in detecting OC at an earlier stage and thereby improving prognosis [4, 5]. Therefore, risk-reducing salpingo-oophorectomy (RRSO) is recommended to lower the risk of OC [6, 7]. After RRSO, the risk of OC is reduced by 80% to 96% [8-10]. The recommended age for RRSO after childbearing in BRCA1 carriers is between 35 and 40 years, and in BRCA2 carriers, between 40 and 45 years. Women from a hereditary breast and ovarian cancer family (≥2 first-degree relatives with OC) are advised to undergo RRSO after childbearing is completed, but no specific age is given [8, 10].

A major side effect of RRSO in premenopausal women is the immediate onset of menopause. This is accompanied by an increase in noncancer-related morbidity, including a range of endocrine symptoms, sexual symptoms, mood disturbance, increased risk of cardiovascular disease, and osteoporosis [11-13]. For example, women generally experience a decline in sexual function after RRSO. Notably, the use of

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hormone replacement therapy (HRT) mitigates some of these symptoms [14-18]. The association of serum sex steroid levels with sexual function has been controversial. Some authors have shown an association between sex steroids and either female sexual dysfunction or hypoactive sexual desire disorder [19, 20]. Others did not find an association between sexual domain scores and sex steroid levels [21, 22]. A better understanding of factors that influence the severity of symptoms following RRSO could improve patient counseling and possibly treatment of symptoms.

In addition, it has also been debated whether the postmenopausal ovary still produces androgens, especially testosterone. Judd et al were the first to demonstrate a decline in concentrations of circulating testosterone and androstenedione in postmenopausal women following bilateral oophorectomy [23]. Their findings were supported by other reports [24, 25]. However, Couzinet et al presented strong evidence that the postmenopausal ovary does not contribute to circulating androgen levels [26]. Notably, most of these studies present an important limitation that scarcely has been addressed. Serum testosterone, and other sex steroids in serum, have been primarily analyzed using immunoassay technology, which tends to lack sensitivity and specificity in low concentration ranges. This limitation has been extensively described in literature and emphasizes substantial discrepancies when serum testosterone is measured in women [27-31]. To increase reliability of these measurements, there has recently been a shift toward routine application of liquid chromatography tandem-mass spectrometry (LC-MS/MS)-based analysis of steroid hormones, including testosterone [32-34]. This technique is currently considered to be the best practice for steroid analysis and is recommended for pediatric and female testosterone analysis [32].

In the present study, our objective was to investigate longitudinal sex steroid levels in pre- and postmenopausal women undergoing RRSO using highly sensitive and specific LC-MS/MS methods. In addition, we investigated differences in steroid levels between naturally postmenopausal women (postmenopausal group before RRSO) and women in which menopause was surgically induced by RRSO (premenopausal group). Finally, we aimed to examine the relationship between changes in these sex steroid levels and both sexual functioning and menopausal symptoms validated questionnaire scores.

Methods

Research Setting and Study Sample

This prospective, observational, multicenter study was performed at the Netherlands Cancer Institute (Amsterdam) and the Leiden University Medical Center in the Netherlands. Participants were included between November 2006 and April 2012 [35]. Pre- and postmenopausal patients undergoing RRSO with a BRCA1 or BRCA2 mutation or women with a familial risk that was estimated to exceed 10% were eligible [36]. Women visiting the gynecology outpatient clinic opting for RRSO were invited to participate in the study. All participants provided written informed consent.

Inclusion criteria for the current study were (1) the absence of malignancies at the time of RRSO and (2) no HRT use or hormonal therapy during the study period. Postmenopausal status was defined by amenorrhea for at least 12 months, and pre- and postmenopausal groups were defined based on menopausal status at baseline. Blood samples and questionnaire scores were obtained within 1 week before (T0), 6 weeks after (T1), and 7 months after (T2) RRSO. The study was in accordance with the Declaration of Helsinki and the institutional review boards of the Leiden University Medical Center and the Netherlands Cancer Institute approved the study. Written consent was obtained from each participant.

Measures

The respondents' age, education, relationship status, parity, body mass index (BMI), comorbidities, mutation status (BRCA1/2), regular menses, history of breast cancer, previous breast cancer treatments, and current menopausal status were obtained by self-report. Women were asked if they had regular menses during the past 3 months. If a negative response was received, inquiries were made about the reason of the absent menses.

Between 9:00 am and 5:00 pm, blood was collected in serum separator tubes, centrifuged (10 minutes at 2500 g) and stored at −30°C until analysis. Serum testosterone, estradiol (E2) and estrone (E1) analysis was performed using previously published methods [37, 38]. A full description of the method and validation for the quantitation of androstenedione in serum is described in Supplementary Data 1 [39]. All steroids were measured using an LC-MS/MS assay. The lower limit of quantitation for each steroid was determined at 0.025 nmol/L (testosterone), 0.35 nmol/L (androstenedione), 8.0 pmol/L (E2), and 6.9 pmol/L (E1).

The perceived intensity of hot flushes was assessed using the Hot Flush Rating Scale (HFRS) [40]. This scale is used to generate a mean of 3 scores (1-10 scale) to rate to what extent hot flushes were bothersome and caused interference with daily life in the preceding week. Lower scores indicated less intense symptoms, whereas higher scores indicated more intense symptoms.

We assessed sexual functioning with the Sexual Functioning Questionnaire (SFQ) [41]. The SFQ consists of 7 domains: desire (SFQ Desire, 6-items), arousal-sensation (SFQ Arousal S, 4 items), arousal-lubrication (SFQ Arousal L, 2 items), orgasm (SFQ Orgasm, 3 items), enjoyment (SFQ Enjoyment, 6 items), pain (SFQ Pain, 3 items), and partner relationship (SFQ Partner, 2 items). Higher scores indicate better sexual functioning [41].

The Functional Assessment of Cancer-Therapy-Endocrine Symptoms was used to monitor menopausal symptoms. The questionnaire consists of 18 items that address a range of menopausal symptoms. Occurrence of each symptom in the past 4 weeks was scored on a 5-point scale, ranging from "not at all" to "very much." Item scores were summed to obtain a total score (range 0-72), with higher values indicating more menopausal symptoms [42].

Statistics

Pre- and postmenopausal groups were analyzed independently. For description of baseline characteristics, data of continuous variables were checked for normality using Q-Q plots and Shapiro-Wilk tests. Normally distributed data were described by mean and SD. In contrast, data that were not normally distributed were described by median and interquartile range. For longitudinal analysis of steroids, differences between time points were checked for normality. In case normality could be assumed, data were

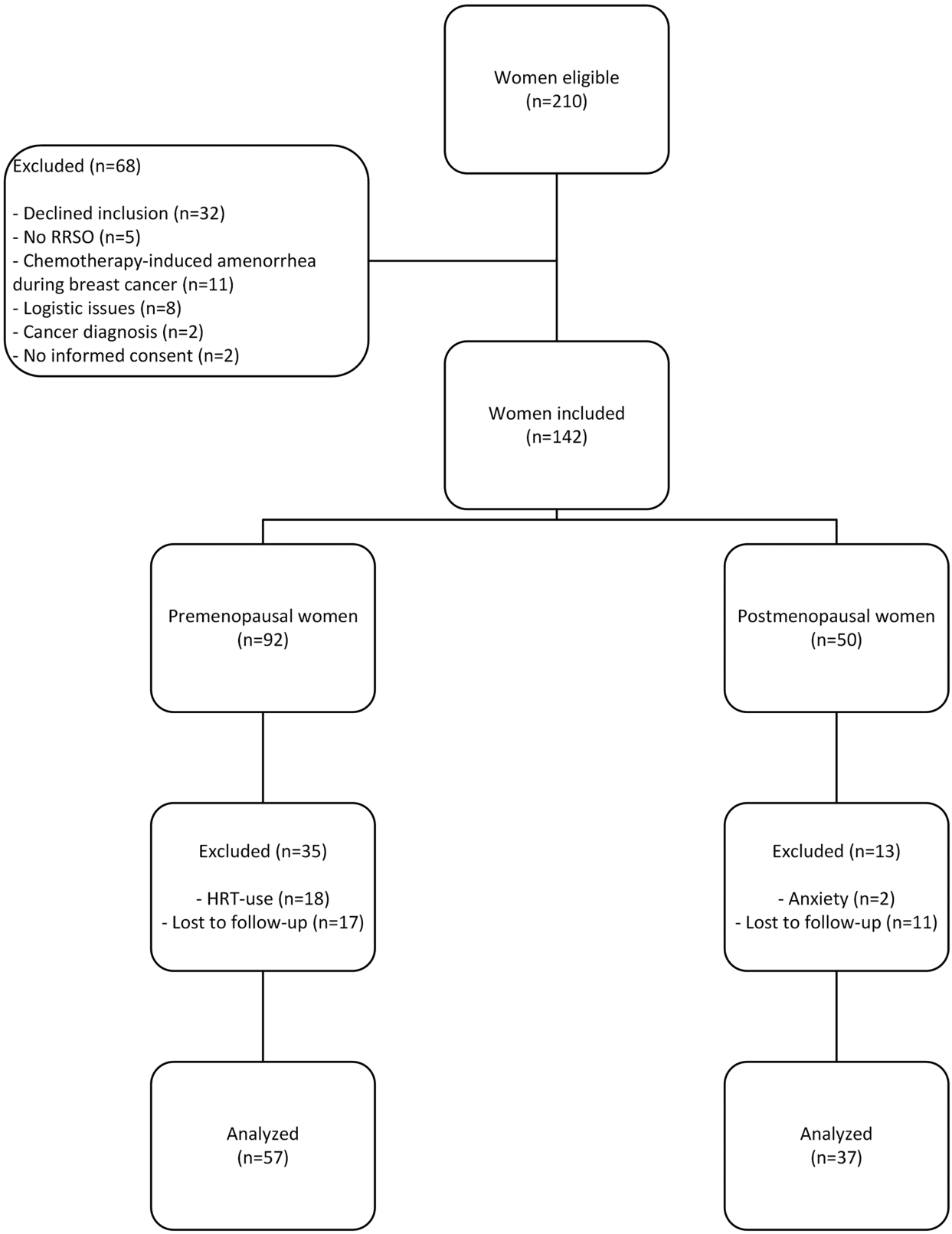

Figure 1. Flow chart displaying participant inclusion and exclusion. Abbreviation: RRSO, risk-reducing salpingo-oophorectomy.

investigated using repeated measures analysis of variance (ANOVA) models to assess within-group (follow-up) effects. Generalized eta-squared (η_g^2)values were calculated to describe the effect size (ES) of the ANOVA output. ES ≤ 0.08 was considered low; 0.09 to 0.16, medium; and ≥0.17, high. Post hoc pair-wise paired *t*-tests (Bonferroni correction) were performed between follow-up moments to evaluate short-term (ΔT0-1), long-term (ΔT0-2), and follow-up (ΔT1-2) differences between steroid levels. In case steroid differences between time points were not normally

distributed, only nonparametric pairwise sign tests were performed. Differences in steroid levels between naturally postmenopausal women (postmenopause T0) and surgically postmenopausal women (premenopause T1 and T2) were assessed using unpaired *t*-tests or Mann-Whitney U tests depending on whether normality could be assumed. Differences in questionnaire scores between follow-up moments were evaluated using paired *t*-tests and Wilcoxon sign rank tests depending on the normality of the differences between the repeated measures. Spearman correlation coefficients (ρ) and its corresponding difference with 0 ($P < 0.05$ was significant) were used to assess associations between changes in steroid levels and questionnaires in 2 follow-up moments (ΔT0-1, ΔT0-2, and Δ T1-2). Correlation coefficients were color coded at a 3-point level: blue indicating $\rho < 0$, white indicating $\rho \approx 0$, and red indicating $\rho > 0$. Significant correlations were marked using asterisk symbols ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$). To adjust for multiple hypothesis testing, heat map interpretation was focused at correlation of either both androgens or estrogens with a questionnaire score between 2 follow-up moments. All statistical analyses were performed using R (version 4.1.0). Spearman's Rho correlation coefficients were color-coded in MS Excel (version 2016) to generate heat maps.

Results

Between November 2006 and April 2012, 142 of the 210 eligible women were enrolled onto the study, of whom 92 and 50 were pre- and postmenopausal at RRSO, respectively (Fig. 1). Of these women, 48 were excluded based on predefined exclusion criteria, predominantly incomplete follow-up (n = 28; lack of interest or unknown reasons) or HRT use during the trial (n = 18). In total, 94 (57 premenopausal, 37 postmenopausal) were included for final data analysis. Baseline characteristics separated for pre- and postmenopausal women are listed in Table 1. Age, DNA status, and comorbidities were significantly different between menopausal groups.

In Figure 2, box plots for each steroid at baseline (T0), 6 weeks (T1), and 7 months (T2) after RRSO are displayed. For testosterone, ANOVA analysis showed that serum concentrations were affected by follow-up ($P < 0.0001$, medium ES,$(\eta_g^2) = 0.1$) (Fig. 2A). Specifically, a decrease in serum testosterone levels was observed both after 6 weeks (premenopause, $P < 0.01$; postmenopause, $P < 0.001$) and 7 months (premenopause, $P < 0.001$; postmenopause, $P < 0.01$) after RRSO. ANOVA analysis showed a similar association of follow-up with serum androstenedione levels ($P < 0.0001$, low ES,$(\eta_g^2) = 0.06$) (Fig. 2B). Herein, only in

Table 1. Baseline characteristics

	Premenopausal	Postmenopausal	*P*-value	Overall *P*-value
n (%)	57 (61)	37 (39)		
Age, year—mean (SD)	44 (4)	57 (6)	<0.0001	
BMI, kg/m^2, median (IQR)	23.2 (22.0-25.7)	25.0 (22.3-27.5)	0.09	
Marital status, n (%)				0.73
Married/cohabitating	49 (86)	30 (81)		
Unmarried/ without partner	8 (14)	7 (19)		
Parity, n (%)				0.26
None	10 (18)	11 (30)		
≥1	47 (82)	26 (70)		
History of breast cancer, n (%)				0.51
Yes	21 (37)	16 (43)		
No	36 (63)	21 (57)		
DNA status, n (%)				0.01
BRCA 1/2 carrier	47 (83)	20 (54)		
Negative	6 (11)	12 (32)		
Unknown	4 (6)	5 (14)		
Comorbidities, n (%)				0.05
Pulmonary disease	0 (0)	3 (8)		
Cardiac disease	0 (0)	0 (0)		
Hypertension	2 (4)	2 (5)		
Stroke	0 (0)	0 (0)		
Renal disease	0 (0)	0 (0)		
Diabetes	0 (0)	0 (0)		
Arthralgia	5 (9)	8 (22)		
Psychological problems	3 (5)	0 (0)		
Malignancies (eg, BC and OC)	2 (4)	0 (0)		
None/unknown	45 (79)	24 (65)		
Days from T0 to RRSO, median (IQR)	3 (2-7)	4 (2-7)	0.89	

Abbreviations: BMI, body mass index; BC, breast cancer; IQR, interquartile range; OC; ovarian cancer; T0, baseline.

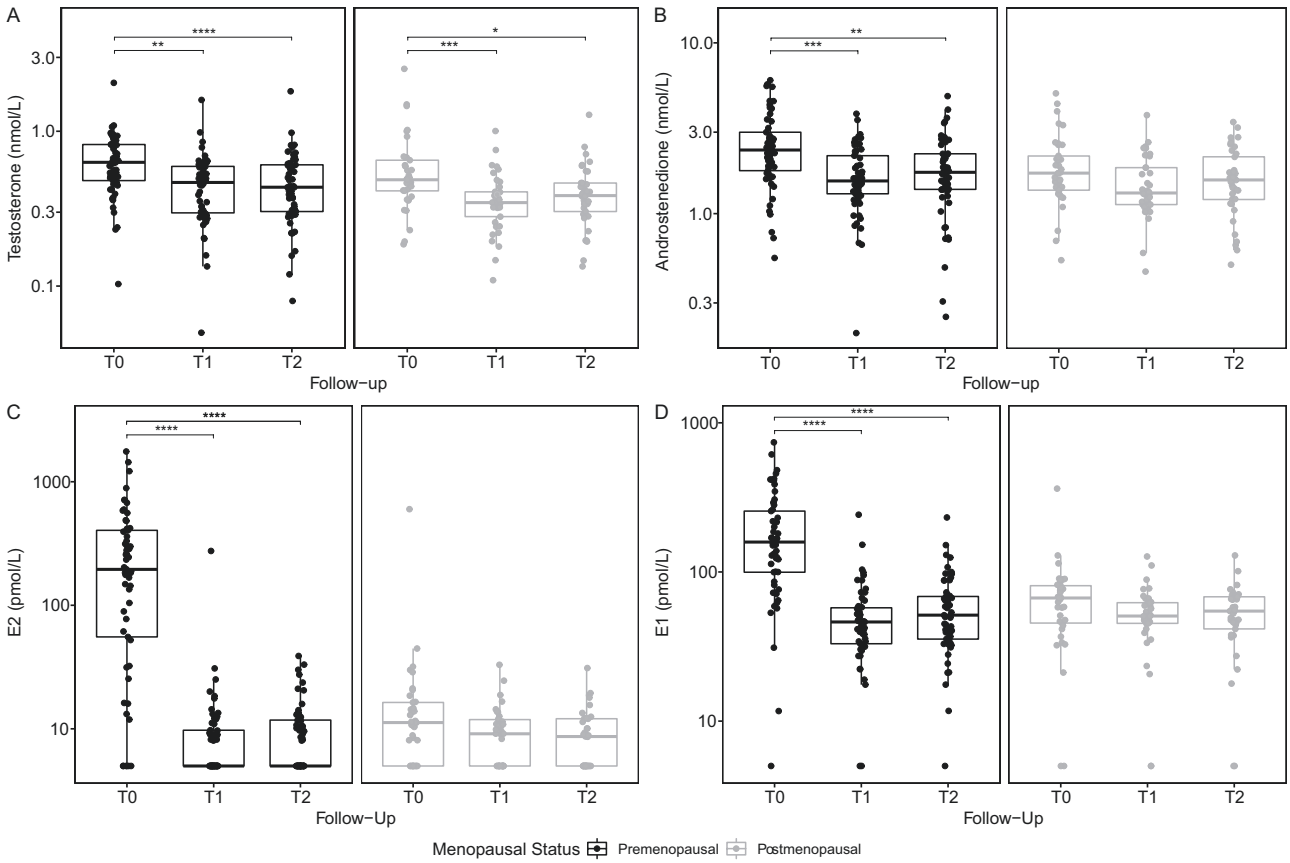

Figure 2. Box plot with log-normal transformed data for testosterone (A), androstenedione (B), E2 (C) and E1 (D) at each follow-up moment. Data were stratified for menopausal status (black, premenopausal; gray, postmenopausal). Significant pair-wise comparisons are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. Abbreviations: ANOVA, analysis of variance; E1, estrone; E2, estradiol; T0, baseline; T1, 6 weeks; T2, 7 months.

premenopausal women, a decrease in serum androstenedione levels 6 weeks ($P < 0.001$) and 7 months after RRSO ($P < 0.01$) was observed. For both E1 and E2 (Fig. 2C and 2D), serum concentrations deviated from a normal distribution, and thus no ANOVA analyses were performed. Pairwise comparisons between follow-up moments revealed decreases 6 weeks ($P < 0.0001$) and 7 months ($P < 0.0001$) after RRSO for both estrogens in premenopausal women. No differences in serum estrogen levels between follow-up moments were observed in postmenopausal women.

Relative individual changes between short- or long-term follow-up after RRSO and baseline serum sex steroid levels are displayed in Figure 3. While most premenopausal women demonstrated relative declines in serum steroid levels (81-93%) (Fig. 3A), a small group (7-19%) (Fig. 3A) had no change or an increase in serum steroid level. In 30% to 46% of the postmenopausal women (Fig. 3B), serum androstenedione, E1, and E2 was similar or increased both short and long term. In contrast, 16% and 27% of postmenopausal women (Fig. 3B) had increases in serum testosterone levels 6 weeks and 7 months after RRSO, respectively.

Figure 4 shows boxplots and differences in serum sex steroid levels between postmenopausal women at T0 (naturally postmenopausal) and premenopausal women at T1 and T2 (surgically postmenopausal). While serum testosterone and androstenedione levels were not different between these groups (Fig. 4A and 4B), E2 levels were lower in premenopausal T1 ($P < 0.01$) and T2 ($P < 0.05$) compared to postmenopausal T0 (Fig. 4C). Furthermore, E1 levels were lower in premenopausal T1 ($P < 0.05$), although not in premenopausal T2 (Fig. 4D) compared to postmenopausal T0.

Box plots of questionnaire scores are displayed in Figure 5 and Supplementary Figure 1 [39]. Herein, all sexual functioning questionnaire and menopausal complaint scores decreased, while HFRS sum scores increased in premenopausal women. In contrast, no differences were observed in postmenopausal women.

Finally, Figure 6 lists correlations of follow-up moment differences (ΔT0-1, ΔT0-2, and Δ T1-2) between steroid levels and questionnaire scores. In postmenopausal women, long-term (ΔT0-2) changes in HFRS sum scores and serum androgens were negatively correlated ($P < 0.05$). In addition, long-term (ΔT0-2) SFQ scores and serum estrogen levels were positively correlated (SFQ Desire, $P < 0.05$; SFQ Arousal L, $P < 0.01$; SFQ Arousal S, $P < 0.01$) in premenopausal women. In contrast, short-term (ΔT0-1) SFQ scores were positively correlated with serum androgen levels (SFQ Arousal L, $P < 0.05$) and serum estrogen levels (SFQ Orgasm, $P < 0.05$) in postmenopausal women. Correlations between steroids and SFQ pain, partner, and enjoyment scores were separately analyzed and are shown in Supplementary Figure 2 [39]. In premenopausal women, both estrogens were associated with SFQ Pain (ΔT0-2, $P < 0.05$) and SFQ Enjoyment (ΔT0-1, $P < 0.05$; ΔT0-2, $P < 0.05$).

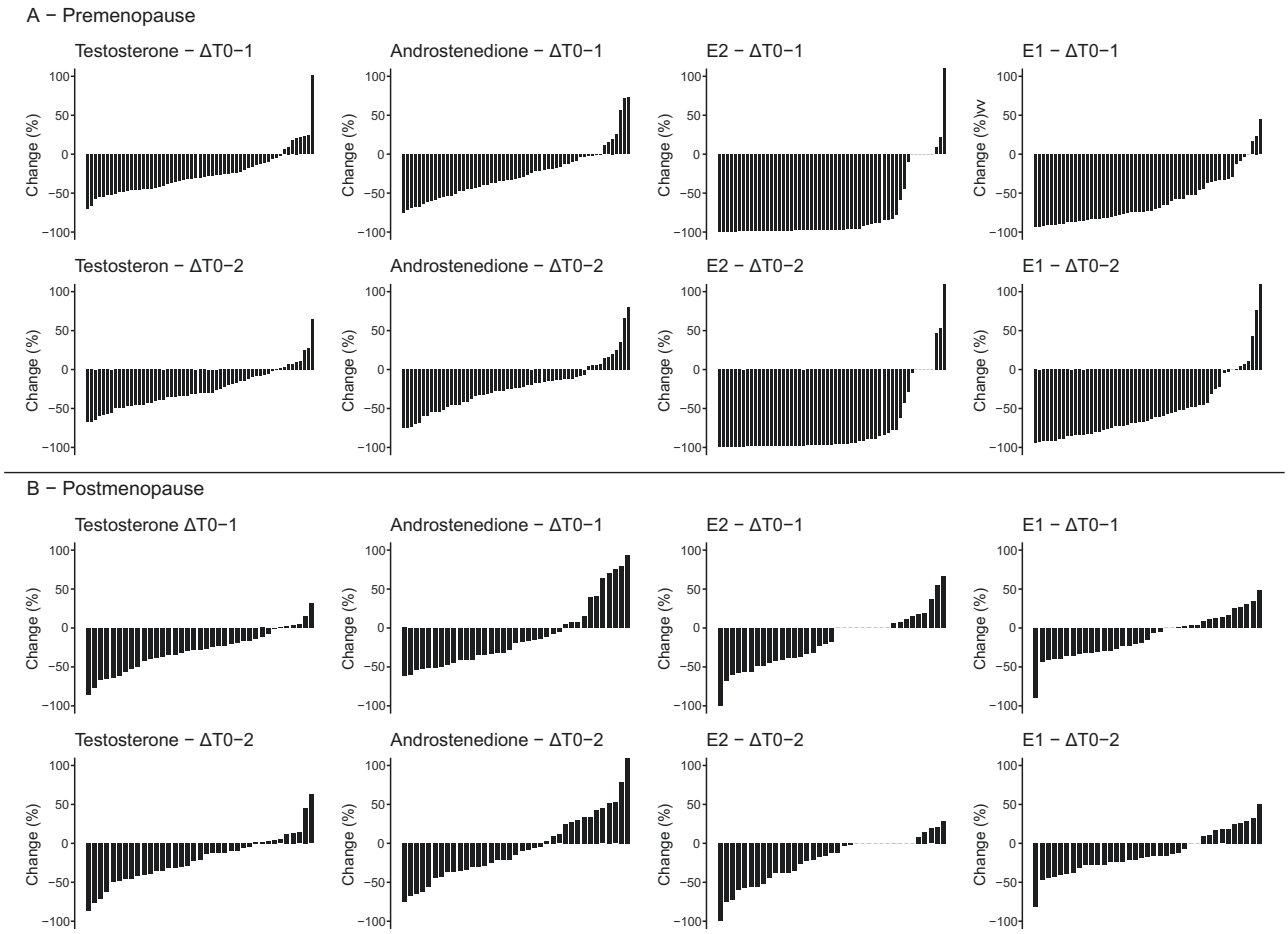

Figure 3. Waterfall plots showing relative individual changes between baseline and 6 weeks or 7 months after RRSO. Abbreviations: E1, estrone; E2, estradiol; RRSO; risk-reducing salpingo-oophorectomy; T0, baseline; T1, 6 weeks; T2, 7 months.

Discussion

The first aim of this study was to investigate the longitudinal changes in steroid levels in pre- and postmenopausal women at increased risk of hereditary or familiar risk of ovarian cancer who underwent RRSO. In premenopausal women, decreases were observed for all serum sex steroids, both short (6 weeks after RRSO) and long term (7 months after RRSO), while in postmenopausal women only serum testosterone levels were decreased after RRSO compared to baseline before RRSO. The results in postmenopausal women for testosterone, androstenedione, and E2 were in line with the findings of Judd et al and Fogle et al, whereas Stanczyk et al observed similar findings for testosterone, androstenedione, and E1 [23, 25, 43]. Notably, the study of Stanczyk et al also investigated circulating sex steroid levels pre- and post-RRSO in premenopausal women and expectedly found decreased levels for all 4 sex steroids. Furthermore, Davison et al and Laughlin et al also found lower circulating testosterone levels in older women (>55 years old) after RRSO, although androstenedione was not decreased [24, 44]. The latter authors also investigated E1 and E2 and found no differences between postmenopausal women with and without ovaries. Interestingly, Couzinet et al found no difference for both testosterone and androstenedione between postmenopausal women and oophorectomized women [26]. Discrepancies of our findings with previous results could be explained by the analytical methodology used. All previous studies depend on radioimmunoassays for the quantitation of circulating sex steroids. While radioimmunoassays, or immunoassays in general, provide considerable sensitivity, they lack specificity in low concentration ranges leading to unreliable results. To this end, mass spectrometry-based assays are regarded as the golden standard. Also, the design of the previous studies could explain these discrepancies with our findings. Notably, most studies were underpowered ($n < 20$) and/or were not prospectively designed, which could have introduced additional variation and bias in the data. Yet another explanation could be that our results are affected by variability in BMI. Although previous studies have demonstrated a relationship between BMI and circulating sex steroid levels [45-48], we did not find differences between baseline BMI and post-RRSO BMI or correlations between changes in BMI and changes in serum sex steroid levels.

In addition, we investigated differences in serum sex steroid levels between postmenopausal women before RRSO (natural menopause, T0) and premenopausal women at 6 weeks (T1) and 7 months (T2) after RRSO (surgically induced menopause). We found higher serum estrogen levels in postmenopausal women before RRSO compared to the premenopausal group with surgically induced menopause after RRSO, although no differences were found for testosterone and androstenedione. For testosterone, these results were in

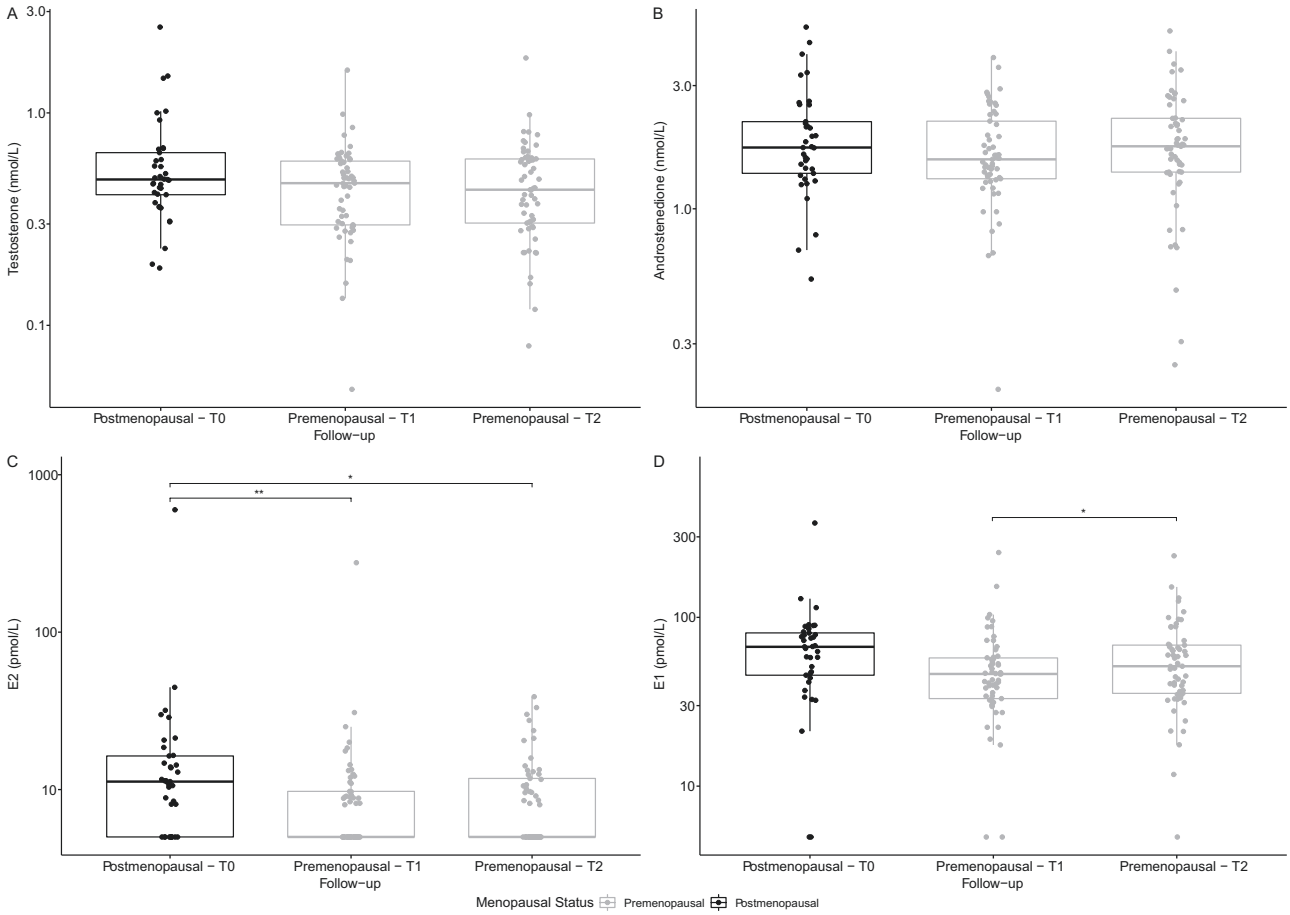

Figure 4. Box plots for comparison of naturally postmenopausal women (black) and surgically postmenopausal women (gray). Graphs for testosterone (A), androstenedione (B), E2 (C), and E1 (D) are displayed. Significant pair-wise comparisons are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. Abbreviations: E1, estrone; E2, estradiol; RRSO; risk-reducing salpingo-oophorectomy; T0, baseline; T1, 6 weeks; T2, 7 months.

line with one previous study [49], while another study found different results [50]. A possible explanation could be that residual testosterone production (eg, in the adrenal glands) slowly declines with increasing age as previously has been described [44, 51]. The difference in serum estrogen levels between naturally menopausal and surgically menopausal women is not in line with a previous study comparing naturally and surgically postmenopausal women, warranting further research into possible underlying mechanisms [49].

Our second aim was to examine the relationship between changes in steroid levels and changes in menopausal symptoms and sexual functioning. Our results suggest that in premenopausal women, larger decreases in estrogens are associated with a worsening of sexual functioning 7 months after RRSO. Furthermore, in postmenopausal women, larger decreases in androgens were associated with worsening of menopausal complaints (HFRS sum scores, ΔT0-T2) and sexual functioning (SFQ Arousal L, ΔT0-T1), although the latter association was not observed at ΔT0-T2. In addition, a decrease in estrogen level was associated with worsening sexual functioning (SFQ Orgasm), albeit only at ΔT0-T1. Notably, additional lowering of testosterone levels in postmenopausal women undergoing RRSO could therefore intensify menopausal complaints after 7 months and shortly (at 6 weeks) decrease sexual functioning. Therefore, these results suggest that postmenopausal women might benefit from testosterone supplements after RRSO. Notably, testosterone supplements are not available in Europe anymore for postmenopausal women experiencing problems with their sexual functioning. Experts increasingly urge on its potential clinical benefits [52, 53], although it should be mentioned that caution should be taken with unwanted aromatization of testosterone to E2 in women with high breast cancer risk (eg, BRCA1/2 mutant carriers).

While several studies have been performed investigating the impact of sex steroid supplements on sexual functioning and menopausal complaints after RRSO [14-17], only few studies investigated the relationship between steroid levels and sexual functioning questionnaire scores [21, 54]. In these studies, no relationship was found between serum sex steroids and sexual functioning after RRSO. The difference in findings could be explained by the used approach to investigate these associations. Specifically, multivariable regression and logistic regression were applied to predict the sexual discomfort score or sexual dysfunction. Furthermore, different questionnaires were used to assess sexual functioning (ie, the Female Sexual Function Index, the Sexual Activity Questionnaire, and the Female Sexual Distress Scale Revised [55-57]). Another factor could be the study design, which was cross-sectional instead of the cohort design in the present study. Also, the included women were grouped by sexual activity, not by menopausal status. While both approaches are statistically valid, this could have led to different outcomes.

Interestingly, no association between serum E2 levels and HFRS-sum was detected, and thus, our findings were not in

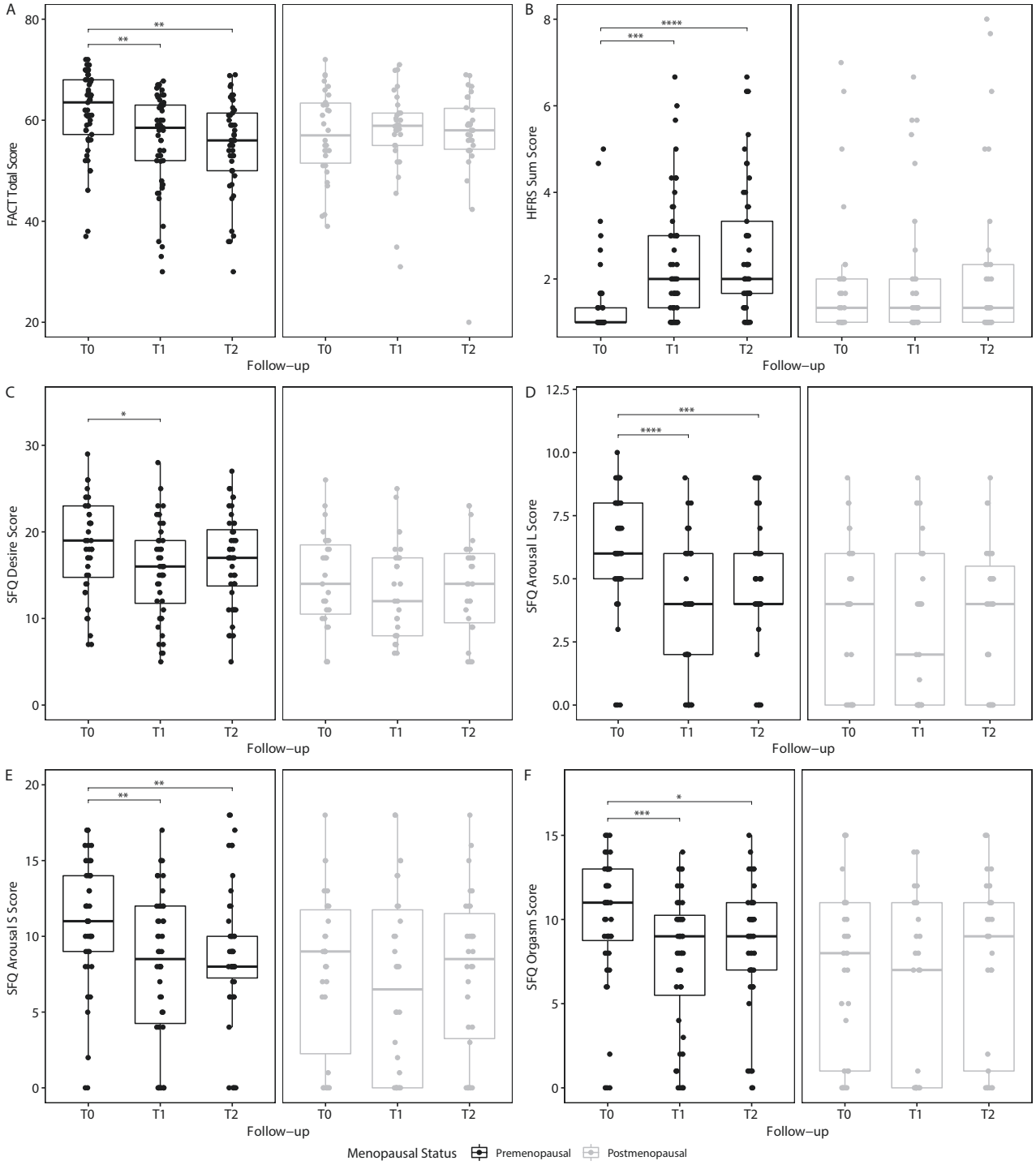

Figure 5. Box plots for FACT total (A), HFRS sum (B), SFQ Desire (C), SFQ Arousal L (D), SFQ Arousal S (E), and SFQ Orgasm (F) scores at each follow-up moment after RRSO. Data was stratified for menopausal status (black, premenopausal; gray, postmenopausal). Significant pair-wise comparisons are indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. Abbreviations: E1, estrone; E2, estradiol; FACT, Functional Assessment of Cancer Therapy; HFRS, Hot Flush Rating Scale; L, lubrication; RRSO; risk-reducing salpingo-oophorectomy; S, sensation; SFQ, Sexual Functioning Questionnaire; T0, baseline; T1, 6 weeks; T2, 7 months.

line with current clinical practice advising estrogen supplements to relieve menopausal complaints. This advice is based on multiple clinical studies investigating the efficacy and safety of estrogen supplements in postmenopausal women [17, 58]. While reports of associations between serum E2 levels and the intensity of menopausal complaints remain scarce, the SWAN study, a large clinical study investigating over 3000 women in menopause transition, found that low circulating E2 levels were associated with a higher prevalence of hot flashes [59]. This effect, however, was marginal, which could explain the absence of this association in our small cohort.

Some limitations were associated with our study. First, the time of blood withdrawal was not standardized. Serum steroid hormone levels are known to fluctuate according to

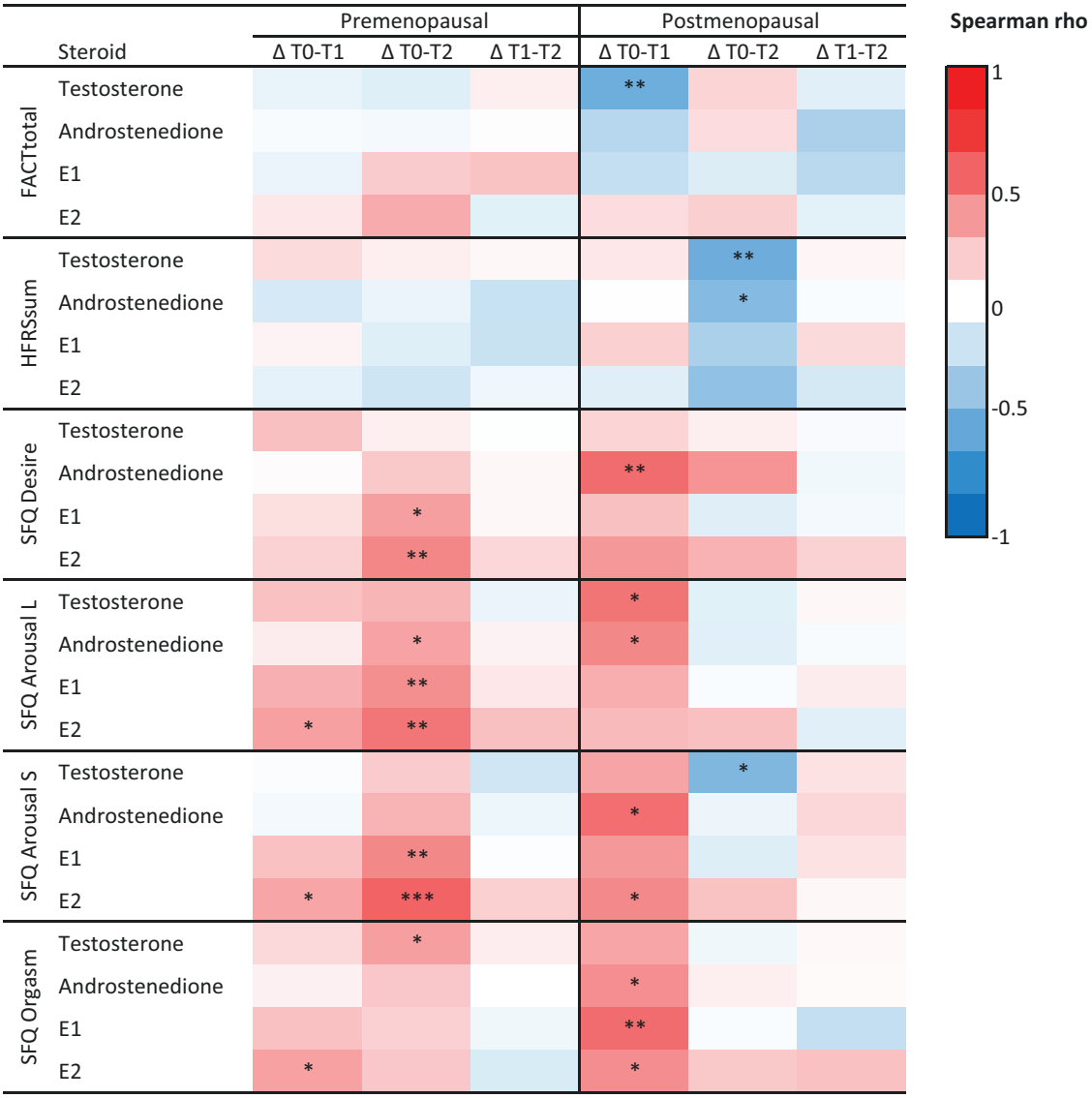

Figure 6. Spearman rho correlation matrix for correlations between changes in sex steroid levels and questionnaire scores. Correlation coefficients are color-coded at three levels from −1 (blue) to 0 (white) to 1 (red). Spearman's correlation coefficients that are significantly different from 0 ($\alpha = 0.05$) are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. Abbreviations: E1, estrone; E2, estradiol; FACT, Functional Assessment of Cancer Therapy; HFRS, Hot Flush Rating Scale; L, lubrication; RRSO; risk-reducing salpingo-oophorectomy; S, sensation; SFQ, Sexual Functioning Questionnaire; T0, baseline; T1, 6 weeks; T2, 7 months.

the circadian rhythm. This could have introduced variation into our data. To this end, we assessed whether sampling times before and after 11 am influenced serum sex steroid concentrations. We only found a significant difference for serum E1 levels in premenopausal women 7 months after RRSO (Mann-Whitney U, $P = 0.02$) indicating variation introduced by time of blood collection was limited. Second, for postmenopausal women at baseline, 1 serum E2 level was abnormally high (622 pmol/L) indicating that this individual (1) was actually premenopausal, (2) received estrogen supplementation, or (3) had an underlying E2-secreting tumor or (4) preanalytical or analytical errors were made. Although luteinizing hormone and follicle-stimulating hormone were within postmenopausal ranges and this individual had amenorrhea for 5 years and did not report estrogen supplementation intake, other factors explaining this high serum E2 level cannot be excluded. Third, participants were not screened for other treatments influencing steroidogenesis, such as corticosteroids and aldosterone antagonists. Although our results do not indicate treatment with these therapies, this could have potentially influenced serum sex steroid levels. Fourth, *P*-values in our correlation analysis were not adjusted for multiple hypothesis testing and could have resulted in type 2 errors. To reduce the prevalence of these errors, our investigation focused on questionnaire scores that correlated with both androgens or estrogens. Lastly, arguably marital status influences sexual functioning, which could have biased our results. However, we found that only SFQ Pain scores were affected by marital status in premenopausal women 6 weeks after RRSO and in postmenopausal women at baseline and 7 months after RRSO. This indicates that although there may have been some bias introduced in our analysis from differences in marital status, it appears to be limited.

In conclusion, our results, derived from a longitudinal prospective cohort using state-of-the-art serum steroid assays, show that in premenopausal women, removal of the ovaries is

accompanied by a decrease in serum androgens and estrogens both 6 weeks and 7 months after RRSO. For estrogens, these decreases were associated with a decline in sexual functioning. In postmenopausal women, our findings show that only serum testosterone is decreased after RRSO, which indicates that postmenopausal ovaries maintain some testosterone production. Furthermore, in these women, our findings suggest that removal of the ovaries, together with a decrease in serum testosterone level, results in more menopausal complaints and a short-term decline in sexual functioning. Therefore, these results support the Global Consensus Position Statement on the Use of Testosterone Therapy for Women [53], although further studies confirming these results are warranted.

Clinical Trial Information

Trial registration: toetsingonline.nl NL11371.031.06.

Disclosures

The authors have nothing to disclose.

Data Availability

The data set that was established from the clinical trial data to produce the results of the present study is not publicly available. The data set can be accessed upon reasonable request.

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